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Comparative gene mapping of lactoperoxidase, retinoblastoma, and α-lactalbumin genes in cattle, sheep, and goats

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Abstract. The lactoperoxidase (*LPO*), retinoblastoma (*RB1*), and α-lactalbumin (*LALBA*) genes have been mapped by fluorescent in situ hybridization respectively to cattle Chromosomes (Chrs) 19, 12, 5; goat Chrs 19, 12, 5; and sheep Chrs 11, 10, 3. The results confirm the homologies among cattle, sheep, and goat chromosomes, previously reported, and provide more information for the comparison between the bovine and human karyotypes and gene maps.

Introduction

The karyotypes of the three main domestic Bovidae species, cattle (*Bos taurus L.*), sheep (*Ovis aries L.*) and goat (*Capra hircus L.*), show a high degree of similarity in banding patterns (Evans et al. 1973; Schnedl and Czaker 1974; Buckland and Evans 1978; Hayes et al. 1991), and the proposed homoeologies among the chromosomes of these three species are given in ISCNDA 1989 (1990). However, these homoeologies were based only on the comparison of the banding patterns of the chromosomes.

The localization of genetic markers on the chromosomes by in situ hybridization makes it possible to verify the proposed homoeologies and also to extend the comparison to other mammalian species, that is, human or mouse. To date, only a few genes have been mapped in all three bovine, ovine, and caprine species: the glucose phosphate isomerase (*GPI*) gene to sheep Chr 14q22-24 and the homoeologous cattle and goat Chr 18q22-24 (Chowdhary et al. 1991); the omega (*IFNO*) and trophoblast (*IFNT*) interferon genes to sheep Chr 2p15 and the homoeologous cattle and goat Chr 8q15 (Iannuzzi et al. 1992, 1993); the β-lactoglobulin (*LGB*) gene and the immunoglobulin M heavy chain-like (IGHML) sequence respectively to sheep Chr 3p28 and 3p23 and to the homoeologous cattle and goat Chr 11q28 and 11q23 (Hayes and Petit 1993); and the α S2-casein (*CASAS2*) to the homoeologous cattle, sheep, and goat Chr 4q32 (Hayes et al. 1993). Also, Gunawardana and colleagues (1992) have localized eight bovine VNTRs on the chromosomes of these three species.

At present, our objective is to have at least one genetic marker on each of the homoeologous cattle, sheep, and goat chromosomes to confirm that the similarities in banding patterns at the chromosomal level do reflect similarities in genetic organization at the DNA level.

In this paper, we report the localization of three additional genes to homoeologous cattle, sheep, and goat chromosomes: lactoperoxidase (*LPO*), retinoblastoma (*RB1*), and α-lactalbumin (*LALBA*) genes.

Lactoperoxidase and α-lactalbumin are respectively one of the minor and one of the major proteins in the milk whey of ruminants (Jenness 1982), and the determination of the chromosomal localization of their genes is part of the studies concerning the genomic structure and organization of lactoprotein genes in cattle, sheep, and goats. To our knowledge, the *LPO* gene has not been mapped in any species, and the *LALBA* gene has been mapped in human (Human Gene Mapping 11 1991) and assigned to cattle (Threadgill and Womack 1990) and sheep (Imam Ghali et al. 1991) chromosomes with panels of somatic cell hybrids.

The gene *RB1* confers susceptibility to retinoblastoma, the most common cancer of the eye in children (Lee et al. 1987) and thus has been the object of many studies in human genetics. *RB1* gene has been mapped in human, primates, mouse, rat (Human Gene Mapping 11 1991), and rabbit (Lemieux and Dutrillaux 1992), but in none of the Artiodactyls. Therefore, it was of interest to determine its localization on cattle, sheep, and goat chromosomes.

Since *RB1* and *LALBA* genes have been mapped to human chromosomes (Human Gene Mapping 11 1991),

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we compared their localization with those on bovine chromosomes. In the case of the *LPO* gene, which has not been mapped in human, we suggest a putative human chromosome on the basis of the results of the comparison between bovine and human chromosome banding patterns and gene maps.

Materials and methods

Chromosome preparations

R-banded chromosome spreads were prepared by synchronization of cattle, sheep, and goat embryo fibroblast cell cultures with a double thymidine block (Hayes et al. 1991) or with a single methotrexate block (Yunis 1976) and by incorporation of 5-bromodeoxyuridine (BrdU) during the second half of the S phase. Slides were stored at −20°C until in situ hybridization was performed.

Human chromosome spreads were obtained according to Viegas-Péquignot and Dutrillaux (1978). RBG bands were stained by the fluorochrome-photolysis-Giemsa method (Viegas-Péquignot et al. 1989).

Probes

The probes used in this set of experiments are all whole plasmids purified by CsCl density equilibrium centrifugation:

- pLPc contains a 1.8-kb cDNA fragment of the caprine *LPO* gene inserted into pUC 18 (Cals 1992).
- pBR4.5 contains the 4.5-kb complete cDNA of the human *RB1* gene inserted into pGEM1 (Lee et al. 1987).
- pLA11.5 contains an 11.5-kb *SalI-PstI* caprine genomic DNA fragment inserted into pPolyIII-I (Vilotte et al. 1991). This fragment includes 5′ upstream sequences and the region hybridizing with *LALBA* cDNA, and it recognizes *LALBA* pseudogenes.

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

In situ hybridization to metaphase chromosomes with the biotin-labeled probes (15 μl/slide; 6.5 ng/μl for pLPc and pBR4.5; and 0.05 ng/μl for pLA11.5), and their detection has been described elsewhere (Hayes et al. 1992). This protocol was followed except for the washing steps, which were modified as follows for all three probes: after hybridization, the slides were rinsed at 38°C for 2 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; and once in 2 × SSC, pH 7. In the case of the *LPO* and *RB1* probes, no competitor DNA was needed, because they do not contain repetitive sequences as generally expected with cDNA sequences. However, in the case of the *LALBA* probe, which does contain repetitive sequences, the conditions of the hybridization had to be adjusted. Addition of sonicated total genomic caprine DNA at a ratio probe/competitor DNA of 1/1000 reduced background but not sufficiently, and it was necessary to decrease the concentration of the probe to 0.05 ng/μl; that is, a 100-fold decrease compared with standard conditions. The R-banding pattern was revealed according to the method described by Lemieux and coworkers (1992). The hybridized DNA fragments (yellow/green) and the R-bands (red and dark), visualized simultaneously, were photographed with Ektachrome 400 ASA Kodak film. Some of the original slides obtained with this film were enlarged with a slide duplicator and a 50 ASA Ilford PAN F black and white film treated with Ilford Microphen developer. The chromosomes were classified according to ISCNDA 1989 (1990).

Results and discussion

Chromosomal localizations

The biotinylated probes were hybridized to cattle, sheep, and goat chromosome spreads, and the total number of metaphases examined together with the number of spreads with a signal on four, three, two chromatids and one chromatid are shown in Table 1. Chromosomes were identified by R-banding, and fluorescent spots were repeatedly detected at precise chromosomal positions for each probe (Fig. 1):

- cattle Chr 19q13, sheep Chr 11q13, and goat Chr 19q13 for pLPc, the probe for the *LPO* gene (Fig. 1 aa′,bb′,cc′)
- cattle Chr 12q13, sheep Chr 10q13, and goat Chr 12q13 for pBR4.5, the probe for the *RB1* gene (Fig. 1 dd′,ee′,ff′)
- cattle Chr 5q21, sheep Chr 3q21, and goat Chr 5q21 for pLA11.5, the probe for the *LALBA* gene (Fig. 1 gg′,hh′,ii′).

As shown in Fig. 1 gg′,hh′,ii′, the hybridization signals corresponding to the *LALBA* probe are very clear and sufficiently intense to be easily detected. Under the conditions described in Materials and methods, the intensity of the background signals owing to the hybridization of the repetitive sequences in the probe is effectively reduced, which allows an unambiguous interpretation of the results. Also, the signal frequencies and their distribution are similar to those obtained

Table 1.

Probe	Species chromosome	Total number of spreads examined	Percentage of spreads with n-labeled chromatids n =					Chromosome number and specific band
			4	3	2[a]	2[b]	1	
pLPc[c]	sheep	44	0	9	37	2	52	11q13
pLPc	cattle	47	0	4	32	2	62	19q13
pLPc	goat	46	0	2	33	11	54	19q13
pBR4.5	sheep	53	0	9	30	8	53	10q13
pBR4.5	cattle	58	0	4	17	5	74	12q13
pBR4.5	goat	49	2	8	14	6	70	12q13
pLA11.5	sheep	74	1	3	22	7	67	3q21
pLA11.5	cattle	59	0	5	17	0	78	5q21
pLA11.5	goat	70	3	6	20	4	67	5q21

[a] On the same homolog; [b]on two different homologs. [c]pLPc, *LPO* gene; pBR4.5; *RB1* gene; pLA11.5; *LALBA* gene.

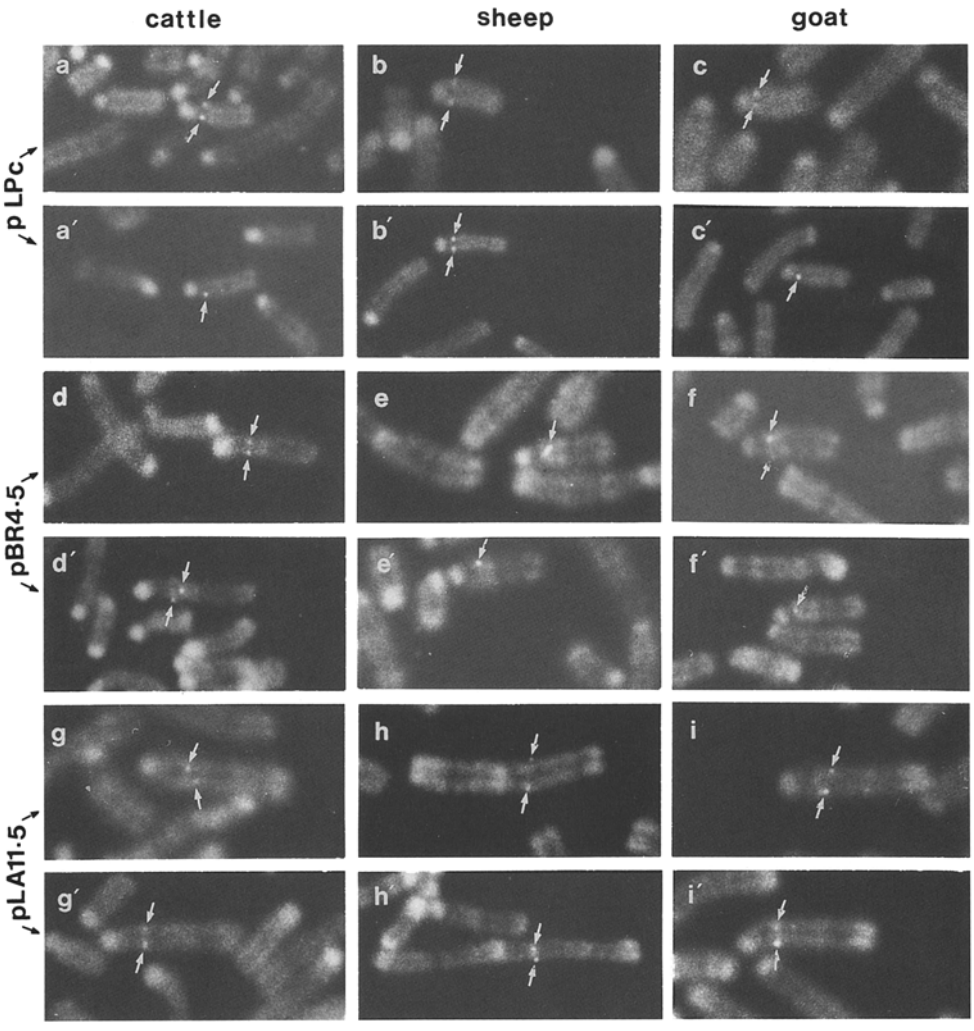

Fig. 1. R-banded partial metaphase spreads after in situ hybridization with the *LPO* gene probe (pLPc) on cattle Chr 19 (**a and a′**), sheep Chr 11 (**b and b′**), and goat Chr 19 (**c and c′**); with the *RB1* gene probe (pBR4.5) on cattle Chr 12 (**d and d′**), sheep Chr 10 (**e and e′**), and goat Chr 12 (**f and f′**); and with the *LALBA* gene probe on cattle Chr 5 (**g and g′**), sheep Chr 3 (**h and h′**), and goat Chr 5 (**i and i′**); **arrows** indicate specific hybridization signals, and chromosomes are numbered according to ISCNDA 89 (1990).

for the two other probes pLPc and pBR4.5 (Table 1), which indicates that even at a concentration as low as 0.05 ng/μl, the quantity of single-copy DNA in the probe is in excess compared with that of the chromosomal target DNA on the slides. In no metaphase spread were specific hybridization signals other than those on cattle and goat Chr 5q21 and sheep Chr 3q21 observed, which suggests that the *LALBA* pseudogenes are clustered together in the same chromosomal region as the *LALBA* gene. This agrees with the fact that the *LALBA* pseudogenes described to date are the result of gene duplications (Soulier et al. 1989) and not processed pseudogenes, because the former would be detected with our probe, but not the latter.

Therefore, the *LPO* gene can be assigned to the first positive R-band q13 of sheep Chr 11 and of cattle and goat Chr 19, the *RB1* gene to the first positive R-band q13 of sheep Chr 10 and of cattle and goat Chr 12, and the *LALBA* gene to q21 of sheep Chr 3 and of cattle and goat Chr 5.

On the basis of banding patterns, it has been shown that respectively sheep Chr 11, cattle and goat Chr 19, sheep Chr 10, cattle and goat Chr 12, the long arm of sheep Chr 3, cattle and goat Chr 5 are homoeologous (Reading Conference 1976; ISCNDA 1989 [1990]), which is confirmed by the in situ hybridization results presented here.

The localization of the *LPO* gene adds another DNA marker to cattle Chr 19 and sheep Chr 11 in their proximal part, which extends the overlapping region between these two chromosomes because the two other genes regionally localized on these chromosomes (*KRT10* and *GH*) are in the distal part (Hediger et al. 1990, 1991). The localization of the *RB1* gene provides a DNA marker for cattle Chr 12 and its homologs in sheep and goat, for which no marker was known so far. The result for the *LALBA* gene confirms the previous assignment to cattle Chr 5 (Threadgill and Womack 1990) and to sheep Chr 3 (Imam-Ghali et al. 1991).

Comparison between bovine and human chromosomes

The *LPO* gene has not been mapped in human. However, all the genes that have been mapped to bovine Chr 19—*POLR2, PRCKA, GH* and *HOX2* (O'Brien et al. 1993) and *KRT10* and *KRT19* (Fries et al. 1991)—

are localized on human Chr 17 and cover both arms (p13.1 to q24). Also, from a karyotypic point of view, these two chromosomes are similar. Therefore, it is likely that the *LPO* gene is located on human Chr 17. The *RB1* gene is localized on human Chr 13q14, and it is the first marker mapped to bovine Chr 12q13. The comparison of RBG-banded human Chr 13 and bovine Chr 12 reveals a high degree of similarity, which is confirmed by the conservation of this gene localization. The *LALBA* gene is localized on human Chr 12q13. Several other genes localized on human Chr 12 have been mapped to bovine Chr 5: *GAPD, GLI, HOX3, IFNG, KRAS2, LDHB, PAH, TPI1* (O'Brien et al. 1993) and *KRT5* and *KRT1* (Fries et al. 1991). In addition, the existence of a conserved syntenic group in cattle containing eight loci from pter to q24 of human Chr 12 has been reported by Threadgill and colleagues (1990), but two other loci found on bovine Chr 5 do not belong to human Chr 12: *PFKM,* human Chr 1cen-q32 and *ACO2,* human Chr 22q11.2-q13.31 (O'Brien et al. 1993). These results show that several intrachromosomal and interchromosomal rearrangements have occurred during evolution of Artiodactyls and primates.

In conclusion, the precise mapping of *LPO, RB1,* and *LALBA* genes confirms the homoeologies among cattle Chr 19, sheep Chr 11, and goat Chr 19; cattle Chr 12, sheep Chr 10, and goat Chr 12; and cattle Chr 5, sheep Chr 3, and goat Chr 5, particularly in the case of sheep Chr 10 and the homoeologous cattle and goat Chr 12, for which *RB1* is the first gene to be localized. These results contribute to the physical bovine, ovine, and caprine gene maps and to comparative cytogenetics between Bovidae and Hominidae.

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References

Buckland, R.A., Evans, H.J. (1978). Cytogenetic aspects of phylogeny in the Bovidae. I. G-banding. Cytogenet. Cell Genet. 21, 42–63.

Cals, M.-M. (1992). Détermination de la structure primaire de la lactoperoxydase bovine. Etude génétique de l'enzyme et localisation cellulaire de sa secrétion. Thèse de Doctorat de l'Université de Paris VII.

Chowdhary, B.P., Harbitz, I., Davies, W., Gustavsson, I. (1991). Chromosomal localization of the glucose phosphate isomerase (*GPI*) gene in cattle, sheep and goat by in situ hybridization—chromosomal banding homology versus molecular conservation in Bovidae. Hereditas 114, 161–170.

Evans, H.J., Buckland, R.A., Sumner, A.T. (1973). Chromosome homology and heterochromatin in goat, sheep and ox studied by banding techniques. Chromosoma 42, 383–402.

Fries, R., Threadgill, D.W., Hediger, R., Gunawardana, A., Blessing, M., Jorcano, J.L., Stranzinger, G., Womack, J.E. (1991). Mapping of bovine cytokeratin sequences to four different sites on three chromosomes. Cytogenet. Cell Genet. 57, 135–141.

Gunawardana, A., Schoeberlein, A., Solinas-Toldo, S., Fries, R., Stranzinger, G. (1992). Comparative gene mapping and cytogenetics in cattle, sheep and goat. 10th Eur. Colloq. Cytogenet. Domest. Anim., Utrecht, The Netherlands.

Hayes, H.C., Petit, E.J. (1993). Mapping of the beta-lactoglobulin gene and of an immunoglobulin M heavy chain-like sequence to homoeologous cattle, sheep and goat chromosomes. Mammalian Genome 4, 207–210.

Hayes, H., Petit, E., Bouniol, C., Popescu, P. (1993). Localization of the alpha S2-casein gene (*CASAS2*) to the homoeologous cattle, sheep and goat chromosomes 4 by in situ hybridization, in press.

Hayes, H., Petit, E., Dutrillaux, B. (1991). Comparison of RBG-banded karyotypes of cattle, sheep and goats. Cytogenet. Cell Genet. 57, 51–55.

Hayes, H., Petit, E., Lemieux, N., Dutrillaux, B. (1992). Chromosomal localization of the ovine beta-casein gene by non-isotopic in situ hybridization and R-banding. Cytogenet. Cell Genet. 61, 286–288.

Hediger, R., Johnson, S.E., Barendse, W., Drinkwater, R.D., Moore, S.S., Hetzel, J. (1990). Assignment of the growth hormone gene locus to 19q26-qter in cattle and to 11q25-qter in sheep by in situ hybridization. Genomics 8, 171–174.

Hediger, R., Ansari, H.A., Stranzinger, G.F. (1991). Chromosome banding and gene localizations support extensive conservation of chromosome structure between cattle and sheep. Cytogenet. Cell Genet. 57, 127–134.

Human Gene Mapping 11 (1991). Eleventh International Workshop on Human Gene Mapping. Cytogenet. Cell Genet. 58, 5–102.

Iannuzzi, I., Gallagher, D.S., Di Meo, G.P., Ryan, A.M., Ferrara, L., Womack, J.E. (1992). Chromosomal localization of omega and trophoblast interferon genes in cattle, river buffalo, goat and sheep by fluorescent in situ hybridization. 10th Eur. Colloq. Cytogenet. Domest. Anim., Utrecht, The Netherlands.

Iannuzzi, L., Gallagher, D.S., Ryan, A.M., Di Meo, G.P., Womack, J.E. (1993). Chromosomal localization of omega and trophoblast interferon genes in cattle and river buffalo by sequential R-banding and fluorescent in situ hybridization. Cytogenet. Cell Genet. 62, 224–227.

Imam-Ghali, M., Saidi-Mehtar, N., Guérin, G. (1991). Sheep gene mapping: additional DNA markers included (CASB, CASK, LALBA, IGF1 and AMH). Anim. Genet. 22, 165–172.

ISCNDA 1989 (1990). International System for Cytogenetic Nomenclature of Domestic Animals, D. Di Berardino, H. Hayes, R. Fries, S. Long, eds. Cytogenet. Cell Genet. 53, 65–79.

Jenness, R. (1982). Interspecies comparison of milk proteins. In: *Developments in Dairy Chemistry,* Vol. 1, *Proteins,* P.F. Fox, ed. (New York: Applied Science Publishers) pp. 87–114.

Lee, W.H., Shew, J.Y., Hong, F.D., Sery, T.W., Donoso, L.A., Young, L.J., Bookstein, R., Lee, E.Y.H.P. (1987). The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. Nature 329, 642–645.

Lemieux, N., Dutrillaux, B. (1992). New gene assignments to rabbit chromosomes; implications for chromosome evolution. Cytogenet. Cell Genet. 61, 132–134.

Lemieux, N., Dutrillaux, B., Viegas-Péquignot, E. (1992). A simple method for simultaneous R or G banding and fluorescence in situ hybridization of small copy genes. Cytogenet. Cell Genet. 59, 311–312.

O'Brien, S.J., Womack, J.E., Lyons, L.A., Moore, K.J., Jenkins, N.A., Copeland, N.G. (1993). Anchored reference loci for comparative genome mapping in mammals. Nature Genet. 3, 103–112.

Reading Conference, 1976 (1980). Proceedings of the First International Conference for the Standardization of Banded Karyotypes of Domestic Animals, Reading, England, 1976, C.E. Ford, D.L. Pollock, and I. Gustavsson, eds. Hereditas 92, 145–162.

Schnedl, W., Czaker, R. (1974). Centromeric heterochromatin and comparison of G-banding in cattle, goat and sheep chromosomes (Bovidae). Cytogenet. Cell Genet. 13, 246–255.

Soulier, S., Mercier, J.C., Vilotte, J.L., Anderson, J., Clark, A.J., Provot, C. (1989). The bovine and ovine genomes contain multiple sequences homologous to the alpha-lactalbumin-encoding gene. Gene 83, 331–338.

Threadgill, D.W., Womack, J.E. (1990). Genomic analysis of the major bovine milk protein genes. Nucleic Acids Res. 18, 6935–6942.

Threadgill, D.W., Adkison, L.R., Womack, J.E. (1990). Syntenic

conservation between humans and cattle. II. Human chromosome 12. Genomics 8, 29–34.

Viegas-Péquignot, E., Dutrillaux, B. (1978). Une méthode simple pour obtenir des prophases et des prométaphases. Ann. Genet. (Paris) 21, 122–125.

Viegas-Péquignot, E., Dutrillaux, B., Magdelenat, H., Coppey-Moisan, M. (1989). Mapping of single copy DNA sequences on human chromosomes by in situ hybridization with biotinylated probes: enhancement of detection sensitivity by intensified fluorescence digital-imaging microscopy. Proc. Natl. Acad. Sci. USA 86, 582–586.

Vilotte, J.L., Soulier, S., Printz, C., Mercier, J.C. (1991). Sequence of the goat alpha-lactalbumin-encoding gene: comparison with the bovine gene and evidence of related sequences in the goat genome. Gene 98, 271–276.

Yunis, J.J. (1976). High resolution of human chromosomes. Science 191, 1268.