Meiotic chromosome behaviour reflects levels of sequence divergence in *Sus scrofa domestica* satellite DNA

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Abstract. We present a general model for the evolution of chromosome-specific satellite DNA subfamilies. *Sus scrofa domestica* has a bimodal karyotype with two autosomal subsets of 12 meta-/submetacentric (Mc) and 6 acrocentric (Ac) chromosome types (Mc and Ac “subgenomes”). We show that the centromeric heterochromatin is characterised by two distinct satellite DNA families designed Mc1 and Ac2. Mc1 is a diverse satellite family of the Mc subgenome of which certain members with a 100 bp repeat unit are found to occur at the pericentromeric regions of each Mc autosome, while others are chromosome-specific, e.g. clone Mc pAv1.5, a higher order repeat variant, which hybridises specifically to chromosome 1. Ac2 is a homogeneous satellite occurring at the subterminal pericentromeric regions of all Ac autosomes. DNA sequence analyses showed that all clones investigated are built up from a 14 bp repeat unit which is highly conserved. In situ hybridisation to meiotic pachytene nuclei revealed a distinct spatial arrangement of the Ac2 centromeric satellite.

Introduction

The detailed study of the so-called alpha satellite DNA families in the human genome has resulted in a much refined model where centromeric repeated sequences consist of multimeric higher-order repeat units which are organised in a highly chromosome-specific fashion (Willard and Waye 1987a; Jørgensen et al. 1987). While in this example the organisation of satellite DNA has been evaluated in great detail at the molecular and chromosomal levels (see Willard and Waye 1987b), a number of questions still remain unanswered such as the forces and modes of generation and maintenance of chromosomal subsets in the alpha satellite DNA family.

In this article, we study the phenomenon of the chromosome-specific patterns of variation in satellite DNA sequence in a different system, *Sus scrofa domestica*, focussing on the latter problem. *S. s. domestica* has a bimodal karyotype with two autosomal “subgenomes”, i.e. it consists of 12 biarmed (meta-/submetacentric) and 6 rod-shaped (acrocentric) pairs of autosomes. All autosomes and the X chromosome have centromeric heterochromatin (C-bands). The Y chromosome, in addition, has heterochromatin on both its arms.

The heterochromatin of *S. s. domestica* is well characterised cytologically (Lin et al. 1982; Schwarzacher et al. 1984); there are, however, with one exception of a Y-specific repeated DNA (McGraw et al. 1988), no reports on the highly repeated satellite DNAs it contains. Here we present detailed information concerning the kind of autosomal satellite DNAs in *S. s. domestica*. It is shown that there are two major families of satellite DNA in this organism which are each confined to a subgenome and which differ in their degree of heterogeneity and their autosomal distribution. Chromosome-specific repeat units were discernible only in one family, namely that of the biarmed subgenome. The two major satellite families of *S. s. domestica* differ also in their spatial arrangement in the male pachytene nucleus. A model is presented in which the implications of constraints exerted by the disposition of meiotic chromosomes for the evolution of tandemly arranged highly repeated DNA sequences are analysed.

Materials and methods

*DNA extraction and satellite separation.* High molecular weight nuclear DNA was prepared from a fresh liver as described by Blin and Stafford (1976). Satellite DNA separation was performed by actinomycin D/CsCl density gradient centrifugation according to Barnes et al. (1976). Absorbance of the fractions was monitored at 260 nm using an LKB Uvicord monitor.

*Recombinant DNA techniques.* After restriction endonuclease digestion and electrophoresis of the satellite DNA fraction, highly repet-
itive DNA fragments were excised from polyacrylamide gels and eluted. Half of the eluted DNA was used for ligation into the SmaI site of pUC18, and subsequent transformation into *Escherichia coli* JM83, while the remaining half of the DNA was used to identify positive clones by colony hybridisation (Grunstein and Wallis 1979). DNA transfer and hybridisation were done as follows: 6–10 μg of digested DNA samples were fractionated on agarose or polyacrylamide gels and subsequently transferred to nylon membranes (Amersham UK) by capillary blotting (Maniatis et al. 1982) or electrophoretic transfer (Bittner et al. 1980). Hybridisation was performed in 5 x SSC, 5 x Denhardt’s solution, 0.1% SDS at 62°C with up to 1 μg of nick-translated plasmid DNA. Sequences were determined following the chain termination method (Sanger et al. 1977) after recloning of the repetitive DNA in the phage mp18. To estimate the copy number of the cloned highly repetitive DNA fragments we used a dot blot hybridisation assay as described by Martinez-Zapater et al. (1986). Our *S. s. domestica* satellite DNA sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession numbers X51555 to X51565.

**In situ hybridisation to fixed chromosome spreads.** Mitotic and meiotic chromosome spreads were prepared as described (Schwarzacher et al. 1984). Hybridisation and detection of biotinylated probes using the horseradish peroxidase system were performed according to Ambros and Schweizer (1987). For examination of the slides we used a Leitz reflection contrast microscopy system (Landegent et al. 1985).

**Results**

We isolated a satellite DNA fraction by isopycnic ultracentrifugation in the presence of the GC-specific drug actinomycin D. Restriction endonuclease digestion with the enzymes AluI, HinII, MboI and PvuII and subsequent polyacrylamide gel electrophoresis (PAGE) separation of this satellite DNA fraction released typical satellite-like ladder formations of approximately 15, 45, 40 and 70 bp repeat units respectively. In contrast to this ladder formation a prominent band of 340 bp could be detected after cleavage of main band DNA with AvaI. The most brilliantly stained bands were excised from the gel, cloned and used for further studies. From the results of a semiquantitative dot blot hybridisation we calculated the average copy number of some of the cloned highly repetitive DNA fragments (Table 1).

![Fig. 1a-f. Non-radioactive in situ hybridisation of highly repeated DNA fragments to mitotic chromosome spreads (a-d) and meiotic nuclei (e, f) of *Sus scrofa domestica*. Clone pAv1.5 was exclusively localized in the pericentromeric heterochromatin of chromosome 1 (a), while pAl7.5 which shares strong homologies to pAv1.5 (cf. Figs. 2 and 4) showed pericentromeric distribution on all metacentric chromosomes (e), and pPv15 could be detected at the centromeres of chromosomes 10–12 (b). All clones of the Ac2 type (see Table 1) gave strong signals at the centromeric heterochromatin of acrocentric chromosomes (d). Hybridisation with a mixture of pAl7.5, pAv1.5 and pPv15 DNA to pachytene nuclei showed that centromeres of metacentric chromosomes remain dispersed during meiosis (e some labelling is arrowed) while in hybridisation with pMb3.4 or any other Ac2 clone member a unique signal could be detected (f arrow) indicating the physical association of centromeres of acrocentric chromosomes (cf. Fig. 5).](image_url)