Assignment¹ of the *MRPS35* gene to bovine chromosome 5q3.2→q3.4 by fluorescence in situ hybridization and confirmation by 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 mitochondrial ribosomal protein S35 (MRPS35) belongs to a group of 15 proteins present in mammalian mitochondrial 28S subunits (MRPS22 through MRPS36) which are specific to mitochondrial ribosomes and help in protein synthesis within the mitochondrion (Cavdar Koc et al., 2001). Proteins in this group have no apparent homologs in bacterial, chloroplast, archaeobacterial, or cytosolic ribosomes. The human *MRPS35* gene consists of 8 exons spanning about 45.5 kb and was mapped on chromosome HSA12p11, inferred following radiation hybrid (RH) mapping (Kenmochi et al., 2001; NCBI map viewer, human genome build 34.3). Pseudogenes corresponding to this gene are found on HSA3p, 5q, and 10q (Zhang and Gerstein, 2003). The murine *Mrps35* gene also consists of 8 exons and is located on mouse chromosome MMU6G3 (NCBI map viewer, mouse genome build 32).

Because of the possible involvement of mitochondrial ribosomal defects in human diseases we have started to characterize the *MRPS35* gene in cattle. This mapping report provides a basis for studying possible roles of *MRPS35* defects in bovine mitochondrial disorders. We report here the assignment of the bovine *MRPS35* gene to BTA5q3.2→q3.4 by fluorescence in situ hybridization (FISH) and RH mapping. This location is

consistent with known conservation of synteny between cattle, human and mouse genomes (e.g. Hayes et al., 2003).

Materials and methods

Isolation and characterization of the bovine MRPS35 clone

A bovine BAC clone with the *MRPS35* gene, was isolated from the bovine BAC library RPCI-42 (Warren et al., 2000). High density BAC colony filters were screened with a ³²P-labeled bovine *MRPS35*-specific bovine PCR product according to the CHORI protocols (<http://www.bacpac.chori.org>). Primers (F 5'-TAA ACT TTC CAG TTT GAA TTT AG-3', R 5'-GTC TTG ATG GTA AGC ACA TC-3') were designed on the basis of a bovine EST sequence (GenBank accession no. CB449694) containing exon 6 of *MRPS35* and amplified a product of 102 bp on bovine genomic DNA. A single positive bovine genomic BAC clone designated RP42-186J2 was isolated and the presence of the *MRPS35* sequence verified by PCR. 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). BAC ends were sequenced using the ThermoSequenase kit (Amersham Biosciences, Freiburg, Germany) and a LI-COR 4200 automated sequencer. The BAC SP6 and T7 end sequences were deposited in the EMBL nucleotide database under accessions AJ781392 and AJ781393, respectively, and subjected to BLASTN analysis against build 34.3 of the human genome.

Chromosome preparation and fluorescence in situ hybridization (FISH) analysis

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 FISH the chromosomes were GTG-banded and well-banded metaphase chromosomes were photographed using a highly sensitive CCD camera and IPLab 2.2.3 (Scanalytics, Inc.). Identification adhered strictly to the ISCNDB 2000 classification (Cribiu et al., 2001). The BAC clone containing the bovine *MRPS35* gene was labeled 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,

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Fig. 1. Chromosomal assignment of the bovine *MRPS35* gene by FISH analysis. The digoxigenin-labeled bovine BAC RP42-186J2 containing the *MRPS35* gene was hybridized on GTG-banded metaphase chromosomes of a normal cow. Double signals are visible on both BTA5q3.2→q3.4 as indicated by arrows. The chromosomes were counterstained with DAPI and propidium iodide and subsequently identified by GTG-banding and DAPI staining.

Germany). The chromosomes were counterstained with DAPI and embedded in propidium iodide/antifade. Previously GTG-banded metaphase spreads were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RP42-186J2

Probe type: bovine genomic BAC clone

Insert size: 190 kb

Vector: pBACe 3.6

Proof of authenticity: PCR and DNA sequencing

Gene reference for the human MRPS35 gene: Cavdar Koc et al. (2001)

Radiation hybrid (RH) mapping

For fine mapping, the bovine BAC clone containing the *MRPS35* 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 SP6 sequence of the BAC clone RP42-186J2 (F 5'-TCT GCA GCC TGT CAC ATA GC-3'; R 5'-ATA TCA CGA TGC TCC CGA AG-3') to give a 530-bp fragment. Two independent PCR reactions were carried out in a 20- μ l reaction containing 25 ng of RH cell line DNA, 15 pmol of each primer and 0.75 U Taq polymerase (Qiogene, 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 60 s at 60 °C and extension for 40 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 *MRPS35* gene.

Results and discussion

The bovine BAC clone RP42-186J2 was retrieved from the BAC library using a bovine *MRPS35* PCR product probe. Sequencing and BLASTN analysis of the T7 BAC end (AJ781393) revealed a significant match over 104 bp on HSA12p11 that is located approximately 65 kb distal of the *MRPS35* gene (BLAST E-value $6e^{-10}$). The chromosomal location of the bovine *MRPS35* gene on BTA5q3.2→q3.4 was determined by FISH of the BAC clone RP42-186J2 to bovine metaphase chromosomes (Fig. 1).

Mapping data

Location: 5q3.2→q3.4

Number of cells examined: 30

Number of cells with specific signals: 0 (4), 1 (3), 2 (10), 3 (4), 4 (9) chromatids per cell

Most precise assignment: 5q3.2→q3.4

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 13%. Two-point analysis revealed close linkage of *MRPS35* to the BTA5 marker BM8230 at a distance of 36.1 cR with a LOD score of 7.1. This RH result confirms the chromosomal location obtained by FISH. The BM8230 microsatellite marker was previously mapped on BTA5 by linkage mapping (Stone et al., 1995). The assignment of the bovine *MRPS35* gene on BTA5q3.2→q3.4 agrees with the comparative mapping data, which shows conserved synteny between BTA5, HSA12 and MMU6 (Hayes et al., 2003; Liu et al., 2003).

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