Assignment¹ of the *PHACTR1* gene to bovine chromosome 23q24 by fluorescence in situ hybridization and radiation hybrid mapping

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¹ To our knowledge this is the first time this gene has been mapped in cattle.

Rationale and significance

The phosphatase and actin regulator (*PHACTR*) proteins represent a family of protein phosphatase 1 (PP1) and actin regulatory proteins. PP1 is a multifunctional enzyme with diverse roles in the nervous system, including regulation of synaptic activity and dendritic morphology. In rat the *Phactr1* gene was cloned for the first time as the prototypical member of this family (Allen et al., 2004). By searching databases for sequences similar to rat *Phactr1*, Allen et al. (2004) identified a family of four *PHACTR* genes in mouse and human. It was shown that human *PHACTR1*, which was designated KIAA1733, has been detected previously by sequencing hippocampus cDNA clones (Nagase et al., 2000). Functional studies determined that recombinant rat *Phactr1* inhibited PP1 enzymatic activity in a concentration-dependent manner and detected highest expression in brain and lower levels in lung, heart, kidney, and testis (Allen et al., 2004). The human *PHACTR1* gene consists of 14 exons spanning about 570 kb on chromosome 6p23 (NCBI map viewer, human genome build 34) and the homologous rat *Phactr1* gene consists of 23 exons and is located on chromosome 17p12 (NCBI map viewer, rat genome build 2). The *PHACTR1* gene has not been identified in cattle, however a bovine fetal kidney EST sequence (Accession AV607621) corresponding to the 3' end of the human homolog cDNA sequence can be found (NCBI map viewer, human genome build 34). We report here the assignment of the bovine *PHACTR1* gene to BTA23q24 by fluorescence in situ hybridization (FISH) and radiation hybrid (RH) mapping.

Materials and methods

Isolation and characterization of the bovine *PHACTR1* clone

BAC DNA of a randomly chosen BAC clone designated CH240-229B8 of the bovine CHORI-240 BAC library from the BACPAC Resource Center (<http://bacpac.chori.org/>) was prepared from 100-ml overnight cultures using the Qiagen plasmid midi kit according to the modified protocol for BACs (Qiagen, Hilden, Germany). This DNA was restricted independently with two enzymes (*SacI* and *XbaI*) and then separated on 0.8% agarose gels. The resulting fragments were partially cloned into the polylinker site of pGEM-4Z (Promega, Mannheim, Germany). Recombinant plasmid DNAs were end sequenced with the ThermoSequenase kit (AmershamBiosciences, Freiburg, Germany) and a LICOR 4200 automated sequencer. The sequences generated were deposited in the EMBL nucleotide database (Accessions AJ810862-AJ810871) and used in BLAST analyses against non-redundant NCBI nucleotide databases and build 34 of the human genome. The BLAST results (described below) showed matches with human *PHACTR*.

Chromosome preparation

Bovine metaphase spreads for FISH on GTG-banded chromosomes were prepared using phytohemagglutinin stimulated blood lymphocytes. Cells were harvested and slides prepared using standard cytogenetic techniques. Prior to fluorescence in situ hybridization the chromosomes were GTG-banded and well-banded spread metaphase chromosomes were photographed using a highly sensitive CCD camera and IPLab 2.2.3 (Scanalytics, Inc.). Identification of chromosomes strictly followed the ISCNDB 2000 classification (Cribiu et al., 2001).

Fluorescence in situ hybridization (FISH) analysis

The bovine BAC clone CH240-229B8 containing the bovine *PHACTR1* gene was labelled with digoxigenin by nick translation using a Nick-Translation-Mix (Boehringer Mannheim, Mannheim, Germany). FISH on GTG-banded bovine chromosomes was performed using 750 ng of digoxigenin-labelled BAC DNA. 1 µg sheared total bovine DNA and 10 µg salmon sperm were used as competitors in this experiment. After hybridization overnight,

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signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with DAPI and propidium iodide and embedded in antifade. Thirty metaphases that had been photographed were reexamined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: CH240-229B8

Probe type: bovine genomic BAC clone

Insert size: 170 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference: Allen et al. (2004)

Radiation hybrid (RH) mapping

To confirm the cytogenetic localization of the bovine *PHACTR1* gene a bovine radiation hybrid panel was analyzed. The Roslin/Cambridge 3,000 rad bovine RH panel was purchased from Research Genetics (Huntsville, Ala., USA). The RH panel of 94 clones was created by exposing a bovine fibroblast primary cell line to 3,000 rad of X-rays and fusing the irradiated cells with non-irradiated HPRT deficient hamster recipient cells (Wg3H). A pair of bovine primers (*PHACTR1*-F 5'-CTC CTG GAA ATG AGG ATG GA-3'; *PHACTR1*-R 5'-TGG ACC AAA GAG GGA GTG AC-3') for PCR amplification was designed based on a subclone sequence (Accession AJ810869) of BAC clone CH240-229B8 to give a 508-bp fragment. PCR reactions were performed in a total of 20 µl containing 25 ng of RH cell line DNA, 10 pmol of each primer and 0.5 U *Taq* polymerase (Qiogene, Heidelberg, Germany). The reaction started with an initial denaturation at 94 °C for 4 min followed by 35 cycles under 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 products were separated on 1 % agarose gels. Two separate PCRs were carried out and scoring of the presence or absence of products was carried out independently by two investigators. The RHMAP3.0 package (Lange et al., 1995) was used for a two-point analysis against approximately 1,200 bovine microsatellite markers typed previously on the panel (Williams et al., 2002).

Results and discussion

Experimental evidence for the gene content of the bovine BAC clone is available for the *PHACTR1* gene. Partial DNA sequencing and BLAST analysis of 10 generated CH240-229B8 BAC subclone sequences revealed 10 independent significant matches over 104 kb within the human *PHACTR1* gene at 13.210 to 13.314 Mb on HSA6. One BLAST hit matched within the exon 5 of human *PHACTR1* at 13.290 Mb (build 34; BLAST E-value 5·10⁻⁴⁴). This bovine sequence (Accession AJ810869) also showed 88% identity over 102 bp with rat *Phactr1* mRNA sequence (Accession AY494977). The chromosomal location of this bovine BAC clone was determined by fluorescence in situ hybridization (FISH) to bovine metaphase chromosomes (Fig. 1).

Mapping data:

Location: 23q24

Number of cells examined: 30

Number of cells with specific signals: 0 (3), 1 (1), 2 (6), 3 (4), 4 (16) chromatids per cell

Most precise assignment: 23q24

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 used for RH mapping was designed from the BAC sub-

Fig. 1. Chromosomal assignment of the *PHACTR1* gene by FISH analysis. The digoxigenin-labeled BAC clone CH240-229B8 containing the entire *PHACTR1* gene was hybridized to GTG-banded metaphase chromosomes of a normal cow. Double signals are visible on both chromosomes 23q14.

clone sequence located in exon 5 of *PHACTR1*. Two-point analysis revealed close linkage of *PHACTR1* to the BTA23 microsatellite marker CSSM024 at a distance of 24.8 cR (LOD score 12.0), which has been placed on the telomeric end of the BTA23 linkage map (Kappes et al., 1997). Thus the RH results for the bovine *PHACTR1* BAC confirmed and refined the results obtained by FISH. The chromosomal assignment of *PHACTR1* to BTA23q24 is in good agreement with known conservation of synteny between HSA6p and BTA23 (Everts-van der Wind et al., 2004).

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