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# Centromeric heterochromatin in the cattle rob(1;29) translocation: a-satellite I sequences, in-situ MspI digestion patterns, chromomycin staining and C-bands

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## Abstract

The centromeric regions and a-satellite I sequence were studied on chromosomes 1, 29 and the rob(1;29) translocation in a Portuguese breed of cattle, Barrosa, carrying the translocation. Rob(1;29) centromeric regions showed heterochromatic bands with propidium iodide but, unlike the acrocentric autosomes, no strong centromeric bands were revealed with chromomycin A3. An a-satellite I sequence was not found at the centromeres of the X, Y and rob(1;29) chromosomes in the breed, although it was present at the centromeres of all acrocentric chromosomes including 1 and 29. Restriction enzyme banding with MspI revealed polymorphisms between different rob(1;29) chromosomes in both centromeric and intercalary regions. The data show that the centromeric region of the rob(1;29) chromosome has lost the a-satellite I sequences, while retaining other heterochromatin, and suggest that this important and widespread translocation has occurred multiple times.

## 1. Introduction

The cattle chromosome complement includes 29 pairs of acrocentric autosomal chromosomes plus submetacentric X and Y chromosomes. Many structural rearrangements have been reported, some being widespread and having little reported effect on either phenotype or fertility (Gustavsson 1974, Iannuzi et al. 1990). The Robertsonian translocation (involving end-to-end fusion of two acrocentric chromosomes with loss of the short arms) between chromosomes 1 and 29, 2n ˆ 58, rob(1;29), is widespread in many commercial breeds of cattle, and heterozygotes are common in populations. Rob(1;29) has been found with high frequency in the Portuguese cattle breed Barrosa (Rangel-Figueiredo & Iannuzzi 1990).

Most mammalian chromosomes have a-satellite DNA (also known as satellite I, or, in mouse, the major satellite) located at or near the centromeres, and these are widely thought to play a role in centromere activity (Lee et al. 1997, Tyler-Smith et al. 1998). The a-satellite sequences are composed typically of many thousands of tandemly arrayed units of a repeat monomer arranged in head-to-tail orientation. However, the satellite sequence is essentially absent from some chromosomes, such as the mouse Y chromosome (Garagna et al. 1993). These a-satellite-like sequences and other DNA motifs present in the pericentromeric regions can be differentially stained by a number of methods, including staining with the GC-enhanced fuorochrome chromomycin A3 (Schweizer 1976), and staining with less base pair speci¢c fluorochromes, in both cases after removal of DNA with barium hydroxide (C-banding) or in-situ restriction enzyme treatment.

In the present study, we aimed to characterize the centromeric regions and a-satellite I sequences in a Portuguese breed of cattle, Barrosa, where some individuals carry the rob(1;29) translocation.

## 2. Materials and methods

Genomic DNA was extracted from the blood of an individual Barrosa bull calf and digested with EcoRI using standard methods. After size separation by electrophoresis, an abundant restriction fragment about

1.9 kb long was cloned and partially sequenced (EMBL Nucleotide Sequence Database accession AJ293510). One clone, named pBtKB5, was homologous to a-satellite I sequences from cattle and other mammals (see Results). The clone was used for Southern hybridization to size-separated BamHI and EcoRI digests of genomic DNA from various Bovidae species using standard non-radioactive methods.

Chromosome preparations were made from short-term lymphocyte cultures of whole blood samples from a total of 68 individuals without synchronization or other pretreatments. Chromosome preparations were hybridized in situ with the pBtKB5 clone labelled with biotin-16-dUTP (Roche) using standard methods (Schwarzacher & Heslop-Harrison 2000). The most stringent post-hybridization washes were at 42 C in2 SSC (2 SSC: 0.3 mol/L NaCl, 0.03 mol/L sodium citrate) and 50% (v/v) formamide followed by washes in 0.1 SSC at 60 C. Biotin label was detected by FITC-conjugated avidin. Chromosomes were counterstained with DAPI. Photomicrographs were taken on Fuji Superia 400 ASA colour print ¢lm, digitized and printed from Adobe Photoshop after contrast, overlaying and colour optimization using functions affecting the whole of the image only.

Metaphase chromosomes prepared by methanol^acetic acid ¢xation and air-drying technique are susceptible to DNases (Al¢ et al. 1983) and bovine chromosomes may show heterogeneity following restriction enzyme digestion (Hidas 1995). Freshly prepared metaphase spreads were incubated with 0.3 U/ml of the restriction enzyme MspI (Gibco) in the appropriate buffer. After 12^16 h of incubation at 37 C, slides were washed in distilled water and air dried. They were then stained with DAPI or C-banded following the standard barium hydroxide procedure of Sumner (1972) but using propidium iodide as a counterstain. The chromomycin A3 banding procedure followed Schweizer (1976).

## 3. Results

The sequence of pBtKB5 showed more than 95% homology with previously isolated bovine a-satellite I sequences (e.g. EMBO mammalian database accessions BTREP15, BTRS715B; Gaillard et al. 1981, Taparowsky & Gerbi 1982), and was related to a-satellite I sequences from other species (including sheep (Reisner & Buchholtz 1983), antelope and deer species (Lee et al. 1997)). Sequence and Southern hybridization (not shown) suggested that the sequence was tandemly organized (a ladder of 1.2 kb was seen in BamHI digests) and abundant in cattle, with related sequences present in sheep and goats.

The karyotypes of the 68 Barrosa individuals studied were 2n ˆ 60 (28 individuals); 2n ˆ 59, rob(1;29) (12 individuals) and 2n ˆ 58, rob(1;29), rob(1;29) (also 28 individuals). We studied three individuals in greater detail: 2n ˆ 60, XY; 2n ˆ 59, XX, rob(1;29) and 2n ˆ 58, XY, rob(1;29), rob(1;29) (not shown and Figures 1^3). Fluorescent in-situ hybridization showed that the pBtKB5 sequence was present at the centromeres of all the acrocentric autosomal chromosomes. Little or no hybridization was detected on the X or Y chromosomes, and we saw no hybridization to the centromeric regions of the rob(1;29) (Figure 1a^f) under the hybridization conditions used where probe^target hybrids with more than 85% homology were stably hybridized. C-banding, and restriction enzyme digestion of chromosomes followed by fluorochrome staining, revealed polymorphisms between the two rob(1;29) chromosomes (e.g. in a 2n ˆ 58, XY individual, Figure 2). C-bands were present at the centromeres of 54 acrocentric chromosomes. The centromere of some rob(1;29) translocations showed C-band fluorescence similar to that of the acrocentric chromosomes, while the other rob(1;29) centromeric regions showed only weak fuorescence (Figure 2a shows one of each type,i and ii). Restriction enzyme digestion revealed polymorphisms between the two rob(1;29) chromosomes (Figure 2b), and it is also notable that the X chromosome was resistant to digestion, perhaps because of its unique chromatin conformation.

Many centromeres of the acrocentric chromosomes stained brightly with chromomycin A3, and weaker subtelomeric to intercalary bands were also present (Figure 3), although bright, chromomycin A3-positive regions were absent on the X or rob(1;29) chromosome in a heterozygous individual.

## 4. Discussion

We have isolated the a-satellite I sequence from the Portuguese cattle breed Barrosa and shown that it is not detectable at the centromere of the rob(1;29) chromosome. Our observations suggest that during the translocation process, the a-satellite I sequences are lost in the rob(1;29) chromosome (Figure 1). Other mammals, including human and other species in the family Bovidae (such as sheep; see also Modi et al. 1996), have the equivalent a-satellite I sequence present at the centromeres of both acrocentric and submetacentric chromosomes, so the absence is not a universal characteristic of submetacentric chromosomes. As in many mammals, we detected little or no hybridization to the Y chromosome, nor to the X chromosome, as in some species (e.g. sheep; D'Aiuto et al. 1997). It is notable that absence of a-satellite I sequence has not been reported on acrocentric chromosomes in Bovidae species. In our experiments, the hybridization sensitivity would detect a few kb of sequence, so a very small region might be present on the X, Y and rob(1;29) chromosomes (consisting of enough sequence elements for centromeric function). Alternatively, based on comparisons with other species, it is more probable that centromeric function is maintained despite the absence of the a-satellite I.

Wurster & Benirschke (1968) suggested that centric fusions were the major mechanism of karyotypic evolution in the Bovidae, and there has been considerable discussion about the contribution of

centric fusions to speciation in the superfamily (Baker & Bickham 1986). It appears that populations differing by a simple centric fusion have little impairment of fertility, as heterozygotes forming trivalents show essentially normal meiotic segregation (Kumamoto et al. 1996). The presence of the rob(1;29) translocation in cattle breeds, where fertility is closely monitored by breeders, suggests that the translocation, or polymorphism for the translocation, may be associated with a genetic bene¢t or characters selected by man. Some authors take its presence in many breeds and stability to indicate that the translocation is ancient (Gustavson 1974), in contrast to newly formed and unstable dicentric chromosomal translocations (Berland et al. 1988). Digestion of chromosomes with the restriction enzyme MspI removed centromeric heterochromatin from the rob(1;29) chromosome, and apolymorphism was evident between the centromeres following propidium iodide staining (Figure 2a): chromomycin A3 stains GC-rich regions, MspI cuts at the sequence CCGG and the satellite I sequence is about 60% GC rich. The widespread occurrence of the translocation in different cattle breeds, each presumably arising from small founder populations, and the clear polymorphisms between the chromosomes in our individuals (Figure 2) suggest that the translocation has occurred many times during evolution of the species. Biarmed chromosome pairs of several species of Bovidae have arisen through Robertsonian translocations during karyotype evolution. These translocations have been accompanied by heterochromatin loss, as indicated by small bands seen in the centromeric regions of these chromosomes (Buckland & Evans 1978) and loss or inactivation of a functional kinetochore (Bunch et al. 1976). Iannuzzi et al. (1992) have surmised that rob(1;29) is still evolving in cattle by progressive losses of centric heterochromatin and was characterized as being of monocentric nature, involving loss of the centromere of chromosome 29 and retention of the centromere of chromosome 1 (Iannuzzi et al. 1987), when it would be expected that one of the chromosomal arms would retain satellite DNA. Giemsa-stained C-band preparations of rob(1;29) translocations in other cattle breeds show that the chromosome has a single block of heterochromatin proximal on the long q-arm (see Rangel-Figueiredo & Iannuzzi 1991), consistent with our results (Figures 2 and 3). Our results suggest that the most likely mechanism of formation of the rob(1;29) chromosome is by reciprocal translocation with loss of the small translocation product including essentially all the a-satellite I sequence from both chromosomes 1 and 29, along with variable parts of the associated C-band-positive material.

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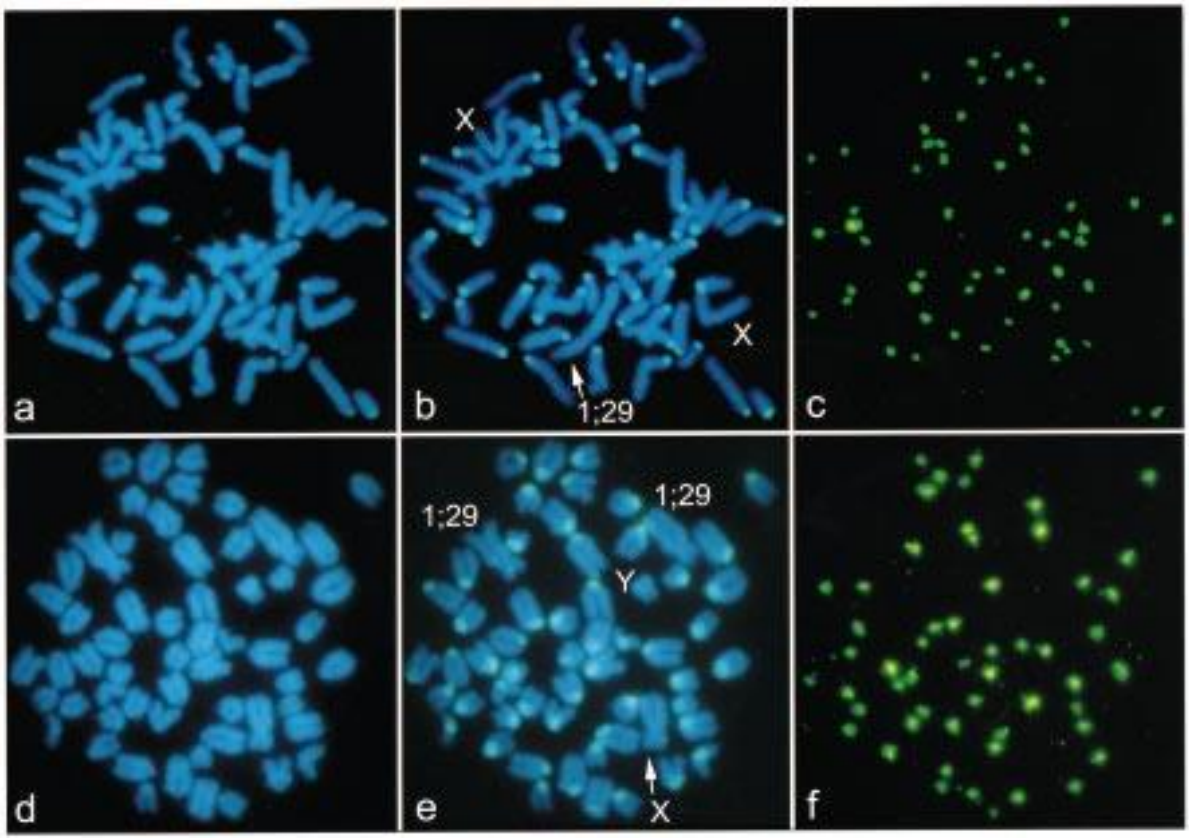


Figure 1. Metaphase chromosome preparations from (a^c) a cow [2n ˆ 59, XX, rob(1;29)] heterozygous for the 1;29 translocation and (d^f) a bull [2n ˆ 58, XY, rob(1;29)] homozygous for the 1;29 translocation. (a, d) Show chromosomes after DAPI staining; (c, f) show in-situ hybridization with the bovine a-satellite I probe pBtKB5; (b, e) overlay of the DAPI staining and in-situ hybridization to show localization of the signal. All 29 autosomal acrocentric chromosome types hybridize with the a-satellite probes at their centromeres, but the metacentric chromosomes, X, Y and rob(1;29) (indicated), show little or no detectable signal. 1000.

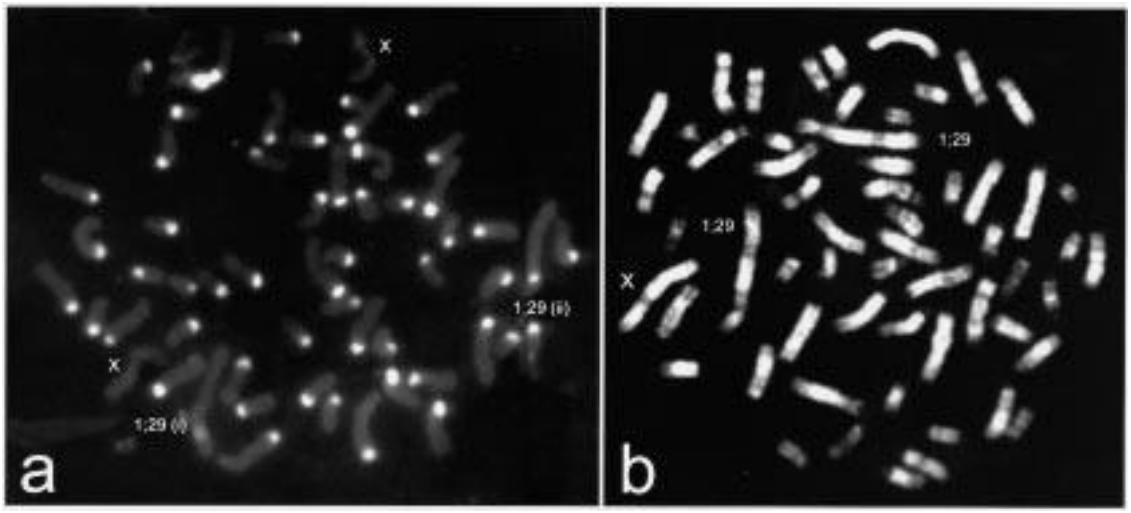


Figure 2. Banding of chromosomes following treatment with MspI restriction enzyme. (a) C-bands revealed with barium hydroxide treatments and staining by propidium iodide in a cow. (b) DAPI staining revealing multiple chromosomal bands in a bull. Polymorphisms in the centromeric region and long arm of the two rob(1;29) chromosomes, which is reproducible between metaphases. 1250.

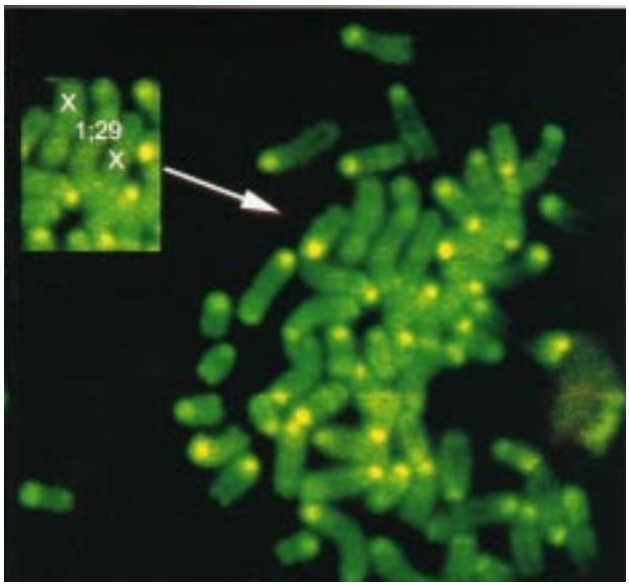


Figure 3. Chromomycin A3 (CMA) staining of heterochromatin in a cow heterozygous for the rob(1;29) translocation. No CMA-positive regions were present on the X or rob(1;29) chromosomes (insert showing identifications made by subsequent banding of the preparation). 1250.