Chromosome Research 1996, **4**, 214–217

Comparative FISH mapping of bovine cosmids to reindeer chromosomes demonstrates conservation of the X-chromosome

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Received 16 November 1995; received in revised form 11 January 1996; accepted for publication by M. Schmid 12 January 1996

Three X chromosome-specific bovine cosmids were used for fluorescence *in situ* hybridization mapping on reindeer (*Rangifer tarandus*) chromosomes, to test whether such large genomic clones could be used for comparative mapping across distantly related species. All three cosmids showed distinct unique hybridization sites on the reindeer X. Comparative map locations of these cosmids, together with the relative C-banding and genome size data on the X chromosomes of the two species, provide preliminary indications that the short and long arms of bovine X correspond, respectively, to the long and short arms of the reindeer X. The study also demonstrates that cosmid clones can be used successfully for comparative mapping across species that diverged 35 million years ago.

Key words: cattle, comparative mapping, fluorescent *in situ* hybridization, reindeer

Introduction

Reindeer (*Rangifer tarandus*) and domestic cattle (*Bos taurus*) belong to the same order (Artiodactyla), but to different families, namely, Cervidae and Bovidae respectively. Karyotypically, these represent two very divergent species. The chromosome number in reindeer is $2n = 70$ (number of major chromosome arms, nombre fondamental, nf = 74 according to Matthey 1945), comprising 33 pairs of acrocentric autosomes, one pair of submetacentric autosomes and a pair of sex chromosomes (Nes *et al.* 1965, Fraccaro *et al.* 1968, Gripenberg 1984). On the other hand, cattle have $2n = 60$ (nf = 62) and all 29 pairs of autosomes are acrocentric. The process of centric fusion/fission apparently fails to explain the evolutionary relationship of these two karyotypes.

A total conservation in the gene content of the ancestral X chromosome in different mammalian species has been hypothesized (Ohno 1967, 1969). However, the giant size of the X chromosome of reindeer has been of special interest, ever since the chromosomes of this species were first known (Makino 1944). This chromosome is remarkably long, comprising about 9% of the haploid female genome (Fraccaro *et al.* 1968), compared with about 5–6% in cattle and in most other mammalian species (Ohno *et al.* 1964). Therefore, the reindeer X chromosome is considered as a representative of a specific type of mammalian X (with almost double the typical contribution to the haploid genome), similar to the X chromosome observed in some rodent species (Ohno *et al.* 1964). Multiple structural rearrangements, not yet fully understood, have greatly changed the morphology of reindeer X chromosome (Gripenberg & Wessman 1993). In addition, acquisition of a substantial amount of heterochromatin, appearing as several distinct C-bands in the long arm, has significantly extended the total length (Gripenberg & Wessman 1993) as compared with other mammalian species. Studying only two major G-positive bands (referred to as A and B) on the X chromosome of over 60 mammalian species, Pathak & Stock (1974) reported a high degree of banding similarity for the mammalian X chromosome. However, comparison of the authors' drawings for the G-banded cattle and reindeer X chromosomes shows no apparent similarity.

Recently, we used bovine microsatellite-containing cosmid clones for comparative mapping studies in goat, sheep and river buffalo genomes. Extensive conservation in the mapping of these loci on homologous chromosomal regions of the four bovidae species was observed (B. Prakash *et al.* 1995, in preparation). In an attempt to test whether such large genomic clones could be used for *in situ* hybridization (ISH) mapping across more distantly related species, we used three bovine cosmid clones on reindeer metaphase chromosomes. The cross-species hybridization experiments, in this initial study, were restricted to clones originating from the X chromosome in cattle.

Materials and methods

Probes: A bovine cosmid library was constructed from a female of the Norwegian Red cattle breed (NRF). Screening of cosmids was performed using a $(GT)_{10}$ oligonucleotide.

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Clones giving a positive signal to this probe were rescreened with bovine satellites 1709, satellite 1715 and bovine C_0t1 DNA. Only clones giving a negative signal with the last three probes were selected and cosmid DNA prepared for *in situ* hybridization. Average insert size in the clones were 40 kb. Three of these cosmid clones were mapped to the bovine X chromosome (I. Olsaker *et al.*, B. Prakash *et al.*, manuscripts in preparation) and have been used in this study.

Probe labelling, hybridization and signal detection: DNA preparations of the three X chromosome-specific cattle cosmid clones were labelled with biotin-14-dATP (BioNick Labeling System, Gibco BRL, 18247-015) using the manufacturer's recommendations. Air-dried metaphase chromosome slides were prepared using standard methods. G-banding of metaphase chromosomes was carried out by trypsin digestion as described previously (Seabright 1971). Fluorescence *in situ* hybridization (FISH), was performed according to Chowdhary *et al* (1995), with minor modifications. Briefly, a higher concentration (9 ng/μl) of labelled probe than usual (5 ng/μl) was used for hybridization. All washings were carried out at 42°C instead of 45°C. Fifty per cent formamide, 2 × SSC, washings were carried out for 3 × 10 min, instead of the usual 3 × 15 min. Probe signals and chromosome images were screened and processed under a Zeiss Axiovert 135M/TV inverted fluorescence microscope equipped with Isee software (Inovation Corporation, Durham, NC, USA), as described previously (Chowdhary *et al.* 1995).

Results and discussion

Hybridization signals appearing as bright double dots on both chromatids of a reindeer chromosome, at a specific chromosomal region, were observed in a majority of the 20 metaphases scored for each of the three cosmids. Based on this analysis, all three cosmids hybridized to the X chromosome, which is morphologically distinguishable as the largest chromosome in the reindeer chromosomal complement. Further, each clone produced specific and unambiguously identifiable hybridization signals on both chromatids of the X chromosome in more than 50% of the metaphase cells analysed. Although relatively more background signal was observed on the reindeer chromosomes than on cattle chromosomes, no specific hybridization signal was observed on any of the autosomes. Representative FISH results for the three bovine cosmid clones cIOBT 314, 945 and 1489 are shown in Figure 1A.

Two of the probes (cIOBT 945 and 1489) hybridized to the short arm, while the third probe (cIOBT 314) hybridized to the long arm of the X chromosome. Since there is no standardized banded karyotype or ideogram of reindeer chromosomes, we developed a G-banded schematic drawing of the X chromosome, to facilitate band assignments of the clones. The ideogram (Figure 1B) was prepared after careful analysis of several G-banded X chromosomes, following trypsin treatment. According to the proposed ideogram, the probes cIOBT 314, 945 and 1489 mapped to reindeer Xq21, Xp21 and Xpter respectively.

Since the probe and chromosomal DNA belonged to very diverse species, the experimental conditions were modified to achieve effective hybridization. The probe concentration was increased to more than double that routinely used for species-specific hybridizations. To suppress hybridization of repetitive sequences contained in the cosmid clones, 300 ng/μl sheared bovine genomic DNA was added to the hybridization mixture and a 15–30 min prehybridization annealing was carried out at 37°C. All washings were carried out at a lower temperature (42°C) than routinely used (45°C). As a result, specific signals for all three probes were obtained consistently, but with background. The relatively higher background observed could be attributed to lower stringency washing, which did not adequately remove the non-specifically bound probe. The conditions were specifically chosen to permit the probe DNA to remain bound to the specific target, in spite of the possible partial differences between the sequences due to evolutionary divergence.

The three cattle cosmids (cIOBT 314, 945 and 1489) used in the present study map to p13–p12, q26–q31 and qter bands, respectively, of the bovine X chromosome (Figure 1B, I. Olsaker *et al.*, in preparation). A comparison of banding patterns between the cattle and reindeer X chromosomes shows no apparent similarity. The linear physical order of the three clones is also inverse in the two species. Further, the relative size of the two chromosomes is considerably different, therefore no definitive conclusion concerning homology could be drawn. However, in the light of the present mapping results, we tried to analyse the available comparative data to propose correspondence between the X chromosomes of the two species.

The short arm of reindeer and the long arm of cattle X chromosomes represent approximately the same fraction of their respective haploid genomes (3.61% and 3.43% respectively, Fraccaro *et al.* 1968, Popescu 1969, Lin *et al.* 1977). The linear order and locations (middle and terminal) of the two cosmids (cIOBT 945 and 1489) on the respective arms of the X chromosome of the two species is also strikingly similar (Figure 1B), suggesting correspondence at the chromosome structure and DNA levels. Further, cIOBT 314 maps to the arm opposite to that of the other two cosmids in both species. However, no comparison for the hybridization site of this clone could be made because of the difference in the length of the long arm of reindeer and the short arm of cattle X chromosomes. The C-banded area of reindeer Xq accounts for about 50% of the chromatin in this arm (Gripenberg & Wessman 1993). Cross-specific mapping of cIOBT 314 suggests that, except for the heterochromatin material, the reindeer long and cattle short arms probably share correspondence. As approximately the proximal and distal one-quarters of the reindeer Xq are mainly heterochromatic (Gripenberg & Wessman 1993), it is most likely that the middle half of this chromosome arm corresponds to the short arm of bovine X chromosome. These preliminary observations will, however, have to be substantiated either with more comparative mapping data between the two species or by applying

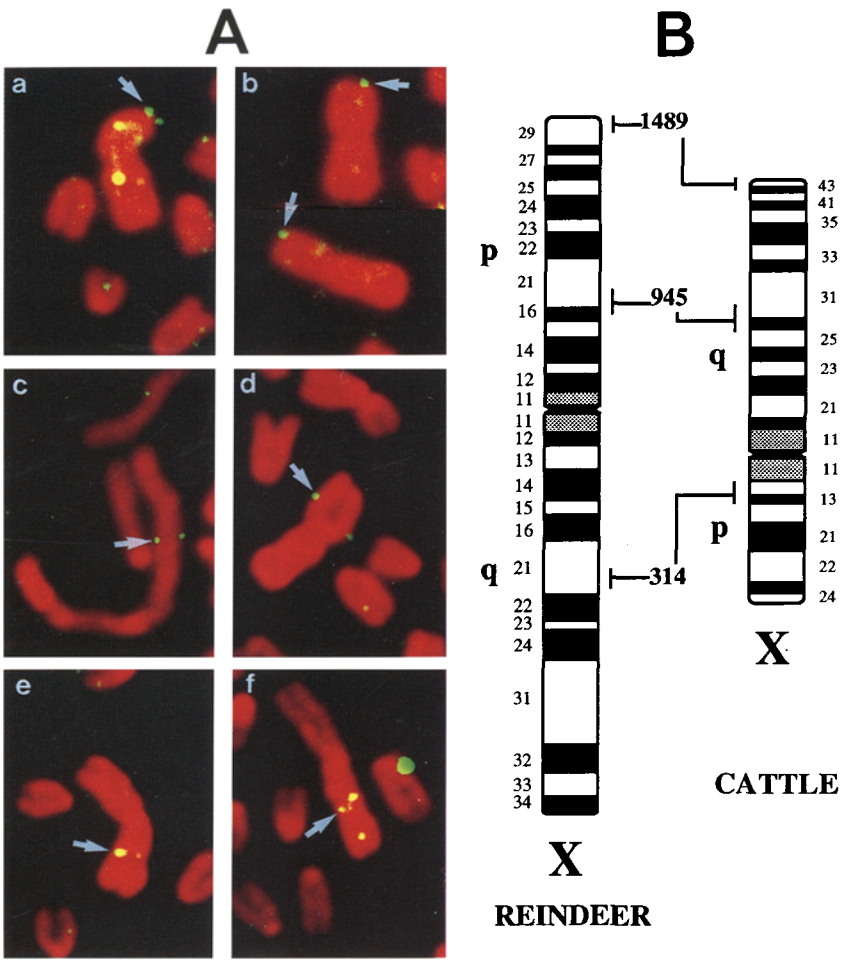

Figure 1. A Partial metaphase cells showing (arrows) fluorescence *in situ* hybridization signals of the cIOBT 1489 (a and b), cIOBT 945 (c and d) and cIOBT 314 (e and f) clones on reindeer X chromosome. Although relatively high background on the X chromosome is visible with cIOBT 1489 (a), consistent signals on Xpter (arrows; a and b) confirm the specificity of the hybridization. **B** Ideograms of G-banded reindeer (left) and cattle (right; upside down) X chromosomes showing the location of the three cosmid clones.

the recently described comparative painting (Zoo-FISH; Scherthan *et al.* 1994) technique using band- or arm-specific libraries from the human or cattle X chromosome.

Cross-species *in situ* hybridization of cosmid clones involves complications because of divergence in both coding and repetitive sequences between the probe and target DNA. The variation is more pronounced in distantly related species than in closely related species such as cattle, sheep and goats. Cattle and reindeer diverged almost 35 million years ago (Colbert 1969), as a result of which significant variability and rearrangements within their genomes are expected. In spite of this divergence, we have been able, in the present study, to use larger genomic fragments for cross-species ISH with success. Further, the findings are the first mapping data on reindeer chromosomes.

Acknowledgements

This work was supported by grants from the Swedish Council of Agricultural Research. Financial assistance from Kupion Luonnon Ystäväin Yhdistys (V. K.) and the Norwegian Council of Agricultural Research (I. O.) is acknowledged. The authors also wish to thank Risto Savolinen, Liisa Nurminen and Dr Auli Mäkinen for help in the work.

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