The Synaptonemal Complex Protein SCP3 Can Form Multistranded, Cross-striated Fibers In Vivo

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Abstract. The synaptonemal complex protein SCP3 is part of the lateral element of the synaptonemal complex, a meiosis-specific protein structure essential for synapsis of homologous chromosomes. We have investigated the fiber-forming properties of SCP3 to elucidate its role in the synaptonemal complex. By synthesis of SCP3 in cultured somatic cells, it has been shown that SCP3 can self-assemble into thick fibers and that this process requires the COOH-terminal coiled coil domain of SCP3, as well as the NH_2-terminal nonhelical domain. We have further analyzed the thick SCP3 fibers by transmission electron microscopy and immunoelectron microscopy. We found that the fibers display a transversal striation with a periodicity of ~20 nm and consist of a large number of closely associated, thin fibers, 5–10 nm in diameter. These features suggest that the SCP3 fibers are structurally related to intermediate filaments. It is known that in some species the lateral elements of the synaptonemal complex show a highly ordered striated structure resembling that of the SCP3 fibers. We propose that SCP3 fibers constitute the core of the lateral elements of the synaptonemal complex and function as a molecular framework to which other proteins attach, regulating DNA binding to the chromatid axis, sister chromatid cohesion, synapsis, and recombination.

Key words: meiosis • cohesion • lateral element • chromosomes • recombination

MEIOTIC cell divisions ensure that haploid germ cells can be produced in diploid organisms. During the first meiotic division, the homologous chromosomes (each consisting of two sister chromatids) pair and recombine, a process essential for the production of genetically distinct haploid germ cells. Two meiosis-specific proteinaceous structures, the synaptonemal complex (SC)[1] and the recombination nodule, have been identified by ultrastructural analysis (Fawcett, 1956; Moses, 1956; von Wettstein, et al., 1984; Loidl, 1990). The recombination nodule has been implicated in recombination and in the establishment of crossovers between homologous chromosomes, resulting in the formation of chiasmata (Carpenter, 1987, 1994). The SC is likely to promote pairing and segregation of homologous chromosomes, influence the number and the relative distribution of cross-overs, and convert crossovers into functional chiasmata (Moens and Spyropoulos, 1995; Roeder, 1995; Heyting, 1996; Roeder, 1997). The SC is found in most sexually reproducing organisms and has a tripartite structure with two lateral elements (LEs) surrounding a central element (CE) (von Wettstein et al., 1984). The LEs and the CE are connected along their entire length by fine fibers, the transverse filaments (TFs), which run perpendicular to the axes of the LEs and the CE (Schmekel and Daneholt, 1995). The sister chromatids are attached to the LEs as a series of chromatin loops (Moens and Pearlman, 1988; Rufas et al., 1992). The SCs disassemble at the end of meiosis I, when the homologous chromosomes segregate. The first meiotic division is immediately followed by a second meiotic division, without intervening DNA replication, resulting in the formation of haploid cells.

Several components of the SC have been isolated and characterized. A putative major constituent of the TF has been described in mammals (synaptonemal complex protein 1 [SCP1]) and in budding yeast (Zip1) (Meuwissen et al., 1992; Sym et al., 1993; Dobson et al., 1994). Three facts strongly suggest that the identified proteins are indeed TF proteins: (*a*) the proteins are filamentous in nature; (*b*) they are associated with the synapsed regions of the homologous

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1. *Abbreviations used in this paper*: CE, central element; GST, glutathione-S-transferase; IF, intermediate filament; LE, lateral element; SC, synaptonemal complex; SCP, synaptonemal complex protein; TEM, transmission electron microscopy; TF, transversal filament; Xlr, X-linked lymphocyte-regulated protein; Xmr, Xlr-regulated, meiosis-regulated.

chromosomes; and (*c*) there are no TFs in yeast cells lacking Zip1 (Meuwissen et al., 1992; Sym et al., 1993; Dobson et al., 1994; Sym and Roeder, 1994, 1995; Moens and Spyropoulos, 1995; Liu et al., 1996; Schmekel et al., 1996).

Four components of the LE have been described in yeast and in mammalian cells. The budding yeast Red1 protein is required for axial/lateral element assembly and binds at discrete positions along the axial/lateral elements of the SC (the LEs are called axial elements before synapsis of the homologous chromosomes) (Smith and Roeder, 1997). Red1 may serve to nucleate the binding of proteins required for the formation of axial/lateral elements (Smith and Roeder, 1997). The budding yeast Hop1 protein is also found at discrete locations along the axial/lateral elements and has been shown to interact with Red1. Hop1, however, is not required for axial/lateral element formation (Hollingsworth et al., 1990; Smith and Roeder, 1997). Two mammalian LE proteins are known, synaptonemal complex proteins 2 and 3 (SCP2 and SCP3) (Offenberg, 1993). SCP3 (the homologous protein is called COR1 in hamster), is ~300 amino acids long, is meiosis specific, and displays no structural similarities to the yeast Red1 or Hop1 proteins (Dobson et al., 1994; Lammers et al., 1994). In contrast to the situation for the two yeast LE proteins, antibodies against SCP3 label the axial/lateral elements in a continuous manner. Since SCP3 appears at the same time and in the same place as LEs during meiosis, it is likely that SCP3 is a basic constituent of the LE (Dobson et al., 1994; Lammers et al., 1994; Moens and Spyropoulos, 1995; Scherthan et al., 1996). Two mammalian proteins related to SCP3 have been described, Xlr (X-linked lymphocyte-regulated protein) and Xmr (Xlr-related, meiosis regulated) (Siegel et al., 1987; Calenda et al., 1994). Like SCP3, Xmr is expressed in meiotic cells, while Xlr is expressed in B- and T-lymphocytes. Since the SCP3, Xmr, and Xlr proteins are expressed selectively in cells undergoing genetic rearrangements, it has been suggested that these proteins take part in various aspects of the recombination process (Siegel et al., 1987; Calenda et al., 1994).

The SCP3, Xmr, and Xlr proteins are most similar in their COOH-terminal regions, which contain several putative coiled coil domains (Siegel et al., 1987; Calenda et al., 1994; Lammers et al., 1994). The coiled coil–forming regions of the hamster SCP3 protein (COR1) have been shown to promote homotypic interactions in vitro (Tarsounas et al., 1997). The coiled coil domain is an important protein dimerization motif that mediates homodimeric and heterodimeric protein–protein interactions through packaging of alternating hydrophobic and hydrophilic amino acids (Lupas, 1996). Proteins that contain coiled coils form mechanically rigid, fibrous structures used as cellular networks, exemplified by intermediate filaments (IFs), and scaffolds to which regulatory complexes are attached, such as tropomyosin and troponin assemblies (Stewart, 1993; Heins and Aebi, 1994). The individual members of the IF protein family contain a central helical rod domain surrounded by two nonhelical end domains (Stewart, 1993; Heins and Aebi, 1994). While the coiled coil domain mediates dimer formation, the ends of the rod domain, together with the nonhelical end domains, contributes to the longitudinal and lateral assembly of oligomers into nuclear and cytoplasmic fibers.

Since the SCP3 protein is strikingly similar to the members of the IF protein family, we have investigated the fiber-forming properties of SCP3 both in vitro and in vivo. We find that SCP3 can self-assemble into conspicuous, transversely striated homopolymeric fibers in vivo in somatic cells. In vitro binding and in vivo expression studies show that the COOH-terminal coiled coil domains in SCP3 mediate homophilic protein–protein interactions. However, the NH_2-terminal nonhelical domain of SCP3 is also required for assembly of the fibers. The striated SCP3 fibers are similar to the highly ordered lateral elements observed in the synaptonemal complexes of *Locusta* (Moens, 1969) and *Neottiella* (Westergaard and von Wettstein, 1970), for example. Our data therefore suggest that SCP3 forms a more or less ordered fibrous core in the LEs. Such a core could function as a molecular scaffold to which other proteins could bind and thereby regulate the synaptic and recombination processes.

Materials and Methods

Primary Antibodies

The affinity-purified rabbit anti-SCP3 and anti-SCP1 antibodies have been described previously (Liu et al., 1996). The monoclonal anti-FLAG (IBI, Eastman Kodak Co., Rochester, NY), the rabbit anti–vimentin (Boehringer Mannheim Corp., Indianapolis, IN) and the rabbit anti–tubulin (Sigma Chemical Co., St. Louis, MO) antibodies were purchased from commercial suppliers.

Plasmid Constructs

The full-length SCP3 sequence (Lammers et al., 1994) was amplified from rat testis cDNA, whereas their full-length SCP1 (Sage et al., 1995) and Xmr (Calenda et al., 1994) sequences were amplified from mouse testis cDNA. All three sequences were cloned into the eukaryotic expression vectors pCMX-pL1 and pCMX-pL1-FLAG. SCP3 was also cloned into the bacterial expression vector pGEX-5X (Pharmacia Biotech, Piscataway, NJ). The amplified sequences to be cloned into pCMX-pL1 contained a 5′ BamHI site, a Kozak translational initiation site, and a 3′ XhoI site. The pCMX-pL1-FLAG vector was constructed by the insertion of a Kozak translational initiation site (Kozak, 1991) and an in-frame FLAG epitope sequence into the EcoRV-BamHI sites. The amplified sequences to be cloned into pCMX-pL1-FLAG or pGEX-5X, forming a FLAG fusion protein or a glutathione-S-transferase (GST) fusion protein, respectively, contained a 5′ BamHI site and a 3′ XhoI site and following digestion were ligated into the BamHI-XhoI sites of these vectors. Truncated versions of the SCP3 cDNA sequence were constructed encoding amino acids 2–130 (SCP3-N) or 125–257 (SCP3-C) of the SCP3 protein, and the amplified sequences were cloned into the BamHI-XhoI sites of the pCMX-PL1-FLAG. Expression of all constructs was tested by coupled in vitro transcription–translation using T7 polymerase and a rabbit reticulocyte system (Promega Corp., Madison, WI). The synthesized proteins were detected by autoradiography or by Western blotting experiments using specific antibodies.

In Vitro Interaction Assays

BL21 bacteria transformed either with the wild-type GST-vector or with the GST-SCP3 construct were grown in bacterial medium, induced by IPTG, and lysed, and the GST proteins were purified from the bacterial debris by addition of glutathione–Sepharose 4B beads (Pharmacia Biotech). The beads were boiled in SDS-reducing buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2.3% SDS, 10 mM DTT) to release the bound proteins, which then were separated on SDS-PAGE and detected by Coomassie blue staining (Laemmli, 1970). ^{35}S-labeled proteins were prepared from different pCMX-SCP3 constructs using an in vitro transcription/translation kit from Promega Corp. Equal amounts of labeled in vitro–translated proteins were mixed with either the GST or GST-SCP3 beads in binding buffer (50 mM Tris, pH 7.9, 120 mM NaCl, 0.2% NP-40, 1 mM PMSF, 1 mM DTT) and incubated for 1 h on a rotary shaker at 4°C. The resin was washed three times with binding buffer at room temperature

and boiled in SDS-reducing buffer, and the supernatant was subjected to SDS-PAGE followed by autoradiography.

Cell Culture and Indirect Immunofluorescence Microscopy

Mouse Swiss-3T3 fibroblasts, mouse L-cells, and Chinese hamster ovary (CHO) cells were cultured in DME (GIBCO BRL, Gaithersburg, MD) containing 10% fetal calf serum (Sigma Chemical Co.). Cells were plated at low density and grown at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air. Cells were fixed in freezing-cold methanol/acetone (50:50) for 5 min and preincubated with 3% BSA before addition of the first antibody. The primary antibodies used were anti-SCP3 (1:10), anti-SCP1 (1:10), anti-FLAG (1:100), anti-vimentin (1:10), and anti-tubulin (1:1,000). The secondary antibodies were a fluorescein isothiocyanate–conjugated swine anti–rabbit IgG (diluted 1:50; DAKOPATTS AB, Älvsjö, Sweden) and a rhodamine-conjugated goat anti–mouse IgG (diluted 1:80; Boehringer Mannheim Corp.). Cells were also stained with 1 μg/ml of Hoechst 33258 for 15 s. Slides were mounted in a 78% glycerol mounting medium containing 1 mg/ml para-phenylene diamine, examined in a Zeiss Axioscope microscope (Thornwood, NY), and photographed with Kodak TMAX 400 film (Rochester, NY).

Automated Microinjection and Lipid-mediated Transfection

DNA constructs (50 ng/μl) were microinjected into the nuclei of asynchronously growing Swiss-3T3 mouse fibroblasts using an automated injection system (Carl Zeiss, Inc.) as described previously (Starborg et al., 1996; Yuan et al., 1996). To label the cells into which DNA was injected, a TRITC-conjugated antibody was also injected. The cells were fixed in freezing-cold methanol/acetone (50:50) 24 h after microinjection and examined by indirect immunofluorescence microscopy.

Alternatively, the DNA constructs were introduced into cells by transfection. In brief, mouse L-cells with a confluency of 40–50% were incubated with a mixture containing 5 μg of the relevant DNA construct, 5 μg of pHook plasmid (Invitrogen, Carlsbad, CA), 15 μl of lipofectamine (Life Technologies, Inc., Gaithersburg, MD), and 0.6 ml of serum-free medium (DME with glutamax; Life Technology). The transfection medium was replaced with DME containing 10% fetal bovine serum (Life Technologies, Inc.) containing gentamycin (0.06 mg/ml), and the cells were cultured for 24 h. Transfected cells (cells containing the pHook plasmid) were selected using a magnetic selection procedure (Invitrogen). In brief, cells were harvested, mixed with magnetic beads, and placed on a magnetic stand. Nontransfected cells were removed by sequential washes with DME medium (with serum).

Transmission Electron Microscopy

Transfected mouse 3T3 fibroblasts collected by the magnetic bead technique were immersed in hypotonic buffer (10 mM Hepes, pH 7.8, 1.5 mM MgCl, 0.5 mM DTT, 10 mM NaCl, and 1 mM PMSF) and homogenized briefly using a Dounce homogenizer. The magnetic beads were removed by placing the suspension on a magnetic stand, and the nuclei were collected by low-speed centrifugation. The pellet was subsequently treated at 4°C in the following way: rinsed in 0.1 M sodium cacodylate buffer, pH 7.2, fixed for 90 min in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, rinsed and incubated overnight in the buffer, postfixed in 1% OsO_4 for 40 min, rinsed in the buffer, and kept in 70% ethanol for 5 min. The nuclear sample was dehydrated at room temperature in a graded series of ethanol solutions, left overnight in a 1:1 mixture of absolute ethanol and Agar 100 Resin mixture, and finally embedded in 100% Agar 100 Resin mixture. The polymerization took place at 45°C for 3 d and subsequently at 60°C for 2 d. Thin sections (60–90 nm) were prepared in a Reichert Ultracut Ultramicrotome (Vienna, Austria) with a diamond knife. The sections were stained at room temperature in a saturated uranyl acetate/ethanol solution (1:1) for 5 min followed by lead citrate for 2 min at high pH. The specimens were inspected and photographed in a JEOL TEM-SCAN 100CX microscope (Peabody, MA) at 60 kV or a Zeiss CEM 902 at 80 kV.

Immunoelectron Microscopy

Immunoelectron microscopy was essentially performed as described by Tokuyasu (1980). Transfected mouse 3T3 fibroblasts were isolated, and nuclei were prepared as described above. The nuclear pellet was fixed in 2% paraformaldehyde and 0.1% glutaraldehyde in PBS and cryoprotected in 2.3 M sucrose at room temperature. After 1–1.5 h in sucrose, the pellet was mounted on a metal pin and frozen in liquid nitrogen. Ultrathin cryosections were picked up on drops of 2.3 M sucrose and deposited on nickel grids coated with formvar and carbon. The sections were blocked with 10% newborn calf serum and incubated with the primary anti-FLAG antibody (1:25) and the 6-nm gold–labeled goat–anti mouse IgG/IgM (1:50) secondary antibody. An anti–proliferating cell nuclear antigen (PCNA) antibody was used as a negative control. The sections were finally stained with 2% aqueous uranyl acetate for 5–7 min and embedded in polyvinylalcohol containing 0.4% uranyl acetate. The specimens were examined in a Phillips CM 120 microscope (Mahwah, NJ) at 80 kV.

Results

In Vivo Expression of SCP3 Results in Nuclear and Cytoplasmic Fibers

We and others have shown by immunoelectron microscopy studies that SCP3 is present in the LE of the SC (Dobson et al., 1994; Lammers et al., 1994; Liu et al., 1996). The occurrence of putative coiled coil motifs in SCP3 suggests that SCP3 could play an important structural role in the LE. To test if SCP3 has the ability to form fibers in vivo, SCP3 was synthesized in cultured mammalian cells (with no expression of endogenous SCP3). A plasmid encoding a full-length SCP3 protein fused to an epitope tag (FLAG) (Fig. 1) was introduced into murine Swiss-3T3 cells by direct microinjection. After 24 h, the cells were fixed and examined for SCP3 expression by immunofluorescence microscopy using an anti-FLAG monoclonal antibody (Fig. 2). The SCP3-positive cells revealed very prominent fiber arrays: a few very thick fibers, often in circular arrays in the nucleus, and thinner fibers of varying diameter in a complex network in the cytoplasm (Fig. 2, *top row*). The thickness of the SCP3 fibers varied up to 0.5 μm, and the fibers seemed to be composed of bundles of thinner fibers. Cells that had been microinjected with a control construct expressing only the FLAG epitope were negative (Fig. 2, *bottom row*).

Three control experiments were carried out (not shown). The fiber structures were revealed by an affinity-purified antibody against SCP3, showing that the fibers do

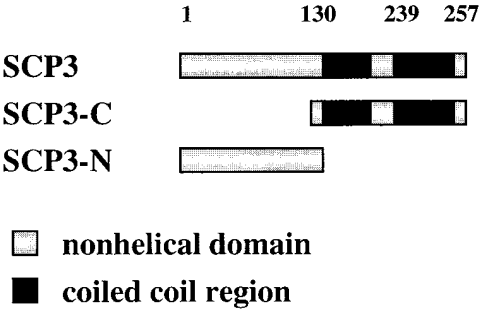

Figure 1. Alterations in the amino acid sequence of SCP3. The full-length rat SCP3 cDNA sequence (257 amino acids) (Lammers et al., 1994) or truncated regions of this sequence were inserted into a mammalian expression vector (pCMX-pL1). All constructs carried identical Kozak translation initiation signals and FLAG epitope sequences upstream of the SCP3 sequence. SCP3-N contains amino acids 2–130 and SCP3-C contains amino acids 125–257.

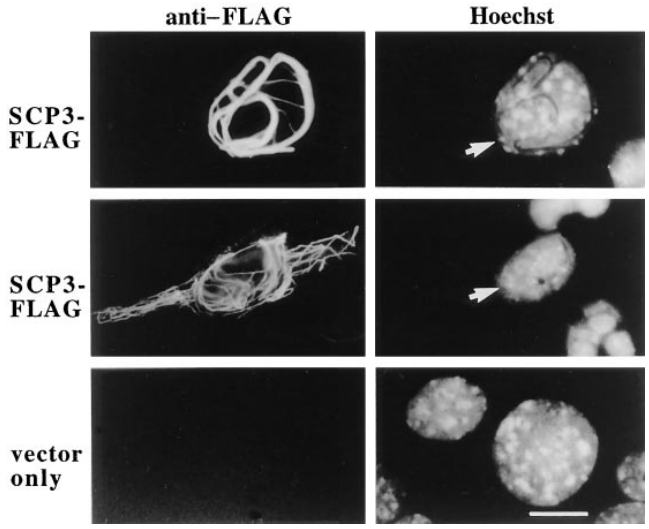

Figure 2. Expression of SCP3 in murine cell culture cells gives rise to nuclear and cytoplasmic fibers. pCMX-SCP3-FLAG plasmid or pCMX-pL1-FLAG vector lacking insert (*bottom row*) were microinjected into cells, and the cells were grown for 24 h. The cells were fixed and labeled with the mouse anti-FLAG antibody and subsequently with a FITC-conjugated anti–mouse antibody. The cells were also stained with Hoechst 33258 to visualize the nuclei. Injected cells were visualized via coinjection of a TRITC antibody (not shown). Cells transfected by SCP3 are indicated with an arrow in the Hoechst pictures. Bar, 10 μm.

contain the SCP3 protein. To ensure that the FLAG epitope tag fused to the SCP3 protein did not contribute to the formation of the fibers, an SCP3 plasmid construct devoid of the FLAG tag was transfected into cells and analyzed by indirect immunofluorescence microscopy. The fibers formed in these cells were indistinguishable from the fibers formed in cells expressing SCP3 fused to FLAG. Finally, the fibers were observed not only in Swiss-3T3 cells but also in CHO cells. We conclude that SCP3 is able to assemble into fibrous structures in somatic cells.

The Fibers Formed in Cells Overexpressing SCP3 Are Multistranded and Transversely Striated

The molecular architecture of the fibers formed by SCP3 was analyzed in more detail using transmission electron microscopy (TEM). Cells transfected either with SCP3 or with a control plasmid were enriched by magnet isolation. The cells were lysed by mild homogenization, and the magnetic beads, which are associated with the plasma membrane, were removed by magnetic separation. The nuclei were then fixed, sectioned, stained with uranyl acetate and lead citrate, and examined by TEM. We observed large nuclear fibrous structures in cells transfected with the SCP3 plasmid (Fig. 3 *A*), whereas no such structures could be observed in cells transfected with a control plasmid expressing only the FLAG epitope (Fig. 3 *D*).

At higher magnification, it is possible to discern that the thick fibers are transversely striated and that they are composed of a large number of closely associated thinner fibers, running in parallel (Fig. 3, *B* and *C*). The elementary fibers have a diameter of 5–10 nm (Fig. 3 *C*, *thin arrows*). The cross-striated pattern shows a periodicity of ~20 nm (Fig. 3 *C*, *rows of arrowheads*). A similar type of cross-striation has been observed in intermediate filaments, which are known to consist of a large number of well-aligned thin fibers, each fiber being composed of polymerized protein subunits forming a distinct molecular arrangement of dimers, tetramers, etc. (see Discussion).

To directly demonstrate that the fibers do contain SCP3 protein, we analyzed the fibers observed in the nuclei of 3T3 cells transfected with the pCMX-SCP3-FLAG plasmid by immunoelectron microscopy using ultrathin cryosections (Tokuyasu, 1980). We have earlier applied this method to determine the localization of the SCP1 and the SCP3 proteins within the SC of murine meiotic cells (Liu et al., 1996). An anti-FLAG antibody was used as primary antibody and visualized by a gold-conjugated secondary antibody. In the cryosections, it was easy to identify the conspicuous large bundles of fibers (Fig. 3, *E* and *F*) that had previously been seen in the plastic sections (Fig. 3, *B* and *C*), although the transverse striation is not well preserved because of the mild fixation used to get a strong immunoreaction. The fibers were strongly labeled with gold particles, demonstrating that they contain large amounts of SCP3. In control experiments, an anti-PCNA antibody was used, and no specific staining was observed (data not shown). It should finally be pointed out that the immunoidentified fibers are present in different regions of the nucleus, sometimes excluding other nuclear material (Fig. 3 *E*), but usually intimately associated with adjacent chromatin (Fig. 3 *F*). In conclusion, the conspicuous bundles of fibers, which appear when SCP3 is overexpressed, are present in the cell nucleus and do contain SCP3.

The Fibers Formed by SCP3 In Vivo in Somatic Cells Seem Not to Be Part of an Endogenous Cellular Network

When investigating the mechanisms by which fiber proteins assemble in vivo, it is important to distinguish if the protein is being incorporated into a preexisting fibrous network or if it is able to self-polymerize. We have tested this by microinjecting the SCP3 construct into Swiss-3T3 cells and staining them with the anti-FLAG antibody and one of a number of antibodies, which decorate known cellular fibers. These other antibodies reacted with vimentin (a predominant intermediate filament protein in Swiss-3T3 cells), actin, tubulin, or lamin (an intermediate filament protein specific for the nuclear membrane). We could in no case see an overlap between the fibers formed by SCP3 and by the endogenous structures labeled by the anti-vimentin (Fig. 4), anti-tubulin (Fig. 4), anti-actin (not shown), or the anti-lamin (not shown) antibodies. We conclude that the fibers observed in cells transfected with SCP3 plasmids result from the self-assembly of SCP3 into fibers.

The Coiled Coil Domain of SCP3 Mediates Protein–Protein Interactions In Vitro

We have investigated regions of SCP3 that are involved in fiber formation by expressing truncated versions of this protein in vitro. The COOH-terminal region of SCP3 contains several coiled coil motifs (Lammers et al., 1994), suggesting that these motifs could take part in protein–protein interactions in vitro. We therefore created truncated versions of the SCP3 plasmid that either expressed the NH_2-ter-

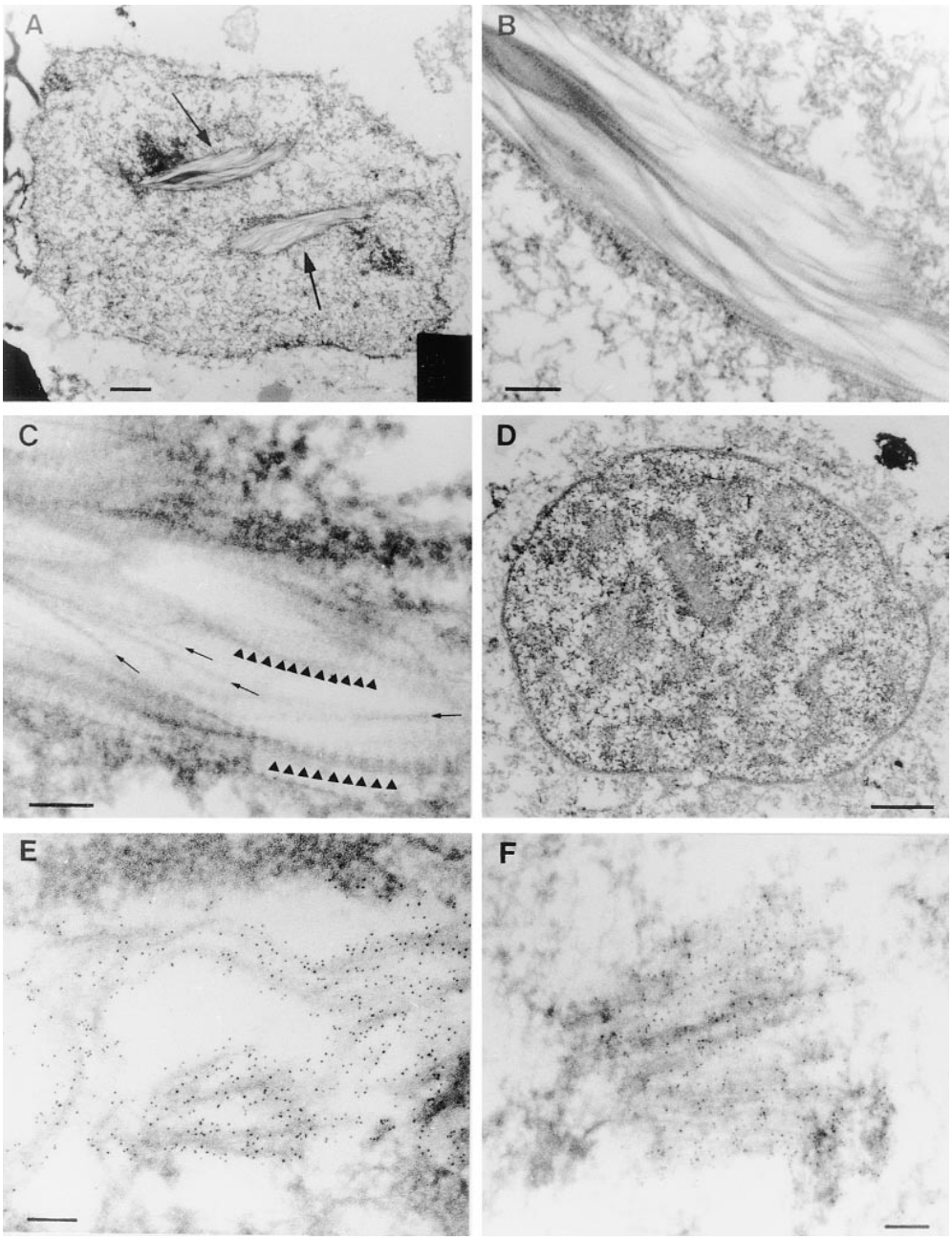

Figure 3. Conventional electron microscopy and immunoelectron microscopy reveal multistranded cross-striated fibers that contain SCP3. The pCMX-SCP3-FLAG plasmid (*A*, *B*, *C*, *E*, and *F*) or a pCMX-pL1-FLAG control plasmid (*D*) lacking insert were transfected into 3T3 cells. The cells were grown for 24 h and isolated by magnet selection. Nuclei were prepared and fixed for conventional electron microscopy or for immunoelectron microscopy. Part of the fiber structure observed in *A* is shown in *B* and *C* at higher magnifications. In *C*, arrows point at thin fibers, 5–10 nm in diameter, and rows of arrowheads indicate the cross-striated pattern. *E* and *F* show fiber bundles in ultrathin cryosections labeled with an anti-FLAG antibody and subsequently with a gold-conjugated secondary antibody. Bars: (*A*, *B*, and *D*) 1 μm; (*C*, *E*, and *F*) 0.1 μm.

minal (the nonhelical part) or the COOH-terminal (including the coiled coil motifs) region of SCP3 (see Fig. 1). The plasmids SCP3, SCP3-C, and SCP3-N (Fig. 1) were expressed in a reticulocyte lysate system, and the proteins were labeled by ^{35}S. We then examined if the labeled proteins could interact in vitro with the full-length SCP3 protein fused to the GST protein. We found that the full-length SCP3 protein interacted more strongly with the SCP3 protein fused to GST than to GST itself (Fig. 5). To identify the region of SCP3 that mediates this protein–protein interaction, the NH_2- and the COOH-terminally truncated versions of SCP3 were tested in a similar manner. We found that the COOH-terminal region interacted strongly with full-length SCP3, while the NH_2-terminal region did not (Fig. 5). This suggests that the COOH-terminal region of SCP3, which includes the coiled coil domains, forms homophilic protein–protein interactions in vitro. These in vitro results strongly support recent data showing that the coiled coil–forming regions of the hamster SCP3 protein (COR1) mediate homotypic interactions in vitro (Tarsounas et al., 1997).

In Vivo Synthesis of a Truncated Form of SCP3 That Includes the Coiled Coil Domain Is Not Sufficient for Fiber Formation

To better understand the process that mediates SCP3 fiber formation, we have expressed the truncated versions of SCP3 fused to a FLAG epitope tag also in vivo. Swiss-3T3 cells were microinjected with the SCP3-N or the SCP3-C constructs and stained with the anti-FLAG antibody to determine whether the truncated SCP3s could form fibers in vivo (Fig. 6 *A*). We did not see any fibers in cells microinjected with either of the two plasmids. We noted, however, that the anti-FLAG antibody gave rise to a strong nuclear

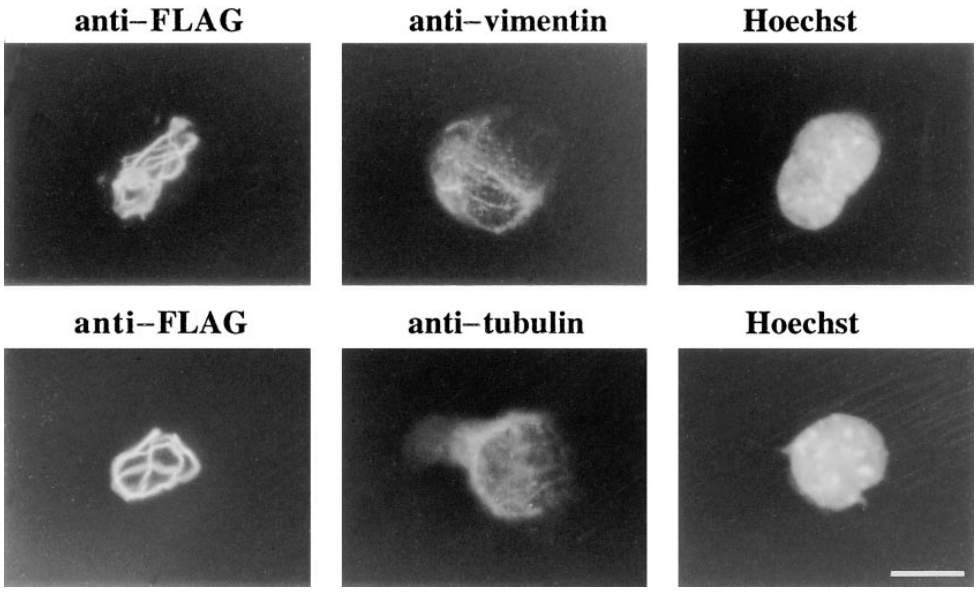

Figure 4. The SCP3 fiber is not part of an endogenous cellular network. The pCMX-SCP3-FLAG plasmid was microinjected into 3T3 cells. Cells were grown for 24 h, fixed, stained with the anti-FLAG antibody to show the SCP3 fibers, and stained with either the anti-vimentin antibody or the anti-tubulin antibody to display the corresponding cytoplasmic network. The cells were also stained with Hoechst 33258. The SCP3 protein fiber was not found to colocalize with either the vimentin or the tubulin networks in these cells. The rounded-up morphology of the cells suggests that overexpression of SCP3 affects their viability. Bar, 10 μm.

and cytoplasmic signal in the injected cells, suggesting that the truncated forms of SCP3 are expressed at a high level but are unable to form fibers. We conclude that the NH_2-terminal, nonhelical part of SCP3 and the COOH-terminal coiled coil region are both required for fiber formation.

Coexpression of the Coiled Coil Domain of SCP3 with the Full-Length SCP3 Protein Inhibits Fiber Formation In Vivo

Coexpression of the nonhelical head or tail domains of intermediate filament proteins with the full-length protein inhibits longitudinal or lateral assembly of intermediate filaments in a dominant-negative manner (Heins and Aebi, 1994). We have used a similar approach to test how the NH_2- and the COOH-terminal regions of SCP3 affect fiber formation in vivo. Plasmids expressing the NH_2- or the COOH-terminal parts of SCP3 (Fig. 1) were coinjected with full-length SCP3 plasmid. We found that coexpression of the coiled-coil domain of SCP3 (SCP3-C) with the full-length SCP3 completely inhibited fiber formation (Fig. 6 *B*). In contrast, coexpression of the NH_2-terminal nonhelical domain of SCP3 (SCP3-N) with the full-length SCP3 protein did not abolish fiber formation (Fig. 6 *B*). We noted, however, that the fibers formed in the latter cells were thinner, suggesting that coexpression of the NH_2-terminal region weakly inhibits the lateral assembly process. We estimate that the levels of expression of the SCP3-C and SCP3-N constructs were about the same, since the observed intensities produced by the anti-FLAG antibody were similar. We therefore do not believe that the difference between SCP3-N and SCP3-C in their abilities to prevent fiber formation is caused by differences in expression level.

This result suggests that the coiled coil domain that is expressed as part of the truncated SCP3-C protein interacts with the coiled coil domain of full-length SCP3 and prevents further polymerization. The less efficient inhibition of fiber formation by the NH_2-terminal nonhelical region is probably due to the inability of this protein region to establish sufficiently strong protein–protein interactions with the full-length SCP3 protein. These in vivo data are supported by the in vitro data presented in Fig. 5, which show that the coiled coil region mediates protein–protein interactions, while the NH_2-terminal nonhelical region does not promote such interactions.

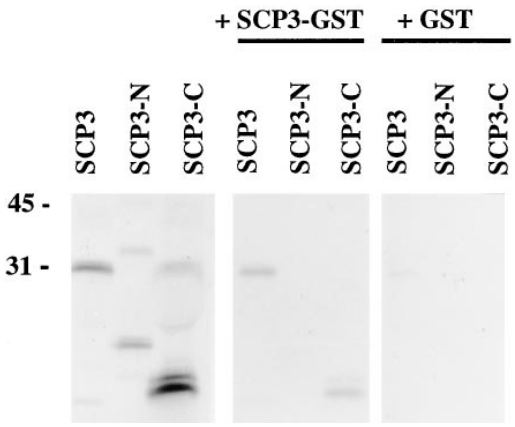

Figure 5. The coiled coil domain of SCP3 mediates protein–protein interactions in vitro. The pCMX-SCP3, pCMX-SCP3-N, and the pCMX-SCP3-C plasmids were translated in vitro, and the ^{35}S-labeled products were separated by SDS-PAGE (*left*). Full-length SCP3 protein synthesized in bacteria and bound to GST-agarose beads was incubated with the ^{35}S-labeled proteins and washed. Proteins bound to the SCP3-GST beads (*middle*) or GST beads only (*right*) were released by boiling the beads in SDS-reducing buffer, separated by SDS-PAGE, and detected by autoradiography. Molecular sizes are given in kD.

The Distribution of the SC Proteins SCP3 and SCP1 Do Not Overlap When Coexpressed in 3T3 Cells

SCP1 is a major constituent of the TF, and it connects the two homologous chromosomes in the SC. The COOH-terminal domain of SCP1 lies close to the LE, as has been shown by immuno-EM (Meuwissen et al., 1992; Dobson et al., 1994; Liu et al., 1996; Schmekel et al., 1996). This suggests that SCP1 could be anchored to the lateral elements by protein–protein interactions. To test if SCP1 in-

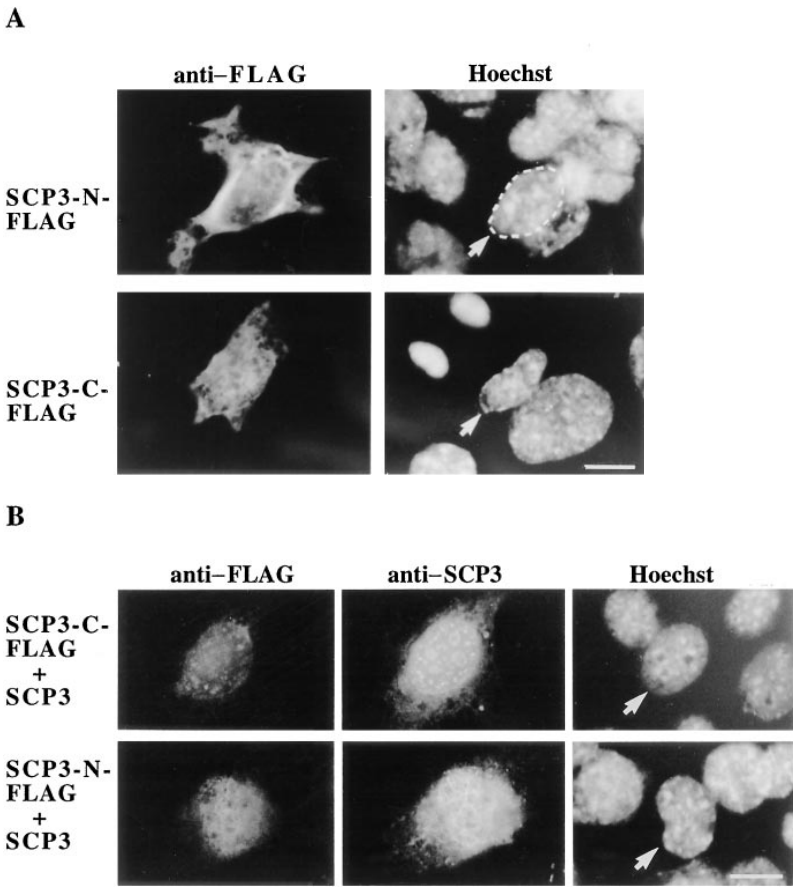

Figure 6. The NH_2-terminal nonhelical region and the COOH-terminal region containing the coiled coil domains are both required for fiber formation in vivo. (*A*) The pCMX-SCP3-N-FLAG and the pCMX-SCP3-C-FLAG were microinjected into cells, which were grown for 24 h, fixed, and stained with an anti-FLAG antibody. The cells were also stained with Hoechst 33258. The nuclei of the cells transfected with SCP3 are demarcated in the Hoechst picture. (*B*) The pCMX-SCP3-C-FLAG or the pCMX-SCP3-N-FLAG plasmids were microinjected into 3T3 cells together with pCMX-SCP3 plasmids lacking FLAG-epitope tag. The cells were grown for 24 h, fixed, and stained in parallel with the anti-FLAG antibody, the anti-SCP3 antibody, and Hoechst 33258. Cells transfected with SCP3 are indicated by an arrow in the Hoechst pictures. Bar, 10 μm.

teracts with SCP3, we coinjected Swiss-3T3 cells with plasmids expressing full-length SCP1 and SCP3 and analyzed these cells with antibodies specific for these proteins. We observed two types of structures: fibers labeled by the anti-FLAG antibody (detecting SCP3) and dots labeled by the anti-SCP1 antibody (Fig. 7). The dot structures detected by the anti-SCP1 antibody have been observed previously in somatic cells transfected only with the SCP1 plasmid (Yuan et al., 1996). We found no overlap between the structures formed by SCP1 and SCP3, suggesting that these proteins do not interact in vivo. Furthermore, no interactions between these two proteins have been observed in vitro using a GST-interaction assay (data not shown).

Discussion

SCP3 has been shown to be part of the lateral elements of the SC, a meiosis-specific proteinaceous structure conserved in most eukaryotic organisms (Dobson et al., 1994; Lammers et al., 1994). We have now analyzed the assembly of SCP3 proteins using both in vivo and in vitro methods. When SCP3 is expressed in vivo in somatic cells, which do not normally contain SC proteins, it self-assembles and forms conspicuous cross-striated fibers both in the cell nucleus and cytoplasm. In transfection studies, it was also shown that the COOH-terminal coiled coil domain of the SCP3 protein, as well as the NH_2-terminal nonhelical domain, is required for the appearance of the

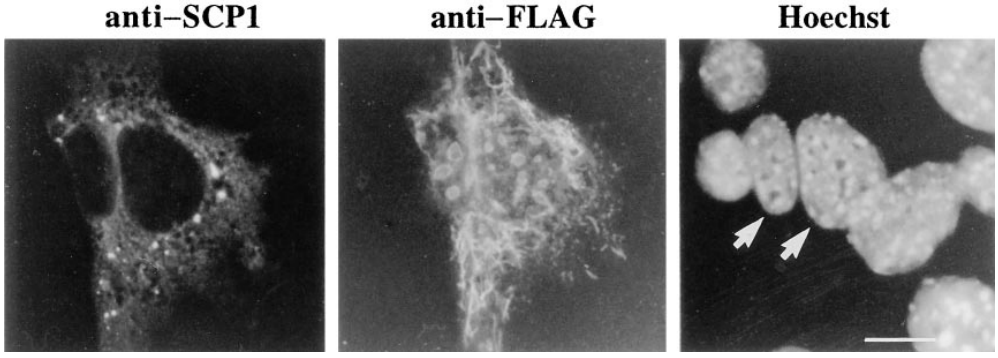

Figure 7. Expression patterns of the SC proteins SCP3 and SCP1 do not overlap when coexpressed in vivo. The pCMX-SCP3-FLAG and the pCMX-SCP1 (lacking the FLAG-epitope tag) plasmids were coinjected into 3T3 cells. The cells were grown for 24 h, fixed, and stained in parallel with the anti-FLAG antibody, the anti-SCP1 antibody, and Hoechst 33258. Nuclei of transfected cells are indicated by arrows in the Hoechst picture. Bar, 10 μm.

fibers. The importance of the COOH-terminal coiled coil domain for the assembly process was further demonstrated both in vitro and in vivo: the COOH-terminal domain was shown to promote protein–protein interactions in vitro, and in vivo it blocks the fiber formation in competition experiments with full-length SCP3 protein.

The SCP3 fibers are multistranded, cable-like structures with a basic fiber ~10 nm in diameter. Furthermore, these fibers exhibit transversal striations with a periodicity of about 20 nm. These features are strikingly similar to those of IFs, which are composed of 10-nm fibers and show cross-striations with a periodicity of 21 nm (for reviews see Stewart, 1993; Heins and Aebi, 1994). Furthermore, the fact that the SCP3 protein contains several putative coiled coil motifs in its COOH-terminal domain suggests that SCP3 is structurally related to members of the IF family (Lammers et al., 1994; Tarsounas et al., 1997). All members of this family have a common structure consisting of a central helical domain with a coiled coil structure, surrounded by nonhelical COOH- and NH_2-terminal domains. Finally, the requirements for SCP3 fiber formation in vitro and in vivo resemble those needed for the assembly of IFs: the coiled coil domain promotes protein–protein interactions in vitro, and this domain, as well as an additional domain of this protein, are essential for IF formation in vivo (Stewart, 1993; Heins and Aebi, 1994). We conclude that the SCP3 fiber and IFs share important features regarding the gross structure of the fibers, the nature of the protein subunits, and the molecular basis for the assembly process.

Since the protein subunits in the SCP3 fiber have a domain organization similar to the subunits in IFs, and the higher order structures in SCP3 fibers resemble those in IFs, it seems likely that the course of the assembly of the SCP3 fibers follows an IF-like pattern (Stewart, 1993; Heins and Aebi, 1994). It has been shown that the assembly of the 10 nm fiber occurs in a hierarchic manner. Protein dimers are formed by coiled coil interactions, and two dimers with opposite orientation interact laterally. Tandem arrays of such paired dimers constitute a 2- to 3-nm protofilament. Two protofilaments then form a 4.5-nm protofibril (an octamer), and four protofibrils form the 10-nm filament (Stewart, 1993; Heins and Aebi, 1994). The resulting 10-nm filament displays a transversal striation with a 21-nm periodicity, a product of the regular alternations of helical and nonhelical domains within the individual protein subunit. These striations are visible as the nonhelical domains of intermediate filament proteins and are more strongly stained by the heavy metals in the TEM method than the helical regions.

In transfected cells, the SCP3 fibers do not appear to be associated with the preexisting fibrous networks, which is suggested from several lines of evidence. First, the SCP3 fibers seem to have a unique organization characterized by exceptionally thick fibers. Second, the SCP3 fibers appear both in the nucleus and in the cytoplasm. Finally, endogenous fiber arrays formed by vimentin, actin, tubulin, and lamin do not overlap with the SCP3 fiber network.

To determine how closely related the SCP3 fibers formed in cultured cells are to the LEs of the synaptonemal complex in meiotic cells, we have compared the structure of the SCP3 fiber with published information. The ultrastructure of the axial/lateral element has been analyzed in different organisms and shown to be conserved during evolution. In some organisms, such as the insect *Locusta* (Moens, 1969) and the ascomycete *Neottiella* (Westergaard and von Wettstein, 1970), the structure of the LE is exceptionally distinct, revealing a cross-striated pattern within the axial/lateral elements with a periodicity of 10 and 19 nm, respectively. Furthermore, ultrastructural analysis of LEs in different eukaryotes has shown that they consist of long, well-aligned fibers, forming cable-like fibrillar structures (Westergaard and von Wettstein, 1970; del Mazo and Gil-Alberdi, 1986). The ultrastructural similarities between the SCP3 fibers and the axial/lateral element described in the literature are therefore striking, especially considering the multistranded organization and cross-striations. The importance of the SCP3 protein for the formation of the axial/lateral element is also supported by protein expression data showing that the accumulation and disappearance of Cor1 (the SCP3 homologue in hamster) in meiotic cells correlates exactly with the formation and the fragmentation of the axial/lateral elements (Moens and Spyropoulos, 1995). Based on these considerations, we propose that SCP3 fibers are key structural components of the LE.

Although the SCP3 fiber observed in the present study has many features in common with the LE, we anticipate differences. Indeed, we find that the thickness and length of the cable-like structures formed by SCP3 in transfected cells are different from the width and length of the LE observed in meiotic cells. One obvious explanation would be the lack of expression of other SC proteins in cultured somatic cells. For example, the yeast Red1 protein has been shown to be required for the formation of axial/lateral elements and to have a discontinuous localization at the axial cores of meiotic chromosomes (Smith and Roeder, 1997). It has been proposed that the Red1 protein is bound to a meiotic chromosome scaffold and serves as nucleation points for the formation of the axial/lateral element (Smith and Roeder, 1997). Another explanation could be that the SCP3 fibers in the heterologous expression system are not restrained by the type of interactions occurring between chromatin and the LE in meiotic cells. Both mitotic and meiotic chromosomes have a scaffold to which chromatin loops are anchored (Moens and Pearlman, 1988; Rufas et al., 1992). It has been suggested that the meiotic chromosome scaffold acts as a framework for the assembly of the LEs (Rufas et al., 1992).

It is interesting to note that SCP3 fibers are recorded in both nucleus and cytoplasm when SCP3 is overexpressed in somatic cells. This feature could be tentatively related to the appearance of prominent, stacked SC-like structures, designated polycomplexes, in meiotic cells of some organisms (Wandall, 1980; Goldstein, 1987). The polycomplexes are formed in the nucleus, where they are not associated with chromosomes, as well as the cytoplasm. The significance of polycomplexes is not known, but their presence suggests a surplus of SC material and perhaps a storage function.

One important question concerns the physiological role of a putative SCP3 structural framework in the LEs of the synaptonemal complex. One role for the SCP3 fiber could be to act as a scaffold to which other meiotic components attach. When synapsis takes place, the axial elements of homologous chromosomes become connected by transverse filaments, and a central element appears. It seems likely that the axial elements are crucial for the initiation of this process. In support of such a model, it has been

shown that yeast mutants lacking axial elements do not assemble an SC (Smith and Roeder, 1997). We have, however, not been able to show a direct interaction between SCP3 and SCP1, the main constituent of the transverse filaments, perhaps suggesting that an additional protein functions as a linker. Furthermore, a growing number of proteins involved in DNA recombination, DNA repair, and checkpoint control have been found to associate with the asynapsed meiotic chromosomes (Ashley et al., 1995; Haaf et al., 1995; Keegan et al., 1997; Scully et al., 1997), suggesting that they interact with the axial element, and maybe with SCP3 directly. Finally, it has also been shown that Cor1 (the SCP3 homologue in hamster) accumulates at the centromere in metaphase and anaphase cells at the first meiotic division, as well as at the centromeres in metaphase cells at the second meiotic division, but is not associated with the centromeres during anaphase of the second meiotic division, indicating that Cor1 is involved in the inhibition of sister chromatid separation at the first meiotic division (Moens and Spyropoulos, 1995). We conclude that the SCP3 fibers in the axial/lateral elements could constitute a molecular scaffold that serves as attachment sites for proteins regulating DNA binding, sister chromatid cohesion, synapsis, and recombination.

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