

ATP-dependent Assembly of a Ternary Complex Consisting of a DNA Mismatch and the Yeast MSH2-MSH6 and MLH1-PMS1 Protein Complexes*

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MSH2 and MSH6 proteins exist as a stable complex, as do the MLH1 and PMS1 proteins. To study the mismatch binding properties of the MSH2-MSH6 complex and to examine its functional interaction with the MLH1-PMS1 complex, these protein complexes were purified to near homogeneity from overproducing yeast strains. As has been reported previously, the purified MSH2-MSH6 complex binds DNA substrates containing a G/T mismatch and insertion/deletion mismatches, but the binding affinity for the latter decreases as the size of the extrahelical loop increases. Addition of ATP or the non-hydrolyzable ATP γ S reduces binding of the MSH2-MSH6 complex to the DNA substrates markedly. Here, we show that MSH2-MSH6 forms a ternary complex with MLH1-PMS1 on a mismatch containing DNA substrate. The formation of this ternary complex requires ATP, which can be substituted by ATP γ S, suggesting that ATP binding alone is sufficient for ternary complex formation. Thus, it appears that ATP binding by the MSH2-MSH6 complex induces a conformation that is conducive for the interaction with MLH1-PMS1 complex, leading to the formation of the ternary complex.

Mismatch repair in yeast and other eukaryotes requires a number of evolutionarily conserved protein factors including three *Escherichia coli* MutS homologs, MSH2, MSH3, and MSH6, and two *E. coli* MutL homologs, MLH1 and PMS1 (1–3). Genetic studies in the yeast *Saccharomyces cerevisiae* have indicated that mutations in the *MSH2*, *MLH1*, and *PMS1* genes result in severe defects in mismatch repair, whereas mutations in the *MSH3* or *MSH6* genes engender a much smaller mismatch repair defect. From these studies, it has been inferred that MSH2 protein functions in mismatch repair in conjunction with MSH3 or MSH6, and in the absence of the latter two proteins, MSH2 is inactive (4–6). Biochemical studies with yeast and human proteins have clarified the role of MSH2, MSH3, and MSH6 in the mismatch recognition reaction. MSH2 dimerizes with either MSH3 or MSH6, and the resulting MSH2-MSH3 and MSH2-MSH6 protein complexes

have somewhat different mismatch binding specificities. MSH2-MSH6 binds the G/T mismatch and small extrahelical DNA loops preferentially, whereas the MSH2-MSH3 complex shows higher binding affinity for larger extrahelical DNA loops (7–11). MSH2 protein alone has little affinity for DNA mismatches (8).

The aforementioned biochemical studies have yielded important information concerning the role of MSH2, MSH3, and MSH6 proteins in DNA mismatch recognition. Despite their indispensability in mismatch repair, relatively little is known about the roles of the MLH1 and PMS1 proteins in the repair process. Recently, we overproduced and purified the MLH1-PMS1 complex from a yeast strain genetically tailored to co-overexpress the two proteins (12). Our biochemical studies indicated that whereas MLH1-PMS1 has no affinity for the DNA mismatch, it forms a ternary complex with MSH2-MSH3 on the DNA mismatch and also enhances the ability of the latter to bind the DNA mismatch (12). The addition of ATP has no effect on the binding of MLH1-PMS1 to MSH2-MSH3 mismatch complex (12). Here we examine the interaction between MLH1-PMS1 and MSH2-MSH6 protein complexes with the DNA mismatch. Interestingly, we find that ternary complex formation among these entities requires ATP.

MATERIALS AND METHODS

Polyclonal Antibodies Specific for MSH2, MSH6, MLH1, and PMS1—The portion of the *MSH6* gene encompassing amino acid residues 38 to 612 of the encoded protein was fused to the *E. coli* transcription terminator rho, and the resulting insoluble rho-MSH6 fusion polypeptide was purified by preparative SDS-polyacrylamide gel electrophoresis, dialyzed against phosphate-buffered saline (10 mM Na₂HPO₄, pH 7.2, 150 mM KCl), and used for raising polyclonal antiserum in rabbits. Antibodies were affinity-purified from the antiserum using a column of cyanogen bromide-Sepharose containing the cross-linked antigen. Antibodies specific for the MSH2, MLH1, and PMS1 proteins were obtained as described (8, 12). All the affinity purified antibodies were dialyzed against phosphate-buffered saline and concentrated to 2 mg/ml using Centricon-30 microconcentrator to be used in the supershifting experiment.

Yeast Plasmids—The *MSH2* protein coding sequence from the ATG translation start codon to 259 nucleotides downstream of the TAA stop codon was placed under the control of the galactose-inducible *GAL-PGK* promoter to yield plasmid pMSH2.1 (2 μ , *GAL-PGK-MSH2*, *URA3*). The *MSH6* gene from the ATG translation start to 1445 bases downstream of the terminator codon was also placed under the control of the *GAL-PGK* promoter to yield plasmid pMMR10 (2 μ , *GAL-PGK-MSH6*, *leu2d*). These plasmids were introduced into the protease-deficient yeast strain BJ5464 (*MAT α* , *ura3-52*, *trp1*, *leu2- Δ 1*, *his3- Δ 200*, *pep4::HIS3*, *prb1 Δ 1.6R*, *can1*, *GAL*). The plasmids pMMR75 (2 μ , *ADC-MLH1*, *TRP1*) and pMMR97 (2 μ , *GAL-PGK-PMS1*, *leu2d*), used for the overexpression of the MLH1 and PMS1 proteins, have been described previously (12).

Purification of the MSH2-MSH6 and MLH1-PMS1 Protein Complexes—Yeast strain BJ5464 harboring the plasmids pMSH2.1 and pMMR10 was grown overnight in synthetic medium lacking both

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leucine and uracil and containing 3% glycerol, 2% lactic acid, and 2% sucrose. The overnight culture was diluted to 0.2 A₆₀₀ in the same medium containing no sucrose and then grown for 16 h. The expression of MSH2 and MSH6 proteins was induced by the addition of 2% galactose to the medium for 7 h. The MSH2-MSH6 complex was purified using a procedure modified from that of Alani (11). Briefly, 3.4 g of cells were broken in 3 volumes of buffer A (25 mM Tris, pH 7.6, 1 mM EDTA, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) containing 0.2 M NaCl using a French press. The extract was clarified by ultracentrifugation (90 min at 100,000 × g), and the supernatant was loaded onto a PBE-94 column (1 × 4-cm). The bound proteins were eluted by a 50 ml 0.2–1 M NaCl gradient in buffer A. The fractions containing the MSH2-MSH6 complex were diluted 3 times before being loaded onto a single-stranded DNA cellulose column (1 × 1.5-cm; United States Biochemical). The proteins were eluted by a 10-ml 0.2–1 M NaCl gradient in buffer A; the fractions containing the MSH2-MSH6 complex were diluted to bring the conductivity of the pool to an equivalent to 100 mM NaCl before being loaded onto Mono S (HR5/5). The bound proteins were eluted with a 20-ml 0.1–1 M NaCl gradient in buffer A. Purified MSH2-MSH6 complex was concentrated in an Amicon-30, divided in small portions, and frozen at -70 °C.

The MLH1-PMS1 complex was purified to near homogeneity as reported by Habraken *et al.* (12). Briefly, 130 g of yeast cells harboring the MLH1 and PMS1 overproducing plasmids were resuspended in phosphate buffer and passed through a French press. The extract was clarified by ultracentrifugation, and the supernatant was fractionated with 0.21 g/ml ammonium sulfate. The precipitated proteins were resuspended, dialyzed, and loaded onto Q-Sepharose. The flow-through fraction from Q-Sepharose was loaded onto an SP-Sepharose column. The proteins eluted from this second chromatography were further purified by passage through hydroxyapatite, Mono S (HR5/5) and Mono Q (HR5/5) as described previously (12).

DNA Mobility Shift Assay—All of the DNA substrates shared the same bottom strand, which was 5'-end-labeled with [γ -³²P]ATP and T4 polynucleotide kinase (Fig. 2A). Because of the shared bottom strand, all of these DNA substrates had the same specific radioactivity (1.2 × 10⁶ cpm/μg). The substrates (5 ng, 6000 cpm) were incubated for 30 min on ice with the indicated amounts of the MSH2-MSH6 and MLH1-PMS1 complexes, and 80 ng of *Hae*III-digested φX174 double-stranded DNA added as nonspecific competitor in 10 μl of reaction buffer (30 mM Tris-HCl, pH 8.0, 1 mM MgCl₂, 50 mM KCl, 1 mM dithiothreitol, 50 μg/ml of bovine serum albumin, and 10% glycerol). After the addition of 3 μl of gel loading buffer (10 mM Tris-HCl, pH 7.5, 3 mM EDTA, 50% glycerol), the reaction mixtures were run in a 4% nondenaturing polyacrylamide gel in 40 mM Tris acetate, pH 7.5, containing 2 mM EDTA. After drying, the gels were exposed to x-ray films and were also subjected to phosphorimage analyses in a Molecular Dynamics PhosphorImager. For the antibody supershifting experiment in Fig. 4, 2 μg of affinity purified antibodies in phosphate-buffered saline were added at the end of the incubation, and the reaction mixtures were kept at 0 °C for 10 min before being subjected to gel electrophoresis.

RESULTS

MSH6 protein exists as a stable complex with the MSH2 protein in both yeast and human (7, 10, 11). To purify the MSH2-MSH6 complex for biochemical studies, we fused the *MSH2* and *MSH6* genes to the *GAL-PGK* promoter to yield plasmids pMSH2.1 (*GAL-PGK-MSH2*, *URA3*) and pMMR10 (*GAL-PGK-MSH6*, *leu-2d*), respectively. These plasmids were introduced into the protease-deficient yeast strain BJ5464, and the overexpression of MSH2 and MSH6 proteins was verified by immunoblot analyses (Fig. 1A) using affinity purified antibodies raised against portions of the two MSH proteins expressed in and purified from *E. coli* (see "Materials and Methods"). For the purification of the MSH2-MSH6 complex, extract from BJ5464 harboring pMSH2.1 and pMMR10 was subjected to chromatographic fractionation in columns of PBE-94, single-stranded DNA cellulose, and Mono S, as described under "Materials and Methods." When the MSH2-MSH6 complex from the last step of purification in Mono S was analyzed on a 7.5% polyacrylamide gel and stained with Coomassie Blue, only the MSH2 and MSH6 protein bands were seen (Fig. 1B), indicating that the protein preparation was nearly homogeneous.

For examining the mismatch binding properties of the puri-

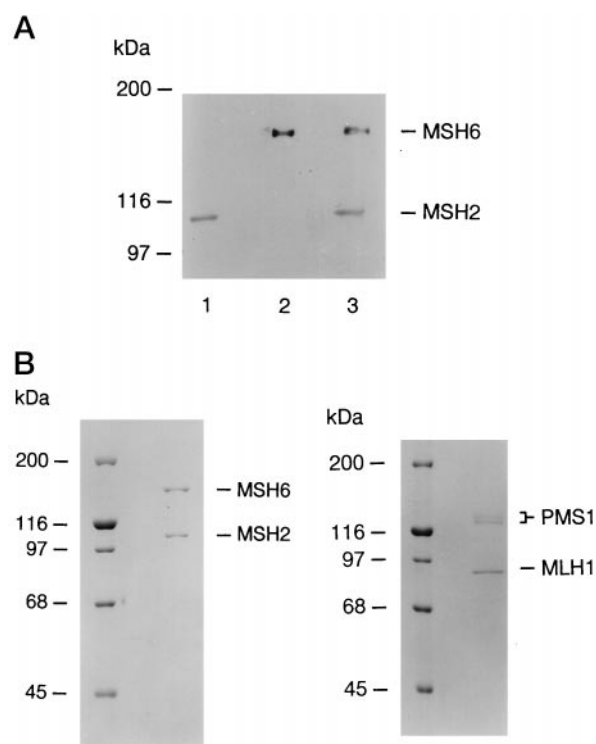


FIG. 1. Overexpression and purification of MSH2-MSH6 and MLH1-PMS1 protein complexes. A, nitrocellulose blot of a 7.5% denaturing polyacrylamide gel was probed with anti-MSH2 and anti-MSH6 antibodies. Lane 1, extract from BJ5464 harboring the MSH2 overexpression vector pMSH2.1; lane 2, extract from BJ5464 harboring the MSH6 overexpressing vector pMMR10; lane 3, extract from BJ5464 harboring both pMSH2.1 and pMMR10. B, Coomassie Blue staining of a 7.5% SDS-polyacrylamide gel containing purified MSH2-MSH6 (600 ng; left panel), purified MLH1-PMS1 (600 ng; right panel) and molecular size markers.

fied MSH2-MSH6 complex, we hybridized oligonucleotides to yield duplex substrates (Fig. 2A) that contained no DNA mismatch (G/C), a G/T mismatch (G/T), and extrahelical loops of 1 base (+1), 2 bases (+2), and 4 bases (+4). These substrates were incubated with the MSH2-MSH6 protein complex, and the reaction mixtures were analyzed on 4% nondenaturing polyacrylamide gels run at 4 °C. Nucleoprotein complexes were visualized by autoradiography of the dried gels, and quantification of binding was done by phosphorimage analysis of the gels. As shown in Fig. 2, B and C, preferential binding of the MSH2-MSH6 complex to the mismatch containing DNA substrates was seen. However, different levels of nucleoprotein complex formation were observed with the DNA substrates, and the results revealed that the MSH2-MSH6 complex has the highest affinity for the +1 substrate, followed by the G/T, +2, and +4 substrates and that only a trace amount of the homoduplex (G/C) was bound (Fig. 2, B and C). Thus, by contrast to the MSH2-MSH3 complex, which has a higher affinity for DNA substrates containing larger extrahelical loops (8, 9, 12), the MSH2-MSH6 complex binds less well to DNA containing larger extrahelical loops. With all the mismatched DNA substrates, MSH2-MSH6 forms two nucleoprotein complexes (Fig. 2B). The reason for these two shifted forms is not known.

One rather intriguing aspect concerning the mismatch recognition ability of the MSH2-MSH6 complex is its marked sensitivity to ATP (7, 10, 11), which is not seen with the MSH2-MSH3 complex (8, 12). For instance, the inclusion of 1 mM ATP to the binding reactions diminishes binding to the G/T and +1 substrates markedly (Fig. 2, D and E). In other experiments, an inhibition of DNA mismatch binding by the MSH2-MSH6 complex was also seen when ATP γ S instead of ATP was

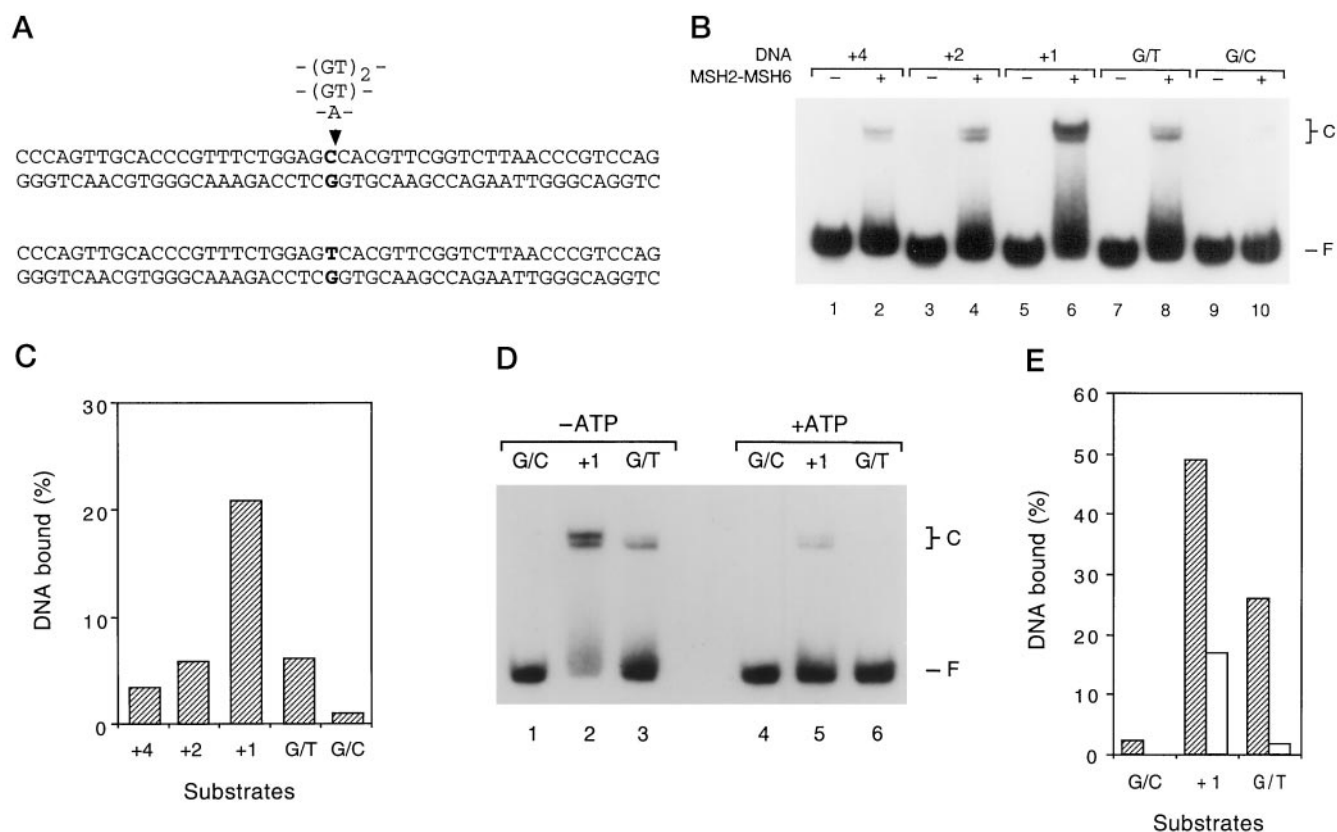


FIG. 2. Binding of MSH2-MSH6 complex to mismatch-containing DNA substrates. *Panel A*, DNA substrates used in this study. All of these substrates share the same bottom strand, which is labeled with ^{32}P at its 5'-end. The arrowhead indicates the site of a 1, 2, or 4 nucleotide insertion. *Panel B*, binding of the homoduplex (G/C) and DNA substrates (5 ng each) containing a G/T mismatch and +1, +2, and +4 loops by 30 ng of purified MSH2-MSH6 complex. Symbols: F, DNA substrate; C, the nucleoprotein complex. *Panel C*, histograms summarizing the results in *panel B*. *Panel D*, the MSH2-MSH6 complex (60 ng) was incubated with the homoduplex (G/C), G/T substrate, or the +1 loop in the absence (*lanes 1-3*) or the presence of 1 mM ATP (*lanes 4-6*). *Panel E*, histograms summarizing the results in *panel D*. Symbols: \square , -ATP; \square , +ATP.

added to the reaction mixture (see Fig. 3C). Thus, it appears that the ATP bound form of the MSH2-MSH6 complex has a much lower affinity for DNA mismatch.

In addition to MSH2, MSH3, and MSH6 proteins, mismatch repair is also dependent on the *MLH1* and *PMS1* gene products. The *MLH1* and *PMS1* proteins also exist as a stable stoichiometric complex in yeast cells, and the complex of these proteins has been purified from a yeast strain genetically tailored to co-overexpress the two proteins (12), and the purified *MLH1-PMS1* complex used in this study was nearly homogeneous (Fig. 1B). As reported in our recent work (12) and reiterated here in Fig. 3A, no binding of *MLH1-PMS1* protein complex to the homoduplex or to any of mismatch-containing DNA substrates (G/T, +1, +2, and +4) was evident. At much higher concentrations of *MLH1-PMS1* than those used in the experiments reported here, a low level of nonspecific binding to all of the DNA substrates occurs, but under no circumstances have we detected preferential binding to the DNA mismatch. Taken together, these results indicate that the *MLH1-PMS1* complex has no mismatch recognition ability.

We showed recently that the *MLH1-PMS1* complex enhances the mismatch binding ability of the MSH2-MSH3 complex and forms a ternary complex with the latter on the DNA mismatch (12). Because of this result, it was of considerable interest to examine the effect of the purified *MLH1-PMS1* complex on the mismatch binding ability of the MSH2-MSH6 complex, and whether a ternary complex between these two protein complexes is formed on the DNA mismatch. As shown in Fig. 3A, the addition of amounts of *MLH1-PMS1* complex stoichiometric to that of MSH2-MSH6 complex had no significant effect on the mismatch binding ability of the latter protein complex. In

Fig. 3A, the +1 substrate was used for testing the effect of the *MLH1-PMS1* complex. We have also used the G/T substrate and found no significant effect of the *MLH1-PMS1* complex on the binding of this DNA mismatch substrate by MSH2-MSH6 or any evidence of the formation of a ternary complex between the DNA mismatch and *MLH1-PMS1* and MSH2-MSH6 protein complexes.

The results in Fig. 3A produced no evidence for a functional or physical interaction between the MSH2-MSH6 complex and the *MLH1-PMS1* complex, which was a somewhat surprising result, given that genetic and biochemical studies have indicated that MSH2-MSH6-dependent mismatch repair also requires the *MLH1-PMS1* complex, and that the latter has been found to interact with the other mismatch recognition protein complex, MSH2-MSH3, in the presence of a DNA mismatch forming a ternary complex (12). Since ATP binding apparently induces a substantial conformational change in MSH2-MSH6 as indicated by a highly reproducible reduction in the binding of DNA mismatches (Fig. 2, D and E), we reasoned that perhaps this ATP-induced conformational change in the MSH2-MSH6 complex is also a prerequisite for interaction with the *MLH1-PMS1* complex. To test this idea directly, we repeated the mismatch binding experiment with both the *MLH1-PMS1* and MSH2-MSH6 protein complexes and the G/T and +1 substrates, but in the presence of 1 mM ATP (Fig. 3B), which as shown previously (Fig. 2, D and E) effects a large reduction in the binding of the MSH2-MSH6 complex to these DNA. Interestingly, a novel nucleoprotein complex (designated by C*) which migrates above the normal MSH2-MSH6-mismatch nucleoprotein complex (designated by C) was formed with both the G/T and +1 substrates in the presence of ATP.

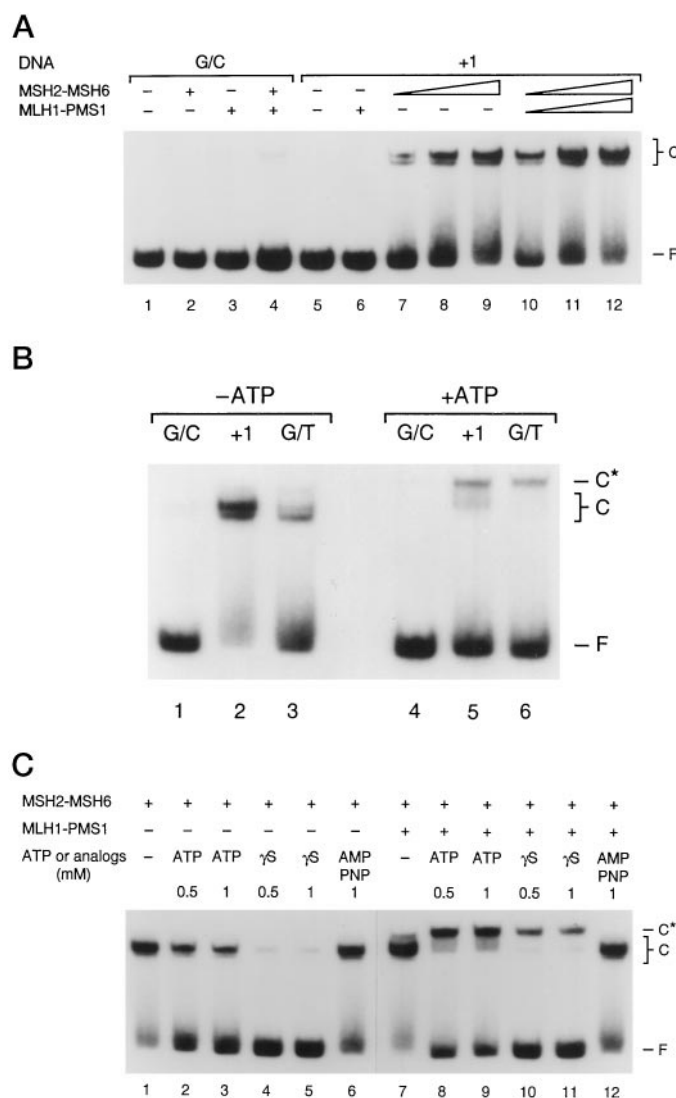


FIG. 3. ATP-dependent ternary complex formation among MSH2-MSH6, MLH1-PMS1, and the DNA mismatch. *Panel A*, binding of the +1 DNA substrate by purified MLH1-PMS1 complex (60 ng, lane 6) by purified MSH2-MSH6 complex (15, 30, and 45 ng, lanes 7–9, respectively) or by both protein complexes together (15, 30, and 45 ng of MSH2-MSH6 with 20, 40, and 60 ng MLH1-PMS1, lanes 10–12, respectively). The homoduplex was incubated either with 45 ng of MSH2-MSH6 (lane 2) or 60 ng MLH1-PMS1 (lane 3) or with these amounts of the two protein complexes (lane 4). *Panel B*, ATP induces the formation of a ternary nucleoprotein complex. The MSH2-MSH6 complex (60 ng) and the MLH1-PMS1 complex (80 ng) were incubated with the homoduplex, the +1 substrate, or the G/T substrate without ATP (lanes 1–3) or with 1 mM ATP (lanes 4–6). Symbols: F, DNA substrate; C, the nucleoprotein complex formed in the absence of ATP; C*, the novel nucleoprotein complex formed in the presence of ATP. *Panel C*, nucleotide binding is sufficient to induce the formation of the ternary complex. Binding of the G/T DNA substrate by the MSH2-MSH6 complex (120 ng, lanes 1–6) or with both MSH2-MSH6 and MLH1-PMS1 complexes (120 and 160 ng, respectively, lanes 7–12). The reaction mixture contains no ATP or its analogue (lanes 1 and 7), 0.5 mM ATP (lanes 2 and 8), 1 mM ATP (lanes 3 and 9), 0.5 mM ATP γ S (lanes 4 and 10), 1 mM ATP γ S (lanes 5 and 11), and 1 mM AMP-PNP (lanes 6 and 12).

To determine if ATP binding alone was sufficient or if ATP hydrolysis was also required for ternary complex formation, we examined whether the novel nucleoprotein complex was formed in the presence of the nonhydrolyzable ATP analogues ATP γ S and AMP-PNP¹ (Fig. 3C). As has been reported previously for

¹ The abbreviation used is: AMP-PNP, adenosine 5'-(β , γ -imino) triphosphate.

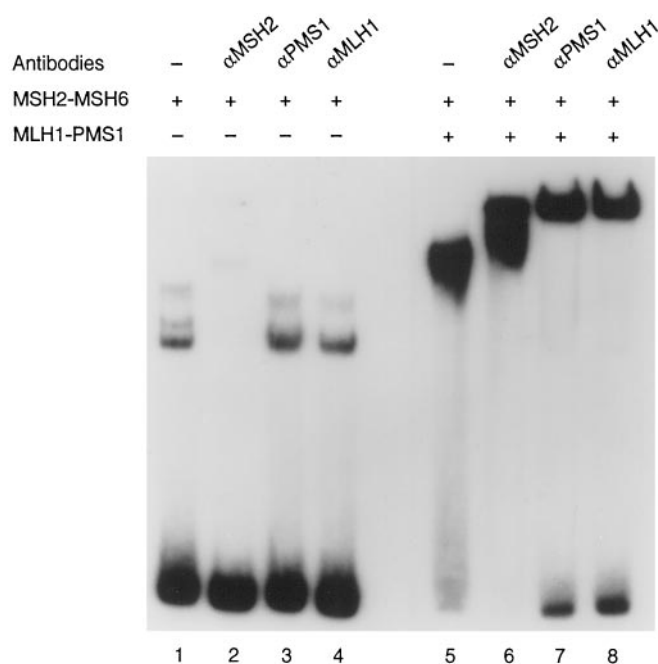


FIG. 4. Antibody-mediated supershifting of the ternary nucleoprotein complex. MSH2-MSH6 complex, 180 ng (lanes 1–8), was incubated with the G/T substrate with 1 mM ATP in the absence (lane 1–4) or presence (lanes 5–8) of 240 ng of MLH1-PMS1 complex. The reaction mixtures in lanes 2 and 6 were treated with anti-MSH2 antibodies (α MSH2) prior to electrophoresis as indicated; in lanes 3 and 7, anti-PMS1 antibodies (α PMS1) were added, and in lanes 4 and 8, anti-MLH1 antibodies (α MLH1) were added.

the human MSH2-MSH6 complex (7), addition of AMP-PNP did not reduce the mismatch binding ability of the yeast MSH2-MSH6 complex (Fig. 3C, lane 6). AMP-PNP also did not promote the formation of a ternary complex of MSH2-MSH6 with MLH1-PMS1 on mismatched DNA (Fig. 3C, lane 12). The lack of any effect of AMP-PNP may result from the inability of MSH2-MSH6 to bind this nucleotide, or binding to this nucleotide may not alter the conformation of MSH2-MSH6 to a form appropriate for interaction with MLH1-PMS1. As shown in Fig. 3C, ATP γ S, however, induced the formation of the novel nucleoprotein complex, albeit at a somewhat lower efficiency than ATP, suggesting that binding of ATP by MSH2-MSH6 is sufficient to promote ternary complex formation.

In a recent study (13), it has been suggested that the human MSH2-MSH6 complex cycles between ADP and ATP bound forms. According to these workers, the ADP bound form of human MSH2-MSH6 binds the DNA mismatch and serves as a nucleation point for the assembly of other mismatch repair proteins. Turnover of the human MSH2-MSH6 complex from the mismatch is triggered upon the exchange of ADP to ATP by the protein complex. Our results with the yeast MSH2-MSH6 heterodimer, however, have indicated that ADP does not induce the formation of a ternary complex of MSH2-MSH6 with MLH1-PMS1 on mismatched DNA. Similar to the effect of AMP-PNP (Fig. 3C), addition of 1 mM ADP had no effect on the mismatch (G/T) binding ability of the MSH2-MSH6 complex, and it did not promote the formation of the ternary nucleoprotein complex (data not shown). Thus, our observations in yeast indicate the involvement of the ATP bound form of MSH2-MSH6 in mismatch repair.

The availability of antibodies against the various mismatch repair proteins has allowed us to directly test for the presence of the MLH1 and PMS1 proteins in the ternary complex by antibody-mediated supershifting. As shown in Fig. 4, treatment of the ternary nucleoprotein complex with either affinity

purified anti-MLH1 or anti-PMS1 antibodies resulted in a retarded mobility of this complex, whereas treatment of the binary nucleoprotein complex formed between MSH2-MSH6 and the DNA mismatch with these antibodies had no effect on its gel mobility. As expected, treatment of the ternary complex with anti-MSH2 antibodies also resulted in retarded mobility of the nucleoprotein complex. In the absence of ATP, no evidence of ternary complex formation was seen, as evidenced by the lack of supershifting by the anti-PMS1 or anti-MLH1 antibodies of the DNA mismatch bound MSH2-MSH6 complex that had been incubated with the MLH1-PMS1 complex (data not shown). Taken together, our results indicate that ATP binding promotes the formation of a ternary complex of MLH1-PMS1 with mismatch bound MSH2-MSH6.

DISCUSSION

We have purified the MSH2-MSH6 protein complex from yeast and carried out biochemical studies to examine the mismatch binding properties of the MSH2-MSH6 complex and explore the functional and physical interactions of this complex with the MLH1-PMS1 protein complex. As has been reported previously (7, 10, 11), the MSH2-MSH6 complex binds with higher affinity to DNA substrates that contain a single-base mismatch and small extrahelical loops than to those that contain larger loops. The mismatch binding preference of the MSH2-MSH6 complex differs from that of the MSH2-MSH3 complex, which has higher avidity for substrates with larger extrahelical loops (8, 9, 12).

The mismatch binding activity of the MSH2-MSH6 protein complex is highly sensitive to ATP or the nonhydrolyzable analogue ATP γ S, suggesting that nucleotide binding induces a marked conformational change in the MSH2-MSH6 complex. One interpretation of these results is that ATP binding by the mismatch bound MSH2-MSH6 complex effects the release of the protein complex from the DNA mismatch. Given that we saw no evidence of ternary complex formation among MSH2-MSH6, MLH1-PMS1, and the DNA mismatch in the absence of ATP, we considered the possibility that the ATP-induced conformational change in the MSH2-MSH6 protein complex may be a prerequisite for the interaction of the former with the MLH1-PMS1 protein complex. Indeed, the addition of ATP promotes the formation of a ternary complex of MLH1-PMS1,

MSH2-MSH6, and the DNA mismatch. We have also shown that ATP γ S can effectively substitute for ATP, suggesting that nucleotide binding by MSH2-MSH6 is sufficient for ternary complex formation. In its dependence upon ATP for ternary complex formation, the MSH2-MSH6 complex resembles the *E. coli* MutS protein, where the binding of the MutS-mismatch complex to MutL also requires ATP binding but not its hydrolysis (14).

In summary, the MLH1-PMS1 complex combines with either the MSH2-MSH3 complex (12) or the MSH2-MSH6 complex (this work) and the DNA mismatch to form a ternary complex. In the case of MSH2-MSH3, ternary complex formation is not dependent on ATP (12), whereas with MSH2-MSH6, ATP binding is required for the assembly of the ternary complex (this work). We suggest that the ternary complexes consisting of MLH1-PMS1 and either MSH2-MSH3 or MSH2-MSH6 would be critical for the recruitment of other components indispensable for mismatch repair including the proliferating cell nuclear antigen (15, 16).

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