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Fluorescent *in situ* hybridization mapping of three fecundity genes on cattle, river buffalo, sheep and goat

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One of the goals of molecular cytogenetics applied to livestock is the extension of their genetic physical maps, especially of loci containing genes related to productions. In this study, a comparative fluorescence *in situ* (FISH) mapping of three genes related to fecundity of cattle, river buffalo, sheep and goat is reported using bovine BAC-clones taking in account the data available on the BovMap database and considering their physical position and the data obtained from banding experiments. The following three gene sequences were mapped: tumor necrosis factor-α (*TNF*), correlated to male fertility; signal transducer and activator of transcription 5A (*STAT5A*), important for its influence on milk production and reproduction activity; melatonin receptor 1A (*MTNR1A*) important for reproductive seasonality. BAC probes containing these gene sequences were assigned by FISH, for the first time, on RB-banded chromosomes of these four important bovids. *TNF* was assigned to BTA/CHI23q21-22, OAR20q21-22 and BBU 2p21-22; *STAT5A* was assigned to BTA/CHI19q17-21, OAR11q17-21 and BBU3p15-21; *MTNR1A* was assigned to BTA/CHI27q14-15, OAR26q14-15 and BBU1p21-22. The three loci were located in homoeologous chromosomes and chromosome bands, underling the high degree of chromosome homologies among Bovids and extending the cytogenetic maps of this economically important species.

Keywords: FISH-mapping, fecundity genes, cattle, sheep, goat, river buffalo

Introduction

Cytogenetic mapping is a method used to construct physical maps which are useful tools for various applications, especially in animal cytogenetics. Indeed, it allows: (a) a precise physical position on single chromosome bands of both type I and type II loci, especially in bovids using FISH mapping on R-banded chromosome preparations (Di Meo et al. 2007); (b) to confirm chromosomes and chromosome regions involved in chromosome abnormalities by using specific molecular markers (Di Meo et al. 2000; Perucatti et al. 2011; Iannuzzi et al. 2013); (c) to study chromosome aneuploidies in sperms and oocytes (Pauciullo et al. 2011, 2012; Hornak et al. 2011); (d) to precisely anchor linkage and RH maps (Stafuzza et al. 2013), as well as genome sequence contigs to specific chromosome regions (Goldammer et al. 2009).

Cattle (*Bos taurus,*2n = 60, BTA), river buffalo (*Bubalus bubalis*, 2n = 50, BBU), sheep (*Ovis aries*, 2n = 54, OAR) and goats (*Capra hircus,* 2n = 60, CHI) are very related species from the evolutionary point of view and, also, the four major domestic bovid species of great economic importance. Although the location of a lot of genes in these species was identified by linkage and RH mapping, a small percentage of those loci were physically assigned to the corresponding bands of specific chromosomal location (Iannuzzi et al. 2003a). So far, several studies on the physical gene mapping using FISH methodology were reported for cattle, river buffalo, sheep, goat and other farm animals (Iannuzzi et al. 2003a, 2003b; Di Meo et al. 2007; Schibler et al. 2009).

In the present study, three important fecundity genes (*TNF, STAT5A* and *MTNR1A)* were comparatively FISH-mapped on cattle, sheep, goat and river buffalo R-banded chromosomes for first time extending the cytogenetic maps of these species. Tumor necrosis factor-α (TNF) is correlated to male fertility (Eggert-Kruse et al. 2007; Kocak et al. 2002); signal transducer and activator of transcription 5A (STAT5A) is important for its influence on milk production and reproduction activity (Yang et al. 2000; Homer et al. 2013); melatonin receptor 1A (*MTNR1A*) is important for reproductive seasonality (Chu et al. 2007; Luridiana et al. 2012).

Materials and methods

Peripheral blood samples from cattle (Agerolese breed), sheep (Laticauda breed), goat (Cilentana breed) and river buffalo were cultured and treated for late BrdU and Hoechst 33258 incorporation according to Iannuzzi and Di Berardino (2008). The bovine BAC clones overlapping studied genes (Table 1) were screened by database searching and ordered from INRA bovine BAC library

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Table 1. BAC-probes, identified DNA sequences of FISH-mapped genes in cattle (BTA), river buffalo (BBU), sheep (OAR), goat (CHI) chromosomes (ISCNDB2000 2001and CSKBB 1994), comparison with human (HSA) chromosomes (HGNC).

BAC FISH Probe	Identified DNA sequence within BAC and locus symbol (HGNC)	Gene name	Cytogenetic localization on RBPI-bands				
			BTA	BBU	OAR	CHI	HSA
BtINRA-81C03	*TNF*	*tumor necrosis factor-α*	23q21-22	2p21-22	20q21-22	23q21-22	6p21.3
BtINRA-243A01	*STAT5A*	*signal transducer and activator of transcription 5A*	19q17-21	3p15-21	11q17-21	19q17-21	17q11.2
BtINRA-448A07	*MTNR1A*	*melatonin receptor 1A*	27q14-15	1p21-22	26q14-15	27q14-15	4q35

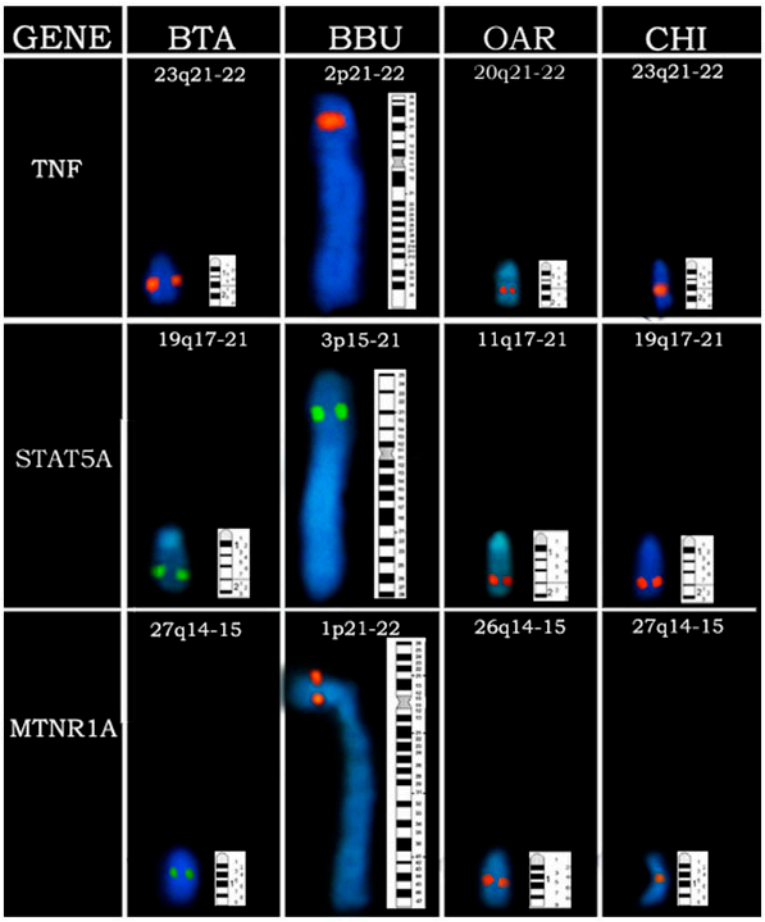

Figure 1. (Color online) Representative FISH results on cattle (BTA), river buffalo (BBU), sheep (OAR) and goat (CHI) chromosomes, using bovine BAC clones containing genes related to fecundity (*TNF, STAT5A, MTNR1A*). FITC and TRIC signals were superimposed on R-banding chromosomes counterstained with DAPI. For each chromosome, the corresponding standard ideogram (ISCNDB2000 2001; CSKBB 1994) it is also reported.

(*CRB- Biological Resources Centre dedicated to livestock genomics –INRA, Jouy-en Josas, France*) (http://locus.jouy.inra.fr/cgibin/bovmap/intro2.pl). Extraction of DNA was done using CHORI (Children's Hospital Oakland Research Institute) recommended protocol. DNA was labeled with biotin and digoxigenin using nick-translation kit (*Roche applied science Inc.*). Slides were then treated for FISH with BAC clones overnight in presence of bovine COT-l DNA and sonicated salmon sperm allocated in a moist chamber. After detection steps with FITC-avidin and anti-digoxigenin antibodies, Chromosomes were counterstained with Vectashield DAPI H1500 in Vectashield H 1000 (Vector Lab) antifade solution. Both RB-banding (R-banding by late incorporation of BrdU) metaphases and fluorescence FITC and TRIC signals were separately captured by a CCD-camera (Photometrics, cool SNAP, Nikon) and processed by superimposing FITC and TRIC signals on RB-banding

preparations. Chromosome identification and banding followed the standard karyotypes for cattle, sheep and goat (ISCNDB2000 2001) and river buffalo (CSKBB 1994).

Results and discussion

Three major fecundity genes (*TNF, STAT5A* and *MTNR1A*), were comparatively physically FISH-mapped on cattle, sheep goat and river buffalo R-banded metaphase chromosomes (Figure 1). Loci FISH-mapped with locus name, symbol, clone identification and chromosome localization are reported in Table 1**.** *TNF* maps on BTA/CHI23q21-22, OAR20q21-22 and BBU 2p21-22; *STAT5A* maps on BTA/CHI19q17-21, OAR11q17-21 and BBU3p15-21; *MTNR1A* maps to BTA/CHI27q14-15, OAR11q17-21 and BBU1p21-22. The three loci were located in homoeologous chromosomes and chromosome bands of the four species extending the cytogenetic maps in these three species chromosomes. FISH-mapping of *STAT5A* agrees with previous localizations performed in BTA/CHI19 and OAR11 by sequential GTG-banding and FISH (Goldammer et al. 1997), while *MTNR1A*, earlier assigned to BBU1 by RH-mapping (Miziara et al. 2007) was now assigned to specific chromosome arms and bands (1p21-22).

During the last fifteen years, FISH techniques have been used in domestic animals research mainly to identify chromosomal rearrangements, gene mapping, comparative mapping, and evolutionary chromosome studies. The localization of *TNF, STAT5A* and *MTNR1A* on homologous chromosomes and chromosome bands in cattle, sheep, goat and river buffalo (Figure 1; Table 1) confirmed the high conservation of autosomal chromosomes among the bovid species and extended the cytogenetic maps of the four economically important domestic species. However, some discrepancies may exist between the localization of loci reported in a reference genome and the localization obtained by FISH physical mapping, as supported by different papers (De Lorenzi et al. 2010; Partipilo et al. 2011; De Lorenzi et al. 2013). These results clearly indicate the idea that physical localization of genomic elements by FISH can further improve the excellent results obtained by genome sequence projects.

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