Positive Role of the Mammalian TBPIP/HOP2 Protein in DMC1-mediated Homologous Pairing*

Rima Enomoto‡, Takashi Kinebuchi‡, Makoto Sato§§, Hideshi Yagi§, Takehiko Shibata‡, Hitoshi Kurumizaka‡‡‡, and Shigeyuki Yokoyama‡§§

From the RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, the Division of Cell Biology and Neuroscience, Department of Morphological and Physiological Sciences, Faculty of Medical Sciences, University of Fukui, 23 Shimoizuki, Matsukawa, Fukui 910-1183, the Solution Oriented Research for Science and Technology, Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-0012, the Cellular and Molecular Biology Laboratory, RIKEN Discovery Research Institute, 2-1 Hirosawa, Wako, Saitama 351–0198, Waseda University School of Science and Engineering, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, the Cellular Signaling Laboratory and Structuralome Research Group, RIKEN Harima Institute at SPring-8, 1-1-1 Kohto, Mikazuki-cho, Sayo, Hyogo 679-5148, the Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

In meiosis, homologous recombination preferentially occurs between homologous chromosomes rather than between sister chromatids, which is opposite to the bias of mitotic recombinational repair. The TBPIP/HOP2 protein is a factor that ensures the proper pairing of homologous chromosomes during meiosis. In the present study, we found that the purified mouse TBPIP/HOP2 protein stimulated homologous pairing catalyzed by the meiotic DMC1 recombinase in vitro. In contrast, TBPIP/HOP2 did not stimulate homologous pairing by RAD51, which is another homologous pairing protein acting in both meiotic and mitotic recombination. The positive effect of TBPIP/HOP2 in the DMC1-mediated homologous pairing was only observed when TBPIP/HOP2 first binds to double-stranded DNA, not to single-stranded DNA, before the initiation of the homologous pairing reaction. Deletion analyses revealed that the C-terminal basic region of TBPIP/HOP2 is required for efficient DNA binding and is also essential for its homologous pairing stimulation activity. Therefore, these results suggest that TBPIP/HOP2 directly binds to DNA and functions as an activator for DMC1 during the homologous pairing step in meiosis.

During meiosis, two successive rounds of nuclear division, meiosis I and meiosis II, are promoted with a single round of DNA replication. As a result, diploid cells produce haploid gametes in eukaryotes. In the cell division at meiosis I, homologous chromosomes are aligned, and a high level of recombination occurs between homologous chromosomes but not between sister chromatids. This preferential recombination between homologous chromosomes, called homologous recombination, ensures their correct segregation at meiosis I through the formation of chiasmata, which physically connect homologous chromosomes.

Homologous recombination is initiated by double strand break (DSB) formation by Spo11 at the initiation sites for recombination (1–3). Then a single-stranded DNA (ssDNA) tail derived from a DSB site invades the homologous double-stranded DNA (dsDNA) to form a heteroduplex. This strand invasion step, called homologous pairing, is the key step in homologous recombination. In Escherichia coli, the RecA protein catalyzes the homologous pairing step (4, 5). Two eukaryotic homologues of RecA, the RAD51 and DMC1 proteins, which are conserved from yeast to human, have been identified (6–9) and have been shown to catalyze homologous pairing in vitro (10–15). The RAD51 gene is expressed in both mitotic and meiotic cells, but the DMC1 gene functions only in meiotic cells. Disruption of the Rad51 gene results in early embryonic lethality in mice (16, 17). In chicken DT40 cells, the RAD51 gene disruption causes the accumulation of spontaneous chromosomal breaks and significantly reduces the recombination frequency between sister chromatids (18, 19). In contrast, disruption of the Dmc1 gene does not cause lethality in mice, but the dmc1 knockout mice are sterile with an arrest of gametogenesis in the first meiotic prophase (20, 21). Therefore, DMC1 is a meiosis-specific homologous pairing protein that may be a central player in meiosis-specific events such as the homologous pairing between homologous chromosomes but not between sister chromatids.

In addition to the DMC1 gene, genetic studies with Saccharomyces cerevisiae have identified the HOP2 gene, which is essential for proper homologous chromosome pairing and recombination during meiosis (22). The HOP2 homologues of Schizosaccharomyces pombe and Arabidopsis thaliana, meu13 and AHP2, respectively, were also identified as meiosis-specific factors that facilitate the pairing of homologous chromosomes and recombination (23, 24). The hop2 deletion mutant in S. cerevisiae fails to sporulate due to a uniform arrest at the pachytene stage of meiosis I with most of the chromosomes engaged in synapsis with nonhomologous partners (22). This meiotic cell cycle arrest by the hop2 mutation is bypassed by the dmc1 mutation, indicating that the meiotic arrest of hop2 is

* This work was supported by the Bioarchitect Research Program (RIKEN), Core Research for Evolutional Science and Technology of JST, the RIKEN Structural Genomics/Proteomics Initiative, the National Project on Protein Structural and Functional Analyses, and grants-in-aid from the Japanese Society for the Promotion of Science and the Ministry of Education, Sports, Culture, Science, and Technology, Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ To whom correspondence may be addressed. E-mail: kurumizaka@waseda.jp.

¶¶ To whom correspondence may be addressed. E-mail: yokoyama@biochem.s.u-tokyo.ac.jp.

‡‡‡ To whom correspondence may be addressed. E-mail: kurumizaka@waseda.jp.

§§ To whom correspondence may be addressed. E-mail: yokoyama@biochem.s.u-tokyo.ac.jp.
due to the action of the Dmc1 protein (25). This genetic interaction between Hop2 and DMC1 suggests that the Hop2 protein function in the strand invasion step, which is promoted by the Dmc1 protein, during meiosis. The mammalian Hop2 homologue, TBPIP, was first identified as a factor interacting with TBP-1, which binds to the human immunodeficiency virus, type 1 Tat protein (26, 27). The TBPIP/Hop2 knock-out mice also display the meiotic cell cycle arrest due to the failure of DSB repair (28).

In the present study, we demonstrated that the mammalian TBPIP/HOP2 protein is an activator that specifically stimulates the homologous pairing catalyzed by DMC1. The homologous pairing stimulation by the TBPIP/HOP2 protein was only observed when TBPIP/HOP2 binds dsDNA, not ssDNA, before the initiation of homologous pairing by DMC1. Deletion analyses of TBPIP/HOP2 showed that the C-terminal basic region, required for efficient DNA binding, is essential for the homologous pairing stimulation activity of TBPIP/HOP2. This is the first biochemical evidence that TBPIP/HOP2 functions with the meiosis-specific homologous pairing protein DMC1.

EXPERIMENTAL PROCEDURES

Overexpression and Purification of the Mouse and Human TBPIP/HOP2 Proteins—The mouse and human TBPIP/HOP2 genes (26, 27) were ligated with the pET-15b expression vector (Novagen) at the Ndel-BamHI sites. The proteins were overexpressed in the E. coli strain BL21-CodonPlus(DE3)-RIL (Stratagene) as N-terminal His-tagged proteins. The cells were grown in 1 liter of LB medium containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol at 30 °C. At the logarithmic phase of growth (A600nm = 0.6), the TBPIP/HOP2 expression was induced with 50 μM isopropyl-1-thio-β-D-galactopyranoside (final concentration). Cells were harvested after an overnight incubation at 18 °C and were lysed by sonication in buffer A (50 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, 10 mM 2-mercaptoethanol, and protease inhibitors (Complete EDTA-free, Roche Applied Science) on ice. The cell lysates were centrifuged at 27,700 × g for 20 min, and the supernatants were gently mixed by the batch method with 4 ml of Ni-NTA-agarose beads (Qiagen) for 1 h at 4 °C. The Ni-NTA-agarose beads with the His6-tagged mouse TBPIP/HOP2 protein were packed into an Econo column (Bio-Rad) and were washed with 30 column volumes of buffer A containing 15 mM imidazole at a flow rate of about 0.3 ml/min. The beads with the His6-tagged human TBPIP/HOP2 protein were washed with buffer A containing 50 mM imidazole. The His6-tagged mouse and human TBPIP/HOP2 proteins were eluted in a 15-column volume linear gradient from 0.2 to 1.2 M KCl in buffer A (50 mM Tris-HCl buffer (pH 8.0) containing 0.5 mM NaCl, 10% glycerol, and protease inhibitors (Complete EDTA-free, Roche Applied Science)) on ice. The lysates were centrifuged at 27,700 × g for 20 min, and the supernatants were gently mixed by the batch method with 4 ml of Ni-NTA-agarose beads (Qiagen) for 1 h at 4 °C. The protein-bound beads were packed into an Econo column (Bio-Rad) and were washed with 30 column volumes of buffer B containing 15 mM imidazole in buffer A. The His6-tag was uncoupled from DMC1 by a digestion with 3 units of thrombin protease (Amersham Biosciences)/mg of DMC1, and the protein was immediately dialyzed against buffer G containing 5 mM imidazole. The DMC1 protein was eluted in a 20-column volume linear gradient from 5 to 300 mM imidazole in buffer B. The Dmc1 mutants were purified by the same method as that for mTBPIP/HOP2. The mTBPIP/HOP2 deletion mutants were purified by the same method as that used for mTBPIP/HOP2. The mTBPIP-HOP2 (1–125), mTBPIP-HOP2 (1–125) and mTBPIP-HOP2 (1–178) mutants were purified by the same method as that used for mTBPIP/HOP2. The mTBPIP (1–162), mTBPIP (1–157), mTBPIP (1–143), mTBPIP (1–125), and mTBPIP (1–105) mutants were purified by the same method as that used for mTBPIP/HOP2. The mTBPIP (1–162) and mTBPIP (1–157) mutants were dialyzed against buffer E (20 mM Tris-HCl buffer (pH 8.0) containing 2 mM 2-mercaptoethanol, 0.25 mM EDTA, and 10% glycerol) after the Ni-NTA column chromatography. The His6 tags were uncoupled by treatments with 3 units for (mTBPIP-HOP2 (1–162) and mTBPIP-HOP2 (1–157)) and 2 units for (mTBPIP-HOP2 (1–178)) of thrombin protease/mg of protein. Then all the mTBPIP/HOP2 mutants were loaded onto a 6-ml heparin-Sepharose (Amersham Biosciences) column previously equilibrated with buffer E. The column was washed with 20 column volumes of buffer E, and the mutants were eluted with 1 column volume linear gradient from 0 to 1 M KCl in buffer E. The peak fractions of the mTBPIP/HOP2 deletion mutants were dialyzed against buffer B. Protein concentrations were determined using a Bio-Rad protein assay kit with bovine serum albumin as the standard.

Overexpression and Purification of the Human DMC1 and RAD51 Proteins—The human RAD51 protein was purified as described previously (29). The human DMC1 gene was inserted into the pET-15b plasmid (Novagen) at the Ndel-BamHI sites, and the protein was overexpressed in the E. coli strain BL21-CodonPlus(DE3)-RIL (Stratagene) as an N-terminal His6-tagged protein (30). The cells were grown in 10 liters of LB medium containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol at 30 °C. When the A600nm of the culture was between 0.4 and 0.6, 1 mM isopropyl-1-thio-β-D-galactopyranoside (final concentration) was added to induce protein expression. Cells were harvested after an overnight incubation and were lysed by sonication in buffer F (50 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl, 10 mM 2-mercaptoethanol, 10% glycerol, and protease inhibitors (Complete EDTA-free, Roche Applied Science)) on ice. The lysates were centrifuged at 27,700 × g for 20 min, and the supernatants were gently mixed by the batch method with 4 ml of Ni-NTA-agarose beads (Qiagen) for 1 h at 4 °C. The protein-bound beads were packed into an Econo column (Bio-Rad) and were washed with 30 column volumes of buffer F containing 5 mM imidazole. The DMC1 protein was eluted in a 20-column volume linear gradient from 5 to 300 mM imidazole in buffer B. The Dmc1 mutants were purified by the same method as that for mTBPIP/HOP2. The mTBPIP/HOP2 deletion mutants were dialyzed against buffer G. Protein concentrations were determined using a Bio-Rad protein assay kit with bovine serum albumin as the standard.

DNA Substrates—In the D-loop formation assay, alkaline treatment of the cells harboring the plasmid DNA was avoided to prevent the dsDNA substrates from undergoing irreversible denaturation. Instead the cells were gently lysed using Sarkosyl as described previously (29). The pGsat4 DNA was created by inserting a 198-base pair fragment of the human α-satellite sequence into the pGEM-T Easy vector (Promega). For the dsDNA substrate used in the D-loop assay, the following high pressure liquid chromatography-purified oligonucleotide was purchased from Roche Applied Science: SAT-1 (50-mer, 5′-ATT TCA TGG TGA ACA GAA GAA TTC TCA GTA ACT TCT TGC TGC TOT GTC TAT-3′ (5′ ends of the oligonucleotides were labeled with [γ-32P]ATP). DNA substrate was added to the DNA binding reactions in the presence of [γ-32P]ATP at 37 °C for 30 min. DNA concentrations are expressed in moles of nucleotides.

The DNA Binding Assay—The dX174 circular ssDNA (40 μM, 5386 bases) is the dX174 circular dsDNA (50 μM, 5386 bases) was mixed with TBPIP/HOP2 in 10 μl of 20 mM Tris- HCl buffer (pH 8.0) containing 0.2 M KCl, 0.25 mM EDTA, and 2 mM 2-mercaptoethanol. Protein concentrations were determined using a Bio-Rad protein assay kit with bovine serum albumin as the standard.

Construction and Purification of the Mouse TBPIP/HOP2 Deletion Mutants—The mouse TBPIP/HOP2 deletion mutants were constructed by polymerase chain reaction and were ligated into the Ndel-BamHI sites of the pET-15b expression vector (Novagen). These deletion mutants were mTBPIP-(1–210), mTBPIP-(1–189), mTBPIP-(1–181), mTBPIP-(1–178), mTBPIP-(1–162), mTBPIP-(1–157), mTBPIP-(1–143), mTBPIP-(1–125), and mTBPIP-(1–105), which lack 7, 28, 36, 39, 55, 60, 74, 92, and 112 amino acids from the C-terminal end, respectively. The mTBPIP-(1–210), mTBPIP-(1–189), mTBPIP-(1–181), mTBPIP-(1–178), mTBPIP-(1–162), mTBPIP-(1–157), mTBPIP-(1–143), mTBPIP-(1–125), and mTBPIP-(1–105) mutants were purified by the same method as that used for mTBPIP/HOP2. The mTBPIP (1–162), mTBPIP (1–157), mTBPIP (1–143), mTBPIP (1–125), and mTBPIP (1–105) mutants were dialyzed against buffer E (20 mM Tris-HCl buffer (pH 8.0) containing 2 mM 2-mercaptoethanol, 0.25 mM EDTA, and 10% glycerol) before the initiation of homologous pairing by DMC1. The homologous pairing catalyzed by DMC1 was displayed due to the failure of DSB repair (28).
RESULTS

Purification of the Mouse and Human TBPIP/HOP2 Proteins—To study the function of TBPIP/HOP2, we overexpressed mTBPIP/HOP2 in the E. coli BL21(DE3) Codon Plus (Stratagene) strain as a fusion protein with an N-terminal hexahistidine tag (His6 tag) containing a cleavage site for thrombin protease. The His6-tagged mTBPIP/HOP2 protein was expressed by induction with isopropyl-1-thio-β-D-galactopyranoside and was purified by chromatography on a Ni2+ -chelating column (Qiagen). The His6 tag was uncoupled with thrombin protease (Amersham Biosciences) from the mTBPIP/HOP2 portion, and mTBPIP/HOP2 was further purified by heparin-Sepharose column chromatography (Amersham Biosciences) (Fig. 1A).

The human TBPIP/HOP2 protein, which shares about 90% amino acid sequence identity with mTBPIP/HOP2, was also overexpressed and was purified by a similar method as that used for mTBPIP/HOP2. The purified human TBPIP/HOP2 preparation contained about 10% of a lead-through product as judged by SDS-polyacrylamide gel electrophoresis analysis. Furthermore degradation products (about 10%) were detected in the fraction containing the purified human TBPIP/HOP2 protein (data not shown), suggesting that the human TBPIP/HOP2 protein is less stable than mTBPIP/HOP2. Therefore, we used mTBPIP/HOP2 in the subsequent biochemical analyses.

The DNA Binding and Homologous Pairing Activities of the TBPIP/HOP2 Protein—The purified mTBPIP/HOP2 protein was tested for its ssDNA and dsDNA binding abilities. As shown in Fig. 1, B and C, mTBPIP/HOP2 bound to both ssDNA and dsDNA. These DNA binding characteristics are similar to those of the homologous pairing proteins such as RAD51, RAD52, and DMC1. Then we tested the homologous pairing activity of mTBPIP/HOP2. To do so, we used the D-loop formation assay, which was used to detect the homologous pairing activity of the human and yeast DMC1 proteins (13, 15). In this assay, a single-stranded 50-mer oligonucleotide and superhelical dsDNA were used as substrates, and the D-loop was formed as a product of the homologous pairing reaction (Fig. 2A). As reported previously (13), the homologous pairing activity of the human DMC1 protein was detected in the D-loop formation assay (Fig. 2, B and C, lane 1). On the other hand, the same amount of mTBPIP/HOP2 (5 µg) itself formed a trace amount of D-loop, which was only detected when the gel was overex-
posed (Fig. 2, B and C). Therefore, mTBPIP/HOP2 shows very little homologous pairing activity in the D-loop formation assay as compared with DMC1.

The TBPIP/HOP2 Protein Enhances the DMC1-mediated Homologous Pairing—We next tested whether mTBPIP/HOP2 affects homologous pairing by DMC1. We used the human DMC1...
protein, which shares 97% amino acid identity with the mouse DMC1 protein. When mTBPIP/HOP2 (5 /H9262 M) was incubated with superhelical dsDNA before mixing with DMC1 (5 /H9262 M) and ssDNA for the initiation of the homologous pairing reaction, the D-loop yield was significantly increased (Fig. 3A, lane 2) as compared with the reaction without mTBPIP/HOP2 (lane 3). The D-loop formation promoted by DMC1 was inhibited in the presence of ADP (Fig. 3B, lanes 9–14) because DMC1 catalyzes D-loop formation in an ATP-dependent manner (13, 15). As shown in Fig. 3B (lanes 3–8), the D-loop formation promoted by DMC1 and mTBPIP/HOP2 was also inhibited in the presence of ADP. On the other hand, the mTBPIP/HOP2 protein contains neither the ATP-binding motif nor the ATPase activity (data not shown). These results further confirmed that DMC1 is the catalytic center for homologous pairing in the presence of DMC1 and mTBPIP/HOP2.

When mTBPIP/HOP2 was mixed with ssDNA before the addition of DMC1, the D-loop formation by DMC1 was significantly inhibited (Fig. 3A, lane 1). Therefore, mTBPIP/HOP2 only enhanced the DMC1-mediated homologous pairing reaction when it was bound to dsDNA, not ssDNA, before the initiation of the homologous pairing reaction.

Intriguingly mTBPIP/HOP2 did not enhance homologous pairing by the human RAD51 protein, another eukaryotic RecA homologue. Although the homologous pairing activity of the human RAD51 protein alone is too weak to be detected by the D-loop formation assay (31, 32), mTBPIP/HOP2 did not enhance the RAD51-mediated D-loop formation to a detectable level (Fig. 3C). In addition, mTBPIP/HOP2 significantly inhibited the RecA-mediated D-loop formation when it was incubated with superhelical dsDNA before mixing with RecA (5 /H9262 M) and ssDNA (Fig. 3D). These results indicate that mTBPIP/HOP2 does not enhance either RAD51- or RecA-dependent homologous pairing. Instead it may specifically function in the DMC1-dependent homologous pairing, which is probably essential for recombination between homologous chromosomes in meiosis.
The mTBPIP/HOP2 Protein Does Not Inhibit D-loop Dissociation—In the present study, we found that the human DMC1 protein formed D-loops in 5–10 min and dissociated them after 10 min (Fig. 4, A and C). Under the same reaction conditions (5 μM RecA, 1 μM 50-mer ssDNA, and 30 μM dsDNA), RecA formed D-loops in 1 min and quickly dissociated them (Fig. 4, D and E). This D-loop dissociation depends on the ATP-dependent branch migration concurrent with the superhelical dsDNA unwinding by RecA (33, 34) and has not been observed in the ATP-independent D-loop formation promoted by the E. coli RecT and RecA proteins.
human RAD52 proteins (29, 35). When mTBPIP/HOP2 was incubated with dsDNA before DMC1 and ssDNA were added, the D-loop yield at 10 min was increased about 2.9-fold relative to that promoted by DMC1 alone, but the D-loop dissociation was still observed (Fig. 4, A and B). These results indicate that mTBPIP/HOP2 stimulates the DMC1-mediated homologous pairing but does not inhibit the D-loop dissociation by DMC1.

During the D-loop formation reaction, about 30% of the dsDNA, corresponding to about 7% of the ssDNA, was incorporated into D-loops by RecA within 1 min (Fig. 4, D and E). On the other hand, DMC1 converted only about 8% of the dsDNA, corresponding to about 1.8% of the ssDNA, to D-loops (Fig. 4, A and E) in the presence of ATP at 5 or 10 min. These results indicate that the ATP-dependent homologous pairing ability of DMC1 is about 25% of that of RecA at the optimal reaction times.

AMP-PNP Significantly Enhances the DMC1-dependent Homologous Pairing and Inhibits D-loop Dissociation—The D-loop formation by the yeast Dmc1 protein is reportedly enhanced by AMP-PNP, which is a non-hydrolyzable analogue of ATP (15). Consist with this previous observation, AMP-PNP significantly stimulated the D-loop formation by the human DMC1 protein. After a 10-min reaction with AMP-PNP, DMC1 formed about 5-fold more D-loops than in the experiment with ATP (Fig. 5, A and B). This D-loop yield with DMC1 and AMP-PNP is almost the same as that with RecA and ATP.

Then we tested whether the mTBPIP/HOP2 protein stimulates the DMC1-dependent homologous pairing in the presence of AMP-PNP. As shown in Fig. 5, A and B, mTBPIP/HOP2 enhanced more than double the D-loop yield by DMC1 alone in the presence of AMP-PNP (Fig. 5, A, lanes 5 and 7, and B). These results indicate that AMP-PNP is a better cofactor than ATP for homologous pairing by DMC1 and does not inhibit the TBP/HOP2 activity during homologous pairing.

In the presence of ATP, DMC1 promoted D-loop formation and dissociation (Fig. 4); however, time course experiments revealed that the D-loop dissociation by DMC1 (with mTBPIP/HOP2) did not occur in the presence of AMP-PNP (Fig. 5, C and D). These results indicate that the D-loop formation (homologous pairing) by DMC1 requires ATP or AMP-PNP binding but not ATP hydrolysis, and its dissociation strongly requires ATP hydrolysis.

The C-terminal Basic Region of mTBPIP/HOP2 Is Required for Efficient DNA Binding—To identify the DNA-binding domain of mTBPIP/HOP2, we performed deletion analyses. The C-terminal region, but not the N-terminal region, of mTBPIP/HOP2 was protease-sensitive as revealed by a mass spectrometric analysis (data not shown). Therefore, we constructed nine deletion mutants, which lacked C-terminal portions, of mTBPIP/HOP2. These mutants are mTBPIP-(1–189), mTBPIP-(1–181), mTBPIP-(1–178), mTBPIP-(1–162), mTBPIP-(1–157), mTBPIP-(1–143), mTBPIP-(1–125), and mTBPIP-(1–105), respectively.

Then we tested whether the mTBPIP/HOP2 protein stimulates the DMC1-dependent homologous pairing in the presence of AMP-PNP. As shown in Fig. 5, A and B, mTBPIP/HOP2 enhanced more than double the D-loop yield by DMC1 alone in the presence of AMP-PNP (Fig. 5, A, lanes 5 and 7, and B). These results indicate that AMP-PNP is a better cofactor than ATP for homologous pairing by DMC1 and does not inhibit the TBP/HOP2 activity during homologous pairing.

In the presence of ATP, DMC1 promoted D-loop formation and dissociation (Fig. 4); however, time course experiments revealed that the D-loop dissociation by DMC1 (with mTBPIP/HOP2) did not occur in the presence of AMP-PNP (Fig. 5, C and D). These results indicate that the D-loop formation (homologous pairing) by DMC1 requires ATP or AMP-PNP binding but not ATP hydrolysis, and its dissociation strongly requires ATP hydrolysis.

The C-terminal Basic Region of mTBPIP/HOP2 Is Required for Efficient DNA Binding—To identify the DNA-binding domain of mTBPIP/HOP2, we performed deletion analyses. The C-terminal region, but not the N-terminal region, of mTBPIP/HOP2 was protease-sensitive as revealed by a mass spectrometric analysis (data not shown). Therefore, we constructed nine deletion mutants, which lacked C-terminal portions, of mTBPIP/HOP2. These mutants are mTBPIP-(1–189), mTBPIP-(1–181), mTBPIP-(1–178), mTBPIP-(1–162), mTBPIP-(1–157), mTBPIP-(1–143), mTBPIP-(1–125), and mTBPIP-(1–105), which lack 7, 28, 36, 39, 55, 60, 74, 92, and 112 amino acids from the C-terminal end, respectively (Fig. 6A). These deletion mutants were designed based on a secondary structure prediction by which the predicted loop regions were selected as the C termini of the mutants. All of the mutants were purified by a method similar to that used for the full-length mTBPIP/HOP2 protein and were analyzed by 15–25% SDS-PAGE (Fig. 6B).

The DNA binding abilities of the mTBPIP/HOP2 deletion mutants were tested by the gel mobility shift assay. As shown in Fig. 1C, mTBPIP/HOP2 bound to both superhelical dsDNA and nicked circular dsDNA with no preference, and the excess amount of mTBPIP/HOP2 formed aggregates, which stacked in the well of the agarose gel. The four mutants mTBPIP-(1–210), mTBPIP-(1–189), mTBPIP-(1–181), and mTBPIP-(1–178) showed the same profiles for dsDNA binding as the full-length mTBPIP/HOP2 protein (Fig. 7A, lanes 1–16). Therefore, the C-terminal 39 amino acid residues are not involved in dsDNA binding. On the other hand, mTBPIP-(1–162) and mTBPIP-(1–157) bound dsDNA but did not form large aggregates (Fig. 7A, lanes 1–16).
dsDNAs are indicated by average of four independent experiments with the full-length mTBPIP/HOP2 protein is shown with the S.D. values.

The DNA binding and homologous pairing stimulation activities of the mTBPIP/HOP2 deletion mutants. A, the dsDNA binding. The 3X174 superhelical dsDNA (10 μM) containing nicked circular dsDNA was incubated with the mTBPIP/HOP2 deletion mutants at 37°C for 10 min. The samples were separated by 0.8% agarose gel electrophoresis in TAE buffer, and the bands were visualized by ethidium bromide staining. The protein concentrations used in the experiments were 0 μM (lanes 1, 5, 9, 13, 17, 21, 25, 29, and 33), 1 μM (lanes 2, 6, 10, 14, 18, 22, 26, 30, and 34), 2 μM (lanes 3, 7, 11, 15, 19, 23, 27, 31, and 35), and 4 μM (lanes 4, 8, 12, 16, 20, 24, 28, 32, and 36). Lanes 2–4, 6–8, 10–12, 14–16, 18–20, 22–24, 26–28, 30–32, and 34–36 indicate experiments with mTBPIP-(1-210), mTBPIP-(1-189), mTBPIP-(1-181), mTBPIP-(1-178), mTBPIP-(1-162), mTBPIP-(1-157), mTBPIP-(1-143), and mTBPIP-(1-105), respectively. Nicked circular and supercoiled dsDNAs are indicated by nc and sc, respectively. B, the ssDNA binding. The 3X174 circular ssDNA (40 μM) was incubated with the mTBPIP/HOP2 deletion mutants at 37°C for 10 min. The samples were separated by 0.8% agarose gel electrophoresis in TAE buffer, and the bands were visualized by ethidium bromide staining. The protein concentrations used in the experiments were 0 μM (lanes 1, 5, 9, 13, 17, 21, 25, 29, and 33), 2 μM (lanes 2, 6, 10, 14, 18, 22, 26, 30, and 34), 4 μM (lanes 3, 7, 11, 15, 19, 23, 27, 31, and 35), and 8 μM (lanes 4, 8, 12, 16, 20, 24, 28, 32, and 36). Lanes 2–4, 6–8, 10–12, 14–16, 18–20, 22–24, 26–28, 30–32, and 34–36 indicate experiments with mTBPIP-(1-210), mTBPIP-(1-189), mTBPIP-(1-181), mTBPIP-(1-178), mTBPIP-(1-162), mTBPIP-(1-157), mTBPIP-(1-143), mTBPIP-(1-125), and mTBPIP-(1-105), respectively. C, stimulation of the DMC1-mediated D-loop formation by the mTBPIP/HOP2 deletion mutants. A 32P-labeled single-stranded 50-mer oligonucleotide (1 μM) was incubated with DMC1 (5 μM) in the standard reaction mixture at 37°C for 5 min. The reactions were initiated by the addition of pGsat4 form I DNA (30 μM) and 10 mM MgCl2 with the mTBPIP/HOP2 deletion mutant (5 μM). Lanes 1 and 10 indicate control experiments with DMC1 alone, and lanes 2 and 11 indicate negative control experiments without protein. Lanes 3 and 12 indicate positive control experiments with both DMC1 and mTBPIP/HOP2. Lanes 5, 6, 7, 8, 9, 14, 15, 16, and 17 indicate experiments with mTBPIP-(1-210), mTBPIP-(1-189), mTBPIP-(1-181), mTBPIP-(1-178), mTBPIP-(1-162), mTBPIP-(1-157), mTBPIP-(1-143), mTBPIP-(1-125), and mTBPIP-(1-105), respectively, instead of the full-length mTBPIP/HOP2 protein. D, graphic representation of the D-loop formation shown in C relative to that formed by DMC1 alone. The average of four independent experiments with the full-length mTBPIP/HOP2 protein is shown with the S.D. values.

7A, lanes 17–24). The mTBPIP-(1-143), mTBPIP-(1-125), and mTBPIP-(1-105) mutants were more defective in dsDNA binding (Fig. 7A, lanes 25–36). Interestingly, when ssDNA was used as the substrate for the gel mobility shift assay, the ssDNA binding profiles of the mTBPIP/HOP2 deletion mutants were quite similar to the dsDNA binding profiles (Fig. 7B). The mTBPIP/HOP2 region from amino acids 118 to 182 may be involved in a basic region flanking the leucine zipper motif.
TBPIP/HOP2 Enhances the DMC1-mediated Homologous Pairing

The C-terminal Basic Region of mTBPIP/HOP2 Is Essential for the DMC1-mediated Homologous Pairing Stimulation—We next tested whether these mTBPIP/HOP2 mutants stimulate the DMC1-mediated homologous pairing. As shown in Fig. 7, C and D, the four mutants mTBPIP-(1–210), mTBPIP-(1–189), mTBPIP-(1–181), and mTBPIP-(1–178), which were proficient in DNA binding, were also completely proficient in D-loop stimulation (lanes 5–8). In contrast, mTBPIP-(1–162), mTBPIP-(1–157), mTBPIP-(1–143), mTBPIP-(1–125), and mTBPIP-(1–105), which were moderately or significantly defective in DNA binding, did not stimulate the DMC1-mediated D-loop formation (Fig. 7, C, lanes 9, 14, 15, 16, 17, and D). These results clearly indicate that efficient dsDNA binding by mTBPIP/HOP2 is essential for stimulation of the DMC1-mediated homologous pairing.

DISCUSSION

Previous yeast genetic studies have shown that the Hop2 protein plays an essential role during meiosis (22, 25). In the putative basic region of mTBPIP/HOP2 is required for efficient association of AMP-PNP and found that the mTBPIP/HOP2 protein significantly enhanced the DMC1-dependent homologous pairing in the presence of AMP-PNP. These results indicate that the mTBPIP/HOP2 protein stimulates the Dmc1-dependent homologous pairing, and even the basal level of homologous pairing by DMC1 is enhanced in the presence of AMP-PNP.

These biochemical observations are consistent with the results from previous genetic studies, which suggested that TBPIP/HOP2 functions together with DMC1, probably in the homologous pairing step (25, 28). Therefore, we conclude that TBPIP/HOP2 is a factor that positively functions with DMC1 in the strand invasion step of homologous pairing. However, we cannot eliminate the possibility that the DMC1 and TBPIP/HOP2 proteins may have another function to ensure the proper pairing of homologous chromosomes during meiosis because the homologous pairing ability of DMC1 is weakly detected in the presence of ATP as compared with that of RecA. Further analyses will be required to clarify this issue.

Recently we determined the crystal structure of the human DMC1 octameric ring, a functional form of DMC1, and revealed the structural basis for octameric ring formation (30). Although the monomeric structures of DMC1, RAD51, and RecA are very similar (30, 39–41), RAD51 and RecA form helical filaments, which are considered to be their functional form. In the present study, we found that TBPIP/HOP2 specifically activated homologous pairing by DMC1 but not by RAD51 or RecA. The TBPIP/HOP2 protein may specifically function with a homologous pairing protein that forms an octameric ring rather than a helical filament.

The yeast Hop2 protein reportedly forms a complex with the Mnd1 protein, which has been identified as a multipurpose suppressor of a temperature-sensitive hop2 mutant allele (37). The MND1 gene is also required for meiotic recombination (38). The mnd1-null mutant exhibits a strikingly similar phenotype to that of the hop2-null mutant (37, 38). This genetic evidence suggests that the Hop2 and Mnd1 proteins function as a complex to promote meiotic chromosome pairing, and the Hop2-MND1 complex may be a cofactor for the RAD51 and DMC1 recombinases. In the present study, we showed that the mammalian TBPIP/HOP2 protein stimulates the DMC1-mediated homologous pairing, indicating that the TBPIP/HOP2 subunit of the HOP2-MND1 complex may be required to activate homologous pairing by the meiosis-specific DMC1 protein. It would be intriguing to study how the DMC1-TBPIP/HOP2-mediated homologous pairing functions to discriminate homologous chromosomes from nonhomologous chromosomes during meiosis. The MND1 protein, like TBPIP/HOP2, is highly conserved from yeast to human. Analyses of the biochemical activities of the MND1 protein and the HOPE2-MND1 complex will be the next critical issue to reveal the molecular mechanism of the meiosis-specific homologous pairing between homologous chromosomes.

Acknowledgments—We thank Y. Matsuo (Waseda University), Y. Takizawa (Yokohama City University), and W. Kagawa (RIKEN Genomic Sciences Center) for technical assistance and W. Kagawa for critical reading of the manuscript.

REFERENCES

1. Keeney, S., Giroux, C. N., and Kleckner, N. (1997) Cell 88, 375–384
2. Romanienko, P. J., and Camerini-Otero, R. D. (2000) Mol. Cell 6, 973–987
3. Baudat, F., Manova, K., Yuen, J. P., Jasin, M., and Keeney, S. (2000) Mol. Cell 6, 989–998
4. Shibata, T., DasGupta, C., Cunningham, R. P., and Radding, C. M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1638–1642
5. McInteer, K., Weinstock, G. M., and Lehman, J. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2615–2619
6. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) Cell 69, 439–456
7. Bishop, D. K., Park, D., Xu, L., and Kleckner, N. (1992) Cell 69, 457–470
8. Shinohara, A., Ogawa, H., Matsuda, Y., Usuki, N., Iken, K., and Ogawa, T. (1993) Nat. Genet. 4, 239–243
10. Sung, P. (1994) Science 265, 1241–1243
11. Baumann, P., Benson, F. E., and West, S. C. (1996) Cell 87, 757–766
12. Gupta, R. C., Bazemore, L. R., Golub, E. I., and Radding, C. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 463–468
13. Li, Z., Golub, E. I., Gupta, R., and Radding, C. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11274–11276
14. Masson, J.-Y., Davies, A. A., Hajibagheri, N., Van Dyck, E., Benson, F. E., Stasiak, A. Z., Stasiak, A., and West, S. C. (1999) EMBO J. 18, 6552–6560
15. Hong, E. L., Shinohara, A., and Bishop, D. K. (2001) J. Biol. Chem. 276, 41906–41912
16. Lim, D.-S., and Hasty, P. (1996) Mol. Cell. Biol. 16, 7133–7143
17. Shibata, T., Ohtani, T., Iwabuchi, M., and Ando, T. (1982) J. Biol. Chem. 257, 13981–13985
18. Noirot, P., and Kolodner, R. D. (1998) J. Biol. Chem. 273, 12274–12280
19. Ko, L., Cardona, G. R., Henrion-Caude, A., and Chin, W. W. (2002) Mol. Cell. Biol. 22, 357–369
20. Gerton, J. L., and DeRisi, J. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 6895–6900
21. Story, R. M., Weber, I. T., and Steitz, T. A. (1992) Nature 355, 318–325
22. Pellegrini, L., Yu, D. S., Anand, S., Lee, M., Blundell, T. L., and Venkitaraman, A. R. (2002) Nature 420, 287–293
23. Shin, D. S., Pellegrini, L., Daniels, D. S., Yelent, B., Craig, L., Bates, D., Yu, D. S., Shvij, M. K., Hitomi, C., Arvai, A. S., Volkman, N., Tsuruta, H., Bulandell, T. L., Venkitaraman, A. R., and Tainer, J. A. (2003) EMBO J. 22, 4566–4576

TBPIP/HOP2 Enhances the DMC1-mediated Homologous Pairing