Physical mapping¹ of *CHX10*, *ALDH6A1*, and *ABCD4* on bovine chromosome 10q34

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¹ To our knowledge this is the first time these genes have been mapped in cattle.

Rationale and significance

Bilateral anophthalmia/microphthalmia represents a rare and severe form of structural eye malformation in different species and has also been observed in cattle (Bähr et al., 2003). Therefore we started to map possible candidate genes for anophthalmia/microphthalmia in cattle. The *ceh-10* homeo domain containing homolog (*C. elegans*) (*CHX10*) gene is an essential component in the network of genes required for the development of the mammalian eye, with profound effects on retinal progenitor cell proliferation and bipolar cell specification or differentiation. The recessive mouse mutation ocular retardation is characterized in the homozygous state by blindness with obvious non-syndromic microphthalmia and this phenotype is caused by a *Chx10* mutation (Burmeister et al., 1996). A similar phenotype has been reported recently in two human families suffering from recessive mutations in *CHX10* (Percin et al., 2000). A considerable number of comparable cases of human and murine anophthalmia/microphthalmia showed chromosomal deletions and translocations in affected individuals (Graw, 2003). For the chromosomal assignment of the bovine *CHX10* gene we developed three locus-specific bovine BAC probes spanning the chromosomal region of

CHX10. The human *CHX10* gene maps at 73.77 Mb on HSA14q24 (NCBI map viewer, human genome build 35) and is flanked proximally by the aldehyde dehydrogenase 6 family, member A1 (*ALDH6A1*) gene at 73.59 Mb, and distal by the ATP-binding cassette, sub-family D (ALD), member 4 (*ABCD4*) gene at 73.82 Mb on HSA14.

This report describes the identification and mapping of a BAC clone that can be used as the basis for cytogenetic studies of possible chromosomal deletions which may occur in bovine anophthalmia/microphthalmia using fluorescence in situ hybridization (FISH). We report here the assignment of the bovine *CHX10* and the flanking *ALDH6A1*, and *ABCD4* genes to BTA10q34 by FISH and radiation hybrid (RH) mapping.

Materials and methods

Isolation and characterization of the bovine *CHX10*, *ALDH6A1*, and *ABCD4* BAC clones

A bovine BAC clone containing the *CHX10* gene, designated RP42-245M5, was isolated from the bovine BAC library RPCI-42 (Warren et al., 2000). High density BAC colony filters were screened according to the CHORI protocols (<http://www.chori.org/bacpac/>), with a heterologous ³²P-labeled insert of a human *CHX10* cDNA clone (Accession NM_182894; Percin et al., 2000) containing the complete ORF of the *CHX10* gene.

From the bovine CHORI-240 library two BAC clones for *ALDH6A1* and *ABCD4* have been identified by BLAST searches of the human reference mRNA sequences (Accessions NM_005589 and NM_005050, respectively). The human *ALDH6A1* exon 4 sequence matched 85 bp of the T7 end sequence of the bovine BAC clone CH240-227P3 (Accession BZ843840; BLAST E-value $3 \cdot 10^{-23}$) and the human *ABCD4* exons 16 and 17 matched 130 bp of the T7 end sequence of the bovine BAC clone CH240-327K10 (Accession CC492996; BLAST E-value $2 \cdot 10^{-21}$). For experimental verification of the gene content of these BAC clones PCR primers (*ALDH6A1*_F 5'-CGT GGG CAG ACA CTT CAA TA-3', *ALDH6A1*_R 5'-GAG GCC TCG AAA CAC ATC TC-3') were designed on the basis of the bovine

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Fig. 1. Chromosomal assignment of the bovine *CHX10* gene by FISH analysis. The digoxigenin-labeled bovine BAC clone RP42-245M5 was hybridized on a GTG-banded metaphase spread of a normal cow (A). Double signals are visible on both BTA10q34 (B).

ALDH6A1 mRNA sequence (Accession NM_175811; Deichaite et al., 1993) spanning intron 4 of *ALDH6A1*. These primers amplified a product of 400 bp on bovine genomic DNA and from BAC clone CH240-227P3. Primers (ABCD4_F 5'-CGA CTC CTG AAG ACC CAG AG-3', ABCD4_R 5'-TGG CTG AAG CAG TTG ATG AG-3') were derived from an alignment of a bovine EST sequence (Accession BE754578) corresponding to human *ABCD4* exon 7 to 11. These primers amplified a product of 600 bp on bovine genomic DNA and from BAC clone CH240-327K10.

BAC ends of RP42-245M5 were sequenced and the RP42-245M5 SP6 and T7 sequences were deposited in the EMBL nucleotide database (Accession AJ810876, AJ810877). A BLAST sequence comparison of the bovine SP6 BAC end sequence with the build 35 of the human genome sequence revealed a significant match (BLAST E-value $5 \cdot 10^{-8}$) over 77 bp (87% identity) starting at 73,676,276 bp of HSA14 approximately 100 kb upstream to human *CHX10*.

Chromosome preparation and fluorescence in situ hybridization (FISH) analysis

Bovine metaphase spreads for FISH on GTG-banded chromosomes were prepared as described by Drögemüller et al. (2001). The BAC clones were labeled using a Nick-Translation-Mix (Boehringer Mannheim, Germany). Identification of chromosomes followed strictly the Reading Conference classification (ISCNDB 2000).

Probe names: RP42-245M5 (*CHX10*); CH240-227P3 (*ALDH6A1*); CH240-327K10 (*ABCD4*)

Probe type: bovine genomic BAC clones for all probes

Insert size: 195 kb (RP42-245M5); 180 kb (CH240-227P3); 175 kb (CH240-327K10)

Vector: pBACe 3.6 (RP42-245M5); pTARBAC1.3 (CH240-227P3, CH240-327K10)

Proof of authenticity: hybridization (RP42-245M5); PCR (CH240-227P3, CH240-327K10)

Gene reference for the human genes: Percin et al. (2000) for *CHX10*; Kedishvili et al. (1992) for *ALDH6A1*; Holzinger et al. (1998) for *ABCD4*

Radiation hybrid (RH) mapping

For fine mapping, the three bovine BAC clones containing the *CHX10*, *ALDH6A1*, and *ABCD4* genes were localized on the 3,000 rad Roslin/Cambridge bovine radiation hybrid panel (Williams et al., 2002). Three pairs of primers were designed based on the SP6 sequence of the BAC clone RP42-245M5 (F 5'-GAT TTT AAG CCA CTG TGA CCA-3'; R 5'-GAT TCC CTG CTG CTG CTA AG-3') to give a 366-bp fragment, on the T7 sequence of the BAC clone CH240-227P3 (F 5'-GCA GGA AGA AAC TGC TGC AT-3'; R 5'-CAG TGG GAA AAA TAA GGA CCT G-3') to give a 189-bp fragment, and on the T7 sequence of the BAC clone CH240-327K10 (F 5'-TGT GCT

CTC CTA CCC GTA CC-3'; R 5'-CAG AGC TTT GGC CTT GTC TC-3') to give a 361-bp fragment, respectively.

Amplification reactions were performed as previously described (Drögemüller et al., 2001). The typing data, obtained in duplicate, were incorporated into the latest radiation hybrid map (<http://databases.roslin.ac.uk/radhyb/intro.py>) using the RHMAP3.0 package (Lange et al., 1995).

Results and discussion

The chromosomal assignment of the bovine *CHX10*, *ALDH6A1*, and *ABCD4* genes on BTA10q34 was determined by FISH of three BAC clones to bovine metaphase chromosomes. As an example Fig. 1 shows the FISH of *CHX10*.

Mapping data

Location: 10q34

Number of cells examined: 30 for each BAC

Number of cells with specific signals: CH240-227P3 0 (0), 1 (0), 2 (2), 3 (2), 4 (26) chromatids per cell; CH240-327K10 0 (0), 1 (3), 2 (6), 3 (4), 4 (17) chromatids per cell; RP42-245M5 0 (0), 1 (0), 2 (5), 3 (5), 4 (20) chromatids per cell

Most precise assignment: 10q34

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

To confirm the FISH mapping of the *CHX10*, *ALDH6A1*, and *ABCD4* BAC clones the Roslin/Cambridge bovine RH panel was analyzed. The three independent STS markers showed a unique retention pattern with a retention frequency of 14.9%. Two-point analysis revealed complete linkage to the BTA10 marker DIK020 at a distance of 0 cR₃₀₀₀ with a LOD score of 17.2, and tight linkage to the BTA10 marker LTBP2 at a distance of 4.4 cR₃₀₀₀ with a LOD score of 14.5, respectively. These RH results confirm the chromosomal locations obtained by FISH. The DIK020 microsatellite marker was previously mapped on BTA10 by linkage mapping (Inoue et al., 1995; Taylor et al., 1997) and the LTBP2 STS marker maps to the

telomeric end of the BTA10 RH map (Band et al., 2000). The assignment of the bovine *ALDH6A1*, *ABCD4*, and *CHX10* genes on BTA10q34 agrees with the comparative mapping data, which shows conserved synteny between BTA10, HSA14 and MMU12 (Band et al., 2000; Hayes et al., 2003).

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