Assignment of the bovine B-cell CLL/lymphoma 2 gene (*BCL2*)¹ to BTA24q27 and the bovine BCL2-like 1 gene (*BCL2L1*)² to BTA13q22 by in situ hybridization

T. Goldammer^a R.M. Brunner^a J. Lyahyai^b P. Zaragoza^b
I. Martín-Burriel^b

^aResearch Unit Molecular Biology, Research Institute for the Biology of Farm Animals Dummerstorf (FBN), Dummerstorf (Germany);

^bLaboratorio de Genética Bioquímica, Facultad de Veterinaria, Universidad de Zaragoza, Zaragoza (Spain)

¹ This is a more precise localization of a gene previously mapped to BTA24 by Band et al. (2000).

² To our knowledge, this is the first time this gene has been mapped in cattle.

Received 6 June 2005; accepted in revised form for publication by M. Schmid, 26 July 2005.

Rationale and significance

The B-cell CLL/lymphoma 2 and BCL2-like 1 proteins, encoded by the genes *BCL2* and *BCL2L1* (alias *BCLX*), respectively, represent two members of the subfamily of anti-apoptotic BCL-2 proteins (Reed, 2000). Both act at the outer mitochondrial membrane and are involved in a wide variety of cellular activities, especially in the regulation of programmed cell death (Denisov et al., 2003). Kurschner and Morgan (1996) suggested the association of the apoptosis pathway with pathways for neurodegenerative disorders such as Alzheimer or Prion diseases in human. This promoted the BCL-2 proteins to be functional candidates for similar metabolic processes and disorders in ruminants like scrapie in sheep or bovine spongiform encephalopathy in cattle. In human, *BCL2* was assigned to HSA18q21.22 and the locus of

BCL2L1 was found on HSA20q11.21. The bovine *BCL2* was first assigned to the distal linkage group of BTA24 with radiation cell hybrids (Band et al., 2000). However, the locus was removed from the improved second-generation cattle RH5000 map (Everts-van der Wind et al., 2004). This report presents the isolation of bovine genomic partial *BCL2* and *BCL2L1* gene sequences and the determination of the cytogenetic loci on cattle chromosome bands by FISH.

Materials and methods

Screening of the bovine BAC library BBI_750 (Resource Center/Primary Database of the German Human Genome Project, <http://www.rzpd.de/>; Zhu et al., 1999) with primers designed from ovine *BCL2* and *BCL2L1* gene sequences (Acc. Nos. AY609317, AY568555) revealed gene-specific genomic DNA clones. Primer annealing temperatures for PCR typing within BAC pools were 62°C for *BCL2* primers and 58°C for *BCL2L1* primers. Identified BACs were labeled with biotin-16-dUTP and hybridized on photographed GTG-banded cattle chromosomes. Karyotyping of chromosomes followed ISCNDB (2000) for GTG-banded chromosomes. FISH was done according to a standard protocol (Pinkel et al., 1986). 200 ng probe DNA were annealed with 10 µg cattle cot-1 DNA and 10 µg salmon sperm DNA for 40 min at 37°C in a pre-hybridization reaction to compete unspecific DNA sequences. The evaluation of hybridization signals was performed on propidium iodide colored chromosomes.

The following primers amplified the bovine DNA:
BCL2-F: 5'-TTCGCCGAGATGTCCAGTCAGC-3'
BCL2-R: 5'-TTGACGCTCTCCACACACATGACC-3'
BCL2L1: F 5'-TTGTGGCCTTTTCTCCTTC-3'
BCL2L1: R 5'-TGGATCCAAGGCTCTAGGTG-3'

Supported by grant GO-896/6-1 from the Deutsche Forschungsgemeinschaft (DFG) and the EET2003-09890 project (CICYT/FEDER). I. Martín-Burriel was supported by the Ramón y Cajal research program and J. Lyahyai by the AEI. The BAC library was constructed by Ross Miller and John L. Williams as a part of the EU framework IV BOREALIS project..

Corresponding author: Dr. Inmaculada Martín-Burriel
Laboratorio de Genética Bioquímica
Facultad de Veterinaria (INGEN, I3A)
Universidad de Zaragoza, Zaragoza (Spain)
telephone: +34 976 761 622; fax: +34 976 761 612; e-mail: minma@unizar.es

Fig. 1. Assignments of *BCL2* to BTA24 and *BCL2L1* to BTA13. Left: GTG-banded chromosomes prior to FISH; Right: same chromosomes after FISH with gene specific bovine BAC probes.

Probe name: BCL2 – BBI_B750 M18138; BCL2L1 – BBI_B750 C13154
Probe type: Bovine genomic DNA-containing BAC clone
Insert size: ~100 kb
Vector: PBACe3.6 (BAC)
Proof of authenticity: PCR, DNA sequencing, NCBI-BLAST
Human gene reference: Boise et al. (1993), Tsujimoto and Croce (1986)

Results

DNA sequencing within the bovine BAC clone BBI_B750 C13154 using *BCL2* primers resulted in the identification of a 733-bp fragment representative of bovine *BCL2*, which is similar to human *BCL2* (Acc. No. NM_000633; Identities = 299/314 bp, 95%, Expect = e-143). Comparison of a 1075-bp fragment found after DNA sequencing of BAC clone BBI_B750 M18138 using *BCL2L1* primers with the corresponding human *BCL2L1* gene sequence identified partial similarity to reference sequence Acc. No. NM_001191 (Identities = 369/397 bp, 92%, Expect = e-160). The sequence data have been deposited with GenBank Data Library under Acc. Nos. DQ001759 and DQ001757. Similarity of the identified bovine sequences for *BCL2* and *BCL2L1* with bovine genome shot gun sequences Acc. Nos. AAFC01288540 (identity: 99%) and AAFC01676990 (identity: 99%) linked the DNA contigs to the corresponding gene-specific chromosome bands.

Mapping data: BCL2/BCL2L1
Most precise location: BTA24q27/BTA13q22
Nucleotide position in human chromosome reference sequence: HSA18/HSA20 contig sequences: NT_026437.11: ~59 Mbp/ NT_025028.13: ~8.7 Mbp
Number of cells examined: 15/12
Number of cells with specific signal: 1 (1/0), 2 (2/1), 3 (2/4), 4 (10/7) chromatids per cell
Location of background signals: none observed

Mapping by FL: BCL2/BCL2L1
Number of chromosomes examined: 27/23
Mean location: 0.97/0.74
Bands encompassed: 24q27/13q22
Range: 100% on 24q27/100% on 13q22
Standard deviation: $\pm 0.015 / \pm 0.021$

References

- Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW, Donovan J, Windish R, Steining C, Mahyuddin P, Womack JE, Lewin HA: An ordered comparative map of the cattle and human genomes. *Genome Res* 10:1359–1368 (2000).
- Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB: *bcl-x*, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74:597–608 (1993).
- Denisov AY, Madiraju MS, Chen G, Khadir A, Beauparlant P, Attardo G, Shore GC, Gehring K: Solution structure of human BCL-w: modulation of ligand binding by the C-terminal helix. *J Biol Chem* 278:21124–21128 (2003).
- Everts-van der Wind A, Kata SR, Band MR, Rebeiz M, Larkin DM, Everts RE, Green CA, Liu L, Natarajan S, Goldammer T, Lee JH, McKay S, Womack JE, Lewin HA: A 1463 gene cattle-human comparative map with anchor points defined by human genome sequence coordinates. *Genome Res* 14:1424–1437 (2004).
- ISCNDB (2000): International System for Chromosome Nomenclature of Domestic Bovids. Di Bernardino D, Di Meo GP, Hayes H, Gallagher D, Iannuzzi L (coordinator) (eds). *Cytogenet Cell Genet* 92:283–299 (2001).
- Kurschner C, Morgan JI: Analysis of interaction sites in homo- and heteromeric complexes containing Bcl-2 family members and the cellular prion protein. *Brain Res Mol Brain Res* 37:249–258 (1996).
- Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc Natl Acad Sci USA* 83:2934–2938 (1986).
- Reed JC: Mechanisms of apoptosis. *Am J Pathol* 157:1415–1430 (2000).
- Tsujimoto Y, Croce CM: Analysis of the structure, transcripts, and protein products of *bcl-2*, the gene involved in human follicular lymphoma. *Proc Natl Acad Sci USA* 83:5214–5218 (1986).
- Zhu B, Smith JA, Tracey SM, Konfortov BA, Welzel K, Schalkwyk LC, Lehrach H, Kollers S, Masabanda J, Buitkamp J, Fries R, Williams JL, Miller JR: A 5 genome coverage bovine BAC library: production, characterization, and distribution. *Mamm Genome* 10:706–709 (1999).