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Sequence organization and cytological localization of the minor satellite of mouse

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ABSTRACT

A complete 120 bp genomic consensus sequence for the mouse minor satellite has been determined from enriched L929 centromeric sequences. The extensive sequence homology existing between the major and minor satellite suggests an evolutionary relationship. Some sequences flanking the minor satellite has also been identified and they provide insight into centromeric DNA organization. Isotopic in situ hybridization analysis of the minor satellite to mouse L929 and Mus musculus metaphase spreads showed that this repetitive DNA class is localized specifically to centromeres of all chromosomes of the karyotype. With the use of high resolution non-isotopic fluorescence in situ hybridization the minor satellite is further localized to the outer surface of the centromere in a discrete region at or immediately adjacent to the kinetochore. Our cytological data suggests that the minor satellite might play a role in the organization of the kinetochore region rather than, as previously suggested, sites for general anchoring of the genome to the nuclear matrix.

INTRODUCTION

The primary constriction (centromere) is both a structurally and functionally distinct domain within the mammalian metaphase chromosome. This region plays an essential role in the segregation of the genome through the regulation of sister chromatid pairing at the inner surface of the centromere (1,2) and the association of the chromosome with microtubules of the mitotic spindle at the outer surface (kinetochore) of the centromere (3,4). While the centromere shows regional variation in terms of function there is little information on the relationship between this functional segregation and the underlying sequence organization. It is clear, however, that the mammalian centromere has a complex sequence organization composed of usually one or several classes of satellite DNA (5). In the mouse centromere, the most prominent tandemly repetitive sequence is the major satellite which represents 5-10% of total genomic DNA (6). In situ hybridization at the electron microscopic level has shown that it is not arranged into a single block but is distributed throughout

the entire centromere (7). Unlike the major satellite that is found on all the mouse chromosomes except the Y, another repetitive mouse centromeric sequence, the minor satellite, has been detected in the centromeric and non centromeric sites of approximately half the chromosomes of the karyotype (8). Recently a complete sequence of the minor satellite basic repeating unit has been inferred from a cDNA clone, pEAT11 (9). In that same report, it was proposed that this repetitive DNA class is involved in anchoring the genome to the nuclear matrix.

To further our understanding of the role of the minor satellite and other sequences in the organization of the mouse centromere, we have taken advantage of the differential susceptibility of mouse metaphase chromosomes to nuclease digestion (10,11) to obtain an enriched fraction of mouse centromeres for investigation. A small plasmid library constructed with the DNA obtained from this fraction has been analyzed. In the course of this analysis it has been possible to develop a direct genomic consensus nucleotide sequence for the minor satellite and it turns out to differ from the one reported previously (8,9). Identification of the sequences flanking the minor satellite DNA have also provided insight into the genomic organization within the centromere. High resolution in situ hybridization with the mouse tissue culture line L929 and Mus musculus metaphase spreads illustrated that the minor satellite DNA is localized specifically to a discrete region on the outer surface of the centromere of all chromosomes, at or immediately adjacent to the kinetochore. The relationship between the association of this sequence with the nuclear matrix and the kinetochore are discussed.

MATERIAL AND METHODS

Isolation and characterization of clones from the library

Samples enriched in centromeric sequences were prepared from nuclease digestion of metaphase chromosomes in mouse L929 cells as previously described (10,11) and randomly inserted into the Sma I site of the multipurpose vector pTZ19U (Pharmacia). The plasmid library (pMK) obtained in this manner was screened by colony hybridization (12) with total mouse genomic DNA and pSAT as probes. pSAT was constructed independently by inserting the gel purified 234 bp major satellite Ava II fragment of mouse into the same vector. The probes were generated by nick-translation with [α-^{32}P] dCTP (800 Ci/mmol, New England Nuclear) as the label using a kit purchased from Betheseda Research Laboratories. The unincorporated

nucleotides were separated from the labelled DNA by Sephadex G-50 spun column chromatography. All standard recombinant DNA manipulations were carried out according to Maniatis et al. (13). Single stranded plasmid DNA templates of pMKB6, pMKC78, pMKC85 and pMKC104 were prepared by infecting the E. coli XL1 blue host cells (Stratagene) with the helper phage M13K07. They were sequenced by the dideoxy-chain termination method (14).

In situ hybridization

Monolayer cultures of mouse L929 cells were grown in Joklik's suspension media supplemented with 10% fetal calf serum. Metaphase chromosomes were prepared from logarithmic cultures that had been grown in the presence of 0.1 μg/ml colcemid for 4 hours prior to harvest. To produce chromosomes with elongated centromeres cultures were grown in the presence of 0.05 μg/ml 33258 Hoechst for 14 hours prior to harvest (15). Cells were removed from the flask by detachment with a rubber policeman and fixed in methanol/acetic acid (3:1, vol/vol). To prepare metaphase spreads from Mus musculus males, the spleens were removed and the cells were teased apart in sterile tissue culture media. The cells were then incubated at 37°C for 24 hours and colcemid (0.1 μg/ml) was added during the last 8 hours of culture. They were fixed as above using standard procedures. In situ hybridization was carried out according to the method of Pinkel et al. (16) except that biotinylated and tritiated riboprobes were used. Since all inserts were placed downstream from the T7 promoter in pTZ19U, run-off in vitro transcripts were made as follows: 1 μg of Sst I linearized DNA template was incubated at 37°C for one hour with 10 mM each of ATP, CTP, GTP, biotin-11-UTP (BRL) or 80 uCi of ^{3}H-UTP (50 Ci/mmol,NEN) as the label, 50 units of RNasin (Boehringer Mannheim), 30 units of T7 RNA polymerase (Pharmacia) in 1X transcription buffer (40 mM Tris-HCl, pH 8.0, 8 mM $MgCl_2$, 25 mM NaCl, 2 mM spermidine-HCl and 10 mM dithiotreitol). The specific activity of radioactive probe was approximately $1x10^8$ dpm/μg. The biotinylated riboprobe was detected by avidin tagged with fluorescein isothiocyanate (FITC). The signal was amplified with biotinylated goat anti-avidin (Dimension) and another layer of avidin-FITC. This amplification procedure has already been described in detail elsewhere (16). Specimens were examined on a Nikon Optiphot microscope equipped with fluorescence attachments and images were recorded on Ilford HP-5 film. The chromosome preparations were counterstained with 4',6-Diamidino-2-phenylindole (DAPI) to provide reference images of the whole chromosome. For autoradiography, the slides were coated with NTB-2 emulsion (Kodak) and

exposed for an appropriate length of time. They were developed, stained with Giemsa and photographed with an 80X objective on a Nikon Optiphot microscope.

Two step labelling protocol

A two step labelling protocol was used to co-localize the kinetochore proteins and the minor satellite probe to the metaphase chromosomes. In this procedure, the mouse L929 metaphase chromosome spreads were fixed in 1% paraformaldehyde. They were first reacted with a CREST autoimmune serum specific for the 19 and 50 kd kinetochore proteins as described by Kingwell and Rattner (17). Immunofluorescence photography of kinetochore staining was recorded and samples were then left under room light to photobleach the fluorescein before they were hybridized with the minor satellite biotinylated riboprobe. The fluorescence detection of the DNA/RNA hybrids was carried out as described in the previous section. The same regions of the metaphase chromosome spreads photographed previously were sought and rephotographed to allow spatial comparison of the immunofluorescent stains due to the kinetochore antibody and the minor satellite in both images.

RESULTS

Minor satellite structure

Centromeric heterochromatin of the mouse is less susceptible to digestion by micrococcal nucleases and restriction enzymes than the euchromatic arms (10,11). By taking advantage of this feature it is possible to obtain a fraction that is enriched in centromeric DNA from mouse L929 cells. DNA from this fraction was randomly sheared by sonication and cloned into the Sma I site of pTZ19U. The construction and full characterization of this library will be published in detail elsewhere. One hundred and four clones were isolated from this library. In a preliminary colony hybridization screening with the major satellite clone pSAT as probe, twenty-two of the clones were found to have inserts containing this repetitive DNA class. Since we did not have probes to the other repetitive DNA classes in mouse, we also screened our library with total mouse genomic DNA and compared the intensity of the hybridization signal to that of the major satellite in order to identify clones that might contain other repetitive families. Several of those clones that gave strong signals on autoradiograms were randomly chosen for sequence analysis. A clone designated pMKB6 was found to contain tandem repeats of the minor satellite DNA.

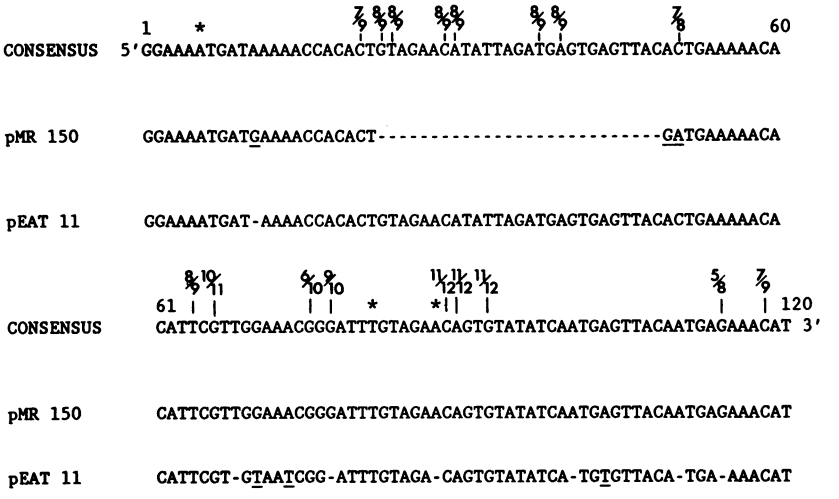


Figure 1. Consensus nucleotide sequence of minor satellite and comparision with pMR150 and pEAT11. The 120 bp genomic consensus sequence was derived from sequencing 14 full and partial monomeric units. The ratio indicates the number of times that a given base appears at that variant position. * indicates that consensus nucleotide is deleted in some monomeric units. Dashes denote the bases are deleted. The underline bases indicate differences among the three sequences.

A restriction fragment containing only minor satellite DNA from pMKB6 was used to probe the library for additional minor satellite clones, pMKC78, C85 and C104 were identified. Although a complete sequence has been derived for the minor satellite from a single cDNA intermediate (9), it may not be representative for the entire genomic population of this DNA class. By sequencing fourteen complete and partial copies of the minor satellite DNA from four independently isolated genomic clones has enable us to establish an unambiguous consensus sequence of 120 bp (Figure 1), 6 and 27 bp longer than pEAT11 (9) and pMR150 (8) respectively. The variant bases in the consensus sequence occurred in only 17 positions. Comparison between our consensus sequence of the minor satellite repeating unit from L929 cell line with that previously reported partial genomic clone pMR150 from Mus musculus and full length cDNA clone pEAT11 from Ehrlich ascite cell line revealed only few changes, suggesting that the minor satellite is highly conserved. Our additional sequence data included the 27 bases (positions 23-29 inclusive) that were deleted in pMR150 (Figure 1) are in exact agreement with that found in pEAT11. The minor satellite monomer is approximately 66% AT rich. Restriction site search of the consensus

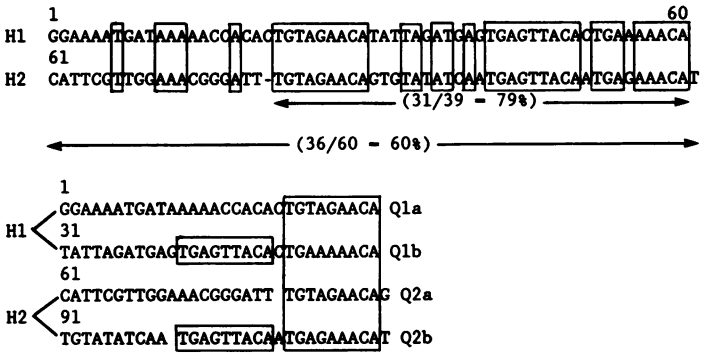


Figure 2. Subrepeat analysis of the minor satellite. A, the consensus sequence is divided into halves, H1 and H2. A base shift in H2 as indicated by a hyphen maximizes homology between the half repeats. The number of matches and percent of homology are given. B, the half subrepeats are further subdivided into quarter subrepeats Q1a, Q1b, Q2a and Q2b. Gaps are introduced to maximize homology. The boxed segments in A and B indicate domains of homology.

sequence indicated no restriction sites. However, Southern blot analysis of the mouse genome digested with several restriction enzymes revealed that Alu I, Mbo I, Pst I, Rsa I, and Msp I sites occasionally appeared within the minor satellite block (data not shown) with only Msp I giving a type B restriction pattern as reported previously (8). The polymorphism that gave rise to the Msp I (CCGG) site was probably due to a G to C transversion or an insertion of a C at position 75. A transversion was observed in three of the repeating units sequenced.

Subunit structures of minor satellite DNA

The 120 bp minor satellite DNA repeating unit could be divided into subrepeats. Figure 2 (Top) shows the 60 bp half repeats, designated as H1 for the first half and H2 for the second half. Although homology between the two half repeats was not observed at first, a base shift in H2 from position 81 to 82 maximized the match within the thirty-nine bases at the 3′ end region of the units (31/39 base match or 79% homology). The 5′ end region, on the other hand, showed considerable divergence. When the half repeats H1 and H2 are further subdivided into quarter repeats Q1a/Q1b and Q2a/Q2b respectively (Figure 2 Bottom), significant homology is again observed at the 3′ end region. When appropriate gaps were introduced, a nine base motif with slight base variations was found to be common among the four quarter repeats. This motif ,TGTAGAACA, in Q1a matches Q2a

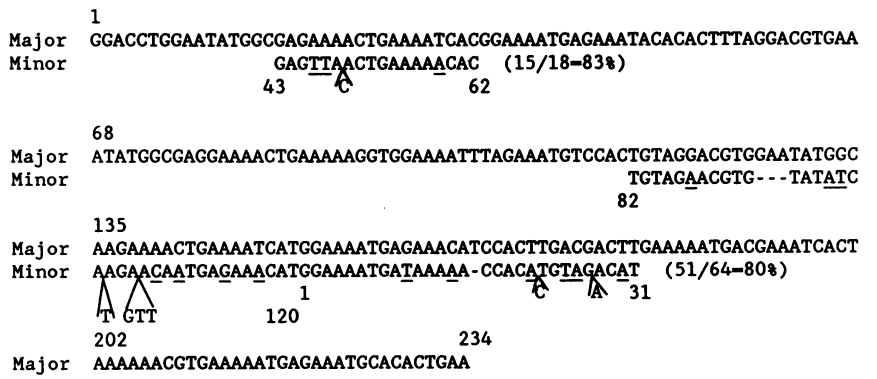


Figure 3. Comparison between the major and minor satellite consensus sequence. Positions 43 to 62 and 82 to 120/1 to 31 of the minor satellite show 83% and 80% homology respectively. Gaps and insertion of bases are included to maximize homology. The underline bases indicate changes between the two satellites.

exactly, whereas there is a one base change, TGA(A/G)AAACA, between Q1b and Q2b. In the middle region of the Q1b and Q2b quarter repeat, a common sequence block, TGAGTTACA was also present. Overall, the quarter subrepeat Q1b and Q2b shared a greater degree of homology. This observation was not unexpected since both of these quarter subrepeats were derived from the 3' end region of the half repeats that exhibited considerable homology. No simple tandem subunit could be observed within the minor satellite repeat from this subdivision analysis.

Comparison between the major and minor satellite DNA sequences

The consensus sequence of the major satellite DNA of mouse is composed of 234 base pairs. This basic component has been further subdivided and a set of nine base ancestral sequence motifs (GAAAAATGA, GAAAAAACT and GAAAAACGT) have been identified (6). As indicated in the previous section, the minor satellite class does not have a similar primordial sequence. However, significant homology can be found in certain regions between the two satellite families. As shown in Figure 3, when proper gaps have been introduced, a stretch at positions 115-183 inclusive of the major satellite overlaps the 3' end (positions 82-120) and 5' end (positions 1 to 31) junction of two minor satellite repeating units (51/64 base matches or 80%). In addition, a smaller region with 83% homology was found at positions 17 to 34 inclusive of the major satellite and positions 43 to 61 inclusive of the minor satellite.

Figure 4. Sequences flanking the minor satellite in pMKB6, pMKC78 and pMKC85. Bold letters represent the minor satellite segment. Only the junctions where the different sequences joined together are illustrated. R and M denote R element and major satellite respectively. Arrows indicate the pentanucleotide inverted repeats. The boxed regions highlight a common sequence motif found at the major and minor satellite junction.

Sequences flanking the minor satellite DNA

Of the four minor satellite clones isolated, three had other sequences flanking the repeating element (Figure 4). These sequences included the truncated 3' end region of a R element (18) or more specifically L1Md (19) and the regions proximal to the Ava II site of the major satellite consensus sequence. While the presence of these flanking sequences was not totally unexpected, since they have been shown to have centromeric localization (20,21), there were certain distinctive and interesting features exhibited at these junctions. In clone pMKB6, the L1Md element flanking the minor satellite has a portion of its 3' end including the adenine tail deleted. In clones pMKC78 and C85, portions of the major and minor satellite repeating unit are found next to one another. The junction at which the two joined together appeared to involve a TGGAA sequence motif which in pMKC78 was derived from the minor satellite and in pMKC85 was derived from the major satellite. In both cases the TGGAA motif was flanked by a five nucleotide stretch that was capable of forming an inverted repeat (Figure 4). The fusion of the two satellites occurred three bases upstream or downstream from the TGGAA motifs. Interestingly, this sequence motif occurred three times in the major satellite and twice in the minor satellite. When a sequence search of other known complex satellites was performed, this motif was found to occur twice within the human alphoid consensus sequence (22) and the Drosophila 1.668 satellite family (23), and also it is similar in sequence to the basic repeating unit of human satellites II and III (24).

In situ hybridization to Mus musculus and L929 metaphase chromosomes

It had been previously reported that the minor satellite clone pMR150 hybridized to some centromeres and to some regions in the arms of metaphase chromosomes prepared from the bone marrow cells of Mus musculus (8). To

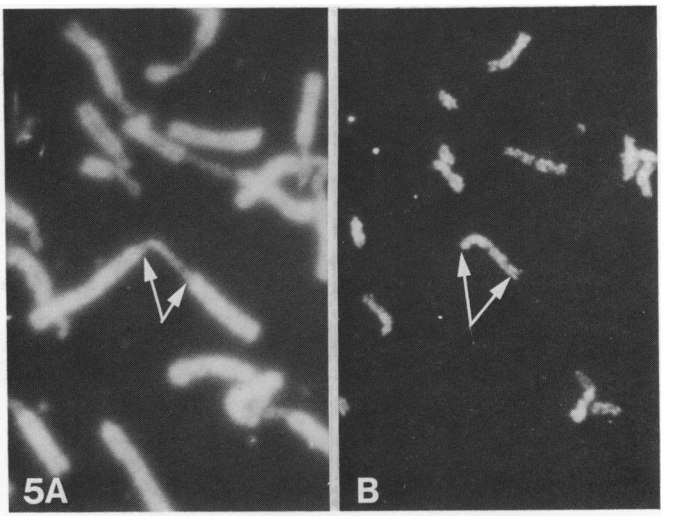


Figure 5. In situ analysis with major satellite. A, DAPI stained metaphase chromosomes from a mouse L929 cell grown in the presence of 33258 Hoechst. The elongated centromere of a metacentric chromosome is denoted by the arrows. B, In situ hybridization using a biotinylated riboprobe made from pSAT and a fluorescence detection system illustrates that major satellite sequences are distributed throughout this and other extended centromere regions of the karyotype.

confirm this observation with our complete minor satellite repeat as probe, in situ hybridization was carried out with mouse L929 and Mus musculus metaphase chromosomes and the hybrids were detected by both immunofluorescence microscopy and autoradiography. The distribution of the hybridized sequences to the centromeres could be more precisely determined when L929 cells were grown in the presence of the dye 33258 Hoechst. This agent binds to the minor groove of the double stranded helix in AT rich regions and interferes with condensation producing centromeres that were 4 to 5 times their native length (15). In a control experiment, hybridization using major satellite biotinylated riboprobe to 33258 Hoechst treated metaphase spreads confirmed that the major satellite is distributed throughout the entire extended centromeric region (Figures 5A and B) (7). When radioactively labeled pMKC104, a clone containing only minor satellite sequences was used as a probe, localization to the centromere region of all the mouse L929 chromosomes was also observed (Figure 6). To our surprise, with appropriate exposure times and reduced amount of tritiated probes, it was possible to occasionally detect the localization of silver grains to

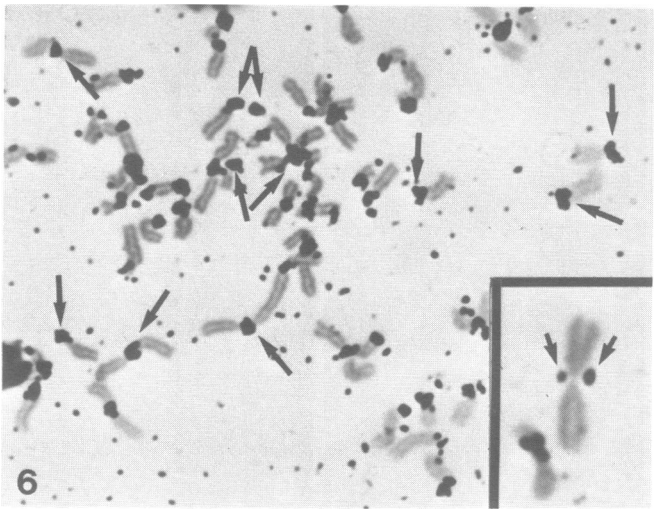


Figure 6. Isotopic in situ analysis with minor satellite. A partial karyotype of a mouse L929 cell hybridized with a ^{3}H-labelled pMKC104 riboprobe. The centromere region (arrows) of each chromosome of the karyotype is labelled in the presence of $1x10^{7}$ dpm of probe. Preparations using only $1x10^{5}$ dpm of probe and three day exposure time display chromosomes in which the label is localized occasionally to two patches at the surface of the centromere (inset).

two patches, each localized to the central lateral surface portion of the centromere encompassing the kinetochore region (Figure 6 inset). To further clarify the position of the minor satellite at the centromere, high resolution fluorescence in situ hybridization was performed with biotinylated riboprobe made from pMKC104. This protocol suggested that the minor satellite was confined only to a site on the outer surface of the centromere, somewhere at or immediately adjacent to the kinetochore, on each chromatid of the karyotype (Figures 7A and B). The elongation of the centromere due to the Hoechst treatment did not affect the size or distribution of the region containing minor satellite sequences (compare Figures 7B and D). The highly localized distribution of the minor satellite sequences and the double dot staining pattern produced by sister chromatids was also detected in interphase nuclei (Figures 7E and F). Since the tissue culture cell line is polyploidy, it is possible that the distribution of minor satellite to all centromeres is an artifact. Therefore, we had also examined metaphase spreads prepared from the spleens of mice, Mus musculus. As shown in Figures 7G and H, the signals of minor

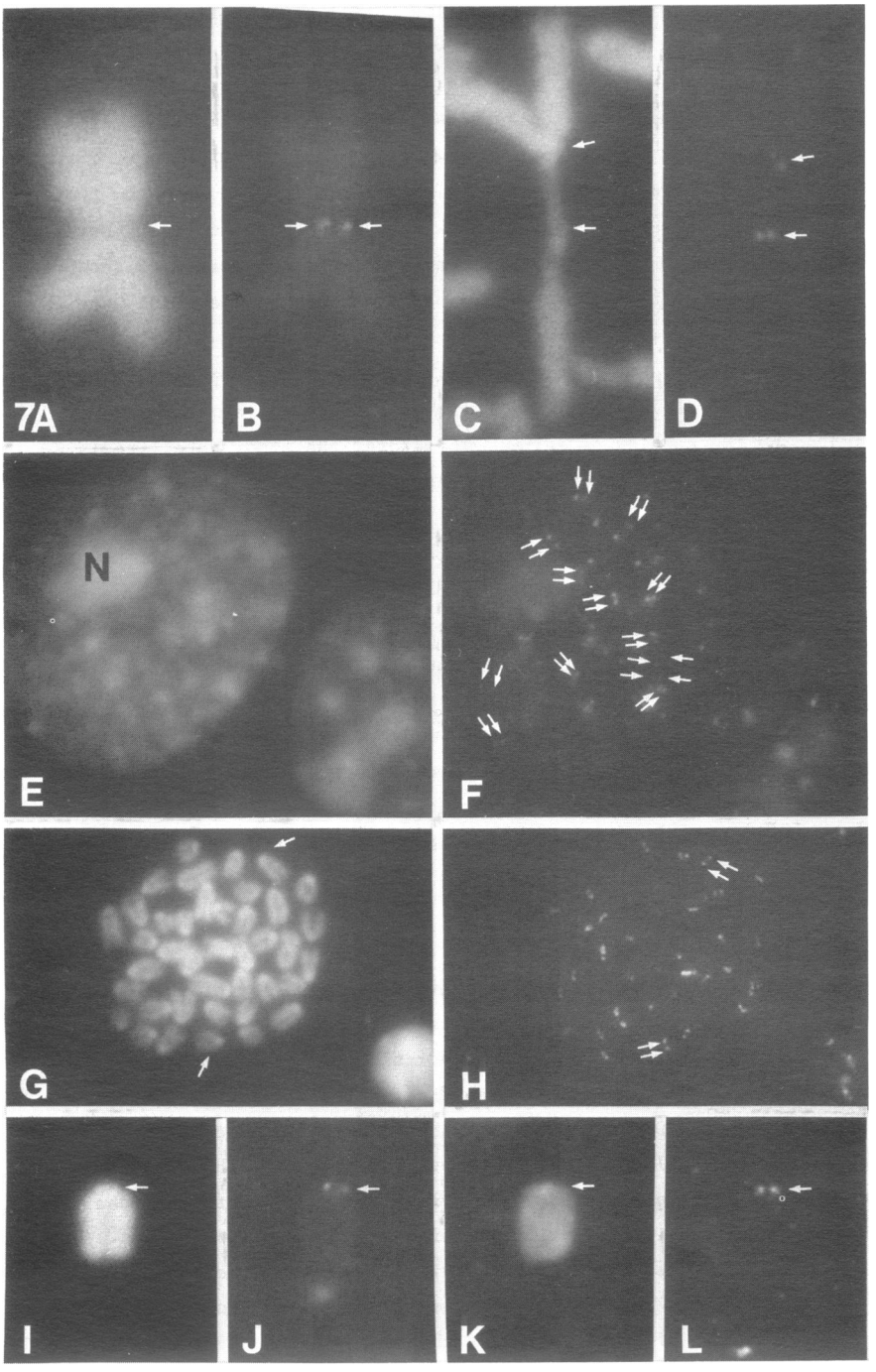
satellite again were observed to localize to all centromeric regions as paired dots. In general this pattern was reminiscence of that produced by kinetochore proteins detected with the CREST autoimmune serum (3).

In order to demonstrate an association between the minor satellite DNA and the kinetochore. We performed a two step labelling experiment in which a metaphase spread was first reacted with a CREST autoimmune serum that contained antibodies specific for the kinetochore proteins (17), therefore provided a reference image for the location of this structure as shown in Figures 7I and J. The metaphase chromosomes from the same slide were then denatured in 70% formamide and hybridized with the biotinylated minor satellite riboprobe. The hybrid signals were detected with avidin-FITC. As shown in Figures 7K and L, the staining pattern was similar to that generated by the autoimmune serum. Therefore, the minor satellite is localized to the outer surface of the centromere at or near the kinetochore region.

Neither autoradiographic nor fluorescence detection systems revealed the presence of minor satellite sequences within the chromosomal arms in mouse L929 cells or spleen cells. This did not appear to be due to limitations in the detection systems since it was possible to visualize the distribution of an R element (L1Md), that is known to disperse throughout the entire chromosome (20). Figures 8A and B illustrate that in addition to the paired dots localized to the vicinity of the kinetochore region, fluorescent staining is also seen throughout the euchromatic arms when the clone pMKB6 containing a minor satellite repeat flanked by an R element was used for in situ hybridization.

DISCUSSION

The centromere represents a relatively large region of the mammalian metaphase chromosome. During cell division the centromere is regionally differentiated in terms of function, mediating sister chromatid pairing and chromosome-spindle interaction. These regional variations may be correlated with the DNA sequence distribution within the centromere. Our observation that a class of middle repetitive elements, the minor satellite, is confined to a single discrete domain supports the idea that there is a site specific distribution of DNA sequences within the centromere. While we have no direct evidence for the function of the minor satellite within the centromere, its discrete localization in the centromeric region of every metaphase chromosome in the L929 cell line and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in Mus musculus seems to suggest its importance. The claim that the minor satellite is present in only half the chromosomes of the mouse karyotype (8) may be an artifactual observation resulting from variations in the sensitivity of the in situ hybridization protocols. We find that the non-isotopic analysis with the avidin/biotin detection system is very precise and informative. It is quite clear that we did not observe any hybridization signals on non-centromeric sites of the Mus musculus metaphase chromosomes when probed with the minor satellite DNA alone. This observation is also true for another mouse species, Mus spretus (data not shown).

On the basis of the in situ hybridization analysis at the light microscopic level, it is not unreasonable to assign the minor satellite to a region at or immediately adjacent to the kinetochore. There are three lines of evidence supporting this tentative assignment. First, if the minor satellite were kinetochore specific, it has to be located in all centromeres and not elsewhere on the metaphase chromosomes. Our cytological data is consistent with this criterion. Second, upon treatment with the DNA binding dye Hoechst, the centromere was decondensed and elongated yet the paired dot distribution of the minor satellite remained undisturbed and localized to the midpoint of a metacentric and the tip of a telocentric chromosome, the regions where one would expect to find the kinetochores. Third, the two step labelling of the same chromosome with the anti-kinetochore antibodies and minor satellite revealed that the staining signals from both probes are localized to the same region of the metaphase chromosome. Also the pair dot characteristics found in interphase nuclei as a result of the minor satellite hybridization are remarkably similar to those observed with specific anti-kinetochore antibodies (3,17).

Figure 7. Non-isotopic in situ analysis with minor satellite and two step labelling experiment. Visualization of the distribution of minor satellite sequences using biotinylated riboprobe from pMKC104 and a fluorescence detection system revealed that these sequences are localized to a discrete patch at or immediately adjacent to the kinetochore region of each centromere. The patches are apparent on metaphase chromosomes from untreated L929 tissue culture (A,B) as well as cells grown in the presence of 33258 Hoechst (C,D). The paired dot characteristic of minor satellite signals on sister chromatids are also detected in L929 interphase nuclei (E,F) and also in spreads prepared from spleen cells of Mus musculus (G,H). (J) is an example of a telocentric chromosome reacted with an anti-kinetochore specific antibody. The same chromosome was hybridized with minor satellite (L). The chromosomes in A,C,F,G,H,I and K have been stained with DAPI. N denotes the nucleolus.

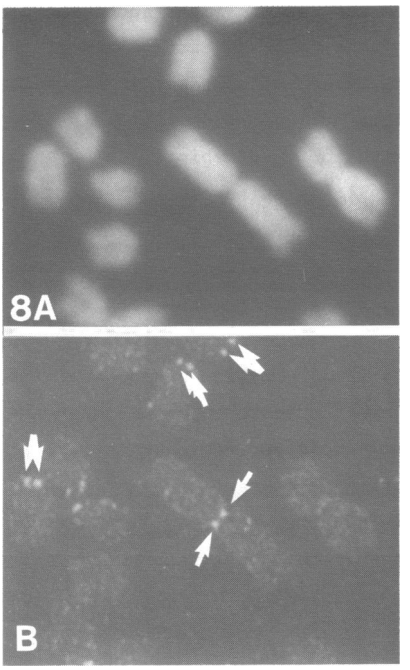

Figure 8. In situ analysis with pMKB6. A biotinylated riboprobe containing minor satellite and an R element sequence hybridized to sites within the centromere (arrows) as well as throughout the arms of the chromosome. A, Partial mouse L929 karyotype stained with DAPI. B, Hybridization pattern detected by a fluorescence detection system.

When we conducted a sequence search of the total DNA data base in Genbank, the minor satellite was found to have a 98% (62/63 bp) homology to domain II of G5, a putative class of nuclear matrix DNA in mouse 3T3 cells (25). In retrospect, these investigators had isolated an unidentified matrix sequence which now is known as the minor satellite DNA. In a very recent publication (9), another group also unaware of this previous observation, independently confirmed that the mouse minor satellite was somehow enriched through DNA and matrix protein interactions. Although both reports (9,25) drew the same conclusion that the minor satellite DNA family probably functions as the nuclear matrix DNA in mouse, other interpretations are possible. Since the kinetochore is highly resistant to nuclease digestion (26), a process commonly used to isolate nuclear matrix DNA, it is conceivable that both groups could have isolated DNA from the kinetochore region in conjunction with DNA that is involved in genome

anchorage. Neuer-Nitsche et al (9), calculated that there are as many as 1000 nuclear matrix attachment sites involving the minor satellite per diploid genome. If these sites are clustered as our data suggest, then there would be approximately 12 attachment sites or chromosomal loop bases for each of the 80 chromatid of the mouse karyotype. This arrangement is compatible with the proposed model for kinetochore organization (26). The idea that the minor satellite is localized to the kinetochore and organizes the nuclear anchorage sites in that specific region would also be in good agreement with the observation that the kinetochore may be part of the metaphase chromosome scaffold (27). Nevertheless, the definitive assignment of the minor satellite to the kinetochore region will have to await high resolution mapping with the use of immunoelectron microscopy.

A number of repetitive sequences are known to map to the centromere but it is not clear how these sequences are distributed or interrelated within this region. Analysis of the sequences flanking the minor satellite indicates that portions of other repetitive DNA families, including the R element (3' end of L1Md) and the major satellite are associated together in the centromeric region. In particular the integration of portions of repeat from both satellite classes seemed to occur through a TGGAA motif flanked by an inverted repeat. Such a unique feature may be indicative of a signal for recombination between the two satellites. The association of minor satellite with other repetitive DNA families might have simply occurred by chance or alternatively their juxtaposition in the genome may be crucial to the organization of the centromere.

The establishment of a complete consensus sequence for the minor satellite has enable us to identify two regions with significant homology to the major satellite. As many as 66 bases of the 120 bp minor satellite basic repeating unit was derived from portions of the major satellite. Since the subunit structure of the minor satellite is highly conserved, its evolution might have occurred recently. The fact that it is confined to a discrete domain within the centromere, suggests evolutionary pressure must have been exerted to maintain its localization. Perhaps this would also reflect a functional role for this particular DNA class. From the subrepeat analysis of the minor satellite, it is clear that certain domains were amplified. It would be of interest to demonstrate that they are involved in binding to proteins in the vicinity of the kinetochore. A number of kinetochore proteins, including a 50 kd polypeptide, have been identified (17). This protein may be similar to the 54 kd polypeptide that appears to

be covalently linked to a fraction of nuclear matrix DNA, presumably to the minor satellite (28,29).

In summary we report a consensus sequence for the minor satellite of mouse and document its location to a specific region at or immediately adjacent to the kinetochore. This represents the first report of a precise localization of a repetitive DNA element to a single discrete domain within the centromeres of all the mouse chromosomes. Lastly our cytological data has raised a possibility that there is a potential association between the kinetochore and the nuclear matrix through the minor satellite.

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