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Cytological and molecular characterization of centromeres in *Mus domesticus* and *Mus spretus*

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Abstract. We have applied EM in situ hybridization (EMISH) and pulsed field gel electrophoresis (PFGE) to samples from diploid primary cell cultures and an established cell line to examine in detail the relative organization of the major and minor satellite DNAs and telomere sequences in the genomes of *Mus domesticus* and *Mus spretus*. EMISH localizes the *Mus domesticus* minor satellite to a single site at the centromere-proximal end of each chromosome. Double label hybridizations with both minor satellite and telomere probes show that they are in close proximity and possibly are linked. In fact, PFGE of *M. domesticus* DNA digested with *Sal* I and *Sfi* I reveals the presence of fragments which hybridize to both probes and is consistent with the physical linkage of these two sequences. The *M. domesticus* minor satellite is the more abundant satellite in *Mus spretus*. Its distribution in *M. spretus* is characterized by diffuse labeling with no obvious concentration near chromosome ends. In addition to this repeat the *M. spretus* genome contains a small amount of DNA that hybridizes to a *M. domesticus* major satellite probe. Unlike the *M. domesticus* minor satellite, it is not telomere proximal but is confined to a domain at the border of the centromere and the long arm. Thus, although both species possess all three sequences, except for the telomeres, their distribution relative to one another is not conserved. Based on the results presented, we propose preliminary molecular maps of the centromere regions of *Mus domesticus* and *Mus spretus*.

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Introduction

All eukaryotic genomes except for the budding yeast *Saccharomyces cerevisiae* contain variable amounts and types of tandemly repeated DNAs usually referred to as satellites. The majority of these sequences are restricted to chromosomal centromeres, giving rise to the C-bands visible in cytological preparations (Pardue and Gall 1970). In addition to these satellites, tandemly repeated hexameric sequences are located at the telomeres (Blackburn and Szostak 1984; Meyne et al. 1989).

The first satellite studied in detail was isolated from the *Mus domesticus* genome. It represents 6–10% of the genome and is distributed throughout the centromere regions of all but the Y Chromosome (Pardue and Gall 1970). A distinct satellite, named the minor satellite, also occurs in this genome (Pietras et al. 1983). It is related to the major satellite by sequence determination and shows a different pattern of centromeric localization (Wong and Rattner 1988). This repeat is the most abundant centromeric repeat in the *Mus spretus* genome.

Although both satellites have been localized to metaphase chromosomes by in situ hybridization followed by autoradiographic and/or fluorescence detection (Pardue and Gall 1970; Pietras et al. 1983; Wong and Rattner 1988), the greater resolution of the electron microscope revealed a more complex organization of the *M. domesticus* major satellite than predicted at the light microscope (LM) level. The in situ signal in the LM suggested a single long tandem array of satellite sequences, whereas in the EM this repeat is apparently nonuniformly distributed throughout centromeric heterochromatin. This result, in conjunction

with the observation that the estimated amount of centromeric DNA is several-fold greater than the amount of satellite DNA (Lica and Hamkalo 1983), suggests that satellite blocks are interspersed with nonsatellite sequences (Radic et al. 1987). The experiments described were carried out in order to determine the distribution of additional centromere-associated sequences at high resolution.

In initial studies, the minor satellite was shown to hybridize to the apparent acrocentric ends of *M. domesticus* chromosomes (Hamkalo et al. 1989), raising the question of where functional telomere sequences reside in a folded chromosome. At about the same time, Moyzis and co-workers (1988) identified the repeat sequence which is found at the telomeres of all vertebrate chromosomes (Meyne et al. 1989). Therefore, it was possible to include these sequences in our mapping studies. PFGE was also used in order to obtain physical linkage data that could be compared with the cytogenetic observations. This paper describes the results of these two types of study in the two species. It presents direct evidence for the probable physical linkage of the telomere and minor satellite sequences on at least some *M. domesticus* chromosomes, in agreement with their close apposition in double label in situ localizations on metaphase chromosomes. Both species possess satellite sequences that are distributed throughout the centromere as well as sequences organized in identifiable domains. However, except for telomere repeats, the distribution of a given repeat is not conserved. Based on these data, preliminary maps are presented of the centromere regions of both species.

Materials and methods

Cell culture

The established mouse cell line L929 was grown at 37°C in Joklik's D-MEM (Gibco) supplemented with 10% FCS and Penicillin/Streptomycin (Gibco). In some experiments, L929 cells were grown in the presence of Hoechst 33258 as described in Radic and colleagues (1987) in order to extend centromere regions and increase spatial resolution.

Diploid *M. domesticus* (Redi et al. 1990) and *M. spretus* cells were obtained from spleen lymphocyte cultures stimulated to divide by recombinant human interleukin-2 (Rosenstein et al. 1984; Narayanswami et al., manuscript in preparation). The *M. domesticus* strain used was C57BL/6 (Charles River) and *M. spretus* were supplied by the Roswell Park Cancer Institute. Female mice were used. Briefly, a pool of 2–3 minced spleens was used to set up a culture after removal of red cells by lysis, and depletion of macrophages based on their adherence to plastic. The nonadherent population was suspended in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine (Sigma), 10 μ/ml penicillin (Sigma), 0.1 mg/ml streptomycin (Sigma), 0.1 mM nonessential amino acids (Gibco), 1 mM sodium pyruvate (Gibco), 5×10^{-5} M 2-mercaptoethanol (J.T. Baker), and 10^3 μm/ml recombinant human interleukin-2 (Hoffman-La Roche). Cultures were seeded at a density of 2×10^6 cells/ml, grown for 4 days at 37°C in an atmosphere of 5% CO_2 and then subcultured every 48 h into fresh medium at an innoculation density of 1×10^6 cells/ml. One week old cultures were subcultured and incubated at 37°C for 24 h prior to mitotic arrest as described below.

Metaphase chromosome preparations for EM in situ hybridization

Metaphase chromosomes for EM in situ hybridization were obtained by arresting cells with 0.15 μg/ml Colcemid (Gibco) for 6 h at 37°C. L929 mitotic cells were collected by selective detachment (Narayanswami et al. 1989). Arrested lymphocyte cultures were used without further enrichment since they grow in suspension. In all cases, cells were lysed in 0.5% Nonidet P40 and released chromosomes were deposited on EM grids by a modification of the Miller procedure as described in Rattner and Hamkalo (1978).

Hybridization probes

The *M. domesticus* major satellite was detected with plasmid pSAT1 (Radic et al. 1987). The *M. domesticus* minor satellite was detected with pMR150 (Pietras et al. 1983). Probes were labeled either by nick-translation in the presence of Bio-dUTP or DNP-dUTP (ENZO Biochemicals) or by direct coupling to N-acetoxy-2-acetylaminofluorene (AAF) as described in Narayanswami and co-workers (1989). Synthetic oligonucleotides corresponding to the vertebrate telomere sequence (the gift of R. Moyzis) were end-labeled by terminal transferase in the presence of Bio-dCTP (Moyzis et al. 1988). In some experiments, biotinylated probes homologous to the telomere sequence were generated using the polymerase chain reaction (PCR; Weier et al. 1990). Primers used were homologous to positions 69–92 and 232–255, respectively, of the published sequence of telomeric clone pHuR143 (Moyzis et al. 1988). Thirty cycles of DNA amplification were carried out using human genomic DNA as template followed by ten cycles in the presence of Bio-11-dUTP. Plasmid probes for PFGE analysis were labeled with ^{32}P by priming with random oligomers (Feinberg and Vogelstein 1983) to a specific activity of at least 10^8 cpm/μg. The synthetic telomere oligonucleotide $(TTAGGG)_7$ was end-labeled with ^{32}P.

In situ hybridization

EM in situ hybridization was performed essentially as described in Narayanswami and co-workers (1989). Preparations were fixed in 0.1% glutaraldehyde, 2× SSC, denatured for 10 min in pH 12 2× SSC and hybridized at 30°C overnight with 800 ng/ml–4 μg/ml denatured biotinylated probe DNA. Hybrid sites were detected via rabbit-anti-biotin antibody (ENZO Biochemicals) followed by goat-anti-rabbit labeled 20 nm colloidal gold (Janssen Pharmaceutica). In the case of the telomere probes, which typically give a small signal, glutaraldehyde fixation was followed by a brief Proteinase K digestion (at 37°C for 30 min) prior to denaturation for 30 min in pH 12 2× SSC. Hybridization to oligonucleotide probes was the same as above except for the final probe concentration (1.2–4.8 μg/ml). In order to enhance a small signal, detection was via one or two successive incubations in goat-anti-biotin antibody (Vector Labs) and biotinylated donkey-anti-goat antibody (Jackson Immunoresearch) followed by labeling with streptavidin-20 nm colloidal gold.

For detection of two probes, chromosomes were hybridized simultaneously to alternately labeled probes (Narayanswami et al. 1989, 1991). Detection was by incubation in mixtures of appropriate antibodies made in different species and, finally, two different sizes of antibody or streptavidin-conjugated colloidal gold particles.

PFGE

Diploid *M. domesticus* and *M. spretus* lymphocytes were washed twice with PBS, resuspended at 10^7 cells/ml in 0.6% low gelling temperature agarose (Sea Plaque GTG Agarose, FMC), and cast into 100 μl molds. The DNA in agarose plugs was purified by incubating in ESP (500 mM EDTA, 1% Sarcosyl, 1 mg/ml proteinase K) at 50°C for two days. Prior to restriction digestion, plugs were washed four times in at least 20 volumes of TE with 1 mM PMSF included in the first two washes. DNAs were digested with several different rare-cutting restriction enzymes. Restriction digests were

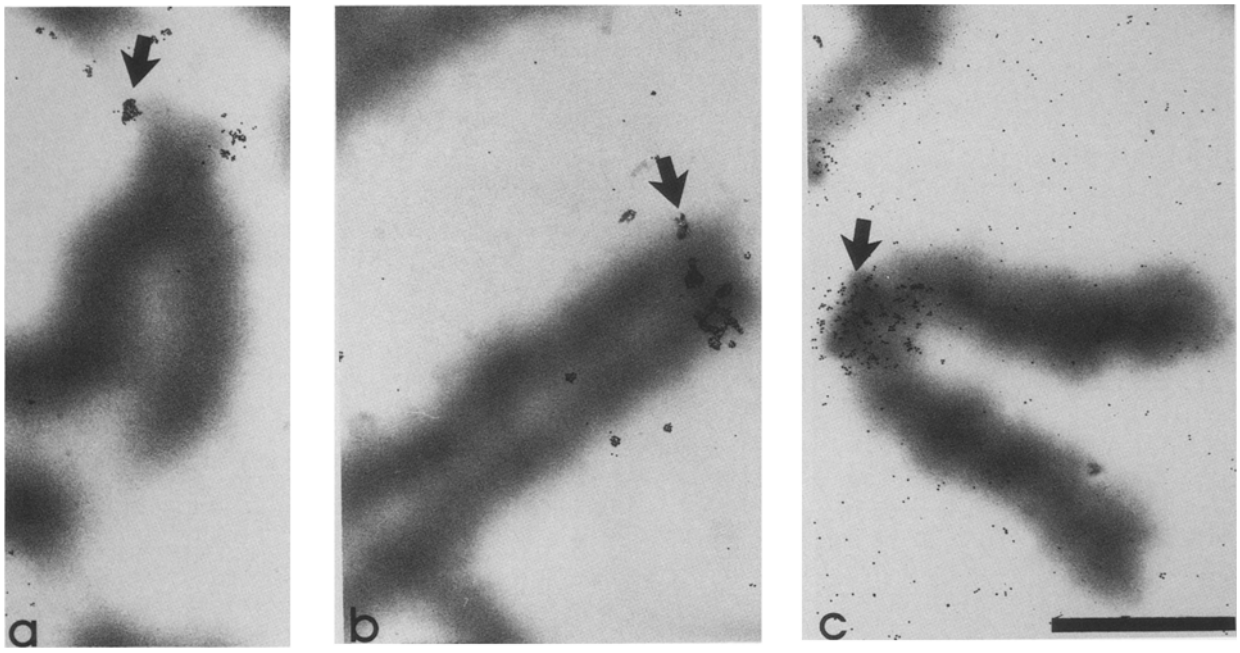

Fig. 1. (a) *M. domesticus* L929 cell metaphase chromosome after EMISH with biotinylated pMR150 and standard detection with goat-anti-rabbit conjugated 15 nm colloidal gold. **Arrows** denotes labeled site. (b) Chromosome labeled as in (a) showing a band of label at the primary constriction (**arrow**). (c) Metaphase chromosome from a diploid *M. domesticus* culture after in situ hybridization with major satellite probe and detection as in (a). **Arrow** denotes patchy labeling over the entire centromere region. Scale bar = 2 μm.

performed with 20 units enzyme/μg DNA in buffer recommended by the supplier for 4 h at 37°C. PFGE was performed on a CHEF-DR II apparatus (BioRad) in a modified 0.5× TBE buffer (50 mM Tris base/50 mM boric acid/0.2 mM EDTA, pH 8.2). One-eighth slice of a plug (approximately 1 μg DNA) was loaded in each lane. λ ladders, *S. cerevisiae* and *S. pombe* chromosomal DNA standards (all from FMC) were used as size standards. Pulse times, run times and the voltage employed for each run are indicated in the figure legends.

After electrophoresis, the DNA in agarose gels was nicked with UV light from a transilluminator (Fotodyne) for 60–90 s, denatured in 0.5 M NaOH/1.0 M NaCl for 1 h, and transferred to nylon membranes (Nytran, Schleicher and Schuell) by capillary action with 10× SSC. Membranes were prehybridized in hybridization buffer (Church and Gilbert 1984) (0.5 M $NaPO_4$, pH 7, 7% SDS, 1 mM EDTA) containing 100 μg/ml sonicated salmon sperm DNA for 2–4 h followed by hybridization to ^{32}P probe (2.5 ng/ml, final concentration), overnight at 65°C. Filters were washed twice briefly in 0.5× SSC, 0.5% SDS at room temperature, followed by four washes for 15 min each in 0.5× SSC, 0.5% SDS at 65°C. The filters were blotted semi-dry, and exposed to X-ray film (Kodak XAR-5) at −70°C between two intensifying screens (Dupont Cronex Lightning-Plus).

Results

Mus domesticus

Figure 1 shows typical examples of the hybridization pattern of the minor satellite probe, pMR150, to telocentric chromosomes from mouse cells. In both diploid and cultured cell chromosomes, hybridization is confined to a small region of the centromere on the majority of the chromosomes and appears either as a discrete patch at the apparent end of the primary constriction (Fig. 1a), or as a diffuse band across this region (Fig. 1b). At least 90% of the chromosomes are labeled (Table 1), in agreement with the fluorescence in situ hybridization results of Wong and Rattner (1988). However, due to differences in sensitivity between LM and EM in situ hybridization this number could actually be greater. The signal size varies among chromosomes as assessed by the number of gold particles in a cluster. This difference probably reflects different amounts of minor satellite among different chromosomes, although we cannot eliminate a contribution by differential accessibility. This labeling pattern is in marked contrast to that obtained with the major satellite which hybridizes throughout the centromere region (Fig. 1c) in both diploid and cultured cell chromosomes.

Although some chromatin decondensation often occurs during the Miller spreading procedure, hybridization with pMR150 shows compact patches of gold particles even in these chromosomes (Fig. 2a), suggesting that the minor satellite exists as a single large block of tandem repeats, unlike the distribution of major satellite (Lica et al. 1986; Radic et al. 1987).

The terminal location of minor satellite sequences raised the question of whether or not they were retained in Robertsonian fusions, chromosomes which

Table 1. Percentages of labeled chromosomes in diploid lymphocytes hybridized with probes for the mouse major and minor satellites. Results are representative of six individual experiments.

Probe	*M. domesticus*	*M. spretus*
pSAT 1	100	>75
pMR150	≥90	100

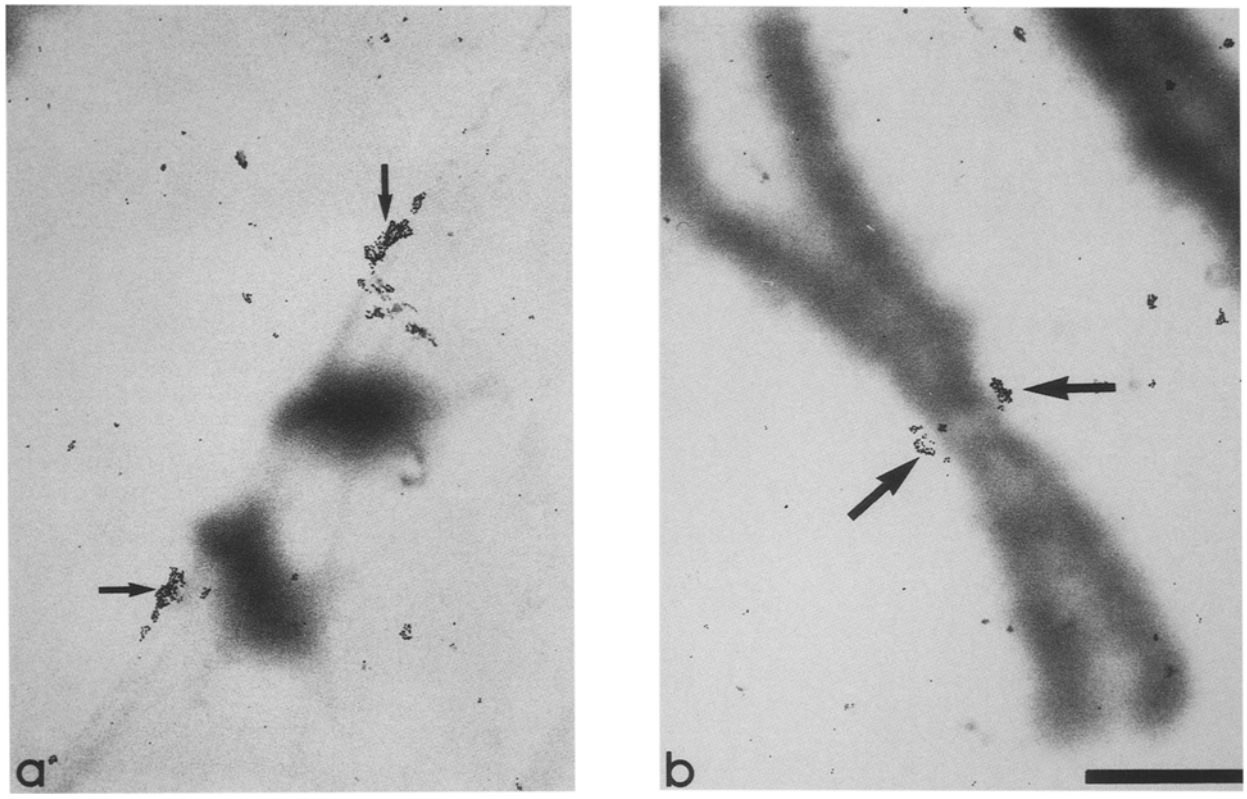

Fig. 2. (a) L929 cell chromosome showing hybridization of minor satellite in a compact pattern to decondensed chromatin (**arrows**). (b) Metacentric chromosome from L929 showing hybridization of minor satellite to the centromere region (**arrows**). Scale bar = 2 μm.

arise by breakage and fusion of telocentric chromosomes. Since fusion chromosomes occur frequently in cultured cells, we examined the hybridization pattern of minor satellite to L929 metacentric chromosomes. Figure 2b shows a typical metacentric chromosome after hybridization with pMR150. This repeat occurs as two patches, often protruding from either side of the primary constriction, a pattern also described by Wong and Rattner (1988). In the case of marker chromosomes containing several constrictions, the minor satellite is present at only subset of the secondary constrictions (Fig. 3a) indicating that most or all can be lost in the course of events which give rise to these chromosomes.

The highly condensed state of centromeric heterochromatin precludes addressing the possibility that the minor satellite exists in several blocks which are brought into close proximity by chromatin folding, as may be the case for the major satellite. However, growth of cells in the presence of the (A-T)-specific ligand Hoechst 33258 prevents complete condensation of centromeric regions and provides a higher resolution view of sequence distribution. Minor satellite localization in extended centromeres was carried out with L929 cells since we were unable to define conditions under which lymphocytes could be treated with Hoechst without massive cell death. Labeling was confined in a majority of the chromosomes to a region closely apposed to the physical end of the primary constriction (Fig. 3b), in agreement with fluorescent localization data from Wong and Rattner (1988). However, a few chromosomes contained two bands of hybridization in the centromere region (Fig. 3c). The second site was clearly separated from the telomere-proximal band, suggesting that a small number of chromosomes contain minor satellite in at least two distinct loci.

The proximity of minor satellite labeling to the apparent ends of chromosomes led us to determine the relative position of telomere functional sequences. Hybridization of metaphase chromosomes from *M. domesticus* lymphocytes and L929 cells with telomere sequences results in labeling of all four chromosome ends (Fig. 4). There is, however, a reproducible difference in the labeling pattern and amount between the centromere-proximal and centromere-distal telomeres. The telomeres at the centromere-proximal ends appear to protrude from the chromosome arms in a pattern very similar to the minor satellite, whereas those at the centromere-distal ends appear to be folded back and embedded in the chromosome arms. In addition, when signal amplification is not included, centromere-distal telomeres contain several foci of label (Fig. 4), whereas the centromere-proximal ends generally show one region of hybridization. This could be a result of differences in accessibility at these telomeres or it could reflect a real difference in telomere sequence distribution. Since the centromere-proximal telomere signal is always about twofold that at the other end of the chromosome, there also may be a

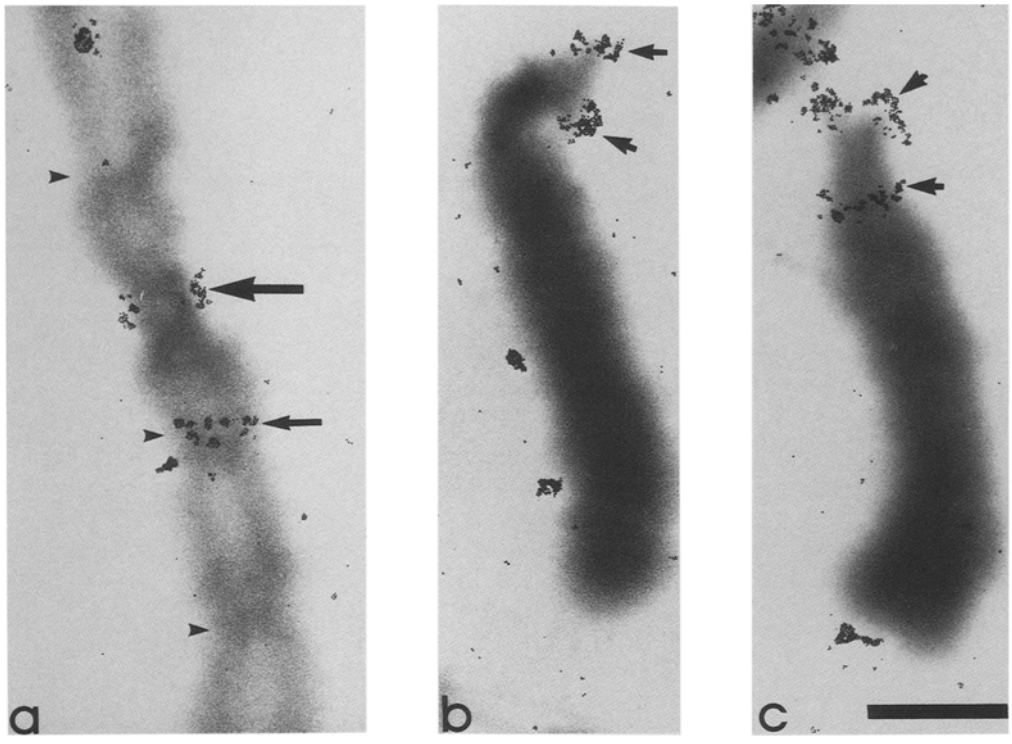

Fig. 3. **(a)** L929 cell marker chromosome showing hybridization of the minor satellite to the centromere **(large arrow)** and one **(small arrow)** of several secondary constrictions **(arrowheads).** **(b)** Metaphase chromosome from L929 grown in the presence of Hoechst-33258 after EMISH with minor satellite. **Arrows** denote labeled sites at the physical end of the centromere. **(c)** Chromosome as in **(b)** showing two regions of hybridization **(arrows).** Scale bar = 2 μm.

difference in copy number between the two ends of the chromosome.

In order to better define the relative arrangement of telomere and minor satellite signals in *M. domesticus,* they were colocalized. Figure 5 shows the centromere region of a typical metaphase chromosome after simultaneous labeling with both the telomere and minor satellite probes. Telomeres are labeled with 20 nm gold and minor satellite is labeled with 5 nm gold. The two sequences are very close, if not adjacent, to each other.

An independent analysis of the long-range organization of the minor and major satellite and telomere sequences in *M. domesticus* was carried out by PFGE.

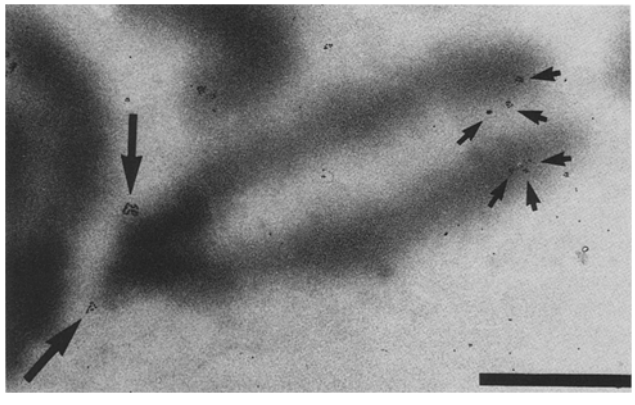

Fig. 4. *M. domesticus* chromosome after EMISH with an oligonucleotide probe for the telomere sequence. **Large arrows** show hybridized sites protruding from the centromere while **small arrows** denote several foci of labeling embedded in the chromosome arm. Scale bar = 2 μm.

Both minor satellite and telomere probes show hybridization to discrete DNA fragments. Furthermore, there are several fragments generated by different enzymes which hybridize to both probes in both separation ranges (a and b, Figs. 6–7). At a 200–500 s pulse ramp common fragments are particularly evident in the *Sfi* I lane (at about 1900, 1640, 1300, and 1000–550 kb), but also are visible in the *Xho* I (1900 kb and larger and at about 1300–1500 kb), *Cla* I (1900 kb and above), *Sal* I, and *Nar* I lanes. At a 1000–3000 s pulse ramp

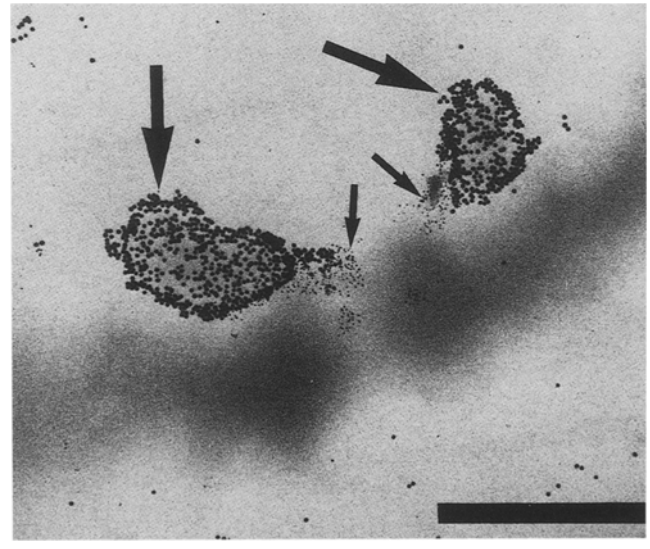

Fig. 5. Centromere region of a typical *M. domesticus* metaphase chromosome after simultaneous labeling with the telomere (20 nm gold, **large arrow**) and minor satellite (5 nm gold, **small arrow**) probes. Labeling is to adjacent sites. Scale bar = 1 μm.

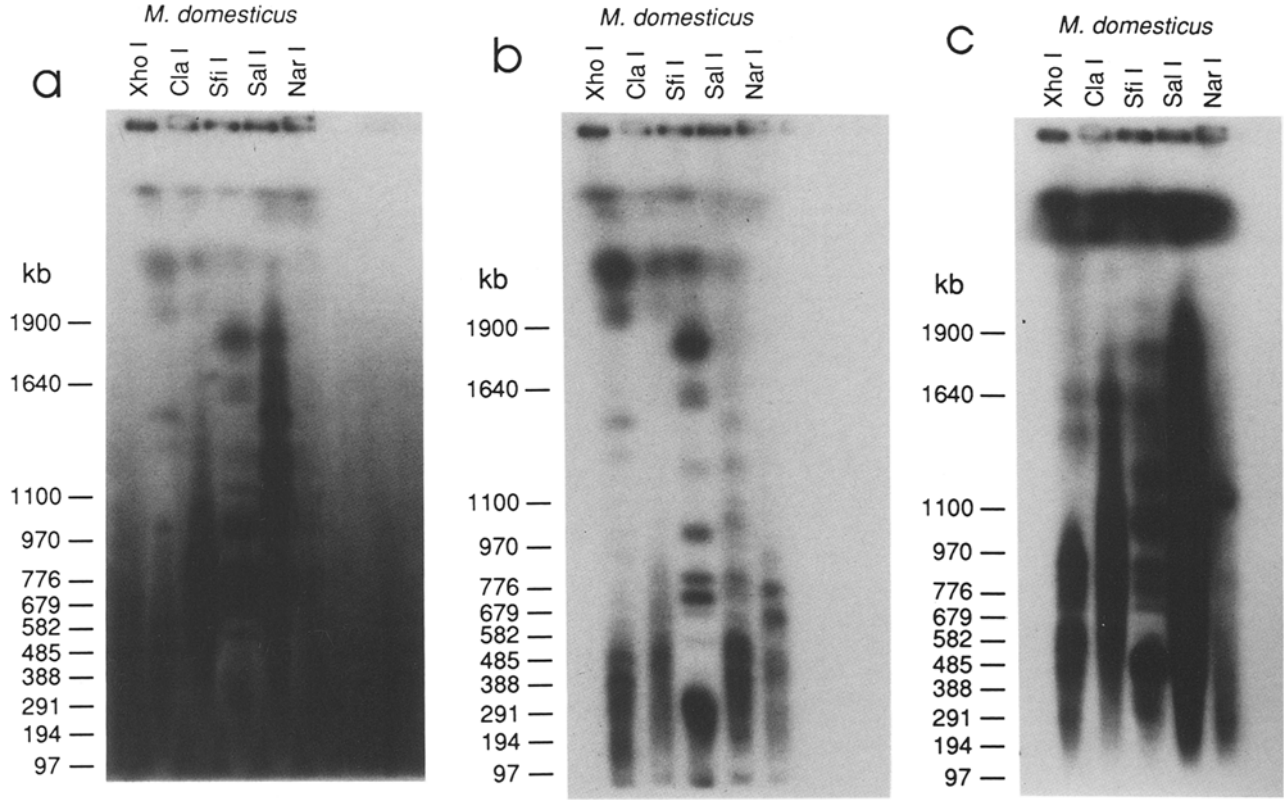

Fig. 6. PFG Southern hybridizations of *M. domesticus* DNA digested with several different restriction enzymes. The restriction enzymes used are indicated above the lanes. Size standards (**left**) were drawn from λ ladders and *S. cerevisiae* chromosomes. PFGE was run for 80 h at 1.5 V/cm with a 200–500 s pulse ramp. **(a)** Hybridization with minor satellite probe pMR150, 16 h exposure. **(b)** Hybridization with the telomere oligonucleotide $(TTAGGG)_7$, exposure 15 d. **(c)** Hybridization with major satellite probe pSAT1; 16 h exposure.

common fragments occur in all five digests above 5000 kb, the first three digests at 3100, 2700, 2300, 1900, and 1100 kb and are also seen in the *Sal* I (1–2000 kb) and *Nar* I (1900 kb, 2700 kb) lanes. Major satellite hybridization, on the other hand, gives a smeared pattern (c, Figs. 6–7) suggesting that these sequences are widely distributed across numerous fragment sizes. However, the presence of several discrete fragments (Fig. 6c, *Xho* I and *Sfi* I lanes), indicates that some proportion exists in the form of long tandem arrays. In addition, there appears to be little or no overlap between major satellite fragments and either minor satellite or telomeric fragments indicating that major satellite is separated from both minor satellite and telomere sequences by considerable amounts of DNA.

Mus spretus

The distribution of major and minor satellites and telomere sequences in *M. spretus* chromosomes was determined to evaluate the similarity or difference in their organization relative to that described above for *M. domesticus*. Figure 8a shows a typical *Mus spretus* lymphocyte chromosome after hybridization with minor satellite, the abundant satellite in this species. Essentially all the chromsomes label with this probe (Table 1). Although labeling is confined to the centromere region as in *M. domesticus*, the nature of the labeling is different. Unlike the *domesticus* pattern of two discrete foci, patchy labeling of the entire primary constriction is observed, and the pattern is similar to that of the *M. domesticus* major satellite on *domesticus* chromosomes. The blocks of satellite in *M. spretus* are clearly interspersed with unlabeled material, a pattern analogous to that seen in *M. domesticus* extended centromeres (Radic et al. 1987). The *M. domesticus* major satellite also shows hybridization to the centromeres of *M. spretus*. The pattern observed is novel: hybridization is to a distinct region at the border of the centromere and the long arm of at least 75% of the chromosomes (Fig. 8b; Table 1). The composition of these sequences is not known; they could be the same as the *domesticus* satellite or merely sufficiently homologous to cross hybridize under the conditions used.

Hybridization of *M. spretus* lymphocyte chromosomes with the telomere probe shows a labeling pattern that is essentially the same as in *M. domesticus* (Fig. 8c). The signal at centromere-proximal telomeres protrudes slightly from the chromosomes, whereas the centromere-distal telomeres appear to be embedded in the chromosome arms. However, under the same conditions of hybridization and detection, fewer *spretus* chromosomes label, suggesting either that the telomeric blocks are smaller or that they are less accessible to detection.

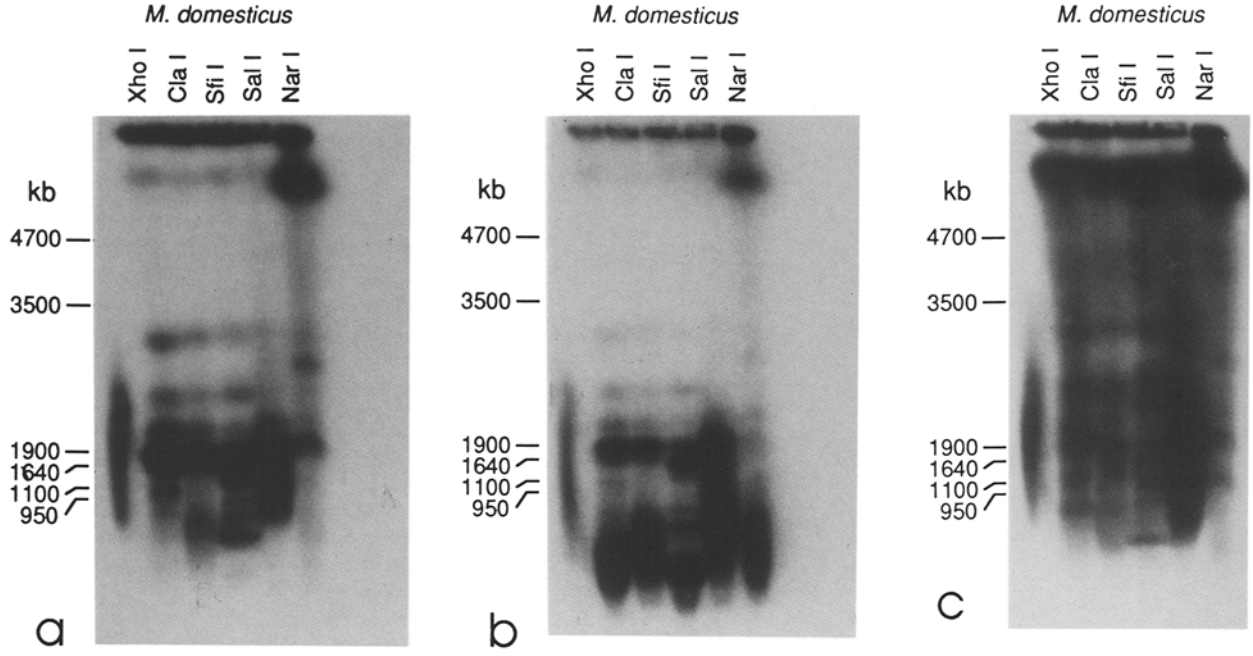

Fig. 7. PFG Southern hybridizations of *M. domesticus* DNA digested with several different restriction enzymes. The restriction enzymes used are indicated above the lanes. Size standards **(left)** were drawn from *S. cerevisiae* and *S. pombe* chromosomes. PFGE was run for 160 h at 1.5 V/cm with a 1000–3000 s pulse ramp. **(a)** Hybridization with minor satellite probe pMR150, exposure for 7½ h. **(b)** Hybridization with the telomere oligonucleotide $(TTAGGG)_7$, 5 d exposure. **(c)** Hybridization with major satellite probe pSAT1. Exposure for 3 h.

PFG analysis of *M. spretus* DNA revealed distinctly different patterns of hybridization with major and minor satellites and telomere sequences than that seen in *M. domesticus* (data not shown). The minor satellite hybridization pattern in *M. spretus* was largely smeary or diffuse with only a few distinct bands, consistent with a wider range of distribution than in *M. domesticus*. Telomere hybridization to *spretus* was also less distinct (fewer bands, and more smearing) than in *domesticus* and was to smaller fragments on average than in the latter species. Because of the diffuse minor satellite and telomere signals no significant cohybridization was found in *spretus*. Major satellite hybridization to *spretus* had clearly the most distinct bands and the least smearing. No overlap was detected between these major satellite PFG fragments and either telomere or minor fragments, as predicted from the EMISH data.

Slot blot experiments were performed to quantitate the relative abundances of major and minor satellites and telomere sequences between *M. domesticus* and *M. spretus*. Major satellite is 200-fold more abundant in *domesticus* than *spretus;* minor satellite is 1.4-fold greater in *spretus* than *domesticus;* and telomere sequence hybridization is tenfold greater in *domesticus* than *spretus* (data not shown).

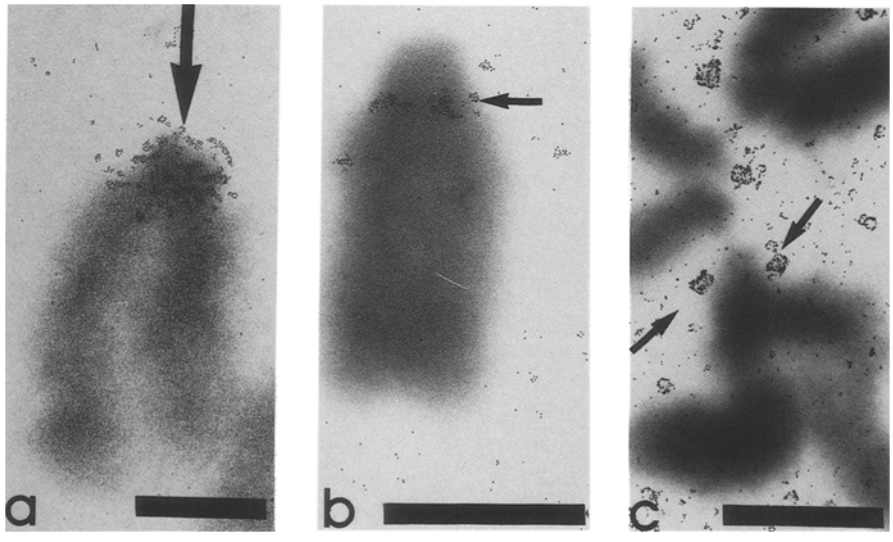

Fig. 8. A typical *M. spretus* metaphase chromosome hybridized with the mouse minor satellite showing patchy hybridization to the entire centromere region **(arrow).** Scale bar = 2 μm. **(b)** Chromosome as in **(a)** hybridized with the mouse major satellite. Labeling **(arrow)** is to the border of the centromere and the long arm. Scale bar = 2 μm. **(c)** Chromosome as in **(a)** and **(b)** hybridized with the telomere probe. Centromere-proximal telomeres protrude from the chromosome **(arrows).** Scale bar = 2 μm.

Discussion

We have combined in situ hybridization and PFGE to examine the organization of centromeric and telomeric sequences in *Mus domesticus* and *Mus spretus*. In situ hybridization at the EM level provides a higher resolution picture of the organization of these sequences than that previously described using fluorescence detection (Wong and Rattner 1988; Wong et al. 1990). Our studies demonstrate that in *M. domesticus* the minor satellite is organized in the form of discrete patches or bands at or near the physical end of the centromere region, in distinct contrast to the major satellite, which is distributed throughout the entire centromere region and may be interspersed with other, yet to be identified, sequences (Lica et al. 1986; Radic et al. 1987). The labeling patterns obtained also suggest that the minor satellite may exist as one or a small number of tandem arrays with little interspersed DNA. These results confirm studies using both autoradiographic (Pietras et al. 1983; Joseph et al. 1989) and fluorescence detection (Wong and Rattner 1988; Wong et al. 1990) which postulate a discrete and terminal location for this sequence on the *M. domesticus* chromosome. Based on the apparent correspondence between the position of minor satellite and kinetochores, Wong and Rattner (1988) suggested this sequence might function as the basis of kinetochore formation. However, more recent work (Wong et al. 1990) as well as data presented here questions this model since one might expect conservation of such a functional sequence in the same genus whereas it is organized very differently in *Mus spretus* chromosomes.

Data from in situ hybridization to Hoechst-treated chromosomes also support the hypothesis that the minor satellite is not extensively interspersed with other sequences, and that it is localized in close proximity to the apparent end of *M. domesticus* chromosomes. In addition, double label hybridization with minor satellite and telomere probes shows that they are essentially adjacent in mitotic chromosomes. Although this suggests physical linkage, these sequences could be separated by a considerable amount of DNA which is invisible as a result of chromosome folding. Further evidence for their probable linkage was provided by the PFG experiments described here. In these experiments, DNA fragments in the size range of 100–5000 kb hybridized to both probes. Since all mouse chromosomes contain both sequences, cohybridization of probes to similar sized fragments is not proof of linkage. However, cohybridization to many fragments across several enzymes and two size separations is consistent with their linkage. Since there are many copies of each sequence it was not possible to determine how close two sequences can be and still be resolved by EMISH (that is, spatial resolution). Nevertheless, it is clearly capable of resolving these sequences whose map positions are indistinguishable by fluorescence.

The telomere repeats at the distal ends of mouse mitotic chromosomes appear to be embedded in chromosome arms and often occur as several foci of label after EMISH. Analysis of PFG separated mouse DNA probed with the telomere probe shows hybridization to discrete bands smaller than those that also contain minor satellite. If these smaller bands derive from the centromere-distal telomeres, it would suggest a distinct organization of telomere sequences at opposite ends of the mouse chromosome. These putative differences in the telomeres at opposite ends of the chromosome may be of biological significance. For example, metacentric mouse chromosomes contain detectable telomere repeats only at chromosome ends (S. Narayanswami, unpublished results); Robertsonian fusion to give rise to metacentric chromosomes appears to be accompanied by the loss of most (possibly all) centromere-proximal telomeric repeats.

Based upon an analysis of telomere lengths in human fibroblasts and cells derived from individuals of different ages, Harley and colleagues (1990) proposed that telomere length and lifespan are directly correlated. However, Kipling and Cooke (1990) disputed this correlation because they found that the short-lived mouse has relatively long telomeres and that there is no effect of age on telomere length in mice. We observed marked differences in the size and a tenfold difference in the amount of the telomere sequences between *M. domesticus* and *M. spretus* despite the fact that the lifespans of these two species are essentially the same. Although these results could be interpreted to support the latter view, it is possible that the analysis of telomere lengths at the level of individual chromosomes would reveal subtle differences that result in chromosome instability which may be more directly correlated with senescence than total telomere length.

Mouse major and minor satellites and telomere sequences all give reproducible labeling patterns in the chromosomes from a variety of *M. domesticus* cell lines (data not shown), suggesting conservation of structural and/or functional domains in the centromere region of a given species. However, the organization is complex and has not yet been defined in extensive molecular detail.

The centromeric distribution of the *M. domesticus* minor satellite in *M. spretus* differs markedly. It is distributed throughout the centromere, similar to the distribution of major satellite in *M. domesticus*. It is not concentrated proximal to chromosome ends and its patchy localization suggests that it occurs in more than one tandem array presumably interspersed with other sequences. Finally, the similarity in amount of minor satellite in the two species coupled with the observed differences in its distribution also lend support to the hypothesis that this sequence is extensively interspersed with other sequences in *M. spretus*. *Mus spretus* also possesses a small amount of centromeric DNA which hybridizes to the *M. domesticus* major satellite. PFGE suggests that this component may be organized predominantly in the form of long tandem arrays. Finally, the presence in each species of mouse of a satellite distributed throughout the centromere

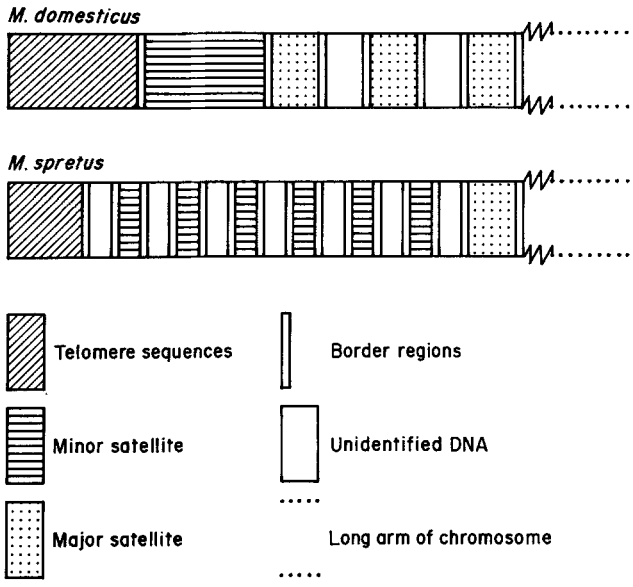

Fig. 9. Schematic representation of the sequence organization of the centromere regions of *Mus domesticus* and *M. spretus*.

may reflect a similar role for these sequences in the higher order organization of centromeric heterochromatin.

The results presented here, in conjunction with previous studies (Lica and Hamkalo 1983; Radic et al. 1987), permit the construction of preliminary maps of the centromere regions of *Mus domesticus* and *M. spretus* (Fig. 9). We propose that in the majority of *M. domesticus* chromosomes telomere sequences are linked to a block or small number of blocks of minor satellite which are, in turn, linked to the first of a series of blocks of major satellite that constitute the main body of the centromere region. Since telomeres appear to be the most distal centromeric sequences in these chromosomes, they are truly telocentric and not acrocentric chromosomes. Based on the similarity of labeling patterns published by Wong and co-workers (1990) we predict a similar centromere organization in telocentric chromosomes from the *Mus musculus* subspecies *M.m. molossinus* and *M.m. poschiavinus*. The *M. spretus* centromere region appears to be organized differently. We propose that in the majority of *M. spretus* chromosomes, telomere sequences are linked to the first of many blocks of minor satellite, which are followed by one or a few blocks of major satellite. As with *M. domesticus*, *M. spretus* chromosomes also appear to be truly telocentric. The compartmentalization of centromeric sequences in the mouse suggests it will be an excellent model system for the analysis of structural and functional relationships in mammalian centromeres.

Note added in proof: In a recent paper, Kipling and co-workers (*Genomics 11:* 235–241, 1991) provided data supporting physical linkage of the minor satellite to the telomere in *M. musculus*.

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