Assignment of the *PAX6* gene to bovine chromosome 15q25→q27 by fluorescence in situ hybridization and confirmation by radiation hybrid mapping

H. Kuiper,^a J.L. Williams,^b O. Distl^a and C. Drögemüller^a^aInstitute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Hannover (Germany)^bRoslin Institute (Edinburgh), Roslin, Midlothian, Scotland (UK)

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Rationale and significance

Paired box gene 6 (*PAX6*), as a member of the paired box gene family, encodes a homeodomain transcription factor involved in oculogenesis and other developmental processes (Gehring, 2002). *PAX6* represents a universal master control gene for eye morphogenesis and in cattle *PAX6* has been shown to be essential for eye development and for tissue-specific gene expression within the eye (Jaworski et al., 1997). Various known mutations in the *PAX6* homologs of man and mouse cause different congenital ocular disorders. Mouse *Pax6* heterozygous mutants have small eyes, whereas homozygous mutants have only remnants of ocular tissues (Graw, 2003). In humans *PAX6* mutations mainly cause aniridia and less commonly cause isolated cataracts (OMIM 607108). The human *PAX6* gene consists of 14 exons spanning about 21.3 kb and has been mapped on human chromosome (HSA) 11p13 (Glaser et al., 1992). A considerable number of the sporadic aniridia cases in man have large chromosomal deletions in 11p13 detectable by fluorescence in situ hybridization (FISH) analysis, and some are also visible with conventional cytogenetic banding (van Heyningen and Williamson, 2002).

Because of the possible involvement of *PAX6* in bovine congenital ocular diseases (OMIA 0049/0050; Nicolas, 2003) we have started to clone the *PAX6* gene in cattle. This report describes the identification and mapping of a *PAX6* BAC clone which provides a basis for cytogenetic studies of possible chromosomal deletions that may occur in bovine anophthalmia/microphthalmia. We report here the assignment of the bovine *PAX6* gene to BTA15q25→q27 by fluorescence in situ hybridization (FISH) and radiation hybrid (RH) mapping. This location is consistent with known conservation of synteny between cattle, human and mouse genomes (Hayes et al., 2003).

Materials and methods

Isolation and characterization of the bovine *PAX6* clone

BLAST search of the human *PAX6* reference mRNA sequence (Accession NM_000280) against the bovine sequences of the genome survey sequence division revealed a significant match of *PAX6* exon 3 (BLAST E-value $6 \cdot 10^{-27}$) over 79 bp to the T7 end sequence of the bovine BAC clone CH240-442K7 (Accession CC567760). Primers (F 5'-GAA TCA GCT CGG TGG TGT CT-3', R 5'-CAG AAT TCG GGA AAT GTC G-3') were designed on the basis of a bovine *PAX6* cDNA sequence (Accession U73855) containing exon 5 of *PAX6*. These primers amplified a product of 115 bp on bovine genomic DNA and a product of this size was also obtained from the BAC clone. The BAC clone CH240-442K7 was obtained from the bovine BAC library CHORI-240 of the BACPAC Resource Center (<http://bacpac.chori.org/>). BAC DNA was prepared from 100-ml overnight cultures using the Qiagen Midi plasmid kit according to the modified protocol for BACs (Qiagen, Hilden, Germany) and the presence of the *PAX6* sequence verified by PCR.

Chromosome preparation and fluorescence in situ hybridization (FISH) analysis

Bovine metaphase spreads for FISH were prepared using phytohemagglutinin-stimulated blood lymphocytes. Cells were harvested and slides prepared using standard cytogenetic techniques. Prior to FISH the chromosomes were GTG-banded and well-banded metaphase chromosomes were photographed using a highly sensitive CCD camera and IPLab 2.2.3 (Scana-

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Corresponding Author: Dr. Cord Drögemüller
Institute for Animal Breeding and Genetics
University of Veterinary Medicine Hannover
Bünteweg 17p, DE-30559 Hannover (Germany)
telephone: +49-511-9538866; fax: +49-511-9538582
e-mail: Cord.Droegemueller@tiho-hannover.de

Fig. 1. Chromosomal assignment of the bovine *PAX6* gene by FISH analysis. The digoxigenin-labelled bovine BAC CH240-442K7 containing the *PAX6* gene was hybridized on GTG-banded metaphase chromosomes from a normal cow. Double signals are visible on BTA15q25→q27.

lytics, Inc.). Identification adhered strictly to the ISCNDB 2000 classification (Cribiu et al., 2001). The BAC clone containing the bovine *PAX6* gene was labelled by nick translation using a Nick-Translation-Mix (Boehringer Mannheim Corp.). Sonicated total bovine DNA and salmon sperm were used as competitor DNA in the FISH experiment. After hybridization overnight, signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with propidium iodide and embedded in DAPI antifade. Previously GTG-banded metaphase spreads were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: CH240-442K7

Probe type: bovine genomic BAC clone

Insert size: 160 kb

Vector: pTARBAC1.3

Proof of authenticity: PCR

Gene reference for the human PAX6 gene: Glaser et al. (1992)

Radiation hybrid (RH) mapping

For fine mapping, the bovine BAC clone containing the *PAX6* gene was localized on the 3,000 rad Roslin/Cambridge bovine radiation hybrid panel (Williams et al., 2002), purchased from Research Genetics (Huntsville, Ala., USA). PCR primers for RH mapping were designed based on the T7 sequence of the BAC clone CH240-442K7 (F 5'-GGT CCT CTG TGC TTC ACC TC-3'; R 5'-CCC AGA GCT ACA CCC ACA TT-3') to give a 247-bp fragment. Two independent PCR reactions were carried out in a 20- μ l reaction containing 25 ng of RH cell line DNA, 10 pmol of each primer and 0.5 U *Taq* polymerase (Qbiogene, Heidelberg, Germany). The reaction conditions started with a denaturing step at 94°C for 4 min followed by 35 cycles using the following conditions: denaturation for 30 s at 94°C, annealing for 30 s at 60°C and extension for 30 s at 72°C. PCR was completed with a final cooling at 4°C for 10 min. PCR products were separated on a 1.5% agarose gel and presence or absence of a PCR signal was scored for each cell. The RHMAP3.0 package (Lange et al., 1995) was used for a two-point analysis against approximately 1,200 bovine markers typed previously on the panel (<http://www.ri.bbsrc.ac.uk/radhyb>) to localize the *PAX6* gene.

Results and discussion

The chromosomal location of the bovine *PAX6* gene on BTA15q25→q27 was determined by FISH of the BAC clone CH240-442K7 to bovine metaphase chromosomes (Fig. 1).

Mapping data:

Location: 15q25→q27

Number of cells examined: 20

Number of cells with specific signals: 0 (0), 1 (0), 2 (5), 3 (3), 4 (12) chromatids per cell

Most precise assignment: 15q25→q27

Location of background signals (site with >2 signals): none observed

To confirm the chromosome location of the BAC clone the Roslin/Cambridge bovine RH panel was analyzed. The STS marker showed a retention frequency of 9.6%. Two-point analysis revealed close linkage of *PAX6* to the BTA15 marker ILSTS027 at a distance of 41.2 cR with a LOD score of 5.4. This RH result confirms the chromosomal location obtained by FISH. The ILSTS027 microsatellite marker was previously mapped on BTA15 by linkage mapping (Kappes et al., 1997). The assignment of the bovine *PAX6* gene on BTA15q25→q27 agrees with the comparative mapping data, which shows conserved synteny between BTA15, HSA11 and MMU2 (Hayes et al., 2003).

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