Evidence That Nucleosomes Inhibit Mismatch Repair in Eukaryotic Cells*

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The influence of chromatin structure on DNA metabolic processes, including DNA replication and repair, has been a matter of intensive studies in recent years. Although the human mismatch repair (MMR) reaction has been reconstituted using purified proteins, the influence of chromatin structure on human MMR is unknown. This study examines the interaction between human MutSα and a mismatch located within a nucleosome or between two nucleosomes. The results show that, whereas MutSα specifically recognizes both types of nucleosomal heteroduplexes, the protein bound the mismatch within a nucleosome with much lower efficiency than a naked heteroduplex or a heterology free of histone proteins but between two nucleosomes. Additionally, MutSα displays reduced ATPase- and ADP-binding activity when interacting with nucleosomal heteroduplexes. Interestingly, nucleosomes block ATP-induced MutSα sliding along the DNA helix when the mismatch is in between two nucleosomes. These findings suggest that nucleosomes may inhibit MMR in eukaryotic cells. The implications of these findings for our understanding of eukaryotic MMR are discussed.

DNA mismatch repair (MMR) is an essential genome surveillance pathway that corrects mismatches generated during DNA replication and repair (1–3). The MMR pathway and its component enzymes are highly conserved from bacteria to humans, and the mechanism of the MMR reaction, although more complex in higher organisms, is related in all organisms studied to date. The steps of the MMR pathway include mismatch recognition by MutS-like proteins, removal of the mispaired nucleotide by nucleases in a manner dependent on MutS- and MutL-like proteins, and DNA repair synthesis by a replicative DNA polymerase in concert with DNA replication factors (3). In comparison with Escherichia coli MMR, human MMR is more complicated, involves more proteins and cofactors, and has more diverse biological roles, including DNA damage signaling (3, 4). It is also highly likely that chromatin structure influences the efficiency of MMR in human and other eukaryotic cells and that the mechanism of MMR in eukaryotic cells reflects this additional layer of complexity.

The nucleosome is the basic structural unit of eukaryotic chromatin, and is comprised of an octamer of histone proteins wrapped with a DNA duplex of ~147 bp. The histone octamer contains a central (H3-H4)2 tetramer flanked on either side by a H2A-H2B dimer. During DNA replication, nucleosomes undergo dynamic disassembly/assembly, such that nucleosomes ahead of the replication fork are disrupted and then rapidly re-assembled on nascent DNA behind the replication fork (5). Increasing evidence suggests that MMR is coupled with DNA replication (3, 6–9) and that active MMR complexes may slide over long distances (i.e. as far as 1000 bp) along DNA during MMR (10–12). Although human MMR has been reconstituted with purified proteins in vitro using naked DNA heteroduplexes (13, 14), the influence of chromatin structure on human MMR has not been analyzed. Therefore, it is not known how human MMR proteins recognize and interact with mismatches in nascent or damaged DNA.

The goal of this study was to analyze the interaction between human MutSα (MSH2-MSH6 heterodimer) and a single mismatch in the context of several simple model nucleosome substrates. The results show that MutSα specifically recognizes a mismatch located in or adjacent to a nucleosome. However, MutSα bound the mismatch with lower efficiency in histone octamer-bound DNA than in histone octamer-free DNA, and ATP- provoked sliding of MutSα along DNA helices is blocked when the mismatch is flanked on each side by two nucleosomes. These observations strongly support a notion that chromatin remodeling and/or modification factors are required for MMR in eukaryotic cells.

EXPERIMENTAL PROCEDURES

DNA Preparation—Two complementary 147-mer fragment oligonucleotides (DNA substrate I, Fig. 1A) containing tandem repeats of a high affinity histone octamer-binding DNA sequence, TATAAAGGCC (15), were synthesized in The Midland Certified Reagent Company (Midland, TX). After high-performance liquid chromatography purification, the synthetic oligonucleotides were annealed and 5′-end-labeled using T4 polynucleotide kinase and [γ-32P]ATP. The resulting substrate I, which contains a centrally located G-T mismatch flanked by BglII and HindIII restriction enzyme cleavage sites (Fig. 1A, substrate I), was further purified by agarose gel electrophoresis.

DNA substrate II (200-bp DNA fragment) was constructed by PCR using a plasmid DNA containing the high affinity nucleosome positioning sequence 601 as a template (16), a gift from Timothy Richmond (Eldgenössiche Technische Hochschule, Zürich, Switzerland). Two alternate forward primers with a 5′-end-labeled biotin were combined with a unique reverse primer, as follows,
with restriction enzyme recognition sites shown in underlined font and the mismatch position in bold font: Forward primer 1, 5′-AAGGTCTGACGTGCAAGCTGACCCGCTGATCATTCAAGCTGAGATCCCGGTCG-3′; Forward primer 2, 5′-AAGGTCTGACGTGCAAGCTGACCCGCTGATCATTCAAGCTGAGATCCCGGTCG-3′; Forward primer 3, 5′-ACAGGATTGTATATCTGGAC-3′.

The PCR products were purified by agarose gel electrophoresis, mixed together, heat-denatured, re-annealed by slow cooling, digested with XhoI to cleave homoduplex products, and re-purified by agarose gel electrophoresis. The XhoI-resistant DNA, which contains either a G-T mismatch or an A-C mismatch, was extracted from the gel and 5′-end-labeled with [γ-32P]ATP by T4 polynucleotide kinase.

Nucleosome subunit III was derived from PCR amplification of M13mp18-UKY derivatives (17) that contain two pieces of the 601 nucleosome positioning sequence, with one 601 sequence within the EcoRI-KpnI region and another within the XhoI-HindIII (for M13mp18-UKY1) or NsiI-HindIII (for M13mp18-UKY2). The resulting PCR products were purified by agarose gel electrophoresis and mixed together, heat-denatured, re-annealed by slow cooling, and followed by digestion with XhoI and NsiI to cleave homoduplex products. The heteroduplexes, which contain either a G-T or an A-C mismatch, were re-purified by agarose gel electrophoresis and 5′-end-labeled with [γ-32P]ATP using T4 polynucleotide kinase.

Protein Purification—Recombinant human MutSα was expressed in insect cells and purified to near homogeneity as described previously (14). Human histone genes (kindly provided by Dr. Jeffrey Parvin, Ohio State University) encoding for H2A, H2B, H3, and H4 were expressed in E. coli, and the purified histone proteins were used to assemble histone octamers as described previously (18).

Nucleosome Assembly and Purification—Nucleosomes were assembled from purified histone octamers and DNA substrates shown in Fig. 1A using the salt-dilution method (19). Reconstituted nucleosomes were separated from free DNA by 5–30% glycerol gradient, and fractions were collected using a Beckman fast-protein liquid chromatography Superdex 200 column (data not shown) and verified by SDS-gel electrophoresis (Fig. 1B). Nucleosomes were assembled using the salt dilution method (19) from purified histone octamers and DNA substrates shown in Fig. 1A. Substrates I (147 bp) and II (200 bp) form mononucleosomes, and substrate III (428 bp) forms dinucleosomes. The resulting nucleosome substrates were purified by glycerol gradient sedimentation and verified by restriction enzyme digestion and gel electrophoresis. As shown in Fig. 2, nucleosomes migrated more slowly than their corresponding DNA substrates as if the latter had gained ~200 bp per histone octamer (Fig. 2, A–C), which has a mass approximately equal to that of 200-bp DNA (23, 24). Restriction enzyme susceptibility of individual DNA substrates and their corresponding mononucleosome were also examined. As expected, the substrate I naked DNA (Fig. 2D, lane 2) and its nucleosome (Fig. 2D, lane 5) were resistant to cleavage by XhoI, because the G-T mismatch is in the XhoI recognition site. Naked substrate I DNA

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FIGURE 1. DNA heteroduplexes and human histone octamers used in this study. A, schematic diagram of DNA substrates. Substrate I is a 147-bp fragment containing tandem repeats of a high affinity histone octamer-binding DNA sequence and a centrally located G-T mismatch. Substrate II is a 200-bp heteroduplex containing the high affinity nucleosome positioning sequence 601 in one terminus and a biotin residue (black sphere) in the other terminus. Substrate III is a 428-bp heteroduplex consisting of two pieces of the 601 nucleosome positioning sequence. Open triangles in each case represent the mismatch. B, analysis of purified recombinant human histone octamer by 18% SDS-PAGE.

RESULTS

Reconstitution and Characterization of Mismatch-containing Nucleosomes—Histone octamers were assembled from purified recombinant human histone proteins and purified by a fast-protein liquid chromatography Superdex 200 column (data not shown) and verified by SDS-gel electrophoresis (Fig. 1B). Nucleosomes were assembled using the salt dilution method (19) from purified histone octamers and DNA substrates shown in Fig. 1A. Substrates I (147 bp) and II (200 bp) form mononucleosomes, and substrate III (428 bp) forms dinucleosomes. The resulting nucleosome substrates were purified by glycerol gradient sedimentation and verified by restriction enzyme digestion and gel electrophoresis. As shown in Fig. 2, nucleosomes migrated more slowly than their corresponding DNA substrates as if the latter had gained ~200 bp per histone octamer (Fig. 2, A–C), which has a mass approximately equal to that of 200-bp DNA (23, 24). Restriction enzyme susceptibility of individual DNA substrates and their corresponding mononucleosome were also examined. As expected, the substrate I naked DNA (Fig. 2D, lane 2) and its nucleosome (Fig. 2D, lane 5) were resistant to cleavage by XhoI, because the G-T mismatch is in the XhoI recognition site. Naked substrate I DNA

serum albumin, 0.5 mM EDTA, and 5% glycerol in the presence or absence of 5 mM MgCl₂. Where specified, nucleosomal DNA duplexes were added 10 min prior to addition of nucleotide. MutSα was mixed with [α-32P]ATP, [γ-32P]ATP, or [α-32P]-ADP and incubated for 10 min. Samples were then subjected to 10 min of UV cross-linking (Stratalinker), followed immediately by electrophoresis through 8% SDS-PAGE gels. Radiolabeled bands were quantified using a PhosphorImager. [α-32P]-ADP was generated by incubating [γ-32P]ATP with hexokinase and purified as described before (22). ATPase activity of MutSα was assayed in 20-µl reactions containing 50 mM Tris–HCl (pH 8.0), 110 mM NaCl, 0.5 mM EDTA, 5 mM MgCl₂, [γ-32P]ATP. After incubation at 37 °C for 10 min, the reactions were terminated and fractionated through a 20% denaturing polyacrylamide gel. 32P-Containing species were detected by a PhosphorImager.
but not its nucleosome was sensitive to cleavage by BgIII and HindIII (Fig. 2D, lanes 3 and 4 compared with lanes 6 and 7), indicating that the histone octamer blocks access of the restriction enzymes to the DNA (i.e. steric hindrance). Both substrates II and III contain the high affinity nucleosome positioning sequence 601 (16). Indeed, nucleosomes assembled with these two DNA substrates are located in the 601 sequence, as judged by the fact that substrate II nucleosome was sensitive to digests by HindIII (Fig. 2E); and substrate III nucleosome was sensitive to cleavage by KpnI, which converts dinucleosomal substrate III into two mononucleosomes (Fig. 2F).

Nucleosome Structure Inhibits Binding of MutSα to a Mismatch—EMSAs were used to examine interactions between human MutSα and the 147-bp DNA heteroduplex (i.e. substrate I in Fig. 1A) or its corresponding nucleosome. As shown in Fig. 3A, MutSα bound the heteroduplex nucleosome (lanes 8 –14) but with lower efficiency than the naked heteroduplex (lanes 1 –7), because a higher minimum amount of MutSα was required to supershift the nucleosome than the 147-bp DNA fragment. This binding reflects a specific interaction with the mismatch, because the heteroduplex nucleosome (Fig. 3A, lanes 19 –22) but not the 147-bp homoduplex nucleosome (Fig. 3A, lanes 15 –18) was supershifted in the presence of 20-fold excess homoduplex competitor DNA. This result shows that, although a histone octamer-bound mismatch is specifically recognized by MutSα, the binding affinity is reduced by the presence of the histone octamer. It is noted that, although the naked DNA substrate and its nucleosome differ obviously in their electrophoresis mobility, it is not obvious to distinguish their complexes with MutSα (Fig. 3A). This is likely due to binding of multiple molecules of MutSα to the naked DNA substrate.

Previous studies showed that ATP and non-hydrolyzable ATP analogs inhibit MutSα binding to heteroduplexes (25), likely by inducing a conformational change in MutS proteins, followed by DNA end-dependent dissociation of MutS proteins from DNA substrates (11, 26). We therefore tested the ATP effect on MutSα binding to nucleosomal heteroduplex. As shown in Fig. 3B, addition of ATP (lanes 6 –9) or ATP analogy AMP-PNP (lanes 10 –13) to EMSA reactions induced rapid dissociation of the MutSα-nucleosome complex, as free nucleosomes appeared in early time reactions. These results suggest
that ATP also inhibits interactions between MutSα and nucleosomal heteroduplexes.

**Nucleosome Acts as a Barrier for MutSα Sliding**—The modeling of MMR (3) suggests that MutS and its homologs move bi-directionally away from the mismatch and dissociate from the DNA when it reaches a DNA terminus (11, 26). However, this process has never been examined on a chromatin-like template, and the impact of nucleosomes on the ability of MutSα to slide and dissociate from heteroduplex DNA has not been explored. To examine this question, mononucleosomes were formed with a 5′-biotin-labeled 200-bp fragment with a unique G-T or A-C mismatch and HindIII restriction site upstream of the 601 nucleosome positioning sequence (Fig. 1A, substrate II). Because the resulting nucleosome (Fig. 2B) remained sensitive to cleavage by HindIII (Fig. 2E), the octamer bound stably and was positioned by the 601 sequence, and the mismatch remained nucleosome-free. This nucleosomal heteroduplex was used to examine DNA sliding by MutSα in the presence or absence of streptavidin and ATP or AMP-PNP (Fig. 4). Because the streptavidin-biotin complex blocks dissociation of MutSα from DNA termini (27–29), this experiment allowed us to test whether the stably bound histone octamer downstream of the mismatch inhibits dissociation of MutSα from the terminus.

The results show that MutSα bound to the nucleosomal heteroduplex with or without streptavidin as efficiently as it bound to the corresponding naked heteroduplex (data not shown). This is not surprising, because the mismatch is in a histone octamer-free region of the substrate. In the presence of ATP, ∼50% of MutSα dissociated from the heteroduplex nucleosome without streptavidin (Fig. 4A, lanes 9–12); however, in the presence of streptavidin, the MutSα-nucleosome complex was stable in the presence or absence of ATP (Fig. 4A, lanes 3–7) or AMP-PNP (Fig. 4B). These data strongly suggest that ATP- or AMP-PNP-induced sliding/dissociation of MutSα can be also blocked by a stably bound histone octamer.

We further tested this idea using a dinucleosome derived from DNA substrate III (Fig. 1A), in which a mismatch is located in a histone octamer-free region between two mononucleosomes as the nucleosomal substrate is sensitive to cleavage by KpnI (see Figs. 1A and 2F). MutSα bound efficiently to nucleosome-free substrate III, and this interaction was inhibited in the presence of ATP (Fig. 5, lanes 7–10), as expected for naked DNA. In contrast, MutSα formed a stable complex with dinucleosomal substrate III, which persisted in the presence of ATP (Fig. 5, lanes 2–5). This result is consistent with the notion that stably bound histone octamers flanking a mismatch inhibit MutSα sliding and subsequent dissociation of the MutSα-DNA complex.

**Effects of Nucleosomal Heteroduplexes on ATPase and Nucleotide Binding Activities of MutSα**—MutSα possesses an ATPase activity essential for MMR. Previous studies have shown that DNA heteroduplexes stimulate MutSα ATPase activity (27, 30). To determine the effect of nucleosome on MutSα ATPase, we examined ATP hydrolysis by MutSα during its interactions with individual DNA heteroduplexes or their corresponding nucleosomes. As expected, all three naked DNA heteroduplexes stimulated MutSα ATPase activity to at least 2.5-fold (Fig. 6, lanes 3–5) as compared with MutSα alone (Fig. 6, lane 2). However, the stimulation by nucleosomal heteroduplexes differs from naked DNA substrates. First, there was essentially no stimulation of the MutSα ATPase activity by substrate I nucleosome (lane 6), which might be due to the weak interaction between MutSα and the nucleosome (see Fig. 3). Second, nucleosomes derived from DNA substrates II and III did enhance ATP hydrolysis by MutSα, but the stimulation was weaker compared with naked DNA substrates (compare lanes 7 and 8 with lanes 4 and 5). Because nucleosome substrate I has a lower affinity for MutSα, and because nucleosomal substrates II and III, although bound normally by MutSα, possess limited
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The presence of Mg\textsuperscript{2+} reflects changes in the ADP-binding ability of MSH2 or simply determines if the differences in ADP binding among reactions identical to the ATP hydrolysis profile shown in Fig. 6. To substrate I (Fig. 7 with the least labeling in the reaction containing nucleosomal naked DNA substrates than those with nucleosomal substrates, were detected by using a Storm PhosphorImager. nucleosomal heteroduplexes and 5 mM MgCl\textsubscript{2}, followed by UV cross-linking and SDS-PAGE indicated, in the presence or absence of the indicated DNA or nucleosomal substrates. The reactions were incubated at 37 °C for 20 min, followed by electrophoresis as described under "Experimental Procedures." The \( ^{32}\text{P} \)-labeled species were detected and quantified by using a PhosphorImager. \( ^{32}\text{P} \)-labeled MutS \( \alpha \) was incubated with \( [\gamma-^{32}\text{P}]\text{ATP} \), \( [\alpha-^{32}\text{P}]\text{ATP} \), or \( [\alpha-^{32}\text{P}]\text{ADP} \), as indicated, in the presence or absence of the indicated DNA or nucleosomal heteroduplexes and 5 mM MgCl\textsubscript{2}, followed by UV cross-linking and SDS-PAGE as described under "Experimental Procedures." The \( ^{32}\text{P} \)-cross-linked subunits were detected by using a Storm PhosphorImager.

### DISCUSSION

Growing evidence supports a role for chromatin structure in MMR. Genetic studies in yeast suggest that local chromatin structure influences MMR efficiency, because mutation rates of an identical polyGT tract varied significantly in different locations of the yeast genome (31). A recent biochemical study revealed that regulatory factor X, a chromatin remodeling factor that regulates gene expression, stimulates MMR in vitro (32). In this study, we directly tested possible effects of nucleosomes on mismatch recognition. The data presented here suggest that (i) human MutS\( \alpha \) displays reduced ATPase- and ADP-binding activities when interacting with nucleosomal heteroduplexes; (ii) MutS\( \alpha \) binds the mismatch within a nucleosome with lower efficiency than a naked heteroduplex or a heterology free of histone proteins but between two nucleosomes; and (iii) nucleosomes flanking a mismatch prevent MutS\( \alpha \) from sliding along the DNA helix.

#### TABLE 1

Relative ATPase and ADP binding activities of MutS\( \alpha \)

| MutS\( \alpha \) activity | No substrate | DNA substrate | Nucleosomal substrate |
|--------------------------|--------------|---------------|-----------------------|
|                          | I            | II            | III                   |
|                          | I            | II            | III                   |
| ATPase                   | 1.0          | 2.7          | 2.9                   |
| ADP\( ^{*} \) binding    | 1.0          | 2.4          | 2.2                   |
| ADP binding              | 1.0          | 3.7          | 3.4                   |

The data shown in this table were obtained from Figs. 6 (ATPase), 7D (ADP\( ^{*} \) binding), and 7F (ADP binding) by dividing radioactive intensity in individual reactions of a given assay with the intensity of their corresponding control reaction containing MutS\( \alpha \) alone (i.e., no DNA or nucleosomal substrate).

FIGURE 7. Effects of nucleosomes on nucleotide binding activities of MutS\( \alpha \). MutS\( \alpha \) was incubated with \( [\gamma-^{32}\text{P}]\text{ATP} \), \( [\alpha-^{32}\text{P}]\text{ATP} \), or \( [\alpha-^{32}\text{P}]\text{ADP} \), as indicated, in the presence or absence of the indicated DNA or nucleosomal heteroduplexes and 5 mM MgCl\textsubscript{2}, followed by UV cross-linking and SDS-PAGE as described under "Experimental Procedures." The \( ^{32}\text{P} \)-cross-linked subunits were detected by using a Storm PhosphorImager.

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length for MutS\( \alpha \) sliding, our data appear to suggest that both the sliding and heteroduplex binding are important factors stimulating MutS\( \alpha \) ATPase activity.

It is also known that MutS\( \alpha \) binds both ATP and ADP, with the MSH6 subunit preferentially binding ATP and the MSH2 subunit preferentially binding ADP (21, 22). We examined the effect of nucleosomal heteroduplexes on MutS\( \alpha \) nucleotide binding by performing nucleotide UV cross-linking experiments (21, 22). As shown in Fig. 7, nucleosomes essentially had little influence on MSH6 ATP binding, as judged by the fact that in the absence of Mg\textsuperscript{2+} (i.e. no ATP hydrolysis), almost equal amounts of the MSH6 subunit were labeled with \( [\gamma-^{32}\text{P}]\text{ATP} \) (Fig. 7A) or \( [\alpha-^{32}\text{P}]\text{ATP} \) (Fig. 7C) in all reactions. However, in the presence of Mg\textsuperscript{2+}, which supports ATP hydrolysis, more labeled MSH2 (by \( [\alpha-^{32}\text{P}]\text{ADP} \)) was observed in reactions with naked DNA substrates than those with nucleosomal substrates, with the least labeling in the reaction containing nucleosomal substrate I (Fig. 7D). Thus, the ADP binding pattern is almost identical to the ATP hydrolysis profile shown in Fig. 6. To determine if the differences in ADP binding among reactions reflect changes in the ADP-binding ability of MSH2 or simply the binding of available ADP (from ATP hydrolysis) to MSH2 in individual reactions, cross-linking experiments were conducted in the presence of \( [\alpha-^{32}\text{P}]\text{ADP} \) (Fig. 7, E and F). In the presence of Mg\textsuperscript{2+} (Fig. 7F), we did observe an ADP binding pattern similar to the one shown in Fig. 7D. Despite the fact that all reactions contained the same amounts of ADP, much less MSH2 labeling was detected in the nucleosomal substrate I reaction (Fig. 7F), indicative of inhibition of MSH2 ADP binding by the nucleosomal heteroduplex. As shown in Table 1, this conclusion was also supported by quantitative analysis of the data shown in Figs. 6, 7D, and 7F. It is clear that both types of substrates (except nucleosomal substrates I) stimulate MutS\( \alpha \) ATPase- and ADP-binding activities, but the nucleosomal heteroduplexes are weaker stimulator than the naked DNA heteroduplexes; there was little (if any) stimulation generated by nucleosomal substrate I (Table 1). Given the importance of the ATPase- and nucleotide-binding activities of MutS\( \alpha \) in MMR, we conclude that nucleosomes inhibit or partially inhibit MMR.

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Relative ATPase and ADP binding activities of MutS\( \alpha \)

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|--------------------------|--------------|---------------|-----------------------|
|                          | I            | II            | III                   |
|                          | I            | II            | III                   |
| ATPase                   | 1.0          | 2.7          | 2.9                   |
| ADP\( ^{*} \) binding    | 1.0          | 2.4          | 2.2                   |
| ADP binding              | 1.0          | 3.7          | 3.4                   |

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FIGURE 6. ATPase analysis of MutS\( \alpha \). Unless otherwise specified, ATPase activity of MutS\( \alpha \) was assayed in reactions containing 50 nM proteins, \( [\gamma-^{32}\text{P}]\text{ATP} \), and 5 mM MgCl\textsubscript{2}, in the presence or absence of the indicated DNA or nucleosomal substrates. The reactions were incubated at 37 °C for 20 min, followed by electrophoresis as described under "Experimental Procedures." The \( ^{32}\text{P} \)-labeled species were detected and quantified by using a PhosphorImager. \( ^{32}\text{P} \)-labeled MutS\( \alpha \) was incubated with \( [\gamma-^{32}\text{P}]\text{ATP} \), \( [\alpha-^{32}\text{P}]\text{ATP} \), or \( [\alpha-^{32}\text{P}]\text{ADP} \), as indicated, in the presence or absence of the indicated DNA or nucleosomal heteroduplexes and 5 mM MgCl\textsubscript{2}, followed by UV cross-linking and SDS-PAGE as described under "Experimental Procedures." The \( ^{32}\text{P} \)-cross-linked subunits were detected by using a Storm PhosphorImager.
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(i.e. a single strand nick), whereas the stationary model (33, 34) suggests that MutS proteins remain bound at the mismatch during the course of MMR. If the moving model is correct, nucleosomes would inhibit MMR, because they block MutS sliding (Figs. 4 and 5). Therefore, chromatin-remodeling or modification activities that disrupt nucleosomes would be required for efficient MMR. In contrast, if the stationary model is correct, nucleosomes may not inhibit MMR, although higher order chromatin structures still could reduce its efficiency. The results presented here are a first step toward unraveling the detailed interaction between the MMR machinery and chromatin substrates, albeit in a simplified in vitro system.

There is precedent for the idea that chromatin-remodeling/modification activities could be required during MMR in eukaryotic cells, because ample evidence demonstrates the importance of chromatin-remodeling or modification factors in the context of eukaryotic gene transcription and DNA replication (5, 35). In fact, chromatin-remodeling factors and histone-modifying enzymes alter higher order chromatin structure by affecting contacts between nucleosomes or interactions between histones and DNA (for a review see Ref. 35). Recent studies identified at least 30 modifications (e.g. acetylation and phosphorylation) in histone proteins, with the majority of the modifications occurring in H3 and H4 (36), which are the core histone proteins responsible for contacting the phosphate backbone of the wrapped DNA. Thus, lysine acetylation of H3 and H4 residues located in the DNA-histone interface may reduce DNA-histone interaction(s) and facilitate nucleosome disassembly. Using a chemical ligation strategy to acetylate recombinant histones at specific residues, several groups have shown that acetylation of H3K115, H3K122, or H4K16 reduces DNA binding and inhibits the formation of higher order chromatin structure (37, 38). The influence of these specific modifications on MMR remains to be examined.

It should be noted that the experiments described here were performed in a simplified in vitro system, including only human MutSα and mono- or dinucleosome substrates. In addition, histone octamers were formed from unmodified histone proteins, and nucleosomes were positioned using a high affinity nucleosome binding sequence (e.g. the 601 positioning sequence). Therefore, it remains possible that MutS proteins (e.g. MutSα) and/or other MMR proteins may possess chromatin-remodeling activity by displacing modified or more natural histone octamers on DNA. Therefore, future studies should evaluate the extent to which higher order chromatin structure and modified histone or non-histone chromosomal proteins, including MMR proteins, promote, inhibit, or modulate MMR in eukaryotic cells.

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