2572–2579 *Nucleic Acids Research, 1998, Vol. 26, No. 11*

SCP2: a major protein component of the axial elements of synaptonemal complexes of the rat

Hildo H. Offenberg, Johanna A. C. Schalk, Ralph L. J. Meuwissen, Mirjam van Aalderen, Henri A. Kester, Axel J. J. Dietrich[1] and Christa Heyting*

Department of Genetics, Agricultural University, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands and [1]Institute of Human Genetics, University of Amsterdam, Meibergdreef 15, NL-1105 AZ Amsterdam, The Netherlands

Received February 26, 1998; Revised and Accepted April 21, 1998 DDBJ/EMBL/GenBank accession no. Y08981

ABSTRACT

In the axial elements of synaptonemal complexes (SCs) of the rat, major protein components have been identified, with relative electrophoretic mobilities (M_rs) of 30 000–33 000 and 190 000. Using monoclonal anti-SC antibodies, we isolated cDNA fragments which encode the 190 000 M_r component of rat SCs. The translation product predicted from the nucleotide sequence of the cDNA, called SCP2 (for synaptonemal complex protein 2), is a basic protein (pI = 8.0) with a molecular mass of 173 kDa. At the C-terminus, a stretch of ~50 amino acid residues is predicted to be capable of forming coiled-coil structures. SCP2 contains two clusters of S/T-P motifs, which are common in DNA-binding proteins. These clusters flank the central, most basic part of the protein (pI = 9.5). Three of the S/T-P motifs are potential target sites for $p34^{cdc2}$ protein kinase. In addition, SCP2 has eight potential cAMP/cGMP-dependent protein kinase target sites. The gene encoding SCP2 is transcribed specifically in the testis, in meiotic prophase cells. At the amino acid sequence and secondary structural level, SCP2 shows some similarity to the Red1 protein, which is involved in meiotic recombination and the assembly of axial elements of SCs in yeast. We speculate that SCP2 is a DNA-binding protein involved in the structural organization of meiotic prophase chromosomes.

INTRODUCTION

During meiotic prophase, chromosomes are arranged in an orderly manner along proteinaceous axes called axial elements (1). These elements differ from mitotic metaphase chromatid scaffolds because: (i) the two chromatids of a meiotic prophase chromosome share a single axial element, whereas the chromatids of a metaphase chromosome have each their own scaffold; (ii) the major protein components of axial elements are meiosis-specific and thus not found in chromatid scaffolds (2); and (iii) axial elements are longer than metaphase chromatid scaffolds, and morphologically better defined. Axial elements and chromatid scaffolds can both be visualized by silver impregnation techniques, at least in certain types of microscopical preparations of spread cells (3–7).

As meiotic prophase proceeds, the axial elements are incorporated in zipperlike structures, called synaptonemal complexes (SCs), which keep homologous chromosomes in close apposition along their length (1). Meiotic recombination probably initiates just before or simultaneously with axial element assembly, and the assembly of full length axial elements appears to depend upon the initiation of meiotic recombination by double-strand DNA scission, at least in yeast (8). At the end of meiotic prophase, the SCs are disassembled at about the same time when recombination intermediates are resolved (9), and within each homolog the two separate scaffolds of the sister chromatids gradually become discernable (5). At the sites of reciprocal recombination between non-sister chromatids of homologous chromosomes, the scaffolds of the recombined chromatids 'cross over' (5,7) to form chiasmata, which contribute to the physical connection between homologs. In most eukaryotes, such connections are essential for the proper orientation of bivalents at metaphase I.

The relation between axial elements and sister chromatid scaffolds remains to be elucidated. In mitotic chromosomes, the sister chromatids do not normally share one axis, although single axes of a similar length to axial elements have been demonstrated by silver impregnation along mitotic chromosomes that were forced to condense from G2 in the presence of a topoisomerase II inhibitor (10). These single G2 axes probably represent the still unseparated sister chromatid scaffolds. Although axial elements of SCs are morphologically more similar to these single G2 chromosome axes than to metaphase chromatid scaffolds, there are also important differences, because axial element assembly is part of normal meiotic chromosome behaviour and does not require cell cycle drugs and topoisomerase II inhibitors, and because the major protein components of axial elements are specific for meiotic prophase (2,11–13). In the rat, these meiosis-specific components have relative electrophoretic mobilities (M_rs) of 30 000–33 000 and 190 000 (2,11–13), of which the 30 000–33 000 M_r components are most probably products of a single gene, *Scp3* (14). In yeast, at least two candidate components of axial elements have been identified, namely Hop1 and Red1 (15–17), which are also meiosis-specific. It is possible that the axial element of a meiotic prophase chromosome is formed after premeiotic S-phase by association of meiosis-specific proteins with the still undivided chromosome scaffold (5). Alternatively, an entirely meiosis-specific axial element

*To whom correspondence should be addressed. Tel: +31 317 482150; Fax: +31 317 483146; Email: christa.heyting@molcelgen.el.wau.nl

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

is assembled at the beginning of meiotic prophase, which is replaced by the two sister chromatid scaffolds when the axial element is disassembled. To distinguish between these possibilities, it is necessary to characterize the axial element components, and analyse their interaction with chromatin, in particular with the special DNA-regions called SARs (scaffold attachment regions, 18), by which chromatin is attached to chromatid scaffolds.

Why meiotic prophase chromosomes should be organized on single, at least partially meiosis-specific axial elements is another unresolved question, although several suggestions have been made: it is possible that the axial elements fix the chromosomes in an elongated state, and enhance the exposure of relevant pairing sites in an ordered, longitudinal array. This could facilitate the alignment of homologous chromosomes (19). Other possible functions of axial elements include the inhibition of sister chromatid exchanges and/or the enhancement of recombination between non-sister chromatids of homologous chromosomes (9,15,20–22), the conversion of the products of interchromosomal reciprocal recombination into stable chiasmata that can ensure the proper orientation of bivalents at metaphase I (16), and/or the generation of sister chromatid cohesiveness (23,24).

In order to learn more about the nature and function(s) of meiotic axial elements/lateral elements (LEs), we study the composition of these structures. In this paper we describe the isolation and characterization of the cDNA encoding the 190 000 M_r component of rat SCs. The protein predicted from the nucleotide sequence of the cDNA, called SCP2, is basic (pI = 8.0) and has features of a protein which is capable of binding to the minor groove of AT-rich DNA. It shares these features with proteins that bind to SARs (25), including topoisomerase II (18,26), which is a major chromosome scaffold component (27). We speculate that SCP2 is involved in the organization of chromatin during meiotic prophase, possibly by temporarily binding to SARs.

MATERIALS AND METHODS

The DDBJ/EMBL/GenBank accession number of the SCP2 cDNA sequence is Y08981.

Antibodies

The Mabs recognizing the 190 000 M_r SC protein in rat were elicited by immunization of mice with rat SCs as described by Offenberg *et al.* (13). Of these antibodies, Mab IX9D5 has been described in detail by Heyting *et al.* (2) and Offenberg *et al.* (13). A polyclonal antiserum (serum 493) against amino acid residues 293–828 of the predicted translation product of the rat cDNA was prepared as follows: a 1600 bp *Pst*I fragment of cDNA clone 5 (which encodes a major part of SCP2 of the rat, see below) was cloned in the pQE31 expression vector (Qiagen, Chatsworth, CA, USA). Expression and isolation of the translation product were performed by means of the Qia expressionist system (Qiagen) according to the instructions of the manufacturer. Antibodies were elicited in a rabbit by eight injections of 60 µg fusion protein in 750 µl PBS, mixed with 750 µl Freund's complete adjuvant (Sigma, St Louis, MO, USA) (first injection), or 750 µl Freund's incomplete adjuvant (Sigma) (all following injections). The rabbit was injected subcutaneously at 2 week intervals. One week after the fourth and the sixth injection, 20 ml bleedings were collected from the ear-veins. After the eighth injection a final bleeding of 80 ml was collected.

Isolation of cDNAs encoding SCP2

For the isolation of cDNAs encoding the 190 000 SC M_r protein of the rat, we screened an expression cDNA library of the rat testis (28) in λzap® (Stratagene, San Diego, USA) with a pool of six independently isolated Mabs, each of which recognizes the 190 000 M_r SC protein, following described procedures (28). Among 3×10^5 phage clones, 10 positive clones were found and purified. The purified clones had overlapping restriction enzyme fragment maps and inserts ranging in length from 1.1 to 3.9 kb. The 5′ *Eco*RI fragment of the longest clone, 3C1, was used for a secondary screening, and this yielded clone 5 with an insert size of 4.4 kb. In search of rat cDNA clones extending further in the 5′ direction than clone 5, we performed a 5′ RACE experiment (29) exactly as described by Van Heemst *et al.* (30), using oligonucleotides complementary to the most 5′ sequences of clone 5 as primers and total rat testis RNA as a template. This yielded a DNA fragment which extended 302 nt further in the 5′ direction than clone 5. We then performed new RACE experiments, using primer sets complementary to this DNA fragment, a higher concentration of total testis RNA, and higher temperatures during cDNA synthesis. PCR performed on the cDNA fragments obtained at 50 and 52°C yielded two major products in each reaction. The longest product of each reaction was cloned in pGEM-T (Promega, Madison, WI, USA) and sequenced. Both (independently obtained) RACE products had identical sequences which extended 32 bp further in the 5′ direction, and contained a stop codon in-frame with the first ATG codon. We therefore concluded that we had isolated and sequenced the full-length SCP2 cDNA.

Sequence analysis

The insert of cDNA clone 3C1 was subcloned into the pBluescript vector SK(–) according to the instructions of the manufacturer (Stratagene). From both ends of the insert of clone 3C1 we generated unidirectional sets of deletions by partial digestion with exonuclease III and S1 nuclease using the erase-a-base kit of Promega. In addition, we subcloned several restriction enzyme fragments of the independently isolated cDNA clone 5 in pBluescript. We determined the nucleotide sequences by the dideoxy chain termination method of Sanger *et al.* (31), using [α-^{35}S]dATP (650 Ci/mmol; Amersham Corp., Buckinghamshire, UK), *Taq* polymerase (Gibco BRL Life Technologies, Paisley, UK or Promega) and oligonucleotide primers complementary to the polylinker sequences of pBluescript. The products of the RACE experiments (see above) were cloned into the pGEM-T vector according to the instructions of the supplier and sequencing reactions were performed in both directions from vector-specific primers, by means of the Dye Deoxy Terminator Cycle sequencing kit from Perkin-Elmer (Norwalk, CT, USA) and the nucleotide sequence was analysed on a 373A stretch 48 cm WAR DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA). The complete cDNA sequence encoding SCP2 was assembled by means of the Wisconsin GCG sequence analysis package (University of Wisconsin, WI, USA).

Immunocytochemical staining

Immunofluorescence staining of frozen sections of the rat testis was carried out as described by Heyting *et al.* (12) and Heyting and Dietrich (32). Ultrastructural localization of the antigen was performed by immunogold labeling of surface spread rat

spermatocytes essentially as described by Moens *et al.* (33) and Heyting and Dietrich (32).

RNA isolation and northern blot hybridization

RNA was isolated from various tissues of 37-day-old rats by the GuTC/LiCl method of Cathala *et al.* (34); poly(A)$^+$ RNA was purified by affinity chromatography on oligo(dT)-cellulose (35). RNA (15 µg/0.5 cm slot) was electrophoresed in the presence of ethidium bromide on formaldehyde/agarose gels, and transferred to Hybond-N$^+$ membranes (Amersham Corp.) by standard procedures (36). After transfer, we verified on the basis of ethidium bromide fluorescence, that all lanes on the northern blot membranes contained a similar amount of RNA. The membranes were washed in 3× SSC, dried and fixed with UV light (312 nm; 200 J/m^2) for 2 min. As probes for northern blot hybridization we used RNA transcripts of a 3′ deletion clone, which had been linearized with *Hin*dIII. Transcription was performed from the T7 promotor in the presence of [α-^{32}P]rATP (3000 Ci/mmol). The northern blot membranes were prehybridized in 50% formamide, 5× SSC, 0.1% sodium pyrophosphate, 1% SDS, 0.2% PVP, 0.2% Ficoll, 5 mM EDTA and 150 µg/ml sheared herring sperm DNA for 6 h at 60°C. Hybridization was performed in the same mixture with 0.07 µg/ml probe (36 × 10^6 c.p.m./µg) for 17 h at 60°C. Subsequently the blots were washed for 30 min at 65°C in, successively, 2× SSC 0.1% SDS, 1× SSC 0.1% SDS, 0.1× SSC 0.1% SDS and 0.1× SSC 0.1% SDS.

In situ hybridization

In situ hybridization was performed on 10 µm thick frozen sections of rat testes, as described by Meuwissen *et al.* (28). As a probe we used RNA that was obtained by transcription from the T7-promoter of a linearized 3′ deletion clone of clone 3C1; probe synthesis was performed in the presence of [α-^{35}S]rUTP (3000 Ci/mM, Amersham Corp.), as described by Meuwissen *et al.* (28). After hybridization and washes, the slides were dipped in Ilford K5 nuclear track emulsion, exposed for 3 weeks at 4°C, developed in Kodak developer D19, and analyzed by dark field microscopy.

Other procedures

SCs were isolated as described by Heyting *et al.* (37) and Heyting and Dietrich (32); SDS–PAGE of proteins was performed according to Laemmli (38), as described by Heyting *et al.* (37); immunoblotting was carried out according to Dunn (39), as described by Heyting and Dietrich (32).

RESULTS

Isolation of cDNAs encoding the 190 000 M_r SC component (SCP2) of the rat

For the isolation of cDNAs encoding the 190 000 M_r component of rat SCs, we screened a rat testis cDNA expression library, using a pool of six independently isolated Mabs, which had been elicited against isolated rat SCs. On western blots carrying SC proteins, each of these Mabs recognizes specifically the 190 000 M_r SC component, and a series of smaller fragments. We interpret the smaller fragments as breakdown products of the 190 000 M_r protein, because different Mabs recognize the same pattern of peptide bands [compare Mab IX1H9 (Fig. 1, lane 6) and IX3E4 (Fig. 1, lane 7)].

Figure 1. Reaction of antibodies used in this study with SC proteins. 1.5 × 10^7 SCs were loaded per cm slot of an SDS-10% polyacrylamide gel and stained with Coomassie blue or transferred to nitrocellulose. The arrows indicate from top to bottom the position of the 190 000, the 125 000, the 30 000 and the 33 000 M_r SC proteins. Lane 1, 0.4 cm wide strip of the gel stained with Coomassie blue; lanes 2–10, immunoblot strips of the same gel incubated in Mab IX8B11 (lane 2), Mab IX2G11 (lane 3), Mab IX9D5 (lane 4), Mab IX8F1 (lane 5), Mab IX1H9 (lane 6), Mab IX3E4 (lane 7), serum 493 (lane 8), pre-immune serum 493 (lane 9), control hybridoma supernatant (lane 10).

Among 3 × 10^5 recombinant phage clones, this pool of Mabs recognized 10 clones, containing cDNA inserts of 1100–3900 nt with overlapping restriction enzyme maps. In order to isolate a full-length clone, we screened the cDNA library with the 5′ *Eco*RI fragment of the clone with the longest insert, clone 3C1. This yielded clone 5, with an insert size of 4437 bp, which extended 500 bp further in the 5′ direction than clone 3C1. By successive RACE experiments, performed on total testis RNA as a template, we identified and sequenced 334 additional nucleotides at the 5′ end.

The nucleotide sequence of clone 3C1 and parts of the sequence of clone 5 were determined, and no discrepancies were found. The nucleotide sequence of the complete cDNA, as assembled from the sequences of clone 3C1, clone 5, and the products of the RACE experiments, contains a single open reading frame of 4515 nt, which encodes a 173 kDa protein consisting of 1505 amino acids (Fig. 2). Nucleotide 154–156 is assigned as the translation start codon because it is the first in-frame ATG codon; 135–133 nt upstream of this ATG codon (position 19–21 in Fig. 2) there is an in-frame stop codon.

We think that the nucleotide sequence in Figure 2 represents the full-length cDNA sequence encoding the 190 000 M_r SC protein of the rat, for the following reasons: (i) the recombinant gene product is recognized by four of the six independently isolated Mabs that were used for screening (not shown). (ii) The predicted pI (8.0) is in good agreement with the pI (8) of the 190 000 M_r component as observed in two-dimensional separations of SC proteins (40). (iii) A polyclonal antiserum, elicited against the translation product of part of the cDNA, serum 493, reacts specifically with the 190 000 M_r SC component on a western blot,

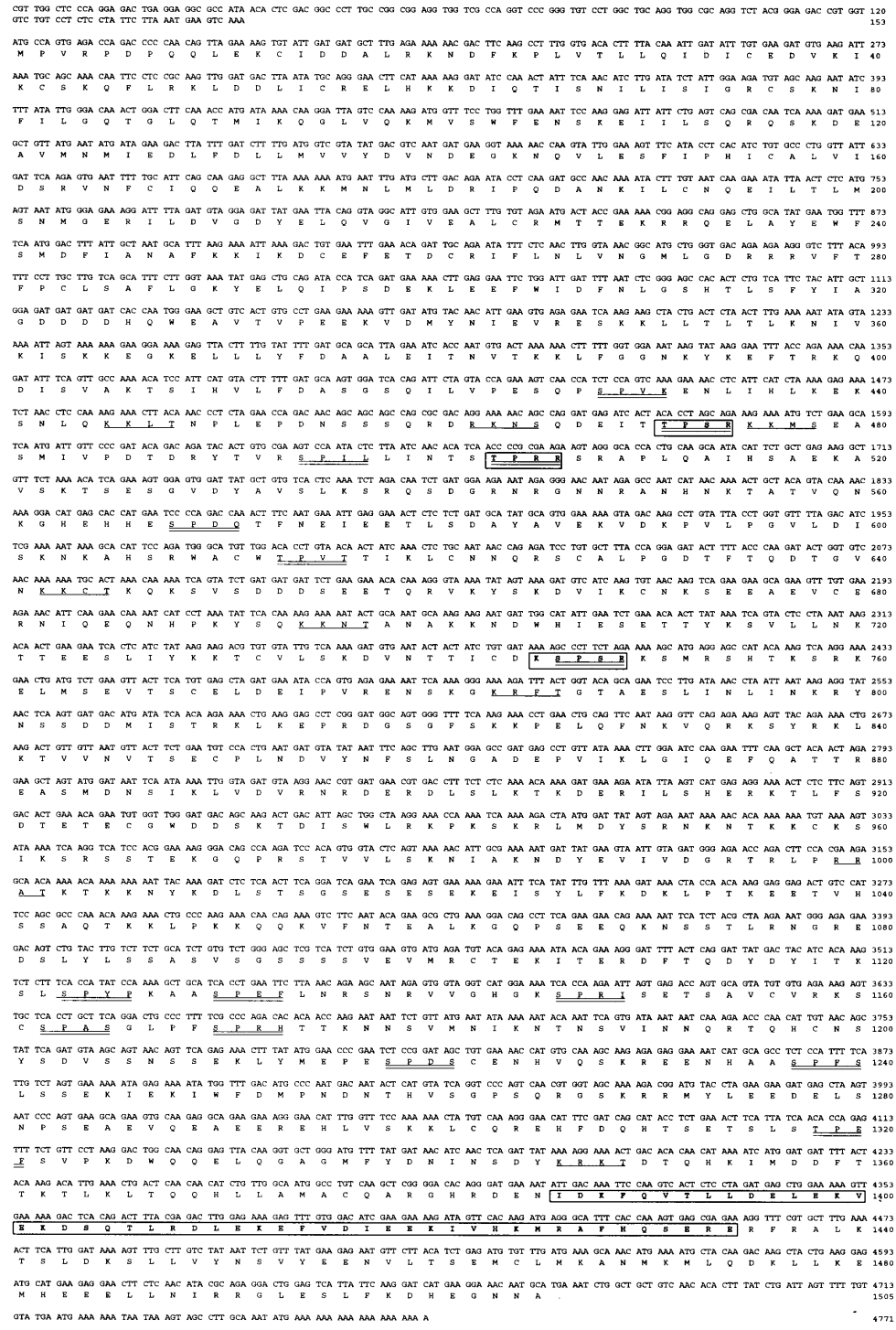

Figure 2. The nucleotide sequence of the cDNA encoding SCP2 and the predicted amino acid sequence in single-letter code. The S/T-P motifs are double underlined. The $p34^{cdc2}$ kinase target sites are boxed. The cAMP/cGMP-dependent protein kinase target sites are underlined. The coiled-coil domain is indicated by a large box.

carrying SC proteins (Fig. 1), and a series of peptide bands, which are also recognized by two of the Mabs (compare Fig. 1 lane 8 with lanes 6 and 7), and which we interpret as breakdown products of the 190 000 M_r SC component. (iv) In frozen sections of the testis, serum 493 reacts specifically with nuclei of meiotic prophase cells (spermatocytes): the immunofluorescence pattern in Figure 3 is virtually identical to the pattern obtained with the Mabs that were used for screening [compare Fig. 3 of this paper with figure 3a in Offenberg *et al.* (13)]. (v) Within spermatocytes, serum 493 recognizes specifically the LEs of SCs, like the Mabs that were used for screening (Fig. 4). We therefore conclude that we have cloned the cDNA encoding the 190 000 M_r component of the LEs of SCs, for which we propose the name SCP2 (synaptonemal complex protein 2). The discrepancy between the

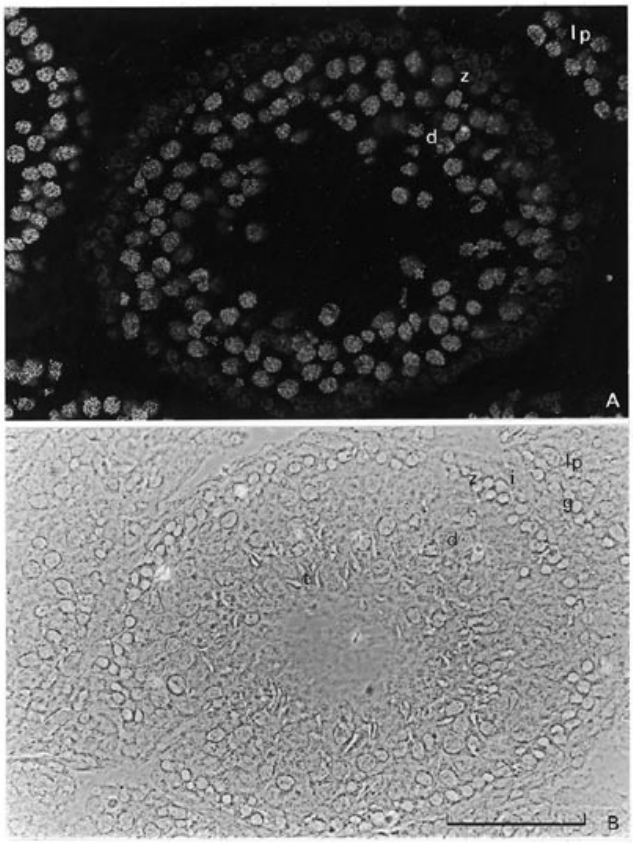

Figure 3. Frozen section of rat testis after immunofluorescence staining with serum 493 (elicited against the translation product of part of the SCP2 cDNA). (**A**) Immunofluorescence, and (**B**) phase contrast of the same section. The centre of the micrographs shows a cross-sectioned tubule which is in developmental stage XIII because it contains two layers of spermatocytes; these are in zygotene (z) and diplotene (d) (74). The cell layer outside the spermatocyte layers contains spermatogonia (g); the cells inside the spermatocyte layers are spermatids (t); between the tubules, there are interstitial cells (i). The upper right corner of the micrographs shows part of a stage VII–IX tubule, because it contains a single layer of relatively large spermatocytes; these are in mid-late pachytene (lp) (74). Bar represents 150 μm.

relative electrophoretic mobility of the 190 000 M_r SC component and the predicted molecular weight is probably due to the fact that SCP2 is a basic protein with a high percentage of proline residues. Proteins with these characteristics will migrate more slowly in SDS–polyacrylamide gels than can be expected on the basis of their molecular weight (41). A similar discrepancy between predicted molecular weight and observed electrophoretic mobility has been found for other proteins with these characteristics, for instance, the proline-rich protein RAP1 (42). Searching libraries of known sequences by the BlastP program (43) revealed a limited amino acid sequence similarity between residues 425 and 478 of SCP2 and residues 564 and 617 of the Red1 protein of *Saccharomyces cerevisiae* (Fig. 5), which has a role in SC assembly and meiotic recombination (16).

SCP2 has features of a DNA-binding protein

Between amino acid residues 1364 and 1499, SCP2 contains a predicted amphipathic α-helical domain, of which, according to the algorithm of Lupas *et al.* (44), the stretch between residues 1386 and 1434 is capable of forming a coiled-coil structure. According to Chou-Fasman analysis (45) SCP2 is rich in β-turns. No other readily identifiable secondary structural motifs were found in SCP2.

SCP2 contains several interesting small scale amino acid sequence motifs: the protein is enriched in S/T-P motifs, which are common in a variety of DNA-binding proteins (46), and allow non-sequence specific binding to DNA, presumably through interaction in the minor groove with the phosphodeoxyribose backbone (47,48). SCP2 has 15 of these motifs and in addition 40 S/T-S/T motifs, which can mimic the conformation of the S/T-P motif (46). The S/T-P motifs occur in two clusters (Fig. 2), which are separated by a hydrophilic and basic domain of the protein with a calculated pI of ~9.5. SCP2 has several potential protein kinase target sites, including one target site for $p34^{cdc2}$ protein kinase which fulfils the consensus K-S/T-P-X-Z (49), and two sites which satisfy the relaxed consensus S/T-P-X-Z (50), and eight cAMP/cGMP-dependent protein kinase target sites [K/R-K/R-X-S/T, where X is any amino acid (51)], which are interspersed with the S/T-P motifs, or are located in the basic central part of the protein. Furthermore, SCP2 contains two nuclear targeting signals [K-R/K-X-R/K, where X is any amino acid (52)].

The gene encoding SCP2 is transcribed specifically during meiosis

The antigens recognized by the anti-190 000 M_r monoclonal antibodies (2,13) and the polyclonal anti-SCP2 serum (serum 493) (Figs 3 and 7) are found exclusively in meiotic prophase cells. Northern blot analysis, performed with antisense RNA probes derived from SCP2, revealed a single transcript of ~4900 nt in poly(A)$^+$ RNA from the testis, but not in RNA from other organs (Fig. 6). Within the testis, the gene encoding SCP2 is transcribed predominantly in meiotic prophase cells (Fig. 7).

DISCUSSION

Meiotic prophase chromosomes are organized in loops along proteinaceous axes, called axial elements, which are incorporated as LEs in the tripartite structure of SCs. The axial elements are distinct from mitotic or meiotic metaphase chromosome scaffolds in that they largely consist of meiosis-specific components (2). Furthermore, each axial element is shared by the two sister chromatids of a meiotic prophase chromosome, whereas the sister chromatids of mitotic or meiotic metaphase chromosomes each have their own scaffold. Elucidation of the relation between axial elements and chromosome scaffolds will provide insight into the structure of chromosomes and the mechanisms of meiotic chromosome pairing and crossover formation. In mammals, major protein components of axial elements have been identified, with M_rs of 30 000, 33 000 and 190 000, respectively (2,11). The 30 000 and 33 000 M_r components are closely related and probably the products of a single gene, *Scp3* (14). The corresponding cDNA of the rat has been cloned, and encodes a 30 kDa protein called SCP3 (14); the cDNAs encoding the homologous proteins of the mouse (SYCP3; 53), and of the hamster (COR1; 24) have also been cloned. In this paper we describe the isolation and sequencing of the cDNA encoding the 190 000 M_r component of the axial elements.

Expression of the gene encoding SCP2

The 190 000 SC M_r component occurs exclusively in meiotic prophase nuclei, in SCs (2,13,54; Figs 3 and 4). The experiments in this paper show that expression of *Scp2* is regulated at the

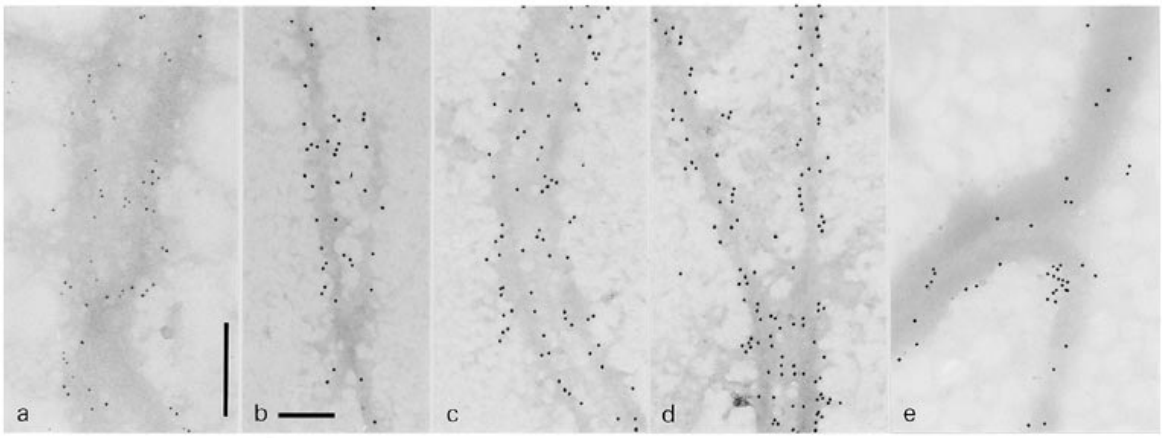

Figure 4. Ultrastructural localization of the antigens of serum 493 by immunogold labeling of surface spread spermatocytes. (**b**) Zygotene, (**c**) pachytene and (**d**) diplotene spermatocytes; (**e**) immunogold labeling of the XY chromosome pair by serum 493; (**a**) immunogold labeling of a pachytene SC by Mab IX9D5, one of the monoclonal antibodies that were used for screening. In (a), the immunogold grains have a diameter of 5 nm, in (b)–(e) 10 nm. Bars represent 200 nm. The magnification in (c)–(e) is the same as in (b).

```
SCP2   425  SQPSPVKENLIHLKEKSNLQKKLTNPLEPDNSSSQRDRKNSQDEITTPSRKKMS 478
            :|||:: |::  | ||| : ||||||     : :||  |:   :  | |   |:|
RED1   564  GQPPSKKQKQFHKKEKKKQQKKLTNFKPIIDVPSQDKRNLRSNAPTKPKSIKVS 617
```

Figure 5. Amino acid sequence comparison of SCP2 and the Red1 protein of yeast (*S.cerevisiae*) by the BLASTP program (43). The numbers to the left indicate for each protein the first amino acid where the similarity begins. Connecting lines indicate identical amino acid residues, and colons similar amino acid residues.

transcriptional level, because northern blot analysis of RNA from various tissues only revealed transcripts of the *Scp2* gene in testis RNA (Fig. 6). A similar result was obtained earlier with respect to two other major components of SCs, namely SCP1 (28) and SCP3 (14). These results corroborate our earlier conclusion (12,13) that SCs originate predominantly by assembly from newly synthesized components rather than by rearrangement of pre-existing nuclear structures. Within the testis, *Scp2* transcripts occurred predominantly in meiotic prophase cells, whereas a low level of transcripts appeared to persist in spermatids (Fig. 7). We cannot exclude that the *Scp2* gene is transcribed at very low levels in other organs. Of the SCP1 gene, low levels of transcripts have been detected in the brain by means of PCR (55), although such transcripts were not detectable on northern blots of brain RNA (28). However, we doubt whether such very low levels of transcription are functionally significant, because no SCP1 or SCP2 protein has been detected in any other tissue than testis and ovary.

Sequence and predicted secondary structure of SCP2

The amino acid sequence of the predicted protein SCP2 contains several motifs of potential interest: the protein contains three potential $p34^{cdc2}$ kinase target sites, which could be important for regulation of the assembly and disassembly of the SC: mutation of the CDC28 gene, which encodes the *S.cerevisiae* protein homologous to $p34^{cdc2}$, causes an arrest in pachytene (56); it is thus possible that the $p34^{cdc2}$ protein kinase plays a role in the regulation of SC disassembly. The major component of the transverse filaments of SCs, SCP1, also has a potential target site for $p34^{cdc2}$ protein kinase (28). Besides the $p34^{cdc2}$ kinase target sites, SCP2 contains eight target sites for cAMP/cGMP dependent protein kinase (51). This protein kinase is possibly also involved in the regulation of SC (dis)assembly: inhibition of phosphorylation of nuclear lamins by cAMP/cGMP-dependent protein kinase plays a key role in the regulation of the disassembly of the nuclear lamina (57). Furthermore, axial element component SCP3 also has two potential target sites for cAMP/cGMP-dependent protein kinase (14). However, it still has to be sorted out whether the potential kinase target sites on SCP2 are actually phosphorylated *in vivo*, and whether this plays any role in the regulation of SC (dis)assembly.

SCP2 has features of a DNA binding protein

SCP2 shares features with several other proteins that have a function in chromatin organization. SCP2 has two large clusters of S/T-P and S/T-S/T motifs, which flank a basic domain of the protein. S/T-P and S/T-S/T motifs are common in a variety of DNA-binding proteins, and allow non-sequence-specific interaction with the minor groove of DNA (47,48). Transverse filament proteins SCP1 of the rat (28) and Zip1 of yeast (58), and meiotic chromosome core component Red1 of yeast (17,59) also contain clusters of S/T-P and S/T-S/T motifs. Several nuclear matrix proteins also contain S/T-P clusters, for example SAF-A, a protein for which *in vivo* binding to matrix attachment DNA-regions has been proven (60). Other nuclear matrix proteins carrying clusters of S/T-P motifs include mammalian nuclear matrix protein NUMA (61,62), lamins (chicken lamin A, B1 and B2, human lamin A and C; 63–66), repressor/activator site binding protein Rap1 of yeast (42), and topoisomerase II (67), which is found in chromosome scaffolds (27). Lamin B-1 (68,69), Rap1 (42), SATB1 (70) and SCP1 (71) have been shown to bind to DNA *in vitro*, although no obvious similarity with conserved features of DNA binding proteins could be identified in these proteins besides the S/T-P and S/T-S/T clusters. Furthermore, Meuwissen (71) showed that SCP1 binds to DNA through interaction with the minor groove, and that its DNA-binding activity is confined to the C-terminal domain, which contains all S/T-P and S/T-S/T motifs. In preliminary southwestern blot experiments, an expression product of a large part of the SCP2 cDNA was also

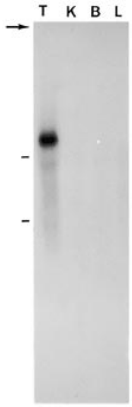

Figure 6. Transcription of the gene encoding SCP2, analysed by northern blot analysis. 15 µg of poly(A)$^+$ RNA from respectively, testis (T), kidney (K), brain (B) and liver (L) was layered per 0.5 cm wide slot of a 1% denaturing agarose gel. After electrophoresis and blotting, hybridization was performed with an RNA transcript of an 850 bp fragment of cDNA clone 3C1. Bars indicate the position of the 28S (4700 nt) and 18S (1900 nt) ribosomal RNA of the rat. The arrowhead indicates the top of the gel.

capable of binding to DNA (K_a = 3.6 × 10^9 M^{-1}, unpublished experiments). However, it remains to be demonstrated that SCP2 binds to DNA *in vivo*.

Possible functions of SCP2

The localization and predicted secondary structural features of SCP2 suggest that this protein is involved in the organization of meiotic prophase chromatin, possibly by temporarily binding to SARs. In detailed immunofluorescence studies (Schalk *et al.*, in preparation), we found that SCP2 first assembles into short stretches of axial element, which fuse to form long, linear, unsynapsed axial elements, which then shorten and thicken as synapsis proceeds. How the assembly of linear axes is accomplished is not known. It is not necessary to suppose a specific function for SCP2 in this respect, because chromosomes that are forced to condense from G2 in somatic cells, which do not contain SCP2, also develop long, linear axes (10). However, (premature) condensation from somatic G2 results in the assembly of one axis for each chromatid; only if condensation proceeds in the presence of a topoisomerase II inhibitor (ICRF), a single, undivided axis is formed for the two chromatids of each chromosome (10); upon recovery from ICRF, the single axis splits, the centromeric regions being separated last. It is possible that in meiotic prophase, SCP2 has a comparable effect as a topoisomerase II inhibitor on the separation of sister chromatids, by competing with topoisomerase II for binding to SARs. The two sister chromatids would then remain unseparated until SCP2 is removed, possibly by phosphorylation, and replaced by topoisomerase II, which gradually congregates onto the axial elements in the second half of meiotic prophase (72). SCP2 persists in the centromeric region during meiotic metaphase I (Schalk *et al.*, in preparation), as has been described earlier for SCP3 (COR1) (24); such a localization would be consistent with a role of SCP2 in sister chromatid cohesion. The ultrastructural localization of SCP2 in the center of the axial element (Schalk *et al.*, in preparation) is also consistent with such a role.

One other possible clue to the function(s) of SCP2 is provided by the work on the yeast Red1 protein. SCP2 shows a limited sequence similarity to the Red1 protein of yeast. SCP2 and Red1 are also

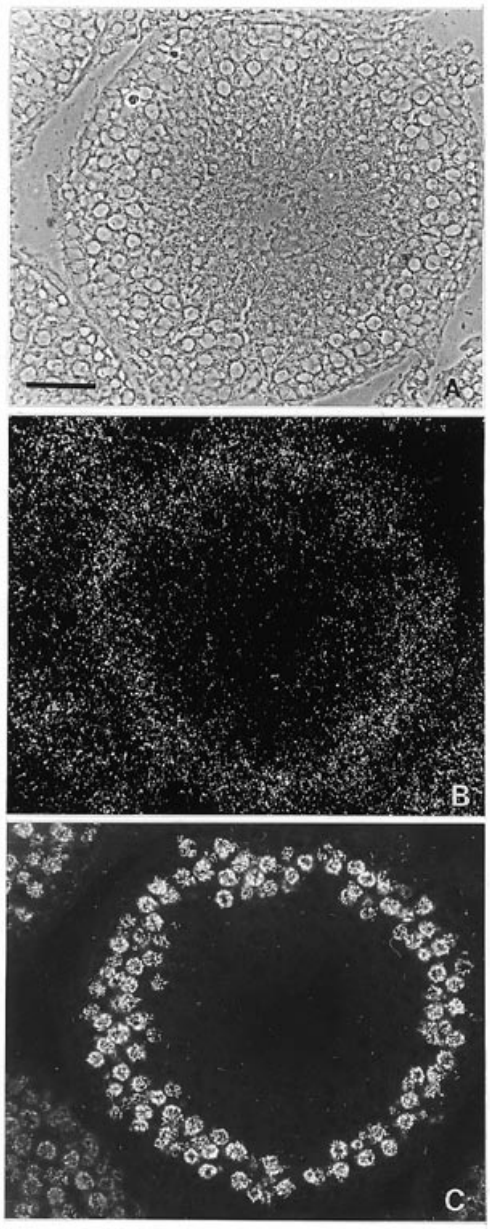

Figure 7. Localization of *SCP2* transcripts in the testis by *in situ* hybridization. (**A**) Phase contrast micrograph of a transverse section of a testicular tubule in developmental stage VII–IX spermatocytes (mid-late pachytene; 74). (**B**) Localization of *SCP2* transcript in the adjacent section by *in situ* hybridization; a ^{35}S-labeled antisense RNA transcript was used as a probe. Note that the transcripts are present in the cytoplasm: nuclei are visible as 'black holes'. (**C**) Localization of SCP2 in the same section by indirect immunofluorescence staining with Mab IX1H9 as primary antibody: this antibody made part of the pool of anti-190 000 M_r Mabs that was used for screening of the cDNA library. Bar represents 50 µm.

similar in that both proteins contain many S/T-P and S/T-S/T motifs [Red1 has six S/T-P motifs and 20 S/T-S/T motifs, (59)], and are predicted to form a short coiled-coil domain at their C-terminus. Although there are also considerable differences (Red1 has a much lower molecular weight and pI than SCP2), the similarities are of interest because Red1 is a candidate component of the axial elements of yeast SCs (17). The Red1 protein localizes to the cores of meiotic prophase chromosomes (17). *red1* mutants do not assemble axial

elements (16), and display a decreased level of meiotic chromosome pairing, heteroduplex formation (73), and interchromosomal gene conversion and crossing over (16). Furthermore, Red1 is required for the formation of crossovers that can ensure a proper disjunction of homologous chromosomes at metaphase I (16) and has a role in monitoring the recombination process (22). The involvement in such a variety of processes can be understood if Red1 is a structural component of the axial elements which interacts with several proteins that function in one or more of these processes. The same could be true for SCP2. The cloning of the cDNA encoding SCP2 will allow us to analyse this by searching for the proteins and DNA sequences that interact with SCP2.

ACKNOWLEDGEMENTS

We thank A.C.G.Vink for expert technical assistance, and M.Nijland for her contribution during her undergraduate study. This work was supported in part by NWO Netherlands Organization for Scientific Research, grant nos 900-501-064 and 900-501-143.

REFERENCES

1 Von Wettstein,D., Rasmussen,S.W. and Holm,P.B. (1984) *Annu. Rev. Genet.*, **18**, 331–413.
2 Heyting,C., Dietrich,A.J.J., Moens,P.B., Dettmers,R.J., Offenberg,H.H., Redeker,E.J.W. and Vink,A.C.G. (1989) *Genome*, **31**, 81–97.
3 Howell,W.M. and Hsu,T.C. (1979) *Chromosoma*, **73**, 61–66.
4 Rufas,J.S., Giménez-Martín,G. and Esponda,P. (1982) *Cell Biol. Int. Rep.*, **6**, 261–267.
5 Rufas,J.S., Santos,J.L., Diez,M. and Suja,J.A. (1992) *Genome*, **35**, 1054–1061.
6 Earnshaw,W.C. and Laemmli,U.K. (1984) *Chromosoma*, **89**, 186–192.
7 Stack,S.M. (1991) *Genome*, **34**, 900–908.
8 Padmore,R., Cao,L. and Kleckner,N. (1991) *Cell*, **66**, 1239–1266.
9 Schwacha,A. and Kleckner,N. (1994) *Cell*, **76**, 51–63.
10 Giménez-Abián,J.F., Clarke,D.C., Mullinger,A.M., Downes,C.S. and Johnson,R.T. (1995) *J. Cell Biol.*, **131**, 7–17.
11 Heyting,C., Moens,P.B., van Raamsdonk,W., Dietrich,A.J.J., Vink,A.C.G. and Redeker,E.J.W. (1987) *Eur. J. Cell. Biol.*, **43**, 148–154.
12 Heyting,C., Dettmers,R.J., Dietrich,A.J.J., Redeker,E.J.W. and Vink,A.C.G. (1988) *Chromosoma*, **96**, 325–332.
13 Offenberg,H.H., Dietrich,A.J.J. and Heyting,C. (1991) *Chromosoma*, **101**, 83–91.
14 Lammers,J.H.M., Offenberg,H.H., van Aalderen,M., Vink,A.C.G., Dietrich,A.J.J. and Heyting,C. (1994) *Mol. Cell. Biol.*, **14**, 1137–1146.
15 Hollingsworth,N.M., Goetsch,L. and Byers,B. (1990) *Cell*, **61**, 73–84.
16 Rockmill,B. and Roeder,G.S. (1990) *Genetics*, **126**, 563–574.
17 Smith,A.V. and Roeder,G.S. (1997) *J. Cell Biol.*, **136**, 957–967.
18 Laemmli,U.K., Käs,E., Poljak,L. and Adachi,Y. (1992) *Curr. Opin. Genet. Dev.*, **2**, 275–285.
19 Scherthan,H., Weich,S., Schwegler,H., Heyting,C., Haerle,M. and Cremer,T. (1996) *J. Cell Biol.*, **134**, 1109–1125.
20 Schwacha,A. and Kleckner,N. (1996) *Cell*, **83**, 783–791.
21 Schwacha,A. and Kleckner,N. (1997) *Cell*, **90**, 1123–1135.
22 Xu,L., Weiner,B.M. and Kleckner,N. (1997) *Genes Dev.*, **11**, 106–118.
23 Maguire,M.P. (1990) *Biochem. Cell. Biol.*, **68**, 1231–1242.
24 Dobson,M., Pearlman,R.E., Karaiskakis,A., Spyropoulos,B. and Moens,P.B. (1994) *J. Cell Sci.*, **107**, 2749–2760.
25 Saitoh,Y. and Laemmli,U.K. (1994) *Cell*, **76**, 609–622.
26 Mirkovitch,J., Mirault,M.E. and Laemmli,U.K. (1984) *Cell*, **39**, 223–232.
27 Earnshaw,W.C. and Heck,M.M.S. (1985) *J. Cell Biol.*, **100**, 1716–1725.
28 Meuwissen,R.L.J., Offenberg,H.H., Dietrich,A.J.J., Riesewijk,A., Van Iersel,M. and Heyting,C. (1992) *EMBO J.*, **11**, 5091–5100.
29 Frohman,M.A., Dush,M.K. and Martin,G.R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8998–9002.
30 Van Heemst,D., Swart,K., Holub,E.F., Van Dijk,R., Offenberg,H.H., Goosen,T., Van Den Broek,H.W.J. and Heyting,C. (1997) *Mol. Gen. Genet.*, **254**, 654–664.
31 Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
32 Heyting,C. and Dietrich,A.J.J. (1991) *Methods Cell Biol.*, **35**, 177–202.
33 Moens,P.B., Heyting,C., Dietrich,A.J.J., Van Raamsdonk,W. and Chen,Q. (1987) *J. Cell. Biol.*, **105**, 93–103.
34 Cathala,G., Savouret,J.F., Mendez,B., West,B.L., Karin,M., Martial,J.A. and Baxter,J.D. (1983) *DNA*, **2**, 329–335.
35 Aviv,H. and Leder,P. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1408–1412.
36 Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) 2nd ed. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
37 Heyting,C., Dietrich,A.J.J., Redeker,E.J.W. and Vink,A.C.G. (1985) *Eur. J. Cell. Biol.*, **36**, 307–314.
38 Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
39 Dunn, S.D. (1986) *Anal. Biochem.*, **157**, 144–153.
40 Offenberg,H.H. (1993) *Identification and characterization of synaptonemal complex proteins of the rat*. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.
41 Hames,B.D. (1990) In Hames,B.D. and Rickwood,D. (eds), *Gel Electrophoresis of Proteins, a Practical Approach*. 2nd edition, IRL Press, Oxford, UK. pp. 19–20.
42 Shore,D. and Nasmyth,K. (1987) *Cell*, **51**, 721–732.
43 Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) *J. Mol. Biol.*, **215**, 403–410.
44 Lupas,A., Van Dyke,M. and Stock,J. (1991) *Science*, **252**, 1162–1164.
45 Chou,P.Y. and Fasman,G.D. (1978) *Adv. Enzymol.*, **47**, 45–148.
46 Suzuki,M. (1989) *J. Mol. Biol.*, **207**, 61–84.
47 Churchill,M.E., and Travers,A.A. (1991) *Trends. Biochem. Sci.*, **16**, 92–97.
48 Green,G.R., Lee,H.J. and Poccia,D.L. (1993) *J. Biol. Chem.*, **268**, 11247–11255.
49 Moreno,S. and Nurse,P. (1990) *Cell*, **61**, 549–551.
50 Draetta,G. (1990) *Trends. Biochem. Sci.*, **15**, 378–383.
51 Feramisco,J.R., Glass,D.B. and Krebs,E.G. (1980) *J. Biol. Chem.*, **255**, 4240–4245.
52 Chelsky,D., Ralph,R. and Jonak,G. (1989) *Mol. Cell. Biol.*, **9**, 2487–2492.
53 Klink,A., Lee,M. and Cooke,H.J. (1997) *Mammalian Genome*, **8**, 376–377.
54 Dietrich,A.J.J., Kok,E., Offenberg,H.H., Heyting,C., De Boer,P. and Vink,A.C.G. (1992) *Genome*, **35**, 492–497.
55 Kerr,S.M., Taggart,M.H., Lee,M. and Cooke,H.J. (1996) *Hum. Mol. Genet.*, **5**, 1139–1148.
56 Shuster,E.O. and Byers,B. (1989) *Genetics*, **123**, 29–44.
57 Lamb,N.J.C., Cavadore,J.C., Labbe,J.C., Maurer,R.A. and Fernandez,A. (1991) *EMBO J.*, **10**, 1523–1534.
58 Sym,M., Engebrecht,J. and Roeder,G.S. (1993) *Cell*, **72**, 365–378.
59 Thompson,E.A. and Roeder,G.S. (1989) *Mol. Gen. Genet.*, **218**, 293–301.
60 Goehring,F. and Fackelmayer,F.O. (1997) *Biochemistry*, **36**, 8276–8283.
61 Compton,D.A., Szilak,I. and Cleveland,D.W. (1992) *Cell Biol.*, **116**, 1395–1408.
62 Yang,C.H., Lambie,E.J. and Snyder,M. (1992) *J. Cell Biol.*, **116**, 1303–1317.
63 Peter,M., Kitten,G.T., Lehner,C.F., Vorburger,K., Bailer,S.M., Maridor,G. and Nigg,E.A. (1989) *J. Mol. Biol.*, **208**, 393–404.
64 Vorburger,K., Lehner,C.F, Kitten,G.T., Eppenberger,H.M. and Nigg,E.A. (1989) *J. Mol. Biol.*, **208**, 405–415.
65 McKeon,F.D., Kirschner,M.W. and Caput,D. (1986) *Nature*, **319**, 463–468.
66 Fisher,D.Z., Chaudhary,N. and Blobel,G. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 6450–6454.
67 Austin,C.A., Sng,J.H., Patel,S. and Fisher,L.M. (1993) *Biochim. Biophys. Acta*, **1172**, 283–291.
68 Ludérus,M.E.E., De Graaf,A., Mattia,E., Den Blaauwen,J.L., Grande,M.A., De Jong,L. and Van Driel,R. (1992) *Cell*, **70**, 949–959.
69 Ludérus,M.E.E., Den Blaauwen,J.L., De Smit,O.J.B., Compton,D.A. and Van Driel,R. (1994) *Mol. Cell. Biol.*, **14**, 6297–6305.
70 Dickinson,L.A., Joh,T., Kohwi,Y. and Kohwi Shigematsu,T. (1992) *Cell*, **70**, 631–645.
71 Meuwissen,R.L.J. (1997) *SCP1, a major protein component of synaptonemal complexes of the rat*. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands.
72 Moens,P.B. and Earnshaw,W.C. (1989) *Chromosoma*, **98**, 317–322.
73 Nag,D.K., Scherthan,H., Rockmill,B., Bhargava,J. and Roeder,G.S. (1995) *Genetics*, **141**, 75–86.
74 Leblond,C.P. and Clermont,Y. (1952) *Ann. N.Y. Acad. Sci.*, **55**, 548–573.