Saccharomyces cerevisiae Hop1 Zinc Finger Motif Is the Minimal Region Required for Its Function in Vitro*

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* The abbreviations used are: SC, synaptonemal complex; BSA, bovine serum albumin; CD, circular dichroism; DTT, dithiothreitol; form I DNA, negatively supercoiled DNA; form II DNA, nicked circular double-stranded DNA; form III DNA, linear double-stranded DNA; WT, wild-type; ZnF, zinc-finger motif; FBA, filter binding assay.

Saccharomyces cerevisiae meiosis-specific HOP1, which encodes a core component of synaptonemal complex, plays a key role in proper pairing of homologous chromosomes and processing of meiotic DNA double strand breaks. Isolation and analysis of hop1 mutants indicated that these functions require Cys371 of Hop1 embedded in a region (residues 343–378) sharing homology to a zinc finger motif (ZnF). However, the precise biochemical function of Hop1, or its putative ZnF, in these processes is poorly understood. Our previous studies revealed that Hop1 is a DNA-binding protein, showed substantially higher binding affinity for G4 DNA, and enhances its formation. We report herein that ZnF appears to be sufficient for both zinc as well as DNA-binding activities. Molecular modeling studies suggested that Hop1 ZnF differs from the previously characterized natural ZnFs. The zinc-binding assay showed that the affinity for zinc is weaker for C371S ZnF mutant compared with the wild type (WT) ZnF. Analysis of CD spectra indicated that zinc and DNA induce substantial conformational changes in WT ZnF, but not in C371S ZnF mutant. The results from a number of different experimental approaches suggested that the DNA-binding properties of ZnF are similar to those of full-length Hop1 and that interaction with DNA rich in G residues is particularly robust. Significantly, WT ZnF by itself, but not C371S mutant, was able to bind duplex DNA and promote interstitial pairing of DNA double helices via the formation of guanine quartets. Together, these results implicate a direct role for Hop1 in pairing of homologous chromosomes during meiosis.

For faithful segregation of homologous chromosomes during meiosis I, each chromosome must recognize, pair, and recombine with its correct partner. This is achieved by a string of rather poorly defined processes. However, it has become increasingly apparent that, prior to segregation, homologous chromosomes undergo a series of biochemical changes that increase their compaction (1), reorganize their orientation in the nucleus (reviewed in Ref. 2), and resolve spatial and topological issues among and within themselves (3–5). In many eukaryotes, pairing culminates in synaptic pairing, whereby homologous chromosomes are physically linked along their entire lengths by a meiosis-specific proteinaceous structure called the synaptonemal complex (SC) (reviewed in Ref. 3). Several lines of evidence indicate that genetic exchange between homologous chromosomes occurs in this context, which is facilitated by the formation of chiasmata (6). Ultrastructural analysis reveals that the SC is composed of two lateral elements, one on each homologue, and a central element that, in turn, are linked by transverse elements (7, 8).

The precise mechanism by which the SC components facilitate pairing of meiotic chromosomes is unclear. However, observations of synaptic alignment and the display of substantial levels of homologue pairing in asynaptic organisms implicate a role for SC in maintaining rather than initiating pairing of homologues (reviewed in Ref. 7). In addition, homologous chromosomes interact with each other during genetic exchange in the absence of SC. These interactions are not prerequisites for pairing; rather, they are believed to contribute to the maintenance of pairing. Consistent with these observations, numerous yeast mutants that are deficient in homologous recombination do not completely lack pairing, although the amount of pairing is decreased and synapsis is defective in these mutants (9–11).

In Saccharomyces cerevisiae, genetic analyses have identified mutants defective in meiotic chromosome synapsis, some of which produce strong asynaptic phenotypes and abnormal SC structures (7, 8). The genes that encode SC components include HOP1, HOP2, RED1, ZIP1, ZIP2, and ZIP3 (12–19). HOP1, which specifies a component of lateral element of SC, is required for proper pairing of meiotic chromosomes (13), whereas the HOP2 gene product quells synapsis between non-homologous chromosomes (19). Like Hop1, Red1 is a major component of SC lateral elements (20). Hop1 colocalizes with Red1 to discrete sites on axial elements, which serve as precursors to lateral elements; however, Hop1 dissociates as these elements become incorporated into mature SCs (20). The Zip1 protein localizes along the lengths of synapsed meiotic chromosomes and serves as a major component of the central regions of SC (16). Zip3 and Zip5 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 (18). However, the mechanisms underlying the functions of any of the SC proteins in chromosome pairing has not been established. In this regard, we reported that Hop1 protein is a structure-specific DNA-binding protein (21) and that it is capable of promoting synapsis between a pair of double-stranded DNA helices (22). Importantly, the interaction between double-stranded DNA helices occurs in a G4 DNA-dependent manner (22). These studies...
revealed a biochemical function for Hop1, first for any SC component, in interstitial pairing of meiotic chromosomes.

Zinc fingers (ZnF) are among the most ubiquitous protein domains found in eukaryotic organisms. The variant forms of ZnFs are distinguished by the arrangement of conserved zinc binding ligands: the most common configurations are CCHH, CCCCH, CCHC, or CHHC motifs. The Cys/His ZnFs fold into β β' structure by Zn2+ binding. The ZnF structure is stabilized by Zn2+ coordination of conserved histidine and cysteine residues and hydrophobic interaction among other residues in the ZnF. ZnFs function as recognition elements for interaction with DNA, RNA, or other proteins with high affinity (reviewed in Ref. 23). Sequence comparisons previously identified a motif [(448)CXnCXmCyC6(CyC)n] (hereafter referred to as ZnF motif) in Hop1 (13, 24), reminiscent of the Cys2/Cys2 type ZnF found in eukaryotic transcription factors (25). The Cys/Cys motif of Hop1 appears to be crucial for its function in meiosis, because a single mutation of C371S within the putative ZnF renders the hop1 mutant allele defective in sporulation and meiosis (13, 24). Because ZnFs are often sequence-specific DNA-binding motifs, we asked whether Hop1 ZnF might bind DNA in a specific fashion. In this study, we have taken a biochemical approach to delineate the role of putative ZnF implicated in Hop1 function. Intriguingly, we find that Hop1 ZnF displays all of the known activities of full-length Hop1 protein. Together, these results implicate a direct role for Hop1 in meiotic chromosome pairing, processing of meiotic double-strand breaks, and recombination.

**MATERIALS AND METHODS**

**Peptides, DNA, and Enzymes**—The wild type Hop1-putative ZnF and its corresponding mutant peptide were obtained from Genemed Synthesis, Inc. The peptides were dissolved in buffer (20 mM Tris-HCl, pH 7.5, and 0.1 mM ZnCl2). The Q5 peptide (50 μM) containing 20 mM Tris-HCl, pH 7.5, 0.1 mM ZnCl2, and indicated concentrations of 32P-labeled DNA (duplex or single-stranded or G4 DNA) and Hop1 WT or mutant peptide. Samples were incubated at 30 °C for 30 min and analyzed as described previously (28). Samples were electrophoresed on either 0.8% agarose or 8% polyacrylamide gels at 4 °C in 45 mM Tris borate buffer (pH 7.5, and 50 mM NaCl) prior to use. Increasing concentrations of the Hop1 WT or mutant peptide and the indicated DNA substrate were mixed in 20 μl of buffer (20 mM Tris-HCl, pH 7.5, and 0.1 mM ZnCl2). After incubation at 30 °C for 30 min, a 10-μl aliquot was applied onto a nitrocellulose filter (for determination of total radioactivity), and the remaining 10 μl was diluted into 0.8 ml of cold FBA buffer. The diluted samples were applied onto KOH-treated filters under vacuum and immediately washed with 5 ml of cold FBA buffer. The filters were dried, and the bound radioactivity was quantified by liquid scintillation.

**Circular Dichroism Measurements**—All the CD measurements were made on a Jasco J-715 spectropolarimeter with constant nitrogen purge. CD spectra were recorded at 25 °C in a 2-mm path length cell with a resolution of 0.2 nm over the wavelength range of 200–250 nm. Each spectrum represented the average of two scans. For Zn2+ titration experiments, increasing concentrations of ZnCl2 were added to a solution containing WT peptide (25 μM) in 20 mM Tris-HCl, pH 7.5. CD spectra were recorded at each point of titration following 5 min of incubation after the addition of Zn2+. The spectra were then corrected by subtraction of spectra obtained with buffer in the presence or absence of ZnCl2. To determine the dissociation constant (Kd) of zinc interaction, the changes in molar ellipticity at a single wavelength were plotted against the total zinc ion concentration. The zinc ion concentration required for half-maximal change in ellipticity yielded the Kd value.

In DNA-binding experiments, increasing concentrations of various DNA substrates were added to a solution containing WT or mutant peptide (50 μM) in 20 mM Tris-HCl, pH 7.5, and 0.1 mM ZnCl2. The spectra were recorded following 10 min of incubation after each addition of DNA. The spectra obtained for peptide-DNA complex were corrected by subtracting the spectra for DNA alone. The molar ellipticity (θ) values were obtained using the conversion factor of 100/θ cm2 (dissociation constant (molar), and l is the path length (centimeters).

**DNA Synapsis Assay**—Reaction mixtures (20 μl) containing 20 mM Tris-HCl (pH 7.5), 0.1 mM ZnCl2, and 10 pmol of indicated 32P-labeled duplex DNA were incubated with Hop1 WT or mutant peptide at the indicated concentrations for 20 min at 30 °C. The reactions were terminated by the addition of protease K (0.2 mg/ml), SDS (0.2%), and KCl (0.1 M). Following incubation at 30 °C for 20 min, samples were loaded onto an 8% non-denaturing polyacrylamide gel and electrophoresed in 45 mM Tris borate buffer (pH 8.3) containing 10 mM KCl, 5 mM MgCl2, and 1 mM EDTA at 10 V/cm for 4 h. The products were visualized by autoradiography.

**Labelled and Unlabelled Satellite Substrates**—Page Partial methylation of 32P-labeled 48-bp duplex DNA containing a G/G stretch was carried out with 0.1% dimethyl sulfate in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) at 30 °C for 2 min (21, 22). The reaction was stopped by the addition of 50 μl of stop buffer (1.5 mM sodium acetate, pH 7, 1 μl 2-mercaptoethanol, and 25 μg of yeast tRNA/ml) and 300 μl of ethanol. DNA was precipitated with 20 mM Tris-HCl, pH 7, 1 mM EDTA, and 0.1% each of bromphenol blue and xylene cyanol. The pellet was washed with 70% ethanol, dried, and resuspended in TE buffer. A synopsis assay was carried out with partially methylated duplex DNA as described above. The duplex DNA and synopsis product were excised from the gel and eluted into TE buffer. DNA was precipitated with ethanol and subjected to cleavage by DpnII. The blots were then washed twice in 0.1× SSC/0.1% SDS at 90 °C for 20 min. Samples were ethanol-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 bromphenol blue and xylene cyanol. Samples were dena-
Zinc Binding by Hop1 ZnF—Because the Hop1 ZnF is shown to be essential for its function in vivo (13), the WT and its corresponding C371S mutant peptides were assessed for their ability to bind $^{65}$Zn in zinc blot assays (34–36). The radioactive zinc blot assays have been used to demonstrate zinc-binding efficiencies to a variety of proteins and their mutants (37). Increasing concentrations of WT Hop1 ZnF and its corresponding C371S mutant were subjected to the zinc blot assay as described under “Materials and Methods.” Briefly, increasing concentrations of the WT Hop1 ZnF and its corresponding C371S mutant were spotted on a strip of nitrocellulose paper. It was then incubated with $^{65}$Zn, washed with excess buffer to remove the unbound $^{65}$Zn, and subjected to autoradiography. The amount of $^{65}$Zn bound to WT and mutant ZnF is shown in Fig. 2A. The results suggest that binding of $^{65}$Zn by the WT ZnF is linear, whereas the C371S mutant ZnF bound increasing amounts of $^{65}$Zn in the 2–10 μM range and then reach a plateau (Fig. 2B). However, it is not clear whether this represents a background activity of nonspecific zinc ion binding, or it possesses a weak but specific zinc-binding activity. The ZnFs often bind other divalent cations (38). The specificity of binding of $^{65}$Zn to the Hop1 ZnFs was examined in the presence of nonradioactive Zn$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Cd$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, or Mn$^{2+}$. Whereas the binding activity was not affected by the presence of Ca$^{2+}$, Mg$^{2+}$, or Mn$^{2+}$, several metal ions that are capable of tetrahedral coordination decreased binding of $^{65}$Zn to the WT Hop1 ZnF to varying extents (Fig. 2C).

Zinc Induces Conformational Changes in Hop1 ZnF—It is possible to assess metal ion binding to synthetic peptides by examining conformational changes in the secondary structure using circular dichroism (CD) spectroscopy in the far-UV region in the range of 175–260 nm (39). To ascertain whether the 36-mer peptides can fold into ZnFs, changes in their secondary structure were monitored in the absence or presence of increasing concentrations of Zn$^{2+}$. In the presence of Zn$^{2+}$, WT Hop1 ZnF displayed significant conformational changes in secondary structure, which led to the conversion of random coil to the ordered state (data not shown). The CD spectrum of the WT Hop1 ZnF$^{Zn^{2+}}$ complex, compared with apo-ZnF, showed a change in negative molar ellipticity at 210 and 222 nm, characteristic of α-helix formation. These observations are reminiscent of zinc ion binding properties of synthetic or natural ZnFs of Rauscher murine leukemia virus nucleocapsid (40), S. cerevisiae transcriptional activator Adr1 (33), or Xenopus laevis TFIIIA (41). The change in ellipticity at 222 nm is plotted as a function of zinc ion concentration (Fig. 2D). CD data was sub-

![Image](https://example.com/image.png)
The peptide concentration. The diagram shown in Fig. 2 used as negative control.

**Fig. 2.** WT Hop1 ZnF displays high affinity while the C371S mutant reduced affinity for $^{65}$Zn. A, $^{65}$Zn blot assay. Increasing concentrations of the WT or mutant peptide was blotted onto nitrocellulose membrane and probed with $^{65}$Zn as described under "Materials and Methods." E. coli single-stranded DNA binding protein (SSB) was used as negative control. B, quantification of zinc binding. The autoradiogram shown in Fig. 2A was scanned, and the data are plotted against the peptide concentration. C, competition of $^{65}$Zn binding by divalent metal ions. Wild type peptide (4 $\mu$g) was blotted onto nitrocellulose filter in multiple sets. Each strip was incubated with $^{65}$ZnCl$_2$ either in the absence (none) or presence of competitor metal ions at indicated concentrations. Blots were washed and visualized by autoradiography. D, determination of the dissociation constant of peptide-Zn$^{2+}$ complex. The ellipticity values at 222 nm are plotted against the Zn$^{2+}$ concentration. The $K_d$ value was obtained from non-linear regression analysis.

Projected to non-linear regression analysis. The results yielded an apparent equilibrium dissociation constant ($K_d$) of $2.5 \times 10^{-5}$ M. Together, these results suggest that WT Hop1 ZnF possesses metal-binding and structural characteristics of correctly folded ZnFs.

**Hop1 ZnF Binds G4 DNA—**We showed previously that full-length Hop1 displayed higher binding affinity for DNA rich in G residues and G4 DNA compared with normal DNA (21). The evidence that the WT Hop1 ZnF was able to bind Zn$^{2+}$ and that the metal ion induced conformational changes in its secondary structure prompted us to investigate the possibility of whether Hop1 ZnF or its corresponding C371S mutant bind DNA. To explore this, we used 36-mer oligonucleotides bearing tracks of four (4G3) or six (6G3) guanine residues. Additionally, we used 39-mer oligonucleotide containing two tracks of four guanine residues (OX-1T). These were either in the monomeric form (Fig. 3A) or folded into G4 DNA (Fig. 3B) (21). Electrophoretic mobility shift assays were performed using various $^{32}$P-labeled oligonucleotides containing increasing arrays of G residues. The assay conditions with regard to pH, temperature, and cofactor requirements were similar to those used for full-length Hop1 (21). The data in Fig. 3A (lanes 2, 5, and 8) show that the wild type Hop1 ZnF was able to bind labeled DNA in a manner similar to that of the full-length Hop1 protein (21). In contrast, the same assay revealed no evidence for binding of the C371S mutant ZnF to DNA (Fig. 3A, lanes 3, 6, and 9). The results obtained by this assay are specific, because a similar behavior was also evident with three different DNA substrates and that the C371S mutant ZnF failed to form detectable nucleoprotein complexes. These data allow us to conclude that the putative ZnF of Hop1 constitutes the minimal DNA-binding domain. Having demonstrated a direct interaction between oligonucleotide substrates and Hop1 ZnF, we investigated whether it can bind G4 DNA, a substrate previously shown to be specific for Hop1 protein (21). We used the same 36-mer substrates that do not share much homology with each other but as shown previously (21), can form G4 DNA. As shown in Fig. 3B, the WT Hop1 ZnF displayed avid binding to G4 DNA, whereas the C371S mutant peptide did not. In parallel experiments, we observed that WT Hop1 ZnF, but not C371S mutant peptide, was able to bind various topological forms of bacteriophage M13 DNA (data not shown). Together, these results suggest that C371S mutant ZnF is broadly defective in DNA-binding activity.

**Hop1 ZnF Promotes the Formation of G4 DNA—**We next investigated the ability of Hop1 ZnF and its corresponding C371S mutant to promote the formation of G4 DNA. Varying
Fig. 4. **Hop1 ZnF promotes the formation of G4 DNA.** A, sequence of the oligonucleotide used. Highlighted letters indicate the array of eight G residues. B, WT Hop1 ZnF and not its C371S mutant promote the formation of G4 DNA. Increasing concentrations of the WT Hop1 or C371S mutant motif was incubated with 10 pmol of 32P-labeled oligonucleotide and analyzed for G4 DNA formation as described (21). Lanes 1–7, DNA incubated in the absence (lane 1) or presence of 1, 2, 5, or 5 μM of WT (lanes 2–4) or C371S mutant (lanes 5–7) peptide, respectively. The position of monomer and G4 DNA is indicated on the left.

Concentrations of WT Hop1 ZnF or its corresponding C371S mutant were incubated with oligonucleotide containing a stretch of eight G residues (Fig. 4A). Subsequently, the peptides were removed by incubation with proteinase K (22). Samples were separated by gel electrophoresis under non-denaturing conditions and visualized by autoradiography (22). As shown in Fig. 4B, the WT Hop1 ZnF was able to promote the formation of G4 DNA, whereas its C371S mutant at the same concentration range did not. The correlation between binding of Hop1 ZnF to G4 DNA and the formation of G4 DNA reinforces the notion that Hop1 ZnF is sufficient for the display of all of the known activities of full-length Hop1 under in vitro conditions.

**Hop1 ZnF Displays Higher Affinity for G4 DNA**—To explore the apparent affinity of Hop1 ZnFs for DNA, we used two types of duplex substrates: 48-bp B-form DNA, an identical DNA fragment containing a mismatched 8-bp G/G sequence embedded at the center, and G4 DNA. The relative affinity of Hop1 ZnF to these DNA substrates was determined using a nitrocellulose filter-binding assay. The 32P-labeled DNA substrates were incubated with increasing concentrations of WT Hop1 ZnF and assayed as described under “Materials and Methods.” Comparison of data revealed that the ZnF was the major determinant for nucleic acid substrates (42). In particular, we chose to analyze as described under “Materials and Methods.” The percentage of DNA bound to ZnF is plotted against the added amount of the peptide. The figure shows the data obtained for the 48-bp duplex DNA of mixed base sequence (panel A), 48-bp duplex DNA containing mismatched 8-bp G/G sequence embedded at the center (panel B), and G4 DNA assembled from OX-1T (panel C) or 4G3 (panel D). Non-linear regression analysis of the data yielded the Kd for various substrates, which are depicted in the inset. The data in each panel represent an average of two independent determinations.

**DNA Induces Conformational Changes in the Hop1 ZnF**—We reasoned that if WT Hop1 peptide functions as a canonical ZnF, then its interaction with DNA might induce conformational changes (43). Conversely, similar changes may not be apparent in the C371S mutant peptide. We tested this possibility by monitoring conformational changes in apo-peptides upon interaction with DNA. To examine ligand-induced changes in the apo-peptides, we used double-stranded DNA, because full-length Hop1 displayed vastly enhanced binding affinity for duplex DNA over ssDNA. The spectra of WT peptide showed a substantial decrease in negative molar ellipticity at 210 and 222 nm in the presence of DNA (Fig. 7A). No changes in the spectrum were apparent at 245 nm or with increasing concentration of DNA (above 2 μM). By contrast, the C371S mutant peptide failed to display similar changes, thereby precluding a similar interaction with double-stranded DNA (Fig. 7B). Together, these results indicate that the Hop1 349CXX3CX19XX2C574 sequence motif is indeed a functional ZnF.

**Hop1 ZnF Promotes Synapsis between Two DNA Double Helices**—The characterization of Hop1 ZnF made it possible to test its biochemical activity using a functional assay that has been developed for Hop1 protein (22). In particular, we chose to investigate whether ZnF can promote synapsis between two DNA double helices. In one set of experiments, increasing concentrations of WT ZnF or its corresponding C371S mutant was incubated with 32P-labeled 48-bp duplex DNA containing a...
centrally embedded 8-bp mismatched G/G region (Fig. 8A). In parallel, reactions were performed in a similar manner, but prior to electrophoresis the samples were deproteinized by incubation with proteinase K as described under "Materials and Methods." As shown in Fig. 8B (lanes 1–6), ZnF was able to form a distinct peptide-DNA complex in a concentration-dependent manner. Most strikingly, ZnF was able to promote synapsis of DNA double helices (Fig. 8B, lanes 8–13), a feature reminiscent of that of the full-length Hop1 protein (22). Quantification of product suggested that the extent of synapsis was ~35% in the presence of 10 μM ZnF (Fig. 8B, lane 13). The formation of synapsis product was in the same concentration range as full-length Hop1, and a further increase in the concentration of ZnF failed to increase the extent of synapsis product formation (data not shown). In contrast to this, the C371S mutant peptide failed to interact with DNA (Fig. 8C, lanes 2–7), and as a result was unable to promote synapsis product formation (Fig. 8C, lanes 8–12).

To extend our observations, we examined the ability of Hop1 ZnF to promote the formation of synapsis product with various mutant DNA substrates (Fig. 9A). As observed for the full-

FIG. 7. Hop1 ZnF displays DNA-induced conformational changes. The decline in negative molar ellipticity (θ) of the WT Hop1 ZnF (A) and its corresponding C371S mutant (B) at 210 and 222 nm is plotted against DNA concentration.

FIG. 8. Hop1 ZnF promotes synapsis between DNA double helices containing mismatched G/G sequences. A, schematic of the DNA substrate. Highlighted bases indicate the mismatched G/G sequences. B, WT peptide promotes synapsis of DNA double helices containing a mismatched 8-bp G/G sequence. Ten picomoles of 32P-labeled duplex DNA was incubated in the absence (lane 7) or presence of WT Hop1 ZnF at concentrations indicated at the top of each lane. One set of samples was assayed for DNA binding (lanes 1–6), whereas the second set of samples was assayed for the formation of synapsis product (lanes 8–13). C, the C371S Hop1 mutant peptide failed to bind DNA and defective in promoting synapsis of DNA double helices. Ten picomoles of 32P-labeled duplex DNA was incubated in the absence (lane 1) or presence of C371S mutant peptide at concentrations indicated above each lane. One set of samples was assayed for DNA binding (lanes 2–7), whereas the second set was analyzed for synapsis product formation (lanes 8–12).
length Hop1 protein (22), control experiments showed that WT ZnF promoted a robust synapsis of the duplex DNA substrate containing an 8-bp mismatched region (Fig. 9B, lanes 2 and 3). Furthermore, substitution of a pair of G/G bp for A/T bp led to marginal decrease in the extent of synapsis, relative to the “WT” substrate (Fig. 9B, lanes 5 and 6). However, when three and two G/G mismatches from each end flanking the central 3-bp G/G array was replaced, synapsis was completely abolished (Fig. 9B, lanes 8 and 9). Notably, ZnF failed to display synapsis with substrates containing either two contiguous or three non-contiguous mismatched G/G bp (Fig. 9B, lanes 10–13). These results suggest that a minimum of four contiguous G residues, alone, are essential and sufficient for synapsis promoted by ZnF. Importantly, under similar conditions, the C371S ZnF failed to promote synapsis with the mutant DNA substrates (Fig. 9C). In additional experiments, we ascertained that the defect in synapsis product formation emanated from the inability of C371S mutant to interact with DNA (data not shown). These results suggest that ZnF, like the full-length Hop1 (22), was able to distinguish the base sequence in the G/G parenthesis denotes the number of contiguous mismatched G/G bp. C, the C371S hop1 mutant fails to promote synapsis of 48-bp duplex DNA substrates containing varying number of G/G bp. Reactions were performed as described for B.

**Fig. 9.** Mutations in the G/G region diminish synapsis between double-stranded DNA helices. **A,** schematic of the DNA substrates. The mismatched G/G bp is shown in boldface. **B,** Hop1 zinc-finger promoted synapsis of DNA bearing mutation in the G/G region. 10 pmol each of the indicated 32P-labeled duplex DNA was incubated with Hop1 ZnF at concentrations as shown above each lane for 20 min at 30 °C. The reactions were deproteinized and analyzed as described under “Materials and Methods.” The subscript to G/G parenthesis denotes the number of non-contiguous mismatched G/G bp. C, the C371S hop1 mutant fails to promote synapsis between two double-stranded DNA helices. The position of each of the duplex DNA and their synapsis product is indicated on the left.
DISCUSSION

In this study, we show that a 36-mer synthetic peptide encompassing the Hop1 putative ZnF \((34\text{CX}_5\text{CX}_{13}\text{CX}_2\text{C}_{37})\) performs all of the known biochemical functions of full-length Hop1 protein. To elucidate the nature and function of Hop1 ZnF, interaction between peptides and metal ions were examined. We observed that the motif was able to bind zinc and, as a consequence, caused significant decrease in negative ellipticity, indicating conformational changes in its secondary structure. In contrast, such changes were not detectable in the C371S mutant peptide, suggesting direct involvement of Cys371 in zinc binding. Although only crystal structure can define the details of secondary structure, theoretical considerations predict that Hop1 ZnF is an atypical ZnF and differs from those in TFIIIA-like DNA-binding proteins (30). Competition experiments showed that a subset of divalent metal ions that are capable of tetrahedral coordination were able to compete with zinc for binding to the Hop1 ZnF. Together, these results suggest that Hop1 ZnF functions as an independent zinc-binding module.

The one or more biochemical roles for any of the SC proteins are not known, simply because samples of pure protein and/or assays have not been developed. These results are consistent with a biochemical role of Hop1 as a DNA-binding protein in interstitial pairing of meiotic chromosomes. We also showed that binding of Hop1 to DNA was sequence-independent but structure-specific (21). In the present study, we show that Hop1 ZnF plays a dual role: robust binding to G4 DNA and catalyst for the folding of DNA into this conformation; therefore, this represents a novel type of ZnF.

How can we reconcile the observation that Hop1 ZnF alone is sufficient for the display of all of the known activities of full-length Hop1 protein? What would be the function of the remaining part of Hop1 protein? One of the answers to this question lies in the fact that the different domains might be involved in its interaction with other SC components. In this regard, Hop1 has been shown to interact genetically with RED1 and forms a complex with its gene product (20, 45, 46). However, the domains involved in interaction between Hop1 and Red1 proteins remain to be identified. In furthering our understanding of the mechanism of Hop1 function, a combination of structural analysis and functional characterization of interaction between ZnF and \(^{65}\text{Zn}\) on one hand, and DNA and ZnF on the other, has been particularly informative. Structural characterization of the binding of \(^{65}\text{Zn}\) to ZnF and its interaction with DNA has not only revealed the physical basis for the defect of C371S hop1 mutant allele \(\text{in vivo}\) but also allowed the identification of novel type of zinc finger.

To investigate the biochemical function of Hop1 ZnF, we studied its binding to various DNA substrates. We used gel mobility shift assays in combination with methylation interference to demonstrate binding of Hop1 to DNA rich in arrays of G residues as well as the formation of G4 DNA. Hop1 ZnF, like the full-length protein, preferentially binds to DNA-containing arrays of G residues and G4 DNA, and it promotes the formation of the latter. In contrast, the C371S mutant is impaired in both \(^{65}\text{Zn}\)-binding and DNA-binding properties, indicating that the integrity of ZnF is very important for Hop1 function \(\text{in vivo}\). Remarkably, interaction of Hop1 ZnF with different DNA substrates was functionally indistinguishable from that of the full-length Hop1 protein (21, 22, 28 and this study). However, ZnF but not the full-length Hop1 bound shorter DNA fragments and promoted synopsis product formation. The observation that Hop1 ZnF displayed all of the activities of full-length Hop1 is analogous to that of \(E\).\text{coli} RecA. In particular, a 20-mer peptide of RecA was able to promote both ATPase and strand exchange activities (47). It is likely that these are biologically relevant, because the same properties and kinetics were also evident with the full-length Hop1 protein (21, 22, 28).

Numerous ZnFs have been characterized from a variety of proteins and sources. Although some are thought to function through binding to nucleic acids, a subset of them has been implicated in protein-protein interaction (23, 48). ZnFs often prefer G-rich sequences in DNA operators and bind through major groove (49). Each of them recognizes a DNA base triplet in its respective binding site (50). An engineered modular ZnF has been shown to bind oligonucleotides containing the human telomeric repeat sequence folded into G4 DNA (51). Several G4 DNA-binding proteins have been isolated from a variety of sources, which display high affinity for DNA sequences (52, 53). The affinity of Hop1 ZnF to DNA (micromolar range) is com-
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