Crystal Structure of MutS2 Endonuclease Domain and the Mechanism of Homologous Recombination Suppression

Kenji Fukui, Noriko Nakagawa, Yoshiaki Kitamura, Yuya Nishida, Ryoji Masui, and Seiki Kuramitsu

From the RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148 and the Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1, Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan

DNA recombination events need to be strictly regulated, because an increase in the recombinational frequency causes unfavorable alteration of genetic information. Recent studies revealed the existence of a novel anti-recombination enzyme, MutS2. However, the mechanism by which MutS2 inhibits homologous recombination has been unknown. Previously, we found that Thermus thermophilus MutS2 (ttMutS2) harbors an endonuclease activity and that this activity is confined to the C-terminal domain, whose amino acid sequence is widely conserved in a variety of proteins with unknown function from almost all organisms ranging from bacteria to man. In this study, we determined the crystal structure of the ttMutS2 endonuclease domain at 1.7-Å resolution, which resembles the structure of the DNase I-like catalytic domain of Escherichia coli RNase E, a sequence-nonspecific endonuclease. The N-terminal domain of ttMutS2, however, recognized branched DNA structures, including the Holliday junction and D-loop structure, a primary intermediate in homologous recombination. The full-length of ttMutS2 digested the branched DNA structures at the junction. These results indicate that ttMutS2 suppresses homologous recombination through a novel mechanism involving resolution of early intermediates.

Homologous recombination is required for a variety of DNA transactions such as DNA repair, the rescue of stalled replication forks, and the creation of genetic diversity (1–4). The reaction begins with the introduction of a double strand break in the homologous region of the donor strand (5–7). The ends of the strand are resected by exonucleases to generate termini with 3'-overhangs. Then, RecA protein (Rad51 in eukaryotes) recognizes the single-stranded region of the DNA and catalyzes the invasion into the target double-stranded DNA to generate the D-loop structure. The sequential DNA synthesis and ligation yield a general intermediate called the Holliday junction. Several junction-specific endonucleases resolve the junction, and DNA ligase reses the nicks to complete the process of homologous recombination. This homologous recombination process is utilized not only for the recovery but also for the alteration of genetic information.

To avoid unfavorable alteration of genetic information, organisms are equipped with a series of enzymes that not only promote but also suppress homologous recombination (4). Recent studies have shown that bacterial and plant MutS2 proteins are candidate anti-recombination enzymes (8, 9). MutS2 is a paralogue of Escherichia coli MutS, which recognizes a mismatched base pair and induces the mismatch repair (MMR) pathway (Fig. 1). Proteins homologous to E. coli MutS are widely distributed and classified into two subfamilies, MutSI and MutSII, on the basis of amino acid sequence comparison (10). The former is responsible for MMR and includes bacterial MutS and eukaryotic MSH2, MSH3, and MSH6 (11–12). The latter, MutSII, is expected to not be involved in MMR but in recombinational events, and includes bacterial MutS2 and eukaryotic MSH4 and MSH5. It had been reported that eukaryotic MSH4 and MSH5 promote homologous recombination (13–15). In contrast, the disruption of Helicobacter pylori mutS2 increases the frequency of homologous recombination, indicating that H. pylori MutS2 suppresses homologous recombination (8, 9). Moreover, it has been shown that purified H. pylori MutS2 binds to a DNA structure mimicking the Holliday junction and interferes with the strand-exchange reaction mediated by RecA in vitro (8). However, the mechanism by which MutS2 inhibits the strand-exchange reaction has been unknown.

Previously, we found that Thermus thermophilus MutS2 (ttMutS2) possesses nicking endonuclease activity (16), and this activity is confined to the C-terminal domain, which does not exist in the other MutS homologues (17). This C-terminal domain is called the small MutS-related (Smr) domain. The amino acid sequence homologous to this domain is widely conserved in a variety of proteins with unknown function from almost all organisms, including Homo sapiens, Drosophila melanogaster, Saccharomyces cerevisiae, Caenorhabditis elegans, and E. coli (18, 19). It was also confirmed that the endonuclease activity of the T. thermophilus Smr domain (ttSmr) is sequence-nonspecific (20). However, it remains unclear how
the endonuclease activity is involved in the suppression of homologous recombination. The role of the N-terminal domain of ttMutS2 has also to be established.

In this study, we determined the crystal structure of ttSmr$_{663}$ (amino acid residues 663–774 of ttMutS2) at 1.7 Å resolution, which revealed the structural similarity between ttSmr and the DNase I-like catalytic domain of E. coli RNase E. The structural relevance of ttSmr to nonspecific endonucleases supports our previous finding of the sequence-nonspecific endonuclease activity of ttSmr. Furthermore, it has been clarified that the N-terminal domain of ttMutS2 recognizes branched DNA structures, including the D-loop structure, which is an early intermediate in homologous recombination. ttMutS2 incised the branched DNA structures at the junctions. These results strongly suggest the novel mechanism of MutS2 inhibiting homologous recombination through endonucleolytic resolution of the primary intermediates. Such a mechanism had previously been unknown.

**EXPERIMENTAL PROCEDURES**

*Prokaryote Preparations*—E. coli strains DH5α, BL21(DE3), and Rosetta-gami(DE3) (Novagen, Madison, WI) were cultured in YT broth (0.8% (w/v) tryptone, 0.5% (w/v) yeast extract, and 0.5% (w/v) NaCl) at 37 °C. Plasmids pET-11a/ttmutS2, pET-11a/N60-ttmutS2, and pET-15b/ttSmr$_{621}$, derived from pET-11a and pET-15b (Novagen), contain the complete, 3′-terminal region-deleted, and 5′-terminal region-deleted ttmutS2 genes, respectively, under control of a T7 promoter (17). ttMutS2, N60-ttMutS2, ttSmr$_{621}$, and *T. thermophilus* MutS1 (ttMutS1) were overexpressed and purified as described previously (17, 22). A DNA fragment expressing the ttSmr$_{663}$ domain was generated by PCR using the *T. thermophilus* HB8 genomic DNA as a template. The following primer pairs were used for amplification of the fragment: 5′-ATGATCATATTGCCGGAAGTGGAAGGAGTG-3′ and 5′-ATGATAGATCTTTATTACAAAGCCGGAAGGCACCACAC-3′. The forward and reverse primers contained Ndel and BgIII restriction sites, respectively (underlined). The amplified ttSmr$_{663}$ fragment was ligated into the Ndel and BamHI sites of pET-11a (Novagen). The *E. coli* smr (ydaL) gene was also amplified by PCR using the *E. coli* genome DNA as a template. The amplified gene fragment was ligated into the Ndel and BamHI sites of the pET-15b (Novagen) generating pET-15b/E. coli smr plasmid. Sequence analyses revealed that the constructions were error-free.

*E. coli* BL21(DE3) was transformed with pET-11a/ttmutsr$_{663}$ and then grown at 37 °C in 1.5 liters of YT medium containing 50 μg/ml ampicillin. When the density of cultures reached 4 × 10$^8$ cells/ml, isopropyl β-D-thiogalactopyranoside was added to 1 mM. The cells were grown at 37 °C for 4 h after induction and then harvested by centrifugation. The cells were then lysed by sonication in buffer I (20 mM Tris-HCl (pH 8.0), and 50 mM NaCl) and then heated to 70 °C for 10 min. After centrifugation at 48,000 × g for 60 min, the supernatant was loaded onto a SuperQ column (40 ml, TOSOH, Tokyo, Japan) pre-equilibrated with buffer I. The flow-through fraction was collected and loaded onto an SP-Sepharose column (40 ml, TOSOH) equilibrated with buffer I. The column was washed with 100 ml of buffer I and then eluted with a 300-ml gradient of 0.05–1 M NaCl in buffer I. The fractions containing ttSmr were detected by SDS-PAGE and concentrated using a Vivaspin concentrator (Vivasience, Hanover, Germany). The concentrated solution was applied to a Superdex 200 HR column (24 ml, GE Healthcare Biosciences, Uppsala, Sweden) pre-equilibrated with buffer I using an AKTA Explorer (GE Healthcare Biosciences). Because the lack of tryptophan and tyrosine residues in ttSmr$_{663}$ prevented us from determining the concentration using the absorbance intensity at 280 nm (23), the concentration of the protein solution was determined by means of the Bio-Rad Protein Assay with bovine serum albumin as the standard. The protein was stored at 4 °C. Peptide mass fingerprinting (24) confirmed that the purified protein was ttSmr$_{663}$. *E. coli* Smr was overexpressed by the same procedure as that for ttSmr$_{663}$ using the pET-15b/E. coli smr plasmid. The harvested cells were lysed by sonication in buffer II (20 mM Tris-HCl (pH 8.0) and 500 mM NaCl). After centrifugation, the supernatant was loaded onto a HiTrap Chelating HP5 column (5 ml) pre-equilibrated with buffer II containing 1 mM NiCl$_2$ using AKTA Explorer. The column was washed with buffer II containing 50 mM imidazole and then eluted with a 60-ml gradient of 0.05–0.5 M imidazole in buffer II. The fraction containing *E. coli* Smr was loaded onto a HiLoad 16/60 Superdex 200-20 pg column (120 ml) pre-equilibrated with the buffer containing 20 mM Tris-HCl (pH 8.0) and 200 mM NaCl. The eluted *E. coli* Smr solution was concentrated to 2.4 mg/ml using a Vivaspin concentrator. N-terminal amino acid sequencing analysis confirmed that the purified protein was *E. coli* Smr, and the protein was stored at 4 °C.

*X-ray Crystallographic Analysis of ttSmr$_{663}$*—The crystallization conditions for ttSmr$_{663}$ were surveyed by the hanging-drop vapor diffusion method by mixing 1 μl of a protein solution with an equal volume of a reservoir solution using crystal screen kits (Crystal Screen, Crystal Screen2 and Index, Hampton Research) at 20 °C. The protein concentration used was 10 mg/ml in 20 mM Tris-HCl (pH 8.0) and 50 mM NaCl. Crystals suitable for x-ray diffraction were obtained under the conditions of 100 mM citric acid and 25% polyethylene glycol 3350 at 20 °C. The crystal in the mother liquor was cryo-cooled in a nitrogen-gas stream. Data were collected at the wavelength of 1.0 Å at BL26B2 at Spring-8 (Hyogo, Japan). Diffraction images were processed with the HKL2000 program (25). Using the solution structure of the human Smr domain (2d9i) as a search model, the structure of ttSmr$_{663}$ was solved by molecular replacement. The program MOLREP was used for the

![FIGURE 1. A schematic representation of the primary structures of ttMutS1 and ttMutS2. Regions represented as mismatch-recognition, dsDNA-binding, and ATP-binding and dimerization domains in ttMutS1 are the counterparts of T. aquaticus MutS whose crystal structure has been solved (35). The dsDNA-binding and ATP-binding domains of ttMutS2 show >30% identity in the respective domains of ttMutS.](image)
rotation and translation searches (26). The model was refined using COOT (27) and REFMAC (28). The final model was refined to an R-factor of 18.7% and an R-free value of 22.2% and examined by PROCHECK (29). The structure coordinates have been deposited in the Protein Data Bank under accession number 2ZQE.

**Gene Disruption and in Vivo Experiments**—The 5’- and 3’-terminal 300-bp regions of ttmu52 were amplified by PCR from the pT7Blue/ttmu52 plasmid. A thermostable kanamycin resistance gene, HTK, was also amplified by PCR from pUC18/HTK plasmid DNA (30). The three DNA fragments were fused by the fusion PCR method (31) to obtain the pT7Blue/ttmu52::HTK plasmid for ttmu52 gene disruption. pT7Blue/ndx8::Hyg (hygromycin resistance gene) was a generous gift from Drs. Yoshinori Koyama and Takushi Ooga and was used for transformation experiments. The culture condition for *T. thermophilus* HB8 was described previously (30).

The rate of homologous recombination between the endogenous ndx8 gene and the exogenous DNA cassette, including the thermostable hygromycin resistance gene flanked by 300-bp of the 5’ and 3’ regions of ndx8, was measured. An overnight culture was diluted 1:60 with TT medium and then shaken at 70 °C for 2 h. An overnight culture was diluted 1:60 with TT medium and then shaken at 70 °C for 2 h and then spread on plates containing 20 μg/ml hygromycin. The plates were incubated at 70 °C for 30 h.

*E. coli* Rosetta-gami (DE3) strain was transformed with pET-11a, pET-11a/ttmu52, or pET-11a/N60-ttmu52 and then plated onto YT medium containing 50 μg/ml ampicillin. The cells were cultured in 3 ml of YT medium containing 50 μg/ml hygromycin. The plates were incubated at 70 °C for 30 h.

The synthesized 60-mer single-stranded DNA (ssDNA), 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, was labeled at the 5’-end with 32P, and then annealed with three synthesized ssDNAs, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, and 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’. To obtain the immobile Holliday junction lacking the homologous core, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, and 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, was labeled at the 5’-end with 32P, and then annealed with three synthesized ssDNAs, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, and 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’. To obtain the free(native) DNA, the synthesized 32P-labeled ssDNA was annealed to the complementary ssDNA (5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’) to obtain dsDNA containing a matched or mismatched base pair (X = T for perfectly matched homoduplex and G-T mismatched heteroduplex; Y = A for homoduplex; and Y = G for G-T mismatched heteroduplex). To obtain the mobile Holliday junction containing a homologous sequence, the synthesized ssDNA, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, was labeled at the 5’-end with 32P, and then annealed with three synthesized ssDNAs, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’, and 5’-GGGTGAACTCTGCGAGTTCGATTGACGTCATAGACAATGGATTGCTAGGACATCTTTGCCCACCTGCAGGTTCACCC-3’.

**Table 1** Data collection and refinement statistics

| Data collection        | Free (native) |
|------------------------|--------------|
| Space group            | p2 2 2       |
| Cell dimensions, a, b, c (Å) | 48.8, 54.2, 30.2 |
| Resolution (Å)         | 50.1-1.7 (1.76-1.7) |
| Rmerge                 | 0.047 (0.137)* |
| I/σI                   | 36.4 (13.2)  |
| Completeness           | 99.8 (100)   |
| Redundancy             | 6.3 (5.2)    |
| Measured reflections   | 58,499       |

Additional 5.5

Disallowed 0

Values in parentheses are for the outermost shell.

The refined model was deposited in the Protein Data Bank under accession number 2ZQE.

**TABLE 1** Data collection and refinement statistics

| Data collection        | Free (native) |
|------------------------|--------------|
| Space group            | p2 2 2       |
| Cell dimensions, a, b, c (Å) | 48.8, 54.2, 30.2 |
| Resolution (Å)         | 50.1-1.7 (1.76-1.7) |
| Rmerge                 | 0.047 (0.137)* |
| I/σI                   | 36.4 (13.2)  |
| Completeness           | 99.8 (100)   |
| Redundancy             | 6.3 (5.2)    |
| Measured reflections   | 58,499       |

Additional 5.5

Disallowed 0

Values in parentheses are for the outermost shell.

The refined model was deposited in the Protein Data Bank under accession number 2ZQE.

**TABLE 1** Data collection and refinement statistics

| Data collection        | Free (native) |
|------------------------|--------------|
| Space group            | p2 2 2       |
| Cell dimensions, a, b, c (Å) | 48.8, 54.2, 30.2 |
| Resolution (Å)         | 50.1-1.7 (1.76-1.7) |
| Rmerge                 | 0.047 (0.137)* |
| I/σI                   | 36.4 (13.2)  |
| Completeness           | 99.8 (100)   |
| Redundancy             | 6.3 (5.2)    |
| Measured reflections   | 58,499       |

Additional 5.5

Disallowed 0

Values in parentheses are for the outermost shell.

The refined model was deposited in the Protein Data Bank under accession number 2ZQE.
TGATCACTGGTAGCGG-3' and 5'-TAAGACTGGACT-CAGCTAGGTCATGGCTAACAT-3'.

Gel Shift Assay—The assay was performed as described previously (17). The 32P-labeled homoduplex and Holliday junction were incubated with 0–1000 nM ttMutS2 or N60-ttMutS2 in 50 mM Tris-HCl, 100 mM KCl, 1 mM dithiothreitol, 0 or 5 mM ADP, and 0.1 mg/ml bovine serum albumin (BSA, Takara), pH 7.5, for 30 min at 37 °C.

Nuclease Assay Using Oligonucleotides—The same substrate as that used in the gel shift assay was incubated with various concentrations of freshly prepared ttMutS2 in a buffer comprising 50 mM Tris-HCl, 100 mM KCl, 1 mM dithiothreitol, 2 mM MgCl2, and 0.1 mg/ml BSA, pH 7.5. Reactions were performed at 37 °C and stopped by the addition of an equal volume of sample buffer (5 mM EDTA, 80% deionized formamide, 10 mM NaOH, 0.1% bromphenol blue, and 0.1% xylene cyanol). The incubation time is indicated in the legends to the figures. The reaction mixtures were loaded onto 25% polyacrylamide gels or 11% polyacrylamide sequencing gels (8 M urea and 1× TBE buffer) and then electrophoresed with 1× TBE buffer.

Competition Experiment—The same substrates as that used in the gel shift assay were used here. The 32P-labeled homoduplex was mixed with 200 nM freshly prepared ttMutS2, N60-ttMutS2, or 500 nM ttSmr in a buffer comprising 50 mM Tris-HCl, 100 mM KCl, 1 mM dithiothreitol, 2 mM MgCl2, or 5 mM ADP and 0.1 mg/ml bovine serum albumin (BSA, Takara), pH 7.5, in the presence of various concentrations of the non-labeled homoduplex, Holliday junction, or D-loop structure (concentrations of competitors are indicated in the legends to figures). The mixtures were imme-
MutS2 Binds to and Incises Branched DNA Structures

FIGURE 4. Specificity of ttMutS2 for the branched DNA structures. A, gel shift assay. The percentage of complexed substrate to total substrate was plotted against the protein concentration. Circles: mobile Holliday junction; triangles: fork structure; squares: homoduplex; diamonds: heteroduplex. B, the unlabeled homoduplex or mobile Holliday junction was added as a competitor during DNA binding of ttMutS2 to 32P-labeled homoduplex in the presence of ADP. The concentrations of competitors were 0, 5, 10, 30, 60, and 120 nM. C, the same experiment as in B was performed using N60-ttMutS2 instead of ttMutS2. D, the relative percentages of the shifted signals to all signals in B and C were determined, assuming that the values at the points without a competitor were 100%. The homoduplex (circles) or mobile Holliday junction (triangles) was used as a competitor. Red: ttMutS2; Blue: N60-ttMutS2. E, the D-loop structure or mobile Holliday junction was used as a competitor during DNA binding of ttMutS2 to the 32P-labeled homoduplex in the presence of ADP. F, the relative percentage of shifted signals in D was plotted against the concentration of the competitor. The D-loop structure (circles) or mobile Holliday junction (triangles) was used as a competitor. G, the competition was performed using ttSmr621. The reaction conditions were the same as in B except for the competitor concentrations of 0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 μM. H, the relative percentage of shifted signals in J was performed using E. coli Smr.

FIGURE 5. ttMutS2 bound to the junction region of the branched structures. A, the 32P-labeled 60-bp homoduplex, mobile Holliday junction, or D-loop structure was digested by DNase I in the presence of various concentrations of N60-ttMutS2. The concentrations of N60-ttMutS2 were 0, 50, 100, and 200 nM, except for in the right panel, where the concentrations were 0, 10, 20, 50, and 100 nM. The reaction solutions were analyzed with a sequencing gel. 32P-labeled ttMutS2 or mobile Holliday junction was added as a competitor during DNA binding of ttMutS2 to 32P-labeled homoduplex in the presence of ADP.

Igor Pro 4.03 (WaveMetrics). Equation 1 was generated by assuming competitive inhibition, as below.

The dissociation constants of the enzyme for the substrate and competitor are given in Equations 1 and 2.

\[ K_d = \frac{[E][S]}{[ES]} \]  
\[ K_i = \frac{[E][I]}{[EI]} \]  

The total enzyme concentration, \([E]_0\), and the free enzyme concentration, \([E]\), are related by Equation 3.

\[ [E] = [E]_0 - [ES] - [EI] \]  

Also, the total competitor concentration, \([I]_0\), and the free competitor concentration, \([I]\), are related by Equation 4.

\[ [I] = [I]_0 - [EI] \]  

The total substrate concentration, \([S]_0\), was supposed to nearly equal to that of the free substrate, \([S]_\text{free}\).

From these equations, Equation 5 was obtained.

\[ \frac{[ES]}{[S]}_0 = \frac{([E]_0 - [I]_0)}{K_d + (K_i[S]_0/K_d + K_i - [E]_0 + [I]_0)^2 + 4(1 - K_d/([S]_0) (K_i[S]_0/K_d))^{1/2}} \]  

\[ K_i[S]_0/(K_i[S]_0/K_d + K_i) \]  

\[ K_d/([S]_0) (K_i[S]_0/K_d)^{1/2} \]  

\[ 2[S]_0(I - K_d/([S]_0)) \]  

\[ \text{Eq. 5} \]
DNase I Footprinting—DNase I footpinting was carried out by modifying the procedure originally described by Galas and Schmitz (32). The $^{32}$P-labeled homoduplex, mobile Holliday junction, or D-loop structure was incubated with various concentrations of N60-ttMutS2 or ttMutS1 in 50 mM Tris-HCl (pH 7.5) buffer containing 100 mM KCl, 5 mM MgCl$_2$, 5 mM ADP, and 0.1 mg/ml BSA at 37 °C for 1 h (concentrations of enzymes are indicated in legends to figures). Then, 1 μl of 0.35 unit/μl DNase I (Takara) was added, followed by incubation at 37 °C for 5 min. Reactions were stopped by the addition of an equal volume of a phenol-chloroform solution and the solutions were centrifuged at 15,000 g for 10 min. The supernatants were mixed with the sample buffer (5 mM EDTA, 80% deionized formamide, 10 mM NaOH, 0.1% bromphenol blue, and 0.1% xylene cyanol) and then heat-treated at 95 °C for 5 min. The length of the DNA product was subsequently determined by analysis on an 11% polyacrylamide sequencing gel containing 8 M urea and 1× TBE buffer.

Nuclease Assay Using Plasmid DNA—The assay was performed as described previously (17). The 5 ng/μl pUC19 (Takara) or pUC(AT) (New England Biolabs) plasmid DNA was incubated with or without freshly prepared ttMutS2 or bovine DNase I (Takara) in 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM MgCl$_2$, 0.1 mg/ml BSA, and 1 mM dithiothreitol at 37 °C. The concentrations of the enzymes and incubation times are indicated in the legends to the figures.

RESULTS

Crystal Structure of the ttMutS2 Endonuclease Domain—We prepared ttSmr$_{663}$ for x-ray crystallographic analysis. The ttSmr$_{663}$ was designed on the basis of the definition of the Smr domain (PF01713) in Pfam (33) and results obtained from limited proteolysis of full-length ttMutS2 and ttSmr$_{621}$ (amino acid residues 621–774 of

---

**Figure A**

DNase I footprinting was carried out by modifying the procedure originally described by Galas and Schmitz (32). The $^{32}$P-labeled homoduplex, mobile Holliday junction, or D-loop structure was incubated with various concentrations of N60-ttMutS2 or ttMutS1 in 50 mM Tris-HCl (pH 7.5) buffer containing 100 mM KCl, 5 mM MgCl$_2$, 5 mM ADP, and 0.1 mg/ml BSA at 37 °C for 1 h (concentrations of enzymes are indicated in legends to figures). Then, 1 μl of 0.35 unit/μl DNase I (Takara) was added, followed by incubation at 37 °C for 5 min. Reactions were stopped by the addition of an equal volume of a phenol-chloroform solution and the solutions were centrifuged at 15,000 g for 10 min. The supernatants were mixed with the sample buffer (5 mM EDTA, 80% deionized formamide, 10 mM NaOH, 0.1% bromphenol blue, and 0.1% xylene cyanol) and then heat-treated at 95 °C for 5 min. The length of the DNA product was subsequently determined by analysis on an 11% polyacrylamide sequencing gel containing 8 M urea and 1× TBE buffer.

**Figure B**

Nuclease Assay Using Plasmid DNA—The assay was performed as described previously (17). The 5 ng/μl pUC19 (Takara) or pUC(AT) (New England Biolabs) plasmid DNA was incubated with or without freshly prepared ttMutS2 or bovine DNase I (Takara) in 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM MgCl$_2$, 0.1 mg/ml BSA, and 1 mM dithiothreitol at 37 °C. The concentrations of the enzymes and incubation times are indicated in the legends to the figures.

**Figure C**

Crystal Structure of the ttMutS2 Endonuclease Domain—We prepared ttSmr$_{663}$ for x-ray crystallographic analysis. The ttSmr$_{663}$ was designed on the basis of the definition of the Smr domain (PF01713) in Pfam (33) and results obtained from limited proteolysis of full-length ttMutS2 and ttSmr$_{621}$ (amino acid residues 621–774 of
ttMutS2) (data not shown). It was confirmed that ttSmr<sub>663</sub> retained the endonuclease activity that converts the covalently closed circular form of plasmid DNA into the open circular form (data not shown).

The crystal structure of ttSmr<sub>663</sub> was determined at 1.7-Å resolution by means of molecular replacement methodology using the solution structure of the human Smr domain (2d9i) as a search model. The statistics for the data collection and model refinement are summarized in Table 1. The overall structure of the ttSmr<sub>663</sub> comprised an α/β-sandwich structure with a βαβαββ fold consisting a four-stranded β-sheet stacked against two α-helices (Fig. 2, A and B). The structures of ttSmr<sub>663</sub> and the human Smr domain are highly homologous to each other (Z-score = 10.65, root mean square deviation (r.m.s.d.) = 2.2 Å, sequence identity = 27%) (Fig. 2C), indicating that the Smr domains from all kingdoms have the same folds. Interestingly, a structural homology search using the MATRAS program (34) revealed that the overall structure of ttSmr<sub>663</sub> significantly resembles that of the catalytic domain of <i>E. coli</i> RNase E (2bx2) (Z-score = 6.63, r.m.s.d. = 2.9, sequence identity = 11%) (Fig. 2D), although there is no detectable sequence similarity between them. It has been pointed out that the structure of the RNase E catalytic domain resembles that of the N- and C-terminal domains of bovine DNase I, and the catalytic sites of these enzymes roughly coincide (35). In good agreement with this, the Dalii program (36) revealed the structural similarities between ttSmr<sub>663</sub> and the N-terminal domain of bovine DNase I (2dnj) (Z-score = 3.4, r.m.s.d. = 3.0 Å, sequence identity = 10%) (Fig. 2D).

Our crystal structure of ttSmr revealed the presence of Asp-669 and Arg-671 at a site spatially corresponding to Asp-303 and Asn-305 in <i>E. coli</i> RNase E (Fig. 2D, green sticks). The Asp-303 in <i>E. coli</i> RNase E is a catalytic residue coordinating a magnesium ion, and Asn-305 is supporting the orientation of Asp-303 through hydrogen bonding. The Asp-669 and Arg-671 form a salt bridge in Smr<sub>663</sub> (supplemental Fig. S1), indicating that Arg-671 fixes the orientation of Asp-669 and strengthens the acidity of the carboxyl group of the side chain. The region around Asp-669 is one of the most conserved regions among Smr sequences. Therefore, it can be expected that this aspartic residue is involved in the endonuclease activity of ttSmr. The crystal structure reported here contains no divalent metal ion probably because of the absence of the ions in the crystallization buffer. Unfortunately, we obtained no crystal when a divalent buffer. Unfortunately, we obtained no crystal when a divalent

FIGURE 6. ttMutS2 preferably digested the DNA containing branched DNA structures. A, pUC19 or pUC1AT was incubated with or without 5 nm DNase I. B, the same experiment as in A was performed with 0, 100, and 200 nm ttMutS2. The reaction solutions were incubated for 10 or 30 min (upper and lower panels, respectively). OC, Linear, and CCC indicate the nicked open-circular, linear, and covalently closed-circular forms of plasmid DNA, respectively. C, the <sup>32</sup>P-labeled homoduplex, mobile Holliday junction, loop, and D-loop structures were incubated with 200 nm ttMutS2 at 37 °C for 20 min. Schematic representations of the DNA substrates used are indicated above the lanes. Circles indicate positions of labels, D, various DNA structures were reacted with 200 nm ttMutS2 at 37 °C for 30 min. Schematic representations of DNA substrates used here are indicated below the graph. Signals yielding on the enzymatic digestions were counted, and the apparent rate constants were determined. HJ: Holliday junction. E, the homoduplex was incubated with 0, 20, or 50 nm ttMutS2 at 37 °C for 30 min, and then analyzed on a sequencing gel. F, the immobile Holliday junction was incubated with 0, 20, or 50 nm T7 endonuclease I or ttMutS2 at 37 °C for 30 min. G, the same experiment as in E was performed using the D-loop structure as a substrate. Black arrows and gray arrows indicate the products generated on the enzymatic digestion and the position of 30-mer ssDNA, respectively. Arrowheads in schematic representations of the DNA structures indicate incision sites in the substrates.

specific endonuclease activity of ttSmr. Our next goal is to identify the substrate of the endonuclease activity of ttMutS2.

ttmutS2 Is Involved in Suppression of Homologous Recombination—The in vivo experiments provided significant clues as to the substrate-specificity. The spontaneous mutation rate of ttmutS2-disrupted <i>T. thermophilus</i> was measured by monitoring the rate of acquisition of streptomycin resistance. The disruption of ttmutS2 did not cause a significant increase in the spontaneous mutation rate (data not shown). The rate of homologous recombination in the wild-type and ttmutS2-disrupted strains was also measured. A slight increase in the homologous recombination rate of ttmutS2-disrupted strain was observed compared with that of the wild-type strain (Fig. 3A). This result indicates that ttMutS2 is involved in the suppression of homologous recombination like <i>H. pylori</i> MutS2 (8, 9). We examined the effect of ttmutS2 disruption on the resistance to mitomycin-C (MMC), which induces both DNA intrastand and, to a greater extent, interstrand cross-links. As shown in Fig. 3B, the ttmutS2-disrupted strain showed higher resistance to MMC than the wild-type strain. It is thought that homologous recombination is responsible for the repair of interstrand cross-links (37). Therefore, the results we obtained here can be interpreted as the result of an increase in homologous recombination caused by ttmutS2 disruption. Furthermore, we examined the effect of ttmutS2 expression in <i>E. coli</i> on its UV light resistance. <i>E. coli</i> cells were transformed with expression vectors carrying wild-type ttmutS2, N60-<i>ttmutS2</i>, or no insert. N-60-ttMutS2 is the Smr-deleted N-terminal 60-kDa region of ttMutS2 and lacks the endonuclease activity. The transformants were exposed to 254 nm UV, and the survival ratio was measured. It is known that UV irradiation causes various DNA lesions, including thymine dimers and 6-4-pho- toproducts (38). Such lesions are repaired by nucleotide excision repair and other repair systems (39, 40). The un-repaired lesions lead to double strand breaks through DNA replication (41). Double strand breaks are generally repaired through homologous recombination; therefore, the suppression of homologous recombination has an effect on the UV resistance. As a result, expression of wild-type ttmutS2 resulted in a decrease in the survival ratio after UV irradiation (Fig. 3C), indicating that ttMutS2 prevented homologous recombination in the repair process. On the other hand, an expression of the N60-ttmutS2 mutant did not give rise to a serious decrease in the survival ratio. We have reported that the N60-ttMutS2 mutant binds to dsDNA as tightly as wild-type ttMutS2, however, N60-ttMutS2 does not retain the endonuclease activity (17). The endonuclease activity of ttMutS2 would be required for complete suppression of homologous recombination. Thus,
MutS2 Binds to and Incises Branched DNA Structures

the results of in vivo experiments implied that the intermediates in the homologous recombination might be the substrates of the ttMutS2 endonuclease activity.

**ttMutS2 Recognized the Branched DNA Structures at the Junction**—To determine the substrate specificity of ttMutS2, a gel shift assay was performed using various substrates, including perfectly matched linear dsDNA (homoduplex), G-T mismatched linear dsDNA (heteroduplex), the mobile Holliday junction, and the fork structure. The Holliday junction is a general intermediate in homologous recombination (5, 42). The mobile Holliday junction contains the homologous sequence at its core region, and spontaneous branch migration will occur. As shown in Fig. 4A, ttMutS2 appeared to bind the branched DNA structures more readily than linear dsDNAs. To determine the precise dissociation constants, a series of competition experiments was also performed using the non-radiolabeled homoduplex and mobile Holliday junction as competitors against the radiolabeled homoduplex. As a result, the Holliday junction was found to behave as a much more effective competitor than the homoduplex (Fig. 4B). The dissociation constants were determined to be 780 and 40 nM for the homoduplex and Holliday junction, respectively (Fig. 4D). The same results were obtained when N60-ttMutS2 was used instead of intact ttMutS2 (Fig. 4C), and its dissociation constants were determined to be 890 and 62 nM for the homoduplex and Holliday junction, respectively (Fig. 4D). The same competition experiment was carried out using T. thermophillus MutS1 (ttMutS1), a DNA mismatch-recognizing protein, as a control, which showed that there was no obvious difference between the dissociation constant of ttMutS1 for the homoduplex and that for the Holliday junction (data not shown). These results confirmed that ttMutS2 tightly binds to branched DNA structures and the Smr domain of ttMutS2 is not essential for the substrate recognition. Further experiments showed that the non-labeled D-loop structure inhibited the binding of N60-ttMutS2 to the radiolabeled homoduplex more potently than that to the Holliday junction (Fig. 4, E and F). The dissociation constant of N60-ttMutS2 determined for the D-loop structure was 15 nM. Because the D-loop structure is a primary intermediate in homologous recombination (7), ttMutS2 is likely to be involved in the early step of the homologous recombination process.

It has also been revealed that the ratio of shifted signals decreases when the reaction mixture does not contain ADP (supplemental Fig. S2A). However, ADP stabilized not only the ttMutS2-branched structure complex but also the ttMutS2-homoduplex one (supplemental Fig. S2, B and C). The same result was obtained when the D-loop structure was used as a competitor (data not shown). Thus, it was concluded that ADP did not affect the specificity of ttMutS2 for the branched DNA structures. It was also clarified that the addition of ATP had no obvious effect on the substrate specificity of ttMutS2 (data not shown).

Surprisingly, the competition experiment involving ttSmr621 revealed that not only the N-terminal domain of ttMutS2 but also ttSmr621 is capable of recognizing the branched DNA structure (Fig. 4, G and H). However, the dissociation constant for the Holliday junction was significantly higher than that of the N-terminal domain (Kd = 260 nM). In addition, the N-terminal domain bound to the branched DNA structures as strongly as full-length ttMutS2 (Fig. 4, B and C). These results indicate that the N-terminal domain mainly contributes to the branched-DNA recognition. We also examined the DNA-binding property of E. coli Smr (YdaL, NP_415856), which is the stand-alone type Smr domain. As a result, the E. coli Smr also recognized the Holliday junction with the dissociation constant of 370 nM (Fig. 4F). The modest affinity to branched DNA structure would be a common feature of all kinds of Smr domains.

We also attempted to determine the location of the N60-ttMutS2-binding site on the branched DNA structure using a DNase I footprinting assay. Junction-specific protection from DNase I digestion was observed when the mobile Holliday junction or the D-loop structure was used as the substrate (Fig. 5A), indicating that N60-ttMutS2 was located on the junction of the substrate DNA. Such junction-specific protection was not detected when a mismatch-recognition protein, ttMutS1, was used (Fig. 5B).

**ttMutS2 Preferably Digested Branched DNA Structures**—Because competition experiments and DNase I footprinting assays revealed that ttMutS2 preferably binds to branched DNA structures, nuclease activity assays of ttMutS2 were carried out using branched DNA structures as substrates. First, a plasmid DNA, pUC(AT), containing a cruciform structure was digested with ttMutS2. pUC(AT) was made by replacing the multiple cloning site sequence of pUC19 with 40-bp A/T repeats and used to assay the junction-resolving or structure-specific nicking endonuclease activity (43). As shown in Fig. 6B, ttMutS2 digested pUC(AT) more preferably than pUC19, a normal plasmid DNA, whereas a negative control DNase I equally digested the two kinds of plasmid DNAs (Fig. 6A). Second, three kinds of branched DNA structures, the immobile Holliday junction, and the D-loop and loop structures, were incubated with ttMutS2. As a result, it was obvious that the D-loop structure was the most preferable substrate for ttMutS2 among the branched DNA structures examined (Fig. 6C). As a control, it was shown that DNase I digested the homoduplex and the D-loop structure equally (supplemental Fig. S3A). Third, among other substrates, including fork, pseudo-Y, flap, and mobile Holliday junction structures, the most preferable substrate was the D-loop structure, although the loop and flap structures were digested at significant rates (Fig. 6D). The preference for the loop and flap structures would be due to the structural analogy of these substrates to the D-loop. ttSmr621 also digested the branched DNA structures more efficiently than the homoduplex (supplemental Fig. S3B).

The reacted solutions were also analyzed by electrophoresis on a sequencing gel to identify the incision sites. Digestion of the immobile Holliday junction (lacking the homologous core region) and the D-loop structure by ttMutS2 yielded specific digests that were not detected when linear dsDNA was reacted (Fig. 6, E–G). In particular, ttMutS2 incised the D-loop structure specifically at the junction sites (Fig. 6G). In contrast, ttMutS2 incised the Holliday junction roughly at the junction compared with a junction-specific endonuclease, T7 endonuclease I (Fig. 6E). These results strongly suggest that ttMutS2 is a branched structure-specific endonuclease and that the D-loop structure is a more favorable substrate than the Holl-
day junction. Resolution of the Holliday junction leads to the completion of homologous recombination, whereas digestion of the D-loop structure should result in inhibition of this process (Fig. 7). Thus, the results of in vitro studies showed good agreement with those of in vivo studies.

**DISCUSSION**

Our in vivo studies strongly suggest that ttmutS2 is, unlike other well known MutS homologues, involved not in DNA repair or promotion of homologous recombination but in suppression of homologous recombination. It is known that the MMR system suppresses homologous recombination by recognizing mismatched bases generated through the hybridization of imperfectly matched sequences (44). However, our previous study revealed that ttMutS2 does not recognize mismatched bases (16). In addition, disruption of ttmutS2 caused an increase in the frequency of recombination between perfectly matched sequences (Fig. 3B). Therefore, it is expected that MutS2 has another suppression mechanism. The expression test on the wild-type and nuclease activity-deficient ttmutS2 in E. coli implied that the endonuclease activity is required for ttMutS2 to suppress the homologous recombination (Fig. 3C). We also clarified that ttMutS2 preferentially incises the branched DNA structures, especially the D-loop structure over the Holliday junction (Fig. 6). The preference for the D-loop structure is in accordance with the results of in vivo experiments. The suppression mechanism through the resolution of an early intermediate (Fig. 7) is expected to be more effective than MMR system. This notion might be supported by the fact that naturally competent bacteria generally possess MutS2 (45).

It should be mentioned that ttMutS2 digested branched DNA structures other than the D-loop structure at the significant rate. We suspect that other factors may be involved in the structure recognition of ttMutS2. ATP/ADP-binding properties and interaction with other proteins can be considered. Although the ATP/ADP-binding properties of ttMutS2 did not affect the substrate specificity in this study, these properties may be correlated with the interaction of ttMutS2 with other proteins such as RecA localizing at the D-loop structure.

The N-terminal domain of ttMutS2 recognized branched DNA structures but ttMutS1 did not. Despite the high level of amino acid sequence similarity in the dsDNA-binding domain between ttMutS1 and ttMutS2, there seems to be a significant difference in the DNA-binding mode between the two proteins. It has been known that the eukaryotic MSH4/MSH5 heterodimer can also recognize the Holliday junction (15). The slight difference in primary structure of dsDNA-binding domain between MutS1 and MutSII would lead to the significant difference in substrate specificity. Structural analyses are needed to elucidate the nature of the substrate specificity.

To our surprise, not only the N-terminal domain of ttMutS2 but also ttSmr621 also recognized the branched DNA structure. It has been known that the configuration of subunits is strictly correlated with the branched DNA-recognition activity of many branched structure-specific endonucleases, including T7 endonuclease I and T4 endonuclease VII (46, 47). Guan and Kumar (43) reported that a single
MutS2 Binds to and Incises Branched DNA Structures

catalytic domain of the junction-resolving enzyme T7 endonuclease I non-specifically nicks the substrate DNA. Because ttSmr, unlike ttMutS2, lacks self-dimerization ability and possesses only weak DNA-binding activity (data not shown), the region between residues 621 and 663 may be involved in the structural configuration of the ttSmr subunits, which recognizes the branched structures. ttSmr, incised not only the D-loop structure but also other branched DNA structures to the same extent (data not shown). This broad substrate specificity also reminds us of that of T7 endonuclease I and T4 endonuclease VII (48, 49). The Smr domain in ttMutS2 may act as a branched DNA-specific endonuclease domain with broad substrate specificity, and the N-terminal domain of ttMutS2 will contribute to the strict recognition of the primary intermediate in homologous recombination.

The ability of ttSmr, to recognize the branched DNA structures indicates that the Smr domains of proteins with unknown function other than MutS2 also exhibit affinity to the DNA structures. This will support functional analyses of these proteins. For instance in S. cerevisiae, a function-unknown protein, Cue2 protein (accession number is NP_012833), has the Smr domain in its C-terminal region. A genome-wide study (51) has shown that Cue2 protein interacts with a subtelomeric protein, Tbf1, which negatively regulates telomerase-dependent elongation of the telomere (52). Although the mechanism underlying this regulation has been unknown, the branched-structure preference of the Smr endonuclease domain may be a clue for understanding it, because the telomere contains the branched DNA structures (53). Human NEDD4-binding protein 2 (N4BP2) also has the Smr domain in its C-terminal region. The human Smr domain possesses apparently nonspecific endonuclease activity (54), however, the function of N4BP2 remains unknown. The substrate specificity of the Smr domain may be utilized to clarify the function of N4BP2.

Acknowledgments—We express our great thanks to Drs. Takushi Ooga and Yoshinori Koyama for providing the tntds8::Hgy gene used in the transformation experiment, to Dr. Tsutomu Mikawa for preparation of proteins, to Drs. Hiroshi Kanzawa, Hisao Masukata, and Akira Shinohara for their critical advice regarding this study, to Naoko Aoki for constructing the expression vector of ttSmr, to Masami Nishida for purifying ttSmr, and to the colleagues in our laboratory for their useful discussions and technical support.

REFERENCES

1. Sonoda, E., Hochegger, H., Saberi, A., Taniguchi, Y., and Takeda, S. (2006) DNA Repair 5, 1021–1029
2. Weaver, D. T. (1995) Trends Genet. 11, 388–392
3. Shinohara, A., and Ogawa, T. (1995) Trends Biochem. Sci. 20, 387–391
4. Thomas, C. M., and Nielsen, K. M. (2005) J. Biol. Chem. 280, 274–280
5. Huang, S., and Blaser, M. J. (2005) J. Bacteriol. 187, 3528–3537
6. Eisen, J. A. (1998) Nucleic Acids Res. 26, 4291–4300
7. Modrich, P. (1989) J. Biol. Chem. 264, 6597–6600
8. Jiricny, J. (1998) Mutat. Res. 405, 107–121
9. Ross-Macdonald, P., and Roeder, G. S. (1994) Cell 79, 1069–1080
10. Hellingworth, N. M., Ponte, L., and Halsey, C. (1995) Genes Dev. 9, 1728–1739
11. Snowden, T., Acharaya, S., Butz, M. B., and Fishel, R. (2004) Mol. Cell 15, 437–445
12. Fukui, K., Masui, R., and Kuramitsu, S. (2004) J. Biochem. 135, 375–384
13. Fukui, K., Kosaka, H., Kuramitsu, S., and Masui, R. (2007) Nucleic Acids Res. 35, 860–870
14. Moreira, D., and Philippe, H. (1999) Trends Biochem. Sci. 24, 298–300
15. Malik, H. S., and Henikoff, S. (2000) Trends Biochem. Sci. 25, 414–418
16. Fukui, K., Takahata, Y., Nakagawa, N., Kuramitsu, S., and Masui, R. (2007) Nucleic Acids Res. 35, e100
17. Dinh, T., Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Takamatsu, S., Kato, R., and Kuramitsu, S. (1996) Nucleic Acids Res. 24, 640–647
19. Kuramitsu, S., Hiromi, K., Hayashi, H., Morino, Y., and Katagamiya, H. (1990) Biochemistry 29, 5469–5476
20. Kumarathasan, P., Mohottalage, S., Goegman, P., and Vincent, R. (2005) Anal. Biochem. 348, 85–89
21. Ortonowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
22. Vagin, A., and Teplyakov, A. (2000) Acta Crystallogr. Sect. D Biol. Crystallogr. 56, 1622–1624
23. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132
24. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–255
25. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
26. Hosoki, J., Yano, T., Koyama, Y., Kuramitsu, S., and Katagamiya, H. (1999) J. Biochem. 126, 951–956
27. Hitazono, A. A., Tohe, B. T., Kaltou, H., Diamant, N., and Kron, S. J. (2002) Yeast 19, 141–149
28. Galas, D. L., and Schmitz, A. (1978) Nucleic Acids Res. 5, 3157–3170
29. Finn, R. D., Mistry, J., Schuster-Bockler, B., Griffiths-Jones, S., Hollich, V., Lassmann, T., Moxon, S., Marshall, M., Khan, A., Dubin, R., Eddy, S. R., Sonnhammer, E. L., and Bateman, A. (2006) Nucleic Acids Res. 34, D247–D251
30. Kawabata, T. (2003) Nucleic Acids Res. 31, 3367–3369
31. Callaghan, A. J., Marcaida, M. J., Stead, J. A., McDowell, K. J., Scott, W. G., and Luisi, B. F. (2004) Nature 437, 1187–1191
32. Holm, L., and Sander, C. (1995) Trends Biochem. Sci. 20, 478–480
33. Noll, D. M., Mason, T. M., and Miller, P. S. (2006) Chem. Rev. 106, 277–301
34. Pfeifer, G. P., You, Y. H., and Besaratinia, A. (2005) Mutat. Res. 571, 19–31
35. Truglio, J. J., Croteau, D. L., van Houten, B., and Kisker, C. (2006) Chem. Rev. 106, 233–252
36. Gillet, L. C. J., and Scharer, O. D. (2006) Chem. Rev. 106, 253–276
37. Garinis, G. A., Mitchell, J. R., Moorhouse, M. J., Hanada, K., de Waard, H., Vanephelite, D., Janes, J., Brand, K., Smid, M., van der Spek, P. J., Hoeijmakers, J. H., Kanaar, R., and van der Horst, G. T. (2006) EMBO J. 25, 3952–3962
38. Duckett, D. R., Murchie, A. I. H., Diekmann, S., van Kitzing, E., Kemper, B., and Lilley, D. M. (1988) Cell 55, 79–89
39. Guan, C., and Kumar, S. (2005) Nucleic Acids Res. 33, 6225–6234
40. Worth, L., Clark, S., Radman, M., and Modrich, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3238–3241
41. Rocha, E. P. C., Cornet, E., and Michel, B. (2005) PLoS Genet. 1, e15
42. Hadden, J. M., Déclais, A. C., Carr, S. B., Lilley, D. M., and Phillips, S. E. (2007) Nature 449, 621–624
43. Bier tumfel, C., Yang, W., and Suck, D. (2007) Nature 449, 616–620
44. Mashal, R. D., Koontz, J., and Sklar, J. (1995) Nat. Genet. 9, 177–183
45. Salo, P. C., Birkenkamp, K., Pfeiffer, P., and Kemper, B. (1993) J. Mol.

33426 JOURNAL OF BIOLOGICAL CHEMISTRY
VOLUME 283 • NUMBER 48 • NOVEMBER 28, 2008
50. Lilley, D. M., and White, M. F. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9351–9353
51. Uetz, P., Giot, L., Cagney, G., Mansfield, T. A., Judson, R. S., Knight, J. R., Lockshon, D., Narayana, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J. M. (2000) Nature 403, 623–627
52. Berthiau, A.-S., Yankulov, K., Bah, A., Revardel, E., Luciano, P., Welling, R. J., Géli, V., and Gilson, E. (2006) EMBO J. 25, 846–856
53. Greider, C. W. (1999) Cell 97, 419–422
54. Watanabe, N., Wachi, S., and Fujita, T. (2003) J. Biol. Chem. 278, 26102–26110
55. Obmolova, G., Ban, C., Hsieh, P., and Yang, W. (2000) Nature 407, 703–710