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# Short Note

# Chromosomal Homology between the Human and the Bovine *DMRT1* Genes*

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*Doublesex* and *mab-3* related transcription factor 1 (*DMRT1*) is considered to be the most conserved gene among loci involved in the molecular pathways of animal sexual development. In the majority of the extensively examined vertebrates, its function is limited to the upstream or downstream testis regulators acting during embryogenesis. Our present study demonstrated the structural homology between *DMRT1* orthologos in human and cattle. A BAC clone with a specific bovine sequence of the gene was used in the FISH mapping experiments. The physical localization of *DMRT1* in cattle (BTA 8q17) was determined and its homology to the human locus was shown (HSA 9p24.3). Furthermore, another BAC probe, containing the sequence of the human homologue (pBACe3.6), generated hybridisation signals on bovine metaphase chromosomes and indicated the physical location of the autosomal bovine *DMRT1* locus. Further investigations of the gene in domestic animals might provide more support for its conservative status and may help in understanding the molecular mechanisms involved in the occurrence of sexual abnormalities often diagnosed in livestock.

Key words: BAC clones, cattle, *DMRT1*, FISH mapping, ZOO-FISH.

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*Doublesex and mab-3 related transcription factor 1* (*DMRT1*) is so far the only exception among genes acting during vertebrate sexual development due to its surprising structural and functional conservation between phyla. First cloned in human (RAYMOND *et al.* 1998), *DMRT1* appears to contain the DM domain, a new zinc finger-like DNA-binding motif, previously identified in two invertebrate sex regulatory genes: *doublesex* in *Drosophila melanogaster* (ERDMAN & BURTIS 1993) and *mab-3* in *Caenorhabditis elegans* (RAYMOND *et al.* 1998). Human *DMRT1* maps to autosomal locus 9p24.3 (RAYMOND *et al.* 1998; FLEJTER *et al.* 1998), a critical deleted region, that, when hemizygous, manifests gonadal dysgenesis and XY feminisation (reviewed in OTTOLENGHI & MCELREAVEY 2000). Further investigations (mainly expression studies in embryonic gonads) undertaken in a wide range of vertebrate species, have determined the role of *DMRT1* in male gona-

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dal development which is limited either to the late sex determination or early testis differentiation period (reviewed in BRATUŚ & SŁOTA 2006). More support for the evolutionarily conserved status of the vertebrate *DMRT1* genes comes from both functional studies (BOYER *et al.* 2002) and comparative cytogenetic and genomic analyses (RAYMOND *et al.* 1998; NANDA *et al.* 1999; NANDA *et al.* 2000; BRUNNER *et al.* 2001; MATSUDA *et al.* 2005).

In the presented FISH mapping studies, we report the chromosomal homology between the human *DMRT1* and its cattle orthologue by using BAC probes containing the human and bovine sequence of the gene.

## Material and Methods

### BAC Probes preparation

The cattle genomic DNA BAC library (INRA) was screened to obtain the BAC clones harbouring the fragment of bovine sequence of *DMRT1* (Ensembl Gene ID: ENSBTAG00000024313/www.enembl.org). The following primer sequences were designed for PCR screening: F: 5'AGAGTCTGCGCTTCTCCTTG 3', R: 5' TCAGGTTGCACTTCTTGCAC 3'). Two positive BAC clones (INRA-1021B04 and INRA-1036E06) were found. DNA of both clones was prepared from 500 ml overnight cultures of the positive

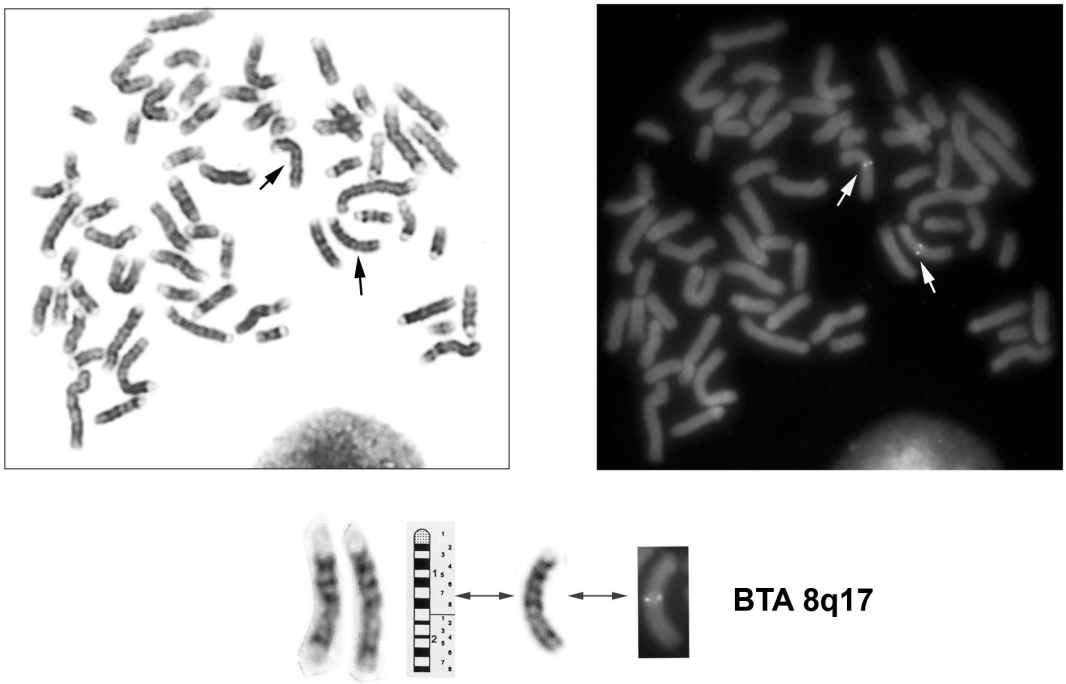


Fig. 1. Chromosomal assignment of cattle *DMRT1* to BTA 8q17. GTG-banded metaphase (left) prior to FISH (right) with BAC clones harboring bovine *DMRT1*. The arrowheads indicate the position of the hybridisation signals.

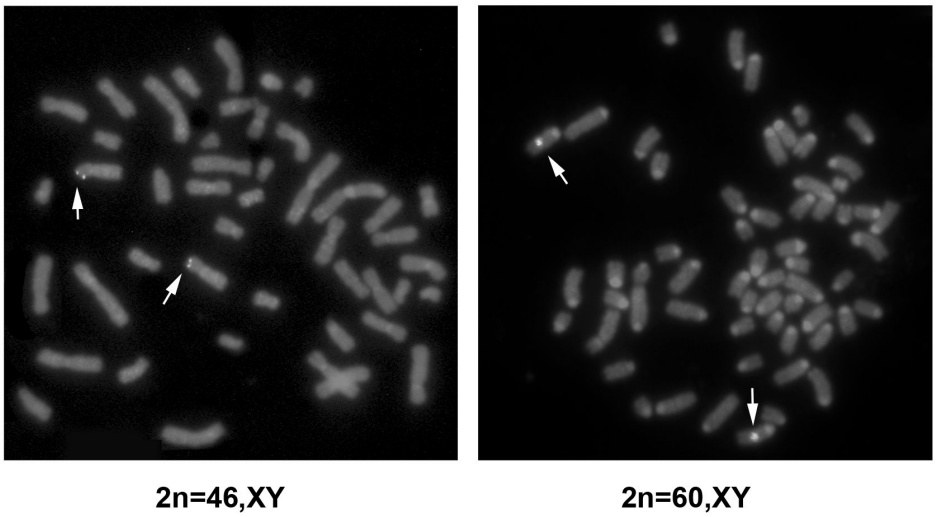


Fig. 2. Human (left) and cattle (right) male metaphase spreads after FISH with human RP11-143M115 clone. Arrows indicate the hybridisation region: HSA 9p23-24.3 (left) and BTA 8q17 (right).

BAC clone using the Qiagen Midi plasmid kit (Qiagen AG, Basel, Switzerland) according to the alkaline lysis protocol for BACs.

The currently available cattle BAC end sequences were subjected to BLAST analysis against the human genome. The SP6 and T7 end sequences of the bovine BAC clones revealed a significant match (BLAST: INRA-1021B04 -SP6- 99% identity over 799 bp; INRA-1021B04-T7-98% identity over 770 bp; INRA-1036E06-SP6- 99% over 560 bp and INRA-1036E06-SP6- 100% over 600 bp) on bovine chromosome 8. Additionally we sequenced both BAC clones with the primers used for BAC library screening. These sequences revealed a significant match (BLAST E-value 6e-66, 85% identity over 258 bp) on human chromosome 9 ending at 832226 bp in the area of the *DMRT1* gene (Accession No. NT_008413.17)**.** The other BAC probe containing the human genomic *DMRT1* sequence (clone RP11-143M115 of chromosome region 9p23-24.3, EMBL accession AL136365.9) was purchased in Peter de Joung's laboratory in BACPAC Resources Center at Children's Hospital, Oakland Research Institute (Oakland, USA) and was used in the ZOO-FISH experiment.

### Chromosome preparation

Mitotic metaphase chromosomes of karyotypically normal humans and cattle were obtained from Pokeweed-stimulated peripheral blood lymphocyte cultures according to standard protocols.

### FISH

A total of 100 ng of BAC DNA was labelled with Biotin- 16-dUTP (Roche) and used for FISH on previously photographed G banded metaphases obtained according to the method described by WANG and FEDOROFF (1972). Chromosomes were identified according to the GTG-banded chromosome nomenclature (DI BERARDINO *et al.* 2001). Hybridisation followed the protocol described by BUGNO (BUGNO & SŁOTA 2007) with some modification. The analysis of FITC fluorescence signals was carried out on propidium iodide-stained slides under a Zeiss Axiophot fluorescence microscope.

## Results

The fluorescence signals on the bovine metaphase spread (Fig. 1) obtained in the FISH experiment with the bovine BAC clone indicate the physical localization of the cattle *DMRT1* homologue: BTA 8q17. The locus of bovine *DMRT1* was additionally determined by FISH mapping with the BAC probe of the human orthologue (pBACe3.6) which was previously tested on human metaphase chromosomes (HSA 9p 23-24.3) (Fig. 2).

## Discussion

In contrast to other sex regulators in vertebrates, *DMRT1* exhibits strong conservation. Although the functional conversation of *DMRT1* in mammals has been demonstrated (BOYER *et al*. 2002), other studies have revealed some variation in the role of *DMRT1* not only in vertebrates (BRATUŚ & SŁOTA 2006), but also within mammalian species (PASK *et al.* 2003).

In order to gain a greater understanding of the conservative status of *DMRT1*, the present study was extended to another species, cattle, and the initial investigations were carried out on the cytogenetic level. We mapped the cattle orthologue by fluorescence *in situ* hybridisation (FISH) to the central region of bovine chromosome 8 (BTA 8q17) which displays homology to the human distal chromosome 9p, according to the comparative human-cattle chromosome painting map (CHOWDHARY *et al*. 1996) as well as the chromosome homology information provided by INRA(http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl). Bovine *DMRT1* appears to be an autosomal locus, the same as in karyotypes of most examined vertebrates including human (RAYMOND *et al.* 1998), mouse (RAYMOND *et al.* 1999), tammar wallaby (EL-MOGHARBEL *et al.* 2005), frog *Rana rugosa* (AOYAMA *et al.* 2003), turtle *Pelodiscus sinensis*, snake *Elaphe quadrivirgata* (MATSUDA *et al.* 2005) and medaka fish (BRUNNER *et al.* 2001), however, this is not the case in the Z-linked avian *DMRT1* homologues in chicken (NANDA *et al.* 2000) and emu (SHETTY *et al*. 2002) or X-linked *DMRT1* in mammalian species: platypus (EL-MOGHARBEL *et al.* 2006).

Furthermore, the ZOO-FISH experiment (the application of human *DMRT1* BAC clone to the bovine chromosomes) revealed hybridisation signals which additionally confirmed both the physical localization of cattle *DMRT1* and the conservation of the gene in mammals. Moreover, the human RP11-143M115 clone contains both *DMRT1* and *DMRT3* (two members of DM domain genes) which are very closely situated to the minimal distal region of human 9p (OTTOLENGHI *et al.* 2000). This may indicate that in the bovine genome these orthologs are in the same cluster as well.

The incorporation of livestock animals in studies concerning the putative autosomal male regulatory gene is important not only because of the conservative nature of the gene, but also due to the as of yet undiagnosed cases of sexual developmental

malformations recognised in domestic animals (VAIMAN & PAILHOUX 2000; VEITIA *et al*. 2001).

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