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Human Synaptonemal Complex Protein 1 (SCP1): Isolation and Characterization of the cDNA and Chromosomal Localization of the Gene

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Synaptonemal complexes (SCs) are structures that are formed between homologous chromosomes (homologs) during meiotic prophase. They consist of two proteinaceous axes, one along each homolog, that are connected along their length by numerous transverse filaments (TFs). The cDNA encoding one major component of TFs of SCs of the rat, rnSCP1, has recently been isolated and characterized. In this paper we describe the isolation and characterization of the cDNA encoding the human protein homologous to rnSCP1, hsSCP1. hsSCP1 and rnSCP1 have 75% amino acid identity. The most prominent structural features and amino acid sequence motifs of rnSCP1 have been conserved in hsSCP1. Most probably, hsSCP1 is functionally homologous to rnSCP1. The hsSCP1 gene was assigned to human chromosome 1p12–p13 by fluorescence *in situ* hybridization.

INTRODUCTION

The life cycle of sexually reproducing organisms is characterized by the alternation of diploid and haploid generations of cells. The transition from the diploid to the haploid state is accomplished at meiosis, which in mammals immediately precedes gametogenesis. At meiosis, a single round of DNA replication is followed by two successive nuclear divisions, meiosis I and II. During the prophase of meiosis I, homologous chromosomes (homologs) condense, pair, recombine, and disjoin. At meiosis II, the chromatids of each chromosome segregate, as in a mitotic division. The chromatin rearrangements of meiotic prophase are accompanied by the assembly and disassembly of synaptonemal complexes (SCs)[2] (reviewed by von Wettstein *et al.*, 1984). SCs consist of two proteinaceous axial cores or lateral elements (LEs), one along each homolog, that are connected along their length by numerous transverse filaments (TFs); a third longitudinal structure, the central element (CE), exists on the TFs, between both LEs (Gillies, 1975; Schmekel *et al.*, 1993).

To analyze the function of SCs, we have started to study their composition. Several protein components of SCs of rodents (Heyting *et al.*, 1987, 1989; Smith and Benavente, 1992; Chen *et al.*, 1992) and yeast (reviewed in Roeder, 1995) have been identified. Among these components were the putative TF proteins Zip1p of yeast (Sym *et al.*, 1993; Sym and Roeder, 1995) and synaptonemal complex protein 1 (SCP1) of the rat (in this paper referred to as rnSCP1) (Meuwissen *et al.*, 1992). The cDNA encoding rnSCP1 was isolated and sequenced (Meuwissen *et al.*, 1992), and the predicted amino acid sequence was analyzed: rnSCP1 consists of an α-helical stretch of 700 amino acid residues, flanked by N- and C-terminal globular domains. rnSCP1 molecules are highly organized within SCs: the C-terminal domains are located in the inner half of the LEs, whereas the N-terminal domains lie in the vicinity of the CE (Schmekel *et al.*, 1996). The C-terminal domain of rnSCP1 contains S/T-P (Ser/Thr-Pro) motifs, which are characteristic of DNA-binding proteins (Suzuki, 1989a; Churchill and Travers, 1991). The DNA-binding capacity of the C-terminal domain was recently demonstrated (Meuwissen *et al.*, unpublished experiments). The gene encoding rnSCP1 is transcribed exclusively in meiotic prophase cells (Meuwissen *et al.*, 1992). If, as seems likely, rnSCP1 is functionally homologous to Zip1p of yeast (Sym *et al.*, 1993; Sym and Roeder, 1995), it probably has a function in the regulation of reciprocal crossing over and chromosome segregation (Sym and Roeder, 1994).

We cloned and characterized the cDNA encoding the human homolog of rnSCP1 (hsSCP1) to identify conserved domains within the protein and obtain a better insight into the importance of structural features and amino acid sequence motifs in SCP1. The predicted amino acid sequence of hsSCP1 has 75% identity to

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[2] Abbreviations used: CE, central element; TF, transverse filament; LE, lateral element; SC, synaptonemal complex; mmSCP1, mouse SCP1; hamsyn1a, hamster SCP1; rnSCP1, rat SCP1; hsSCP1, human SCP1; SSC, standard saline citrate (150 m*M* NaCl, 15 m*M* sodium citrate, pH 7.0); PBS, phosphate-buffered saline (150 m*M* NaCl, 50 m*M* Na_2HPO_4/NaH_2PO_4, pH 7.4).

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rnSCP1; all prominent predicted structural features and most amino acid sequence motifs were conserved in hsSCP1. The gene encoding hsSCP1 was localized on human chromosome 1p12–p13 by fluorescence *in situ* hybridization (FISH). No human meiotic phenotype could be correlated with abnormalities in the chromosome 1p12–p13 region.

MATERIALS AND METHODS

Isolation of cDNAs encoding human SCP1. A human testis cDNA library in λgt11 (Huynh *et al.*, 1985) was screened with a polyclonal anti-rnSCP1 antiserum (Meuwissen *et al.*, 1992) and the monoclonal anti-rnSCP1 antibody IX5B2 (Offenberg *et al.*, 1991). Screening of 4 $\times 10^5$ phage yielded two positive clones with inserts of 1.5 and 1.8 kb, respectively. A 5′ end probe of 350 bp derived from the 1.8-kb insert was used for screening a human testis cDNA library in λgt10 (Huynh *et al.*, 1985) (Clontech Laboratories Inc., Palo Alto, CA). This yielded a clone with a 2.7-kb insert that overlapped with the cDNAs from the λgt11 library. We obtained the missing 5′ end of the human cDNA from the λgt10 library by means of PCR, for which we used two nested oligonucleotides homologous to the 2.7-kb cDNA insert and one oligonucleotide homologous to the λgt10 vector as primers: about 2×10^8 plaque-forming units of the λgt10 library were resuspended in 75 μl deionized water and heated for 5 min at 70°C. Subsequently, the sample was adjusted to 1.5 m*M* $MgCl_2$, 0.2 m*M* dATP, dGTP, dCTP, and dTTP, and 50 pmol of each of both primers and 2.5 units *Taq* polymerase (Pharmacia Biotech Europe) were added. The sample was incubated according to the following schedule: 1 cycle of 5 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 52°C, and 2 min at 72°C; and 1 cycle of 1 min at 95°C, 1 min at 52°C, and 15 min at 72°C. The PCR yielded a 750-bp fragment, which, after Southern blot analysis (Sambrook *et al.*, 1989), hybridized with the 5′ end probe of rnSCP1 cDNA. The 750-bp fragment was gel purified and sequenced with the use of oligonucleotide primers derived from the 5′ end of the human 2.7-kb insert sequence and the λgt10 vector. The nucleotide sequence of the 2.7-kb insert was determined as follows: we subcloned the 2.7-kb cDNA insert into the pBluescript SK(+) (Stratagene Inc., San Diego, CA) vector and generated unidirectional sets of deletions from both ends of the 2.7-kb insert by partial digestion with exonuclease III and S1 nuclease, using an Erase-a-base kit (Promega, Madison, WI). We performed the sequencing reactions on the deletion clones and the PCR products, using the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Perkin–Elmer Inc., Palo Alto, CA) and analyzed the nucleotide sequences on an ABI 373A automatic DNA sequencer (Applied Biosystems Inc., Foster City, CA). The nucleotide sequence of human SCP1 (hsSCP1) was assembled by means of the GCG sequence analysis software package (University of Wisconsin, Madison, WI). Sequence similarity searches of GenBank, EMBL, Swissprot, and PIR databases were carried out with several BLAST programs (Altschul *et al.*, 1990), FASTA and tFASTA (Pearson, 1990). Prediction of secondary structure was performed by means of a program based on Chou-Fasman algorithms (Chou and Fasman, 1978). Amino acid sequence alignments were determined using the Pile-Up program from the GCG sequence analysis software package (University of Wisconsin, Madison, WI).

Fluorescence in situ hybridization analysis. A 2700-bp fragment of the human SCP1 cDNA was labeled with biotin-11–dUTP, whereas a human subtelomeric repeat probe, D1Z2, specific for chromosome 1 (Buroker *et al.*, 1987), was labeled with digoxigenin-11–dUTP. Both labeling reactions were performed by nick-translation, and both probes were mixed together for hybridization with the metaphase chromosomes. The metaphases were accumulated in EBV transformed human lymphocytes by the thymidine synchronization method (Viegas-Péquignot, 1993) and spread onto slides as described previously (Dauwerse *et al.*, 1992). *In situ* hybridization was performed as described by Dauwerse *et al.* (1992). Briefly, the procedure was as follows: the metaphase chromosomes on slides were aged overnight at 60°C. After this, they were incubated for 1 h at 37°C in 100 μg/ml RNase in 2× SSC, washed for 3× 2 min in 2× SSC at 37°C, incubated for 10 min in 0.01% pepsin in 10 m*M* HCl at 37°C, washed for 2× 5 min with PBS at room temperature, fixed for 10 min in 3.7% formaldehyde in PBS, washed for 2× 5 min in PBS, dehydrated successively in 70, 96, and 100% ethanol (2 min per step), and air-dried. The chromosomes were then denatured for 5 min at 80°C in 60% formamide, 2× SSC, 50 m*M* NaH_2PO_4/Na_2HPO_4, pH 7.0, dehydrated, and fixed by incubation for 2× 5 min in 70% ethanol at −20°C, for 5 min in 96% ethanol at room temperature, and for 5 min in 100% ethanol at room temperature, and air-dried. The D1Z2 probe was dissolved in 50% formamide, 50 m*M* Na_2HPO_4/NaH_2PO_4, pH 7.0, 10% dextran sulfate, and a 50-fold excess of Cot-1 DNA, denatured for 5 min at 70°C, and prehybridized by incubation for 30 min at 37°C. The hsSCP1 probe was dissolved in 50% formamide, 50 m*M* Na_2HPO_4/NaH_2PO_4, pH 7.0, and 10% dextran sulfate, and denatured for 5 min at 70°C before it was mixed with the prehybridized D1Z2 probe. After mixing, the final concentration of D1Z2 was 5 ng/μl, and the final concentration of hsSCP1 was 10 ng/μl. The hybridization mixture was added to the pretreated slides for an overnight hybridization at 37°C in a moist chamber. After hybridization the slides were washed: for 3× 5 min at 42°C in 50% formamide, 2× SSC, pH 7.0; for 3× 5 min at 60°C in 0.1× SSC; and for 5 min at room temperature in 4× SSC, 0.05% Tween. Hybridized human SCP1 probe was detected by successive incubation rounds in avidin-FITC conjugate and biotinylated goat anti-avidin antibodies. The hybridized subtelomeric repeat probe D1Z2 was detected by successive incubation rounds in mouse anti-digoxigenin, sheep anti-mouse antibodies, and anti-sheep IgG-TRITC conjugates. Counterstaining of the chromosomes was performed with DAPI in a Vectashield (Vecta Laboratories Inc.) antifade solution. Slides were examined in a Zeiss Axioplan epifluorescence microscope. For digital imaging microscopy the Cytovision Probe System (Applied Imaging Inc., New Castle, UK) was used.

RESULTS

Isolation and Sequencing of Human SCP1 cDNAs

A mixture of an affinity-purified polyclonal anti-rnSCP1 antiserum (Meuwissen *et al.*, 1992) and a monoclonal anti-rnSCP1 antibody, IX5B2 (Offenberg *et al.*, 1991), was used for screening 4×10^5 recombinant phage of a human testis λgt11 cDNA library. This yielded two clones with insert sizes of 1.5 and 1.8 kb, respectively. Both cDNA inserts had overlapping restriction enzyme maps and displayed nucleotide sequence homology with the rat SCP1 cDNA sequence. We performed a secondary screening of a human testis λgt10 cDNA library to isolate the remaining part of the human cDNA (see Materials and Methods). Extensive screening of the independent λgt10 and λgt11 cDNA libraries yielded only a single type of cDNA clones, i.e., we found only one type of human cDNA clones homologous to the rat SCP1 cDNA. The complete human SCP1 (hsSCP1) cDNA sequence contained an open reading frame of 2928 nucleotides, which encoded a protein of 976 amino acids (Fig. 1). The translation initiation codon in the human cDNA sequence (Fig. 1, nucleotide positions 95–97) was the first ATG in the open reading frame and is preceded by a consensus sequence for eukaryotic translation initiation (Kozak, 1986). Furthermore, in the amino acid sequence alignment of hsSCP1 and three rodent SCP1 proteins (Fig. 2), the first 6 amino acid residues are identical.

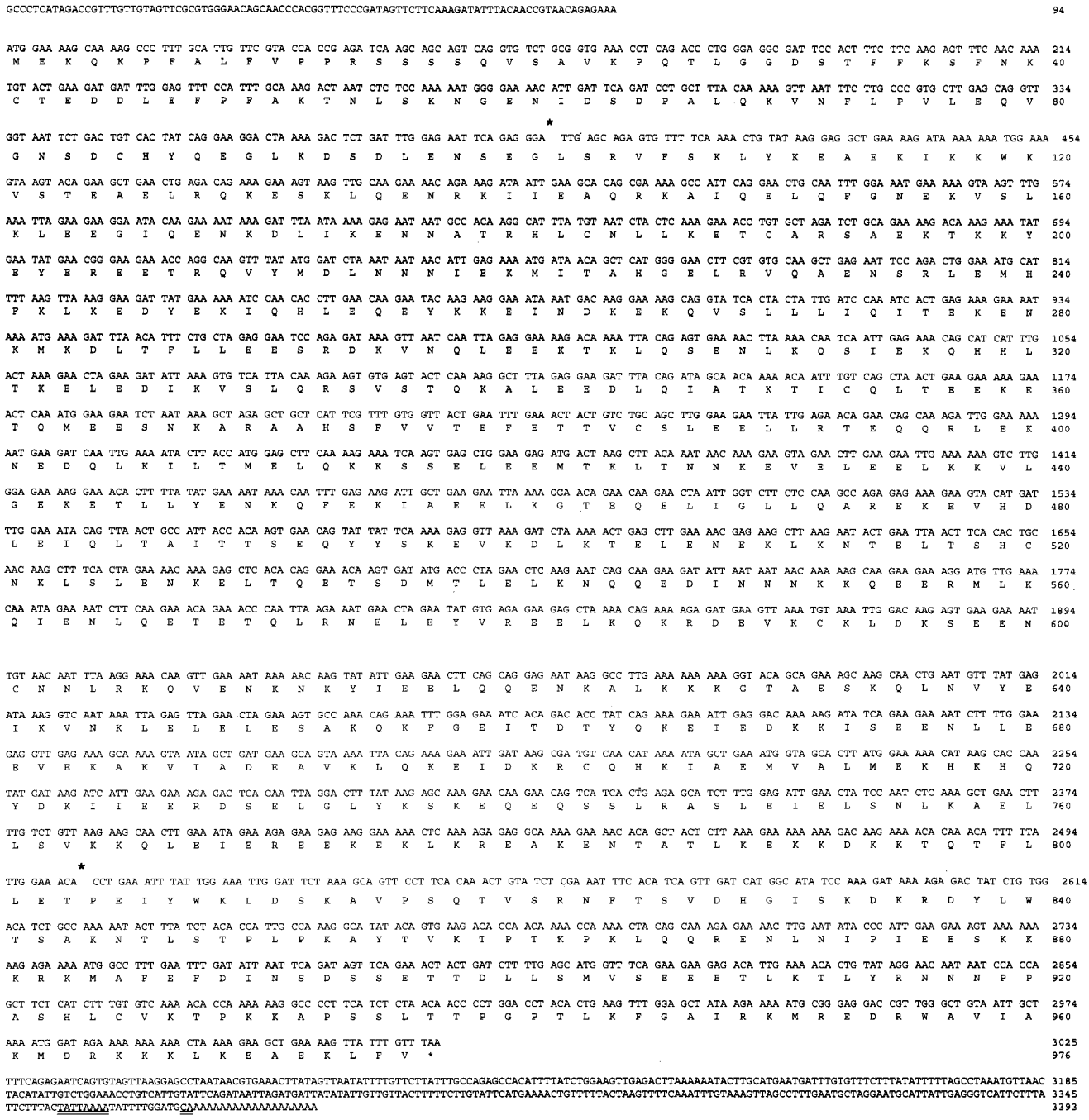


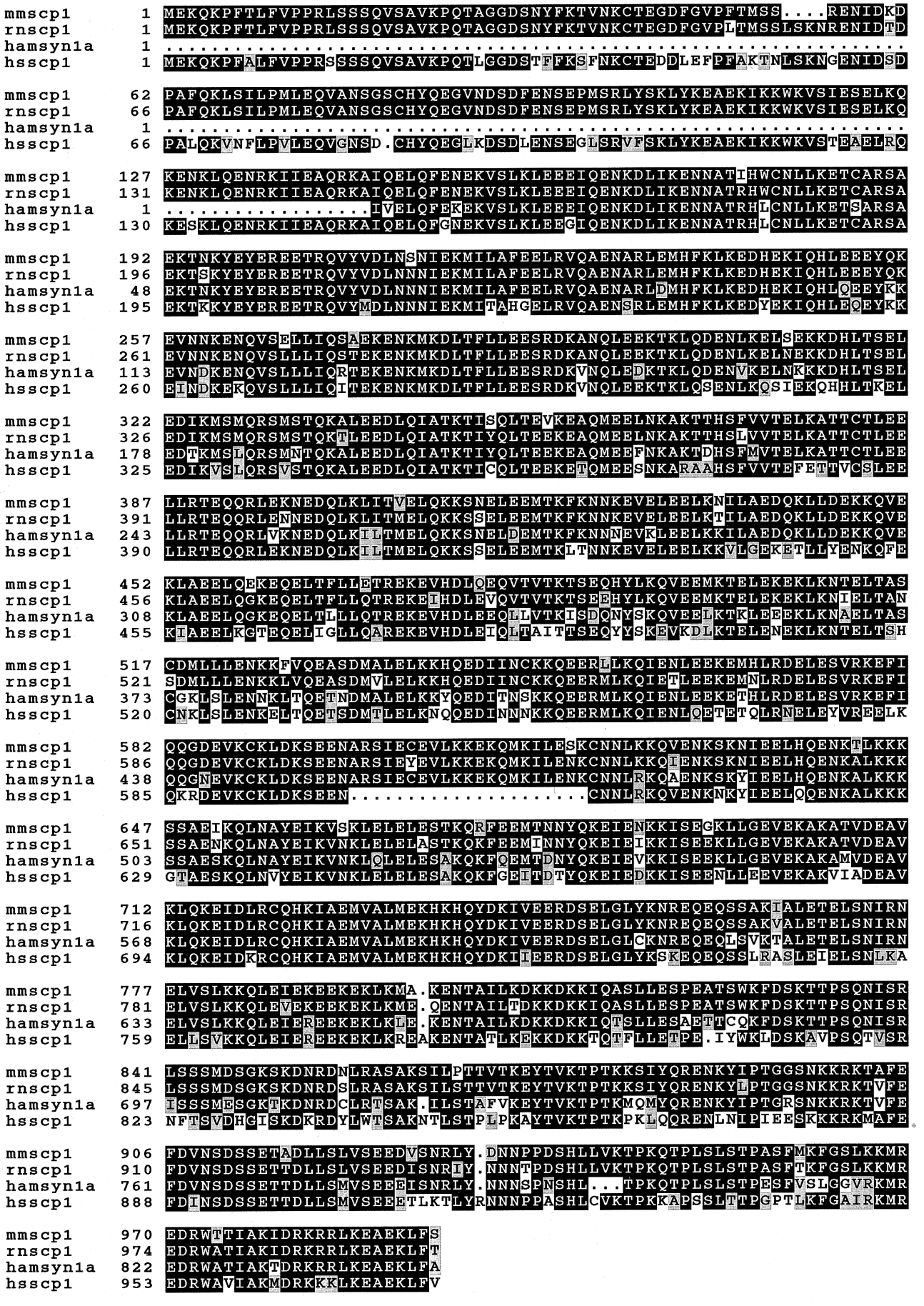
FIG. 1. Nucleotide sequence and derived amino acid sequence of the cDNA encoding hsSCP1. The predicted translation product is presented below the first nucleotide of each codon. The putative poly(dA) addition signal and addition site are underlined. The ends of the coiled-coil region as predicted according to Lupas *et al.* (1991), with a window of 28 amino acids, are indicated by asterisks. The name and Accession No. for the human SCP1 cDNA sequence in the EMBL database are hsSCP1 and X95654, respectively.

Amino Acid Sequence Homology between the Different SCP1 Proteins

The amino acid identity between hsSCP1 and SCP1 of the mouse (Sage *et al.*, 1995), hamster (Dobson *et al.*, 1994), and rat (Meuwissen *et al.*, 1992) is 74.6, 74.4, and 75.7%, respectively. The amino acid identity is distributed evenly over hsSCP1, except for the C-terminal 174 amino acid residues, from hsSCP1 positions 802 to 976, which show only 64.5% amino acid identity. However, the level of amino acid sequence identity in the hsSCP1 alignment (Fig. 2) is sufficient to imply a structural and functional similarity between hsSCP1 and the rodent SCP1 proteins (Sander and Schneider, 1991).

Amino Acid Sequence Features of SCP1

The structural organization of the SCP1 proteins is very similar. Like the rodent SCP1s, hsSCP1 has three distinct domains, each having its own characteristic secondary structure as predicted by the algorithm of


mmscp1 1 MEKQKPFTLFVPPRLSSSQVSAVKPQTAGGDSNYFKTVNKCTEGDFGVPFTMSS....RENIDKD
rnscp1 1 MEKQKPFTLFVPPRLSSSQVSAVKPQTAGGDSNYFKTVNKCTEGDFGVPLTMSSLSKNRENIDTD
hamsyn1a 1 .....
hsscp1 1 MEKQKPFALFVPPRSSSSQVSAVKPQTLGGDSTFFKSFNKCTEDDLEFPFAKTNLSKNGENIDSD
mmscp1 62 PAFQKLSILPMLEQVANSGSCHYQEGVNDSDFENSEPMSRLYSKLYKEAEKIKKWKVSIESELKQ
rnscp1 66 PAFQKLSILPMLEQVANSGSCHYQEGVNDSDFENSEPMSRLYSKLYKEAEKIKKWKVSIESELKQ
hamsyn1a 1 .....
hsscp1 66 PALQKVNFLPVLEQVGNSD.CHYQEGLKDSDLENSEGLSRVFSKLYKEAEKIKKWKVSTEAELRQ
mmscp1 127 KENKLQENRKIIEAQRKAIQELQFENEKVSLKLEEEIQENKDLIKENNATIHWCNLLKETCARSA
rnscp1 131 KENKLQENRKIIEAQRKAIQELQFENEKVSLKLEEEIQENKDLIKENNATRHWCNLLKETCARSA
hamsyn1a 1 .....IVELQFEKEKVSLKLEEEIQENKDLIKENNATRHLCNLLKETSARSA
hsscp1 130 KESKLQENRKIIEAQRKAIQELQFGNEKVSLKLEEGIQENKDLIKENNATRHLCNLLKETCARSA
mmscp1 192 EKTNKYEYEREETRQVYVDLNSNIEKMILAFEELRVQAENARLEMHFKLKEDHEKIQHLEEEYQK
rnscp1 196 EKTSKYEYEREETRQVYVDLNNNIEKMILAFEELRVQAENARLEMHFKLKEDHEKIQHLEEEYQK
hamsyn1a 48 EKTNKYEYEREETRQVYVDLNNNIEKMILAFEELRVQAENARLDMHFKLKEDHEKIQHLQEEYKK
hsscp1 195 EKTKKYEYEREETRQVYMDLNNNIEKMITAHGELRVQAENSRLEMHFKLKEDYEKIQHLEQEYKK
mmscp1 257 EVNNKENQVSELLIQSAEKENKMKDLTFLLEESRDKANQLEEKTKLQDENLKELSEKKDHLTSEL
rnscp1 261 EVNNKENQVSLLLIQSTEKENKMKDLTFLLEESRDKANQLEEKTKLQDENLKELNEKKDHLTSEL
hamsyn1a 113 EVNDKENQVSLLLIQRTEKENKMKDLTFLLEESRDKVNQLEDKTKLQDENVKELNKKKDHLTSEL
hsscp1 260 EINDKEKQVSLLLIQITEKENKMKDLTFLLEESRDKVNQLEEKTKLQSENLKQSIEKQHHLTKEL
mmscp1 322 EDIKMSMQRSMSTQKALEEDLQIATKTISQLTEVKEAQMEELNKAKTTHSFVVTELKATTCTLEE
rnscp1 326 EDIKMSMQRSMSTQKTLEEDLQIATKTIYQLTEEKEAQMEELNKAKTTHSLVVTELKATTCTLEE
hamsyn1a 178 EDTKMSLQRSMNTQKALEEDLQIATKTIYQLTEEKEAQMEEFNKAKTDHSFMVTELKATTCTLEE
hsscp1 325 EDIKVSLQRSVSTQKALEEDLQIATKTICQLTEEKETQMEESNKARAAHSFVVTEFETTVCSLEE
mmscp1 387 LLRTEQQRLEKNEDQLKLITVELQKKSNELEEMTKFKNNKEVELEELKNILAEDQKLLDEKKQVE
rnscp1 391 LLRTEQQRLENNEDQLKLITMELQKKSSELEEMTKFKNNKEVELEELKTILAEDQKLLDEKKQVE
hamsyn1a 243 LLRTEQQRLVKNEDQLKILTMELQKKSNELDEMTKFKNNNEVKLEELKKILAEDQKLLDEKKQVE
hsscp1 390 LLRTEQQRLEKNEDQLKILTMELQKKSSELEEMTKLTNNKEVELEELKKVLGEKETLLYENKQFE
mmscp1 452 KLAEELQEKEQELTFLLETREKEVHDLQEQVTVTKTSEQHYLKQVEEMKTELEKEKLKNTELTAS
rnscp1 456 KLAEELQGKEQELTFLLQTREKEIHDLEVQVTVTKTSEEHYLKQVEEMKTELEKEKLKNIELTAN
hamsyn1a 308 KLAEELQGKEQELTLLLQTREKEVHDLEEQLLVTKISDQNYSKQVEELKTKLEEEKLKNAELTAS
hsscp1 455 KIAEELKGTEQELIGLLQAREKEVHDLEIQLTAITTSEQYYSKEVKDLKTELENEKLKNTELTSH
mmscp1 517 CDMLLLENKKFVQEASDMALELKKHQEDIINCKKQEERLLKQIENLEEKEMHLRDELESVRKEFI
rnscp1 521 SDMLLLENKKLVQEASDMVLELKKHQEDIINCKKQEERMLKQIETLEEKEMNLRDELESVRKEFI
hamsyn1a 373 CGKLSLENNKLTQETNDMALELKKYQEDITNSKKQEERMLKQIENLEEKETHLRDELESVRKEFI
hsscp1 520 CNKLSLENKELTQETSDMTLELKNQQEDINNNKKQEERMLKQIENLQETETQLRNELEYVREELK
mmscp1 582 QQGDEVKCKLDKSEENARSIECEVLKKEKQMKILESKCNNLKKQVENKSKNIEELHQENKTLKKK
rnscp1 586 QQGDEVKCKLDKSEENARSIEYEVLKKEKQMKILENKCNNLKKQIENKSKNIEELHQENKALKKK
hamsyn1a 438 QQGNEVKCKLDKSEENARSIECEVLKKEKQMKILENKCNNLRKQAENKSKYIEELHQENKALKKK
hsscp1 585 QKRDEVKCKLDKSEEN.....CNNLRKQVENKNKYIEELQQENKALKKK
mmscp1 647 SSAEIKQLNAYEIKVSKLELELESTKQRFEEMTNNYQKEIENKKISEGKLLGEVEKAKATVDEAV
rnscp1 651 SSAENKQLNAYEIKVNKLELELASTKQKFEEMINNYQKEIEIKKISEEKLLGEVEKAKATVDEAV
hamsyn1a 503 SSAESKQLNAYEIKVNKLQLELESAKQKFQEMTDNYQKEIEVKKISEEKLLGEVEKAKAMVDEAV
hsscp1 629 GTAESKQLNVYEIKVNKLELELESAKQKFGEITDTYQKEIEDKKISEENLLEEVEKAKVIADEAV
mmscp1 712 KLQKEIDLRCQHKIAEMVALMEKHKHQYDKIVEERDSELGLYKNREQEQSSAKIALETELSNIRN
rnscp1 716 KLQKEIDLRCQHKIAEMVALMEKHKHQYDKIVEERDSELGLYKNREQEQSSAKVALETELSNIRN
hamsyn1a 568 KLQKEIDLRCQHKIAEMVALMEKHKHQYDKIVEERDSELGLCKNREQEQLSVKTALETELSNIRN
hsscp1 694 KLQKEIDKRCQHKIAEMVALMEKHKHQYDKIIEERDSELGLYKSKEQEQSSLRASLEIELSNLKA
mmscp1 777 ELVSLKKQLEIEKEEKEKLKMA.KENTAILKDKKDKKIQASLLESPEATSWKFDSKTTPSQNISR
rnscp1 781 ELVSLKKQLEVEKEEKEKLKME.QENTAILTDKKDKKIQASLLESPEATSWKFDSKTTPSQNISR
hamsyn1a 633 ELVSLKKQLEIEREEKEKLKLE.KENTAILKDKKDKKIQTSLLESAETTCQKFDSKTTPSQNISR
hsscp1 759 ELLSVKKQLEIEREEKEKLKREAKENTATLKEKKDKKTQTFLLETPE.IYWKLDSKAVPSQTVSR
mmscp1 841 LSSSMDSGKSKDNRDNLRASAKSILPTTVTKEYTVKTPTKKSIYQRENKYIPTGGSNKKRKTAFE
rnscp1 845 LSSSMDSGKSKDNRDSLRASAKSILSTTVTKEYTVKTPTKKSIYQRENKYLPTGGSNKKRKTVFE
hamsyn1a 697 ISSSMESGKTKDNRDCLRTSAK.ILSTAFVKEYTVKTPTKMQMYQRENKYIPTGRSNKKRKTVFE
hsscp1 823 NFTSVDHGISKDKRDYLWTSAKNTLSTPLPKAYTVKTPTKPKLQQRENLNIPIEESKKKRKMAFE
mmscp1 906 FDVNSDSSETADLLSLVSEEDVSNRLY.DNNPPDSHLLVKTPKQTPLSLSTPASFMKFGSLKKMR
rnscp1 910 FDVNSDSSETTDLLSLVSEEDISNRIY.NNNTPDSHLLVKTPKQTPLSLSTPASFTKFGSLKKMR
hamsyn1a 761 FDVNSDSSETTDLLSMVSEEEISNRLY.NNNSPNSHL...TPKQTPLSLSTPESFVSLGGVRKMR
hsscp1 888 FDINSDSSETTDLLSMVSEEETLKTLYRNNNPPASHLCVKTPKKAPSSLTTPGPTLKFGAIRKMR
mmscp1 970 EDRWTTIAKIDRKRRLKEAEKLFS
rnscp1 974 EDRWATIAKIDRKRRLKEAEKLFT
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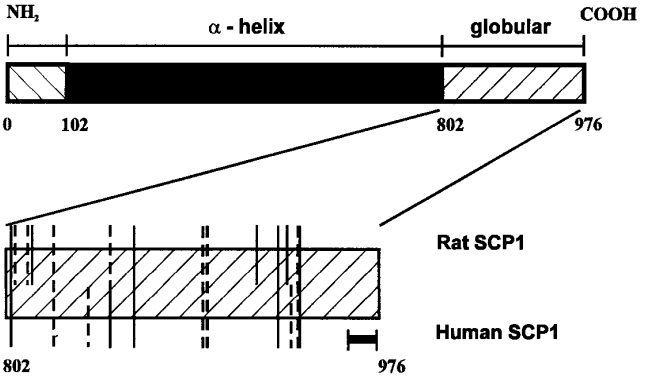

FIG. 3. Comparison of the localization of the S/T-P motifs in SCP1 from rat and human. A diagrammatic presentation of SCP1 is shown. The sizes of the three different domains are indicated by the amino acid residue numbers for hsSCP1. An enlargement of the C-terminal end of SCP1 is shown, in which the S/T-P motifs are indicated by solid vertical bars and the S/T-S/T motifs by broken vertical bars. The basic C-terminal domain is indicated by a horizontal bar.

Chou and Fasman (1978). In hsSCP1, the N-terminal domain includes amino acid residues 1–102. It is rich in acidic amino acids and does not contain any structural motifs. From position 102 to 802, hsSCP1 has a predicted amphipatic α-helical domain; within this domain hsSCP1 has a deletion of 21 amino acid residues compared to rodent SCP1s (Fig. 2). We found the corresponding deletion in three independently isolated cDNA clones, two of which originated from the human testis cDNA library in λgt11 and one from the cDNA library in λgt10. The deletion of 21 amino acids does not disturb the heptad repeat frame of the predicted amphipatic α-helix. The C-terminal domain of hsSCP1 extends from positions 803 to 976, is enriched in basic amino acids, and has a predicted p*I* of 9.8, compared with a p*I* of 5.9 for the entire hsSCP1 protein. Another feature of the C-terminal domain of hsSCP1 is the presence of 5 S/T-P and 7 S/T-S/T motifs. The S/T-P motifs are common in various DNA-binding proteins (Suzuki, 1989a; Churchill and Travers, 1991) and are believed to contribute to DNA binding (Suzuki, 1989b; Green *et al.*, 1993). S/T-S/T motifs can adopt a conformation similar to that of the S/T-P motif (Suzuki, 1989a). The localization of both motifs is shown in Fig. 3. Although the presence of S/T-P and S/T-S/T motifs is conserved in SCP1, their number and exact position are not. One S/T-S/T motif of rnSCP1 has turned into an S/T-P motif in hsSCP1, namely on position 850 of hsSCP1 (Fig. 3). Other amino acid sequence motifs that are conserved in the four SCP1 proteins analyzed in this paper and their amino acid positions in hsSCP1 are: two potential nuclear target sites at the positions 120–124 and 879–883 (consensus K-R/K-X-R/K, where X is any amino acid; Roberts, 1989); a $p34^{cdc2}$ kinase target site at positions 928–933 (Z-(S/T)-P-X-Z, where X is polar and Z is generally basic; Langan *et al.*, 1989); a leucine zipper motif (Landschulz *et al.*, 1988) at positions 391–419; a tyrosine kinase target site at positions 728–735 (R/K-X(2)-D/E-X(3)-Y, Cooper *et al.*, 1984); three cAMP/cGMP-dependent protein kinase target sites at positions 414–417, 627–630, and 671–674 (R/K(2)-X-S/T, Glass *et al.*, 1986), and 12 protein kinase C target sites (S/T-X-R/K, Kishimoto *et al.*, 1985) that are dispersed over the hsSCP1 protein. The small basic domain at the C-terminus in all four SCP1 proteins (hsSCP1 positions 948–976) shows 29% amino acid sequence identity with the DNA-binding domain of a protein-tyrosine phosphatase of the rat (Radha *et al.*, 1993). All SCP1 proteins show amino acid sequence similarity to several filamentous proteins like keratin or myosin, but this similarity does not exceed that expected on the basis of an amphipatic α-helical structure. No sequence homology to other proteins was detected.

Chromosomal Localization of the Human SCP1 Gene

Two-color fluorescence *in situ* hybridization was used to localize the human SCP1 gene to chromosome 1p12–p13 (Fig. 4). A biotinylated cDNA probe for hsSCP1 was detected with FITC, and a subtelomeric repeat probe for the short arm of chromosome 1 (D1Z2) (Buroker *et al.*, 1987) was labeled with digoxigenin and detected with TRITC. Analysis of 25 informative, DAPI-banded metaphases with two signals on both chromosomes 1 enabled us to localize SCP1 to chromosome 1p12–p13.

DISCUSSION

In this paper we describe the isolation of the cDNA encoding the human protein (hsSCP1) homologous to synaptonemal complex protein 1 of the rat (rnSCP1). The overall amino acid sequence identity of hsSCP1 to rodent SCP1 is about 75%. The three distinct domains that are found in rodent SCP1s also occur in hsSCP1. No domains can be discerned in SCP1 that are consid-

FIG. 2. Amino acid sequence alignment of the known rodent SCP1 proteins with the hsSCP1 protein. Alignments were performed by means of the Pile-Up program (GCG software package, University of Wisconsin, Madison, WI), and the results are presented using the Boxshade program (Bioinformatics Group, ISREC, Lausanne, Switzerland). Identical amino acids are highlighted in black; similar amino acids are highlighted in gray. The following amino acids were considered similar: (M, V, I, L), (D, E, Q, N), (S, A, T, G), and (H, R, K). Abbreviations: mmscp1, mouse SCP1 (Sage *et al.*, 1995); rnscp1, rat SCP1 (Meuwissen *et al.*, 1992); hamsyn1a, hamster SCP1 (Dobson *et al.*, 1994); hsscp1, human SCP1. The complete amino acid sequence of the hamster SCP1 is not known (Dobson *et al.*, 1994). The dots at the N-terminal part of the hamster SCP1 represent the unknown amino acid sequence. The remaining dots represent the gaps in the amino acid sequence alignment. The predicted amino acid sequence of rnSCP1 in this figure extends 51 amino acids further in the N-terminal direction than the originally published sequence (Meuwissen *et al.*, 1992). Due to a sequencing error, the codon CCC for a proline residue was read as CC. This resulted in a frameshift by which the first six amino acid residues of rnSCP1 were missed. Thereupon the start codon was erroneously located at amino acid position 52 of rnSCP1 (Sage *et al.*, 1995; Meuwissen, unpublished results).

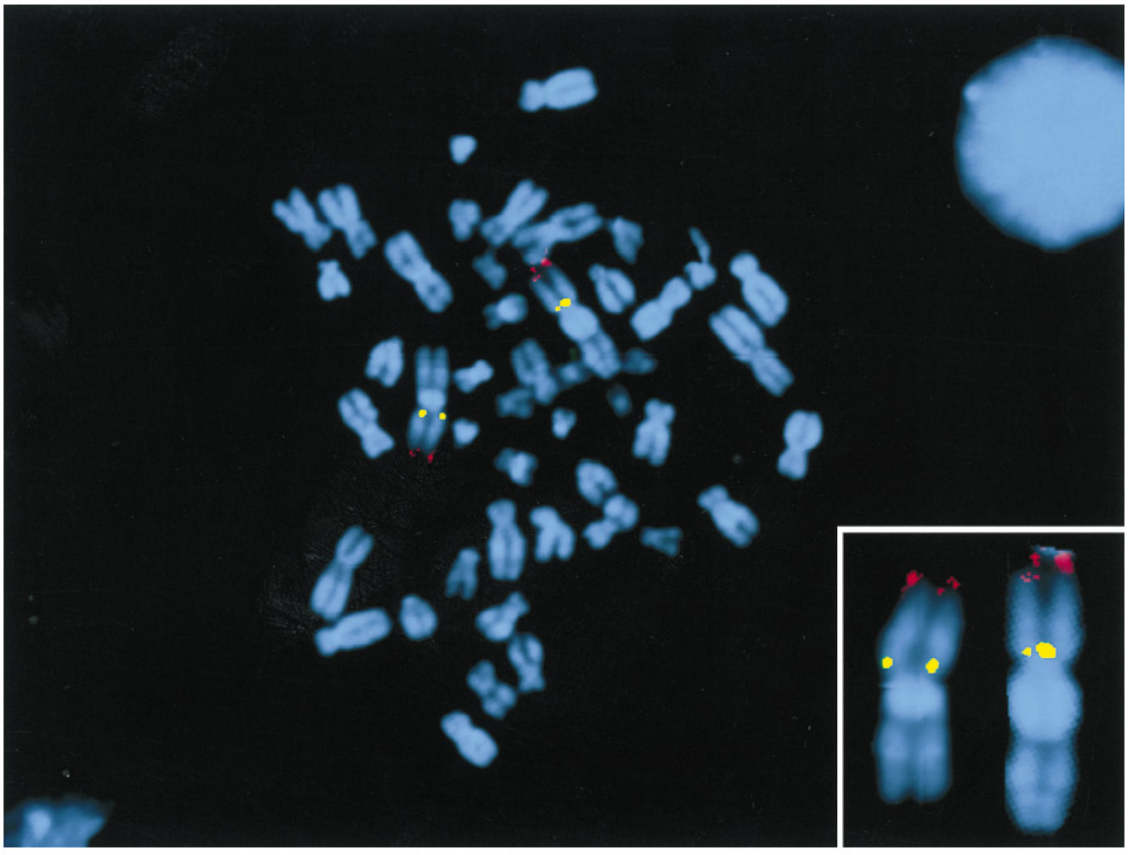

FIG. 4. Localization of the human SCP1 gene to chromosome 1p12–p13. Metaphase chromosomes from human lymphocytes were hybridized with a mixture of two probes: a biotin–dUTP labeled probe derived from hsSCP1 cDNA (detected with FITC, yellow pseudo-color and a digoxigenin–dUTP labeled human subtelomeric repeat probe D1Z2 (detected with TRITC, red pseudo-color). The chromosomes were counterstained with DAPI (blue). We considered the presence of a paired signal as a positive localization. (Inset) Enlargement of the individual chromosomes #1 of the same metaphase.

erably more strongly conserved than the protein as a whole. The C-terminal domain is somewhat less well conserved than the whole SCP1 molecule: 64% versus 75% amino acid identity. Because no other human cDNAs encoding proteins homologous to rnSCP1 were found, despite extensive screening with anti-rnSCP1 antibodies and probes derived from the cDNA for rnSCP1, we believe that hsSCP1 is the human functional homolog of rnSCP1. This is further supported by the fact that various motifs have been conserved. With respect to the S/T-P and S/T-S/T motifs, it appears as if the presence of these motifs in the C-terminal domain is important, but their exact position is not. hsSCP1 has five S/T-P and seven S/T-S/T motifs, whereas rnSCP1 has seven S/T-P and seven S/T-S/T motifs. Only nine of these motifs are at exactly corresponding positions. The S/T-P and S/T-S/T motifs are thought to cause β-turns in peptide chains so that these chains get a "kinky" conformation; such chains are thought to fit into the minor groove of DNA and to make contact with the phosphoribose backbone at the β-turns (Suzuki, 1989a; Churchill and Suzuki, 1989). The precise positions of the S/T-P and S/T-S/T motifs are not very crucial for that (Suzuki, 1989a). Some S/T-S/T motifs in rnSCP1 are S/T-P motifs in hsSCP1 (e.g., at position 850 in hsSCP1), which indicates that the presence of the β-turn is important. *In vitro,* the C-terminus of rnSCP1 binds to DNA (Meuwissen *et al.*, unpublished experiments).

Another conserved amino acid sequence motif that deserves attention is the $p34^{cdc2}$ protein kinase target site. This site also occurs in nuclear lamins A and C, where it is involved in the regulation of the disassembly of the nuclear lamina at mitosis (Heald and McKeon, 1990). In budding yeast (*Saccharomyces cerevisiae*), mutations of *CDC28*, which is the gene equivalent to *cdc2* of fission yeast (*Schizosaccharomyces pombe*), results in a block in the pachytene stage of meiosis: SCs are not disassembled in these mutants (Davidow and Byers, 1984; Shuster and Byers, 1989). Two other conserved potential phosphorylation sites are the cAMP/cGMP dependent protein kinase (PKA) target sites. Inhibition of phosphorylation by PKA is important for the disassembly of the nuclear lamina at mitosis (Lamb, 1991). It will therefore be of interest to discover if the potential $p34^{cdc2}$ and PKA phosphorylation sites in SCP1 are phosphorylated *in vivo.*

Mutation of SCP1 will most probably result in defects in meiosis, such as chromosomal nondisjunction (Sym *et al.*, 1993; Sym and Roeder, 1994) and infertility. As

yet, no human phenotypes have been identified that are linked with defects in meiosis and that are correlated with chromosomal abnormalities in human chromosome 1p12–p13 (Weith *et al.*, 1996). Further analysis of the human 1p12–p13 chromosomal area together with mutational analysis of SCP1 in rodents should provide more information about the meiotic function of SCP1.

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