Stimulation of DNA Strand Exchange by the Human TBPIP/Hop2-Mnd1 Complex*

Received for publication, June 15, 2005, and in revised form, December 20, 2005
Published, JBC Papers in Press, January 9, 2006, DOI 10.1074/jbc.M506506200

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

From the ‡RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, the §Division of Cell Biology and Neuroscience, Department of Morphological and Physiological Sciences, Faculty of Medical Sciences, University of Fukui, 23-3 Shimoaizuki, Matsuoka-cho, Fukui 910-1193 Japan, the ¶Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology Agency (JST), 4-1-8 Honcho Kawaguchi-shi, Saitama 332-0012, Japan, the ¶Graduate School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan, the §RIKEN Harima Institute at SPring-8, 1-1-1 Kohto, Mikazuki-cho, Sayo, Hyogo 679-5148, Japan, and the ¶¶Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

In Saccharomyces cerevisiae, the Hop2 protein forms a complex with the Mnd1 protein and is required for the alignment of homologous chromosomes during meiosis, probably through extensive homology matching between them. The Rad51 and Dmc1 proteins, the eukaryotic RecA orthologs, promote strand exchange and may function in the extensive matching of homology within paired DNA molecules. In the present study, we purified the human TBPIP/Hop2-Mnd1 complex and found that it significantly stimulates the Dmc1- and Rad51-mediated strand exchange. The human Hop2-Mnd1 complex preferentially binds to a three-stranded DNA branch, which mimics the strand-exchange intermediate. These findings are consistent with genetic data, which showed that the Hop2 and Mnd1 proteins are required for homology matching between homologous chromosomes. Therefore, the human TBPIP/Hop2-Mnd1 complex may ensure proper pairing between homologous chromosomes through its stimulation of strand exchange during meiosis.

In meiosis, a high level of homologous recombination occurs only between homologous chromosomes but not between sister chromatids. This meiotic homologous recombination is initiated by the formation of a double strand break (DSB), which is introduced by the SPO11 protein (1–3). On the other hand, in mitosis, homologous recombination functions to repair DSBs, which are introduced by DNA damaging agents, such as ionizing radiation, DNA cross-linking reagents, oxidative stress, and replication errors. This mitotic homologous recombination repair mainly occurs between sister chromatids or between the abundant intra- and inter-chromosomal homologous repeat sequences.

After the DSB formation, a single-stranded DNA (ssDNA) tail derived from a DSB site invades the homologous double-stranded DNA (dsDNA). This strand-invasion step, called homologous pairing, primes heteroduplex formation, in which new Watson-Crick base pairs are formed between the invading strand and its complementary strand of parental dsDNA. Then, the heteroduplex region is expanded by the subsequent strand-exchange step. This strand-exchange step may be important for the extensive matching of homology between paired chromosomes to ensure that homologous recombination occurs between the proper partners. The Escherichia coli RecA protein is known to catalyze the homologous pairing and strand-exchange steps (4–7), and two RecA homologues, the Rad51 and Dmc1 proteins, have been identified in eukaryotes (8–11). The Rad51 and Dmc1 proteins have been shown to catalyze strand exchange in vitro, but their strand-exchange activities are low as compared with that of RecA (12–14).

Genetic studies with Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Arabidopsis thaliana have identified the HO2P, meu13, and AHP2 genes, respectively, as being essential for meiotic homologous recombination (15–17). The HO2P, meu13, and AHP2 genes are orthologs, and the human and mouse TBPIP proteins have been identified as mammalian orthologs of Hop2 (18, 19). The hop2 deletion mutant in S. cerevisiae fails to sporulate, due to a uniform arrest at the pachytene stage of meiosis I (15). The TBPIP/Hop2 knock-out mice also display meiotic cell cycle arrest, due to the failure of DSB repair (20). Interestingly, in the hop2 deletion mutant cells, most of the chromosomes are engaged in synopsis with nonhomologous partners but not with homologous chromosomes (15), suggesting that the Hop2 protein functions to align homologous chromosomes during meiosis. The S. cerevisiae Hop2 protein reportedly forms a complex with the Mnd1 protein, which has been identified as a multicopy suppressor of a temperature-sensitive hop2 mutant allele (21). The S. cerevisiae MND1 gene and its S. pombe ortholog, mcp7, are also required for meiotic recombination (21–23). The mnd1-null mutant exhibits a strikingly similar phenotype to that of the hop2-null mutant (21, 22). These results suggest that the Hop2 and Mnd1 proteins function as a complex to promote meiotic chromosome pairing. Biochemical analyses also revealed that the yeast and mouse Hop2 (TBPIP/Hop2) and Mnd1 proteins form a complex that can stimulate the strand-invasion step promoted by their cognate Dmc1 proteins (24, 25).

In the present study, we found that the purified human TBPIP/Hop2-Mnd1 complex (hTBPIP/Hop2-hMnd1) significantly stimulates the Dmc1- or Rad51-mediated strand exchange, which may be required for the extensive matching of homology within homologous chromosomes. Therefore, hTBPIP/Hop2-hMnd1 may function in the strand-exchange step, which occurs just after homologous pairing, to ensure that the correct partner is involved in meiotic homologous recombination.

References

8 This work was supported by the RIKEN Structural Genomics/Proteomics Initiative (RSGI); the National Project on Protein Structural and Functional Analyses; grants-in-aid from the Japanese Society for the Promotion of Science (JSPS); the Ministry of Education, Sports, Culture, Science, and Technology, Japan; and the Japan Health Sciences Foundation. 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.

9 To whom correspondence may be addressed. Tel.: 81-3-5286-8189; Fax: 81-3-5292-9211; E-mail: kurumizaka@waseda.jp.

10 To whom correspondence may be addressed. Tel.: 81-45-303-9197; Fax: 81-3-503-9195; E-mail: yokoyama@biochem.s.u-tokyo.ac.jp.

11 The abbreviations used are: DSB, double strand break; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; h, human; Ni-NTA, nickel-nitrilotriacetic acid; JM, joint molecule; NC, nicked circular dsDNA; HPLC, high performance liquid chromatography; RSGI, the National Project on Protein Structural and Functional Analyses; grants-in-aid from the Japanese Society for the Promotion of Science (JSPS); the Ministry of Education, Sports, Culture, Science, and Technology, Japan; the Japan Health Sciences Foundation. 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.

‡* This work was supported by the RIKEN Structural Genomics/Proteomics Initiative (RSGI); the National Project on Protein Structural and Functional Analyses; grants-in-aid from the Japanese Society for the Promotion of Science (JSPS); the Ministry of Education, Sports, Culture, Science, and Technology, Japan; and the Japan Health Sciences Foundation. 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.
Biochemical Activity of the Human Hop2-Mnd1 Complex

**EXPERIMENTAL PROCEDURES**

Overexpression and Purification of the Human TBPIP/Hop2-Mnd1 Complex—The human TBPIP/Hop2 gene was previously cloned as a homologue of the mouse TBPIP gene (18, 19). The human Mnd1 gene (GenBank™ accession number NM_032117) was cloned from a human testis cDNA pool (Clontech). The human TBPIP/Hop2 and Mnd1 genes were ligated into the NdeI-BamHI sites of the pET15b and pET11a vectors, respectively. Then, the pET11a vector containing the Mnd1 gene was digested with BglII and BamHI, and the resulting fragment was ligated into the NdeI-BamHI sites of the pET15b and pET11a vectors, respectively. The resulting construct containing pET15b vector. In this construct, the human Mnd1 protein (hMnd1) and the N-terminally His$_6$-tagged human TBPIP/Hop2 protein (hTBPIP/Hop2) were coexpressed in the E. coli strain BL21-CodonPlus(DE3)-RIL (Stratagene). The cells expressing both hMnd1 and hTBPIP/Hop2 were grown at 37 °C in 10 liters of LB medium, containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. At the logarithmic phase of growth (A$_{600}$ = 0.6), both hTBPIP/Hop2 and hMnd1 were produced in the presence of 500 μM isopropyl 1-thio-β-D-galactopyranoside (IPTG). Then, the cells were harvested after a 4 h incubation at 37 °C and were disrupted by sonication in buffer A (50 mM Tris-HCl buffer (pH 7.5) containing 0.5 mM NaCl, 10% glycerol, 5 mM 2-mercaptoethanol, and protease inhibitors (Complete EDTA-free; Roche Diagnostics)) on ice. The cell lysate was centrifuged at 27,700 × g for 20 min, and then the proteins in the supernatants were precipitated with 0.24 g/ml ammonium sulfate (40% saturation). The precipitate was removed by centrifugation at 27,700 × g for 20 min, and the proteins in the supernatant were further precipitated by the addition of 0.13 g/ml ammonium sulfate (60% saturation). The precipitate was dissolved in 30 ml of buffer A and was gently mixed with 4 ml of Ni-NTA-agarose beads (Qiagen) for 1 h at 4 °C. The Ni-NTA-agarose beads bound to the His$_6$-tagged hTBPIP/Hop2 protein were packed into an Econo-column (Bio-Rad) and were washed with 20-column volumes of buffer A containing 7 mM imidazole, at a flow rate of about 0.3 ml/min. The His$_6$-tagged hTBPIP/Hop2 protein complexed with the hMnd1 protein was eluted in a 15-column volume linear gradient from 7 to 400 mM imidazole in buffer A. The His$_6$ tag was uncoupled from the hTBPIP/Hop2 portion by a digestion with 1 unit of thrombin protease (Amersham Biosciences) per mg of the hTBPIP/Hop2-hMnd1 proteins. After the thrombin treatment, the hTBPIP/Hop2-hMnd1 proteins were immediately dialyzed against buffer B (20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM NaCl, 5 mM 2-mercaptoethanol, 0.25 mM EDTA, and 10% glycerol) for more than 12 h at 4 °C. The hTBPIP/Hop2-hMnd1 proteins were loaded onto a 6-ml heparin-Sepharose (Amersham Biosciences) column previously equilibrated with buffer B and were washed with 20-column volumes of buffer A containing 7 mM imidazole, at a flow rate of about 0.3 ml/min. The His$_6$-tagged hTBPIP/Hop2 protein complexed with the hMnd1 protein was eluted in a 15-column volume linear gradient from 7 to 400 mM imidazole in buffer A. The His$_6$ tag was uncoupled from the hTBPIP/Hop2 portion by a digestion with 1 unit of thrombin protease (Amersham Biosciences) per mg of the hTBPIP/Hop2-hMnd1 proteins. After the thrombin treatment, the hTBPIP/Hop2-hMnd1 proteins were immediately dialyzed against buffer B (20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM NaCl, 5 mM 2-mercaptoethanol, 0.25 mM EDTA, and 10% glycerol) for more than 12 h at 4 °C. The hTBPIP/Hop2-hMnd1 proteins were loaded onto a 6-ml heparin-Sepharose (Amersham Biosciences) column previously equilibrated with buffer B. The column was washed with 10-column volumes of buffer B, and the proteins were eluted with a 10-column volume linear gradient from 0.2 to 1.2 mM NaCl in buffer B. The peak fractions of the hTBPIP/Hop2-hMnd1 proteins were dialyzed against buffer C (20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM NaCl, 5 mM 2-mercaptoethanol, and 0.25 mM EDTA). The hTBPIP/Hop2-hMnd1 proteins eluted from the heparin-Sepharose column were loaded onto a HiLoad 26/60 Superdex 200 column (Amersham Biosciences) previously equilibrated with buffer C. The purified hTBPIP/Hop2-hMnd1 proteins were eluted from the Superdex 200 column with buffer C, at a flow rate of about 0.8 ml/min. Protein concentrations were determined using a Bio-Rad protein assay kit with bovine serum albumin as the standard.

The Human Replication Protein A (RPA), Dmc1, and Rad51 Proteins—The human replication protein A (RPA) was purified as described previously (26). The human Dmc1 and Rad51 proteins (hDmc1 and hRad51, respectively) were expressed in the E. coli strain BL21-CodonPlus(DE3)-RIL (Stratagene) and were purified to homogeneity as described previously (27–29). The His$_6$ tags of these proteins were removed by thrombin protease digestion during the purification process. 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) (30). For the ssDNA substrate used in the D-loop assay, the following high performance liquid chromatography (HPLC)-purified oligonucleotide was purchased from Roche Diagnostics. SAT-1 (50-mer, 5'-ATT TCA TGC TAG ACA GAA GAA TTC TCA GTA ACT TCT TTG TG TGT GTG TA-3'). The 5’ ends of the oligonucleotides were labeled with T4 polynucleotide kinase (New England Biolabs) in the presence of [γ-32P]ATP at 37 °C for 30 min. DNA concentrations were expressed in moles of nucleotides.

The DNA substrates used in the competitive DNA binding assay were identical to those in the synthetic Holliday junction (I) designed by Iwasaki et al. (31). The DNA substrates containing the three-stranded DNA branch, the Y-form DNA, and the single-stranded oligonucleotide were produced with three 49-mer single-stranded oligonucleotides, 1, 2, and 3, with the sequences 5'-ATCGA TGTC TGAGC GCTCA GGATT GATCT GTAAAT GGCC GTAAG-3' and 5'-GTCCCC AGGCC ATTAC AGATC AATCC TGAGC AGGC TGATTG-3', and 5'-TGATC AGTCG ATCTG CGTCG AATCC TGAGC AGCTG TCTAG AGACA TCGA-3', respectively. The three-stranded DNA branch substrate contained a 12-bp mobile region at the center, and the Y-form DNA substrate was composed of a 30-bp duplex region and two single-stranded tails (18 and 19 bp). All of the oligonucleotides were purified by HPLC, and the DNA concentrations were expressed in moles of nucleotides.

The DNA Binding Assay with ssDNA and dsDNA—The φX174 circular ssDNA (40 μM; 5,386 bases) and the PstI-linearized ϕX174 dsDNA (40 μM; 5,386 base pairs) (New England Biolabs) were mixed with hTBPIP/Hop2-hMnd1 (0.25, 0.5, 1, 2, and 4 μM) in 10 μl of 20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM NaCl, 0.25 mM EDTA, and 5 mM 2-mercaptoethanol. The reaction mixtures were incubated at 37 °C for 10 min, and the protein-DNA complexes were resolved by 0.8% agarose gel electrophoresis in 1× TAE (40 mM Tris acetate and 1 mM EDTA) buffer at 3.3 V/cm for 2 h. The bands were visualized by ethidium bromide staining.

Competitive DNA Binding Assays with the Three-stranded DNA Branch, the Y-form DNA, and the Single-stranded Oligonucleotides—The 32P-labeled oligonucleotide 1 (0.16 μM) was mixed with the unlabeled oligonucleotides 2 (0.16 μM) and 3 (0.16 μM), and the 32P-labeled DNA substrates containing the three-stranded DNA branch, the Y-form DNA, and the single-stranded oligonucleotide were produced by annealing. The DNA sample used in this assay contained the three-stranded DNA branch, the Y-form DNA, and the single-stranded oligonucleotide at 1:2:2.9 ratios. The indicated amounts of hTBPIP/Hop2-hMnd1 were incubated with the DNA substrates (total 64 nM) at 37 °C for 10 min in 10 μl of 20 mM Tris-HCl buffer (pH 8.0), containing 1 mM ATP, 1 mM MgCl$_2$, 100 μg/ml bovine serum albumin, 2 mM creatine phosphate, and 75 μg/ml creatine kinase. The products were analyzed by 10% polyacrylamide gel electrophoresis in 1× TBE buffer (90 mM

5576 JOURNAL OF BIOLOGICAL CHEMISTRY
VOLUME 281 • NUMBER 9 • MARCH 3, 2006
Tris borate and 2 mM EDTA) and were visualized by autoradiography of the dried gel.

**Analytical Ultracentrifugation**—Sedimentation equilibrium experiments were performed in a Beckman ProteomeLab XL-1 instrument. The hTBPIP/Hop2-hMnd1 proteins (0.5 mg/ml) were spun in a Beckman An-50Ti rotor with a 6-sector centerpiece. Equilibrium distributions were analyzed after 20 h of centrifugation at 9,000 rpm and 20 °C. For the molecular weight analysis, a partial specific volume of 0.675 cm³/g and a solution density of 1 g/cm³ were used.

**Size Exclusion Chromatography**—hTBPIP/Hop2-hMnd1 (100 μM, 500 μl), hDmc1 (50 μM, 500 μl), and hRad51 (24 μM, 300 μl) were subjected to Superdex 200 HR 10/300 GL chromatography (Amersham Biosciences) at 4 °C in 20 mM Tris-HCl buffer (pH 7.5), containing 0.2 M NaCl, 0.25 mM EDTA, and 5 mM 2-mercaptoethanol. In the experiments with hTBPIP/Hop2-hMnd1 and hDmc1, or hTBPIP/Hop2-hMnd1 and hRad51, hTBPIP/Hop2-hMnd1 was incubated with hDmc1 or hRad51 at 4 °C with a hTBPIP/Hop2-hMnd1:hDmc1 or hRad51 ratio of 2:1.

**The ATPase Assay**—The ATPase activities of the proteins were analyzed by the release of 32P from [γ-32P]ATP. The proteins were mixed with 40 μM φX174 linear dsDNA or 40 μM φX174 circular ssDNA, in 10 μl of 20 mM Tris-HCl buffer (pH 8.0), containing 0.1 mM ATP, 5 nCi of [γ-32P]ATP, 1 mM MgCl₂, and 100 μg/ml bovine serum albumin. The protein concentrations were 6.5 μM for hDmc1 and hRad51 and 0.2 or 1.3 μM for hTBPIP/Hop2-hMnd1. The reaction mixtures were incubated at 37 °C for 30 min, and the products were separated by thin layer chromatography on polyethyleneimine-cellulose in a 0.5 M LiCl and 1 M formic acid solution.

**The D-loop Formation Assay**—The hDmc1 protein (6.5 μM) and 32P-labeled SAT-1 ssDNA (1.4 μM) were incubated in 80 μl of the standard reaction mixture (20 mM Tris-HCl buffer (pH 7.5) containing 2 mM ATP, 5 mM MgCl₂, 100 μg/ml bovine serum albumin, 8 mM creatine...
Biochemical Activity of the Human Hop2-Mnd1 Complex

RESULTS

**Purification of the Human TBPIP/Hop2-Mnd1 Complex**—To obtain the purified human TBPIP/Hop2-Mnd1 complex, we constructed a TBPIP/Hop2 and hMnd1 co-expression system, in which the human TBPIP/Hop2 and Mnd1 genes were tandemly ligated into the PET15b vector (Novagen). In this system, TBPIP/Hop2 was produced as a fusion protein, with an N-terminal His8 tag containing a cleavage site for thrombin protease, while hMnd1 was expressed without any additional sequence. Both hMnd1 and His8-tagged TBPIP/Hop2 were overexpressed in the *E. coli* BL21-CodonPlus (DE3)-RIL (Strategene) strain by an induction with IPTG (Fig. 1A, lanes 2 and 3). hMnd1 co-eluted with His8-tagged TBPIP/Hop2 from a Ni2+ chelating column (Ni-NTA agarose, Qiagen) (Fig. 1A, lane 6), indicating that hMnd1 and TBPIP/Hop2 formed a complex, like the yeast and mouse homologues (24, 25). The His8 tag was uncoupled with thrombin protease (Amersham Biosciences) from the TBPIP/Hop2 portion (Fig. 1A, lane 7), and the TBPIP/Hop2 and hMnd1 proteins were further purified by heparin-Sepharose column chromatography (Amersham Biosciences) followed by Superdex 200 gel filtration chromatography (Amersham Biosciences) (Fig. 1A, lanes 8 and 9).

The TBPIP/Hop2 and hMnd1 proteins co-purified through all of the purification steps in about 1:1 stoichiometry, and no fraction containing TBPIP/Hop2 or hMnd1 alone was found in any chromatographic step, indicating that these proteins formed a stable complex. Purified TBPIP/Hop2-hMnd1 bound to both ssDNA and dsDNA (data not shown). A competitive DNA binding experiment with circular ssDNA and linearized dsDNA showed that TBPIP/Hop2-hMnd1 preferentially bound to dsDNA (Fig. 1B). This DNA binding property of TBPIP/Hop2-hMnd1 is the same as that of the yeast (*S. cerevisiae*) Hop2-Mnd1 complex containing the C-terminally His8-tagged Mnd1 protein (24). However, the mouse TBPIP/Hop2-Mnd1 complex containing the C-terminally His8-tagged TBPIP/Hop2 protein reportedly binds to ssDNA and dsDNA without any preference (25), suggesting
that the DNA binding properties of the human and mouse TBPIP/Hop2-Mnd1 complexes may be somewhat different.

The apparent molecular mass of hTBPIP/Hop2-hMnd1 was estimated by analytical ultracentrifugation (~49 kDa) and gel filtration chromatography (~47 kDa) (Fig. 1, C and D). Therefore, hTBPIP/Hop2-hMnd1 is a heterodimer, with a molecular mass of 49 kDa (25 kDa for hTBPIP/Hop2 and 24 kDa for hMnd1). The gel filtration analyses also indicated that hTBPIP/Hop2-hMnd1 did not affect the Dmc1- and Rad51-polymer formation, when it was mixed with Dmc1 or Rad51 (Fig. 1D).

hTBPIP/Hop2-hMnd1 Stimulates Homologous Pairing Mediated by hDmc1—Next, we tested whether hTBPIP/Hop2-hMnd1 stimulates the homologous pairing promoted by hDmc1, like the yeast and mouse orthologs (24, 25). The D-loop formation assay was employed to test the

![Image of a diagram showing the experiments and results]
Biochemical Activity of the Human Hop2-Mnd1 Complex

Dmc1-mediated homologous pairing (Fig. 2A). In this assay, hDmc1 formed D-loops within 5 min, and the D-loops were dissociated by the subsequent strand exchange promoted by hDmc1 (Fig. 2F). ATP. The reaction mixtures contained 6.5 μM hDmc1 (lanes 2, 4, 6, and 8) and 0.2 μM hTBPIP/Hop2-Mnd1 (lanes 3, 4, 7, and 8). Lanes 1 and 5 indicate the negative control experiments without proteins. The samples were separated by thin layer chromatography, and were visualized with the BAS2500 image analyzer. The reactions were conducted using the same methods as shown in A, except hRad51 was used instead of hDmc1. The reaction mixtures contained 6.5 μM hRad51 (lanes 2, 4, 6, and 8) and 1.3 μM hTBPIP/Hop2-Mnd1 (lanes 3, 4, 7, and 8). Lanes 1 and 5 indicate the negative control experiments without proteins.

hTBPIP/Hop2-hMnd1 Preferentially Binds to a Three-stranded DNA Branch—We next tested the DNA binding specificity of hTBPIP/Hop2-hMnd1. A competitive DNA binding assay with a 49-mer Y-form DNA (a two-stranded DNA branch), and a 49-mer ssDNA, was performed. Intriguingly, hTBPIP/Hop2-hMnd1 significantly enhanced the NC yield in the Dmc1-mediated strand-exchange reaction, indicating that this complex stimulates the strand exchange promoted by hDmc1. Robust enhancement of the NC yield was detected in the presence of 0.5 or 1 μM hTBPIP/Hop2-hMnd1 with 15 μM hDmc1 (Fig. 3B, lanes 5 and 6, and C). At this optimal hTBPIP/Hop2-hMnd1 concentration, the decrease in the JM yield was concomitant with the increase in the NC yield, suggesting that hTBPIP/Hop2-hMnd1 stimulates the conversion from JM to NC in the Dmc1-mediated strand-exchange reaction (Fig. 3, B and C). In addition, hTBPIP/Hop2-hMnd1 also stimulated the strand-exchange promoted by hRad51 (Fig. 3, D and E). In both the Dmc1-mediated and Rad51-mediated reactions, NC formation was inhibited in the presence of an excess amount of hTBPIP/Hop2-hMnd1 (Fig. 3, B–E). Time course experiments showed that the NC yield by the Dmc1- and Rad51-mediated reactions was significantly stimulated by hTBPIP/Hop2-hMnd1 at the early reaction times (10–30 min) (Fig. 3, F and G).

Since the Dmc1- and Rad51-mediated strand exchange is an ATP-dependent reaction, we tested whether hTBPIP/Hop2-hMnd1 enhances the ATP hydrolyzing activities of hDmc1 and hRad51. As shown in Fig. 4, the ATP hydrolyzing activities of Dmc1 and Rad51 were observed in the presence of ssDNA or dsDNA. However, hTBPIP/Hop2-hMnd1 did not enhance the DNA-dependent ATP hydrolyzing abilities of hDmc1 and hRad51 (Fig. 4).

hTBPIP/Hop2-hMnd1 Preferentially Binds to a Three-stranded DNA Branch—We next tested the DNA binding specificity of hTBPIP/Hop2-hMnd1. A competitive DNA binding assay with a 49-mer three-stranded DNA branch, which mimics an intermediate for strand exchange, a 49-mer Y-form DNA (a two-stranded DNA branch), and a 49-mer ssDNA, was performed. Intriguingly, hTBPIP/Hop2-hMnd1 preferentially bound to the three-stranded DNA branch rather than the Y-form DNA (Fig. 5, A and B). hTBPIP/Hop2-hMnd1 also preferred to bind the Y-form DNA rather than the canonical dsDNA (data not shown), indicating that it has a binding preference for the branched DNA structure. These findings support the idea that hTBPIP/Hop2-hMnd1 preferentially binds to the three-stranded DNA branch to stimulate the JM–NC conversion during the strand-exchange process promoted by the Dmc1 and Rad51 proteins.
At the beginning of homologous recombination, an ssDNA tail derived from a DSB site invades the homologous dsDNA. This initial strand-invasion step is called homologous pairing. Just after homologous pairing, the heteroduplex region produced by homologous pairing is expanded by strand exchange. These two homologous pairing and strand-exchange steps may play distinct roles. Homologous pairing mediates the initial contact in a short tract and may be important for finding homologous sequences between chromosomes. Only short homologous sequences are required for homologous pairing. In fact, bacterial RecA promotes homologous pairing with short oligonucleotides (32, 33), and as shown in the present and previous studies, Rad51 and Dmc1 also promote homologous pairing with short oligonucleotides (34–38). These short homologous sequences are frequently found between nonhomologous chromosomes, such as repetitive DNA sequences (39).

On the other hand, strand exchange is required to search for extensive spans of homology between paired DNA molecules, by extension of the heteroduplex region. Rad51 is known to promote strand exchange between circular ssDNA and linear dsDNA substrates in the presence of RPA and ATP (12, 13). Recently, it was reported that the human Dmc1 protein also promotes strand exchange over at least several thousand base pairs in the presence of RPA and ATP (14). The strand-exchange activities of Dmc1 and Rad51 may function to search for extensive DNA homology around the homologous pairing site and may play an essential role to ensure the correct pairing between homologous chromosomes during meiosis.

In the present study, we found that hTBPIP/Hop2-hMnd1 significantly stimulates the strand exchange mediated by hRad51 or hRad51. This finding suggests that hTBPIP/Hop2-hMnd1 functions in the search for extensive homology between homologous chromosomes, through its strand-exchange stimulation activity. This idea is consistent with the genetic analysis of the S. cerevisiae hop2Δ strain. In this strain, the absence of the Hop2 protein leads to pairing between nonhomologous chromosomes, but not between homologous chromosomes, during meiosis (15). This inappropriate chromosome pairing in the hop2Δ strain may be due to a defect in the extensive homology search along paired chromosomes by the Dmc1- and/or Rad51-mediated strand exchange. These biochemical and genetic studies indicated that TPBP1/Hop2-Mnd1 is important for the proper alignment of homologous chromosomes, probably through its stimulation of strand exchange.

The hTBPIP/Hop2-hMnd1 complex also stimulates the Dmc1-mediated homologous pairing. Previously, the yeast and mouse Hop2 (TPBP1/Hop2)-Mnd1 complexes were shown to promote the stimulation of the Dmc1-mediated homologous pairing (24, 25). Our previous study indicated that the mouse TPBP1/Hop2 protein itself enhances the Dmc1-mediated homologous pairing without Mnd1, but a large amount of the TPBP1/Hop2 protein was required (40) (5 μM TPBP1/Hop2 for 5 μM Dmc1). In contrast to the TPBP1/Hop2 protein alone, hTBPIP/Hop2-hMnd1 significantly enhances the Dmc1-mediated homologous pairing, with a small amount of protein (0.2 μM hTBPIP/Hop2-Mnd1 for 6.5 μM hDmc1). This fact suggests that both TPBP1/Hop2-hMnd1 complex functions in a catalytic manner. We found that the human TPBP1/Hop2 protein was extremely unstable in the absence of the hMnd1 protein (data not shown). This is consistent with genetic results that the overexpression of the Mnd1 gene suppressed a temperature-sensitive hop2 mutant allele in S. cerevisiae (22). Therefore, the enhancement of the Dmc1-mediated homologous pairing by the mouse TPBP1/Hop2 protein was only detected in the presence of an excess amount of the protein, probably due to its extreme instability in the absence of the Mnd1 protein.