Synaptonemal Complexes from DNase-Treated Rat Pachytene Chromosomes Contain $(GT)_n$ and LINE/SINE Sequences

Ronald E. Pearlman, Nora Tsao and Peter B. Moens

Department of Biology, York University, Downsview, Ontario, Canada M3J 1P3

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ABSTRACT

Purified chromosome cores (synaptonemal complexes) of rat pachytene chromosomes, from which the chromatin is removed by extensive DNase II digestion, retain a residual class of DNA, presumably the bases of chromatin loops. This synaptonemal complex-associated DNA, isolated by proteinase digestion and phenol extraction of purified DNase-treated synaptonemal complexes, and cloned in plasmid vector pEMBL18, has a length distribution of 50–500 bp. From a library of these fragments, 21 fragments were sequenced. Present in this sample are short 40–200-bp segments with >80% identity to "long" and "short" interspersed repeated elements (LINE/SINEs), an excess of GT/CA tandem repeats and a number of unidentified sequences. The LINE/SINE segments may play a role in homology *vs.* nonhomology recognition during meiosis and the alternating purine-pyrimidine sequences have been implicated in genetic recombination. Their enrichment in synaptonemal complexes may be related to the synapsis and recombination functions of meiosis.

AT pachytene of meiotic prophase, the synaptonemal complex (SC) exists at the interface of homologously paired chromosomes and it is the attachment site of the chromatin loops (see Figure 1). If SC-associated DNA is to be characterized, the SCs must first be separated from the nuclear matrix. Conventional nuclear isolation and extraction procedures, when applied to pachytene spermatocytes, cause the SCs to become an "integral" part of the nuclear matrix (IERARDI, MOSS and BELLVÉ 1983). Thus the DNA isolated from high salt and DNase-extracted rat spermatocyte nuclei and characterized by LI *et al.* (1983) probably consists mostly of matrix-associated DNA rather than SC-associated DNA.

Methods to isolate SCs from other nuclear components by cell lysis and DNase digestion in the absence of Mg^{2+} ions were reported by HEYTING *et al.* (1985). Subsequently, we reported that such SCs, although devoid of surrounding chromatin, still contain a fraction of DNA that is apparently protected from DNase digestion. That DNA fraction can be isolated only after proteinase digestion and phenol extraction of the SCs (MOENS and PEARLMAN 1988b). With these SC isolation and proteinase digestion methods, KARPOVA *et al.* (1989) have shown by Southern blot analysis that the SC-associated DNA is enriched in GT/CA sequences and in long interspersed repeated sequences.

The Southern blot analysis of SC-associated DNA is restricted by the choice of probes. To obtain a more precise representation of SC-associated DNA sequences, we have cloned and sequenced DNA fragments from DNase-treated, proteinase-digested and phenol-extracted rat pachytene spermatocyte SCs. We confirm the presence of GT/CA repeats and "long" and "short" interspersed repetitive elements (LINE/SINEs). The SC-associated DNA LINE/SINEs, however, are unusually short segments flanked by unrelated sequences. The presence of 5 clones with GT/CA repeats in about 10 kbp of sequenced SC-associated DNA fragments suggests a 25-fold enrichment over an average of one GT/CA segment per 50 kbp genomic DNA in mammals (HAMADA, PETRINO and KAKUNAGA 1982). These SC-associated DNA sequences and additional, unidentified sequences, bear no obvious resemblance to the scaffold- or matrix-associated regions of DNA (SAR/MARs) that have been isolated from extracted mitotic chromosomes or nuclei (IZAURRALDE, MIRKOVITCH and LAEMMLI 1988; COCKERILL, YUEN and GARRARD 1987; BLASQUEZ *et al.* 1989). The lack of similarity may reflect the meiosis-specific functions of pachytene chromosomes. The LINE/SINE segments may play a role in homology *vs.* nonhomology recognition (RADMAN 1989) during meiosis, and the alternating purine-pyrimidine sequences have been implicated in genetic recombination (reviewed by BLAHO and WELLS 1989).

MATERIALS AND METHODS

SC isolation: Rat SCs were isolated according to HEYTING *et al.* (1985). Briefly, spermatocytes were purified by centrifugal elutriation and by density centrifugation in Percoll, then lysed in Tris buffer with Triton X-100, EDTA and protease inhibitors. The nuclear pellets were incubated in 8 mM Tris-HCl buffer, pH 7, with 800 Kunitz units of DNase II per 1×10^8 nuclei for 30 min, and then an additional 800 units of enzyme were added. Incubation continued for

30 min. Following DNase II incubation, the SCs were collected by centrifugation through 1.5 M sucrose.

The methods for immunocytology of surface-spread pachytene chromosomes have been reported in detail for topoisomerase II antibodies and preimmune controls (MOENS and EARNSHAW 1989).

Isolation of SC-associated DNA: We designate the DNA extracted from DNase-treated, proteinase-digested, phenol-extracted SCs operationally as "SC-associated DNA" without implying that it is unique to SCs. SCs from three rats were dialyzed against TE (10 mM Tris HCl, pH 8.0, 1 mM EDTA) to remove sucrose, and digested in 20 mM EDTA and 0.5% sodium dodecyl sulfate (SDS) with proteinase K at a final concentration of 50 μg/ml for 1 hr at 65°. DNA was extracted once with phenol saturated with TE and three times with chloroform. DNA was precipitated with two volumes of 95% ethanol, washed with 70% ethanol and dried under vacuum at room temperature. For size estimation, this SC-associated DNA was electrophoresed through a 2% agarose gel and was detected by ethidium bromide staining. The size standard was *Hin*fI-digested pBR322 DNA.

Cloning and nucleotide sequence analysis of SC-associated DNA: Restriction endonucleases and DNA-modifying enzymes were purchased from a variety of suppliers (GIBCO/BRL; Pharmacia; Boehringer Mannheim; New England Biolabs; US Biochemical Corp.; Amersham) and used according to the distributor's instructions. Recombinant DNA manipulations were done according to SAMBROOK, FRITSCH and MANIATIS (1989). SC-associated DNA was treated with the large (Klenow) fragment of DNA polymerase I from *Escherichia coli* and all four unlabeled dNTPs to ensure all termini were completely filled in. This DNA was ligated into *Sma*I-digested pEMBL18 (DENTE, CESARENI and CORTESE 1983), transformed into JM 101 cells (MESSING, CREA and SEEBURG 1981) and grown on plates containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal). Miniplasmid DNA prepared by the method of BIRNBOIM and DOLY (1979) was isolated from white, ampicillin-resistant colonies and analyzed by restriction endonuclease digestion. Single-strand DNA was prepared by superinfection of plasmid-carrying strains with IR1 phage (DENTE, CESARENI and CORTESE 1983). Nucleotide sequence analysis was by the dideoxy method (SANGER, NICKLEN and COULSON 1977) using the 17-bp universal primer and modified T7 DNA polymerase (Sequenase, version II, US Biochemical Corp.) according to the suppliers' directions.

High molecular weight DNA from rat liver was sonicated until the size distribution of the majority of the fragments was between 100 and 800 bp. This DNA was treated exactly as the SC-associated DNA to prepare a library and determine nucleotide sequences.

Hybridization analysis: Colonies from the SC-associated DNA or from sonicated rat DNA libraries were grown, transferred to nitrocellulose filters (Amersham), denatured, and hybridized according to GRUNSTEIN and HOGNESS (1975) as described in SAMBROOK, FRITSCH and MANIATIS (1989). Filters were washed three times at room temperature in 0.5 × SSC (1 × SSC is 0.015 M sodium citrate, 0.15 M NaCl, pH 7), 0.1% SDS and once in the same solution at 60°, dried and exposed to Kodak XAR film at −70°, using two Quanta III intensifying screens. Probes were labeled with [^{32}P]dATP using the random priming protocol of FEINBERG and VOGELSTEIN (1983). Unincorporated nucleotides were removed from the probe by centrifugation through Sephadex G25 or G50 (SAMBROOK, FRITSCH and MANIATIS 1989).

Analysis of nucleotide sequence data: The complete nucleotide sequence data base in the GenBank or EMBL libraries were searched for alignment with SC-associated DNA or sonicated rat DNA sequences using a FASTA search according to PEARSON and LIPMAN (1988). Both complementary strands of all sequences were analyzed in this way.

***In situ* hybridization:** The localization of $(GT)_n$ and LINE sequences in the mouse pachytene chromosome was investigated with *in situ* hybridization and with the "primed *in situ* labeling" (PRINS) technique of KOCH *et al.* (1989). The methodology has been reported previously (MOENS and PEARLMAN 1990). Briefly, for *in situ* hybridization surface-spread pachytene spermatocytes on coverslips were fixed in 1% paraformaldehyde, denatured at 70° in 70% formamide and hybridized with denatured biotinylated probe (PM13, SCA7; see Figure 4) at 37° overnight. Following washes the cells were incubated with streptavidin conjugated to fluorescein isothiocyanate (FITC). For *in situ* labeling, the primer $(GT)_{10}$, biotinylated nucleotides, and DNA polymerase (Klenow fragment) were added to the denatured spread spermatocytes and incubated at 43° for 30 min, followed by washes and incubation with streptavidin-FITC. The FITC signal and 4,6-diamidino-2-phenylindole (DAPI)-stained chromosomes were observed and recorded with epifluorescence microscopy.

RESULTS

Evidence for SC-associated DNA: At meiotic prophase each bivalent (a set of paired homologous chromosomes) contains an axial structure, the SC, which consists of the parallel aligned chromosome cores to which the chromatin loops are attached (Figures 1 and 3a) (WEITH and TRAUT 1980). The DNA component of the bivalents is visualized with fluorescent microscopy of the DNA binding dye, DAPI (Figure 2a), and by indirect immunostaining with anti-DNA antibody (Figure 2b). In both figures, the axial elements are intensely stained relative to the surrounding chromatin loops, a general indication for the presence of DNA in association with the SC.

More direct evidence for the existence of SC-associated DNA is contributed by the presence of DNA in DNase-treated SCs. Following isolation and DNase treatment of meiotic prophase chromosomes, essentially naked SCs, devoid of chromatin, are collected in the pellet by centrifugation through 1.5 M sucrose (Figure 3a). The lack of chromatin surrounding the SC, and the presence of DNA associated with the SC itself is demonstrated in Figure 3, b and c, by staining of the isolated SCs with DAPI and anti-DNA antibody, respectively.

The most direct evidence for the existence of SC-associated DNA is provided by the extraction of 50–500-bp DNA fragments from the SCs in the pellet, whereas the supernatant from the centrifugation following DNase II digestion has no detectable oligonucleotides. Thus, under conditions of complete chromatin digestion, the SCs retain a fraction of DNA that is protected from DNase digestion.

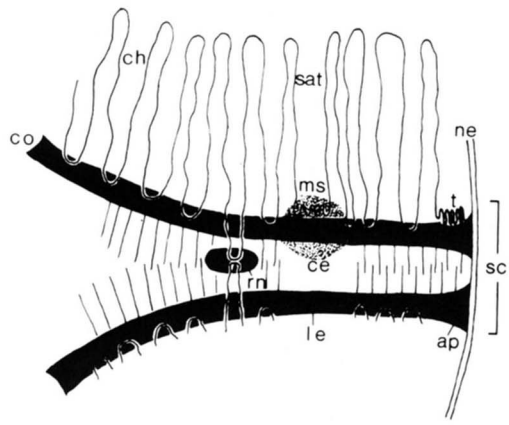

FIGURE 1.—Diagram of a synaptonemal complex with associated chromatin to illustrate SC terminology. During meiotic prophase the two chromatids (only one drawn) of a chromosome become associated with a structurally well defined core, *co*, which is attached to the nuclear envelope, *ne*, at the attachment plaque, *ap*. The cores of a pair of homologous chromosomes synapse to form the parallel aligned lateral elements, *le*, of the synaptonemal complex, *sc*. Following SC isolation procedures and DNase treatment, the chromatin loops, *ch*, are digested but a DNase-resistant DNA fraction remains associated with the SC as illustrated in the lower lateral element. It is this fraction that is characterizsed in this report. Previously, it was shown that rat satellite 1 DNA, *sat*, occurs in the chromatin loops of the centromeric region. In the mouse, a minor satellite, *ms*, is present in the centromere, *ce*. Telomere DNA, *t*, was located at the attachment plaques. These classes of DNA have so far not been recovered from isolated SCs. The presumed involvement of the SC in recombination is depicted by a recombination nodule, *rn*, where it is found in *Allium fistulosum*, next to the centromere, coincident with the localized position of the chiasma.

SC-DNA cloning and characterization: Electrophoresis through a 2% agarose gel of SC-associated DNA released from purified SCs by proteinase digestion and phenol extraction, reproducibly shows a size distribution of fragments of approximately 50–500 bp (results not shown). Libraries were prepared from two separate isolates of SC-associated DNA by cloning into the *Sma*I site of pEMBL18. For further analysis, 49 clones were selected at random. For sequencing, 19 clones were selected at random from the 49 clones, and 2 clones from the remaining 30 were selected by positive hybridization with a probe of total rat DNA made radioactive by random priming. The nucleotide sequences of 21 SC-DNA fragments ranging in size from 63 to 438 bp were determined and the sequences of both complementary strands were computer aligned with all sequences present in the GenBank or EMBL libraries. A summary of these data is presented in Figure 4. Thirteen of the 21 sequenced fragments contain elements of characterized repetitive sequence families. Of these, five are of the LINE type and two of the SINE type. Four sequences contain $(GT)_{14-26}$ tandem repeats, one has a tandemly repeated $(GCGT)_6$ element immediately 5′ to tandem repeats of GT, and one contains the sequence element $(GGGA)_{13}$. Eight sequences do not contain any obvious internal repeats nor do they show any significant similarity with any sequence in the data library. In no case does the repeat element, including LINE or SINE segments, extend the full length of the SC-DNA fragment.

The extent and location of the LINE fragments recovered from the SC-associated DNA are diagrammed in the context of *RAT LINE 3A* and *4A* prototype sequences (D'AMBROSIO *et al.* 1986) in Figure 5. The names of the fragments are the numeral part of the names in Figure 4. The short tails on one or both sides of the fragment indicate the presence of non-LINE sequences. Fragments 61 and 64, obtained from sonicated genomic DNA (see below), do not have tails. Fragment SCB9 has two LINE elements in opposite orientation from different regions of *LINE 3A* which are indicated by 9a and 9b in Figure 5. When the SC-associated DNA LINE fragments are used to search the GenBank and EMBL libraries, they typically recognize *RAT LINE 3A* and *4A* elements with high homology scores as well as LINE elements associated with genes such as *CYP2A1* and *2A2* (MATSUNAGA *et al.* 1990) (Figure 5). All of the elements found in the library are much longer than the matching portion of the SC-associated DNA LINE fragment. For example, the LINE element in intron 8 of *CYP2A1* is 1253 bp in length, and contains four segments with homologies to SC-associated DNA fragments. Incidentally, the same LINE element is not present in *CYP2A2* which has a shorter eighth intron.

The occurrence of GT/CA repeats in *RAT LINE 4A* and *CYP2A1* are also shown in Figure 5. In the second intron of *CYP2A1*, there is a region of complex short repeats; $(CT)_{19}(\mathbf{CA})_{13}[CAAA(\mathbf{CA})_5]_6$. The same repeats are not present in *2A2*, but 1.5 kbp of the second intron was not sequenced. *RAT LINE 4A* has a $(\mathbf{GT})_{16}$ repeat at the 3′ end about 80 bp downstream of the poly A tail.

Of the sequences recovered from the SCs and recognized in the data bases, the (GGGA) repeat is the only one that occurs in transcribed regions. The (GGGA) repeat is found in the rat rDNA spacer regions and in the exons of a large number of genes, frequently in conjunction with GA repeats.

Genomic DNA fragment cloning and characterization: If, following DNase digestion, nucleotide sequences had been recovered from both the SC pellet and the supernatant, a direct comparison could have been made between the DNA sequences associated with the SCs and the residual sequences of the chromatin. Since digestion of the chromatin was complete, SC-associated DNA sequences could not be compared directly with genomic DNA of the same preparation. To obtain an estimate of the occurrence of SC-associated DNA type sequences in the genome, high molecular weight rat liver DNA was sonicated to yield a

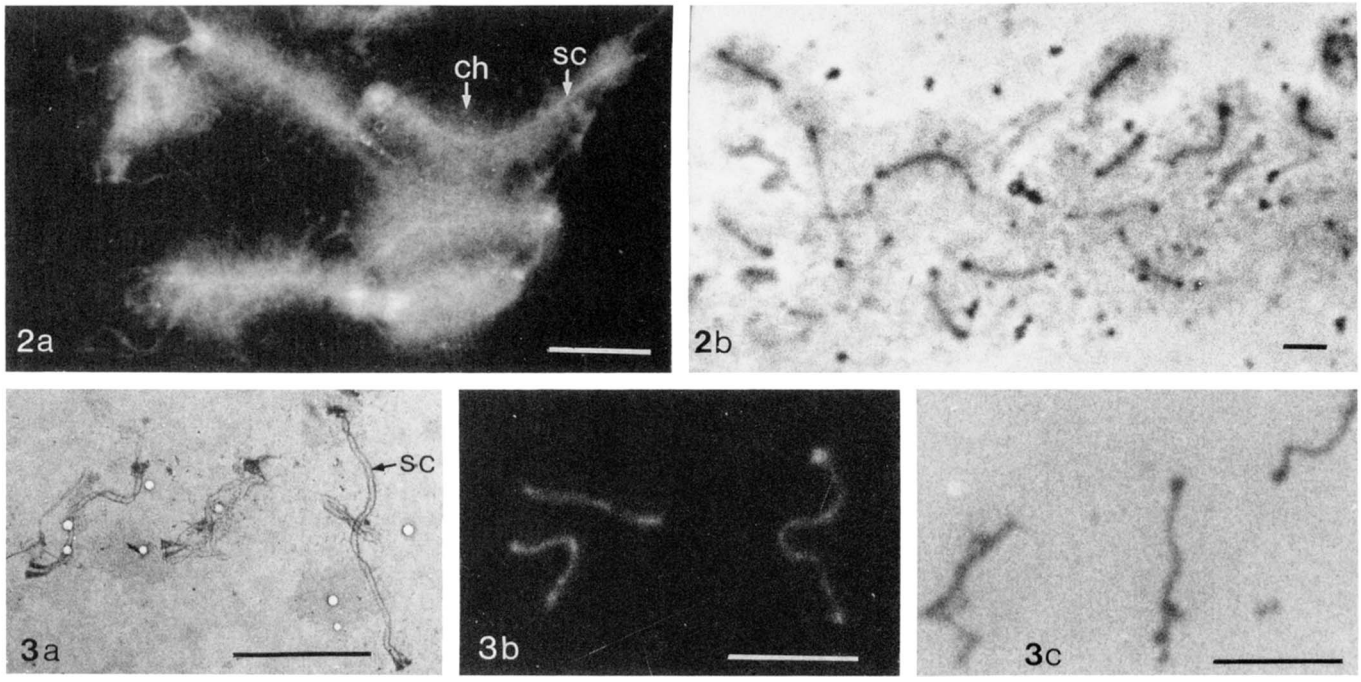

FIGURE 2.—Surface-spread rat pachytene chromosomes. (a) Fluorescent image of chromosomes stained with the DNA-binding dye, DAPI. The chromatin loops, *ch*, and the axial elements, *sc*, fluoresce brightly against the dark background. (b) The chromosomes were incubated with anti-DNA antibody and the antigen visualized with diaminobenzidine coloring of the secondary peroxidase-conjugated antibody. The DNA of the chromatin loops and of the axial element are stained dark against a light background. Bars represent 5 μm.

FIGURE 3.—Isolated SCs. (a) Electron micrograph of a purified, DNase-treated SC preparation, *sc*. The parallel aligned cores of the paired homologous chromosomes are evident. There is little chromatin adhering to the SCs. (b) Fluorescent image of DAPI-stained, purified SCs. The fluorescence indicates the presence of residual DNA associated with the SC after extensive DNase treatment. (c) The positive reaction of the purified SC with anti-DNA antibody further demonstrates the absence of chromatin and shows the presence of residual DNA in these structures. Bars represent 5 μm.

Name	Type	% ID	Structure	Accession #
SCB9	LINE	94,85	0 59 - LINE - 272 320 - ENIL - 360	X61782
SCB10	LINE	80	0 80 - LINE - 240 438	X61783
PM13	LINE	94	0 - LINE - 46 156	X61793
SC3	LINE	86	0 161 - LINE - 366	X61772
SC8	LINE	97	0 28 - LINE - 140 - TCTCTCACTCACTCACT - 158 187	X61774
PM11	SINE		0 - SINE - 80 198	X61792
SCC12	SINE		0 30 - SINE - 120 330	X61790
SCB9I	GT		GTAT$(GT)_7$AT$(GT)_{17}$CCGTA$(GTGC)_3$ - 72 186	X61785
SCA7	GT		0 97 - $(GT)_{22}$ - 142 194	X61778
SCB4I	GT		0 170 - $(GT)_{26}$ - 223 233	X61784
SC18	GT		0 45 - $(GT)_{14}$ - 74 - GCGCGTGC	X61776
SCD1	GTGC		TTAGTGCCTATGTGCAT$(GCGT)_6$GTGTCTGTGTGT - 54 277	X61791
SCB2	GGGA		0 63 - $(GGGA)_{13}$ - 115	X61780
8 SC-associated DNA fragments with no significant similarity				*
			RANDOM GENOMIC 50-500 NT SEQUENCES	
Rat 64	LINE	88	0 . . . . . LINE 250	X61907
Rat 61	LINE	90	0 . . . . . LINE 96	X61906
Rat 74	SAT I		0 . . . . . SAT 163	X61913
10 fragments			no significant similarity	**

FIGURE 4.—A summary of 21 nucleotide sequences of DNA from purified synaptonemal complexes (SC-associated DNA). From a sample of cloned genomic fragments generated by sonication of rat liver DNA, 13 were sequenced. The sequences were compared with all sequences of the EMBL and GenBank libraries. Numbers represent nucleotide position arbitrarily designating one end of the fragment as zero. % ID: percentage identity relative to *RAT LINE 3A*.

*Accession numbers X61773, X61775, X61779, X61781, X61786 → X61789.

**Accession numbers X61901 → X61905, X61908 → X61912.

size distribution of fragments similar to that of SC-DNA fragments. These were cloned into the *Sma*I site of the pEMBL18 vector. Gel electrophoretic analysis of 27 of these fragments confirmed a size distribution between 100 and 500 bp.

The nucleotide sequence of 13 of these sonicated genomic fragments were determined and aligned with all sequences in the GenBank and EMBL data libraries. Two matches to LINE type sequences, *RAT 61* and *64* (Figure 5) and one to rat satellite I DNA were found (Figure 4). The genomic LINE fragments were notably different from the SC-associated DNA LINE fragments. Whereas the genomic LINE fragments consist entirely of LINE sequences, the SC-associated DNA fragments have only truncated, short, 100–200-bp LINE sequences and are mostly flanked by non-

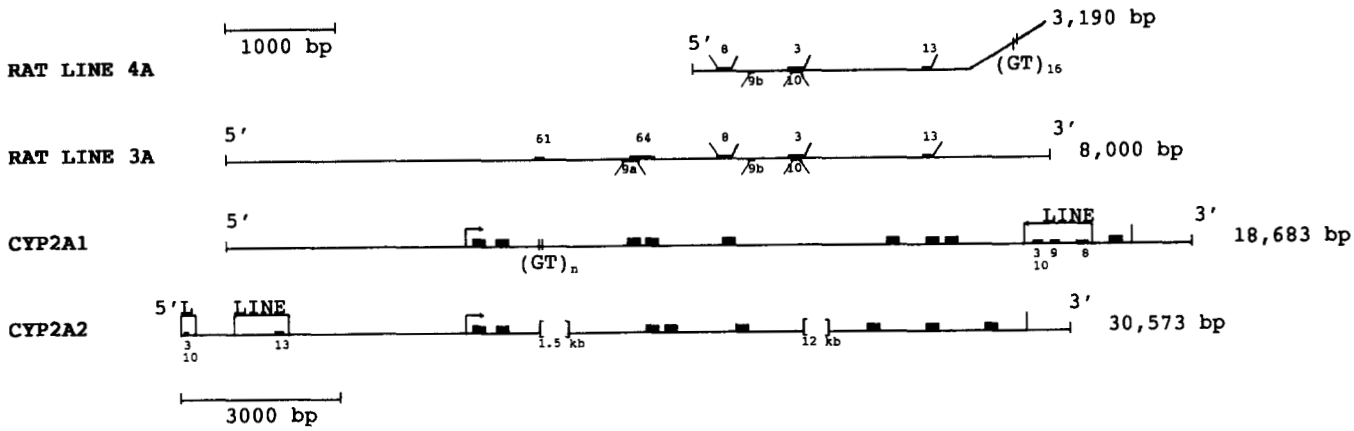

FIGURE 5.—Distribution of isolated LINE fragment and (GT)$_n$ sequences (Figure 4). All SC-associated DNA LINE fragments have homology to regions of the 7-kbp RAT LINE 3A sequence and some have homology to the 5′-truncated RAT LINE 4A sequence. The fragments are the small solid blocks and are numbered according to the numeral part of the name in Figure 4. The non-LINE sequences that flank the SC-associated DNA LINE fragments are indicated by slanted tails on one or both sides of the fragment. Fragments 61 and 64 were obtained from sonicated genomic DNA and do not have non-LINE flanking sequences. The portions of the LINE and (GT)$_n$ sequences are illustrated for two arbitrarily chosen genes, CYP2A1 and CYP2A2. The solid blocks represent CYP exons. The genomic LINE elements as present in the CYP genes are longer than those recovered from the SC-associated DNA and may contain one or more sites homologous to SC-associated DNA LINE fragments.

LINE sequences. The remaining 10 genomic sequences showed no significant similarity with sequences in the data libraries nor did they contain any obvious internal repetitive elements.

Hybridization: To further compare the SC-associated DNA clones with the genomic clones, they were hybridized *in situ* with random primed labelled SC-associated DNA and with labeled genomic DNA. These labeled probes should hybridize only to colonies containing abundant repetitive sequences which are also present in the probes. The same four of the 49 SC-associated DNA clones hybridized *strongly* with the SC-associated DNA and the genomic probes (weakly hybridizing clones were not sequenced). These four clones contained tandemly repeated (GT)$_n$ sequences where $n > 13$. Clone SCD1, which contains few GT repeats, did not hybridize strongly with either of the probes. None of the 27 genomic clones hybridized strongly with either probe. This is consistent with the absence of (GT)$_n$ repeats in the 13 sequenced genomic clones. Although these numbers are too small for statistical comparison, they suggest a higher frequency of (GT)$_n$ repeats in the SC-associated DNA than in the genomic DNA. In general, the recovery of one short and four long (GT)$_n$ segments in about 12.5 kbp of 49 random SC-associated DNA clones is well in excess of one (GT)$_n$ sequence per 30–100 kbp in mammalian genomic DNA (HAMADA, PETRINO and KAKUNAGA 1982). These observations suggest an enrichment of (GT)$_n$ sequences in SC-associated DNA as compared to genomic DNA.

Sequence localization: *In situ* hybridization gave no conclusive results on the localization of (GT)$_n$ or LINE sequences in pachytene chromosomes. On the assumption that the sequences were too short and too far interspaced to give a detectable signal with *in situ* hybridization, an alternative method, primed *in situ* labeling (PRINS) was attempted with a (GT)$_{10}$ oligonucleotide. Previously, a 17-bp sequence from a centromeric mouse minor satellite had produced a clear centromeric signal with the PRINS method (MOENS and PEARLMAN 1990). The PRINS procedure with (GT)$_{10}$ as primer on rat pachytene chromosomes results in bright fluorescence from the SCs and general fluorescence of the chromatin. However, this cannot be taken as a confirmation of the (GT)$_n$ enrichment in the SC. In the absence of (GT)$_{10}$ primer, a similar pattern develops, be it less bright. Technical refinement of the PRINS method is required for more definitive conclusions.

DISCUSSION

In an earlier attempt to characterize rat SC-associated DNA, LI *et al.* (1983) digested spermatocyte nuclei with DNase I in the presence of 3 mM $MgCl_2$ and removed histones from those nuclei with 2 M NaCl. The authors noted that the residual structures have SCs as well as membranes and matrix, and contain 1–2% of the genomic DNA. No special characteristics could be detected for the DNA isolated from the residual structure as compared to genomic DNA. Subsequently, HEYTING *et al.* (1985) succeeded in the large-scale separation of SCs from other nuclear components. These purified, DNase-treated SCs were found to retain a residual fraction of DNA detectable by DNA staining dyes, immunocytology, and DNA isolation from proteinase-digested SCs (MOENS and PEARLMAN 1988a,b; this report). Dot blot hybridization analysis of mouse SC-associated DNA obtained using similar methods (0.5% of nuclear DNA) (KAR-

POVA *et al.* 1989), showed no difference between genomic and SC-associated DNA with a β-globin gene probe, but did show a 1.5–2× enrichment of GT/CA sequences and a 1.4–2× enrichment of long interspersed repeated B_1 sequences in SC-associated DNA. To clarify in more detail the structure of SC-associated DNA, we undertook the sequencing of SC-associated DNA fragments and random fragments of sonicated rat liver genomic DNA.

From a library of cloned SC-associated DNA fragments, 49 were chosen at random. For sequencing, 19 clones were selected at random from the 49 clones and of the remaining 30, two clones were selected by positive hybridization with a total rat genomic DNA probe. In the first sample of 19 clones, 3 contained (GT) repeats. An additional two GT-containing fragments were identified in the remaining 30 clones by colony hybridization and they were also sequenced. Since the SC-associated DNA fragments are on average 250 bp long, the 49 clones represent about 12.5 kbp of DNA recovered from SCs. Of these, 5 are GT rich (Figure 4): an average of 1 $(GT)_n$ region per 2.5 kbp. In mammals, with a haploid genome size of 3×10^9 bp, HAMADA, PETRINO and KAKUNAGA (1982) report approximately 5×10^4 copies of GT/CA for the human genome, 3×10^4 for calf and 1×10^5 for mouse. In the absence of published measurements on the rat genome we assume an intermediate value of 5×10^4 copies for the rat, an average of 1 copy per 60 kbp. Thus, the SC-associated DNA appears to be 24× enriched in GT/CA sequences. The CYP and LINE elements in Figure 5, which were selected for a different parameter (homology to SC LINE fragments), have 2 GT repeats in 45 kbp. None of the 27 genomic clones, about 6.75 kbp, had GT repeats. It is unlikely that the excess of GT repeats in the SC-associated DNA is an artifact of the isolation procedures for several reasons: (1) The supernatant from the DNase-digested pachytene chromosomes did not have residual GT/CA fragments or other oligonucleotides, (2) DNase II has not been shown to preferentially generate GT/CA fragments (DOSKČ and SORM 1961) and (3) The "alternating-*B*" conformation of the GT/CA tandem repeats does not cause preferential excision of those repeats by S_1 nuclease (GROSS, HUANG and GARRARD 1985). The fact that GT repeats as well as LINE sequences and unidentified fragments were recovered from the SCs further argues against an isolation artifact.

The recovery of GT/CA repeats from SCs is in contrast to the type of MAR/SAR sequences from somatic nuclear matrixes and chromosome scaffolds. Since the MAR/SARs are suggested to regulate transcriptional activity of chromatin loop domains (STIEF *et al.* 1989), the lack of MAR/SARs in the SC-associated DNA would imply that pachytene chromatin loops are formed by different mechanisms and serve different functions. In the context of meiosis, the GT repeats are of interest because the repeats and the SCs both show correlations with genetic recombination. The repeats have been found near sites of somatic and germ line recombinants and the positions of the repeats are evolutionarily conserved (BULLOCK, MILLER and BOTCHAN 1986; BOEHM *et al.* 1989; METZENBERG *et al.* 1991). The distribution of the repeats among chromosomes correlates positively with the frequency of meiotic recombination in *Drosophila* species (LOWENHAUPT, RICH and PARDUE 1989), and in *Saccharomyces cerevisiae*, meiotic reciprocal recombination is enhanced by the insertion of GT/CA repeats on both homologs (TRECO and ARNHEIM 1986). The proposed mechanisms whereby the sequences exert their recombinogenic effects vary, and include the consequences of Z-DNA formation (BLAHO and WELLS 1989), binding of the rec 1 protein to Z-DNA (KMIEC and HOLLOMAN 1984), the preferential binding of topoisomerase II to purine-pyrimidine repeats (SPITZNER, CHUNG and MULLER 1989), and the consequences of the "alternating-*B*" conformation of GT/CA tracts (GROSS, HUANG and GARRARD 1985). The correlations between the GT/CA repeats and recombination suggest that they may serve, in an as yet unspecified manner, recombinational functions in the context of the SC. However, the GT repeats are not likely to be the only factor. Other sequences such as poly(dA·dT) tracts enhance meiotic gene conversion in yeast (SCHULTES and SZOSTAK 1991), and a 300-bp heterologous sequence creates a hotspot for meiotic reciprocal recombination and gene conversion in yeast (STAPLETON and PETES 1991).

Seven of the 19 SC-associated DNA clones that were chosen at random and sequenced, contain elements homologous to interspersed repeated sequences, five with homology to LINEs, and two with homology to SINEs (D'AMBROSIO *et al.* 1986; HUTCHISON *et al.* 1989; DEININGER 1989). As with the GT repeats, these elements have no homology to MAR/SARs sequences and they further indicate that chromatin loop organization at meiosis is different from that in somatic cells. The estimated 40,000 copies of the LINE 1 family which have an average length of 6.7 kbp make up about 10% of the rat genome (D'AMBROSIO *et al.* 1986). The incomplete LINEs are usually truncated at the 5′-end. The SC-associated LINE/SINE sequences are of a different type in that the average length of fragments that are bordered by non-LINE/SINE sequences is only 120 bp in length, and they are not 5′-truncated segments.

The interspersion of very short LINE fragments with non-LINE DNA is probably a real characteristic of SC-associated DNA rather than a cloning artifact arising from the ligation of several small fragments,

four in the case of SCB9. If such ligations were common, we would have found cloned inserts longer than 500 bp. As well, the genomic library, cloned with identical procedures, would have had similar ligations but none was found (Figure 4).

Because the biology of LINE/SINEs is incompletely understood, there are relatively few speculations that relate their presence to possible meiotic functions. Genomes with a high DNA content have numerous and different repeated sequences which are distributed over several or all chromosomes and which might lead to synapsis between nonhomologous chromosomes and recombination between homologous but nonallelic genes or pseudogenes. However, if the repeated sequences at different loci are divergent, they may prevent effective synapsis at other than allelic sites. RADMAN (1989) argues that divergent repeated elements and other noncoding sequences, by aborting strand exchanges with mismatches, act as anti-recombination elements, thereby stabilizing the chromosomes.

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