Robertsonian metacentrics of the house musk shrew 
(*Suncus murinus*, Insectivora, Soricidae) lose 
the telomeric sequences in the 
centromeric area.

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The house musk shrew, *Suncus murinus*, is polymorphic for five Robertsonian translocations (Rb8.17, 9.13, 10.12, 11.16, 14.15). Fluorescence *in situ* hybridisation with a biotin–labelled oligonucleotide, (TTAGGG)_n, was performed to localise the telomeric DNA sequences at Rb chromosomes of heterozygous shrews. Hybridisation signals were observed at both ends of all chromosomes, but not at the pericentromeric areas of any of the Robertsonian metacentrics. Our results indicate a complete loss of the telomeric sequences at the fusion points of the Rb metacentrics in *S. murinus*.

INTRODUCTION

Telomeric DNA of all vertebrates has been shown to contain tandem repeats of the sequence TTAGGG (Meyne et al., 1989). This sequence has been found at the telomeres of the chromosomes of all vertebrate species studied so far using fluorescence *in situ* hybridisation (FISH). Moreover, in many species this telomeric sequence has been detected outside the true telomeres. Most of the non-telomeric sites of the telomeric repeats are located at pericentric areas within or at the borders of the regions of constitutive C-heterochromatin or at interstitial chromosome locations (Meyne et al., 1990). It has been suggested that these interstitial telomeric sequences are the vestiges of true telomeres which remained after chromosome rearrangements such as Robertsonian (Rb) and tandem fusions and pericentric inversions (Meyne et al., 1990). These types of chromosome rearrangements often distinguish closely related species and populations and seem to have played an important role in the karyotype evolution of mammals (Holmquist and Dancis, 1979; King, 1993; Searle, 1998).

Analysis of the chromosomal location of telomeric sequences in polymorphic species, in which rearrangements have occurred recently, may shed a light on the evolutionary role of the non-telomere telomeric repeats. So far only a few species polymorphic for Rb fusions have been studied: *Okapia johnstoni* (Vermeesch et al., 1996), *Akodon cursor* (Pagundes et al., 1997); *Blarina carolinensis* (Qumsiyeh et al., 1997) and *Mus musculus* (Garagna et al., 1995; Nanda et al., 1995).

The aim of our study was to determine the distribution of the telomeric sequences (TTAGGG)_n on the Rb chromosomes of the house musk shrew, *Suncus murinus* (Insectivora, Soricidae), which was shown to be polymorphic for five Rb fusions (Rogatcheva et al., 1997a).

MATERIALS AND METHODS

In this study we used shrews of the SK hybrid stock originated from the crosses and intercrosses of two laboratory strains of *S. murinus*: KAT and SRI. The KAT strain was derived from a wild population in Kathmandu, Nepal (Oda et al., 1992) and has been shown to have 2n = 40 (Rogatcheva et al., 1996). The SRI strain was derived

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from animals captured on the West Coast of Sri Lanka (Ishikawa et al., 1989). The karyotype of the SRI strain differed from that of the KAT strain by the presence of five Rb fusions (Rogatcheva et al., 1997a). SK shrews have various combinations of these variant chromosomes.

Metaphase chromosomes were prepared from primary cultures of fibroblasts initiated from skin biopsies of one female (38XX) and one male (36XY) of the SK hybrid stock as previously described (Rogatcheva et al., 1996).

For localisation of the telomeric DNA sequences, fluorescence in situ hybridisation (FISH) was performed using a mixture of biotin–labelled oligonucleotides (TTAGGG)$_7$ and (CCCTAA)$_7$ as a probe, according to the method described by Ono and Yosida (1993). Hybridisation of biotin-labelled telomeric probe was detected with fluorescein-conjugated avidin. Photographs were taken on Fujicolor 400 ASA film.

RESULTS AND DISCUSSION

Fig. 1 shows metaphase spreads of the male (36XY), which was heterozygous for four Rbs (8.17; 9.13; 11.16 and 14.15) (Fig. 1a), and the female (38XX), which was heterozygous for two Rbs (10.12 and 11.16) (Fig. 1b). Hybridisation signals were observed only at both ends of all chromosomes. About 97% of telomeres were visible at each metaphase.

Being the largest metacentrics in the karyotype, Rbs were easy to identify by their morphology (Fig. 1). No telomeric sequences were detected at pericentromeric sites of any of the five Rb metacentrics. On the Rb chromosomes, as well as on their twin acrocentrics, the telomeric hybridisation signals were observed only at both ends of the chromosomes.

In some species, telomeric sequences are associated with nucleolus organizer regions (Meyne et al., 1989; Liu and Fredga, 1999). Therefore we paid special attention to those chromosome regions where genes for 18+28S rRNA genes of S. murinus were located (Rogatcheva et al., 1997b): 5p15-pter; 9q64-qter; 13q38-qter. However, no difference in the strength of the hybridisation signals between these loci and other chromosomal termini was detected.

Thus, we demonstrated complete loss of the telomeric sequences at the fusion points of the Rb metacentrics in S. murinus. They were also found to be lost at the centromere regions of Rb metacentrics in some polymorphic species, in which rearrangements have occurred recently, for example, in the house mouse (Garagna et al., 1995; Nanda et al., 1995) and short-tailed shrew (Qumsiyeh et al., 1997). Also Meyne et al. (1995) did not detected interstitial sites of either vertebrate or putative insect telomeric sequences in the chromosomes of several species of ants from the genus Myrmecia. However, in okapi (Vermeesch et al., 1996), Akodon cursor (Fagundes et al., 1997) and many other vertebrate species (Meyne et al., 1990) interstitial telomeric repeats have been detected near the centromeres of Rb metacentrics. This may indicate that two different mechanisms were responsible for Rb formation in these two groups of species. Sljibepevic (1998) suggested that a prerequisite for the formation of Rb fusions should be either telomere loss or telomere inactivation. Complete loss of p-arm telomeres by chromosome breakage within minor satellite sequences leads to Rb-like fusion or whole-arm reciprocal translocation between the acrocentrics involved. Thus, we could assume that in the former group of species, including S. murinus, Rb fusions happened due to telomere loss, whereas in the latter group due to telomere inactivation.

Although it is generally accepted that in karyotype evolution Rb-fusions are more common events than fissions (for a review see Qumsiyeh (1994)), Imai (1993) and Meyne et al. (1995) provided arguments that fissions could also participate in karyotype evolution. Meyne et al. (1995) interpreted the absence of interstitial sites in the chromosomes of several species of ants from the point of view that chromosome fissions, but not fusions, played an important role in chromosome evolution of this taxon.

In the case of S. murinus, we believe that Rb-fusions, but not fissions, have caused chromosome polymorphism in this species. Indeed, the most common karyotype of S. murinus has 2n=40 (Yosida, 1982; Rogatcheva et al., 1997a). It is present throughout the area of distribution of this species (Yosida, 1982). The only locality with 2n=30–32 is Sri Lanka Island (Ishikawa et al., 1989). Polymorphic populations of Southern India with 2n=30–32, 37, 40 (Aswatharanayana and Krishna, 1979) and Malaysia with 2n=35–40 (Sam et al., 1979) have apparently occurred due to hybridisation between the common karyomorph and that arrived from Sri Lanka or Southern India (Yosida, 1982; Rogatcheva et al., 1997a). For this reason, we consider the Rb metacentrics to be derived chromosomes that originated from ancestral acrocentrics.

Thus, we could assume that Rb fusions in S. murinus occurred due to telomere loss.

Meyne et al. (1990) discussed a possible role of interstitial telomeric repeats in the context of spatial genome organisation in interphase nuclei. Another interesting question is how loss or retention of the telomeric vestiges in a Robertsonian metacentric may affect its pairing with the twin acrocentrics in meiotic prophase of heterozygotes.

Telomeres play an important role in chromosome alignment in meiosis. The telomere region of the chromosome is assumed to be associated with the nuclear membrane. At leptotene, the telomeres come close together (Gillies et al., 1974; Gillies, 1975) and this presumably facilitates pairing initiation in the terminal regions of the homolo-
gous chromosomes. If vestiges of telomeres at the centromeric region of Rb metacentric retained such a function, they might facilitate alignment of Rb metacentric with the centromeres of the twin acrocentrics. In this case, homologous synapses might be initiated at both ends of each arm of the Rb chromosome. Therefore, the arms of the trivalent would be completely and homologously ‘zipped up’ by synaptonemal complex (SC) and the recombination in pericentromeric region would not be suppressed.

If the centromeric region of the Rb metacentric does not have interstitial telomeric repeats, it becomes aligned with the centromeres of the twin-acrocentrics rather late, when the whole arms have been ‘zipped up’ by SC. Up to this time, the p-arm telomeres and therefore the centromeres of the twin-acrocentrics have already been roughly aligned and might pair with each other non-homologously, forming a side arm. If the twin acrocentrics have failed to pair...
by their short arms, an asynapsis in a pericentromeric area is observed. In both cases, homologous pairing in pericentromeric region of such Rb trivalent is delayed and recombination is suppressed.

The patterns of chromosome pairing in *S. murinus* and *M. domesticus*, which do not contain interstitial telomeric repeats at the fusion points of Rbs, have already been analysed in detail. Indeed, in the mouse, Rb trivalents often display extensive asynapsis in a pericentromeric area (see de Boer and de Jong, 1989 for a review). The Rb trivalents in *S. murinus* demonstrate regular formation of side arms and complete, but apparently non-homologous pairing (Borodin et al., 1998). It would be very interesting to compare chromosome pairing in Rb heterozygotes with and without interstitial telomeric repeats at Rb fusion points.

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**REFERENCES**


