

Differential distribution and association of repeat DNA sequences in the lateral element of the synaptonemal complex in rat spermatocytes

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Abstract The synaptonemal complex (SC) is an evolutionarily conserved structure that mediates synapsis of homologous chromosomes during meiotic prophase I. Previous studies have established that the chromatin of homologous chromosomes is organized in loops that are attached to the lateral elements (LEs) of the SC. The

characterization of the genomic sequences associated with LEs of the SC represents an important step toward understanding meiotic chromosome organization and function. To isolate these genomic sequences, we performed chromatin immunoprecipitation assays in rat spermatocytes using an antibody against SYCP3, a major structural component of the LEs of the SC. Our results demonstrated the reproducible and exclusive isolation of repeat deoxyribonucleic acid (DNA) sequences, in particular long interspersed elements, short interspersed elements, long terminal direct repeats, satellite, and simple repeats. The association of these repeat sequences to the LEs of the SC was confirmed by *in situ* hybridization of meiotic nuclei shown by both light and electron microscopy. Signals were also detected over the chromatin surrounding SCs and in small loops protruding from the lateral elements into the SC central region. We propose that genomic repeat DNA sequences play a key role in anchoring the chromosome to the protein scaffold of the SC.

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Introduction

The synaptonemal complex (SC) is an evolutionarily conserved meiosis-specific structure that contributes to homologous chromosome synapsis, meiotic recombination, and proper chromosome segregation (for reviews, see Moses 1968; Sotelo 1969; Moens 1994; Maguire 1995; Kleckner 1996). The SC shows a characteristic tripartite organization, composed of a central region (CR) and two lateral elements (LEs). The CR is made up of the core element and the transverse filaments, which run perpendicular

to the core element. This central component bridges the space between LEs and holds the homologous chromosomes together (Page and Hawley 2004). The major protein components of mammalian SC are the LE proteins SYCP2 and SYCP3 (Lammers et al. 1994; Dobson et al. 1994; Offenberg et al. 1998) as well as the following proteins, SYCP1, SYCE1, SYCE2 and TXE12, which are associated with the CR (Meuwissen et al. 1992; Costa et al. 2005; Hamer et al. 2006).

The chromatin of the homologous chromosomes is organized in loops surrounding the SC, and it has been suggested that the LEs provide attachment sites for those chromatin loops (Rattner et al. 1980). Previous electron microscopy studies have shown that SCs also contain deoxyribonucleic acid (DNA) and that most of the SC-associated DNA is located in the LEs. The CR of the SC is mainly devoid of DNA. However, there are some exceptions where the recombination nodules as well as small noncharacterized loops protrude from the LEs into the CR (Vázquez-Nin and Echeverría 1976; Vázquez-Nin et al. 1993; Ortiz et al. 2002). Previous biochemical studies have shown that SC-associated DNA sequences correspond to GT/CA repeats, short interspersed elements (SINEs), and long interspersed elements (LINEs, Karpova et al. 1989, 1995; Pearlman et al. 1992). Furthermore, based on *in silico* studies, it has been proposed that DNA, which is highly repeated in the genome (such as *Alu* repeat sequences), may be anchoring the chromosomes to the LEs of the SC (Dadashev et al. 2005). However, the specific identity of genomic DNA associated with the LEs has not been characterized.

To understand the role of LE-associated DNA sequences, we performed a detailed characterization and cytolocalization of these sequences. To this end, we performed chromatin immunoprecipitation (ChIP) assays in rat pachytene cells using an antibody against the SYCP3 protein, which is a major structural component of the LEs. This yielded a large set of genomic sequences. Sequence analysis of the DNA and *in situ* hybridization demonstrated a specific enrichment of different types of repeat sequences in the LEs of the SC. Our results suggest that repeated genomic sequences participate in the SC conformation and most likely are involved in recombination events.

Materials and methods

Chromatin immunoprecipitation

In the present study, we used testes of 22-day-old Wistar rats. There is an enrichment of pachytene-stage spermatocytes in this tissue. To isolate germ cells, seminiferous tubules were excised and digested in phosphate-buffered saline (PBS) containing 0.01% trypsin (GIBCO) for 15 min

at 37°C. After digestion, cells were pelleted and resuspended in Dulbecco minimal essential medium supplemented with 2% fetal bovine serum, to inhibit trypsin. The ChIP assay was performed as previously described (Weinmann et al. 2002; Rincón-Arango et al. 2007). The following antibodies were used: SYCP3 antibody (Alsheimer and Benavente 1996), which we purified using high-affinity columns, anti-Z-DNA (Abcam), anti-RNA polymerase II (COVANCE), and a mock IP using normal IgG. Immunoprecipitated DNA was purified by the MinElute PCR Purification Kit (QIAGEN) and eluted in 50 μ l and 1 μ l was used for each polymerase chain reaction (PCR) reaction. For duplex PCRs, 25-mer primers were designed to amplify the representative repeat sequences and a coding sequence (Table 1). The final concentration of each primer was chosen to achieve amplification products of almost similar intensity when the whole genome is used as a template (input chromatin; Supplementary Fig. 1). The amplified fragments were electrophoretically separated in a 6% acrylamide gel, digitally imaged using a phosphorimager (Typhoon scanner 8600), and the ImageQuant program was used for quantification of PCR bands.

DNA cloning

DNA samples obtained from ChIPs using anti-SYCP3, anti-RNA polymerase II, and anti-Z-DNA antibodies were treated with 0.1 U of Mung Bean nuclease/volume sample (New England BioLabs) to remove the remaining single strands. The DNA was then ligated into the pCR-Blunt II-TOPO cloning vector (Invitrogen). Positive clones were isolated, purified, and sequenced.

Sequence analysis

All the isolated sequences were designated “M” and numbered as they were sequenced and are available on request. The general features of the isolated sequences were analyzed using the BLAT program from University of California Santa Cruz Genome Bioinformatics (<http://genome.ucsc.edu/cgi-bin/hgGateway>), BLAST program from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST/), and the Repeat Masking program (www.repeatmasker.org/). For the search of a high-scoring segment pair (HSP), BLASTN analysis was performed on a database, which was generated from the 100 identified sequences (Bedell et al. 2003). The database was formatted to create the alignment matrix using nine- and six-word size, respectively. The BLASTN program was run comparing the 100 independent sequences among themselves, and the HSP were analyzed. The ClustalW program was used to search for putative consensus sequences (Chenna et al. 2003).

Immunolocalization and fluorescent in situ hybridization

Rat seminiferous tubules were fixed in acetone at -20°C for 10 min, included in Jung Tissue Freezing medium (Leica Instruments), frozen, and sectioned in a cryostat at -14°C . Sections were mounted on slides covered with poly-L-lysine (SIGMA). For immunolocalization, samples were blocked with Tris-buffered saline (0.01 M Tris-HCl pH 7.5, 0.15 M NaCl, 3 mM CaCl_2 , 2 mM MgCl_2 , 5 mM sodium butyrate pH 8.0) for 30 min then incubated with the anti-SYCP3 antibody (1/100 in PBS) at 4°C overnight. The next day, slides were washed in PBS-Tween 0.05% (PBST) and incubated with fluorescein isothiocyanate (FITC)-conjugated anti-Guinea pig (Jackson ImmunoResearch) secondary antibody (1/100 in PBS) for 1 h at room temperature in the dark. All of the following steps were carried out in the dark. Slides were washed once with PBST and then with PBS. The secondary fixation was performed with 4% paraformaldehyde for 10 min at room temperature, and sections were washed with PBS. For in situ hybridization, the endogenous peroxidase was quenched with 3% H_2O_2 in PBS for 15 min at room temperature and washed twice with PBS. RNase treatment was performed by incubating the samples with RNase A (SIGMA) in $2\times$ sodium chloride-sodium citrate (SSC) for 1 h at 37°C in a humid chamber and washing three times with $2\times$ SSC. The DNA was denatured with a NaOH solution (pH 12) for 2 min (Brown 2002), and the NaOH was then neutralized by washing the slides three times with cold PBS. The representative DNA probes (LINE-M8, SINE-M9, and small cytoplasmic ribonucleic acid [ScRNA] repeat sequences, respectively) were placed on the sections, and the hybridization was carried out at 65°C overnight under a coverslip in a humid chamber. The probe had been

previously labeled with biotin (Bio Nick Labeling System, GIBCO BRL) and denatured at 75°C for 5 min just before hybridization. The next day, the coverslip was removed, and slides were by washed in $2\times$ SSC for 10 min at 45°C . Two cycles of signal amplification were performed using the TSA Biotin System (Perkin Elmer). The slides were then incubated for 30 min at room temperature with Texas Red coupled to Streptavidin (Perkin Elmer; 1/400 in PBS) and counterstained with 4',6-diamidino-2-phenylindole (100 $\mu\text{g}/\text{ml}$). The slides were rinsed with PBST, mounted using Vectashield mounting medium (VECTOR) and analyzed by epifluorescence and optical sectioning in an Apotome microscope (Carl Zeiss) and by a laser scanning microscope (Olympus).

Ultrastructural in situ hybridization

Small fragments of rat testis were fixed in 4% paraformaldehyde for 1 h, rinsed with PBS, dehydrated, and Lowicryl K4M embedded. The polymerization was performed using UV light for 24 h at 4°C . The resin blocks were cut with a Leica ultra microtome (Leica Instruments). The sections were collected on formvar-coated 200-mesh nickel grids, treated with RNase at 37°C for 1 h, and rinsed twice with $2\times$ SSC. The DNA was denatured with NaOH (pH 12) for 30 min, and then the grids were washed three times with cold PBS. The representative biotinylated DNA probe (LINE-M8) was placed on the grids, and the hybridization was carried out at 40°C overnight in a humid chamber. For probe detection, the grids were incubated with streptavidin coupled to 10-nm colloidal gold particles (SIGMA) for 30 min at 37°C . The grids were contrasted with uranyl acetate for 10 min and with lead citrate for 5 min and then observed in a JEOL 1010 transmission electron microscope (JEOL).

Table 1 Oligonucleotide sequences and primer concentrations for duplex PCR's

Amplification product	Primer sequence	Size (bp)	Concentration for duplex PCRs (pmol)
LINE-M44 sequence (Family L1)	L1: 5'-3', GGCTAGAAAACAATTTAAAGGAATC L1rev: 5'-3', GGCTCACCATTTGATACTACATAAC	108	1.5
SINE-M9 sequence (Family <i>Alu</i>)	S1: 5'-3', GCAATGGTGGTGCATGCCTTTAATC S1rev: 5'-3', CAAGGATTCTCTATGTAGCTCTGGT	119	2.5
LTR-M157 sequence	LTR: 5'-3', CCCTTAACCAAATGAGCATGAGTCA LTRrev: 5'-3', GGGAGGACATGTGATATTGGAGAGA	210	2.5
Satellite-M68 sequence	Sat: 5'-3', GGGACATGCTTGTATGAGTTTGCTT Satrev: 5'-3', GGGAACTACTGAATTCACCATGAT	89	0.5
Rat Actin (intron-exon2 and intron-exon3)	Act: 5'-3', GGCCCTGTGTTCACTTTGTATTTTCG Actrev: 5'-3', CCATCACTAGCCACTTCTGCTTCCTC	353	3.5 (with LINE and LTR primers) 2.5 (with SINE primers) 4.5 (with Satellite primers)
ScRNA (Small Cytoplasmic RNA)	ScRNA: 5'-3', GGGGTTGGGGATTAGCTCA ScRNArev: 5'-3', CCGGAGCTGGGGACCGAACC	93	

Table 2 Repeat sequences associated with SYCP3, classification (International Human Genome Sequencing Consortium, 2001) and distribution in rat genome databases

Repeat sequences Class and family	Frequency on SYCP3-IP chromatin ($n=100$)	Size range (bp)	Distribution on rat genome
Transposon-derived repeats			
LINE (family L1)	39	94–801	Broadly distributed, intergenic
SINE (family <i>Alu</i>)	8	67–816	Broadly distributed, intergenic and intronic
(family ID)	1	299	Broadly distributed, intergenic and intronic
(family MIR)	4	308–618	Broadly distributed, intergenic and intronic
(family B1-B4)	7	199–790	Broadly distributed, intergenic and intronic
LTR	22	82–835	Broadly distributed, intergenic
DNA transposons	6	79–785	Middle distribution
Simple sequences repeats (SSRs)			
Satellite	3	81–353	Centromeric and sub-telomeric distribution
Simple repeat	10	226–834	Middle distribution

Results

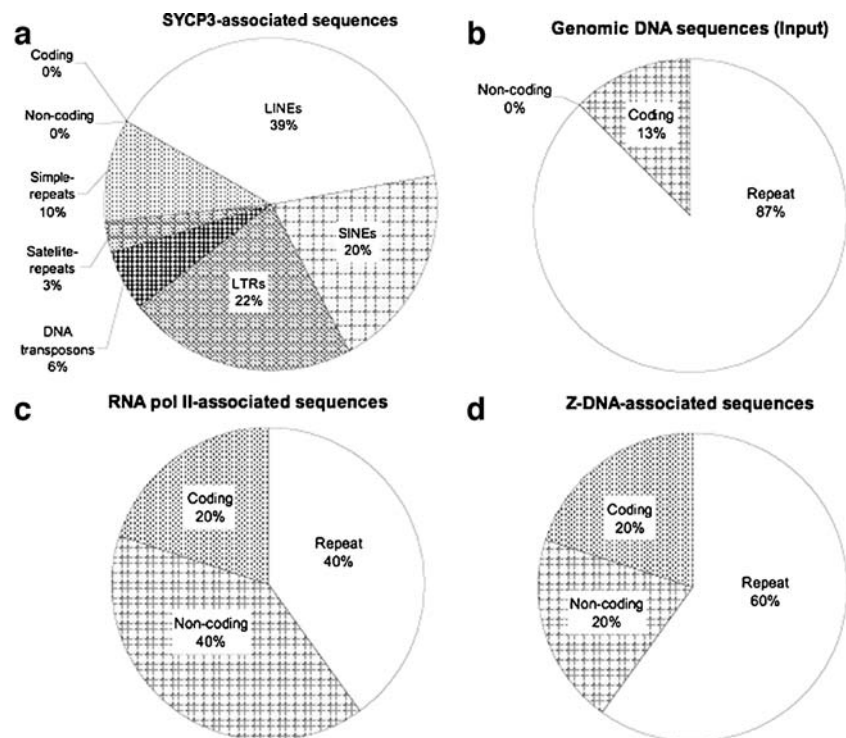
Isolation and characterization of SC-associated DNA sequences

Previous biochemical studies isolated a small subset of repeat DNA sequences associated with the SC (Pearlman et al. 1992). In addition, our group described the presence of DNA within the LEs of the SC (Vázquez-Nin et al. 1993; Ortiz et al. 2002). These observations suggest that a particular subtype of genomic sequences interacts with

highly specific components of the SC. The systematic characterization of such sequences would allow a better understanding of the organization of meiotic chromosomes.

To analyze the DNA associated with the LEs of the SC, we performed ChIP assay on rat spermatocytes using an anti-SYCP3 antibody. The immunoprecipitated DNA fragments were cloned, sequenced, and analyzed using bioinformatics programs and web-based databases. We found that the 100 independent resulting sequences correspond exclusively to different types of the repeat sequences present in eukaryotic genomes (International Human Genome

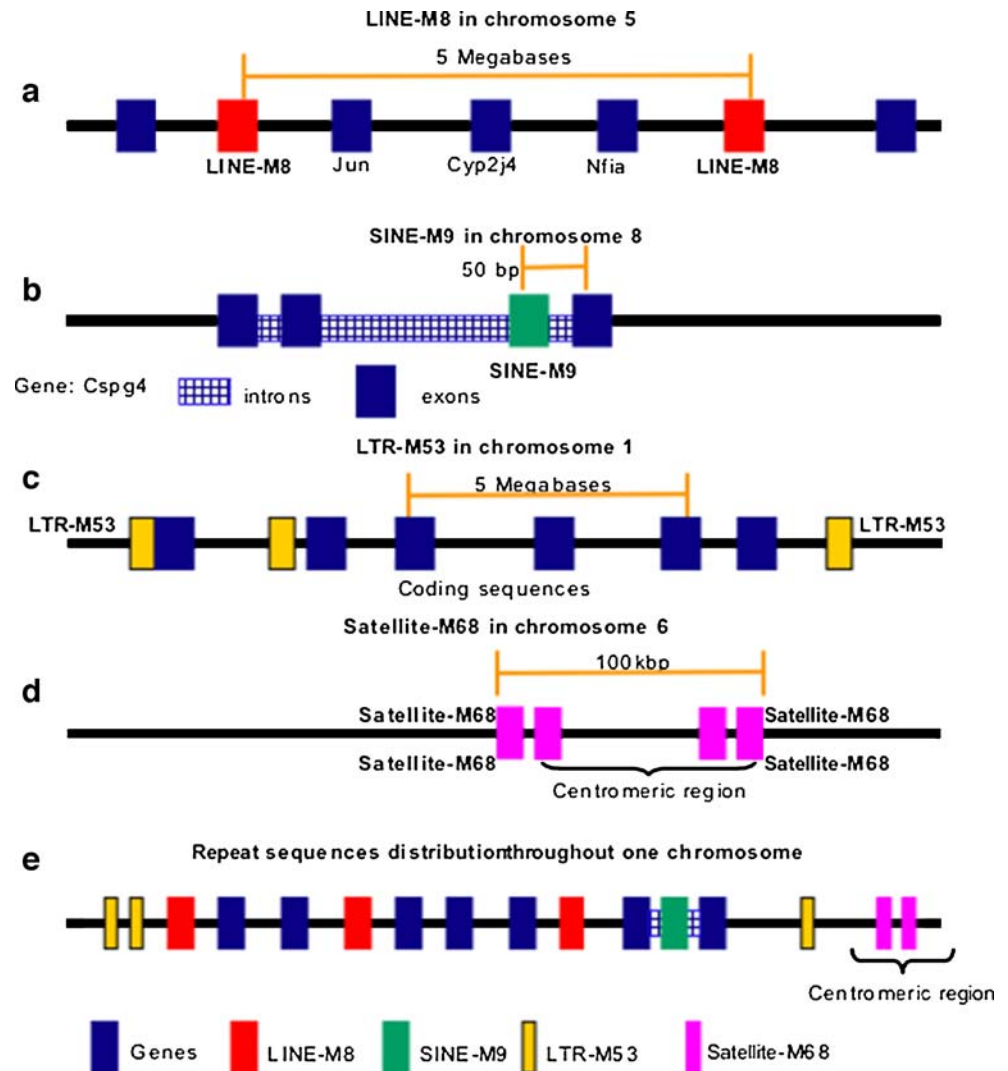
Fig. 1 Graphics showing the profile of the isolated sequences by ChIP. **a** Percentages of DNA sequences immunoprecipitated with anti-SYCP3. Coding sequences were absent (0%). **b** Percentages of DNA sequences from genomic DNA (*input chromatin*). **c** Percentages of DNA sequences immunoprecipitated with anti-RNA polymerase II. **d** Percentages of DNA sequences immunoprecipitated using anti-Z-DNA



Sequencing Consortium 2001). Most of these sequences correspond to transposon-derived repeats: 39 LINES, 20 SINEs, 22 long terminal direct repeats (LTRs), and 6 DNA transposons. The simple sequence repeats class is also represented, with three satellite repeats and ten simple repeats (Table 2). Notably, in a sample of 100 independent isolated sequences we were unable to identify a single coding sequence (Fig. 1a). Database searches revealed that the identified LINE, SINE, and LTR sequences are broadly distributed throughout the rat genome, while the satellite sequences are confined to centromeric and subtelomeric regions; alternatively, simple repeats are under-represented in the rat genome (Table 2). In agreement with our findings, two simple repeat sequences identified in our study (M2 and M3) were previously reported to be associated with the SC (Pearlman et al. 1992). The distribution of identified LINE sequences reveals that the great majority of those sequences are located on intergenic genomic regions, frequently flanking coding sequences in the rat genome.

As a representative example, the isolated LINE-M8 sequence is found in several locations all along chromosome V. It is interesting to note that LINE-M8 repeats are distributed on each side of a cluster of three genes encompassing a 5-Mb genomic region (Fig. 2a). If the two matches with LINE-M8 repeats correspond to anchorage points, the 5-Mb genomic region would represent a single chromatin loop surrounding the SC. Moreover, the identified SINE sequences are distributed along intergenic and intronic genomic sequences in the whole rat genome. For instance, one match with the identified SINE-M9 sequence is located in the intron of the chondroitin sulfate proteoglycan 4 gene on chromosome 8 (Fig. 2b), indicating that the anchorage point may be located in introns as well. The isolated LTR sequences are distributed over intergenic regions in the rat genome, and matches corresponding to the LTR-M53 sequence are found near coding regions (Fig. 2c). As predicted, satellite repeats sequences are confined to centromeric and subtelomeric domains of all

Fig. 2 Schemes showing representative location of the repeat sequences. **a** Matches of the LINE-M8 sequence are located in intergenic and flanking coding regions, as in chromosome 5, where repeat sequences are flanking a 5-Mb coding region composed of three known rat genes. **b** Matches of the isolated SINE-M9 sequence are located in intergenic and intronic regions. One match shows the SINE-M9 sequence located in an intron of the chondroitin sulfate proteoglycan 4 gene. **c** Matches of the identified LTR-M53 sequence are found in intergenic regions flanking coding sequences. **d** The identified matches of the Satellite-M68 are confined to centromeric and subtelomeric domains. **e** Scheme showing the distribution of the isolated repeat sequences along a chromosome



chromosomes, as we observed on chromosome 6 (Fig. 2d). Figure 2e depicts the location of the identified repeat sequences throughout a chromosome and demonstrates a broad distribution of the repeat sequences along the chromosome. These widespread distributions of repeat sequences may represent multiple anchorage sites throughout the entire chromosomes to the SC.

Chromatin immunoprecipitation assay corroboration

The repeat DNA sequences comprise 44% of rodent genomes (Martens et al. 2005); thus, a bias produced by their high abundance is possible. To verify the specificity of the ChIP assay, we sequenced DNA fragments from the input chromatin (genomic DNA), from the immunoprecipitations with anti-RNA polymerase II and with Z-DNA antibodies, which is able to detect DNA after it has been transcribed (Cerna et al. 2004). The genomic DNA obtained in these immunoprecipitations corresponds to 87% repeated

and 13% coding sequences (Fig. 1b). Meanwhile, from the bulk of RNA polymerase II-associated chromatin, we found 40% repeated, 40% noncoding, and 20% coding sequences (Fig. 1c). It is interesting that immunoprecipitations using the RNA polymerase II isolated not only coding sequences but also repeat sequences. This may occur because some repeats are transcribed or may simply be due to nonspecific immunoprecipitation. In contrast, when an antibody specific to SYCP3 is used, repeat sequences are exclusively recovered, and no coding sequences were isolated (Fig. 1a). This fact supports the specificity of our approach, and the highly frequent association of SYCP3 to repeat sequences as an important component of the LEs. Finally, from the Z-DNA-associated chromatin, 60% were repeated, 20% were noncoding, and 20% were coding sequences (Fig. 1d), indicating the transcription status of such sequences. With these corroboration tests, we confirmed once more that the enrichment of repeat sequences in the SYCP3-IP chromatin is due to the specificity of the SYCP3-repeat sequence

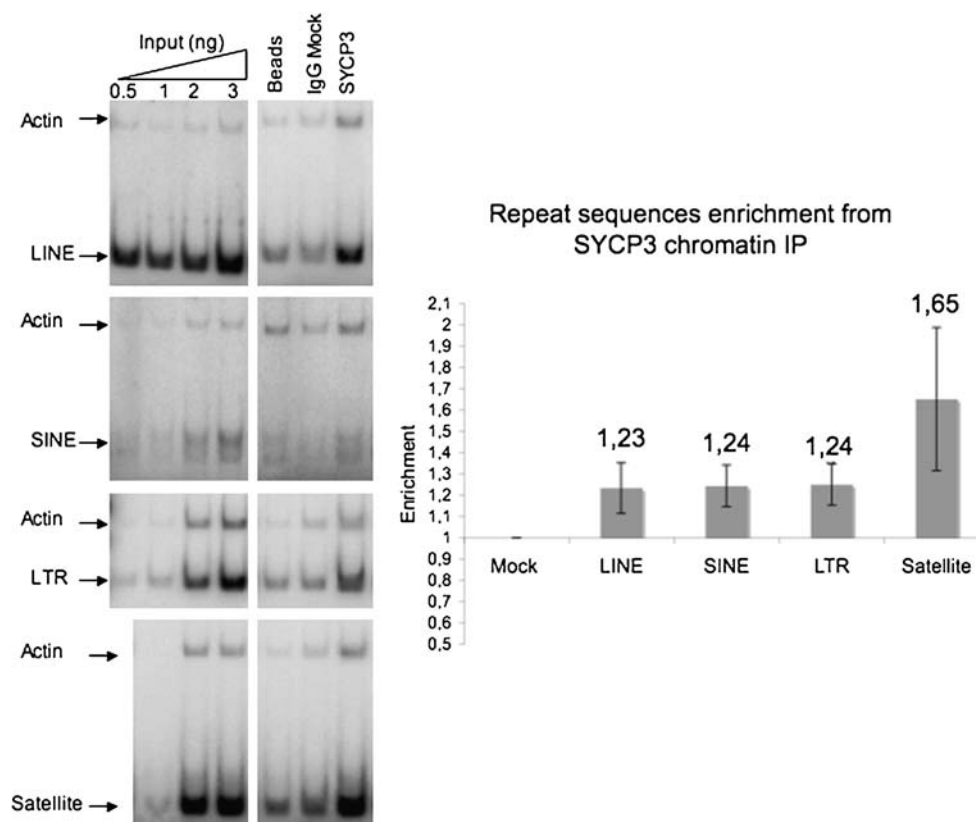


Fig. 3 Chromatin immunoprecipitation validation. Radioactive duplex PCRs and quantification. To obtain the linear range of amplification, duplex PCRs of repeat sequences (LINE, SINE, LTR, and satellite repeats), and coding sequence (Actin) were performed using increasing concentrations of genomic DNA (*Input*; gels on the left side of each repeat sequence panel, respectively). Duplex PCRs were then performed using the chromatin IPs with anti-SYCP3, anti-IgG (*Mock*), and with the immunoprecipitation reagent (*beads*). The

repeat/coding sequence ratio from SYCP3-bound fraction was standardized by dividing by the repeat/coding ratio from the IgG-bound material to determine enrichment of repeat sequences during the immunoprecipitation. Enrichment of the repeat sequence over the actin gene is determined to be greater than 1, which indicates no enrichment. Radioactive PCRs were done in triplicate, and three independent ChIP assays were performed. Standard errors are shown

interactions and not to a bias produced by the high percentage of these sequences in the rat genome (Martens et al. 2005).

To semiquantitatively evaluate the enrichment of repeat sequences compared to coding sequences in the immunoprecipitated chromatin (IP), we performed radioactive duplex PCRs. We designed the PCR reaction to obtain a one-to-one ratio between representative repeat sequences (LINE-M44, SINE-M9, LTR-M157, and Satellite-M68) and a coding sequence (Actin) using genomic DNA as a template

(Supplementary Fig. 1). Radioactive PCR was performed on templates from chromatin IPs using anti-SYCP3 and mock chromatin IPs using IgG. The amplification of representative repeat sequences was performed in the linear range of the PCR reaction. The ratio of products obtained with repeat and coding sequences was determined for SYCP3 and IgG antibodies, respectively (Fig. 3). We obtained a modest but reproducible enrichment of all the repeat sequences previously found (LINE, SINE, LTR, and satellite repeats) when SYCP3-IP chromatin was used as a

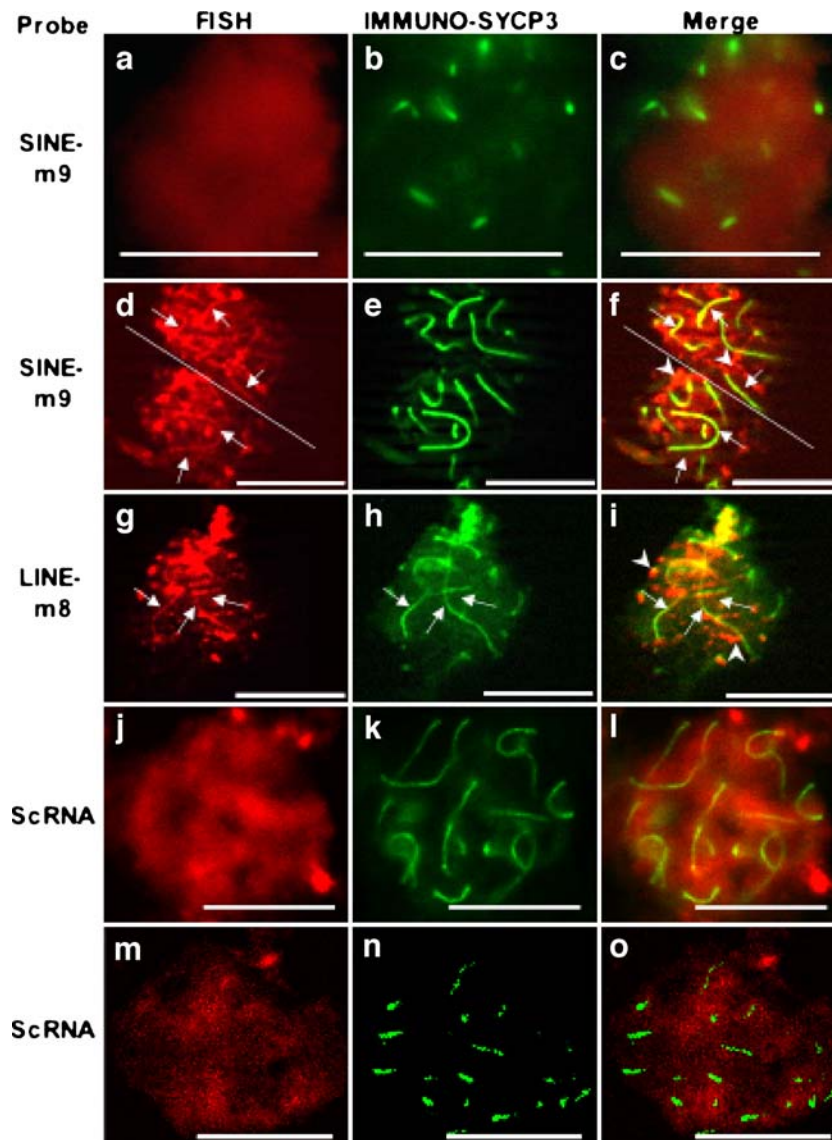


Fig. 4 Epifluorescent and optical sections of Immuno-FISH assays in rat pachytene cells. Probes for LINE, SINE and ScRNA sequences (LINE-M8, SINE-M9, and ScRNA) were used to detect the DNA repeat sequences (*red*). Synaptonemal complexes are labeled by anti-SYCP3 (*green*). **a–c** Epifluorescence of a prepachytene cell using SINE-M9 repeat as a probe. The signal is diffuse, and no thread-like structures are found. The SINE-M9 probe is associated with linear structures (*arrows* in **d**), which correspond to those labeled by SYCP3 (*arrows* in **e**). The *merge* shows that both signals colocalize (*arrows* in

f). **g–i** Sequence LINE-M8 is also localized to linear structures stained with anti-SYCP3 (*arrows* in **i**). Both SINE and LINE sequences are associated with the chromatin not in direct contact with the SC as demonstrated by the diffuse red fluorescence (*arrowheads* in **f**, **i**). **j–l** Epifluorescence analysis of ScRNA in pachytene cells: The signal is found surrounding the SC. **m–o** In an optical section of the FISH, using ScRNA as probe, no colocalization with SYCP3 is observed. *Bar* represents a 10 μ m scale

template (Fig. 3). This reproducible and constant enrichment confirms that repeat sequences are associated specifically with the SYCP3 protein.

Repeat DNA sequences are associated to the LEs of the SC

To survey the cytolocalization of the isolated repeat sequences, we performed fluorescence in situ hybridizations (FISH) coupled to SYCP3 immunolocalization (immuno-FISH; Fig. 4a–i). SYCP3 antibody was used for immuno-detection of the SC. We chose repeat sequences the SINE-M9 and LINE-M8 as representative and used them as probes for hybridizations. As a control probe, we incorporated the ScRNA repeat sequence (obtained from rat genome databases available at (www.genome.ucsc.edu/)). This sequence was not one of those recovered in our ChIP assays. Optical sectioning was performed (see “Materials and methods”). When LINE-M8 and SINE-M9 probes were used, the signal in meiotic cells localizes to thread-like structures in the majority of the pachytene nuclei (arrows in Fig. 4d,g). These structures correspond to the SC as confirmed by the anti-SYCP3 labeling (arrows in Fig. 4f,i). By contrast, the ScRNA probe does not colocalize with the SYCP3 signal (Fig. 4j–o). In prepachytene cells, where SYCP3 dots are discernible, SINE-M9 probe is diffusely observed, and no thread-like structures are evident (Fig. 4a–c).

No signal above background was detected when the empty vector is used as a probe (Fig. 5a–c). To eliminate the possibility of a leakage of green FITC emission into the red channel, we used SYCP3-FITC-labeled nuclei (with no M8 or M9 probe hybridization), and the images were captured in the red channel under the same exposure conditions as in immuno-FISH. We conclude that the hybridization signal of

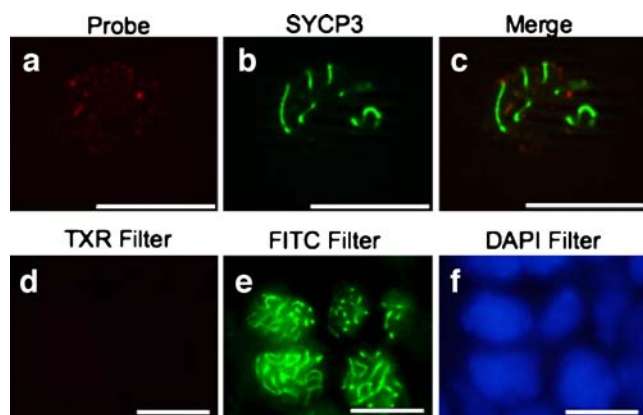


Fig. 5 Immunostaining controls. **a–c** As a negative control, the cloning vector was labeled and hybridized with the pachytene cells. **d–f** To discount a leakage of FITC emission into the red channel, an anti-SYCP3-FITC-labeled nucleus (which is not hybridized with LINE-M8 or SINE-M9) was visualized in the green and in the red channel. A picture was obtained in the red channel under the same exposure conditions as in **a–c**. No signal in the red channel could be detected. Bar represents a 10- μ m scale

the M8 and M9 probes is due to Texas red-coupled probes and not to a leakage of FITC fluorescence into the red channel (Fig. 5d–f). Furthermore, in the pachytene cells, the M8 and M9 probes hybridized throughout the surrounding chromatin of the SCs (arrowheads in Fig. 4f,i).

In summary, association of isolated repeat sequences LINE- M8 and SINE- M9 with LE is specific and not due the high frequency of these sequences in the rat genome. Moreover, in pre-pachytene stages the SINE-M9, did not form thread-like structures suggesting, that these sequences do not precede the SC formation. These results indicate that in pachytene cells, the repeat sequences are specifically recruited to the SC, but a portion of those repeats remained diffusely distributed throughout the nucleus.

Ultrastructural analysis of LE-associated repeat sequences

To further characterize these associations, we investigated the localization of repeat sequences among the components of the SC using ultrastructural in situ hybridization, using the LINE-M8 sequence as a probe. The hybridization signal was localized to the LEs of the SC (arrows in Fig. 6a–c). Components of the LE and the prominent signal are more

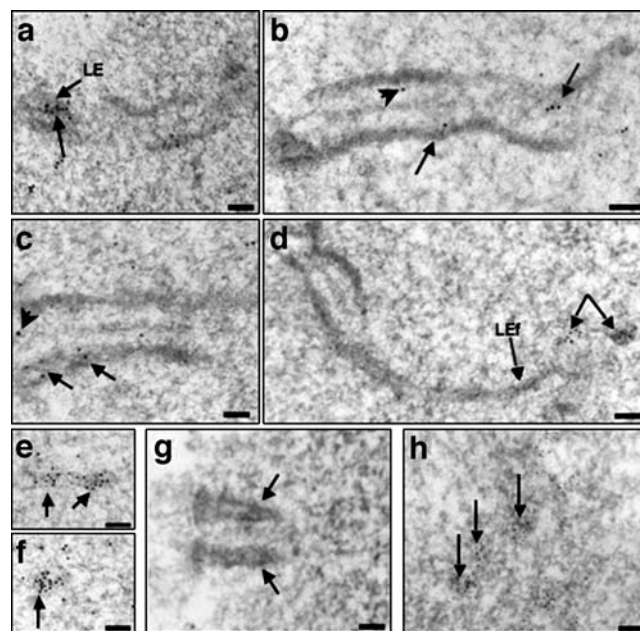


Fig. 6 Ultrastructural in situ hybridization. The LINE-M8 sequence coupled to 10-nm gold grains was used as a probe. **a–c** The signal is present on the LEs of the SC (arrows). Gold particles are also associated with filaments protruding into the central space (CE; arrowheads in **b** and **c**). **e, f** Transverse sections of the SC show that the lateral elements are densely labeled. Gold grains are present in the lateral element of the SC in the process of formation (arrows in **d**). **g** When the cloning vector was used as a probe, no hybridization signal was detected on the LEs of the SC. **h** The gold grains were also located in some heterochromatin clumps in prepachytene cells (arrows). Bar represents a 100 nm scale

prominent in cross-sections because of an increased accessibility of LE-associated DNA to the probes at the surface of tissue sections (Fig. 6e,f). Evidence demonstrating that these structures, contrasted with uranyl-lead, correspond to LE cross-sections of the SCs is presented in Supplementary Fig. 2. Together, these results support the view that repeat sequences are involved in chromatin anchoring to the SC. It is noteworthy that clusters of gold particles were also observed over less electro-dense structures that are in contact with the highly contrasted LEs at the sites on which SCs are formed during zygotene (arrows in Fig. 6d). Within SCs, gold particles are present over noncharacterized loops protruding from the LEs into the CR of the SC (arrowheads in Fig. 6b,c). We speculate that these repeat sequences might have functions other than anchoring the LEs. As expected, the signal of repeat sequences was also found in heterochromatin clumps (Fig. 6h; Martens et al. 2005). When the cloning vector was used as a probe, no signal was detected in the LEs of the SCs (Fig. 6g). Together, these results demonstrate the association of distinct types of repeat sequences to the LEs of the SC. We suggest that they are involved in the anchorage of the chromatin loops to the SC. Furthermore, the presence of repeat sequences in chromatin loops protruding toward the CR of the SC advocate a function in the meiotic recombination, which takes place in the recombination nodules (see “Discussion”).

Repeat sequences do not share a consensus sequence

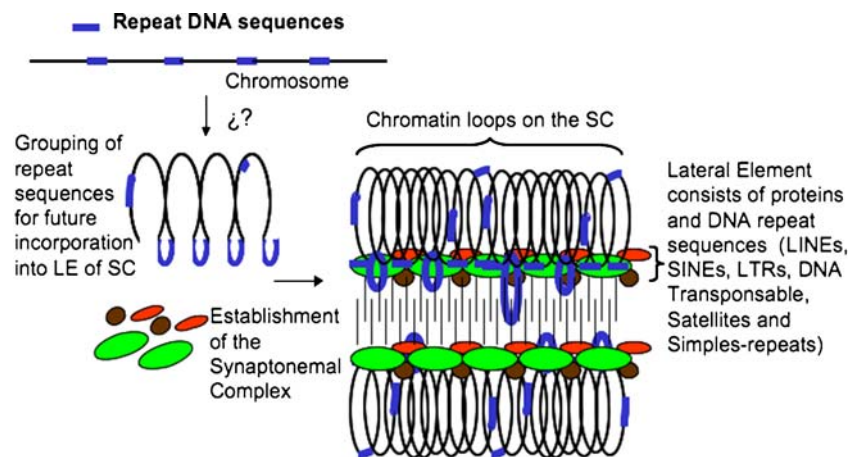
In an effort to elucidate the features of repeat sequences associated to the LE, the 100 isolated sequences were analyzed by means of BLASTN (Bedell et al. 2003) and ClustalW programs (Chenna et al. 2003). BLASTN did not find a HSP in either the leading strand or the lagging strand, only between the sequences of the same class of repeat element (i.e., LINE with LINE; data not shown). Further-

more, analysis with ClustalW confirmed that there are no consensus sequences or HSP that could be involved in the recruitment of the repeat sequences to the LE of the SC. In conclusion, we believe that the repeat sequences themselves associated with the LEs of the SC do not follow a primary sequence identity consensus. Instead, it seems reasonable to predict that their particular chromatin structure conformation represents a key aspect of these repeat sequences at the onset of SC formation (Dadashev et al. 2005; Peng and Karpen 2007).

Discussion

During the pachytene stage of meiotic prophase I, the chromatin of homologous chromosomes is anchored to the LEs of the SC. The requirements and mechanisms that govern chromosome attachment to the SC are unknown. Because LE-associated DNA is a good candidate for chromosome anchoring to the SC, we decided to isolate and characterize the associated genomic sequences. Using a ChIP assay with an antibody against the major LE protein SYCP3, we were able to isolate and characterize 100 independent DNA sequences. All the isolated sequences were repeat elements like LINE, SINE, LTR, satellite, and simple repeats. Remarkably, coding sequences were not immunoprecipitated with the SYCP3 antibody. Bioinformatic analysis revealed that repeat sequences have a widespread distribution throughout the whole genome (Fig. 2 and Table 2). Our in situ hybridization demonstrated that the isolated repeat sequences are present in the LEs. This is the first molecular and cytological systematic characterization of the association of repeat DNA sequences to the LE of the SC. These results strongly support that these repeat elements may play an important role in anchoring meiotic chromosomes to the SC during meiotic prophase I in rat pachytene cells.

Fig. 7 A model of the protein–DNA scaffold of the SC in pachytene rat spermatocytes. The repeat sequences (*blue lines*) are broadly distributed throughout the rat genome; some of these sequences are recruited to the lateral elements where they become associated with the structural proteins of these elements (*green, brown, and red circles*). Small loops of DNA containing these sequences protrude into the central space of the synaptonemal complex



As a first effort to address how repeat elements are attached to the LEs of SCs, we analyzed the isolated DNA sequences using the bioinformatics methods. No consensus sequences emerged from these analyses, which could suggest some functional property. The repeat element seems to share some characteristics in their chromatin organization, such as particular histone post-translational modifications (Kondo and Issa 2003; Martens et al. 2005; Peng and Karpen 2007). Thus, our experiments support the functional significance of repeat sequence association with the LEs of the SC in conferring specific chromatin conformations. Currently, we are interested in understanding whether precise histone post-translational modifications are needed for the association of repeat DNA to the LEs of the SC. There is evidence that certain protein components of the SC are likely to be involved in chromatin attachment. Sequence analysis of major SC proteins predicted that SYCP1 and SYCP2 but not SYCP3 contain putative DNA-binding motifs (Meuwissen et al. 1992; Lammers et al. 1994; Dobson et al. 1994; Offenberget al. 1998). Whether or not these proteins can specifically bind repeat DNA sequences such as those described here is currently unknown. Recent studies indicate that cohesins may also play an important role in chromatin anchoring (Revenkova et al. 2004). Mice lacking meiosis-specific cohesin SMC1 β appear to contain less chromatin in the LEs. They show an altered LE-to-loop ratio, with chromatin loops that are twice as long as those found in wild-type mice (Revenkova et al. 2004). It has been previously shown that cohesins can bind SYCP2, and this protein can bind SYCP3 (Pelttari et al. 2001; Eijpe et al. 2003); thus, the emerging picture points to a complex network of DNA–protein and protein–protein interactions mediating chromosome anchoring to the SC.

We propose a model for the protein–DNA scaffold formation at the SC (Fig. 7). In this model, the chromatin loops surrounding the SC are anchored to the LEs by means of short DNA loops, which are composed solely of DNA repeat sequences, such as LINE, SINE, LTR, satellite, and simple repeats. Some of these repeat sequences may protrude into the central space of the SC, with a predicted role in meiotic recombination. It is worth mentioning that not all the repeated sequences are associated with the LEs of the SC, although how certain repeat sequences are targeted to LEs and the kinetics of these associations remain a attractive topics of research.

Future studies are required to ascertain whether LE-associated repeat sequences possess a characteristic chromatin structure. Our study raises the question of whether the repeat elements described here are required, directly or indirectly, for recombination events. Several studies have shown that recombination hot spots are associated with repeat sequences such as *Alu* (i.e., a member of the SINE

elements), minisatellite (Jeffreys et al. 2004, 2005), and LTRs (Myers et al. 2005). The only correlation among most hot spots is their high G+C content (Gerton et al. 2000). It is interesting to note that others have described the presence of G+C rich regions in the LINE, SINE, LTR, and satellite repeat sequences (Scott et al. 1987; Meneveri et al. 1995). For this reason, we are interested in better understanding the role of distinct types of repeat sequences in meiotic-specific function and meiotic recombination.

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