Analysis of Y Chromosome Rearrangements between

*B. Taurus* and *B. Indicus*

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Abstract

The significantly high similarity of karyotypes were shown between Bos taurus and *B. indicus* except for morphology of the Y-chromosome. The B. taurus-Y (BTA-Y) is submetacentric whereas the *B. indicus*-Y (BIN-Y) is acrocentric. Based on the hybridization of the chromosome painting technique, the difference between Y chromosomes in *B. taurus* and *B. indicus* was proposed to be originated from the pericentric inversion between these two species. However, hybridization of SRY to the sub-centromeric end of BIN-Y indicated a double pericentric inversion found between *B. taurus* and *B. indicus*. We suggested that BTA-Y and BIN-Y were originated from a common ancestor submetacentric Y chromosome with a short p-arm. The BTA-Y was formed by pericentric inversion of a major segment of q-arm and a centromere of the ancestor chromosome whereas the BIN-Y was generated from the ancestor Y chromosome by pericentric inversion of the complete p-arm together with the centromere. A proposed model of a common ancestor sub-metacentric Y of *B. taurus* and *B. indicus* had provided a new insight into the evolution of the Y chromosome in the family of Bovidae.

**Keywords**: *B. taurus, B. indicus, Y-chromosome, SRY, pericentric inversion*

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Introduction

The diploid chromosome numbers of the family Bovidae varied ranging from 30 to 60. The autosomal fundamental number was relatively constant ranging from 58 to 60 due to the events of fusion and fission occurred during the chromosome evolution while the intrachromosomal rearrangement appeared common in the sex chromosomes (Gallagher and Womack, 1992).

Karyotypes of Bos taurus and Bos indicus have a high similarity except for the morphology of the Y-chromosome. The morphology of BTA-Y is submetacentric while BIN-Y is acrocentric (Reading Conference, 1980). In mammals, the Y-chromosome is indicative for the male sexing and it has been demonstrated that SRY (sex determining region on Y) is responsible for the male development (Daneau, 1995). In Bos taurus, the SRY gene was mapped to show the location to be near the telomere of q-arm (Cui et al., 1995; Iannuzzi et al., 2001; Liu and Ponce de Lion, 2004).

Due to the small size of the Y chromosome, it is difficult to map the chromosome showing the distinct using the banding patterns. There were the event of chromosome rearrangement occurred between BTA-Y and BIN-Y. However, the event of pericentric inversion would have been the easiest and the most probable intrachromosomal rearrangement. To confirm the occurrence of pericentric inversion, the painting probes from BTA-Y p and q arms had hybridized BIN-Y, and the proximal part of BIN-Y on the BTA-Y. The paint from BTA-Yp12 had hybridized to a segment near the telomere of BIN-Y, and the proximal half of the BIN-Y was completely painted with BTA-Yq12.1-qter (Goldammer et al., 1997). Conversely, the paint from the proximal part of BIN-Y had hybridized to the segment BTA Yq12.1-qter. In addition, two Y specific repetitive sequences λES 6.0 and BC1.2 were mapped by the hybridization. The λES 6.0 mapped to BTA-Yp12-11 and subtelomeric region of BIN-Y. The BC1.2 had hybridized to BTA-Yp13-12 and close to the telomere of...
BIN-Y. These hybridization data have supported the theory of pericentric inversion occurred between the Y chromosome of *Bos taurus* and *Bos indicus*. To further support this theory we hybridized BAC clone containing SRY gene to the entire BIN-Y chromosome.

**Materials and Methods**

**Chromosome preparation**

Metaphase spreads were prepared from lymphocyte cultures of *B. taurus* (Brown Swiss cattle) and *B. indicus* (Thai native cattle) by standard cytogenetic techniques.

**DNA probe preparations**

Two kinds of the DNA probes, SRY BAC clone and λES 6.0 satellite probe, were used in this study. The SRY BAC probe was a bovine 3.5 kb genomic DNA fragment cloned in the Eco RI site of plasmid p422. The probe was amplified by bacteria cloning and PCR amplification using bovine SRY specific primers as 5'-GTC AAC TTT CAA GTT TGC CTT ATG G-3' (forward) and 5'-GTC CAT GGT GAA ACT GTA TGA-3' (reverse). The recombinant plasmid containing bovine SRY gene was digested with Sau 3AI for *in situ* hybridization. The λES 6.0 satellite probe was amplified using bovine genomic DNA and the following primers as 5'-GAA TTC GGT AGA GCC CGC ATC TCG GTG-3' (forward) and 5'-GAA TTC TTG AAG CAG CCA AGC CCC GCG-3' (reverse) (Schwerin et al., 1992). The SRY BAC clone and λES 6.0 satellite probes were labeled with biotin-16-dUTP (Boehringer Mannheim, GmbH) using Prime-it Fluor® Fluorescence Labelling Kit (Stratagene, La Jolla, CA) for detection.

**FISH and probe detection**

Hybridization of the DNA probes to bovine chromosomes was performed as described by Suwattana et al. (2000). In brief, RNA and proteins of the chromosome were eliminated using RNAase and pepsin in concentrated HCl prior to the denaturation of double-stranded DNA on the chromosome. Hybridization mixture containing SRY and/or λES 6.0 probes were applied to metaphase spreads and post hybridization washings, signal amplification and counter staining using 4, 6 Diaminido-2-Phenylindol (DAPI) were followed. The hybridization sites on metaphase bovine chromosomes were detected under a fluorescence microscope and photographed with a CCD camera (Quantix II, Tuscon, AZ).

**Results and Discussion**

The λES 6.0 satellite probe and SRY BAC clone were hybridized to *B. indicus* and *B. taurus* metaphases separately and together (Figure 1 and 2). At least 15 metaphases were analyzed for detection of the hybridization. No specific signals (2 dots) were observed on the chromosomes other than Y.

*Number of cells with specific signal, relative chromosome length*

Our results of the hybridization site of SRY on the subtelomeric region of Yq of the submetacentric BTA-Y is in agreement with those previously reported by Cui et al. (1995) and Iannuzzi et al. (2001). Similarly, the location of the λES 6.0 which was found on BTA-Yp in the present study confirms the result reported (Schwerin, 1992; Goldammer et al., 1997) (Figure 1). The λES 6.0 satellite probe hybridized to subtelomeric segment of BIN-Y (Figure 2), which agreed to the previous report (Goldammer et al., 1997). The result indicated that the chromosomal location contain λES 6.0 satellite did not involve in neither a pericentric inversion nor chromosome Y evolution. SRY hybridized close to the centromere of BIN-Y (Figure 2). However, in terms of the theory of pericentric inversion between submetacentric BTA-Y and acrocentric BIN-Y, signal of SRY hybridization on BIN-Y was expected to be approximately in the middle of the Y chromosome (Figure 3). Mapping of SRY to subcentromeric region of BIN-Y indicates more complicated sequence of rearrangements than single pericentric inversion. We are proposing a model of a
common ancestor sub-metacentric Y-chromosome with short p-arm where BTA-Y was generated by pericentric inversion of the centromere and approximately half-2/3 of q-arm. The BIN-Y locus originates from a pericentric inversion of a complete p-arm (Figure 4). This model agreed with the painting data (Goldammer et al., 1997). BTA-Yp12 paint which was hybridized to a segment near telomere of BIN-Y is not involved in inversion event and BTA-Yq12.1-pter paint mapped to the proximal half of the BIN-Y is too large to be detected in a small pericentric inversion of the ancestor chromosome p-arm. Hybridization of the paint from the BIN-Y proximal half to the segment BTA Yq12.1-pter is also in agreement with the proposed model (Figure 5). Mapping of SRY to sub-centromeric end of B. indicus Y and proposed a model of a common ancestor sub-metacentric Y of B. taurus.
Figure 5. Homologous regions on BTA-Y, BIN-Y and the ancestor Y with the paint-probes. BTA-Yq12-pter paints are shown as grey blocks. The BTA-Yp12 paints are shown in solid lines and BIN-Y proximal half paints in interrupted lines.

and *B. indicus* provides a new insight into evolution of Y chromosome in family Bovidae.

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**References**


