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N. L. Lòpez-Corrales
Roslin Institute (Edinburgh)

T. S. Sonstegard
USDA-ARS

T. P. L. Smith
USDA-ARS, tim.smith@ars.usda.gov

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Physical mapping of the bovine, caprine and ovine homologues of the paired box gene PAX8

N.L. López-Corrales,^a T.S. Sonstegard^b and T.P.L. Smith^c

^aRoslin Institute (Edinburgh), Roslin, Midlothian (UK);

^bUSDA-ARS Beltsville Agricultural Research Center, Beltsville MD;

^cUSDA-ARS U.S. Meat Animal Research Center, Clay Center NE (USA)

Abstract. The PAX8 gene, a member of the human paired box gene family, was mapped by FISH to chromosome 11 in cattle and goat and to the short arm of chromosome 3 in sheep. The cytogenetic position of PAX8 on BTA 11 and on its homologue OAR 3p lies in the region where the interleukin beta (IL1B) gene has been previously located, (BTA 11q22.1→q22.3 and OAR 3p25→q26 respectively; López-Corrales et al., 1998). The results indicated that PAX8 as well

as interleukin beta and interleukin alpha (IL1B and IL1A) genes detected on the human chromosome segment HSA 2q13→q21 maintain a similar order and location in these three related species. In addition, the breakpoint in conserved synteny can now be narrowed to a position between the protein C (PROC) and PAX8 genes, which lie in close proximity on HSA 2.

PAX8 is a member of a family of human paired box genes. The nine known human paired box genes encode transcription factors that are thought to play a fundamental role in early embryonic development. Some of the PAX genes are oncogenes, at least six human phenotypes are caused by loss-of-function mutations in three different PAX genes, Waardenburg syndrome (WS), and kidney and retinal defects (PAX3, PAX6 and PAX2 respectively; Read, 1995). In mice *Pax8* encodes a sequence-specific transcription factor expressed in the developing secretory system and in both developing and adult thyroid (Poolev et al., 1992). Recently Dehbi and Pelletier (1997) provided evidence that PAX8 may function to induce the tumour suppressor gene WT1, which is involved in the development of Wilms tumors (WTs) and plays a role in normal urogenital development (Pelletier et al., 1991). PAX8 has been located in humans in the distal part of chromosome 2, specifically HSA 2q12→q14. (Poolev et al., 1992, Stapleton et al., 1993). Comparative mapping of humans, cattle and sheep has shown a significant conservation of synteny between HSA 2q and bovine and ovine chromosome 2 (BTA 2 and OAR 2q). However, the gene order within this overall synteny has been significantly

altered. In fact four small conserved segments resulting from two major rearrangements involving COL3A1 and INHBB have been reported (Sonstegard et al., 1997). In addition to these rearrangements, a breakpoint in the conservation of synteny has been identified in the vicinity of the centromeres. Accordingly, PROC, IL1B, IL1R1 and IL1R2 (interleukin receptors I and II) lie near the HSA 2 centromere (HSA 2q13→q21), while their bovine counterparts lie on BTA 2 (PROC) or BTA 11 (ILR1, IL1B; Yoo et al., 1994; Sonstegard et al., 1997). These findings have been extended to sheep and goat (López-Corrales et al., 1998). PAX8 has been located on a human YAC contig containing the IL1A and IL1B genes, lying slightly telomeric between IL1B and PROC (Spurr et al., 1996). Therefore it was not possible to predict whether PAX8 is located on BTA 2 or BTA 11, and similarly on OAR 2q or OAR 3p. We report the determination of the cytogenetic location of PAX8 in three bovid species to further refine the comparative map in this region and narrow the position of the evolutionary breakpoint.

Materials and methods

Isolation and analysis of genomic probes for FISH

The isolation of the PAX8 cosmid has been described (Sonstegard et al., 1998) which used an iterative screening process and primers designed from the human PAX8 cDNA sequence (GenBank accession X69699). The primer sequences used to obtain the cosmid clone for PAX8 were: TTGGCAGG-TACTACGAGACTGGCAAACATGGTAGGGTTCTG. DNA was isolated

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Request reprints from T.P.L. Smith, USDA-ARS U.S. Meat Animal Research Center, PO Box 166, Clay Center NE 68933 (USA); telephone: (402) 762-4369; fax: (402) 762-4390; e-mail: smith@email.marc.usda.gov

Fig. 1. Chromosomal localization of PAX8 by FISH. (Right) Biotinylated DNA of the cosmid clone containing the PAX8 gene was hybridized to metaphase chromosome spreads and the corresponding signals were detected with FITC on propidium iodide counterstained chromosomes showing the R-banding pattern. (Left) hybridization pattern of PAX8 in cattle (**a**), goat (**b**), and sheep (**c**) chromosomes showing a conserved location for the gene.

by ion exchange columns according to the manufacturer's protocol (Qia-gen).

Chromosome preparation and FISH analysis

Probes were labeled with biotin by nick translation using a BioNick kit (Gibco) and purified with commercial Sephadex columns (5prime-3prime, Boulder CO). FISH analysis was according to the original method of Lichter et al. (1990) and Ponce de Leon et al. (1996). Chromosome spreads were obtained from peripheral whole blood cell cultures and R-banding of metaphase preparations were obtained according to Lemieux et al. (1992).

Digital imaging and probe localization

Digital images were obtained using a Zeiss Axioscope epifluorescence microscope coupled to a cooled CCD Camera. Fluorescein isothiocyanate (FITC) and propidium iodide (PI) signals were detected with BP 450–490 filter (Zeiss), recorded and photographed from the display monitor. Hybridization signals were assigned to specific bands according to the standard sheep and cattle R-banding pattern (ISCNDA 1989; Iannuzzi et al., 1995; Di Berardino and Iannuzzi, 1989).

Results and discussion

The presence of the target genes in the cosmid clones was detected by sequence analysis and PCR with other exon-specific primers. Cosmid probes were first hybridized to cattle chromosome spreads and revealed signals in the BTA 11q22.1→q22.3 region (Fig. 1a). The same probe and conditions were then used to perform FISH on sheep and goat chromosomes. The signals were detected at OAR 3p26→p25 and on goat chromosome 11, (CHI 11q22.1→q22.3), and cattle (BTA 11, Figs. 1b and 1c, respectively). PAX8 is located in a narrow region to which IL1B (human interleukin beta), IL1A (interleukin alpha) and its receptor IL1R1 have also been mapped in cattle and sheep (O'Brien et al., 1993; López-Corrales et al., 1998). This area is part of a segment of HSA 2q (segment IV in Sonstegard et al., 1997), which has been rearranged on BTA 11 (Smith et al., 1997; López-

Corrales et al., 1998). The comparison of PAX8 location in human, cattle, sheep and goat is presented in Fig. 2.

The cytogenetic positioning of PAX8 reported here demonstrates that the change in synteny which occurred relative to HSA 2q12→q14.3 has been conserved in the three bovid species examined. This result suggests that the evolutionary events leading to the observed differences must have occurred either in the primate lineage after the split with bovids, or in the bovid lineage after the split with primates but prior to speciation of the three livestock species. The shuffling of gene order seen between HSA 2q and BTA 2 is also observed on BTA 11, since PAX8 and IL1R1 lie in close proximity on HSA 2q, but are 51.7 cM apart on BTA 11 (66 and 14.3 cM respectively) (USDA-ARS, MARC Cattle Linkage Map, <http://www.niarc.usda.gov>). The results reported here add more information to support the idea that most bovid species share similar patterns of synteny, which permits the use of data from the more developed cattle and sheep maps to be applied in goat.

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Fig. 2. Human chromosome 2q compared with the three species in this study. The chromosome rearrangement involving the human PAX8, IL1R1, IL1A and IL1B genes is indicated. The relative location of these genes has been rearranged without modification in cattle and sheep. In addition the INHBB and PROC flanking genes also maintain a similar position in human, cattle and sheep.

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