

Cytogenetic alignment of the bovine chromosome 13 genome map by fluorescence *in-situ* hybridization of human chromosome 10 and 20 comparative markers

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Received 10 September 1998; received in revised form and accepted for publication by B. Hamkalo 9 December 1998

Key words: bovine, cattle, comparative gene mapping, fluorescence *in-situ* hybridization

Abstract

A bovine bacterial artificial chromosome (BAC) library was screened for the presence of six genes (*IL2RA*, *VIM*, *THBD*, *PLC-II*, *CSNK2A1* and *TOP1*) previously assigned to human chromosomes 10 or 20 (HSA10 or HSA20). Four of the genes were found represented in the bovine BAC library by at least one clone. The identified BAC clones were used as probes in single-color fluorescence *in-situ* hybridization (FISH) to determine the chromosomal band location of each gene. As predicted by the human/bovine comparative map and comparative chromosome painting analysis, the four genes mapped to bovine chromosome 13 (BTA13). Dual-color FISH was then used to integrate these four type I markers into the existing BTA13 genome map. These FISH results anchor the BTA13 genome map from bands 14–23, and confirm the presence of a conserved HSA10 homologous synteny group on BTA13 centromeric to a HSA20 homologous segment.

Introduction

Approximately 400 genes have been assigned to specific chromosomes of cattle (Womack & Kata, 1995). Many of these genes represent select anchor loci (O'Brien *et al.* 1993) or other conserved genes (type I) previously mapped in man. This comparative mapping has revealed extensive conservation of synteny between the human and cattle genomes (Womack 1987). In addition, human on bovine ZOO-FISH studies (Solinas-Toldo *et al.* 1995, Hayes 1995, Chowdhary *et al.* 1996) have provided insights into the cytogenetic boundaries of homology between cattle and man. As the bovine gene mapping effort continues, comparative gene mapping in cattle rela-

tive to man will play an increasingly important role due to its strategic function in the 'positional candidate cloning' of economic trait loci (Collins 1995). It is becoming evident that comparative mapping within bovine must result in a knowledge of the linear order of genes within conserved syntenies given that preliminary studies indicate that gene order has been altered by intrachromosomal rearrangements (Yang *et al.* 1998, Johansson *et al.* 1995).

A 5000-rad whole-genome radiation hybrid (WG-RH) panel has been developed for the purpose of constructing integrated and ordered comparative genome maps of each cattle chromosome (Womack *et al.* 1997). A 13 loci framework map and 27 loci comprehensive map of cattle chromosome 13 (BTA13) has

already been constructed through analysis of this bovine WG-RH panel (Schlöpfer *et al.* 1997). In addition to 16 microsatellites, the comprehensive RH map includes the following 11 type-I loci which had been previously assigned to BTA13 through hybrid somatic cell analysis: arginine-vasopressin, neurophysin II (**AVP**); prepro-oxytocin, neurophysin I (**OXT**); casein kinase 2, α_1 -polypeptide (**CSNK2A1**); guanidine nucleotide binding protein, α subunit (**GNAS1**); hemopoietic cell kinase (**HCK**); prion protein (**PRNP**); interleukin 2 receptor α (**IL2RA**); vimentin (**VIM**); superoxide dismutase-1, soluble like (**SOD1L**); thrombomodulin (**THBD**); and phosphatidylinositol-specific phospholipase C (**PLC-II**). The majority of these comparative loci have also been mapped to human chromosomes 10 or 20 (HSA10 or HSA20), which is consistent with ZOO-FISH analyses showing BTA13 as composed of a medial segment homologous to a portion of HSA10 flanked by segments homologous to portions of HSA20 (Solinas-Toldo *et al.* 1995, Hayes 1995, Chowdhary *et al.* 1996).

Subsequent to the WG-RH analysis, six type-I (indicated above in bold type) and two microsatellite markers were cytogenetically mapped by chromosomal FISH of bovine bacterial artificial chromosome (BAC) clones (Schlöpfer *et al.* 1998). At the time of this initial FISH analysis, the bovine BAC DNA library of Cai *et al.* (1995) was estimated to have 75% genome coverage; thus several genes comprising the RH map were not identified within the library. The library has since been expanded, consisting of 60 000 clones and an estimated 93% probability of containing a unique DNA sequence. The goal of this study was to exploit the expanded coverage of the bovine BAC library and extend cytogenetic alignment of the bovine chromosome 13 (BTA13) comparative genome map (Schlöpfer *et al.* 1997) by targeting additional type-I loci to BTA13. This was accomplished by screening the bovine BAC library for BTA13 genes previously absent from the library. The identified BAC clones were assigned to a chromosome band location by single-color chromosomal FISH, and then integrated into the previous cytogenetic map by two-color FISH analysis.

Materials and methods

The same primers and PCR conditions for *IL2RA*, *VIM*, *THBD*, *PLC-II* and *CSNK2A1* were used in screening

the bovine BAC library (Cai *et al.* 1995) as when constructing the BTA13 radiation hybrid framework map (Schlöpfer *et al.* 1997). Primers were also designed from the human DNA sequence for the HSA20 marker, *TOP1* (GenBank accession #M60705) using the MacVector Version 4.1 Sequence Analysis Software. The *TOP1* forward primer lies on human exon 19 from base 168 to 191 (CGGAGAGACCTGAAAAGTGCTAAG), and the reverse primer on exon 20 from base 483 to 506 (GCTTGAACCTCCAGCTTCATCAAC). The annealing temperature used with the *TOP1* primer pair was 58°C; otherwise, the PCR conditions were as for the other genes. Isolated BAC clones were grown overnight in 50 ml LB medium with 12.5 mg/ml chloramphenicol at 37°C with shaking. DNA was extracted and purified using a commercially available kit (Qiagen) modified for large insert clones.

Single- and dual-color FISH to mitotic or meiotic chromosomes followed standard protocols (Pinkel *et al.* 1986) as previously described (Gallagher *et al.* 1998). Following fluorescence detection of the probes, FISH preparations were mounted in antifade solution containing the chromosome counterstain Hoechst 33258 to induce a Q-band pattern. Photographs of probe signal relative to counterstain were taken on color print film using an epifluorescence microscope equipped for simultaneous visualization of probe and counterstain fluorescence. Q-banding alone was visualized using a filter set appropriate for optimal excitation of Hoechst 33258. Bovine chromosomes were identified according to the domestic cattle standard nomenclature (Reading Conference 1980, ISCNDA 1990) as modified by Popescu *et al.* (1996). BAC clones were first assigned to a bovine chromosome band location by single-color FISH analysis. Sublocalization of each clone to a chromosome band location was accomplished by shifting between simultaneous excitation of Cy3 and Hoechst versus excitation of Hoechst alone. This analysis was conducted both at the microscope and from color prints. Dual-color FISH to meiotic chromosomes was then used as necessary to integrate the newly assigned genes with loci (i.e. *PRNP*, *HCK*, *CSSM30*) that had been previously sublocalized to BTA13 (Schlöpfer *et al.* 1998).

Results and discussion

Four of the six genes (*IL2RA*, *VIM*, *THBD*, and *TOP1*) were found represented in the bovine BAC

library by one or more clones. As predicted by somatic cell and radiation hybrid mapping of BTA13 (Schlöpfer *et al.* 1997), and/or human on bovine ZOO-FISH analyses, each clone was shown to hybridize exclusively to BTA13 under hybridization suppression conditions. Single-color FISH analysis resulted in our assigning the BAC clones to the following BTA13 band locations: BAC487R1C10 and BAC518R5C3, *IL2RA*, band 14 (Figure 1a);

BAC515R1C9, *VIM*, the boundary of bands 16–17 (Figure 1b); BAC10R7C9, *THBD*, band 17 (Figure 1c); BAC372R1C2 and BAC466R4C1, *TOPI*, the boundary of bands 22–23 (Figure 1d). Our mapping HSA10 marker *IL2RA* to BTA13 band 14 is consistent with the previous assignment of this gene to BTA13 bands 13–14 by Johnson *et al.* (1995). HSA10 marker *VIM* maps telomeric to *IL2RA* and centromeric to HSA20 marker *THBD*. The placement

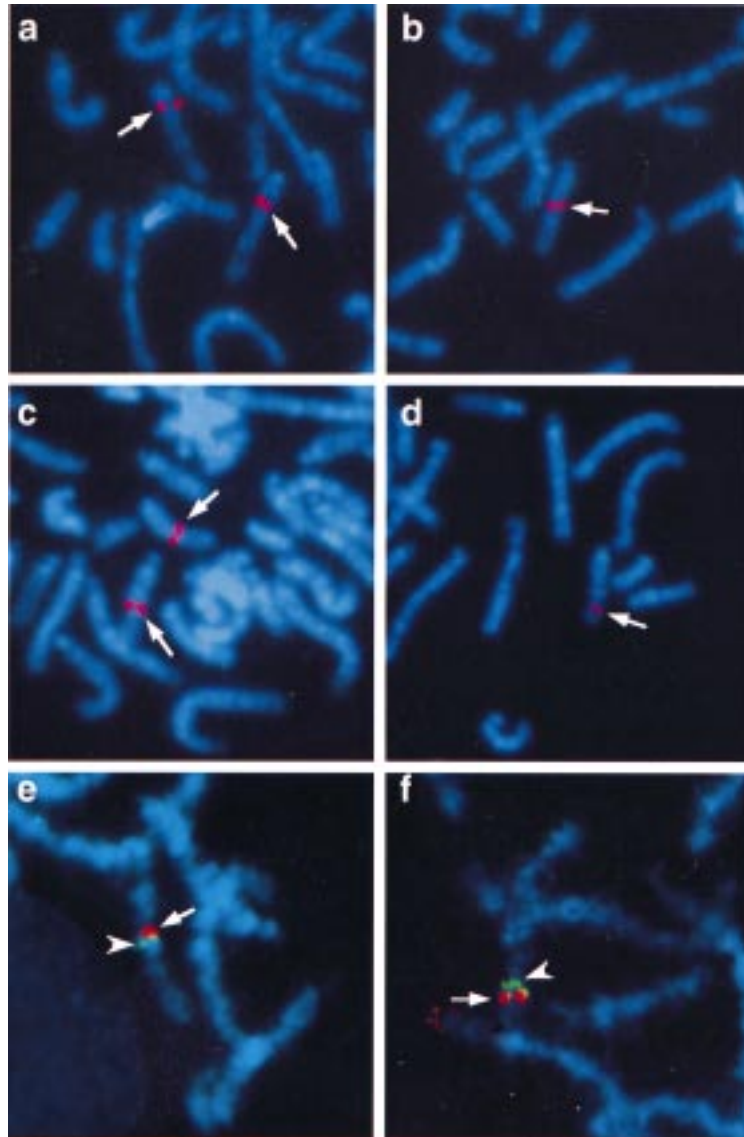


Figure 1. Chromosomal FISH results for BTA13 BACs; (a) *IL2RA*; (b) *VIM*; (c) *THBD*; (d) *TOPI*; (e) *THBD* (red signal) and *PRNP* (green signal); (f) *CSSM30* (green signal) and *TOPI* (red signal). Labeled chromosomes are positioned with centromeres up. Red (arrows) and green (arrowheads) probe signals result from streptavidin-Cy3 and antidigoxigenin-FITC fluorescence detection of biotin- and digoxigenin-labeled probes, respectively.

of *THBD* at BTA13 band 17 is proximate to HSA20 marker *PRNP*, which we previously FISH mapped to BTA13 at the boundary of bands 17–21 (Schläpfer *et al.* 1998). Finally, HSA20 marker *TOP1* appears to be the most telomeric among comparative loci that have been cytogenetically assigned to BTA13, although its cytogenetic placement at the boundary of bands 22–23 overlaps with our previous assignments of *HCK* and microsatellite CSSM30 at band 22 (Schläpfer *et al.* 1998).

Because single-color FISH analysis did not always provide clear chromosome band separation among loci mapped to BTA13, we were able to confirm locus order by dual-color FISH of select BAC clones to pachytene chromosomes. This analysis clearly showed that *THBD* maps centromeric to *PRNP* (Figure 1e), and *TOP1* is telomeric to *HCK* (data not shown) and microsatellite CSSM30 (Figure 1f). These new FISH assignments integrated with those previously made (Schläpfer *et al.* 1998) result in a cytogenetic map order of BTA13 loci as centromere-*IL2RA-VIM-THBD-PRNP*-(*SOD1L/AVP/OXT*)-(BL42/*GNAS1*)-*HCK-CSSM30-TOP1* (loci in parenthesis could not be ordered by chromosomal FISH; microsatellite markers are underlined). This locus order is consistent with the RH framework map and most probable comprehensive map, and provides cytogenetic anchorage of the BTA13 genome map from bands 14–23.

TOP1 had not been previously physically mapped in bovine by either somatic cell or RH analysis because the bovine segregation profile for *TOP1* could not be established relative to the rodent genetic background. *TOP1* was previously mapped to HSA 20q12-13.2 (Kunze *et al.* 1989). Our assignment of *TOP1* to BTA13 at the boundary of bands 22–23 is consistent with the human/bovine comparative map and joins markers *HCK* and *PLCII* in defining a conserved syntenic block between bovine and human (see Schläpfer *et al.* 1997 for an examination of conservation of gene order involving HSA10/20 and BTA13 markers). As previously mentioned by Schläpfer *et al.* (1997), no HSA20 genes have been assigned to the pericentromeric region of BTA13 despite three ZOO-FISH studies that show this region is homologous to a portion of HSA20. Our chromosomal FISH results show that comparative markers assigned to BTA13 do not span the entire chromosome so the current state of the HSA10 and 20/BTA13 comparative map does not refute the conjecture that BTA13 consists of two conserved HSA20

syntenic blocks separated by a HSA10 block. The assignment of additional HSA20 genes to BTA13 will likely extend the comparative map of this bovine chromosome to its centromeric and telomeric ends.

Acknowledgements

J.S. was supported by Swiss National Foundation grant 81BE-043886 and by the Ciba-Geigy-Jubiläums-Stiftung. J.E.W. was supported by USDA NRICGP grant 95-37205-2190 and by Texas Agricultural Experiment Station project 6718. J.F.T. and S.K.D. were partially supported by a grant from the Cattleman's Beef Association and by USDA NRICGP grants number 94-37205-1224 and number 95-37205-2273. We thank Sue Ann Berend for her technical help in collecting the meiotic material.

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