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# Temporal and spatial distribution of Rad51 protein in spermatocytes of the common shrew *Sorex araneus* L. (Soricidae, Eulipotyphla)

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ABSTRACT. Chromosome pairing and recombination at meiosis involves scheduled formation and repair of double-strand breaks of DNA. Rad51, the eukaryotic homologue of the bacterial RecA protein, plays a crucial role in these processes. We used antibodies against human Rad51 to examine the temporal and spatial distribution of Rad51 in the spermatocytes of the common shrew *Sorex araneus* (Eulipotyphla, Soricidae). We found that hundreds of Rad51 foci appeared at leptotene. At the beginning of zygotene their close association with the axial elements of the synaptonemal complex became apparent. From early to late zygotene the number of Rad51 foci gradually decreased. At pachytene we observed a further dramatic decrease in the number of foci. They were distributed irregularly along autosomal bivalents. We detected a prevalence of Rad51 signals on the original X and autosomal arms of the sex trivalent at late pachytene. We did not detect a preferential association of Rad51 foci with unpaired or non-homologously paired regions of lateral elements of synaptonemal complexes in Robertsonian multivalents.

KEY WORDS: *Sorex araneus*, common shrew, recombination, Rad51, synaptonemal complex.

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# Временное и пространственное распределение белка Rad51 в сперматоцитах обыкновенной бурозубки *Sorex araneus* L. (Soricidae, Eulipotyphla)

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РЕЗЮМЕ. Спаривание и рекомбинация хромосом в мейозе обусловлена упорядоченным формированием и репарацией двунитевых разрывов ДНК. Rad51 — эукариотический гомолог бактериального белка RecA – играет решающую роль в этих процессах. Мы использовали антитела против Rad51 человека для исследования временного и пространственного распределения Rad51 в сперматоцитах обыкновенной бурозубки *Sorex araneus* (Eulipotyphla, Soricidae). Мы нашли, что сотни фокусов Rad51 возникают в лептотене. В начале зиготены становится заметной их тесная связь с осевыми элементами синаптонемального комплекса. Число фокусов Rad51 постепенно уменьшается от ранней зиготены к поздней. В пахитене мы наблюдали значительное уменьшение числа фокусов. Они были распределены нерегулярно по аутосомным бивалентам. Мы обнаружили преобладание сигналов Rad51 на аутосомном и "истинном X" плечах полового тривалента в поздней пахитене. Мы не нашли преимущественной ассоциации фокусов Rad51 с неспаренными или негомологично спаренными участками боковых элементов СК в Робертсоновских мультивалентах.

КЛЮЧЕВЫЕ СЛОВА: *Sorex araneus*, обыкновенная бурозубка, рекомбинация, Rad51, синаптонемный комплекс.

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## Introduction

Meiosis is the process of formation of haploid gametes from diploid germ cells. This process involves pairing, recombination and segregation of homologous chromosomes. The initial two processes take place at meiotic prophase, which is subdivided into several stages. At the first stage, called leptotene, the chromosomes form axial elements. The main component of the axial elements is a core protein SCP3. At zygotene, homologous chromosomes align and pair with each other. At pachytene, all homologous chromosomes are completely paired and appear as bivalents, connected by the central element of the synaptonemal complex. At diplotene, the final stage of prophase, as well as at diakinesis and metaphase I, the bivalents remain attached at the points of recombination, which are visible as chiasmata. At anaphase I, homologous chromosomes segregate to opposite poles.

Although many details of chromosome pairing and recombination (such as timing, sequence of events and molecular mechanisms involved) remain unknown it is clear that both processes are initiated by scheduled formation of DNA double strand breaks (DSBs) at specific sites (Wu & Lichten, 1994; Ohta *et al.*, 1999). DSBs result in molecules with single-stranded tails which bind Rad51, the eukaryotic homologue of bacterial RecA protein (Padmore *et al.*, 1991; Schwacha & Kleckner, 1995).

Analysis of the distribution of Rad51 protein in meiotic cells of yeast, lily, chicken, mouse and human indicates that it is associated with sites for the initiation of strand invasion and chromosome pairing and recombination (Ashley *et al.*, 1995; Plug *et al.*, 1996, 1998; Anderson *et al.*, 1997; Barlow *et al.*, 1997). Rad51 has been found to form discrete nuclear foci from leptotene in mouse and from early zygotene in human spermatocytes. These foci are co-localised with axial elements of meiotic chromosomes. During zygotene, the foci are predominantly present in regions undergoing synapsis. The level of autosome labelling with Rad51 becomes reduced at pachytene, although asynapsed segments of XY remain intensely labelled. This indicates that the Rad51 protein plays an important role in interhomologue interactions during meiotic prophase I.

Here we present the results of an immunocytogenetic study of Rad51 distribution in spermatocytes of the common shrew *Sorex araneus* (Eulipotyphla, Soricidae) and compare them with earlier data obtained on mouse and human spermatocytes.

The common shrew is a very interesting model for meiotic studies, because it has an XX/XY$_1$Y$_2$ sex chromosome system. The "X" in *Sorex araneus* represents a fusion between the true mammalian X and an autosome (Sharman, 1956, 1991; Fredga, 1970; Pack *et al.*, 1993). It has been shown that sex chromosomes in mammals differ from autosomes both in the timing and pattern of pairing and recombination (Ashley, 2002). In the common shrew we are able to analyse meiotic behaviour of the "true" X and in autosomal arms in the sex trivalent. Another peculiarity of the common shrew is that it shows some of the most remarkable chromosomal variation in mammals. To date, 68 chromosome races have been described (Wójcik *et al.*, 2003) and the actual number of distinct races probably goes far beyond 100. The source of this chromosomal variation is Robertsonian fusions with further possible modification by whole-arm reciprocal translocations (see Searle, 1993). Analysis of chromosome pairing, recombination and segregation in hybrids between the chromosome races which are heterozygous for several rearrangements is of particular interest.

## Material and methods

Two juvenile male shrews used in this study were trapped during the field season of 2004 in the section of the hybrid zone between the Novosibirsk and Tomsk chromosome races located 30 km southeast from Novosibirsk city (N 54°47', E 83°25') (for more details on these chromosome races see Polyakov *et al.*, 1996). G-banded metaphase chromosomes were prepared according to Král & Radjabli (1974). Following the adopted nomenclature for the chromosomes of the common shrew *Sorex araneus* (Searle *et al.*, 1991), the karyotypes of the male of Novosibirsk chromosome race and the hybrid male are 21, XY$_1$Y$_2$, *af*, *bc*, *go*, *hn*, *ik*, *jl*, *mp*, *qr*, *tu* and 24, XY$_1$Y$_2$, *af*, *bc*, *jl*, *o*/*og*/*gk*/*ki*/*ih*/*hn*/*nm*/*m*, *p*, *q*/*r*, *tu*, respectively.

Spermatocyte spreads were prepared using the technique of Peters *et al.* (1997). Immunostaining was performed as described by Moens *et al.* (1997) with several modifications. Slides were incubated with a rabbit polyclonal anti-human Rad51 antibody (Calbiochem) at 1:1000 dilution, overnight, followed by detection with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG antibody (Jackson) at 1:200 dilution for 1 h. Then after rinsing the slides were incubated with a rabbit antibody to rat SCP3 (a component of lateral elements of synaptonemal complex, a gift from C. Heyt-

Figure 1. Spermatocytes of the common shrew immunostained with antibodies to Rad51 (black) and SCP3 (grey).

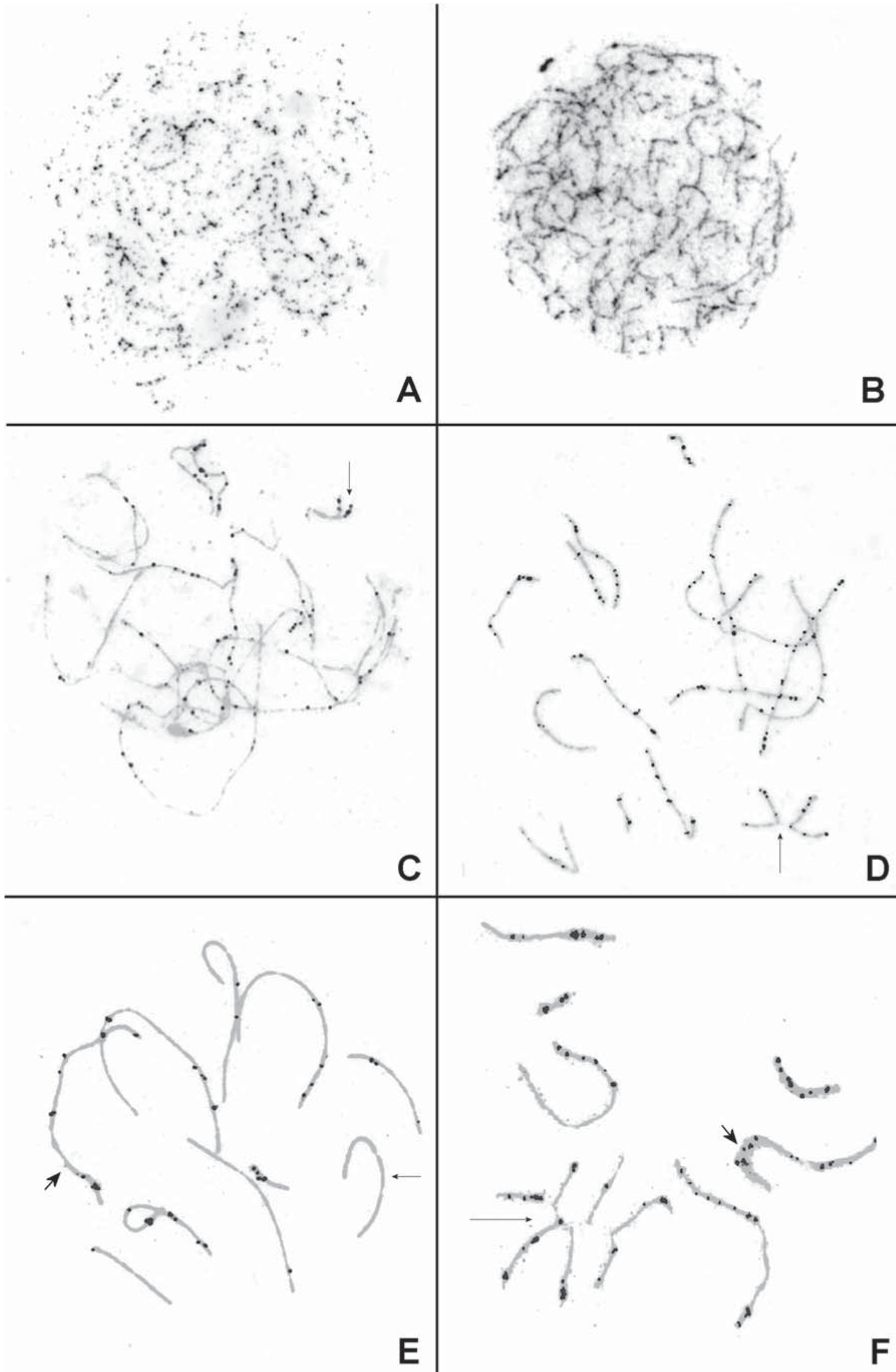
A. Leptotene. Rad51 foci are dispersed throughout the nucleus.

B. Leptotene-zygotene transition. Rad51 forms multiple branching fibres co-localised with axial element material.

C and D. Zygotene. Nuclei contain linear arrays of Rad51 foci. Arrows indicate asynapsed homologues showing symmetry of Rad51 labelling.

E. Pachytene in the shrew of the Novosibirsk chromosome race. Some autosomal bivalents are not labelled (arrow), while the XY$_1$Y$_2$ trivalent (arrowhead) has several Rad51 foci.

F. Pachytene in the inter-racial hybrid. Unpaired regions of the multivalent (arrow) do not show any Rad51 signals. The XY$_1$Y$_2$ trivalent (arrowhead) is intensively labelled.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ing) at 1:1000 dilution for 2 h. This primary antibody was detected with donkey anti-rabbit Cy3 (Jackson) at 1: 400 dilution for 1 h. Finally, the slides were counterstained with DAPI (2 mg/ml) and analysed using an Axioskop 2 (Zeiss) microscope equipped with a CCD camera, filter set, and the ISIS4 image processing package of Metasystems GmbH.

## Results and discussion

The human Rad51 protein shows 99.1%, 98.8% and 95.6% identity to that of the dog (Ochiai *et al.*, 2001), the mouse (Morita *et al.*, 1993) and the chicken (Bezzubova *et al.*, 1993), respectively. Although the shrew Rad51 has not been analysed yet, there is no reason to expect that it is an exception to this high level of conservation. We were able to use polyclonal antisera raised against human Rad51 (hRad51) to study the distribution of Rad51 in shrew spermatocytes.

In contrast to human males, but similar to male mice, shrew males form fragmentary axial elements at leptotene (Borodin, 1991). At this stage, when the axial elements just started to assemble and SCP3 appeared as a small number of isolated foci, numerous Rad51 foci occurred throughout the nuclei (Fig. 1A). This represents the earliest stage at which we were able to detect Rad51 in male shrews. At the leptotene-zygotene transition Rad51 formed multiple branching fibres which were co-localised with axial element material (Fig. 1B).

Zygotene nuclei contained numerous linear arrays of Rad51 foci. The number of foci was maximal at early zygotene and gradually decreased to late zygotene. Again, in contrast to the results obtained with human zygotene nuclei and similar to mouse zygotenes, heavy Rad51 labelling was found in both synapsed and asynapsed regions of the shrew synaptonemal complexes. The majority of asynapsed homologues of the shrew did not show the obvious symmetry of labelling as was previously described by Ashley *et al.* (1995) and Plug *et al.* (1996) in human spermatocytes, although in some bivalents the pattern of distribution of Rad51 foci appeared symmetric (Fig. 1C, D).

In the shrew spermatocytes, the labelling of fully synapsed bivalents appeared to diminish markedly from early to late pachytene (from about 50 to 0 foci per nucleus). At mid-pachytene some bivalents had several foci while others were unlabelled in the same nucleus (Fig. 1E).

At pachytene the number of Rad51 foci on the sex trivalent correlated with total number of foci per cell ($r=0.48$, $n=33$, $p<0.05$). However, the average density of bright Rad51 foci on the axes of the sex trivalent was significantly higher than on the axes of the autosomal bivalents *af* and *bc*, which were approximately of the same length as the sex trivalent (0.45±0.22 and 0.28±0.12 foci per μm, respectively, $t=1.97$, d.f.=31, $p=0.05$). There was no difference in labelling between the original X (arm *e*, 0.48±0.26 foci per μm) and autosomal (arm *d*, 0.42±0.25 foci per μm) parts of the $XY_1Y_2$ trivalent ($t=0.618$, d.f.=31, $p=0.53$).

This indicates that both original and autosomal parts of the $XY_1Y_2$ trivalent display the same delayed pattern of Rad51 labelling characteristic to sex bivalents of mammals (Ashley *et al.*, 1995). In this respect the sex trivalent in the male shrews behaves as a coordinated unit. This is rather different from the behaviour of the shrew X chromosome in female somatic cells. Fredga (1970) and Pack *et al.* (1993) demonstrated that in somatic cells of female shrews the arm *e* was late replicating and apparently inactivated as an unfused X chromosome in other species, but inactivation did not spread to the autosomal arm *d*.

Studies in mice heterozygous for reciprocal translocations demonstrated an intensive delayed Rad51 labelling of unpaired and non-homologously paired regions of the heteromorphic synaptic configurations at pachytene (Plug *et al.*, 1998). In the male hybrid between the Novosibirsk and Tomsk chromosome races we found a chain-of-eight in 54% of pachytene cells. In such a synaptic configuration we often observed relatively large gaps in the lateral elements of the synaptonemal complex around the breakpoints, i.e. the centromeres (Fig. 1F). We did not find Rad51 foci on these gaps. The remaining pachytene nuclei of the hybrid contained incompletely and non-homologously paired elements. We did not find an excess of Rad51 labelling in the regions involved in non-homologous pairing. Even univalents demonstrated the same pattern of labelling as normally synapsed bivalents.

It is intriguing to consider whether the two peculiarities of the Rad51 distribution in the common shrew (i.e. X-like labelling of the sex trivalent and normal labelling of unpaired and non-homologously paired regions of autosomes) are evolutionarily connected. The X-autosome fusion is characteristic of the common shrew and several other related species of the genus *Sorex* (Zima *et al.*, 1996). Searle (1986) supposed that during and after fixation of the X-autosome fusion selection would have acted to reduce anaphase I nondisjunction of the sex trivalent in males and this may facilitate correct segregation of autosomal trivalents as well and thereby increase the probability of fixation of Robertsonian fusions. It is possible that this selection may also have affected earlier stages of meiosis as well. It appears that Rad51 processes the sex trivalent as a coordinated unit and ignores pairing irregularities occurring in autosomal Robertsonian trivalents and multivalents.

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