MOLECULAR AND CELLULAR BIOLOGY, Feb. 1994, p. 1137–1146 Vol. 14, No. 2
0270-7306/94/$04.00+0

The Gene Encoding a Major Component of the Lateral Elements of Synaptonemal Complexes of the Rat Is Related to X-Linked Lymphocyte-Regulated Genes

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Received 15 July 1993/Returned for modification 26 August 1993/Accepted 11 November 1993

The lateral elements of synaptonemal complexes (SCs) of the rat contain major components with relative electrophoretic mobilities (M_rs) of 30,000 and 33,000. After one-dimensional separation of SC proteins on polyacrylamide-sodium dodecyl sulfate gels, these components show up as two broad bands. These bands contain closely related proteins, as judged from their peptide maps and immunological reactivity. Using affinity-purified polyclonal anti-30,000- and anti-33,000-M_r component antibodies, we isolated a cDNA encoding at least one of the 30,000- or 33,000-M_r SC components. The protein predicted from the nucleotide sequence of the cDNA, called SCP3 (for synaptonenal complex protein 3), has a molecular mass of 29.7 kDa and a pI value of 9.4. It has a potential nucleotide binding site and contains stretches that are predicted to be capable of forming coiled-coil structures. In the male rat, the gene encoding SCP3 is transcribed exclusively in the testis. SCP3 has significant amino acid similarity to the pM1 protein, which is one of the predicted products of an X-linked lymphocyte-regulated gene family of the mouse: there are 63% amino acid sequence similarity and 35% amino acid identity between the SCP3 and pM1 proteins. However, SCP3 differs from pM1 in several respects, and whether the proteins fulfill related functions is still an open question.

Synaptonemal complexes (SCs) are nuclear structures that are formed between homologous chromosomes during prophase of the first meiotic division. They are assumed to contribute to the two major effects of meiosis, the reduction of the ploidy level and the generation of new combinations of genes. These effects are accomplished during the first of two meiotic divisions as homologous chromosomes condense, pair, recombine, and segregate. SCs consist of two proteinaceous axes, one along each homolog, that are connected by transverse filaments. On the transverse filaments, between the axial cores, there is a third longitudinal element, the central element; both axial cores together with the central element make up the tripartite structure of the SC (65). The axial cores are called the lateral elements (LEs) of SCs where they make part of the tripartite SC.

The assembly and disassembly of SCs correlate with the successive rearrangements of chromatin. Early in meiotic prophase (leptotene), the axial cores are formed along the chromosomes, presumably between the sister chromatids; during zygotene, they are connected by the transverse filaments, and a central element is formed between them. In pachytene, the homologous chromosomes are connected (synapsed) by the tripartite structure along their entire length. Subsequently, the SCs are disassembled (diplotene), the chromosomes condense (diakinesis), and chiasmata, which result from the physical exchange between non-sister chromatids of homologs (67), show up as physical connections between homologs. In metaphase I, bivalents (paired homologs) orient themselves in the spindle. The chiasmata are essential for this because they hold the bivalents together. There is circumstantial evidence that exchange between non-sister chromatids per se is not sufficient to generate a mature chiasma that can fulfill this function (46, 53). For the proper orientation of bivalents, sister chromatid cohesiveness, which persists until anaphase I, is also essential (24, 30, 41).

At the DNA level, the major events of meiotic prophase result in a high rate of homologous recombination and gene conversion. Meiotic and mitotic recombination differ in several respects. (i) The rate of meiotic reciprocal exchange (and of gene conversion) is several orders of magnitude higher than that of mitotic exchange. (ii) With few exceptions (62), there is positive interference between meiotic reciprocal exchanges (reviewed in references 50 and 67), whereas no evidence has been obtained for interference between mitotic reciprocal exchanges (32). (iii) In several organisms, hot spots for meiotic reciprocal recombination (and gene conversion) have been identified (6, 18, 22, 57), but most of these are not hot spots for mitotic recombination (reviewed in reference 50). (iv) Meiotic reciprocal recombination occurs preferentially between non-sister chromatids of homologous chromosomes (19, 64), whereas the sister chromatid is preferred for mitotic exchange (31). Thus, there is a meiosis-specific chromatin organization by which some sequences (hot spots) are preferentially exposed for homology searching and recombination, positive interference between reciprocal exchanges is effected, reciprocal exchanges between sister chromatids are avoided (or exchanges between non-sister chromatids are preferentially enhanced), sister chromatid cohesiveness is brought about, and reciprocal exchanges between non-sister chromatids result in the formation of functional chiasmata.

It seems likely that SCs, particularly the LEs, play a role in one or more of these aspects of meiosis-specific chromatin

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organization, because SCs are assembled from meiosis-specific components (25, 44, 48, 60, 63) and because the meiotic prophase chromatin appears to be organized on the LEs and axial cores. However, as yet, no direct evidence has been obtained for this.

In order to get more information about SCs, we developed a procedure to isolate these structures from rat spermatocytes (26, 28), elicited monoclonal and polyclonal anti-SC antibodies (27, 29, 48), and, using these antibodies, identified major components of SCs with M_rs of 30,000, 33,000, 125,000, and 190,000 (27, 29). In this paper, we concentrate on the 30,000- and 33,000-M_r SC components, which occur specifically in meiotic prophase cells, on the SCs (15, 25); they are localized on the LEs and axial cores, irrespective of whether these are synapsed or unsynapsed (29, 47). We describe the isolation of a cDNA encoding at least one of the 30,000- or 33,000-M_r proteins by means of affinity-purified anti-30,000 and anti-33,000-M_r protein antibodies. The protein predicted from the nucleotide sequence, called SCP3 (for synaptonemal complex protein 3), is basic (pI 9.4) and has a molecular mass of 29.7 kDa; it has a potential nucleotide binding site. SCP3 has sequence homology to an X-linked lymphocyte-regulated mouse protein called pM1 (20).

MATERIALS AND METHODS

Antibodies. The monoclonal antibodies (MAbs) used in this study were elicited and isolated as described by Offenberg et al. (48); they are described in detail by Heyting et al. (27) and Offenberg et al. (48). A polyclonal anti-30,000- and anti-33,000-M_r antiserum (serum 175) was elicited by immunization of a rabbit with rat SCs according to the same schedule used for the immunization of mice (48). Serum samples (20 ml each) were collected at 2-week intervals, starting 1 week after the third injection of antigen. Although this serum was elicited against whole rat SCs, it recognizes specifically the 30,000- and 33,000-M_r SC components (48). For the experiments in this study, we affinity purified the anti-30,000- and anti-33,000-M_r antibodies from this serum as follows: from preparative immunoblots of SC proteins, we excised strips containing the 30,000- and 33,000-M_r SC components; these strips were incubated in blocking buffer (10 mM Tris-HCl [pH 7.4], 250 mM NaCl, 0.05% [wt/vol] Tween 20, containing 5% [wt/vol] nonfat dry milk and 5% [vol/vol] normal goat serum) for 1 h at 37°C, washed three times for 5 min each in phosphate-buffered saline, and then incubated for 1 h at 37°C in the polyclonal anti-30,000- and anti-33,000-M_r antiserum, diluted 1:100 in blocking buffer supplemented with 0.02% (wt/vol) NaN_3, *Escherichia coli* lysate (1 mg of *E. coli* protein per ml), and 1 mM phenylmethylsulfonyl fluoride. Bound antibodies were eluted from the strips by two successive incubations for 1 h each at room temperature in 10 ml of 0.1 M glycine (pH 2.7). The glycine in the eluted fractions was neutralized with an equimolar amount of Na_2HPO_4, and normal goat serum was added to a final concentration of 10% (vol/vol). Only the second eluted fraction was used. A polyclonal antiserum against the fusion protein of clone 2A4.7 (serum 448) was raised by immunization of a rabbit with inclusion bodies from bacteria harboring clone 2A4.7, containing the fusion protein, exactly as described by Meuwissen et al. (44).

Isolation of cDNAs encoding SCP3. For the isolation of cDNAs encoding 30,000- and 33,000-M_r SC proteins, we subcloned the inserts of about 10^8 phage of an expression cDNA library of rat testis (44) into the pBluescript vector according to the instructions of the manufacturer (Stratagene, San Diego, Calif.). Colonies of *E. coli* XL1 Blue cells carrying these pBluescript vectors with inserts were transferred to nitrocellulose filters. Two replica filters were made, and expression of the cloned cDNA was induced as described by Sambrook et al. (55). Subsequently, the colonies were lysed on the filters by the following incubations at room temperature: 20 min in 5 mg of lysozyme per ml in 50 mM Tris-HCl (pH 7.6)–150 mM NaCl–0.1% Tween 20 (TBST), 1 min in 0.5 M NaOH–1.5 M NaCl, and 5 min in 1 M Tris-HCl (pH 7.4)–1.5 M NaCl. Filters were then washed in TBST, and bacterial debris was wiped off. We screened the lysed colonies by means of the affinity-purified anti-30,000- and anti-33,000-M_r SC protein antibodies as primary antibodies and a goat anti-mouse alkaline phosphatase conjugate as secondary antibody by using the Western blot (immunoblot) incubation procedure described before (16, 25). We performed a secondary screening of the cDNA library by plaque hybridization with the *Hin*cII fragment of clone 2A4 (see Fig. 3) as a probe. Labelling of the probe by random primed labelling and screening were performed according to procedures described by Sambrook et al. (55).

Sequence analysis. We generated unidirectional sets of deletions from both ends of the cDNA insert of clone 2A4 by partial digestion with exonuclease III and S1 nuclease with an Erase-a-base Kit (Promega, Madison, Wis.). In addition, fragments 2, 5, 6, 7, and 9 (see Fig. 3) were subcloned in pBluescript SK^- for nucleotide sequence determination. We determined the nucleotide sequences by the dideoxy-chain termination method of Sanger et al. (56) with the double-stranded DNA Cycle Sequencing System (GIBCO BRL, Gaithersburg, Md.), [γ-^{32}P]ATP (>5,000 Ci/mmol [Amersham]), and oligonucleotide primers complementary to the polylinker sequences of pBluescript. The sequence was assembled with the University of Wisconsin Genetics Computer Group sequence analysis package. Sequence similarity searches of the GenBank, EMBL, Swissprot and PIR data banks were carried out with the FASTA (49) and BLAST (3) programs. Prediction of secondary structure was performed with a program based on Chou-Fasman algorithms (10); coiled-coil regions were identified with an algorithm developed by Lupas et al. (40). Alignment of sequences was performed with the BESTFIT and GAP programs, which are included in the Genetics Computer Group sequence analysis package.

RNA isolation and Northern blot hybridization. RNA was isolated from various tissues of 37-day-old rats by the guanidinium-LiCl method of Cathala et al. (7). RNA was electrophoresed on formaldehyde-agarose gels and transferred to Hybond-N^+ membranes (Amersham) by standard procedures (55). After transfer, the membranes were washed in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), dried, and fixed by baking at 80°C for 2 h. We used five DNA probes (see Fig. 3) for Northern blot (RNA) hybridization. Labelling of the probes with [α-^{32}P]dATP was performed by random primed labelling according to the method of Sambrook et al. (55). The Northern blot membranes were prehybridized in 50% formamide–6× SSC–0.5% SDS–0.01% Na-pyrophosphate–5× Denhardt's solution–200 μg of denatured herring sperm DNA per ml for at least 6 h at 42°C. Hybridization was performed in the same mixture with 1.4 ng of probe (1.1×10^6 cpm/μg) per ml for 17 h at 42°C. The blots were washed subsequently for 30 min in, successively, 2× SSC–1% SDS at 42°C (twice) and 1×SSC–0.1% SDS and 0.1× SSC–0.1% SDS at 65°C.

Characterization of the protein encoded by the cDNA insert

of 2A4. The open reading frame of the insert of cDNA clone 2A4 had a shift of −2 with respect to the reading frame of the *Lac*I fragment to which it was fused. To characterize the protein encoded by the cDNA insert of clone 2A4, we put the insert in frame with respect to the *Lac*I fragment of the pBluescript vector as follows: we digested the vector containing the insert with *Xba*I and *Sma*I, both of which cut the polylinker sequence of the pBluescript vector between the *Lac*I fragment and the *Eco*RI site into which the cDNA was inserted; subsequently the *Xba*I site was filled by means of Klenow polymerase, and the blunt ends of the *Sma*I site and the filled *Xba*I site were ligated; this resulted in a deletion of 16 nucleotides and did not generate a stop codon. *E. coli* XL1-Blue cells were transformed with the resulting construct. The clone containing this construct was designated 2A4.7. A 10-ml culture of clone 2A4.7 was grown overnight at 37°C in Luria-Bertani medium containing 100 μg of ampicillin per ml. A total of 250 ml of prewarmed Luria-Bertani medium containing 100 μg of ampicillin per ml was inoculated with 2.5 ml of the overnight culture. After incubation at 37°C for 2 h, synthesis of the fusion protein was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. After incubation for another 3 h at 37°C, the cells were harvested. The inclusion bodies were purified as described by Sambrook et al. (55) and washed according to the method of Harlow and Lane (23). A total of 2 mg of inclusion body protein was then loaded per 1-cm slot of a 7 to 18% linear gradient polyacrylamide–SDS gel; subsequently, electrophoresis, Western blotting, and immunodetection of translation products of the cDNA insert by means of the polyclonal anti-30,000- and anti-33,000-M_r antibodies were performed as described by Heyting et al. (25).

Peptide mapping. Peptide mapping of the 30,000- and 33,000-M_r SC proteins and of the full-length fusion protein was performed as described by Cleveland et al. (11). Subsequent blotting and immunodetection were performed as described by Heyting et al. (25).

Other procedures. SCs were isolated as described by Heyting et al. (28) and Heyting and Dietrich (26); SDS-polyacrylamide gel electrophoresis of proteins was performed according to the method of Laemmli (35) as described by Heyting et al. (28). Before electrophoresis, the protein samples were boiled in sample buffer (2 M urea, 5% SDS, 125 mM Tris-HCl [pH 6.8], 10% glycerol, 0.5 mM EDTA, 0.1 M β-mercaptoethanol) for 10 min; immunoblotting was carried out according to the method of Dunn (16) as described by Heyting and Dietrich (26).

Nucleotide sequence accession number. The EMBL accession number of the SCP3 cDNA is X75785.

RESULTS

The 30,000- and 33,000-M_r SC components are closely related. One-dimensional SDS-polyacrylamide gel electropherograms of proteins from purified rat SCs show two broad prominent bands with M_rs of 30,000 and 33,000 (28, 29 [Fig. 1A]). These bands contain protein components of SCs, because several MAbs that recognize these bands bind specifically to SCs (25, 27, 29, 48). The 30,000- and 33,000-M_r SC components are immunologically related, because 18 independently isolated anti-SC MAbs recognize both components (Fig. 1A [27]), whereas, as yet, no anti-SC MAbs that can discriminate between these components have been identified. The similarity of the 30,000 and 33,000-M_r SC components is also evident from their peptide maps.

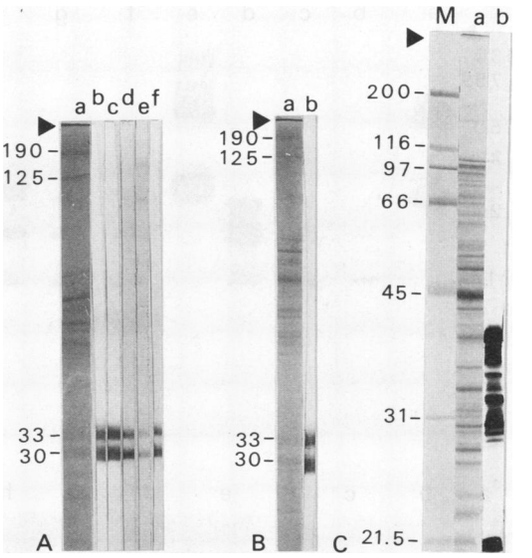

FIG. 1. Reaction of antibodies used in this study with SC proteins. Proteins of 2 × 10^7 SCs were loaded per cm slot of an SDS–10% polyacrylamide gel and stained with Coomassie blue or transferred to nitrocellulose. (A) Reaction of antibodies elicited against the 30,000- and 33,000-M_r components of SCs with SC proteins: Lanes: a, Coomassie blue-stained strip; b to f, immunoblot strips of the same gel incubated in MAb IX3H3 (b), MAb IX4D4 (c), MAb IX8G9 (d), MAb IX7B12 (e), and affinity-purified polyclonal anti-30,000- and anti-33,000-M_r antibodies from serum 175 (f). (B) Reaction of antibodies elicited against the fusion protein of cDNA clone 2A4.7 with SC proteins. Lanes: a, Coomassie blue-stained gel; b, immunoblot strip of the same gel probed with the antiserum elicited against the fusion protein of clone 2A4.7 (serum 448). In panels A and B, the positions of the 190,000-, 125,000-, 33,000-, and 30,000-M_r SC protein bands are indicated. (C) Immunoblot analysis of the translation products of cDNA clone 2A4.7; 2 mg of inclusion bodies of clone 2A4.7 was applied per cm slot of a 7 to 18% linear gradient polyacrylamide-SDS gel, electrophoresed, and stained with Coomassie blue (a) or blotted onto nitrocellulose and probed with affinity-purified anti-30,000- and anti-33,000-M_r antibodies from serum 175 (b). Lane M contains the following molecular mass markers: myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 31 kDa; and trypsin inhibitor, 21.5 kDa. The arrowheads indicate the tops of the gels.

Figure 2A shows the peptides that are produced from the 30,000- and 33,000-M_r protein bands by digestion with *Staphylococcus aureus* V8 protease (SV8) (11) and that are recognized by the antibodies directed against the 30,000- and 33,000-M_r SC components. Lanes a and e of Fig. 2A show the undigested input controls of the 30,000- and 33,000-M_r components. These lanes contain extra bands with M_rs of ~65,000 that are not detected on immunoblots of standard polyacrylamide-SDS gels (Fig. 1). We suppose that these extra bands contain dimers of 30,000- or 33,000-M_r molecules, because they originate from excised 30,000- or 33,000-M_r protein bands (see Materials and Methods) and because they are recognized by the anti-30,000- and anti-33,000-M_r antibodies. Apparently these dimers arise during the lengthy stacking gel phase of the peptide mapping procedure (11); it is possible that they are not easily formed from proteolytic cleavage products (Fig. 2A, lanes b to d and f to h).

Isolation of cDNAs encoding a 30,000- or 33,000-M_r component of SCs. For the isolation of cDNAs encoding 30,000-

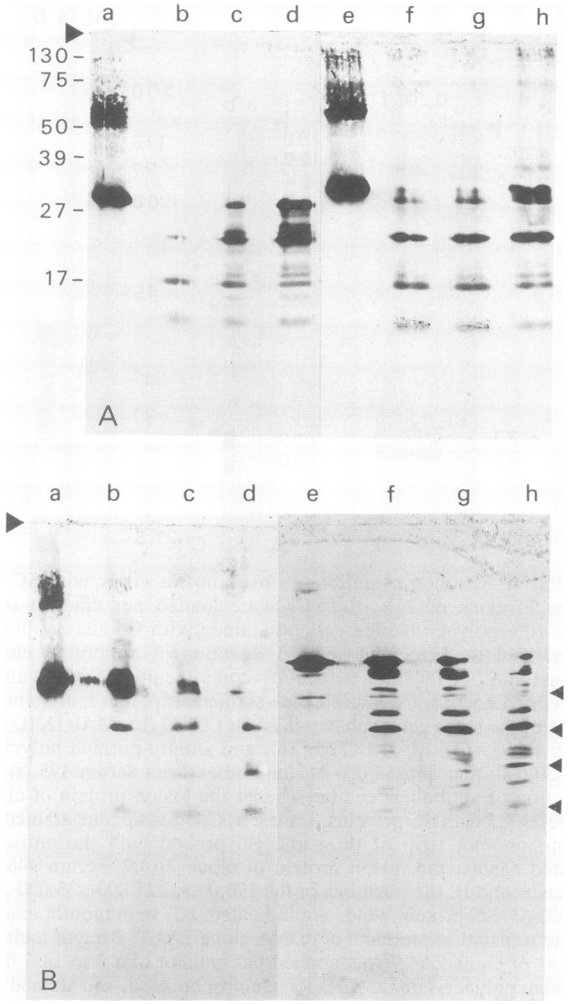

FIG. 2. Characterization of the 30,000- and 33,000-M_r SC components and of the translation products of cDNA clone 2A4.7 by peptide mapping. (A) Proteins of 10^8 SCs were loaded per cm slot of an SDS–10% polyacrylamide gel and electrophoresed. The 30,000- and 33,000-M_r protein bands were excised, digested with SV8, and electrophoresed according to the method of Cleveland (11) (see Materials and Methods); subsequently, the resulting peptides were transferred to nitrocellulose, and the immunoblot was probed with the polyclonal anti-30,000- and anti-33,000-M_r antibodies from serum 175. Lanes: a, undigested 30,000-M_r SC protein; b to d, peptides obtained by digestion of the 30,000-M_r protein with 1,050, 350, and 125 ng of SV8, respectively; e, undigested 33,000-M_r SC protein; f to h, peptides obtained by digestion of the 33,000-M_r SC protein with 1,050, 350, and 125 ng of SV8, respectively. The positions of the prestained marker proteins are indicated by their apparent molecular masses (in kilodaltons). (B) SV8 digests of the 33,000-M_r SC protein (lanes b to d) and the full-length fusion protein product of cDNA clone 2A4.7 (lanes f to h); the 33,000-M_r SC protein and the full-length fusion protein product of cDNA clone 2A4.7 were excised from one-dimensional SDS-polyacrylamide gels and digested with 125 (lanes b and f), 350 (lanes c and g), or 1,050 (lanes d and h) ng of SV8; lane a contains the undigested 33,000-M_r SC component, and lane e contains the undigested full-length fusion protein of cDNA clone 2A4.7. The undigested proteins and the digestion products were electrophoresed according to the method of Cleveland (11), transferred to nitrocellulose, and analyzed with the affinity-purified polyclonal anti-30,000- and anti-33,000-M_r antibodies from serum 175. Arrowheads to the left indicate the positions of the tops of the gels; arrowheads to the right indicate the bands that the digests of the 33,000-M_r SC component and the fusion protein of clone 2A4.7 have in common.

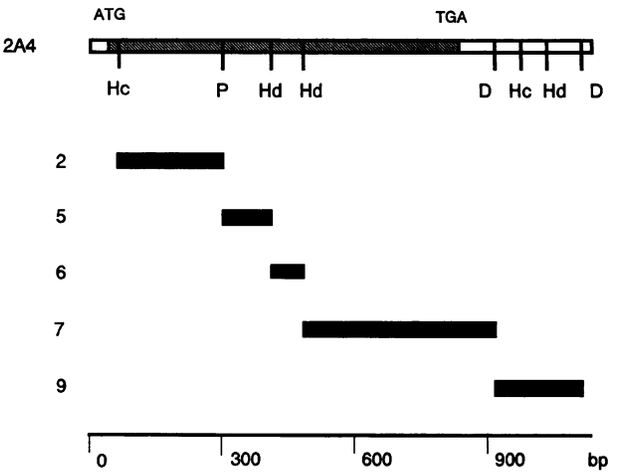

FIG. 3. Restriction map of cDNA clone 2A4. The shaded segment indicates the coding region. The black bars indicate the restriction fragments that were subcloned and used for Northern blot analysis and nucleotide sequence determination. Hc, *Hin*cII; P, *Pst*I; Hd, *Hin*dIII; D, *Dra*I.

and 33,000-M_r SC proteins, we screened an expression cDNA library of the rat testis with affinity-purified polyclonal anti-30,000- and anti-33,000-M_r antibodies. One colony, harboring clone 2A4, reacted relatively strongly with the antibodies. Figure 3 shows the restriction map of the cDNA insert of this clone. Using the *Hin*cII fragment of clone 2A4 as a probe, we screened about 2×10^5 recombinant phage of the cDNA library by plaque hybridization. This yielded 30 positive clones, all with cDNA inserts that were colinear with that of clone 2A4, as judged from their restriction maps. No clones extending further in the 5′ direction than clone 2A4 were found by this secondary screening.

The nucleotide sequence of clone 2A4 was determined as described in Materials and Methods (Fig. 4). Clone 2A4 contains a single open reading frame that encodes a 29.7-kDa protein consisting of 257 amino acids (counted from the first ATG codon) but which is out of frame with respect to the fragment of the *Lac*I gene of the pBluescript vector to which the cDNA insert was fused. The major translation product of clone 2A4 that is recognized by anti-30,000- and anti-33,000-M_r antibodies is a 19,000-M_r peptide (not shown). The synthesis of this peptide probably initiates at the ATG codon at positions 337 to 339, because this codon is preceded by an almost perfect Shine-Dalgarno consensus sequence (GGAGG, at positions 319 to 323). Only small amounts of longer translation products are detected in bacteria harboring clone 2A4 (not shown). However, if the insert of clone 2A4 is put in frame with the fragment of the *Lac*I gene (to produce clone 2A4.7 [see Materials and Methods]), the major translation product of the insert that is recognized by anti-30,000- and anti-33,000-M_r antibodies is a 37,000-M_r peptide (Fig. 1C). This is in good agreement with the expected size of the full-length translation product (4.2 kDa of the *Lac*I fragment plus 31.9 kDa from the cDNA insert). We think that the insert of clone 2A4 encodes at least one of the 30,000- and 33,000-M_r SC components for the following reasons. (i) The predicted molecular mass (29.7 kDa) is in good agreement with the M_rs (30,000 and 33,000) of the SC proteins that are recognized by the antibodies that were used for screening. (ii) The fusion protein encoded by the insert of

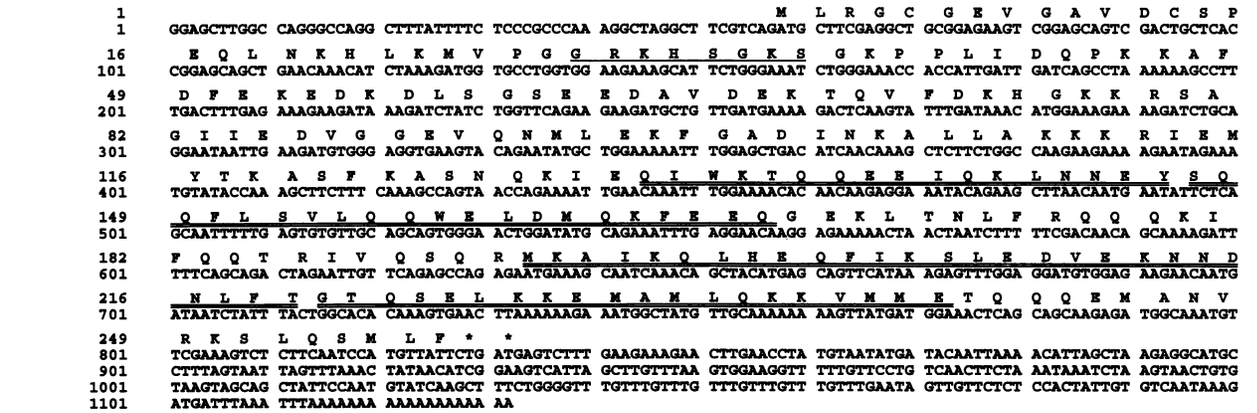

FIG. 4. Nucleotide sequence and predicted amino acid sequence of SCP3. The predicted translation product is shown above the nucleotide sequence. The purine nucleotide binding motif A is underlined. Double underlining indicates amino acid sequences that have a probability of >0.5 of forming coiled-coils, as calculated according to Lupas et al. (40) with a window of 14 amino acid residues. The asterisks indicate the first two stop codons.

clone 2A4 is recognized by at least four independently isolated anti-30,000- and anti-33,000-M_r MAbs that were not used for screening (not shown). (ii) The peptide maps of the supposed full-length fusion protein and of the 33,000-M_r SC component have four bands in common that are recognized by the polyclonal anti-30,000- and anti-33,000-M_r antibodies (Fig. 2B). The SV8 digests of the fusion protein also contain several peptides that do not occur in the digests of the 33,000-M_r SC component. These peptides probably consist of part of the *Lac*I fragment linked to part of the translation product of the cDNA. (iii) The antiserum elicited against the translation products of clone 2A4.7 (serum 448) recognizes specifically the 30,000- and 33,000-M_r SC components, like the antibodies that were used for screening (Fig. 1B), whereas the preimmune serum of serum 448 does not produce any signal (not shown). (iv) In frozen sections of the testis, serum 448 reacts specifically with the nuclei of meiotic prophase cells (spermatocytes); the immunofluorescence staining pattern obtained with serum 448 (Fig. 5A) is identical to the staining pattern obtained with antibodies against the 30,000- and 33,000-M_r SC components (see Fig. 1a in reference 25 and Fig. 3e in reference 48); the preimmune serum is negative in this test (not shown). (v) Within spermatocytes, serum 448 recognizes specifically the lateral elements of SCs (Fig. 6 and 7), like the antibodies against the 30,000- and 33,000-M_r SC components (compare Fig. 6 with Fig. 4a, c, and e in reference 29 and Fig. 5d to f in reference 27). (vi) The gene encoding the predicted 29.7-kDa protein is transcribed specifically in the testis; probes derived from SCP3 cDNA (Fig. 3) detect two transcripts of nearly equal size (about 1.1 kb) in testis RNA, whereas no transcripts are detected in RNA from other tissues and organs (Fig. 8). Thus, we conclude that we have isolated a cDNA encoding at least one of the 30,000- and 33,000-M_r SC components. For this, we propose the name SCP3.

Sequence of the SCP3 cDNA. The complete nucleotide sequence of cDNA clone 2A4, together with the amino acid sequence of the encoded protein, is shown in Fig. 4. The first ATG codon is found at nucleotide position 58. There is a polyadenylation signal (AATAAA) at nucleotide positions 1094 to 1099. The predicted protein SCP3 is rich in glutamine (11.6%) and lysine (13.6%). The glutamine residues are concentrated in the C-terminal half of the protein and often occur in clusters of two or three residues. For other proteins, it has been suggested that such glutamine clusters serve as spacers within the protein molecule (9).

Prediction of the secondary structure according to the method of Chou and Fasman (10) did not reveal large-scale structural motifs. However, analysis of the amino acid sequence according to the method of Lupas et al. (40) revealed four stretches in the C-terminal half of the protein that are likely to form coiled-coil structures (Fig. 4). These are associations of two or three amphipathic α-helices; the amphipathic character of the α-helices is the result of a heptad repeat pattern, with hydrophobic amino acid residues in the first and fourth positions of the heptad repeat. Because seven consecutive residues will form two turns of an α-helix, the hydrophobic residues at positions 1 and 4 will form a hydrophobic ridge on one side of the molecule (43). Such amphipathic α-helical stretches are capable of forming coiled-coil structures through (homotypic or heterotypic) hydrophobic interactions with other amphipathic α-helices (13). It is possible that such interactions were responsible for the appearance of presumed dimers of the 30,000- and 33,000-M_r SC components and, to a lesser extent, for the appearance of the full-length translation product of clone 2A4.7 on Cleveland gels (Fig. 2B).

The amino acid sequence of SCP3 has some other interesting features: there is a potential nuclear targeting signal (consensus, K-R/K-X-R/K, where X is any amino acid [8]) at amino acid positions 109 to 112, and there are potential cyclic AMP [cAMP]-cyclic GMP [cGMP]-dependent protein kinase target sites (consensus, K/R-K/R-X-S/T [17]) at positions 29 to 32 and 77 to 80. In addition, there are four potential target sites for protein kinase C(S/TX-R/K [33]) and five potential target sites for casein kinase II (S/T-X-X-D/E [51]) (not indicated). Furthermore, SCP3 shares the so-called "motif A" (G/AXXXXGKS/T [66]) with several nucleotide binding proteins (amino acid positions 28 to 35). This motif is found in a loop that is involved in the binding of phosphate groups of purine nucleotide molecules (5, 61). The secondary structure, as predicted by Chou-Fasman analysis (10), also displays other features that are supposed to be essential for nucleotide binding, namely a β-strand and an α-helix flanking the loop containing the A motif and a β-strand with a negatively charged amino acid at its C terminus (designated B motif by Walker et al. [66]). This negatively charged amino acid is probably involved in the binding of Mg^{2+}, which is

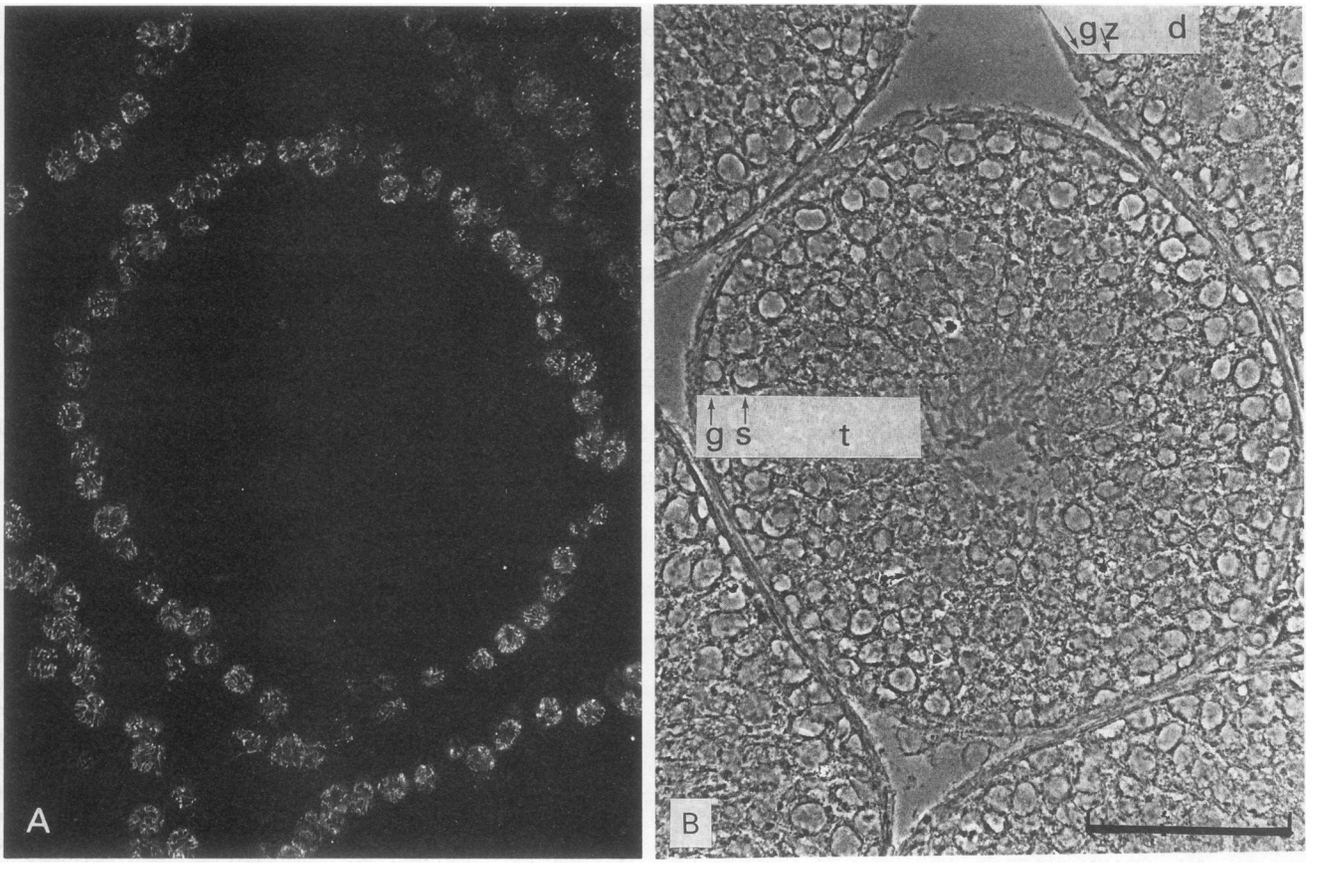

FIG. 5. Frozen section of rat testis after immunofluorescence staining with the polyclonal antiserum elicited against the fusion protein of clone 2A4.7 (serum 448). (A) Immunofluorescence; (B) phase-contrast micrograph of the same section. The centre of the micrograph shows a cross section of a testicular tubule. In panel B, the cell layers containing spermatogonia (g) and spermatocytes (s) are indicated. The seminiferous epithelium in the central tubule is in developmental stages VII to IX (37), because it contains a single layer of relatively large spermatocytes; these are in pachytene (25, 37); the cells inside the layer of spermatocytes are spermatids (t). The upper right corner shows part of a stage XIII tubule (37), with two successive layers of spermatocytes, which are in diplotene (the large cells to the right [d]) and zygotene (z) (25, 37); the layer of spermatogonia (g) is also indicated in this tubule. Bar, 50 μm.

thought to interact with β- and γ-phosphates (5, 61). In SCP3, the A motif is preceded by a segment predicted to form a β-strand (residues 21 to 25) and is followed by a region predicted to form an α-helical structure (residues 40 to 43). There are four additional segments capable of forming a β-strand, and two of these have a negatively charged amino acid at their C terminus (D at position 73 and E at position 96). Amino acid residues 73 to 76 match the consensus for a second sequence element that is characteristic for GTP-binding proteins, namely DXXG at a distance of 40 to 80 residues from motif A (14). However, SCP3 lacks the third consensus sequence that has been identified for GTP-binding proteins, namely NKXD at 40 to 80 residues from the DXXG sequence (14). Thus, although it seems likely that SCP3 can bind purine nucleotide molecules, the nucleotide specificity is uncertain.

Comparison of amino acid sequences with libraries of known sequences (see Materials and Methods) revealed a large-scale similarity to an X-linked lymphocyte-regulated mouse protein called pM1 (59). The amino acid sequence of pM1 can be aligned along its entire length with that of SCP3 in such a way that there is 35.3% identity between the aligned sequences (Fig. 9). Despite this overall similarity, there are also significant differences between pM1 and SCP3. SCP3 carries a stretch of 43 additional residues N terminal to the region of alignment with pM1. The motif A consensus sequence for nucleoside triphosphate-binding proteins and one of the cAMP-cGMP-dependent protein kinase target sites lie within this stretch; motif B and the other cAMP-cGMP-dependent protein kinase target site of SCP3 have not been conserved in pM1. Furthermore, pM1 has a much lower calculated pI (5.0) than SCP3 (9.4). The nuclear localization signal has been conserved in pM1, which is consistent with the nuclear localization of this protein (21), and pM1 has one amphipathic α-helical stretch that is likely to form a coiled-coil structure (pM1 residues 120 to 142). There is also significant nucleotide sequence similarity between the cDNAs encoding SCP3 and pM1 (54.6% identity, as determined after alignment of the nucleotide sequences by means of the GAP program). No other sequence similarities were detected at either the amino acid or nucleotide sequence level.

DISCUSSION

During meiotic prophase, chromosomes are organized in loops on proteinaceous axial cores that later become the LEs of SCs. These cores are distinct from mitotic or meiotic

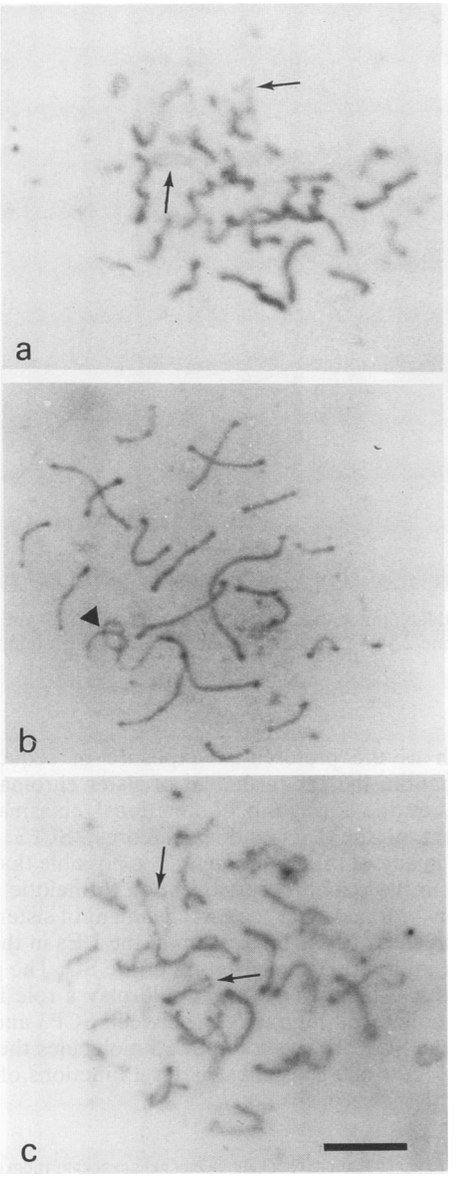

FIG. 6. Light micrographs (bright-field illumination) of agar filtrates of lysed spermatocytes after indirect immunoperoxidase staining with the polyclonal antiserum elicited against the fusion protein of cDNA clone 2A4.7 (serum 448). (a) Zygotene; (b) pachytene; (c) diplotene. Note the staining of the unpaired axes of the zygotene and diplotene bivalents (arrows); the arrowhead indicates the XY bivalent. Bar, 10 μm.

metaphase chromosome scaffolds and from interphase nuclear matrices because they consist largely of components that are specific for meiotic prophase (27, 29, 47). It is still largely unknown which functions are fulfilled by the LEs or their components, although several possible functions have been suggested. LEs could play an inhibiting or enhancing role in recombination (34, 39, 54); they might be involved in chiasma maintenance (53) or in sister chromatid cohesiveness (41). In order to study the nature and possible functions of LEs, we have elicited MAbs that recognize specifically components of the LEs of SCs. In this paper, we show that two major components of the LEs, with M_rs of 30,000 and 33,000, are closely related, and we describe the isolation and characterization of a cDNA encoding at least one of these components, SCP3.

The 30,000- and 33,000-M_r SC components are closely related. The LEs of SCs contain 30,000- and 33,000-M_r components that, upon one-dimensional gel electrophoresis, are resolved in two broad bands with M_rs of 30,000 and 33,000. We think that these components are closely related because 18 of 18 independently isolated anti-30,000- and anti-33,000-M_r MAbs recognize both the 30,000- and 33,000-M_r protein bands and because the SV8 digestion patterns of the 30,000- and 33,000-M_r proteins are very similar to each other. What causes the difference with respect to M_r still has to be sorted out. It is possible that the M_r variants are translation products of different transcripts of the same gene, because various probes derived from SCP3 cDNA (Fig. 3) hybridize with two transcripts of nearly equal size on Northern blots of testis RNA (Fig. 8). Hybridization of the same probes with rat genomic blots indicate that these transcripts are encoded by a single gene (not shown). However, the relationship between the 30,000- and 33,000-M_r variants and the relationship between the two transcripts are still under investigation, and other possible explanations for the difference with respect to M_r, such as proteolytic breakdown, have not yet been excluded.

Sequence and predicted secondary structure of SCP3. The predicted amino acid sequence of SCP3 has several interesting features. First, there are two domains in the C-terminal half of the protein (residues 130 to 175 and 205 to 245 [Fig. 4]) that each contain two stretches that are likely to form coiled-coil structures. It is possible that these domains facilitate the formation of dimers of 30,000- and/or 33,000-M_r molecules. It is also possible, however, that the coiled-coil domains serve to anchor the 30,000- and 33,000-M_r SC components to coiled-coil proteins of the nuclear matrix or the SC (12, 44, 45, 63, 70). In that case, SCP3 might have similar functions, as has been suggested for another nuclear protein with a relatively short coiled-coil domain, namely the yeast Rep1 protein (69), which is involved in plasmid segregation. Another interesting feature of SCP3 is the potential purine nucleotide binding site; it seems likely that SCP3 can bind ATP. Numerous proteins involved in DNA repair and recombination bind ATP or at least have the consensus sequence for purine nucleotide binding, G/AXXXXGKS/T (66). Among the proven ATP-binding proteins are the *E. coli* RecA protein (42) and type II DNA topoisomerases (38, 68). For these proteins, it has been proposed that hydrolysis of bound ATP causes conformational changes that alter the DNA binding properties of the protein molecules (52, 61). The consensus for purine nucleotide binding has also been found in a number of yeast proteins that are involved in meiotic recombination and chromosome synapsis—for instance, the RecA-like Rad51 and Dmc1 proteins (4, 58) and the Rad50 protein (1). However, whether SCP3 binds ATP and whether it is capable of hydrolyzing it remain to be proven.

SCP3 has two cAMP-cGMP-dependent protein kinase target sites; one of these overlaps with the consensus sequence for nucleotide binding. It is possible that these potential phosphorylation sites are important for the regulation of the assembly and disassembly of the LEs of SCs. For nuclear lamins, it has been shown that inhibition of phosphorylation by cAMP-cGMP-dependent protein kinases is essential for the disassembly of the nuclear lamina at mitosis (36). cAMP-cGMP-dependent protein kinase target sites also occur in two other SC proteins, namely SCP1 and SCP2 (44, 48a).

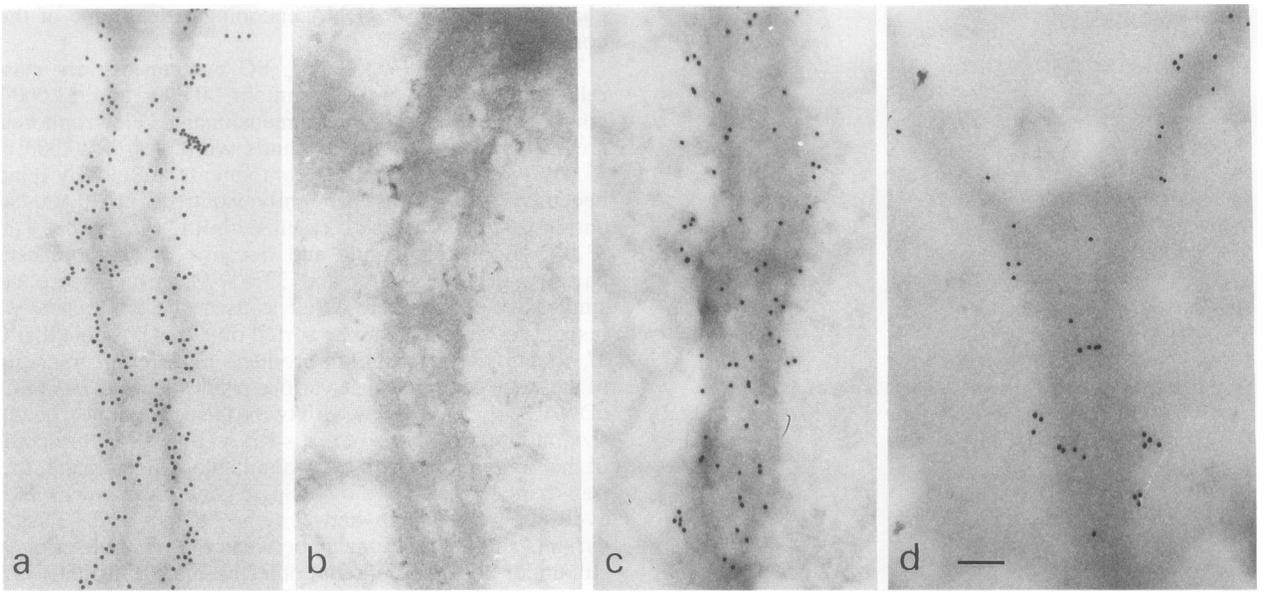

FIG. 7. Electron micrographs of surface-spread spermatocytes after immunogold labeling with antiserum against the 30,000- and 33,000-M_r SC components (serum 175 [a]), antiserum against the fusion protein of cDNA clone 2A4.7 (serum 448 [c and d]), or the preimmune serum of serum 448 (b). (a to c) Pachytene; (d) diplotene. Bar, 100 nm.

To summarize the main features of SCP3, it is a basic protein with a potential nucleotide binding site, it has two domains predicted to be capable of forming coiled-coil structures, and it has target sites for cAMP-cGMP-dependent protein kinases. Can these features be related to possible functions of SCP3? As was argued in the Introduction, the LEs and axial cores of SCs are probably involved in the structural organization of meiotic prophase chromatin and in transient, meiosis-specific modulations of chromatin function such as the preferential exposure of sequences for recombination, the establishment of sister chromatid cohesiveness, or the formation of functional chiasmata. As a component of the LEs and axial cores, SCP3 could be involved in any of these functions. It is possible that SCP3 is involved in the structural organization of meiotic prophase chromatin—for instance, the establishment of sister chromatid cohesiveness or the integration of the LEs in the nuclear matrix or in the tripartite structure of the SC. The predicted amphipathic α-helical stretches could play a role in this by allowing coiled-coil interactions between SCP3 and components of the SC or between the SCP3 molecules themselves. With respect to the possible structural functions of SCP3, it

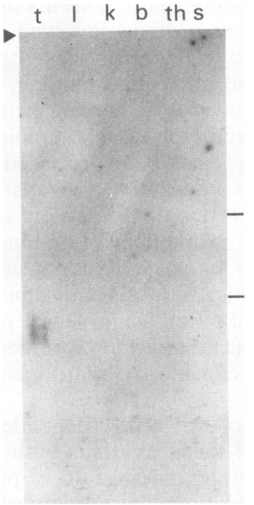

FIG. 8. Transcription of the gene encoding SCP3, analyzed by Northern blot hybridization. Thirty micrograms of total RNA from testis (t), liver (l), kidney (k), brain (b), thymus (th), and spleen (s) was loaded per 0.75-cm-wide slot. For this experiment, fragment 2 (see Fig. 3) was used as a probe; hybridization with probes 5, 6, 7, and 9 (see Fig. 3) yields essentially the same result (not shown). The bars indicate the positions of the rat 28S (4,700 nucleotides) and 18S (1,900 nucleotides) rRNA; the arrowhead indicates the top of the gel.

```
SCP3   1 MLRGCGEVGAVDCSPEQLNKHLKMVPGGRKHSGKSGKPPLIDQPKKAFDF 50
                                                  ...:|:
 PM1   1 ..........................................MENWDL  6

SCP3  51 EKED.KDLSGSEEDAVDEKTQVFDKHGKKRSAGIIEDVGGEVQNMLEKFG 99
         ..:: .| .:.| |.::|...| ..:. ...::::|. :.|||:|:||:
 PM1   7 SSDEMQDGNAPELDVIEEHNPVTRDDENANPEEVVGDTRSPVQNILGKFE 56

SCP3 100 ADINKALLAKKKRIEMYTKASFKASNQKIEQIWKTQQEEIQKLNNEYSQQ 149
         :|||| |  |:||:| |.|.|||.|| |:||:|||..:| .|:||.:::|
 PM1  57 GDINKRLHIKRKRMETYIKDSFKDSNVKLEQLWKTNKQERKKINNKFCEQ 106

SCP3 150 FLSVLQQWELDMQKFEEQGEKLTNLFRQQQKIFQQTRIVQSQRMKAIKQL 199
         ::..:|.:::|:|||:|:.|| .| :..:|. :. .:. ||| :.|||::
 PM1 107 YITTFQKFDMDVQKFNEEQEKSVNNYQKEQQALKLSKCSQSQTLEAIKDM 156

SCP3 200 HEQFIKSLEDVEKNNDNLFTGTQSELKKEMAMLQKKVMMETQQQEMANVR 249
         ||.::.:| ::|.|| |:: :.::||:|||.::.|.:|..|      . .
 PM1 157 HENYMEGLMNLETNNYNMLFDVDGELRKEMSVFKKDLMKHT.....LKYS 201

SCP3 250 KSLQSMLF 257
         .|:.|
 PM1 202 SSFPSSD. 208
```

FIG. 9. Alignment of the predicted amino acid sequences of SCP3 and the mouse pM1 protein. Vertical lines indicate identical amino acids; colons and dots indicate similar amino acids, as defined by the BESTFIT sequence alignment program. Amino acids are numbered, starting with 1 at the first Met in the coding sequence.

should be kept in mind that SCP3 is no longer immunologically detectable after meiotic prophase (15, 25), apart from some clumps of antigenic material in part of the early spermatids (25). Thus, although it is possible that SCP3 establishes sister chromatid cohesiveness, other factors must be responsible for the maintenance of cohesiveness during dictyotene (for results with females, see reference 15), diakinesis, and metaphase I.

Similarity of SCP3 to the pM1 protein. SCP3 has considerable amino acid sequence homology to the pM1 protein, which is one of the predicted products of an X-linked lymphocyte-regulated (*Xlr*) gene family of the mouse (59). Mouse genomic DNA contains 50 to 75 *Xlr* sequences (59) that probably have arisen by amplification during recent evolution of the mouse; the closely related species *Coelomys pahari* and *Rattus norvegicus* have only one or two *Xlr*-related sequences (20). Most of the *Xlr* sequences of the mouse do not encode functional transcripts, and only two potentially functional transcripts have been identified. One, encoding the pM1 protein, is produced during the most mature stages of differentiation of T- and B-cell lines (20, 59); the other one, which has been described preliminarily (20), occurs specifically in the testis, but is shorter (0.9 kb) than the transcript encoding SCP3 (1.1 kb). The rat homolog of this transcript (if there is one) is not detected on Northern blots with any of the probes derived from SCP3 cDNA that we have tested (Fig. 8). pM1 is a nuclear protein (21), like SCP3, and part of the predicted amphipathic α-helical stretch of SCP3 has been conserved in the pM1 protein. However, there are also significant differences between the proteins; the pM1 protein can be extracted from the nucleus by chelating agents like EDTA (21), whereas SCP3 remains stably integrated in the LEs and axial cores in the presence of EDTA (28, 29). Furthermore, the pM1 protein lacks the potential nucleotide binding site and the potential cAMP-cGMP-dependent protein kinase target sites of SCP3, and it has a much lower pI value. Therefore, it seems doubtful that the proteins fulfill related functions. One function that could be considered for both proteins is the inhibition of recombination. The pM1 protein is expressed in the most mature stages of differentiation of B cells, where allelic exclusion has been established (2); SCP3 is localized in the LEs and axial cores of SCs and could have a role in the prevention of recombination between sister chromatids or in the inhibition of the initiation of recombination between non-sister chromatids in later stages of meiotic prophase. However, given the considerable differences between the pM1 protein and SCP3, it is quite possible that these proteins, although they are obviously evolutionarily related, are involved in completely different functions.

ACKNOWLEDGMENTS

We thank W. van Raamsdonk (University of Amsterdam) for much practical advice, M. A. W. Peters (animal facilities, Agricultural University) and W. van Raamsdonk for performing immunizations, and S. Maassen for his contribution to the characterization of SCP3 cDNAs during the course of his undergraduate study.

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