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A comparative radiation hybrid map of sheep chromosome 10

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Abstract. Comparative radiation hybrid (RH) maps of individual ovine chromosomes are essential to identify genes governing traits of economic importance in sheep, a livestock species for which whole genome sequence data are not yet available. The USUoRH5000 radiation hybrid panel was used to generate a RH map of sheep chromosome 10 (OAR10) with 59 markers that span 1,422 cR over an estimated 92 Mb of the chromosome, thus providing markers every2 Mb (equivalent to every 24 cR). The markers were derived from 46 BAC end sequences (BESs), a single EST, and 12 microsatellites. Comparative analysis showed that OAR10 shares remarkable conservation of gene order along the entire length of cattle chromosome 12 and that OAR10 contains four major homologous synteny blocks, each related to segments of the homologous human chromosome 13. Extending the comparison to the horse, dog, mouse, and chicken genome showed that these blocks share conserved synteny across species.

The current sheep map is one of the least advanced for livestock (Cockett, 2003). To date, a genome wide medium-density sheep linkage map exists (Maddox et al., 2001). However, the density of mapped loci in sheep is significantly less than in other important livestock species such as cattle and pig, where whole genome sequencing is under way or completed (http://pre.ensembl.org/). As a first step towards sequencing the sheep genome, more than 375,000 BAC end sequences (BES) of the CHORI-243 ovine BAC library have been deposited in public databases (http://www.ncbi.nlm.nih.gov/). A first virtual whole genome map was constructed in silico using this resource in combination with human/cow and vertebrate synteny, and the sheep linkage map (Dalrymple et al., 2007). Early bidirectional Zoo-FISH studies established extensive synteny among sheep and human chromosomes (Hayes, 1995; Schibler et al., 1998; Iannuzzi et al., 1999). Despite serving as an important foundation for understanding the organization of the ovine genome, the low resolution of these sheep-human comparative maps has been inadequate for rapid discovery of markers closely linked to traits of interest in the sheep. Among the most apparent drawbacks associated with these maps were the presence of regions on several sheep chromosomes that lack adequate numbers of mapped gene-specific markers and the absence of uniformity in the distribution of markers over individual chromosomes. Therefore, experimentally derived physically ordered comparative maps that align the sheep map with its mammalian counterparts are needed to confirm the in silico derived BES based virtual genome map and to serve as a tool in assembling the sequence data of the ovine genome.

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The recent development of an ovine whole-genome radiation hybrid (RH) panel (USUoRH5000) (Wu et al., 2007) and the high-resolution RH maps for sheep chromosomes (OAR) 9 and 23 (Tetens et al., 2007) represent the first steps in developing a dense genome map for the sheep. The human-sheep synteny information offers the opportunity to use the recently developed sheep BES resource to increase marker density and to determine whether chromosomal synteny is conserved at the level of individual gene order by comparative mapping of markers derived from these BES. In this study, we report the construction of an RH map of OAR10, which is homologous to HSA13. We undertook the development of an RH map of the chromosome with the goal of defining markers at 2 Mb intervals. This study presents the third comprehensive map for a single sheep chromosome that integrates all available mapping data and adds new information about ovine BAC clones that span the chromosome. The map has been integrated with the latest ovine linkage map. The relationship of OAR10 with genomes of human and a number of other species enhances identification of candidate loci for ovine traits mapped to this chromosome. For example, the locus controlling horn development has been mapped to OAR10 (Montgomery et al., 1996; Beraldi et al., 2006). The identification of the correct order of markers and the detailed multi-species comparisons are essential to the research community and help us to understand the relative organization and hence evolution of homologues of this chromosome in different mammals/vertebrates. Furthermore, the map could also be used for assembling the sequence data of the ovine genome and to verify the order of loci.

Materials and methods

Sequence processing and bioinformatics

A total of 376,493 NCBI (http://www.ncbi.nih.gov/) entries derived from BES of the ovine BAC CHORI-243 library (http://bacpac.chori.org/library.php?id=162) were downloaded. Repetitive sequences were masked with RepeatMasker (http://www.repeatmasker.org). Masked sequences were subjected to BLASTN searches against the HSA13 sequence of build 36.2 of the human genome (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9606) using a cutoff of $E \leq 10^{-5}$ and a minimum hit length of 150 bp. This procedure revealed 4,340 ovine BESs showing hits on HSA13. BESs with hits on more than one human chromosome were filtered out. Therefore, a reverse BLASTN strategy using the 4,340 ovine BESs as queries against the whole human genome sequence was performed. After filtering 3,133 BESs with multiple hits within the human genome, the remaining 1,207 unique and significant hits were termed as HSA13 specific hits. Primers were designed from single copy sequences using the online oligonucleotide design tool Primer 3 (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The primer sequences and their related information are shown in supplementary Table 1 (www.karger.com/doi/10.1159/000124379). Primer pairs were tested with hamster and sheep genomic DNA; those primers that did not amplify the same size fragment in sheep and hamster were typed on the USUoRH5000 RH panel.

RH mapping

The genotyping procedure was performed as described previously (Tetens et al., 2007). The RH maps of OAR10 were constructed using the Cartha Gene Software (de Givry et al., 2005). Initially, the best marker order was determined under the haploid equal retention model applying the Lin-Kerningham heuristics implemented in Cartha Gene. The best map according to log10-likelihood was then improved using the 'greedy' command which applies a taboo search algorithm. The map was further tested using the 'flips' algorithm, which checks all possible permutations in a sliding window of fixed size, using window sizes of seven and four markers. Finally, the best map was checked with the 'polish' algorithm, which tries to improve the map by sequentially removing every marker from the map and trying to insert it at every possible position. The final map distances were calculated under the diploid equal retention model. Additionally, two comparative RH maps were computed using the marker order on BTA12 (build 4.0) and HSA13 (build 36.2) determined by BLAST search as reference orders. Therefore, the traditional maximum multipoint log10-likelihood criterion was replaced by the comparative mapping criterion using the 'dsbplambda' command with lambda set to 1. The RH mapping data was merged to the human and bovine marker orders, respectively, and the two comparative RH maps were generated as described above.

Comparative analysis with genome sequences of different species

The BES and EST sheep specific sequence entries of RH mapped markers (supplementary Table 3) were used for BLASTN sequence comparisons (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). To obtain sequence surrounding microsatellites, ovine or bovine primer sequences were used to extend (using 1 kb flanking sequence from the bovine genome assembly) prior to performing the comparison with other genome sequences. The two microsatellite markers *OarDB3* and *CSRD287* were not included in the cross-species comparison because the corresponding ovine or bovine sequences were not available.

Results and discussion

Marker selection and construction of an RH map of OAR10

Primarily we used BES markers for RH mapping because of their availability from the ovine genome project and their assigned positions in the human genome sequence, which provided information for the human-sheep comparative map. Ovine BESs were anchored to HSA13 with unique and significant hits in BLASTN analysis as previously described for the horse (Leeb et al., 2006). A total of 1,207 comparatively anchored BAC clones were fairly evenly distributed on the HSA13q sequence and on average about 13 BAC clones per Mb could be identified (supplementary Table 2). Only the short arm and the centromeric region of HSA13 showed no matching ovine BES. Similar to the other human acrocentric chromosomes 14, 15, 21, and 22, the short arm of HSA13 is heterochromatic and contains families of repeated sequences (Dunham et al., 2004). A total of 46 ovine BESs at approximately 2 Mb intervals on the human chromosome sequence were successfully scored on the USUoRH5000 panel (supplementary Table 1). The selected BESs span the entire HSA13q sequence of approximately 92 Mb. In addition, a single ovine EST corresponding to the HSA13q32.3 gene *DOCK9* and 12 microsatellite markers from the latest ovine genetic linkage map (supplementary Table 1) at approximately 8 cM intervals were genotyped on the USUoRH5000 panel. The average retention frequency for all the mapped markers on OAR10 was 20.3% and varied from 6.8% for HH41 to 40.9% for DU254603. Markers at both chromosome ends showed higher retention frequencies

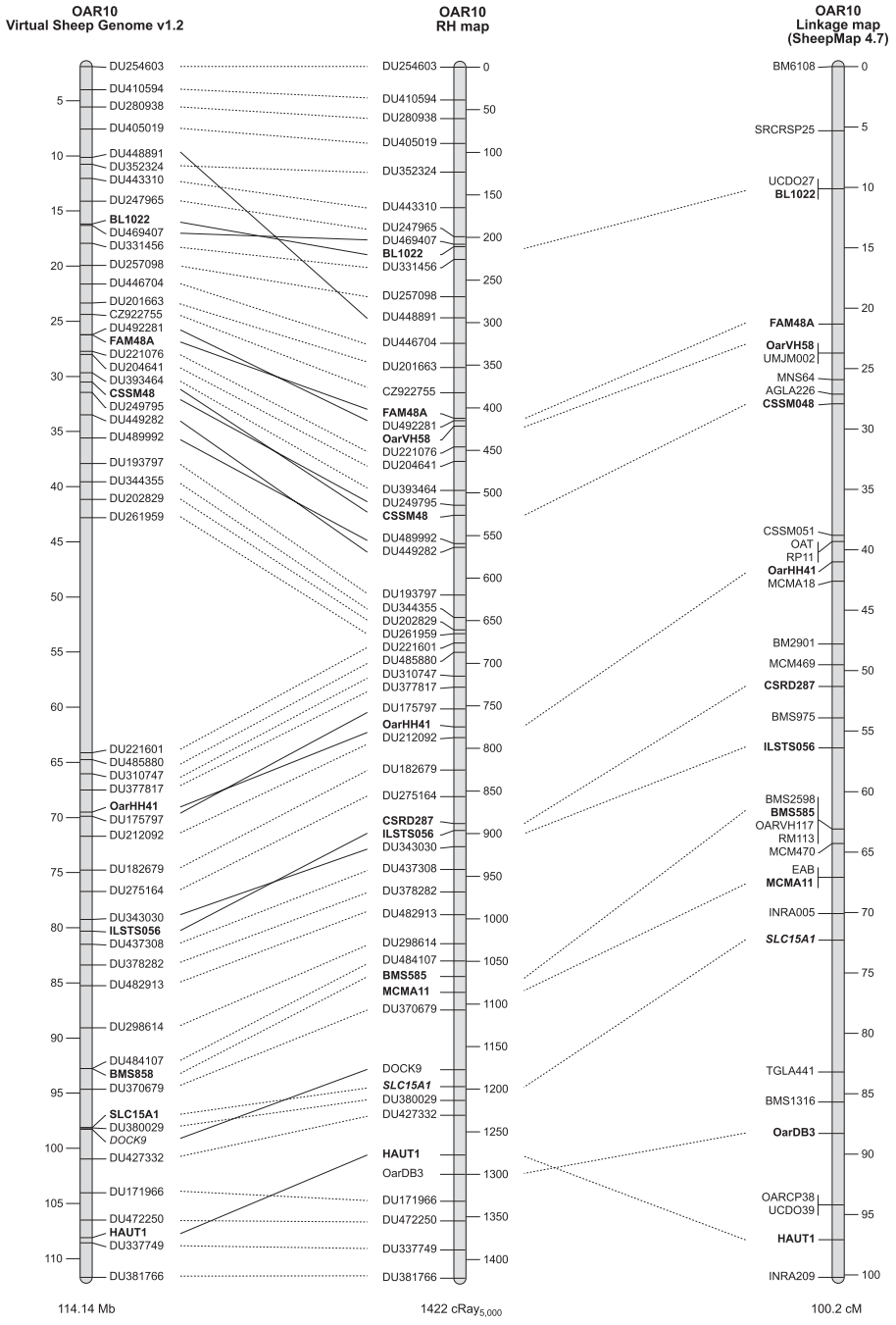

Fig. 1. RH map for OAR10 (center), compared with the genetic linkage map (right) and the sheep virtual genome map version 1.2 (left). Microsatellite markers mapped on the three maps are bold typed. Lines are used to indicate locations of common markers across the maps.

(supplementary Table 3). The overall average retention of 70 markers on OAR23 and 47 markers on OAR9 averaged 28.8% and 25%, respectively (Tetens et al., 2007; Wu et al., 2007). This indicated a slightly lower representation of OAR10 fragments within the RH panel. Previous studies in cattle have reported that the pattern of retention frequencies varies markedly between chromosomes (Williams et al., 2002; Weikard et al., 2006).

The total length of the RH map of OAR10 without applying a reference order extended to 1,422 $cR_{5,000}$ and its log10-likelihood was –550.58 (supplementary Table 3). The traditional approach of RH mapping is to heuristically produce a so-called framework map, incorporating only a fraction of typed markers which are reliably ordered. However, a major disadvantage of building framework maps is that the remaining unplaced markers are placed into bins of confidence, which may not be of true order. In this study, we have also tried to construct a map of OAR10 using a comparative RH mapping approach recently introduced in Cartha Gene (de Givry et al., 2005). This approach is based on a probabilistic Bayesian model integrating the usual RH probabilistic model with a probabilistic model of breakpoint occurrences

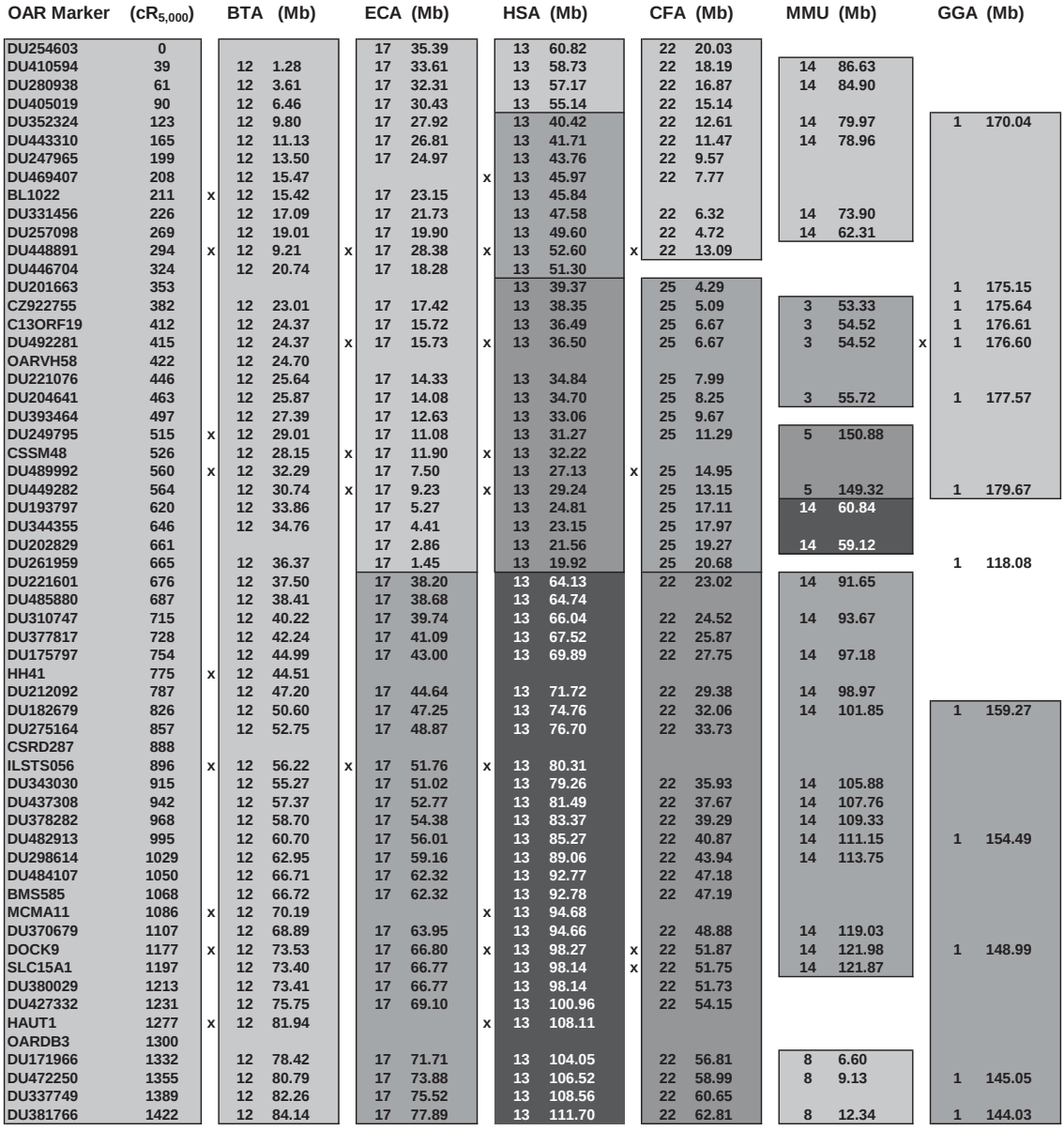

Fig. 2. A BLASTN comparative map of the OAR10 RH map to orthologous chromosome regions in cattle (BTA), horse (ECA), human (HSA), dog (CFA), mouse (MMU) and chicken (GGA). Orthologs showing conserved order compared to the derived order of ovine loci are grouped vertically in grey shaded rectangles. Single loci within these rectangles which are apparently inverted are indicated by 'x' on the left.

with a reference order, typically obtained from the position of orthologous markers in a related sequenced genome (Faraut et al., 2007). In this probabilistic model, breakpoints induced by chromosomal rearrangements are considered as rare events, which follow a Poisson law. Equivalently, we believe genome assembly errors create rare spurious breakpoints between the RH map order and the current assembly order. Therefore, Cartha Gene was used to produce RH maps integrating the ovine RH data with the current human and bovine genome assemblies, respectively. Exact distances were evaluated under a pure diploid RH model using all markers that had a match on the closely related bovine (build 4.0) and the evolutionarily more distant human (build 36.2) sequences. The map built using the bovine sequence order had a log10-likelihood of –565.96 and extends up to 1,495.5 $cR_{5,000}$, whereas the map built according to the human sequence order had a log10-likelihood of –563.77 and extends up to 1,449.3 $cR_{5,000}$ (data not shown). Thus, the maps derived from the bovine and human genome sequences were not significantly less likely than our RH map order without applying a reference order (log10-likelihood ratio differences of –15 and –13, respectively).

The OAR10 RH map has been integrated with the recently introduced virtual physical genome map and the lat-

est published linkage map. A comparison between the calculated RH map and the latest sheep genetic linkage map v4.7 (http://rubens.its.unimelb.edu.au/~jillm/jill.htm) and the sheep virtual genome map v1.2 (http://www.livestockgenomics.csiro.au/perl/gbrowse.cgi/vsheep1.2/) showed good congruency (Fig. 1). Twelve of the 59 ordered markers on the OAR10 RH map have also been positioned on the OAR10 linkage map. The order of these microsatellite loci in the new OAR10 RH map was identical except an inversion between the two distal markers OarDB3 and HAUT1. Fifty five markers were common between the RH and virtual maps, with only minor map discrepancies (Fig. 1). In six cases, orders of adjacent markers were flipped. Three markers were assigned on the OAR10 RH map differently than what was predicted by the virtual genome map (DU448891, DOCK9 and HAUT1). Under the assumption of extensive synteny conservation across species (see below) this could indicate a limited resolution of the ovine RH panel leading to misassignments or may reflect newly detected evolutionary chromosomal micro-rearrangements. However, these inconsistencies cannot be resolved because there are no other published maps of OAR10.

Assuming a similar length of OAR10 to the homologous HSA13q sequence of 92 Mb, one ovine $cR_{5,000}$ corresponds to a length of approximately 65 kb in the genomic sequence in sheep which is similar to previously estimated 69 and 128 kb/$cR_{5,000}$ for OAR23 and OAR9, respectively (Tetens et al., 2007; Wu et al., 2007). This resolution indicates that the USUoRH5000 panel is a valuable tool that can be used to order markers in fine mapping studies.

Multispecies comparison

A comparative map of OAR10 to cattle, human, dog, mouse, and chicken was developed using the order of 59 loci on the ovine RH map (supplementary Table 3). 54, 51, 55, 48, 30 and 13 loci were aligned against cattle (build 4.0), horse (build 2.0), human (build 36.2), dog (build 2.1), mouse (build 37.1), and chicken (build 1.1) genome data, respectively. Megabase positions of orthologs were obtained for the different species and maximally contiguous chromosomal regions with identical gene content order were identified by clustering the contiguous megabase locations of individual loci (Fig. 2).

The comparative analysis confirms and refines knowledge about chromosomal conservation and rearrangements between sheep, cattle and human. The comparison of our OAR10 RH map with the HSA13 sequence, identified three major breakpoints for intrachromosomal rearrangements (supplementary Figure 1), supporting previous Zoo-FISH bidirectional chromosome painting results suggesting conservation between OAR10 and HSA13 (Iannuzzi et al., 1999). The resulting comparative map shows internal rearrangements represented by four major homologous synteny blocks (HSB, $\geq$2 markers). The positions of these breakpoints were equivalent to those previously shown for the alignment of the syntenic BTA12 with HSA13 (Everts-van der Wind et al., 2005; Schibler et al., 2006). There are two major intrachromosomal inversions (supplementary Fig. 1), with the first inversion occurring in the 19.9–39.4 Mb region on HSA13, and the second inversion occurring in the distal region of 55.1–60.8 Mb that corresponds to the centromeric region of OAR10. The three gaps between evolutionary breakpoints in the comparative map were narrowed to regions of 1.05 Mb (HSB I/II), 2.54 Mb (HSB II/III), and a gap of 3.31 Mb between HSB III and IV. The latter large gap resulted from a paucity of sheep BES with sequence corresponding to this region of HSA13 (supplementary Table 2). In contrast, in the virtual map the equivalent gaps are 0.16, 0.16 and 20 Mb, respectively (Dalrymple et al., 2007). The much larger size of the latter is due to the methodology which assigned all human genome sequence to a location on the virtual sheep genome. Combining the two approaches narrows the likely breakpoints down to very small regions.

We did not find evidence of a syntenic region of OAR10 on HSA14 as reported for BTA12 (Everts-van der Wind et al., 2005). Also in horse a one-to-one homology with HSA13 occurred on the RH map of ECA17 (Lee et al., 2004), which could be confirmed by our BLASTN based alignments (Fig. 2). In dog and horse there are only two intrachromosomal breakpoints in comparison to the human genome sequence. In ruminants, an additional third evolutionary chromosomal breakpoint leads to the obvious fragmentation of the sheep-human HSB III and IV (supplementary Figure 1). In dog and mouse, the homologies are distributed over two and four different autosomes, respectively (Fig. 2). Despite these rearrangements the intrasegmental order of the loci is quite similar to that seen in sheep for all conserved chromosomal segments. This is also visible in the sheep-chicken comparison (Fig. 2), where all orthologous loci map to chromosome 1, indicating a quite low number of intrachromosomal rearrangements have occurred between chickens and mammals. In the mouse genome, a large chromosome segment conserved in human and sheep (HSB I) occurs in three fragmented smaller synteny blocks on MMU3, 5, and 14, respectively. The entire human-sheep HSB II and III and a large segment of human-sheep HSB IV is located non-divided on MMU14 (Fig. 2). The distal segment of human-sheep HSB IV could be retrieved as an isolated syntenic block on MMU8, indicating a different evolutionary intrachromosomal breakpoint during rodent development.

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