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Meiosis-specific yeast Hop1 protein promotes synapsis of double-stranded DNA helices via the formation of guanine quartets

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ABSTRACT

In most eukaryotes, genetic exchange between paired homologs occurs in the context of a tripartite proteinaceous structure called the synaptonemal complex (SC). Genetic analyses have revealed that the genes encoding SC proteins are vital for meiotic chromosome pairing and recombination. However, the number, nature and/or the mechanism used by SC proteins to align chromosomes are yet to be clearly defined. Here, we show that *Saccharomyces cerevisiae* Hop1, a component of SC, was able to promote pairing of double-stranded DNA helices containing arrays of mismatched G/G sequences. Significantly, pairing was rapid and robust, independent of homology in the arms flanking the central G/G region, and required four contiguous guanine residues. Furthermore, data from truncated DNA double helices showed that 20 bp on either side of the 8 bp mismatched G/G region was essential for efficient synapsis. Methylation interference indicated that pairing between the two DNA double helices involves G quartets. These results suggest that Hop1 is likely to play a direct role in meiotic chromosome pairing and recombination by its ability to promote synapsis between double-stranded DNA helices containing arrays of G residues. To our knowledge, Hop1 is the first protein shown to promote synapsis of DNA double helices from yeast or any other organism.

INTRODUCTION

Meiosis is a fundamental biological process responsible for genetic exchange between paternal and maternal genomes in all sexually reproducing species. In most eukaryotes, the pairing of homologs along their lengths is facilitated by a meiosis-specific structure: the synaptonemal complex (SC) (1,2). In addition, SC has been shown to play a crucial role in the regulation of crossover frequency (3). Ultrastructural analysis of SC reveals a tripartite structure with two parallel lateral elements, held together by the central element, which, in turn are interconnected by the transverse elements (1,2). The significance of recombination and chromosome synapsis to genome segregation is underscored by the existence of checkpoints that monitor defects in these processes and, consequently, arrest cells at the pachytene stage of meiotic prophase (4–6). Although SC was discovered close to five decades ago (7,8), the number, nature and/or the mechanism used by SC proteins to align meiotic chromosomes have yet to be clearly defined.

While the SC is conserved at the ultrastructural level across eukaryotic kingdom, some core components of this structure have as yet been characterized only in yeast and mammals (1,2). Genetic analyses in *Saccharomyces cerevisiae* have identified mutants defective in meiotic chromosome synapsis, some of which produce strong asynaptic phenotypes and abnormal SC structures (1,2). In *S.cerevisiae*, the genes that encode SC components include *HOP1*, *RED1*, *ZIP1*, *ZIP2, ZIP3* and *MEK1* (9–15). *HOP1*, which encodes a component of lateral element of SC, plays an important role in pairing of meiotic chromosomes (10), whereas *HOP2* gene product prevents synapsis between non-homologous chromosomes (16). Red1 is a major component of SC lateral elements and the axial elements that serve as precursors to lateral elements (17). Hop1 colocalizes with Red1 to discrete sites on axial elements; however, Hop1 dissociates as these elements become incorporated into mature SCs (17). The Zip1 protein localizes along the lengths of synapsed meiotic chromosomes and serves as a major component of the central regions of SC (13). Zip2 and Zip3 are present on meiotic chromosomes at discrete foci that correspond to the sites where synapsis initiates, and these proteins are required for the proper assembly of Zip1 along meiotic chromosomes (17). Mek1 is a meiosis-specific kinase that colocalizes with Red1 on meiotic chromosomes and phosphorylates Red1, and is required for wild-type levels of meiotic sister chromatid cohesion (18,19).

Hop1 contains a putative zinc-finger motif (9,10), analogous to those that exist in proteins containing strong nucleic acid binding activity. The importance of this motif for Hop1 function was established *in vivo* by the isolation of loss-of-function mutations in the conserved Cys within this region (9,10). Consistent with this, we showed that Hop1 functions as an oligomeric, structure-specific, DNA-binding protein (20,21). While Hop1 is known to be essential for normal SC formation and pairing of meiotic chromosomes, the mechanism by which Hop1 contributes to these processes is not clear.

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Here, we show that Hop1 is likely to play a direct role in meiotic chromosome pairing and recombination by its ability to promote pairing between double-stranded DNA helices containing arrays of G residues. To our knowledge, Hop1 is the first protein shown to promote synapsis of DNA double helices from yeast or any other organism.

MATERIALS AND METHODS

DNA and proteins

The oligonucleotides were obtained from Microsynth, Switzerland. The top strand of the duplex DNA was labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase or the 3′ end with terminal deoxy nucleotidyl transferase (22). The labeled strand was annealed with an equimolar amount of unlabeled complementary strand. The annealing mixture was electrophoresed on a 6% non-denaturing polyacrylamide gel in 89 mM Tris-borate buffer (pH 8.3) containing 1 mM EDTA at 10 V/cm for 3 h. Duplex DNA was excised from the gel, eluted into TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA), precipitated with 0.3 M sodium acetate (pH 5.2) and 95% ethanol. The pellet was washed with 70% ethanol, dried and resuspended in 20 µl of TE buffer. *Saccharomyces cerevisiae* Hop1(20) and *Mycobacterium tuberculosis* RecA (MtRecA) (23) were purified and their concentration was determined (20). Histone H1 (from calf thymus) was obtained from Sigma-Aldrich, St Louis, MO, USA.

Electrophoretic mobility shift assays

Reaction mixtures (20 µl) contained 0.5 µM of ^{32}P-labeled duplex DNA, 20 mM Tris–HCl (pH 7.5), 0.1 mM $ZnCl_2$ and indicated concentrations of Hop1. Samples were incubated at 30°C for 30 min and processed as described (20). Samples were separated on a native 8% polyacrylamide gel by electrophoresis at 4°C in 45 mM Tris-borate buffer (pH 8.3) containing 1 mM EDTA at 10 V/cm for 4 h.

Synapsis assay

Reaction mixtures (20 µl) containing 20 mM Tris–HCl (pH 7.5), 0.1 mM $ZnCl_2$ and 0.5 µM of indicated ^{32}P-labeled duplex DNA, were incubated with Hop1 or histone H1 for 20 min at 30°C. The reaction was terminated by the addition of proteinase K (0.2 mg/ml), SDS (0.2%) and KCl (0.1 M). After incubation at 30°C for 20 min, samples were loaded onto an 8% polyacrylamide gel and electrophoresed in 45 mM Tris-borate buffer (pH 8.3) containing 10 mM KCl, 5 mM $MgCl_2$ and 1 mM EDTA at 10 V/cm at 24°C for 4 h. The products were visualized by autoradiography. Quantification of bands was performed with the UVItech gel documentation system (England) using UVI-BANDMAP software and the values obtained were plotted using Graph pad Prism software. In the case of RecA, reaction mixtures contained 0.5 µM of ^{32}P-labeled 8 bp mismatched G/G substrate in 30 mM Tris–HCl (pH 7.5), 1.5 mM ATP and 2 mM $MgCl_2$. Following incubation at 30°C for 20 min, samples were deproteinized and analyzed as described above. Competition assays were performed with 5 pmol of ^{32}P-labeled 48 bp DNA containing 8 bp G/G array in the presence of increasing amounts of the same unlabeled DNA, and assayed as described above.

Cleavage with restriction enzymes

^{32}P-labeled synapsis product labeled at either the 5′ or 3′ end was excised from the gel and eluted into 10 mM Tris–HCl buffer (pH 7.5) containing 1 mM EDTA and 100 mM KCl. Cleavage reactions were performed with 0.5 µM of ^{32}P-labeled duplex DNA or synapsis product with 20 U XhoI or BamHI in the assay buffers provided by the manufacturer. After incubation at 37°C for 2 h, samples were deproteinized, loaded onto 12% non-denaturing polyacrylamide gel, and electrophoresed in 45 mM Tris-borate buffer containing 1 mM EDTA at 10 V/cm at 24°C for 2 h. The gels were dried and visualized by autoradiography. Quantification was performed using the UVItech gel documentation system, and the data were plotted using Microsoft Excel software.

DMS interference assay

Methylation of ^{32}P-labeled 48 bp duplex DNA was performed with 0.1% dimethyl sulfate (DMS) in TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) at 30°C for 2 min (21). The reaction was stopped by the addition of 50 µl of stop buffer [1.5 M sodium acetate (pH 7), 1 M 2-mercaptoethanol and 25 µg of yeast tRNA/ml] and 0.3 ml of ethanol. DNA was precipitated with ethanol followed by centrifugation at 14 000 r.p.m. at 4°C for 30 min. The pellet was washed with 70% ethanol, dried and resuspended in TE buffer. The synapsis assay was performed with partially methylated DNA as described above. Substrate DNA and synapsis products were excised from the gel and eluted into TE buffer. DNA was precipitated with ethanol and subjected to cleavage by incubation with 1 M piperidine at 90°C for 20 min. Samples were evaporated to dryness and the pellets were resuspended in 90 µl of water. This procedure was repeated three times. The pellets were dissolved in a solution containing 80% formamide, 10 mM NaOH and 0.1% each of bromophenol blue and xylene cyanol. Samples were denatured at 100°C for 10 min, chilled to 4°C, and equal amounts of DNA (based on radioactivity) were loaded onto a 10% polyacrylamide gel containing 8 M urea. The gel was electrophoresed at 1800 V for 2 h, dried and subjected to autoradiography. The band intensity was quantified in the UVItech gel documentation system by using UVI-BANDMAP software.

RESULTS

Rationale

The complexity of SC, and the lack of functionally well characterized SC proteins, has limited, so far, the *in vitro* investigations on the mechanistic aspects of their function. With the availability of purified Hop1, our goal was to test the hypothesis that SC proteins might be involved in interstitial pairing of meiotic chromosomes, and use the results of this analysis to discern the mechanism of meiotic chromosome synapsis and recombination. To this end, we adopted the approach developed to detect synapsis between DNA double helices by a non-enzymatic reaction (24).

Hop1 promotes efficient synapsis between DNA double helices containing mismatched G/G sequences

Previously, we showed that Hop1 binds efficiently to duplex DNA with little, if any, sequence specificity, but binding was markedly higher to oligonucleotides containing G-rich sequences (21). To gain insights into whether Hop1 is able to promote pairing of DNA double helices, we used 48 bp synthetic duplexes with 8 bp mismatched G/G sequences embedded centrally in the Watson–Crick duplex DNA (Fig. 1A). Similar DNA substrates have been used to demonstrate synapsis between DNA double helices by a non-enzymatic reaction (24). Although several hydrogen-bonding schemes for G/G mismatch base-pairing have been proposed, recent investigations indicate a zipper-like conformation for the mismatched G/G base pairs, in which the base pairing is disrupted, and the unpaired bases are intercalated with each other (25).

To assess whether Hop1 interacts with 48 bp duplex DNA containing an 8 bp G/G array (Fig. 1A), a mobility shift assay was used to monitor binding with increasing concentrations of Hop1. Consistent with previous findings (20), Hop1 generated several protein–DNA complexes (Fig. 1B). With increasing Hop1/DNA ratios the complexes were shifted towards a higher molecular mass. In parallel experiments, deproteinized samples were separated by non-denaturing gel electrophoresis, and the products were visualized by autoradiography (Fig. 1C). This analysis showed that Hop1 generated two distinct bands: one band with a mobility corresponding to the size of free DNA and the second displayed a size of 150 bp. Restriction cleavage experiments excluded the possibility that the latter resulted from end-to-end joining of duplex DNA by Hop1 (data not shown), indicating that the product of the reaction is a non-tandem dimer (henceforth this process is termed synapsis). Quantification suggested that the extent of synapsis was ~30% in the presence of 10 μM Hop1 (Fig. 1D). Hop1 failed to generate synapsis product with control substrates lacking an array of G/C or mismatched G/G sequences (data not shown).

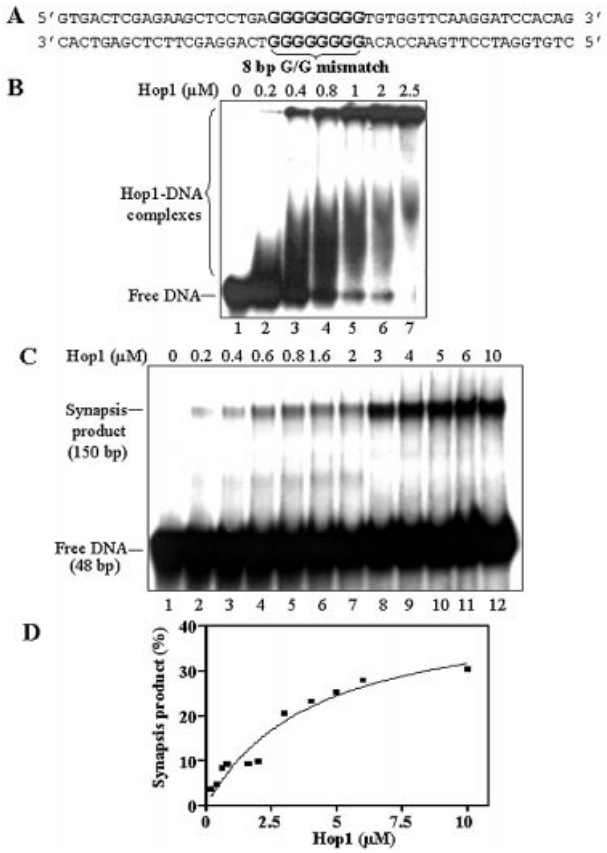

Figure 1. Hop1 binds to and promotes synapsis between double-stranded DNA containing mismatched G/G sequences. (**A**) Schematic of the DNA substrate. (**B**) Binding of Hop1 to 48 bp duplex DNA containing a mismatched G/G region. Reactions were performed with 0.5 μM of ^{32}P-labeled 48 bp duplex DNA containing 8 bp mismatched G/G sequence in the absence or presence of indicated amounts of Hop1. (**C**) Hop1 promotes interstitial synapsis between double-stranded DNA helices containing 8 bp mismatched G/G sequence. Samples were deproteinized and analyzed as described in Materials and Methods. (**D**) Quantification of Hop1-promoted synapsis. The autoradiogram shown in (C) was scanned and the data were plotted as described in Materials and Methods.

Kinetics and specificity of Hop1-promoted synapsis

To determine the kinetics of synapsis of double-stranded DNA helices containing G/G array, we incubated a fixed concentration of labeled DNA substrate with Hop1. At the indicated time intervals, samples were deproteinized and analyzed by gel electrophoresis. Figure 2A shows that synapsis occurred maximally with the briefest incubation time tested. On the other hand, there was no detectable synapsis product in the absence of Hop1 (Fig. 2A). Quantification suggested that synapsis was rapid and occurred maximally within 2.5 min of incubation time (Fig. 2B). The specificity of the formation of synapsis product was explored by competition assays using specific, but unlabeled DNA. In these experiments, addition of a 50-fold molar excess of unlabeled DNA was able to completely abolish synapsis (Fig. 2C).

Histone H1 and RecA failed to promote synapsis of DNA double helices

To investigate the specificity further, and to test whether DNA strand exchange proteins such as RecA can promote synapsis of DNA double helices, we used *M.tuberculosis* RecA, whose genome is especially rich in GC content, and therefore might be expected to favor this mode of interaction. In addition, it has been shown that RecA and Rad51 proteins display higher affinity for substrates containing GT sequences (26). Under conditions of strand exchange (23), *M.tuberculosis* RecA failed to promote synapsis between DNA double helices (Fig. 3A). Next, we tested whether positively charged chromosomal protein, histone H1, could promote synapsis between DNA double helices. These experiments showed that histone H1 failed to promote synapsis of duplex DNA even at a 24-fold higher concentration relative to Hop1 (Fig. 3B).

Mutations in the mismatched G/G region affect synapsis promoted by Hop1

In light of our previous observations that Hop1 was able to form G quartets (21), we wished to test how many of the eight mismatched G/G residues in the duplex DNA were actually essential for synapsis. To explore this, we assayed Hop1-promoted synapsis using a group of mutant substrates containing a varying number of mismatched G/G base pairs

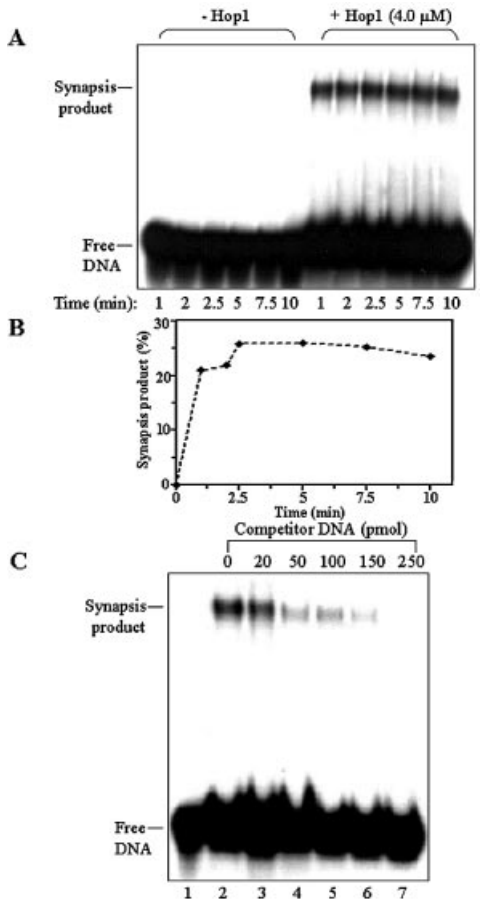

Figure 2. Specificity of synapsis promoted by Hop1. (**A**) Kinetics of synapsis. ^{32}P-labeled 48 bp duplex DNA containing an 8 bp G/G array (0.5 μM) was incubated in the absence (lanes 1–6) or presence (lanes 7–12) of Hop1 (4 μM) for varying time periods. Samples were deproteinized and analyzed as described in Materials and Methods. (**B**) Quantification of the kinetics of Hop1-promoted synapsis of DNA double helices. (**C**) Competitive inhibition of Hop1-promoted synapsis. Reaction mixtures contained 5 pmol of ^{32}P-labeled 48 bp duplex DNA harboring an 8 bp G/G array, 4 μM Hop1 and an amount of unlabeled homologous duplex DNA as indicated above each lane.

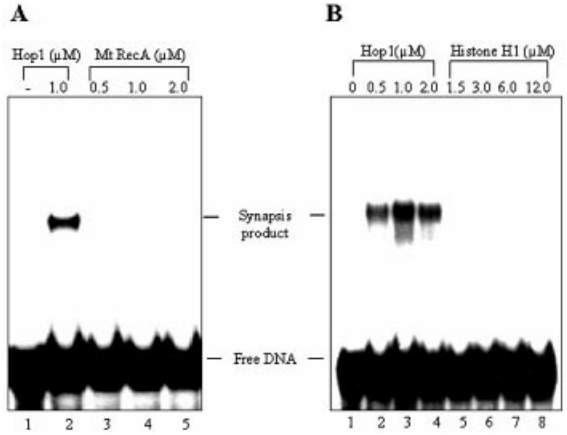

Figure 3. RecA or histone H1 fail to promote synapsis of 48 bp duplex DNA containing an 8 bp G/G array. (**A**) Synapsis assay with Hop1 protein in comparison with MtRecA. ^{32}P-labeled 48 bp duplex DNA containing an 8 bp G/G array (0.5 μM) was incubated in the absence (lane 1) or presence of Hop1 (lane 2) or MtRecA protein (lanes 3–5). Samples were deproteinized and analyzed as described in Materials and Methods. (**B**) Histone H1 fails to promote synapsis of 48 bp duplex DNA with a mismatched G/G array. Reactions were performed as described for (A). Lanes 1–8 represent reactions performed in the absence (lane 1) or presence of Hop1 (lanes 2–4) or histone H1 (lanes 5–8).

(Fig. 4A). Analysis of the extent of synapsis indicated that substitution of one G/G base pair for one A/T base pair led to a marginal decrease, relative to the 'wild-type' substrate (Fig. 4B, lanes 4–6). However, when three and two G/G mismatches from each end flanking the central 3 bp G/G array were replaced, synapsis was completely abolished. Similarly, Hop1 failed to display synapsis activity with substrates containing either two contiguous or three non-contiguous mismatched G/G base pairs. These results suggest that a minimum of four contiguous G residues, alone, are essential and sufficient for synapsis. Moreover, these results allowed structure requirements to be distinguished from the effects of normal base sequence in the duplex DNA, indicating the formation of G quartets during synapsis.

Determination of the minimal length of DNA double helix required for Hop1-promoted synapsis

To study further the effect of the length of DNA duplex on the activity of Hop1, we constructed a set of truncated substrates in which the length of the centrally embedded mismatched G/G sequence was kept constant while the length of the duplex on either side of the 8 bp mismatched G/G region was decreased up to 28 bp (Fig. 5A). As shown in Figure 5B, reducing the duplex region on each side by 5 bp led to complete abolition of the formation of synapsis product, suggesting that the length required for Hop1-promoted synapsis is in excess of 38 bp. With truncated substrates, increased levels of Hop1 or prolonged incubation failed to promote synapsis (data not shown). More importantly, these results are reminiscent of what is known about the minimum sequence requirements for binding of Hop1 to form a stable protein–DNA complex (20).

Hop1 promotes interstitial synapsis between heterologous DNA double helices

The foregoing observations suggested that Hop1 is capable of interacting simultaneously with two DNA double helices involving the mismatched G/G region to establish synapsis. A crucial question that follows from the above results is whether Hop1 can promote synapsis between heterologous double-stranded DNA. To test this idea, we used an approach similar to that described by Venczel and Sen (24). If, for example, Hop1 promotes synapsis between heterologous DNA double helices of different length, mixed reactions should generate three bands; one for each of the homodimers and a third resulting from the formation of a heterodimer. Accordingly, incubation of Hop1 with either 48 or 58 bp duplex DNA, followed by deproteinization, produced higher order complexes corresponding to the size of predicted products (Fig. 6A). An interesting finding emerged when varying molar ratios of 48 and 58 bp DNA double helices were incubated with Hop1. These reactions contained 48 and 58 bp duplex substrates; however, the total mass of DNA remained constant. We detected three distinct synapsis products. Two

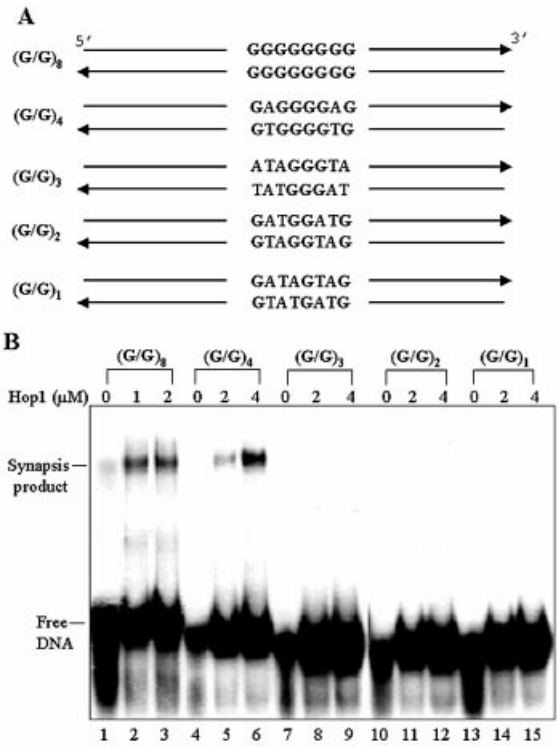

Figure 4. Mutations in the G/G region affect synapsis between double-stranded DNA helices. (**A**) Schematic of the DNA substrates. The mismatched G/G base pair is shown in bold face. (**B**) Hop1-promoted synapsis of DNA bearing a mutation in the G/G region. Each of the indicated ^{32}P-labeled duplex DNAs (0.5 μM) was incubated with amounts of Hop1 as shown above each lane, at 30°C for 10 min. The reactions were deproteinized and analyzed as described in Materials and Methods. The subscript to the G/G parenthesis denotes the number of contiguous mismatched G/G base pairs.

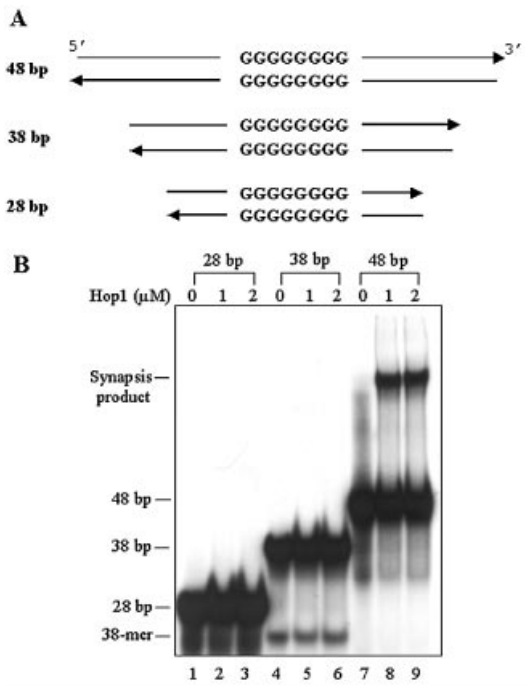

Figure 5. Minimum sequence required for Hop1-promoted synapsis between two double-stranded DNA helices. (**A**) Schematic of the DNA substrates. (**B**) Synapsis between duplex DNA of varying length. Reaction mixtures contained 0.5 μM of ^{32}P-labeled duplex DNA and Hop1p as indicated above each lane. Samples were incubated at 30°C for 10 min, deproteinized and analyzed as described in Materials and Methods. The position of duplex DNA and synapsis product are indicated on the left.

products migrated at positions corresponding to the homodimers arising from 48 or 58 bp, and a heterodimer containing both 48 and 58 bp duplex DNA substrates. At equimolar ratios of 48 and 58 bp duplex substrates, all the three synapsis products were formed in a ratio of 2× heterodimer and 1× each of the homodimers (Fig. 6A, lane 3). These results suggest that only the G/G region is involved in synapsis because the substrates are fully heterologous in nature.

Synapsis does not involve the sequences flanking the G/G array

To investigate whether the flanking sequences were involved in synapsis, we used restriction enzymes as probes to explore this aspect further. The 48 bp DNA described above contains a single cleavage site for XhoI at the 5′ end, and BamHI at the 3′ end, respectively. In both cases, cleavage results in the generation of a 5 bp fragment. The isolated synapsis product and 48 bp DNA, was cleaved with XhoI or BamHI. Figure 6B compares the extent of cleavage by both the enzymes. The 48 bp DNA was cleaved, generating 5 bp products. Similar results were also obtained for the synapsis product (Fig. 6B). However, the substrate and the synapsis product were not fully cleaved by each of the two restriction enzymes. Quantification of the autoradiogram allowed us to assess the extent of cleavage, which was in the range of 40–50% in multiple experiments. However, the lack of complete cleavage is consistent with many observations that cleavage close to the ends of DNA fragments by restriction enzymes is rather inefficient (22).

Methylation interference suggests formation of G4 DNA

Methylation interference is most appropriate to identify the guanine residues important for the formation of G4 DNA (27). The assay was performed by incubation of partially methylated ^{32}P-labeled 48 bp mismatched G/G DNA and the indicated concentrations of Hop1. After deproteinization, each of the products was isolated from the gel, and its methylation pattern analyzed. As shown in Figure 7A, in the control all guanines were methylated to the same extent, indicating the availability of N7 guanine for methylation (Fig. 7A, lane 2). In contrast to this, in the case of DNA incubated with Hop1, the guanines (G7–G14) were relatively inaccessible to methylation though the guanines (G6, G16 and G17) flanking the central 8 bp G/G region were uniformly methylated (Fig. 7A, lanes 3 and 4). The tracing of the autoradiogram indicated ~3-fold protection in the mismatched G/G region, relative to the reaction performed in the absence of Hop1 (Fig. 7B). These results suggest that the guanine residues in the mismatched G/G region are involved in interstitial synapsis of double-stranded DNA helices via the formation of guanine quartets.

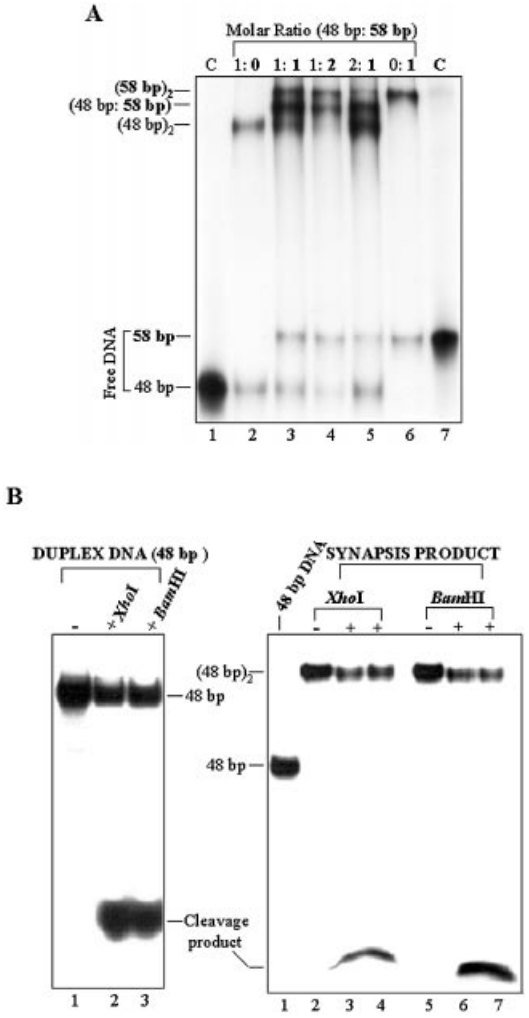

Figure 6. Hop1 promotes synapsis between heterologous double-stranded DNA helices. (**A**) Synapsis promoted by Hop1. Reaction mixtures containing Hop1 and ^{32}P-labeled 48 bp and/or 58 bp DNA fragments were incubated at the specified molar ratios as indicated above each lane. Samples were deproteinized and analyzed as described in Materials and Methods. The positions of duplex DNAs and synapsis products are indicated on the left. (**B**) Synapsis between duplex DNA does not involve arms flanking the G/G region. Assays were performed in the presence or absence of specified restriction enzymes according to the manufacturer's instructions. The cleavage products in lanes 3–4 and 6–7 have merged, resulting in a single band.

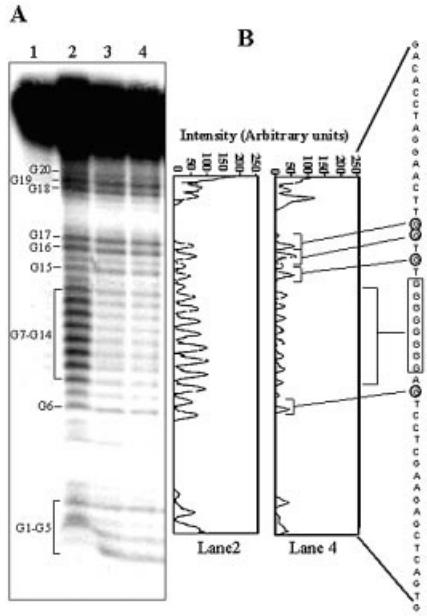

Figure 7. (**A**) Methylation interference implicates involvement of G residues in Hop1-promoted synapsis of DNA double helices. The assay was performed as described in Materials and Methods. In each lane, an equivalent amount of radioactivity was loaded to avoid differences in loading of the samples. Lane 1, reaction in which the duplex DNA was not treated with piperidine; lane 2, methylation pattern of the 48 bp duplex DNA; lanes 3 and 4, cleavage pattern of synapsis product generated in the presence of 2.5 and 5 μM Hop1, respectively. (**B**) The relative band intensity in lanes 2 and 4 in (A) was traced and plotted as arbitrary units. The protected guanines in the G/G region are boxed, and those in the flanking region are circled.

DISCUSSION

In this study, we provide evidence in favor of four conclusions. First, Hop1 was able to promote synapsis between two double-stranded DNA helices, generating a branched DNA species. Secondly, competition assays, synapsis between heterologous duplex DNA, and mutations in the mismatched G/G region implicate G residues in synapsis, and exclude the involvement of flanking sequences. Interestingly, Hop1 requires four contiguous G residues for robust synapsis, and sequence in the range of 38–48 bp was necessary to generate a synapsis product. Thirdly, RecA and histone H1 failed to catalyze synapsis between DNA double helices. Fourthly, methylation interference revealed the formation of G quartets in the 'synapsed' DNA structure. To our knowledge, Hop1 is the first protein to do so from yeast or any other organism.

It has been demonstrated that templates containing G/G lesions undergo DNA synthesis very efficiently in mammalian cell extracts (28). *In vitro* studies have shown synapsis between two double-stranded DNA helices via mismatched G/G base pairs in the absence of proteins (24). These reactions are characterized by slow kinetics of formation and lesser efficiency. *In vivo*, the possibility of the existence of mismatched G/G base pairs is not far-fetched, these have been discovered in the sheared purine-rich strand of the human centromeric satellite DNA (29). Structural studies provide good evidence that the presence of centrally embedded mismatched G/G sequences do not distort duplex DNA more than other types of lesions (25,29). Recent studies have shown that parallel and anti-parallel quadruplex DNA structures coexist and can interconvert under physiological conditions (30).

How does Hop1 establish synapsis between two double-stranded DNA helices? The model in Figure 8 illustrates synapsis promoted by Hop1. Though many details of this process are unknown, it is clear that for synapsis to occur, a second DNA double helix must bind through a mechanism dependent on the array of guanine residues. We speculate that this process might involve a second DNA binding site of Hop1 or a second monomer in the Hop1 dimer. Because of the specificity of interstitial synapsis, Hop1 must recognize a

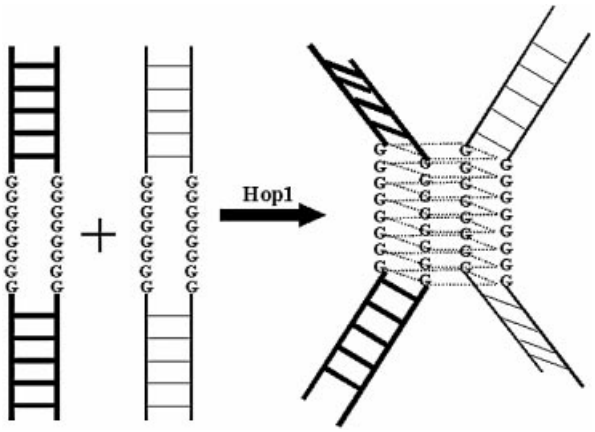

Figure 8. Schematic of synapsis promoted by Hop1. In the presence of Hop1, four G residues interact to form a G quartet by Hoogsteen base pairing. The proposed scheme is similar to the one proposed by Venczel and Sen (24). For details, see text.

particular aspect of DNA structure that facilitates its ability to form G quartets.

Several proteins have been shown to promote G quartet formation from single-stranded DNA containing a stretch of at least four guanines. For example RAP1, a yeast telomere binding protein (TBP) (31), and the β subunit of *Oxytricha* TBP (32), and human Topo I (33) promote the formation of G quartets by telomeric DNA. Whereas RAP1 and TBP increase the rate of formation of inter- or intramolecular quadruplexes by single-stranded DNA, topo I promotes quadruplex formation between duplex and single-stranded DNA (31–33). Although the ability of Hop1 to promote G quartet formation is consistent with such a function, the distinction is that it is the first shown to do so between DNA double helices. This is an important finding, because despite intense efforts, it has not been possible to identify the factor(s) involved in interstitial pairing of homologous chromosomes during meiosis (1,2).

Our results rekindle earlier speculations (27,34) concerning the involvement of G quartets in various phenomena associated with nucleic acid metabolism, including chromosome synapsis and the initiation of recombination (35). A strong correlation exists between the GC content, pairing and crossing over of meiotic chromosomes. Specifically, a relationship between recombination and GC content has been found in *S.cerevisiae* (36), *Drosophila melanogaster*, *Caenorhabditis elegans* (37), *Lillium* (38) and in humans (39). Similarly, Yu *et al*. (40) have reported a weak, albeit significant, correlation between estimated recombination rates and GC content of the human genetic map.

The biological relevance of Hop1-promoted synapsis between double-stranded DNA helices is borne out by a wealth of genetic and cytological data (1,2). In yeast, several genes are essential for the formation of normal SC, including *HOP1*, *HOP2*, *RED1*, *ZIP1*, *ZIP2, ZIP3* and *MEK1*. Null mutants for all the above genes are recombinationally defective in meiosis and produce inviable spores. A question that arises from this work is whether Hop1 is involved in the establishment of synapsis between two double-stranded DNA helices, to maintain it once established, or both. The results of the present and previous work (21), showing that Hop1 promotes G quartet formation between DNA helices as well as displays higher affinity for G4 DNA and remains bound to it, suggest the possibility that it is involved in both the processes. Is Hop1 a true component of SC in eukaryotic species? Recent results suggest that homologs of *HOP1* are present in organisms as divergent as *Kluyveromyces lactis* (41), *Arabidopsis thaliana* (42) and *C.elegans* (43). However, the biochemical activity of Hop1 homologs remains to be determined.

The biological relevance of interstitial pairing of DNA double helices by Hop1 recapitulates many genetic and cytogenetic data. Several lines of evidence indicate that pairing of homologous chromosomes begins at the telomeres and extends inward to the centromeres (44,45). The clustering of telomeres is required for meiotic chromosome pairing and recombination (44,45). The pairing is thought to involve *cis*-acting sequences, called locus-specific sites, along the chromosome (1,2). One characteristic of such DNA is the ability to hold the homologs together at periodic intervals. Examination of G-rich isochors in yeast chromosome III showed that G-rich repeats were most frequently clustered around the sites of DSBs (46). The distribution of G-rich isochors shows a strong correlation with recombinational 'hotspots' and formation of programmed DNA double-strand breaks along the chromosomes (46). Given that Hop1 exhibits high affinity for G4 DNA, we speculate that the locus-specific pairing sites may correspond to arrays of guanine residues. Our data are in most part consistent with models and cytogenetic data suggesting locus-specific pairing of homologs during meiosis (1,2). Furthermore, it is possible that Hop1 functions directly in chromosome synapsis, and that this function is promoted, via formation of G quartets. Finally, we speculate that Hop1 may provide non-sister chromatids the flexibility to align in juxtaposition and eliminate promiscuous interactions with sister chromatids, thus offering a failsafe mechanism to ensure proper genetic exchange between maternal and paternal genomes.

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