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Meiosis-specific yeast Hop1 protein promotes pairing of double-stranded DNA helices via G/C isochores

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Abstract

In eukaryotes, genetic exchange between homologs is facilitated by a tripartite proteinaceous structure called the synaptonemal complex (SC). Several lines of evidence indicate that the genes that encode components of SC are essential for meiotic chromosome pairing and recombination. However, the molecular mechanism by which SC proteins promote these processes is obscure. Here, we report that *Saccharomyces cerevisiae* Hop1 protein, a component of SC, promotes pairing between two double-stranded DNA helices containing a centrally located G/C isochore. Significantly, pairing was rapid and robust, and required four contiguous G/C base pairs. Using a series of truncated DNA double helices we show that 20 bp on either side of 8 bp target G/C sequence is essential for pairing. To our knowledge, Hop1 is the first protein shown to do so from yeast or any other organism. These results indicate that Hop1 protein is likely to play a direct role in meiotic chromosome pairing and recombination.

Keywords: *Saccharomyces cerevisiae*; Hop1 protein; Chromosome synapsis; Recombination; Synaptonemal complex; G/C isochore

In eukaryotes, the pairing of homologs along their lengths is facilitated by a meiosis-specific structure: the synaptonemal complex (SC) [1–3]. In addition, a component of SC has been shown to play a crucial role in the regulation of crossover frequency [4]. Ultrastructural analysis indicates that SC is a tripartite structure with two parallel lateral elements, held together by the central element, which, in turn, is interconnected by the transverse elements [1–3]. The significance of recombination and chromosome synapsis to chromosome segregation is emphasized by the existence of checkpoints that sense defects in these processes and, consequently, arrests cells at the pachytene stage of meiotic prophase [5–7]. Fawcett [8] and Moses [9] independently discovered SC; but 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 kingdoms, the isolation and characterization of SC components has been possible only in yeast and mammals [1–3]. A number of genetic analyses in *Saccharomyces cerevisiae* have identified mutants defective in meiotic chromosome synapsis, some of which produce strong asynaptic phenotypes and abnormal SC structures [1–3]. In *S. cerevisiae*, the genes that encode SC components include *HOP1*, *RED1*, *ZIP1*, *ZIP2*, and *ZIP3* [10–16]. *S. cerevisiae HOP1* encodes a component of lateral element of SC, which plays a key role in pairing of meiotic chromosomes [12], whereas *HOP2* gene product prevents synapsis between non-homologous chromosomes [17]. In *S. cerevisiae*, Red1 protein appears to be a major component of SC lateral elements and the axial elements that serve as precursors to lateral elements [18]. Also, Hop1 protein (Hop1p) has been shown to colocalize with Red1 protein to discrete sites on axial elements; however, Hop1 dissociates as these elements become incorporated into mature SCs [18]. The

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Zip1 protein localizes along the lengths of synapsed meiotic chromosomes and serves as a major component of the central regions of SC [14]. Zip2p and Zip3p 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 Zip1p along meiotic chromosomes [13]. Mek1p is a meiosis-specific kinase that colocalizes with Red1p on meiotic chromosomes and phosphorylates Red1p, and is required for wild-type levels of meiotic sister chromatid cohesion [19,20].

Genetic studies suggest that *HOP1* is essential for normal SC formation and pairing of meiotic chromosomes; however, the molecular mechanism by which Hop1p facilitates these processes is obscure. Here, we show that Hop1p possess the ability to promote pairing between two double-stranded DNA helices via G/C isochors. These findings have significant implications for understanding of interstitial pairing of homologous chromosomes during meiosis.

Materials and methods

DNA and proteins. The oligonucleotides were from Microsynth, Switzerland. The top strand of the duplex DNA was labeled at the 5′ end using [γ-^{32}P]ATP and T4 polynucleotide kinase [21]. The labeled strand was annealed to an equimolar amount of unlabeled complementary strand. The 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), and 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 TE buffer. *S. cerevisiae* Hop1p was purified and its concentration was determined as described [22].

Electrophoretic mobility shift assays. Reaction mixtures (20 μl) contained 0.3 μM ^{32}P-labeled duplex DNA, 20 mM Tris–HCl (pH 7.5), 0.1 mM $ZnCl_2$, and Hop1p at the indicated concentrations. After incubation at 30 °C for 30 min, samples were separated by electrophoresis on an 8% polyacrylamide gel at 4 °C in 45 mM Tris–borate (pH 8.3) containing 1 mM EDTA at 10 V/cm for 4 h [22,23].

DNA pairing assay. Reaction mixtures (20 μl) containing 20 mM Tris–HCl (pH 7.5), 0.1 mM $ZnCl_2$, and 0.3 μM of indicated ^{32}P-labeled duplex DNA were incubated with indicated concentrations of Hop1p for 20 min at 30 °C. 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 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 UVItech gel documentation system (England) using UVIBANDMAP software and the values obtained were plotted using Graphpad Prism software. Following incubation at 30 °C for 20 min, samples were deproteinized and analyzed as described above. Competition assays were performed with 5 pmol ^{32}P-labeled 48 bp DNA containing 8 bp G/C array in the presence of increasing amounts of the same unlabeled DNA and assayed as described above.

Results

Rationale

The absence of well-characterized SC proteins has limited, so far, the in vitro investigations on the mechanistic aspects of their function. With purified Hop1p, our goal was to test the hypothesis that SC proteins might be directly involved in pairing of DNA double helices, and use the results of this analysis to discern the mechanism of meiotic chromosome synapsis and recombination. To this end, previous studies have shown that Hop1p promotes pairing of double-stranded DNA helices (48 bp) containing centrally embedded 8 bp mispaired G/G sequence [23]. However, the biological significance of specific pairing between two double-stranded DNA helices via mispaired G/G sequence has remained unclear.

Hop1p promotes pairing of DNA double helices containing a run of G/C base pairs

The genome of eukaryotes is organized into large genomic regions of relatively homogeneous base composition (referred to as isochores). Assuming that arrays of G-residues are important for Hop1p function in vivo, we performed a comprehensive analysis of *S. cerevisiae* genome to map the density of G residues. Strikingly, such an analysis revealed that *S. cerevisiae* contains a high density of G/C isochores throughout its genome. Fig. 1 depicts the landscape of G/C isochores on *S. cerevisiae* chromosome III. In addition, the clustering of G/C isochores around some of the known hot-spots for meiotic recombination indicates that G/C isochores might play an important role in homolog pairing [24].

To explore the relationship between Hop1p and G/C isochores in meiotic chromosome synapsis and recombination, we designed a 48 bp synthetic duplex with 8 bp G/C sequence embedded centrally in the Watson–Crick duplex DNA (Fig. 2A). Incidentally, the longest tract of G/C sequence in the *S. cerevisiae* genome is 8 bp. The formation of Hop1p-DNA complexes was monitored by mobility shift assays and visualized by autoradiography as described under Materials and methods. Consistent with previous findings [22], Hop1p formed distinct protein–DNA complexes with 48 bp duplex (Fig. 2B). To explore whether Hop1p can pair two DNA double helices in juxtaposition, in parallel experiments, deproteinized samples were separated by electrophoresis on a 10% polyacrylamide gel under non-denaturing conditions, and the products were visualized by autoradiography (Fig. 2C). This analysis showed that Hop1p generated a single DNA band, which is denoted as the synapsis product, with a mobility corresponding to a size of 150 bp. Control experiments excluded the possibility that the latter resulted from end-to-end joining of duplex DNA (data not shown). Therefore, we interpret these results to mean that the product of the reaction is a non-tandem dimer. Previous studies with double-stranded DNA helices containing a centrally embedded mispaired G/G sequence have established that Hop1p was to pair duplex DNA into a four-stranded branched structure [23].