



Comparative fluorescence *in situ* hybridisation (FISH) mapping of class I and II MHC genes on *Bos indicus* and *Bubalus bubalis* chromosomes

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Received: 4 November 2012; Accepted: 10 March 2015

Key words: Cattle, Comparative FISH, MHC Class I & II genes, River buffalo

Study on comparative gene mapping is one of the alternative approaches to easily locate the genes among the related species using the DNA sequences or probes derived from well-explored species. This would also enable to understand better the conservation of divergence of known sequences and thereby help determine the evolutionary lineage between them. Among the species in family Bovidae, lots of molecular and cytogenetic investigations have been carried out in cattle, unlike in other related species such as buffalo. MHC class I sequences had already been regionally assigned using isotopic *in situ* hybridization in cattle (Fries *et al.* 1986).

The cross-species hybridization of probes were demonstrated by many scientist in livestock species. Echard *et al.* (1986) assigned the major histo-compatibility complex to p1.4–q1.2 region of chromosome 7 in the pig by *in situ* hybridization using a human DNA probe for HLA-B7. Similarly, equine major histo-compatibility complex was assigned to chromosome 20 by using human class I cDNA probe (Makinen *et al.* 1989). Therefore, in this study, cross-species hybridization of bovine MHC class I and class II probes were tried on Indian river buffalo chromosomes, besides zebu cattle, to map bovine major histo-compatibility complex having the relevance in immune response modulation. Further, attempts were also made to obtain strong signals using tyramide amplification step.

Probes: The probes used in the hybridization experiments were pBoLA-19 (the MHC class I probe) and DRA clone B24 (MHC class II probe) of cattle origin, obtained from Roslin Institute, United Kingdom. The class I probe comprised a 1 kb *SalI* – *BamHI* promoter fragment and a 7 kb *BamHI* class I gene fragment, cloned between the *SalI* and *BamHI* sites of pBR322 vector. The probe, DRA clone B24, was a 4.5 kb genomic PCR product of the complete DRA gene, cloned in the vector pCR3. These probes were labelled using biotin-14 dATP by nick translation as per manufacturer's recommendations.

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Chromosome preparations and hybridization: The investigation was carried out in 2 zebu cattle (Kangayam and Red Sindhi) and 2 river buffalo (Murrah and Surti) breeds of India. Blood samples (55) were collected and metaphase chromosome spreads were obtained from the pokeweed stimulated blood lymphocytes using the standard technique (Moorehead *et al.* 1960). For carrying out fluorescence *in situ* hybridization, metaphase chromosomes were denatured by treating the slides with 70% formamide, 2 × SSC (3 M sodium chloride and 0.3 M sodium citrate pH 7.0) at 70°C for 1 min followed by an ice-cold ethanol series (70%, 90% and absolute ethanol) for 2 min each step and then, air dried. Simultaneously, the probe mixture was also denatured at 94°C for 5 min and snap cooled. Approximately 200ng of the labeled probe DNA in a volume of 10 ml was placed over the slide, covered with the coverslip (22 mm²) and sealed with DPX mountant on all 4 sides. The slides were incubated at 37°C in a moist chamber for 16–18 h.

At the end of incubation period, the slides were washed twice in 2 × SSC at 37°C for 2 min, followed by two changes in each of 1 × SSC and 0.1×SSC solutions at 50°C for 2 min, for high stringency. The slides were then incubated with 100 µl of blocking reagent (supplied with the kit) in 1 × PBS (phosphate buffered saline- blocking; PBSB) for 30 min in the moist chamber. After removing the blocking buffer, 100 µl of streptavidin-HRP (1:50 dilution in PBSB) was applied over the slide and covered with cover slip and incubated at 37°C for 30 min. In this study, signal amplification was carried out with biotinyl tyramide and kit. The deposited biotins were detected using avidin-FITC (fluorescein isothiocyanate) and washed in 0.1% Tween 20 (poly oxyethylene sorbitan monolaurate) in 1 × PBS containing at 50°C with agitation.

Counterstaining of the chromosomes was carried out with propidium iodide (0.2 µg/ml) and 15 µl of anti fading reagent (DABCO 10 mg/ml). The fluorescent signals were screened under epifluorescent microscope fitted with mercury lamp HG 100 W. A minimum number of 20 spreads per slide bearing hybridization signals were screened and photographs were obtained.

The biotinylated class I and class II MHC probes

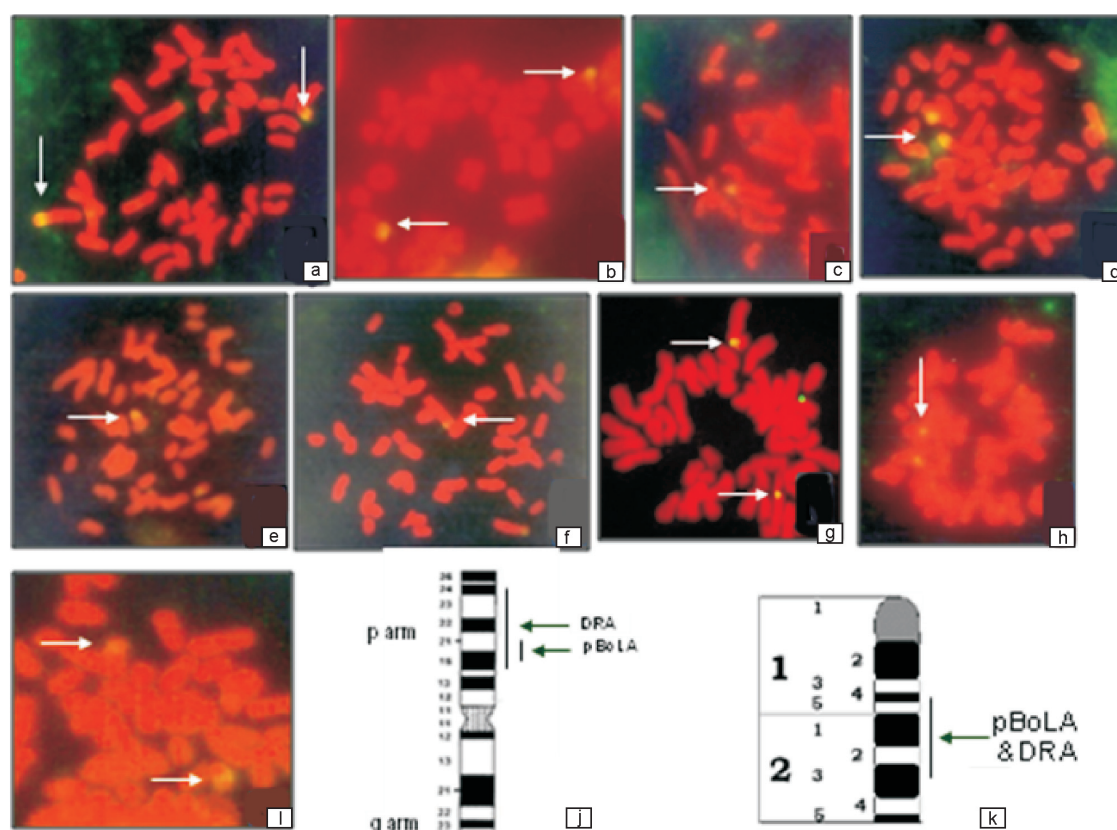


Fig. 1 (a-k). (a) pBoLA in Murrah buffalo; (b) pBoLA in a partial metaphase spread of buffalo; (c) single hybridization spot in Surti buffalo 2p; (d-e) pBoLA in cattle chromosomes; (f-g) DRA gene in buffaloes; (h-i) DRA in cattle; (j) Standard G-banded chromosome 2p of buffalo; (k) standard G-banded cattle chromosome 23 indicating the MHC class I and II genes.

hybridized to cattle and river buffalo chromosomes are represented in Fig. 1. Strong fluorescent signals were noticed in both chromatids of small acrocentric chromosome 23 of cattle and large submetacentric chromosome 2 of buffalo.

The class I MHC gene, pBoLA was assigned to cattle and buffalo chromosome bands 23q15–22 and 2p 22 respectively. While the class II MHC gene, DRA, was assigned in similar location to 23q15–21 and 2p 21–24 in cattle and buffaloes respectively. The localization of genes on to the metaphase chromosomes have been confirmed from the standard karyotypes reported earlier for cattle (ISCNDA 1990) and buffaloes (Iannuzzi 1994). In the study, the signals of hybridizations corresponding to pBoLA and DRA were very clear, large and more intense to be detected easily on the small metaphase chromosomes. This is due to the additional amplification of up to 1000-fold using tyramide molecule which uses streptavidin-HRP to catalyze the deposition of numerous biotin labelled tyramide on to the target surface of the chromosome. In general, the variation observed in hybridization efficiency between cattle and buffaloes for the two probes, is probably owing to limited accessibility of the target (hindrance), degree of sequence specificity, non-specific background signals, cross hybridization to the target molecule, non-specific repeats and stringency of washing. Hybridisation signals observed in all the slides supports the efficiency of the technique and

tyramide detection system

Cattle and buffalo chromosome arms showed the exact location of the pBoLA and DRA genes on the homologous bands in both the species (Fig. 1 - j and k). Our results indicated homologies between cattle chromosome 23 and river buffalo chromosome 2p. The hypothesis of chromosome and banding homology among bovine species were tested by many gene mapping studies carried out earlier. In a study by Chowdhary *et al.* (1991), chromosomal localization of glucose phosphate isomerase gene was confirmed on q22-proximal part of q24 segment of chromosomes 18, 14 and 18 in cattle, sheep and goat, respectively, which indicated high degree of homology in the DNA nucleotide sequence at these sites in the three species. Similarly, Hediger *et al.* (1991) defined extensively conserved chromosome structure in cattle and sheep through mapping of β -subunit of follicle stimulating hormone gene to cytogenetically homologous chromosome 15.

Solinas-Toldo *et al.* (1995) compared the mapping data and standard chromosome banding patterns and proposed a change in the ISCNDA nomenclature. This discrepancy was attributed to a difference in numbering of chromosomes between the Reading Conference (1980) and International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDA 1989). Comparative gene mapping using zinc finger proteins 164 (ZNF164) and 146 (ZNF146), alpha-

galactosyltransferase I (GGTA1), SRY-related HMG-box 2 (SOX2), prolactin receptor (PRLR) and elongation factor 2 (EEF2) on bovine and caprine chromosomes 17, 18, 11, 1, 20 and 7 and on ovine chromosomes 17, 14, 3, 1, 16 and 5 also confirmed the chromosomal homoeologies (Hayes 1996). Iannuzzi *et al.* (1996) discussed extensive chromosome band conservation among Bovidae and inconsistencies in domestic cattle chromosome nomenclatures in the light of β -defensin gene assignment among cattle, river buffalo and sheep chromosomes. Similarly, Prakash (1997) had compared FISH mapping of 4 bovine cosmids (cIOBT 705, cIOBT 962, cIOBT 264 and cIOBT 1447) containing microsatellites and ribosomal RNA gene cluster between cattle and buffalo karyotypes. He showed that river buffalo chromosomes 7 and 8 were homologous to cattle chromosomes 4 and 6 respectively. Based on the study, he also pin pointed interchange in the positions of buffalo chromosomes 7 and 8 in the standard karyotype. For each autosome of the river buffalo, the corresponding cattle chromosome(s) was identified (Iannuzzi 1994, Amaral *et al.* 2008). Because of the close relationship between chromosomes of cattle and buffaloes, a marker that had been located on a given cattle chromosome was expected to be present on the corresponding buffalo chromosome.

Thus, the homology observed in cattle and buffaloes with respect to bands corresponding to MHC genes indicated the genetic homology and evolutionary closeness of these species. This result would also contribute information to the comparative physical map of bovine species.

SUMMARY

The class I and class II major histocompatibility complex (MHC) genes were non-isotopically mapped to Indian zebu cattle breeds and river buffaloes. In cattle, the pBoLA (class I) and DRA (class II) genes were localized to chromosome 23q 15–22 and 23q 15–21 and in buffaloes 2p 22 and 2p 21–24 respectively. The comparative gene mapping revealed the similarity in size and homology in banding pattern between cattle chromosome 23 and p arm of buffalo chromosome 2 with respect to the major histocompatibility complex genes.

ACKNOWLEDGEMENT

The authors are thankful to Dr George Russell, Division of Molecular Biology, Roslin Institute, UK who provided the MHC probes for carrying out the work. The authors wish to thank Dr Rajiva Raman, Department of Zoology, Banarus Hindu University, Varanasi 221 005 for the help rendered in carrying out the hybridisation work successfully.

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