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Comparison of the human with the sheep genomes by use of human chromosome-specific painting probes

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Abstract. Human chromosome specific painting probes were hybridized on sheep (*Ovis aries*, 2n = 54) chromosomes by FISH. The painting results on sequentially stained RBA-banded preparations demonstrated high degree of conserved regions between human and sheep genomes. A total of 48 human chromosome segments were detected in sheep chromosomes. Comparisons with sheep gene mapping data available and previous Zoo-FISH data obtained in sheep, cattle, and river buffalo were performed.

Introduction

The sheep (*Ovis aries*, 2n = 54) is one of the most important bovid species in economic terms. Standard karyotypes at the 250 (Reading Conference 1976), 300 (Long 1988), and 450 (ISCNDA89 1990) band level are available, although discrepancies in the numbering of some chromosomes were noticed (Ansari et al. 1993, 1994; Iannuzzi and Di Meo 1995; Iannuzzi et al. 1998), and G- and R-banding chromosome comparisons between sheep and cattle were performed (Hayes et al. 1991; Ansari et al. 1993; Kaftanovskaya and Serov 1994; Iannuzzi and Di Meo 1995). More recently, the Texas nomenclature (1996) assigned molecular markers (and corresponding human chromosomes) to each sheep and cattle chromosome on the basis of available gene mapping data and the standard karyotypes (Reading Conference 1980; ISDNDA89 1990). However, the 1314 loci currently mapped in sheep includes only 334 designated genes (SheepBase, December 21, 1998).

Much genetic information can be transferred from well-mapped genomes, like those of humans and mice, to sparsely mapped ones like those of bovids. The use of a human chromosome-specific painting probe technique, called Zoo-FISH (Schertan et al. 1994), allows regions to be identified in non-related mammalian chromosomes that are conserved or not conserved. This is an important step for genetic improvement, especially in economically important species, allowing more careful genetic analysis in particular animal chromosome regions where particular genes, well known in the human genome, can be explored and used for livestock production or for animal disease resistance.

Zoo-FISH by using human specific chromosome probes has been applied in cattle (Hayes 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996), pig (Fronicke et al. 1996; Rettenberger et al. 1995), horse (Raudsepp et al. 1996), cat (O'Brien et al. 1997) and river buffalo (Iannuzzi et al. 1998), but it is still lacking in other important domestic species such as sheep. However, in sheep indirect comparison with human chromosomes was performed by Burkin and associates (1997). These authors hybridized specific sheep chromosome painting probes on Indian muntjac (*Muntiacus muntjak vaginalis*, 2n = 6,7) chromosomes and indicated the corresponding human chromosome paintings on the basis of a previous study that painted the Indian muntjac chromosomes with human probes (Yang et al. 1997).

In the present study, we extend our knowledge of differences between human and sheep genomes by using direct human chromosome (HSA)-specific painting probes on R-banded sheep chromosomes (OAR), allowing a detailed description of painted regions along the sheep ideogram.

Materials and methods

Commercially available human specific chromosome libraries (Painting kit 1089-KB, Cambio, England) were used for this study. Concavalin A (Sigma)-stimulated blood lymphocyte cultures were treated for the late incorporation of both BrdU (10 μg/ml) and Hoechst 33258 (20 μg/ml) to obtain enhanced R-banding patterns after in situ hybridization. Slide treatment, in situ hybridization, probe detection, RBH- and RBA-banding, as well as metaphase image processing, were recently reported (Iannuzzi et al. 1998). At least 10 early-metaphases and prometaphases were studied for each probe. Sheep chromosome identification followed the RBA-standard karyotype (ISCNDA89 1990) and the Texas nomenclature (1996), while the R-banded ideogram previously reported (Iannuzzi et al. 1995) was used to show the human chromosome paintings and the sheep gene mapping data (only gene loci mapped by ISH) by using the SheepBase. Comparisons with previous Zoo-FISH data reported by Burkin and colleagues (1997) were performed, while comparisons with previous Zoo-FISH made in cattle (Hayes 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996) and river buffalo (Iannuzzi et al. 1998) were discussed on the basis of the cattle and sheep standard nomenclatures (Reading Conference 1980; ISDNDA89 1990; Ansari et al. 1994; Texas nomenclature 1996).

Results and discussion

Figure 1 shows some representative partial sheep chromosome preparations (both early- and prometaphases) sequentially treated for FISH with human chromosome-specific painting probes and RBA-banding. The resulting clear banding patterns allowed not only easy identification of chromosomes, but also of chromosome regions and bands painted by human probes. Furthermore, for all the probes we used, paintings appeared much more intense in positive R-bands than in positive G-bands (Fig. 1). The same was found in river buffalo (Iannuzzi et al. 1998).

Figure 2 shows the sheep R-banded ideogram with the corresponding painted regions after hybridization with human probes. The lines indicate which human-specific probes paint sheep chromosome regions. A total of 48 human segments were detected, of which 42 were in agreement with the total number of segments reported by Burkin and coworkers (1997). In this figure, we also report the mapped gene loci in specific chromosome regions or bands. Essentially, the painting results with HSA probes agree with the gene mapping data (Fig. 2) and with previous Zoo-FISH conducted in cattle (Hayes 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996) and in river buffalo (Iannuzzi et al. 1998),

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* Technical assistance, image processing, and computerized ideogram.

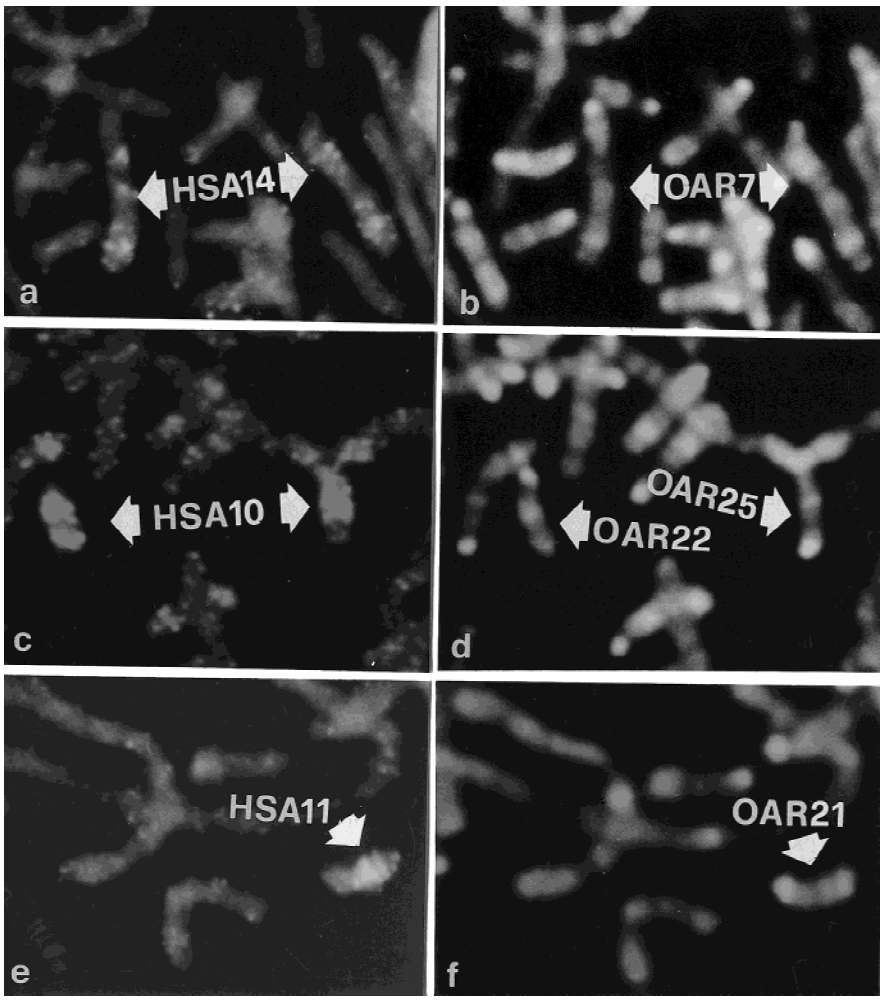

Fig. 1. Representative partial sheep chromosome preparations sequentially treated for FISH (left) and RBA-banding (right) by human chromosome painting probes. OAR25 (Fig. 1c-d) is in NOR-association with two other chromosomes. Notice that the paintings in all chromosomes appear much more intense in the positive R-bands than in the positive G-bands.

as well as with those obtained in Indian muntjac by using sheep painting probes (Burkin et al. 1997). Some differences between sheep and both cattle and river buffalo Zoo-FISH were found concerning the extension of homologous regions painted by HSA probes and some chromosomes that occupy different positions in different standard nomenclatures (Table 1). Indeed, the inverted position between OAR 4 and 6 as well as between OAR 8 and 9 it is well know when comparing the Reading Conference (1980) and ISCNDA89 (1990) standards (Ansari et al. 1994; Texas nomenclature 1996).

HSA7 + 16 and HSA8 paint OAR 26 and 24, respectively, when referring to this study and ISCNDA89; and OAR24 and 26, respectively, when referring to the Texas nomenclature (1996) and Burkin et al. (1997).

HSA10 paints all OAR25 (Fig. 1), as reported in the homologous BTA28 (Hayes 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996), while in river buffalo the HSA10 painting was limited to the bands 4p14-17 (Iannuzzi et al. 1998). HSA10 also paints OAR22 (Fig. 1; homologous to BTA26), while in Indian muntjac Chr 2 only one chromosome region, which includes both OAR25 and OAR22, has been painted by HSA10 (Burkin et al. 1997).

On OAR2p, the entire arm was painted with HSA9, as reported in the homologous BTA8 (Hayes 1995) and BBU3q (Iannuzzi et al. 1998), and no signals were detected when using HSA8, as found in the BTA8 distal part (Solinas-Toldo et al. 1995; Chowdhary et al. 1996) or in BBU3q22-25 bands (Iannuzzi et al. 1998), although gene mapping data between HSA8 and OAR2 are available (Burkin et al. 1997).

HSA21 painted both the proximal and telomeric regions of OAR1q, as found in the homologous BTA1 (Hayes 1995) and BBU1q (Iannuzzi et al. 1998). This agrees with Burkin and associates (1997), who found two corresponding HSA21 segments in Indian muntjac Chr 1 and X + 3, and agrees only partially with Solinas-Toldo and colleagues (1995) and Chowdhary and coworkers (1996), who reported that BTA1 was painted by HSA21 only in the proximal region. However, since the TF-locus (HSA3q21; HGM11 1991) has been reported to map in the telomeric region of OAR1q (Broad et al. 1995), further studies are necessary to improve the knowledge in this sheep chromosome region by using comparative FISH-mapping between OAR1q311-313 (Fig. 2) and HSA21 loci.

For OAR7, homologous to BTA10, our results fully agree with those reported in cattle (Hayes 1995) and in river buffalo (Iannuzzi et al. 1998). In fact, starting from the centromere, this chromosome was painted by HSA5 (OAR7q13) and, alternatively, by HSA15 and HSA14. In the Burkin and colleagues (1997) study, OAR7 was painted only by HSA15 and HSA14. The small region of sheep Chr 7 that was painted in our study by HSA5 (Fig. 2) is consistent with the gene mapping of HEXA to the pericentromeric region of OAR7 (SheepBase; OAR7q13 in the present study), although HEXA maps in HSA15q23-24 (HGM11, 1991). Certainly, a comparative FISH-mapping between the human and sheep (and cattle) genomes by using appropriate probes that map along OAR7 (and BTA10) and HSA5, HSA14, and HSA15 may clarify the complex chromosome rearrangements occurring in OAR7 (and BTA10) when compared with the corresponding human chromosome regions.

In OAR13 only two human segments were detected with HSA10 and HSA20, as observed in both cattle (Hayes 1995; So-

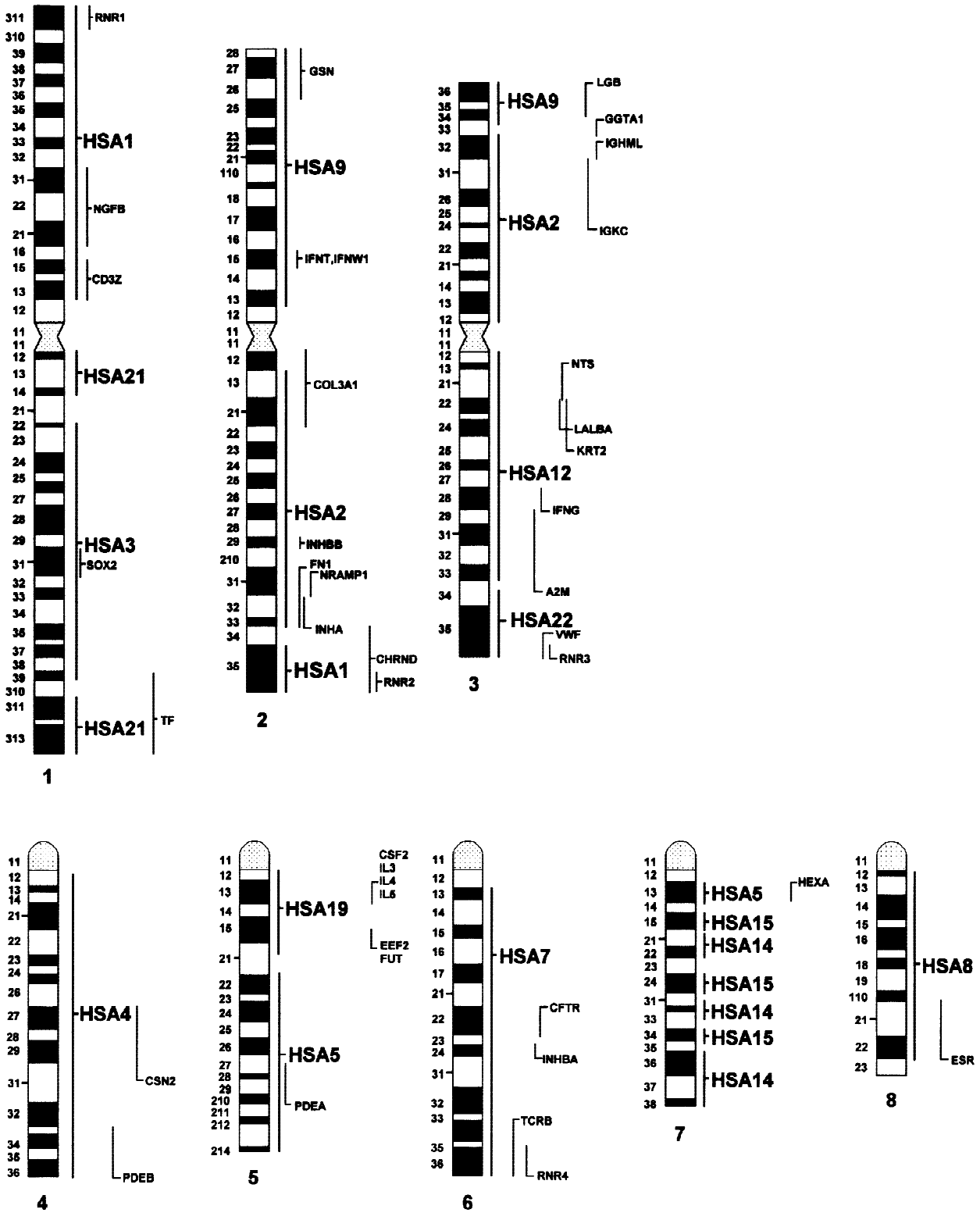

Fig. 2. Sheep R-banded ideogram with corresponding human chromosome painting regions (HSA). Also, the ISH-mapped loci in specific regions or bands were reported to better compare the painting results with gene mapping data. (*Fig. 2 continued on next page.*)

linas-Toldo et al. 1995; Chowdhary et al. 1996) and river buffalo (Iannuzzi et al. 1998), while Burkin and coworkers (1997) found two HSA20 segments separated by a small HSA10 segment in Indian muntjac Chr 2.

OAR14 is painted by HSA16 and HSA19, and the same seems to be occurring in the Indian muntjac Chr 2 (Burkin et al. 1997).

In OAR17 the three detected HSA4, HSA12, and HSA22 segments seem to be conserved in Indian muntjac Chr 1. The same occurred for OAR18 (HSA15 and HSA14 painting) and OAR26 (OAR24 in Burkin et al. 1997) with HSA16 and HSA7.

HSA22 paints OAR3q and OAR17 (present study and Texas nomenclature 1996), in agreement with the painting results ob-

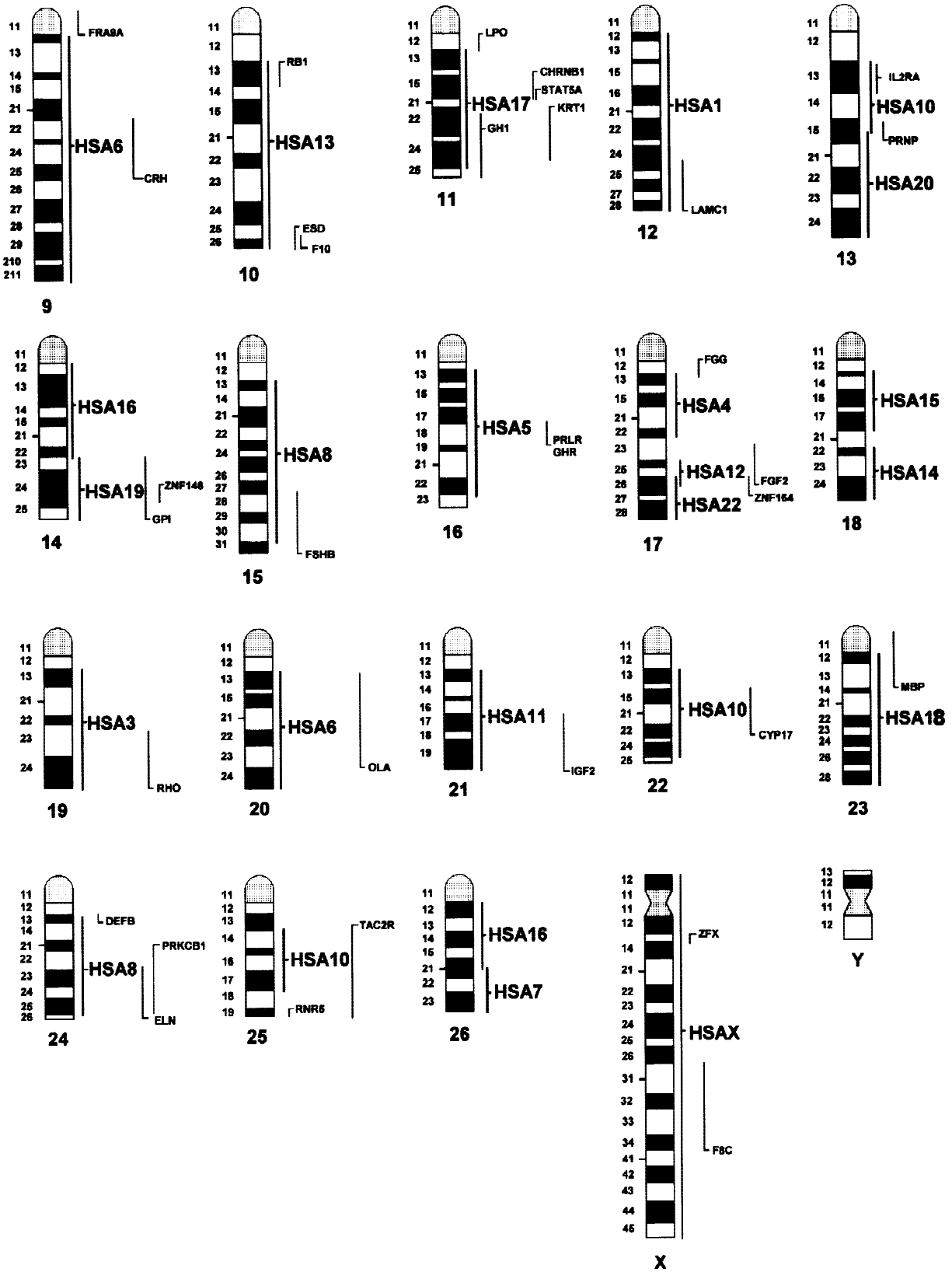

Fig. 2. *Continued.*

Table 1. Correspondences between human (HSA) and sheep chromosomes (this study; ISCNDA89 1990; Texas nomenclature 1996; Burkin et al. 1997) as revealed by Zoo-FISH. Each line in OAR chromosomes reports the same chromosome in the different systems, and the numbers underlined indicate the chromosomes that occupy a different position in the three different systems. OAR23 (last line) in the Burkin et al. (1997) system is reported in bold and underlined to distinguish it from OAR3q (non-homologous).

	OAR		
HSA	Present Study, ISCNDA89	Texas Nomenclature	Burkin et al.
1	1p,2q,12	1p,2q,12	1, 2,12
2	2q, 3p	2q, 3p	2, 3
3	1q, 19	1q, 19	1, 19
4	4, 17	6, 17	6, 17
5	5, 7,16	5, 7,16	5, 16
6	9, 20	8, 20	8, 20
7	6, 26	4, 24	4, 24
8	8, 24	9, 26	9, 26
9	2p, 3p	2p, 3p	2, 3
10	13,22,25	13,22,25	13,22,15
11	15, 21	15, 21	15, 21
12	3q, 17	3q, 17	3, 17
13	10	10	10
14	7, 18	7, 18	7, 18
15	7, 18	7, 18	7, 18
16	14, 26	14, 24	14, 24
17	11	11	11
18	23	23	23
19	5, 14	5, 14	5, 14
20	13	13	13
21	1q	1q	1
22	3q, 17	3q, 17	17, **23**
X	X	X	X

tained in the homologous Chrs 5 and 17 of cattle (Hayes et al. 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996) and 4q and 17 of river buffalo (Iannuzzi et al. 1998). Burkin and associates (1997) reported the painting results with HSA22 on OAR17 and OAR23. However, since no comparative gene mapping data between HSA22 and OAR17/OAR23 are available (SheepBase), these results need to be confirmed.

As observed in cattle and river buffalo, the sheep Y Chr did not show any signal with the human Y Chr painting probe. The same result was obtained by Burkin and colleagues (1997).

Our results extend the Zoo-FISH data obtained by Burkin and coworkers (1997; Table 1) by indicating the specific sheep chromosome arms and regions painted by HSA probes (Fig. 2), allowing a more precise comparison between human and sheep genomes.

The 48 human segments found in sheep chromosomes show a high degree of conserved human chromosome regions in the sheep genome and further confirm the high degree of chromosome similarity among related bovids.

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