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Mapping of the β-lactoglobulin gene and of an immunoglobulin M heavy chain-like sequence to homoeologous cattle, sheep, and goat chromosomes

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Abstract. With a combination of non-isotopic in situ hybridization and simultaneous fluorescent R-banding, this study presents the first map of the short arm of sheep Chromosome (Chr) 3 and of the homoeologous cattle and goat Chrs 11 with two DNA sequences: the β lactoglobulin gene (*LGB*) and an immunoglobulin M heavy chain-like sequence (IGHML). The results are in agreement with the high degree of banding pattern similarity, previously reported, among cattle, sheep, and goat karyotypes.

Introduction

The identification and the cloning of loci controlling physiological and quantitative traits in the genomes of the three main domestic species of the *Bovidae* family (cattle, *Bos taurus L.;* sheep *Ovis aries L.;* goat, *Capra hircus L.*) are currently major goals. Our objective is to participate in this project by increasing the number of markers mapped to each chromosome of these three species. This will also improve our knowledge about their phylogenetic relationships.

In this paper, we report the first markers mapped to the short arm of sheep Chr 3 and the homeologous cattle and goat Chrs 11 (Reading conference 1976; ISCNDA 1989). We applied the nonisotopic in situ hybridization technique of Viegas-Pequignot and coworkers (1989) and the direct fluorescent R-banding technique of Lemieux and colleagues (1992), which have been shown to be useful for gene mapping in Bovidae (Hayes et al. 1992).

The two sequences that have been localized are the ovine β-lactoglobulin gene (*LGB*) and a bovine immunoglobulin M heavy chain-like sequence (IGHML).

β-Lactoglobulin is the major component of the milk whey of ruminants, dogs, and pigs, but not of humans (Pervaiz and Brew 1985). The determination of the chromosomal localization of the β-lactoglobulin gene is part of the studies concerning the genomic structure and organization of the six main lactoprotein genes (αS1-, αS2-, β- and κ-casein, α-lactalbumin, and β-lactoglobulin) in cattle, sheep, and goat.

The second marker, the bovine IGHML sequence, was assigned to cattle Chr 9 q22-23 (Ponce de Leon et al. 1991), which was later renumbered Chr 11 q22-23 (Hayes and Ponce de Leon, personal communication). This is explained by the fact that the original localization was made before publication of the R-banded standard karyotypes in *Bovidae* (ISCNDA 1989). This sequence is considered to be an immunoglobulin M heavy chain-like sequence for the following reasons: (1) it hybridizes to an IGHM cDNA probe (Ponce de Leon, personal communication) and (2) it is not localized on the same cattle chromosome as the *IGHM* gene (Tobin-Janzen and Womack 1992) and the *IGHG2, IGHG3,* and *IGHA* genes (Ponce de Leon et al. 1992), which are all on cattle Chr 21. It is known that all the *IGH* genes studied until now are closely linked in mammalian species (Tobin-Janzen and Womack 1992). To confirm the chromosomal localization of the IGHML sequence, we decided to map this sequence in all three domestic species of Bovidae, bearing in mind that the sheep homoeolog of cattle and goat Chr 11 is the short arm of the metacentric Chr 3, which is easily detected.

Materials and methods

Chromosome preparations

R-banded chromosome spreads were prepared by synchronization of cattle, sheep, and goat embryo fibroblast cell cultures with excess

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thymidine and by incorporation of 5-bromodeoxyuridine (BrdU) during the second half of the S phase, as described by Hayes and colleagues (1991). Slides were stored at −20°C until in situ hybridization was performed.

Probes

The probe for the β-lactoglobulin (*LGB*) gene is a plasmid pB4, which was constructed as follows: plasmid pSS1tgX-S, which contains the cloned ovine *LGB* gene (Simons et al. 1987), was digested with *Sal*I and *Sph*I, and the 4-kb *Sal*I-*Sph*I fragment (B4) corresponding to the 5′ end of the *LGB* gene (Ali and Clark 1988) was inserted into the plasmid vector pUC 18 to produce pB4. This eliminated repetitive sequences present in the 3′ end of the gene. Control hybridizations of fragment B4 to sheep, cattle, and goat total genomic DNA digested with *Bam*HI (Hayes, unpublished) indicated the absence of repetitive DNA and produced two bands for each species, as expected from the restriction map of the *LGB* gene (Ali and Clark 1988). Therefore, pB4 should give a single localization site on cattle, sheep, and goat chromosomes. The probe for the immunoglobulin M heavy-chain constant region-like sequence (IGHML) is a recombinant λ phage vector containing a 14-kb bovine genomic DNA insert.

Probe labeling, in situ hybridization, probe detection, and R-banding

Nonisotopic in situ hybridization to metaphase chromosomes with biotin-labeled probes (15 μl/slide; 6.5 ng/μl for pB4 and 6.5 or 3 ng/μl for the IGHML probe; no difference in signal frequency was observed between the two concentrations) and their detection has been described elsewhere (Hayes et al. 1992). This protocol was followed except for the addition of competitor total genomic DNA to the hybridization mixture (1/500, probe/competitor DNA) and for the washing steps, which were modified as follows: after hybridization, the slides were rinsed at 38°C for 3 min, once in 2 × SSC, pH 7 (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7); twice in 50% formamide, 2 × SSC, pH 7; once in 2 × SSC, pH 7; and once in 1 × SSC, pH 7. The R-banding pattern was revealed according to the method of Lemieux and co-workers (1992), and the chromosomes were classified according to ISCNDA 1989.

Results and discussion

The biotinylated probes (vector plus insert) were hybridized to chromosome spreads of each species. Spots were observed recurrently at certain chromosomal positions in all cases: sheep 3p28, cattle and goat 11q28 for pB4, the probe for the *LGB* gene (Fig. 1a) and sheep 3p23, cattle and goat 11q23 for the IGHML probe (Fig. 1c). The total number of metaphases photographed and the number of spreads with a signal on four chromatids, three chromatids, two chromatids of the same homolog or of two different homologs and on one chromatid are shown in Table 1.

Background was very low in this set of experiments (Fig. 1b and d). In our hands, we find that the amount of background, that is, nonspecific spots, depends on the preparation of the probe before labeling, on the quality of the slides, and on the amount of competitor total genomic DNA added to the hybridization mixture. The intensity of the hybridization signal was similar with both probes on most metaphases, although we expected a stronger signal with the longer IGHML probe, at least on the cattle chromosomes.

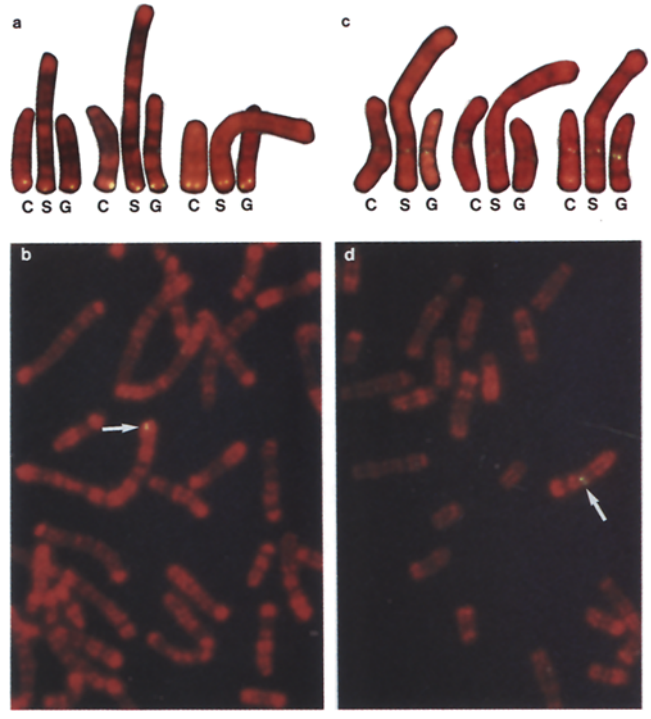

Fig. 1. **(a)** and **(b)** Localization of the *LGB* gene, **(a)** selected examples of cattle (C), sheep (S), and goat (G) Chrs 11, 3, and 11 respectively, and **(b)** partial R-banded sheep metaphase spread **(arrow)**. **(c)** and **(d)** Localization of the IGHML sequence, **(c)** selected examples of cattle (C), sheep (S), and goat (G) Chrs 11, 3, and 11 respectively, and **(d)** partial R-banded goat metaphase spread **(arrow)**.

This absence of intensity difference could be owing to the addition of competitor total genomic DNA at a high concentration to reduce nonspecific hybridization. Lichter and co-workers (1988) have reported an apparent decrease of signal intensity when high concentrations of competitor DNA are used, especially if the ratio probe/competitor DNA is higher than 1:200. We used the ratio probe/competitor DNA. 1:500 because it produced the best results and the signal was still readily detected.

The fluorescent R-banded sheep Chr 3 and the cattle and goat Chrs 11 carrying the hybridization signals were easy to identify on most metaphase spreads.

Therefore, the *LGB* gene can be assigned to the telomeric bands p28 of sheep Chr 3 and q28 of cattle and goat Chrs 11 and the IGHML sequence to the proximal side of p23 of sheep Chr 3 and of q23 of cattle and goat Chrs 11.

The result for the IGHML probe confirms the previous localization on cattle Chr 11 q22-23 (Hayes and Ponce de Leon, personal communication).

Comparative cytogenetic studies have shown the existence of a high degree of similarity of banding patterns among cattle, sheep, and goat RBG-banded chromosomes (Hayes et al. 1991) and between cattle and sheep GTG and QFQ-banded chromosomes (Hediger et al. 1991). The equivalences among cattle, sheep, and goat chromosomes were given in ISCNDA 1989, but a few discrepancies remain (Hediger et al. 1991), and gene mapping comparisons are the best tool to

Table 1.

Probe	Species chromosome	Total number of metaphases photographed	Number of metaphases with n-labeled chromatids. n = 4	3	2[a]	2[b]	1	Chromosome number and specific band
pB4	sheep	28	2	1	7	1	17	3p28
pB4	cattle	34	0	1	10	0	23	11q28
pB4	goat	40	1	2	13	0	24	11q28
IGHML	sheep	24	3	0	7	1	13	3p23
IGHML	cattle	29	0	0	11	1	17	11q23
IGHML	goat	29	1	1	7	2	18	11q23

[a] On the same homolog.
[b] On two different homologs.

solve this problem. The sheep metacentric Chr 3 is probably the result of a Robertsonian translocation between ancestral equivalents of cattle or goat acrocentric Chrs 5 and 11 (Reading conference 1976; ISCNDA 1989). Hence, our results on the localization of the *LGB* gene and the IGHML probe on the short arm of sheep Chr 3 and on cattle and goat Chrs 11 provide further evidence for a close evolutionary relationship among these three species. Also, with the *LGB* gene located on the telomeric band and the IGHML sequence on a median band, they delimit a region covering about half the chromosome.

Until now, the only other genes mapped in all three species are the glucose phosphate isomerase (*GPI*) gene to sheep Chr 14q22-24 and the homoeologous cattle and goat Chrs 18q22-24 (Chowdhary et al. 1991) and the omega (*IFNO*) and trophoblast (*IFNT*) interferon genes to sheep Chr 2p15 and the homoeologous cattle and goat Chrs 8q15 (Iannuzzi et al. 1992). Also, Gunawardana and colleagues (1992) have localized eight bovine VNTRs on the chromosomes of these three species. More information is available on comparative mapping between cattle and sheep (Fries et al. 1986; Hediger et al. 1989, 1990a,b, 1991; Mahdy et al. 1989; Georges et al. 1991; Threadgill and Womack 1990; Hayes et al. 1991; Ansari et al. 1992) and between goat and sheep (Fries et al. 1988; Simi et al. 1989). Most of the genes or VNTR markers have been mapped to homoeologous chromosomes at the same position, and, together with the results presented here, they confirm that similarity in banding patterns at the chromosomal level for these three species reflects similarity in genetic organization at the DNA level.

In addition, the localization of the *LGB* gene on cattle Chr 11q28 allows the assignment of the cattle unassigned synteny group U16 which contains the following loci (Fries 1991): blood group J (*J*), beta lactoglobulin (*LGB*), Abelson oncogene homolog (*ABL*), adenylate kinase 1 (*AK1*), argininosuccinate synthetase (*ASS*), DU16S1, glucose related protein (*GRP78*), immunoglobulin mu-like 1 (*IGHML 1*), proopiomelanocortin (*POMC*) and spectrin, α, non-erythrocytic 1 (*SPTAN1*).

In conclusion, the localization of the *LGB* gene and the IGHML sequence provide the first mapping data for cattle Chr 11 and its homoeologs in sheep and goat. Our results contribute to the preparation of a physical gene map of these three economically important livestock Bovidae species.

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