Zip1-induced Changes in Synaptonemal Complex Structure and Polycomplex Assembly

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Abstract. The yeast Zip1 protein is a component of the synaptonemal complex (SC), which is an elaborate macromolecular structure found along the lengths of chromosomes during meiosis. Mutations that increase the length of the predicted coiled coil region of the Zip1 protein show that Zip1 influences the width of the SC. Overexpression of the *ZIP1* gene results in the formation of two distinct types of higher order structures that are found in the nucleus, but not associated with chromatin. One of these structures resembles the polycomplexes that have been observed in many organisms and are thought to be aggregates of SC components. The second type of structure, which we have termed "networks," does not resemble any previously identified SC-related structure. Assembly of both polycomplexes and networks can occur independently of the Hop1 or Red1 protein, which are thought to be SC components. Our results demonstrate that Zip1 is a structural component of the central region of the SC. More specifically, we speculate that Zip1 is a component of the transverse filaments that lie perpendicular to the long axis of the complex.

One of the unique features of meiosis is the formation of a proteinaceous scaffold between homologous chromosomes called the synaptonemal complex (SC).[1] The earliest detectable intermediates in SC morphogenesis can be seen as short stretches of protein cores called axial elements that form along each pair of sister chromatids. As these cores elongate, intimate associations between homologues begin to form in the context of SC. In some organisms, full-length axial elements are formed before the initiation of synapsis (von Wettstein et al., 1984). However, in *Saccharomyces cerevisiae*, synapsis initiates before axial element formation is complete (Dresser and Giroux, 1988; Alani et al., 1990). Once axial elements are incorporated into the SC, they are referred to as lateral elements. During the pachytene stage of prophase I, each pair of homologues is fully synapsed, and the SC appears as a ribbon-like structure along the entire length of each chromosome pair.

The SC is morphologically conserved across a wide variety of species (von Wettstein et al., 1984). The lateral elements of the SC are separated by ~100 nm throughout the length of each pair of homologues (Fig. 1). The space between two lateral elements is referred to as the central region. In organisms that are particularly favorable for cytological study, two distinct substructures can be observed within the central region (e.g., Solari and Moses, 1973; Schmekel et al., 1993*a*). The central element is a protein core that runs longitudinally along each complex, equidistant between the lateral elements. Transverse filaments lie across the central region, perpendicular to the lateral elements.

In addition to SCs, aggregates of SC-like material called polycomplexes have been observed in meiotic cells of many different organisms (for review, see Goldstein, 1987). Although polycomplexes vary somewhat in morphology and temporal distribution, they all appear as multiple layers of SCs stacked in parallel. These structures are most commonly seen after dissolution of the SC, but they have also been observed before SC formation and even outside the nucleus (e.g., Dudley, 1973; Zickler, 1973; Esponda, 1977; Bogdanov, 1977; Solari and Vilar, 1978). It is generally assumed that polycomplexes are aggregates of proteins that have dissociated from the SC or storehouses of SC precursors that are subsequently used during SC formation (Fiil and Moens, 1973; Zickler and Olson, 1975; Fiil et al., 1977; Bogdanov, 1977).

Several components of the SC have been identified in rats and hamsters (Heyting et al., 1987; Moens et al., 1987; Chen et al., 1992; Meuwissen et al., 1992; Smith and Benavente, 1992; Dobson et al., 1994). In *S. cerevisiae*, antibodies against the Hop1 protein localize specifically to meiotic chromosomes; therefore, Hop1 is thought to be a component of the SC (Hollingsworth et al., 1990). In *hop1* null mutants, short stretches of axial elements are formed, but no SC has been observed (Hollingsworth and Byers, 1989; Loidl et al.,

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1. *Abbreviations used in this paper*: DAPI, 4'-6' diaminophenylindole; SC, synaptonemal complex.

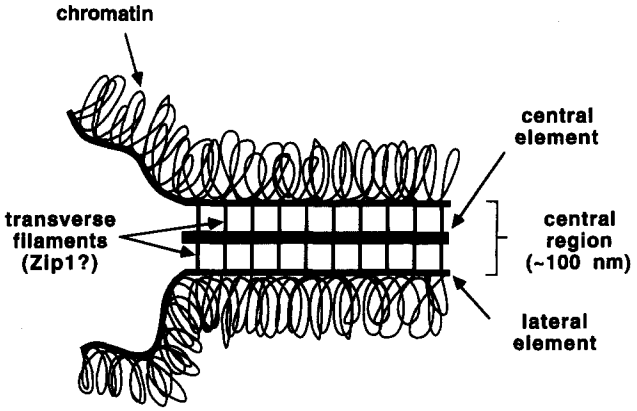


Figure 1. A schematic diagram of the SC. The unsynapsed region to the left depicts the incorporation of transverse filaments. Zip1 may be a component of the transverse filaments that lie across the width of the SC, perpendicular to the lateral elements.

1994). In *red1* null mutants, no obvious SCs or SC precursors have been detected (Rockmill and Roeder, 1990). Overexpression of the *RED1* gene leads to allele-specific suppression of *hop1*, suggesting that Red1 and Hop1 directly interact with each other (Hollingsworth and Johnson, 1993).

Previous studies suggested that Zip1 is a component of the central region of the SC in *S. cerevisiae* (Sym et al., 1993). In *zip1* null mutants, full-length axial elements are formed and homologously aligned, but they fail to synapse. Antibodies against the Zip1 protein localize to the SC, but not to unsynapsed axial elements. DNA sequence analysis revealed that an extensive region of the internal region of the Zip1 protein is predicted to form an α-helical coiled coil, a dimerization motif found in intermediate filament proteins (Albers and Fuchs, 1992; Stewart, 1993). Thus, the Zip1 protein is predicted to form a dimer consisting of a long rod-shaped domain flanked by globular domains (Sym et al., 1993). The predicted length of the Zip1 dimer approximates the width of the SC, based on the number of residues in the coiled coil. This observation led to the hypothesis that Zip1 dimers lie perpendicular to the long axis of the SC and span the distance between lateral elements.

In this study, the length of the Zip1 protein was altered by changing the number of residues in the coiled-coil region. These experiments were suggested by the results of Kilmartin et al. (1993), who altered the length of the coiled-coil region of the yeast Spc110/Nuf1 protein, a component of the spindle pole body. Mutations that decrease the length of the Spc110/Nuf1 protein resulted in a proportionate decrease in the distance between the inner and central plaques of the spindle pole body, suggesting that the protein acts as a spacer that bridges the gap between the two plaques. Our results demonstrate that Zip1 influences the width of the SC and confirm our original hypothesis that Zip1 is a component of the central region.

Overexpression of the *ZIP1* gene revealed that the protein is capable of aggregating into two distinct types of higher order structures, both of which are found in the nucleus, but are devoid of chromatin. One type is strikingly similar to the polycomplexes described in a number of organisms including yeast; however, the yeast strain used in this study does not normally produce polycomplexes. The other structure is an extensive network of densely staining filaments that are also composed of parallel staining lines, but are different in overall morphology from polycomplexes. The ability of Zip1 protein to assemble into higher order structures reinforces our conclusion that Zip1 is a structural protein.

Table I. Yeast Strains

Strain	Genotype
BR2495	*MATa leu2-27 his4-280 arg4-8 thr1-4* *MATα leu2-3,112 his4-260,519 ARG4 thr1-1* *ade2-1 ura3-1 trp1-1* *ade2-1 ura3-1 trp1-289*
MY152	BR2495, except *zip1::URA3* *zip1::URA3*
MY153	MY152, plus pMB156 (*ZIP1* 2μ)
MY187	MY152, plus pMB164 (*zip1-ΔH2* 2μ)
MY154	MY152, plus pMB157 (*zip1-2XH2* 2μ)
MY224	MY152, plus pMB185 (*zip1-3XH2* 2μ)
MY129	BR2495, except *zip1::LEU2* *zip1::LEU2*
MY183	MY129, plus pMB162 (*ZIP1* CEN)
MY185	MY129, plus pMB166 (*zip1-ΔH2* CEN)
MY156	MY129, plus pMB141 (*zip1-2XH2* CEN)
MY226	MY129, plus pMB183 (*zip1-3XH2* CEN)
MY231	BR2495, except *red1::URA3* *red1::URA3*
MY233	MY231, plus pMB156 (*ZIP1* 2μ)
MY273	MY231, plus pMB164 (*zip1-ΔH2* 2μ)
MY234	MY231, plus pMB157 (*zip1-2XH2* 2μ)
MY275	MY231, plus pMB185 (*zip1-3XH2* 2μ)
BR2498	BR2495, except *hop1::TRP1* *hop1::TRP1*
MY265	BR2498, plus pMB156 (*ZIP1* 2μ)
MY269	BR2498, plus pMB164 (*zip1-ΔH2* 2μ)
MY267	BR2498, plus pMB157 (*zip1-2XH2* 2μ)
MY271	BR2498, plus pMB185 (*zip1-3XH2* 2μ)

Plasmids MB141, MB156, MB157, MB162, MB164, MB166, MB183, and MB185 are described in Materials and Methods.

Materials and Methods

Strains and Genetic Procedures

Genotypes of yeast strains are listed in Table I. Strains BR2495 (Rockmill and Roeder, 1990) and BR2498 were provided by Beth Rockmill (Yale University, New Haven, CT). Yeast manipulations were performed and media were prepared using standard procedures (Sherman et al., 1986). Yeast transformations were carried out by the lithium acetate procedure (Ito et al., 1983). All substitutive transformations were verified by Southern blot analysis (Southern, 1975). To monitor sporulation efficiencies, cells were freshly grown on synthetic complete plates lacking the appropriate amino acid to select for plasmids. The plates were then replica-plated onto sporulation medium and incubated at 30°C for 4 d.

Plasmid Constructions

The 4.0-kb XbaI fragment containing *ZIP1* (Sym et al., 1993) was subcloned into the XbaI site of the 2μ plasmid YEp351 (Hill et al., 1986) to generate pMB156. This fragment was also subcloned into the XbaI site of the CEN plasmid pUN70 (Elledge and Davis, 1988) to generate pMB162. An 804-bp HincII fragment was removed from the 4.0-kb XbaI fragment to create a deletion (ΔH2) within the region that encodes the coiled-coil domain. The resulting 3.2-kb XbaI fragment was subcloned into the XbaI sites in YEp351 and pUN70 to generate pMB164 and pMB166, respectively. The 804-bp HincII fragment was inserted into the 5' HincII site of the wild-type

ZIP1 gene to generate two copies of the HincII fragment in a continuous open reading frame (2XH2). The resulting 4.8-kb XbaI fragment was subcloned into the XbaI sites of YEp351 and pUN70 to create pMB157 and pMB141, respectively. Similarly, pMB185 and pMB183 contain three contiguous copies of the HincII fragment (3XH2) in YEp351 and pUN70, respectively. A *zip1::URA3* deletion/disruption plasmid was constructed as follows. A 2.1-kb HpaI-BamHI fragment containing most of the *ZIP1* coding region was removed from pMB96 (Sym et al., 1993) and replaced with the SmaI-BamHI fragment of YCp50 containing the *URA3* gene (Johnston and Davis, 1984). The resulting plasmid (pMB117) was targeted for substitution with XbaI. The *red1::URA3* deletion/disruption plasmid (pV180) was constructed by Karen Voelkel-Meiman (Yale University, New Haven, CT) as follows. The EcoRI-XbaI fragment containing *RED1* (Thompson and Roeder, 1989) was cloned into the same sites in pHSS6 (Seifert et al., 1986). The HindIII fragment within the *RED1* coding sequences was then replaced with the HindIII fragment carrying *URA3*. The resulting plasmid was targeted for substitution with NotI. The *hop1::TRP1* deletion/disruption plasmid (pNH32-1) was provided by Nancy Hollingsworth (Hollingsworth and Byers, 1989), and was targeted for substitution with SacI.

Cytology

All meiotic chromosome preparations were spread according to Dresser and Giroux (1988). For electron microscopy, spreads were generally stained with silver nitrate as described by Dresser and Giroux (1988), but the staining procedure was modified according to Loidl et al. (1991) in one experiment to visualize the central element. Immunofluorescence procedures were performed according to Sym et al. (1993).

Strains carrying 2μ or CEN plasmids were grown to saturation in 2 ml of synthetic complete medium lacking leucine or uracil, respectively, to maintain the plasmids. 1 ml of the saturated culture was then resuspended in 2 ml of rich medium (YPAD) and grown at 30°C for ~10 h. Although some cells lost the plasmid during this period (~10%), growth in rich medium was necessary to ensure efficient sporulation. To induce meiosis, 1.5 ml of each YPAD culture was resuspended in 10 ml of 2% potassium acetate. Unless otherwise stated, spreads were prepared after 17 h in sporulation medium.

To determine the dimensions of SCs, polycomplexes, and networks in cells carrying wild-type or mutant versions of the *ZIP1* gene, spread nuclei were prepared and measurements were taken in a double-blind fashion. Photographs of spreads printed at magnifications of 37,800–50,400 were used to trace the lateral elements (for SCs) or parallel lines (for polycomplexes and networks) onto transparent paper. Distances between lateral elements of SCs were measured at regular intervals along tracings of different stretches of SC from several nuclei. A total of 100 points along the SCs were measured for each strain, representing a total of 10 μm of SC. Distances between adjacent parallel lines were similarly measured in polycomplexes and networks. For polycomplexes, the distance given for each strain represents an average of at least 50 intervals derived from at least six different polycomplexes. For networks, the distance given for each strain is an average of at least 200 intervals derived from four different nuclei.

Results

Zip1 Affects the Width of the SC

If Zip1 is a component of the central region of the SC, and it lies perpendicular to the longitudinal axis of the complex, then one might predict that a deletion within the Zip1 coiled-coil region would decrease the width of the SC, and an extension of the coiled coil would increase the width. Three different constructs were made to test this hypothesis (Fig. 2). In one mutant, an 804-bp HincII fragment was deleted (ΔH2), and the length of the protein is predicted to be 40 nm shorter than wild-type, based on the number of residues removed from the coiled coil. In two other mutants, the HincII fragment was either duplicated or triplicated (2XH2 and 3XH2); these constructs are predicted to increase the length of the protein by 40 and 80 nm, respectively. Length predictions are based on a 1.485-Å change in length per residue of the coiled coil (Fraser and MacRae, 1973). All three mutant

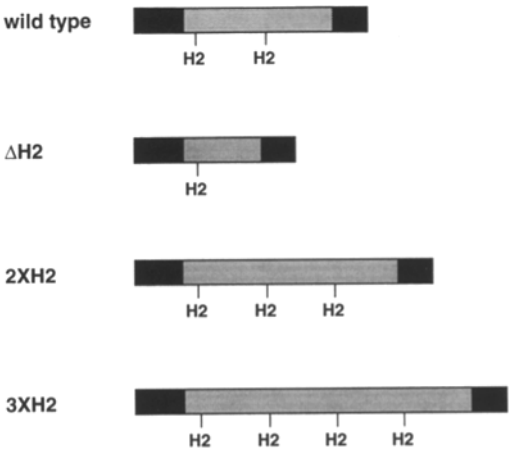


Figure 2. Alterations in the coiled-coil region of Zip1. Regions in black denote the globular domains of Zip1, whereas the shaded regions represent the coiled-coil domain. Three different constructs were made that change the number of residues in the coiled-coil region of Zip1. A deletion mutant (*zip1-ΔH2*) eliminates amino acids 243–511. The other constructs increase the coiled-coil region by duplicating (*zip1-2XH2*) and by triplicating (*zip1-3XH2*) amino acids 243–511.

constructs were introduced into yeast on either single-copy (CEN) or multicopy (2μ) plasmids. Each of the three mutant proteins are incorporated into the SC since, in many spread nuclei, meiotic chromosomes appeared fully synapsed (Fig. 3) and antibodies against the Zip1 protein localized to the SC (Fig. 4, *a* and *b*).

Measurements were taken from photographs of SCs in which the lateral elements could be clearly distinguished as shown in Fig. 5. In the deletion mutant, the width of the SC did not change relative to wild type (Table II). However, the 2XH2 and the 3XH2 constructs increased the width of the SC by amounts that correspond well to the predicted increases in the length of a Zip1 dimer (Table II). Furthermore, the standard deviations for the two longer versions of Zip1 do not overlap with each other or with wild type, indicating that the differences in width are significant. In the unsynapsed chromosomes of the *zip1* null mutant, the distance between homologous axial elements varies greatly from one region to another on the same pair of chromosomes (~100–400 nm). This variation is quite different from the mutant constructs described above, which all show a relatively small standard deviation. Thus, chromosomes in the *zip1* coiled-coil mutants are fully synapsed and the width of the SC is fairly constant.

In the strain background used in this study, *zip1* null mutants fail to sporulate and they arrest before the meiosis I division as described previously (Sym et al., 1993). On both single-copy and multicopy plasmids, the *zip1* coiled-coil mutants partially complement the sporulation defect of a *zip1* null mutant (Table III). Strains carrying the mutant genes sporulate 4- to 18-fold more efficiently than the null mutant. However, none of the mutant genes increases sporulation to the wild-type level. Furthermore, overexpression of the wild-type *ZIP1* gene decreases sporulation efficiency by about twofold relative to a strain containing a single copy of the *ZIP1* gene.

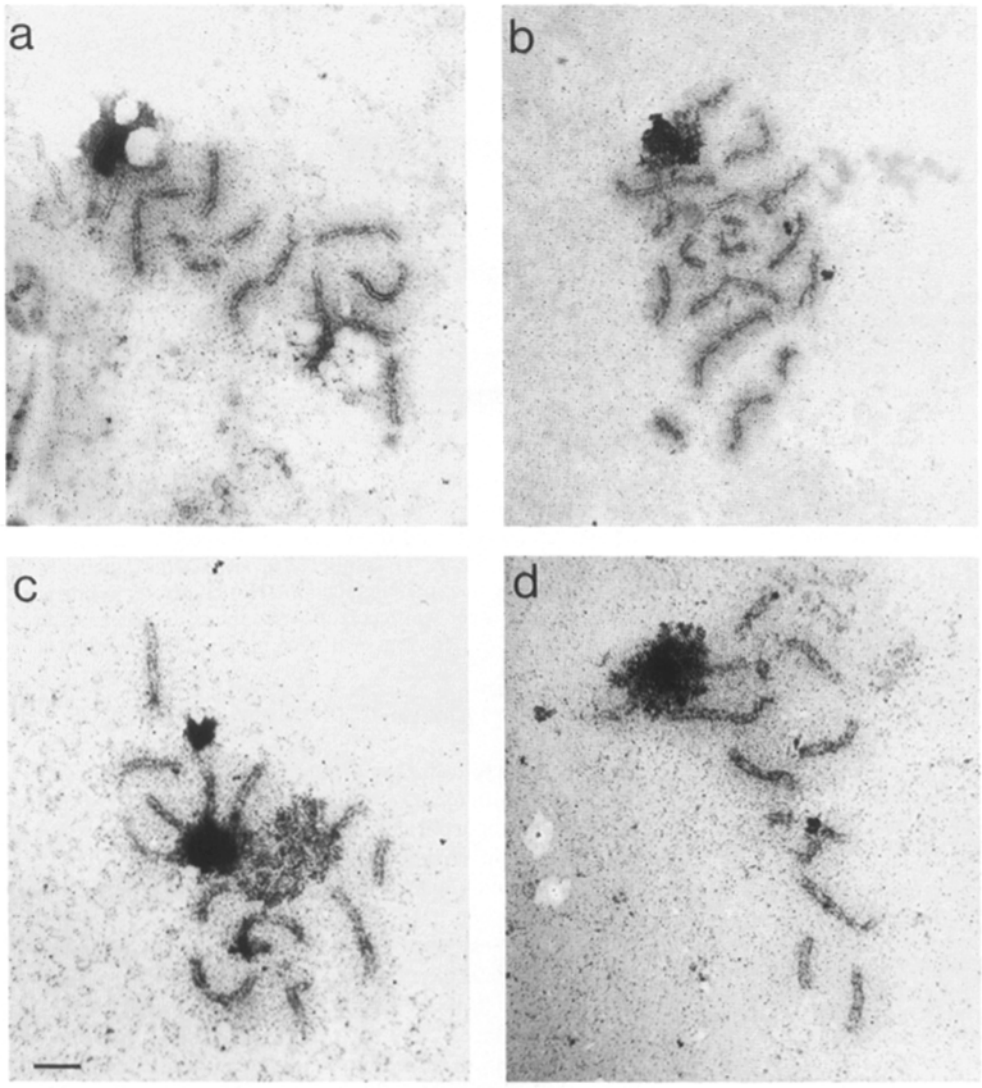


Figure 3. Electron micrographs of meiotic chromosomes during pachytene. Cells overexpressing a wild-type copy of *ZIP1* (*a* = MY153), ΔH2 (*b* = MY187), 2XH2 (*c* = MY154), or 3XH2 (*d* = MY224) show fully synapsed chromosomes at pachytene. The large, darkly stained mass in each panel is the nucleolus. Bar, 1 μm.

Polycomplex-like Structures Are Formed by Overexpressing ZIP1

Overexpression of the *ZIP1* gene on a multicopy plasmid results in the assembly of nuclear structures that are similar to polycomplexes. Although polycomplexes have been described in *S. cerevisiae* (Engels and Croes, 1968; Moens and Rapport, 1971; Zickler and Olson, 1975; Horesh et al., 1979; Alani et al., 1990; Loidl et al., 1994), the wild-type strain used in this study does not form polycomplexes. Other studies have also shown that both the frequency and nature of polycomplexes are highly dependent on yeast strain background (Zickler and Olson, 1975; Horesh et al., 1979; Alani et al., 1990; Loidl et al., 1994).

In strains overproducing the wild-type Zip1 protein, each polycomplex resembles a stack of SCs arranged in parallel (Fig. 6, *a–c*). In some of these polycomplexes, the parallel lines appear in pairwise repeating units (Fig. 6 *c*). The distance between adjacent lines is similar to the distance between lateral elements in the SC (Table II). Polycomplexes were also observed when the Zip1 proteins of varying length were overproduced (Fig. 6, *d–l*). All three versions formed similar polycomplexes when compared to each other, but they are different from wild type in a number of ways. The mutant polycomplexes tend to form either circular or curved arrays, whereas the wild-type Zip1 polycomplexes are usually linear. In the mutant Zip1 polycomplexes, three to five parallel lines are typical, whereas 10–20 lines are common in polycomplexes containing the wild-type Zip1 protein. The distance between adjacent lines in polycomplexes corresponds reasonably well to the distance between lateral elements in the SC for the insertion mutants, but not for the deletion mutant (Table II).

In the electron micrographs described thus far, silver staining was carried out such that the predominant stained structures are the lateral elements of the SCs and the densely staining lines in the polycomplexes. An alternate staining technique has been described for yeast that facilitates detection of the central element, in addition to the lateral elements of the SC (Loidl et al., 1991). Using this technique, a lightly staining protein core was observed equidistant between the densely staining cores of polycomplexes in wild type and the mutants (Fig. 6, *m–o*).

It is not clear whether the polycomplexes observed are physically associated with any nuclear structures, but they

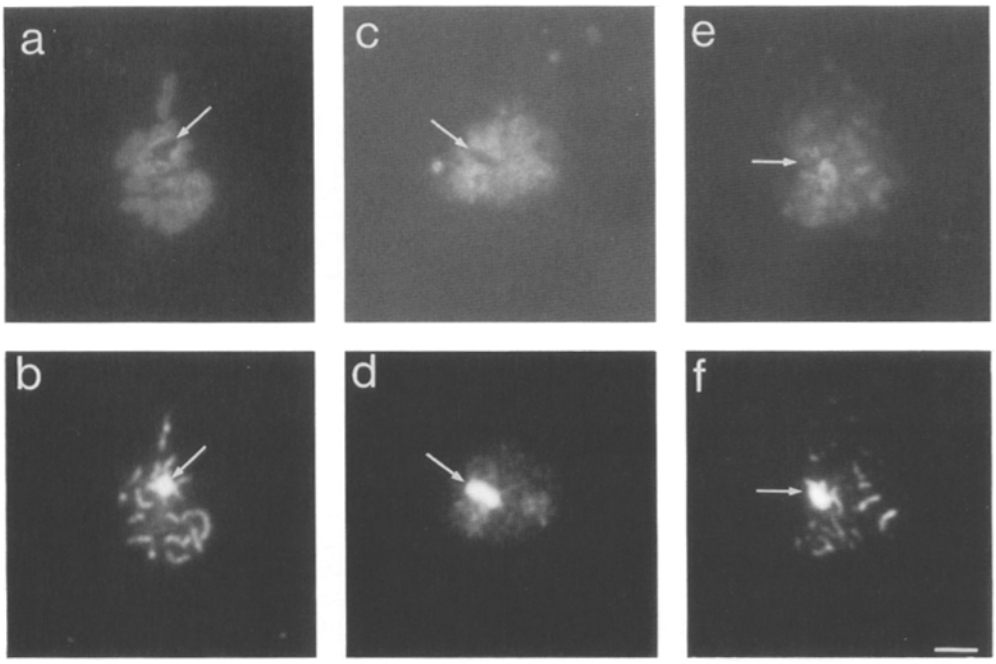


Figure 4. Zip1 localization to polycomplexes in cells overexpressing *ZIP1*. (*a*) DAPI staining of pachytene chromosomes from a strain overexpressing *zip1-2XH2* (MY154) and the corresponding staining with anti-Zip1 antibodies is shown in *b*. (*c* and *e*) DAPI staining of the chromosomes found in *hop1* (*c*, MY265) and *red1* (*e*, MY233) mutants overexpressing *ZIP1* with the respective Zip1 staining shown in *d* and *f*. Zip1 staining of chromosomes in both mutants usually appears punctate (*d*), but more continuous staining is occasionally observed (*f*). Arrows in *b*, *d*, and *f* point to polycomplexes stained with anti-Zip1 antibodies. Arrows in *a*, *c*, and *e* point to regions that correspond to the polycomplexes and appear to be free of chromatin based on the lack of DAPI staining. Bar, 1 μm.

are frequently found near the nucleolus, as observed by Zickler and Olson (1975). Furthermore, they are almost always associated with a very densely staining region. In the mutants, this densely staining region coincides with the center of a circular array or one end of a curved array (Fig. 6, *d–l*). This region may correspond to the so-called dense body or round body, which has been described previously in yeast (Moens and Rapport, 1971; Zickler and Olson, 1975) and is sometimes found in association with polycomplexes (Horesh et al., 1979).

To determine if polycomplexes contain the Zip1 protein, anti-Zip1 antibodies were used to stain spread meiotic

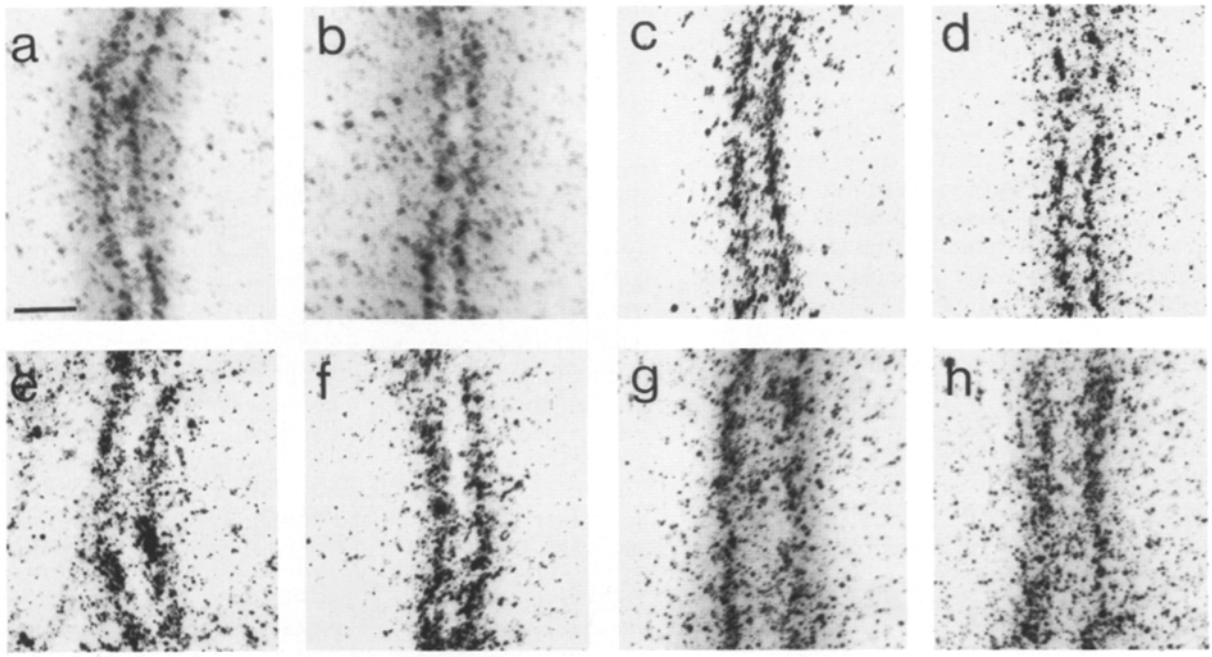


Figure 5. Regions of SC from wild-type and coiled-coil mutants of Zip1. The SC in strains carrying Zip1 proteins of varying lengths are shown at the same magnification for comparison. Two examples are shown for each strain: wild-type (*a* and *b* = MY153), ΔH2 (*c* and *d* = MY187), 2XH2 (*e* and *f* = MY154) and 3XH2 (*g* and *h* = MY224). Bar, 200 nm.

Table II. Measurements of SCs, Polycomplexes, and Networks

	Zip1 dimer	SCs	Polycomplexes	Networks
	nm	*nm*	*nm*	*nm*
Wild type		114 ± 10	113 ± 22	49 ± 9
zip1-ΔH2	−40	115 ± 12	177 ± 19	79 ± 14
zip1-2XH2	+40	153 ± 13	158 ± 21	78 ± 16
zip1-3XH2	+80	189 ± 14	179 ± 20	80 ± 16

The predicted change in the length of the Zip1 dimer in the mutants is indicated. The distances between lateral elements of the SC and between adjacent parallel lines in polycomplexes and networks were measured in spread nuclei from strains producing wild-type and mutant Zip1 proteins. The distances given are averages with standard deviations.

Table III. Sporulation in Strains Carrying zip1 Coiled-coil Mutations

Strain	Sporulation	Strain	Sporulation
zip1::LEU2	<2%	*zip1::URA3*	<2%
" + *zip1-ΔH2* (CEN)	7%	" + *zip1-ΔH2* (2μ)	17%
" + *zip1-2XH2* (CEN)	20%	" + *zip1-2XΔH2* (2μ)	11%
" + *zip1-3XH2* (CEN)	23%	" + *zip1-3XΔH2* (2μ)	7%
" + *ZIP1* (CEN)	60%	" + *ZIP1* (2μ)	35%

Sporulation was assessed in *zip1::LEU2* (MY129) and *zip1::URA3* (MY152) null mutants with wild-type and mutant *ZIP1* genes present on either single-copy (CEN) or multicopy (2μ) plasmids. The following plasmid-bearing strains were used (single-copy, multicopy): *zip1-ΔH2* (MY185, MY187), *zip1-2XH2* (MY156, MY154), *zip1-3XH2* (MY226, MY224), and *ZIP1* (MY183, MY153).

nuclei. During pachytene, polycomplexes show intense Zip1 staining, and they appear to be free of chromatin since they fail to stain with 4′-6′ diaminophenylindole (DAPI), a DNA-binding dye (Fig. 4, *a* and *b*). Polycomplexes were observed in nuclei containing varying degrees of Zip1 staining along the chromosomes (data not shown), indicating that polycomplexes are present at several different stages of SC morphogenesis.

Overexpression of ZIP1 Generates Extensive "Networks"

In addition to polycomplex-like structures, other elaborate aggregates are formed in cells overexpressing the *ZIP1* gene. These aggregates can be classified into two categories, which will be referred to as "dots" and "networks." In a given nucleus, ~10–30 dots that stain with anti-Zip1 antibodies appear in a background of relatively diffuse chromatin (Fig. 7, *a* and *b*). These dots stain very densely with silver nitrate as observed in the electron microscope (Fig. 8 *a*). In other nuclei, extensive networks of Zip1 are evident and the chromatin is sequestered into a smaller region, often well separated from the networks (Fig. 7, *e* and *f*). Electron microscopy revealed that these networks are large arrays of parallel lines that branch out into many different directions (Fig. 8, *c–f*). Some nuclei showed a combination of dots and networks (Figs. 7 *d* and 8 *b*), suggesting that they are related structures. Both dots and networks were found in cells overexpressing the wild-type *ZIP1* gene and all three mutant versions of *ZIP1*. There are no obvious differences among the dots found in wild type and the three mutants. However, the networks in wild type are generally less extensive compared to those in the mutants. In addition, the distance between adjacent lines within a network in wild type is ~50 nm, whereas the lines are separated by ~80 nm in the mutants (Table II).

Frequencies of SC-related Structures in Strains Overproducing Zip1

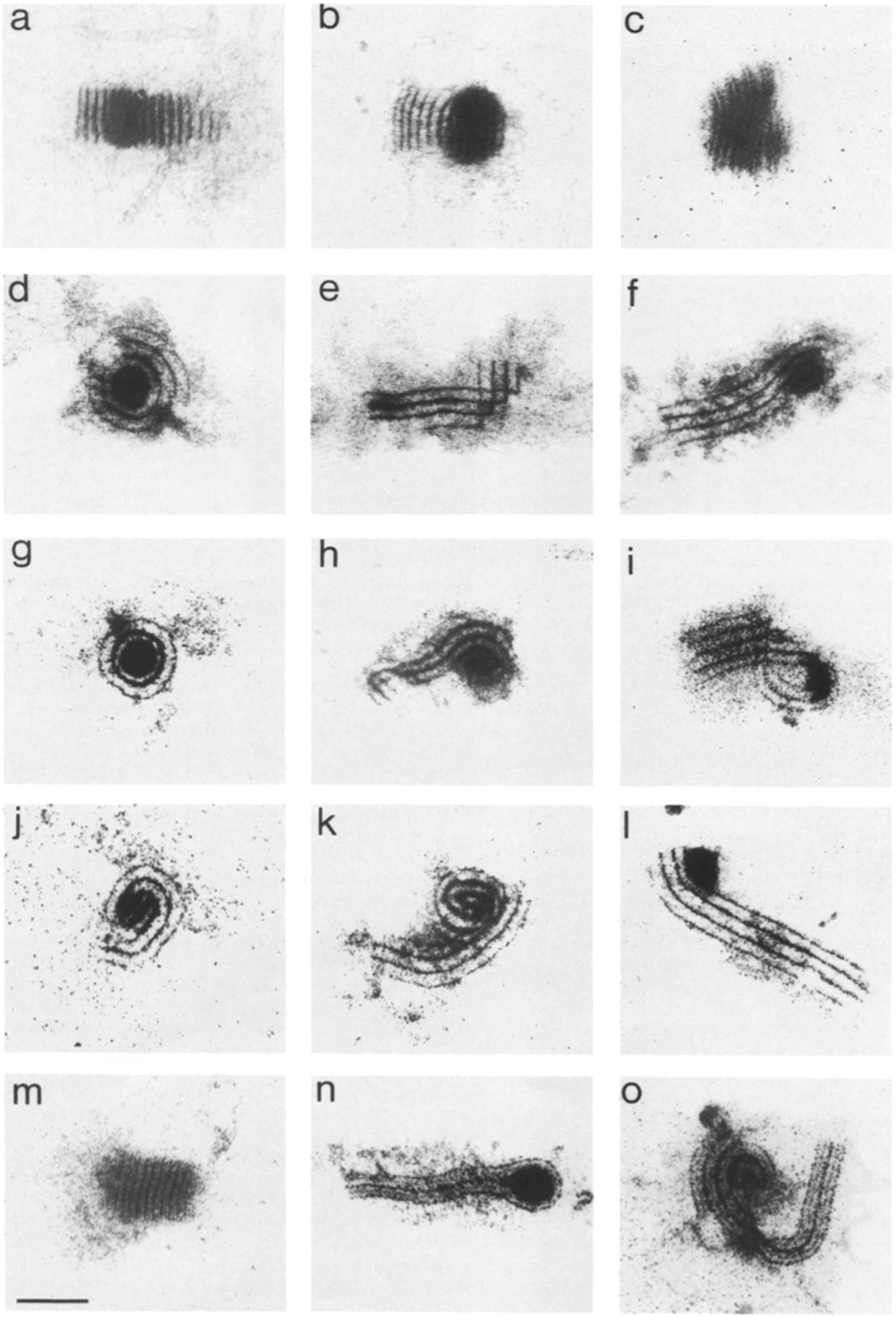
To determine the relative abundance of SCs, polycomplexes, dots and networks, anti-Zip1 antibodies were used to stain both whole meiotic cells and spread chromosome preparations. Localization of Zip1 was observed in uninucleate cells, but not in binucleate and tetranucleate cells, which are indicative of the meiosis I and II divisions, respectively. About 10% of the uninucleate cells showed no staining with anti-Zip1 antibodies, reflecting the frequency with which the plasmid bearing *ZIP1* is lost under the nonselective growth conditions that precede induction of meiosis (see Materials and Methods). In most of the nuclei undergoing prophase I, anti-Zip1 antibodies localize to the SC and polycomplexes (Table IV). None of the mutants differed from wild type more than twofold with respect to either the fraction of nuclei showing any SC staining or the percentage of SC-containing nuclei in which chromosomes appeared fully synapsed. Dots and networks were found in a smaller subset of nuclei that were distinct from those containing SCs and polycomplexes, suggesting there may be two different pathways of Zip1 assembly. The frequencies of these higher order structures is similar for wild-type, *zip1-ΔH2*, and *zip1-3XH2* strains, but the *zip1-2XH2* strain shows a two- to threefold higher frequency of dots and networks compared to the others (Table IV).

Polycomplexes and Networks Are Formed in the Absence of Red1 and Hop1

Polycomplexes are thought to be aggregates of SC proteins that self-assemble into large arrays when they are not associated with chromosomes (Goldstein, 1987). As mentioned above, the Hop1 protein is thought to be a component of the SC and genetic studies have suggested that Red1 interacts with Hop1. In the absence of either of these proteins, Zip1-induced polycomplexes, dots, and networks (both wild-type and mutant versions) are still made (Fig. 4, *c–f*). Furthermore, electron microscopy of the polycomplexes and networks formed in a *red1* or *hop1* background shows no obvious structural differences from those described above (data not shown).

In *red1* and *hop1* mutants, no obvious SC formation has been observed (Rockmill and Roeder, 1990; Hollingsworth and Byers, 1989; Loidl et al., 1994). It is interesting to note

Figure 6. Electron micrographs of Zip1-induced polycomplexes. Overexpression of wild-type *ZIP1* (MY153) produces polycomplex-like structures, which resemble stacks of SC material (*a–c*). Some of these polycomplexes appear in pairwise organization (*c*). The overexpression of *zip1* coiled-coil mutants also produces polycomplexes: ΔH2 (*d–f* = MY187), 2XH2 (*g–i* = MY154), and 3XH2 (*j–l* = MY224). Using a particular staining technique (see Materials and Methods), an additional, less densely staining protein core is apparent between the heavily staining cores in both wild-type (*m* = MY153) and mutant (*n* = MY154, *o* = MY224) polycomplexes. Bar, 1 μm.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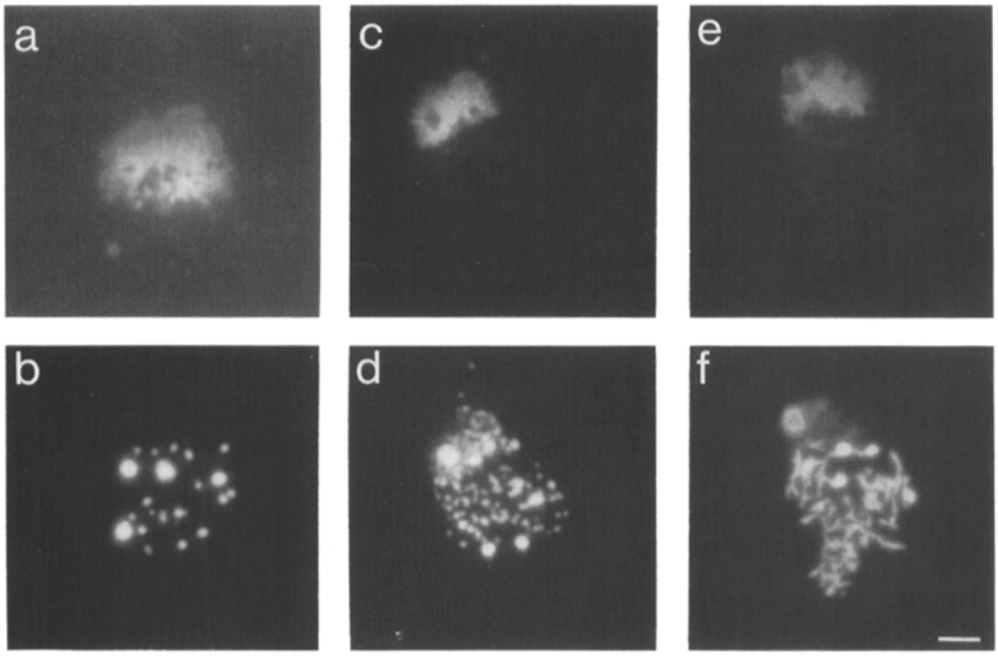

Figure 7. Zipl antibody localization to dots and networks. A nucleus in which anti-Zipl antibodies localize to discrete dots (*b*) shows diffuse chromatin staining with DAPI (*a*). A nucleus in which Zipl localizes to networks is shown (*f*) along with the corresponding DAPI staining (*e*). A nucleus containing both dots and networks is shown (*d*) along with the corresponding DAPI staining (*c*). The chromatin in *c* and *e* appears to be sequestered into a subregion of the spread. Note that in *c* and *e*, faint staining with the DAPI filter is observed in regions corresponding to Zipl staining. These regions do not represent bona fide chromatin staining since the faint signal reflects the intense Zipl staining that bleeds through the DAPI filter. All spreads shown are from strain MY154. Bar, 1 μm.

that Zipl antibodies localize to chromosomal regions in *red1* and *hop1* mutants overexpressing *ZIP1*. The nuclei in these strains typically stain in a punctate fashion (Fig. 4, *c* and *d*); however, in a small percentage of the cells overexpressing the *ZIP1* gene (wild type or mutants), short stretches of Zipl staining were observed, and these corresponded to areas of condensed chromatin (Fig. 4, *e* and *f*). Thus, localization of Zipl to at least some chromosomal regions can occur in the absence of the Red1 or Hop1 protein.

Discussion

Zipl Is a Structural Component of the Central Region of the SC

In this paper, we have shown that changes in the length of the Zipl protein can lead to changes in the width of the SC. Furthermore, overproduction of the Zipl protein results in the formation of Zipl-containing, higher order complexes that have many structural features in common with the SC. These results reinforce our conclusion that Zipl is a structural protein of the SC, and they provide compelling evidence that Zipl is a component of the central region.

Cytological studies of the central region of the SC have shown that the organization of transverse filaments and the central element varies somewhat between organisms. In a number of insect species, the transverse filaments appear in highly ordered, regular intervals, and the central element exhibits a prominent, ladder-like structure (Solari and Moses, 1973; Schmekel et al., 1993*a*; Schmekel et al., 1993*b*). In rats and hamsters, the transverse filaments criss-cross the central region in a much less organized fashion, and the central element appears as a thin, amorphous protein core along the middle of the SC (Solari and Moses, 1973; Schmekel et al., 1993*a*). Electron microscope tomography of the SC in *Blaps cribrosa* has shown that most or all of the transverse filaments extend uninterrupted across the entire central region from one lateral element to the other (Schmekel et al., 1993*b*). However, in other organisms, transverse filaments that end in the central element and therefore traverse only half of the width of the SC are observed (Moens, 1968; Rasmussen, 1976; Schmekel et al., 1993*b*). Each transverse filament may consist of a number of different molecules and/or multiple copies of the same molecule.

The Syn1 protein in hamsters and the homologous Scp1 protein in rats are similar to Zipl in that they contain a long coiled-coil region, and antibodies against the proteins localize exclusively to synapsed regions of wild-type meiotic chromosomes (Dobson et al., 1994; Meuwissen et al., 1992). Furthermore, epitope mapping of the Syn1 protein has shown that antibodies against the amino terminus localize near the central element, whereas antibodies against the carboxy terminus localize just outside the lateral elements (Dobson et al., 1994). Therefore, it is thought that two Syn1 dimers lying end-to-end span the width of the SC.

The results shown here indicate that the width of the SC is increased by lengthening the coiled-coil region of Zipl. Furthermore, increasing the length of the Zipl protein leads to proportionate increases in the width of the SC. These results argue that the Zipl protein lies perpendicular to the long axis of the SC, and they are consistent with the hypothesis that Zipl is a building block of the transverse filaments of the SC (Sym et al., 1993). One interpretation of the increase in SC width in Zipl coiled-coil mutants is that a single Zipl dimer spans the width of the SC from one lateral element to the other. Alternatively, Zipl may fold in half such that both the amino and carboxy termini of a given dimer are attached to the same lateral element (or to the central element). Thus, twofolded dimers that are directly opposed might constitute a transverse filament. The effect of *ZIP1* in-

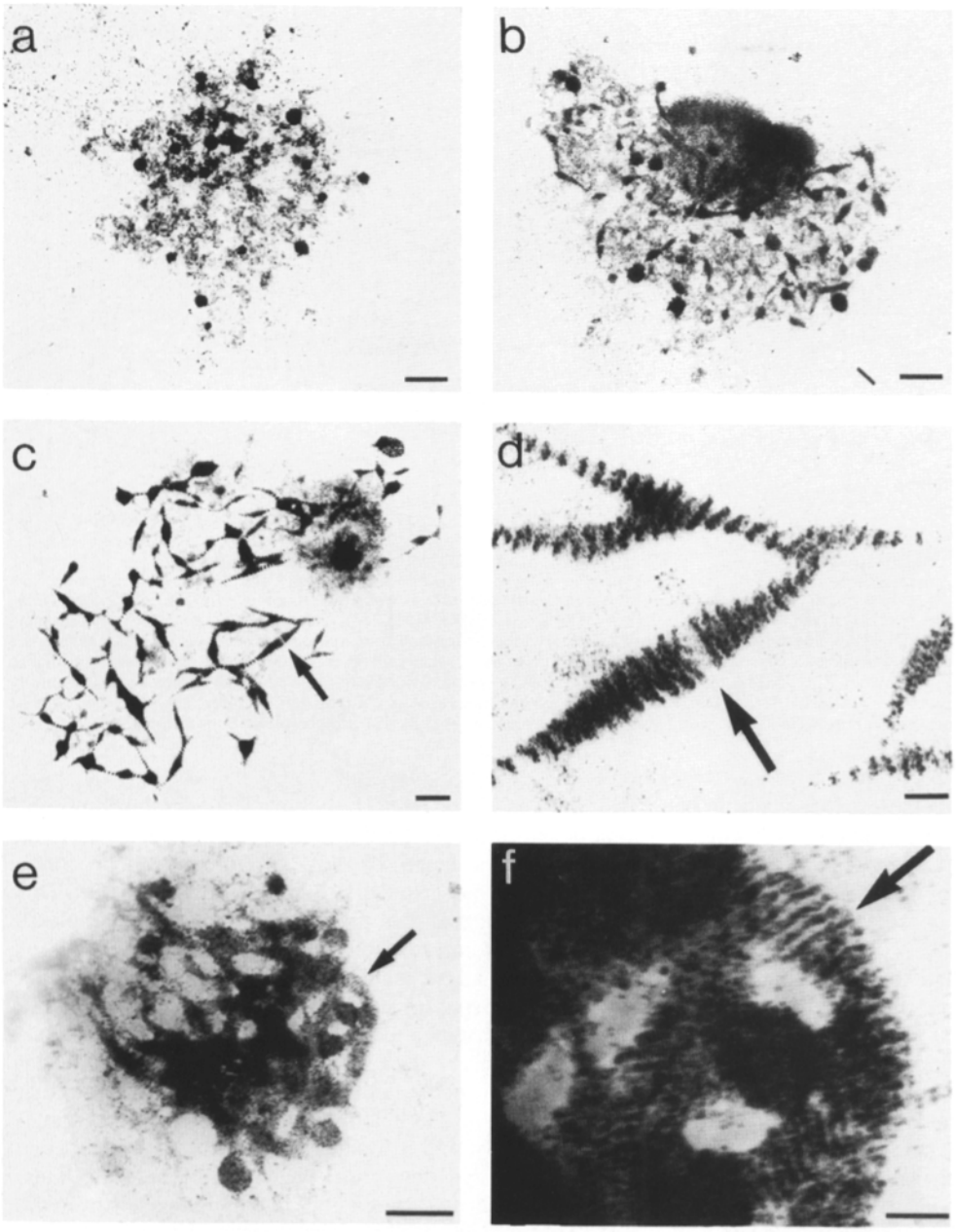

Figure 8. Electron micrographs of dots and networks. (*a*) An example of the dots produced in a strain overexpressing *zip1-2XH2* (MY154). (*b*) A nucleus from MY154 containing both dots and networks. (*c* and *e*) Examples of networks observed in strains producing *zip1-3XH2* mutant (MY224) and wild-type (MY153) Zip1, respectively. Higher magnifications of these networks are shown with arrows indicating a reference point for the enlarged regions (*d* and *f* correspond to *c* and *e*, respectively). Bars in *a–c* and *e*, 1 μm. Bars in *d* and *f*, 200 nm.

sertion mutations on SC width is inconsistent with the model proposed from studies of Syn1 in which two extended dimers lie end-to-end. If this were the case for Zip1, then lengthening the coiled coil should increase the width of the SC by twice the length added to the protein.

Studies of the *ZIP1* deletion mutant indicate that the Zip1 protein is not the sole width-determining component of the SC. Deletion of residues within the Zip1 coiled coil does not lead to a corresponding decrease in the width of the SC or of the distance between densely staining elements in polycomplexes. The fact that the distance between lateral elements does not decrease suggests that there are other proteins in the central region of the SC that prohibit a decrease in width. This is not surprising since biochemical studies have estimated there to be ≥10 meiosis-specific proteins that make up the SC (Heyting et al., 1989). The effect of Zip1 coiled-coil mutations on SC width are not reflected in the dimensions of networks, providing additional evidence for the existence of other proteins that influence the dimensions of SC-related structures.

The Zip1 protein made in the deletion mutant is not long enough to span the SC from one lateral element to the other. Since this mutant makes SC of normal width, the ability of a single Zip1 dimer to span the SC is evidently not required for synapsis. Perhaps two truncated Zip1 dimers interact with each other to maintain the integrity and width of the SC. Alternatively, there may be other proteins in addition to Zip1 that hold lateral elements together.

Zip1 Polycomplexes

Overproduction of Zip1 induces the formation of polycomplexes, and immunofluorescence experiments have shown that Zip1 is a major component of these structures. Although

Table IV. Frequencies of SCs and SC-related Structures in Strains Overproducing Zip1

	Zip1 localization in uninucleate cells (% of total cells)							
	SC no PC		SC + PC					
	Partial SC	Full SC	Partial SC	Full SC	Dots	Networks	No staining	MII (%)
WT:								
15 h	2	3	34	40	3	7	9	2
17 h	2	2	15	41	2	6	12	10
19 h	2	1	16	15	1	2	4	40
ΔH2:								
15 h	3	3	50	26	3	4	12	0
17 h	3	2	31	32	4	8	12	5
19 h	3	2	41	15	3	8	12	10
2XH2:								
15 h	4	3	30	23	12	18	10	0
17 h	2	4	9	36	11	23	9	3
19 h	2	2	21	17	8	22	7	12
3XH2:								
15 h	5	4	35	31	6	8	13	0
17 h	2	5	19	43	5	8	10	5
19 h	3	4	30	26	5	9	8	9

Zip1 antibody localization in nuclear spreads was classified at three different time points during meiosis in strains overexpressing wild-type *ZIP1* (WT = MY153), the deletion mutant (ΔH2 = MY187), and the two longer versions of *ZIP1* (2XH2 = MY154 and 3XH2 = MY224). The maximum number of cells showing full-length SC formation (pachytene) was found at the 17-h time point. Spreads from 200 uninucleate cells for each strain (per time point) were classified as follows: *SC no PC*, SC but no polycomplex staining; *SC + PC*, SC plus conspicuous polycomplex staining; *Dots*, spherical bodies of staining; *Networks*, large assemblies of Zip1 in a meshlike network; *No staining*, no obvious Zip1 localization. Nuclei containing SC were subdivided into those in which chromosomes were fully synapsed (*Full SC*) and those in which synapsis was incomplete (*Partial SC*). Binucleate and tetranucleate cells did not stain with anti-Zip1 antibodies. To monitor progression through meiosis, intact cells from each culture were stained with DAPI, and the percentage of cells that had completed both meiotic divisions (MII) was determined.

it has long been assumed that polycomplexes are made up of SC material, this is the first time that a known SC protein has been demonstrated to be a component of polycomplexes. The ability of Zip1 to assemble into extensive, macromolecular arrays is reminiscent of intermediate filament proteins (Steinert and Roop, 1983).

Antibodies against Hop1 and Red1 localize to the SC and to unsynapsed axial elements, suggesting that these proteins are components of, or closely associated with, the axial/lateral elements (Hop1: Hollingsworth and Byers, 1990; Klein, F., personal communication; Red1: Smith, A., personal communication). The polycomplexes induced by Zip1 overproduction do not require the presence of the Hop1 or Red1 protein. It is possible that these complexes are composed of only central region components or the Zip1 protein exclusively. This is consistent with observations in a number of yeast mutants that make axial elements, but fail to form SC. In these mutants, the abundance of polycomplex-like structures increases significantly over wild type, leading to the suggestion that the unincorporated central region components aggregate into polycomplexes (Alani et al., 1990; Bishop et al., 1992; Loidl et al., 1994). Furthermore, a central element-like core was observed between the densely staining lines in the polycomplexes. Perhaps this core represents an additional central region protein(s) that is physically associated with Zip1. Alternatively, the central element may simply be a region of interdigitation between transverse filaments projecting from opposing lateral elements (Moens, 1968; Rasmussen, 1976).

In strains producing the wild-type Zip1 protein or Zip1 proteins of increased length, the distance between densely staining lines in polycomplexes is similar to the distance between lateral elements of the SC. (For the *zip1-3XH2* mutant, the distance derived from polycomplexes is slightly less than that derived from SCs, but this difference may not be significant, based on the standard deviations observed.) The increases in the length of the Zip1 dimer result in corresponding increases in the dimensions of polycomplexes. This observation suggests that the organization of polycomplexes is similar in some respects to that of SCs, with the Zip1 dimer serving as the major determinant of the distance between densely staining elements. However, in the *zip1-ΔH2* mutant, the distance between lines in polycomplexes is significantly larger than the distance between lateral elements in SCs from the same mutant. This finding argues that polycomplexes in the deletion mutant are organized somewhat differently, and it implies the existence of at least one other protein that determines the distance between lines. Since the distances between lines in polycomplexes from the *zip1-3XH2* and *zip1-ΔH2* mutants are not different, polycomplexes in these two mutants might be organized similarly to each other, but different from wild type and the *zip1-2XH2* mutant.

Wild-type and mutant polycomplexes show striking morphological differences. Wild-type polycomplexes are stacks of linear units, whereas mutant polycomplexes often display curved or circular arrangements. Also, wild-type polycomplexes tend to have many more lines than mutant polycomplexes, but the lines in mutant polycomplexes usually extend further than those in wild type such that the size of the entire polycomplex is roughly the same in all cases. These observations suggest that the mutant proteins aggregate differently from the wild type.

Zip1 Dots and Networks

In a subset of cells overproducing Zip1, extensive networks of densely staining material are observed, which contain

striations similar to those found in polycomplexes. However, these networks, or any structures similar to them, have not been observed previously in meiotic cells. The lines in these networks are not uniform in length or density. The more dense regions are associated with longer lines, whereas the lines flanking these dense regions become gradually shorter and less densely staining (Fig. 8, *c* and *d*). Formation of these networks might be nucleated by spherical aggregates of Zip1 dots (Figs. 7 *b* and 8 *a*), which may correspond to the more densely staining regions of the networks. As observed for polycomplexes, the formation of dots and networks does not require Red1 or Hop1.

In a few nuclei, structures resembling both dots and networks have been observed (Fig. 8 *b*), perhaps representing an intermediate stage in network assembly. Alternatively, the formation of dots versus networks may reflect the concentration of Zip1 protein in the cell. The copy number of the *ZIP1* plasmid varies from cell to cell since it is not stably maintained throughout the growth period that precedes meiotic induction (see Materials and Methods). It may be that small excesses of Zip1 protein induce dot formation, whereas larger excesses induce polymerization of the more elaborate networks.

Overproduction of Zip1 Results in Two Mutually Exclusive Patterns of Assembly

Several observations suggest that polycomplexes and networks are independent pathways of Zip1 assembly. First, networks appear to be mutually exclusive of polycomplexes since these two structures have not been observed in the same nucleus. Second, a distinction between polycomplexes and networks can be made with regard to the behavior of the chromosomes. In most of the nuclei that contain polycomplexes, a substantial amount of SC can be seen along condensed chromosomal regions. In contrast, the SC is not observed in nuclei that contain networks, and the chromosomes in these nuclei appear as a diffuse mass of chromatin. Finally, the overall organization and dimensions of networks are quite different from those found in polycomplexes. Networks appear as multiple interconnected arrays of striped "filaments," whereas polycomplexes are found as single, continuous arrays of repeating units. Although networks and polycomplexes are both composed of densely staining parallel lines, the distance between lines in polycomplexes is approximately twice the distance between lines in networks. Perhaps these dimensions reflect a fundamental difference in how proteins are packed in polycomplexes versus networks. For example, Zip1 dimers may be arranged in register in polycomplexes, but staggered in networks such that each globular domain of a Zip1 dimer apposes the coiled-coil region of adjacent dimers.

If polycomplexes and networks represent distinct pathways of Zip1 assembly, then what factors determine which pathway is taken? Perhaps the initial nucleating event determines how subsequent Zip1 molecules will polymerize. For example, the first few Zip1 dimers may associate end-to-end such that polycomplex formation is favored, or they may associate in a staggered pattern such that network assembly is favored. Once a given pathway is established, it appears to be incompatible with the other since polycomplexes and networks are mutually exclusive.

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