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Characterization of Bovidae sex-determining gene SRY

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Abstract – In mammals, testis determination is under the control of the sex-determining gene SRY. This Y-linked gene encodes a protein with a DNA binding domain similar to those found in high-mobility-group proteins. Here we report the cloning and sequences of the SRY genes of yak and Chinese native cattle. Our data show that SRY genes in Bovidae are less divergent, especially in the coding and 3′ regions.

sex determination / SRY / evolution / Bovidae

1. INTRODUCTION

In mammals, the Y chromosome-linked SRY gene is responsible for male sex determination [17]. SRY encodes a protein with a central HMG-box present in a wide variety of proteins that bind and bend DNA, suggesting that SRY functions as a transcription factor. Mutation in the SRY gene can result in male-to-female sex reversal [3,6]. The observation that the Sry transgenic XX mouse develops a male phenotype strongly suggests that SRY is the dominant gene in sex determination [9]. However, the role of SRY as a transcription factor in sex determination in mammals remains elusive and other genes including SOX9, DMRT1, WNT1, AMH, SF1, DAX1, GATA4, LIM1, Fra1 and aromatase also seem to be involved in the sex-determining pathway [8,13, 16,18]. The evolutionary analysis of the SRY coding region among primates

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and rodents suggests that this gene is rapidly evolving [19, 20]. In contrast, results from wallaby and domestic ruminants appear to indicate that sequence evolution of the SRY gene is less rapid [12, 14]. It remains a challenge how the amazingly complex sex determination pathways evolve in various animal systems, especially in the Bovidae family. Here, we report the identification of SRY gene sequences of another two Bovidae species, yak and Chinese native cattle, and the high degree of sequence conservation of the SRY genes in the Bovidae family.

2. MATERIALS AND METHODS

2.1. Preparation of DNA samples

Yak (Bos grunniens) and Chinese native bovine (Bos taurus) were collected from the Himalayan Mountains in Qinghai, China and Wuhan, China, respectively. Genomic DNAs were isolated from blood samples of both male and female individuals of the two species using standard molecular biology protocols.

2.2. Cloning and sequencing analysis

PCR primers were designed based on the SRY sequence of Japanese bovine (Bos taurus) [7]. The sequences of primer sets used in this study are listed in Table I. Primer sets 1, 2, 3 and 6 were used to amplify the SRY gene of Chinese native cattle, whereas primer sets 1, 2, 4, and 5 to amplify the yak SRY gene, which covers the full length of the SRY gene. PCR was carried out as follows: genomic DNA was denatured for 5 min at 94 °C, then 30 s at 94 °C, 40 s at 48 °C and 1 min at 72 °C for 35 cycles. PCR products were subcloned into pUC18 and sequenced with an ABI automatic sequencer. The SRY sequences from other species have been reported previously under the accession numbers of Z30265 and AF026566, and reference [15] for sheep, Z30646 for goats, Z30321 for Bison, and AB039748 for Japanese cattle. All sequences obtained were aligned using the Clustalw program. Alignment was optimized manually with aid of DNAsis software. Amino acid identity calculations were as previously described [21].

3. RESULTS AND DISCUSSION

3.1. The 5’ and 3’ regions of the Bovidae SRY genes

Three portions (the 5’ region, the coding sequence and the 3’ region) of the Bovidae SRY genes are aligned separately for comparison. Figure 1 presents a comparison of the 5’ and the 3’ regions of SRY genes from Chinese native cattle,
### Table I. Primer sets used in this study.

| Primer set No. | Primer sequences | Region amplified (nucleotide number) \((a)\) | Amplification |
|---------------|------------------|---------------------------------------------|---------------|
| 1             | 5’ CAACTTTCAAGTTTGCCCTTATGG 3’ | 6–634 | + \((b)\) + |
|               | 5’ ACAGCCCAATCCTGTTATATA 3’     |       |               |
| 2             | 5’ GTATCTGAGATTTGCCTGT 3’ | 427–1091 | + + |
|               | 5’ CACCTGTCGTTCAATACTC 3’     |       |               |
| 3             | 5’ GCTGACTGCCAGGACGTA 3’ | 885–1713 | + – |
|               | 5’ TTGTACAGGGAAAGTCC 3’     |       |               |
| 4             | 5’ TTCATAACCCTAGACATCTG 3’ | 673–1511 | – + |
|               | 5’ CATGCTCGGATGGTCGATA 3’     |       |               |
| 5             | 5’ ACAGTCCAGCTGTGGTAC 3’ | 1095–1713 | – + |
|               | 5’ TTGTAGGAAAGTCC 3’     |       |               |
| 6             | 5’ GCATGTAGAGACATTGCA 3’ | 1507–2793 | + + |
|               | 5’ TACAGTTTCCACCATGGAC 3’     |       |               |

\((a)\) Based on the nucleotide number of Japanese bovine SRY;
\((b)\) The primer set were used (+) or not used (−) to amplify in this species.
Species abbreviations are: Chinese native cattle (CNB), Yak.

Yak, Japanese cattle and sheep. In the 5’ region, the alignment shows a high level of sequence conservation among these species except for four variable regions due to insertion/deletion (motif A-D), (Fig. 1a). Sequences in the 3’ region are more conserved with the exception of two insertion/deletion of four bases (Fig. 1b). These insertion/deletion variations are observed between the Bovinae and Caprinae subfamilies, but not within members of the Bovinae subfamily. Thus, the sequence identity appears higher within-subfamily than between-subfamilies. In the 5’ region of the SRY gene, sequences that are potentially important for transcriptional regulation of the gene, such as CAAT-box, TATA-box, SRY-binding site and Sp1-binding site, are conserved, even though the listed sequences are from four species that belong to different subfamilies. These data are consistent with the theory that SRY genes are functionally conserved for sex determination and regulation within the Bovidae family.

The regions containing insertion/deletions in the SRY genes from related species suggest that these regions may not be important for the regulation of SRY expression. Interestingly, however, two of these insertion/deletion regions (motif A and D) corresponded to two motifs (E1 and E4) of four conserved regions from C. elegans (Fig. 1a). BLAST search revealed another
**Figure 1.** Comparisons of the 5′ region (a) and the 3′ region (b) of the SRY sequences from Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE). Dots indicate identical nucleotides. The insertion/deletion motifs are shaded. The regions boxed are shared motifs between Bovidae and *C. elegans*, and motif E1-E9 are from the cosmids of *C. elegans*: C16E9, T01B11, R10E8, T04C12, M01F1, F53A2, C07E3/F20D12, F52F10 and C03G5 respectively.
five conserved motifs (E5-E9) in the 3′ region of the SRY that are also conserved between Bovidae and C. elegans (Fig. 1b). Appearance of these conserved motifs flanking the SRY coding region implies that SRY might originate from an ancient gene. Evidences to support this hypothesis include: (1) the Y chromosome-linked SRY gene appears to originate from an ancient X-linked SRY-box family member, SOX3 gene [5,10]. SOX genes have been found in both vertebrates and invertebrates. (2) Sex chromosomes are thought to have appeared about 240 to 320 Mya (million years ago), shortly after divergence of the mammalian and avian lineages [10]. (3) Our recent finding suggests that the X chromosome occurred much earlier at about 440 Mya when fish appeared (Yi et al., unpublished data). Finally (4) another sex-determining gene DMRT1 is conserved among humans, chickens, nematodes and flies [16], suggesting that at least some aspects of sexual regulation have a common evolutionary origin.

3.2. The coding region of the Bovidae SRY genes

The alignment of the SRY coding regions from yak, Chinese native cattle and other species in Bovidae shows much higher amino-acid identities along the entire coding region (Fig. 2). The sequence conservation is not only confined to the HMG box as observed in rats and mice [19], but also evident in the C-terminal non-box portion of the SRY gene in Bovidae, although the N-terminal region among the ruminants investigated was reduced by 11 amino acids in Bovinae. We calculated amino acid (aa) identities of the SRY gene from different Bovidae species. The Bovidae family shares 84.7% aa identity, and much higher identities (> 95%) were observed within different subfamilies. In the genus Bos, the aa identity reaches to over 99%. Based on the aa identities of the aligned coding regions, an evolutionary tree was constructed, which is consistent with the classic taxonomic relationship (Fig. 3).

Less divergence of SRY in Bovidae suggests a close relationship between the members of this family. Fertile hybrids were obtained: between Bos × Bison, goat × sheep, and yak × cattle [2,4]. Parents of these fertile hybrids were from members of different genera. Based on sequence comparison, we found that the divergence of the SRY gene is related to reproductive isolation in Bovidae. Fertile hybrids could be obtained if the aa identity in the coding region of the SRY gene is more than 95%. No hybrid between the subfamilies with aa identity of 84.7% was described. If hybrids are obtained between two genus members, male hybrids are always sterile, which supports that the Y chromosome may be mainly, at least partly, responsible for the sterility. Our finding is further supported by the rate of base substitution in the flanking region of the SRY genes in the Bovidae family (Tab. II): Fertile hybrids are possible when the transition/transversion rate in the 5′ or 3′ regions of SRY is less than 1%. A hybrid between cattle × sheep or goats has not
Figure 2. Alignment of predicted SRY protein sequences from Chinese native cattle, yak, Japanese cattle, bison, sheep and goats. The HMG box is shaded. Dots indicate identical amino acids and dashes denote gaps. Species abbreviations are: Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE).

Figure 3. Evolutionary tree based on the amino acid identities among Bovidae SRY proteins. The numbers are percentage amino-acid identities.

been obtained, where the transition/transversion rate in the 5' or 3' regions between them were around 4%. These observations suggest that the extent of sequence divergence in the SRY gene correlates with reproductive isolation in Bovidae, although the unbalanced chromosome pairing in hybrids and recessive
Molecular cloning of *Bovidae SRY*

Table II. Substitutions per 100 bases for the 5′ and 3′ regions.

|                    | 5′ region |            | 3′ region |            |
|--------------------|-----------|-----------|-----------|-----------|
|                    | Transition| Transversion| Transition| Transversion|
| Species (CNB-JPB)  | 0.2       | 0         | 0.9       | 0.4       |
| Genus (CNB-Yak)    | 0.8       | 0.6       | 0.8       | 0.5       |
| Family (CNB-SHE)   | 4.4       | 4.1       | 3.9       | 3.8       |

Species abbreviations are: Chinese native cattle (CNB), Yak, Japanese cattle (JPB) and sheep (SHE).

X chromosome epistatic interactions with autosomes appears to be a main factor for the isolation.

In natural populations, some head males often have the priority to mate with a majority of females, especially in large animals including cattle and sheep. Since a few males contribute to the next generation, the effective population size for males will be smaller than that of females [11]. Thus unequal effective population size between the sexes would reduce the variation of the Y chromosome, therefore making the genes on the chromosome, such as *SRY*, less divergent. Another reason for the observed lower divergence of *SRY* may be that rapid cladogenesis in the *Bovidae* family offer little time to accumulate mutation [1]. During a series of cladogeneses, however, some key features of ancient genes must be preserved, which may explain the high degree of sequence conservation in the SRY gene in the *Bovidae* family.

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