Cytogenetic Characterization of a Small Evolutionary Rearrangement Involving Chromosomes BTA21 and OAR18

Cytogenetic characterization of a small evolutionary rearrangement involving BTA21/OAR18 chromosomes

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Abstract

Both cattle (*Bos taurus*) and sheep (*Ovis aries*) belong to the *Bovidae* family but to the different subfamilies *Bovinae* and *Caprinae*, respectively. From a chromosomal point of view, beyond the already known centric fusions (that occurred during the evolutionary process of the *Bovidae* family) and the small differences in the chromosome classification, the two karyotypes are very similar in banding.

In this study, the combination of bioinformatics techniques and physical mapping of DNA markers enabled the identification of a micro-rearrangement, a small inversion involving the bovine chromosome 21 (BTA21) and the corresponding sheep chromosome 18 (OAR18). The aim of this study is to conduct a cytogenetic characterization of this difference in genomic assemblies between cattle and sheep in this single chromosome region.

To verify the inversion in FISH experiments, we used 442H08 and 222H03 BACs from the INRA Library and 134H22 and 436P08 BACs from the sheep-specific CHORI Library. The results confirmed the presence of the inverted fragment in sheep compared to the cattle genome.

Genomic rearrangements may have consequences depending on their influence on gene activity, but the analyses conducted in this case seem to show that no gene or transcribed DNA portion is involved.

In conclusion, we showed for the first time, concerning autosome, that there are other differences between bovine and sheep karyotype besides the already-known centric fusions. Furthermore, we demonstrated how the combination of a bioinformatics approach to physical mapping is a valid tool for the identification of currently unknown rearrangements between related species.

Introduction

Both cattle (*Bos taurus*) and sheep (*Ovis aries*) belong to the *Bovidae* family, which appeared for the first time at around 23 MYA (Vrba 1979; Kingdon, 1989). However, these two species belong to different subfamilies: the first belong to the *Bovinae* subfamily, whereas the latter belong to the *Caprinae* subfamily, which also includes goats (*Capra hircus*). The closest ancestor of cattle and sheep dates back to between 19.7 and 21.5 MYA (Hassanin et al., 2012).

From a chromosomal point of view, excluding the sex chromosomes, cattle and sheep have the same fundamental chromosomal number (FN= 58) but different diploid numbers: 2n=60 for cattle and 2n=54 for sheep. This difference is due to three autosomal centric fusions that occurred during the evolutionary process of the *Bovidae* family. As a matter of fact, in sheep, chromosome 1 originated from the fusion of the homologous bovine chromosomes BTA1 and BTA3; chromosome 2 from BTA2 and BTA8 fusion, and chromosome 3 from BTA5 and BTA11 fusion (Iannuzzi et al., 2009; Pauciullo et al., 2014). A similar situation has been reported in buffalo (*Bubalus bubalis, 2n=50*). In this species, the first five chromosomes result from the fusion of 10 different cattle chromosomes (Iannuzzi, 1994). It is a common opinion that centric fusions represent the most frequent karyotype evolutionary mechanism in the *Bovidae* family. Beyond the centric fusions and the small differences in the chromosome classification, the bovine and ovine karyotypes are very similar according to their banding (Iannuzzi and Di Meo, 1995). Nevertheless, cytogenetic analysis by high-resolution banding is no longer considered sufficiently detailed for the presence of some small chromosomal rearrangements, such as the inversions of the small regions of genome. Conversely, the combination of different bioinformatics techniques and physical mapping of DNA markers allows for more precise analyses. In fact, this kind of approach has recently demonstrated the existence of a small karyotype divergence between cattle and goat (De Lorenzi et al., 2015).

In this study, we applied the same methodology (bioinformatics techniques and physical mapping) in comparing the complete bovine and sheep genomes. The results obtained highlighted a possible divergence in a small region involving the bovine chromosome 21 (BTA21) and the corresponding sheep chromosome 18

(OAR18). The aim of this study is the cytogenetic characterization of this difference in genomic assemblies between cattle and sheep in this single chromosome region.

Materials and methods

Cell cultures

Peripheral blood lymphocyte cultures were performed following standard methods (Iannuzzi and Di Berardino, 2008) to obtain bovine, goat, sheep, and water buffalo metaphases. Cultures were incubated for 72 hours at 37 °C, and colcemid was added 60 minutes before the cells were harvested. The sheep metaphases were also obtained from fibroblast cultures following the method reported by Iuso et al. (2015).

FISH experiments

The lists of BACs from both the INRA Bt library (Eggen et al., 2001), CHORI CH-243 library, and CHORI CH-240 (Osoegawa et al., 1998) were used as probes, as reported in Table 1. DNA was extracted according to the method described on the CHORI website (http://bacpac.chori.org/) after an overnight growth at 37 °C in 3 ml Luria Broth (LB) supplemented with 15 µg chloramphenicol. For each FISH experiment, 250 ng DNA was labeled and FISH was performed as reported in De Lorenzi and colleagues (2017).

Bioinformatics analysis

The data used to identify the supposed inversion were obtained by carrying out the protocol described by De Lorenzi and colleagues (2015). Briefly, ENDs sequences (BES) from the INRA Bt BAC library (Eggen et al., 2001) were used as e-probes to compare the cattle, sheep, and goat genomes. The initial analysis was performed with 24,743 BACs and the corresponding 49,486 BES. Sequences were downloaded from the NCBI GSS database. The considered genomic assemblies were: cattle – UMD3.1.1 and sheep – Oar_v4.0. These sequences underwent stringent quality control to eliminate those sequences that could have produced incongruent results. The localization of e-probes on the genomes was performed using the BLAST-like Alignment Tool (BLAT) software (Kent, 2002). Identification of the regions involved in the Evolutionary Break Points (EBPs) at the molecular level was carried out using BLAT software (Kent, 2002).

Results and Discussion

The bioinformatics analysis highlighted the presence of a small inversion between the cattle and sheep genomes. The supposed 1.2 Mb inversion would have involved the BTA21 24.2-25.4 Mb region. This region appeared to be inverted in the homologous OAR18 23.6-22.6 Mb genomic region (Figure 1a); the genomic position of the BACs, considered in the bioinformatics analysis, are reported in Figure 1b. Furthermore, this supposed discrepancy between cattle and sheep are also visible by analyzing recent genomic assemblies (Table 1). Considering the bovine database, the supposed inversion involving 13 BACs and two Evolutionary Break-Points of the inverted fragment in sheep included the regions between 22,800 kb (BAC 449D06) and 24,300 kb (442H08) for the proximal EBP and between 25,400 kb (BAC 222H03 and 234F10) and 25,700 kb (BAC 328E02) for the distal EBP (Figure 1b).

To verify or refute the inversion highlighted by the bioinformatics approach, we used two 442H08 and 222H03 BACs from the INRA Library in the FISH experiments. The results confirmed the presence of the inverted fragment in sheep compared to the cattle genome (Figure 1c-d). Moreover, we tested the same BACs on goat and water buffalo (*Bubalus bubalis*) but found no variation (Figure 1e-f). Considering the positions of the BACs in the genome assemblies of water buffalo (UOA_WB1) and goat (CHI_1 and ARS1), it was observed that the assembly of this genomic portion in goat coincides with those observed in FISH experiments, but this was not observed in the buffalo. It is suggested that the assembly of this region is not correct in the buffalo. As a further confirmation of the accuracy of our data, we decided to prove the presence of the inversion using BACs belonging to the sheep-specific library CHORI-243 (134H22 and 436P08). FISH experiments using these last probes gave results comparable to the previous ones (Figure 1g-h).

Considering the importance of the result obtained, we carried out another approach with the purpose of identifying the position of the EBPs at the molecular level.

Using several BLAT analyses (additional information available on request), we defined the presumed position of the two EBPs (Figure 2). Considering the cattle genome (UMD 3.1.1 genome assembly) *vs.* the sheep genome

(Oar 4.0 genome assembly), the proximal EBP is between 23,799 kb and 23,829 kb. Considering the distal region, the EBP is between 25,529 kb and 25,530 kb (Figure 2a).

The BACs 6F18 and 319I16 have also been identified in the distal EBP area (Figure 2b). These two BACs have been identified by BLAT analysis, and their positions are reported in the Table 1. They are partially overlapping (28 kb) and the 6F18 breaks as a consequence of the evolutionary event. Using BACs simultaneously on cattle and sheep metaphases, it appears that in cattle, the hybridization signals are practically overlapping (Figure 1i) while in sheep (in which the breaks and inversion event occurs), the signals are visible separately (Figure 1l).

Excluding the centric fusions described above, cattle, sheep, and goat have presented the following autosomal divergences: i) translocation of a small subcentromeric portion of cattle chromosome 9 translocated to the proximal region of goat chromosome 14 and sheep homologous OAR9 (de Gortari et al., 1998, Iannuzzi et al., 2001, 2009); this translocation was also reported and characterized by Iannuzzi et al. (2001) and by De Lorenzi et al. (2015); ii) a 7.4 Mb chromosomal inversion in goat chromosome 13 compared with the homologous BTA13 (De Lorenzi et al. 2015), and iii) a small inversion involving BTA21 and the corresponding homologous OAR18, as reported in the present study. Much more complex were the divergences which differentiated the sex chromosome, especially the X-chromosome, in bovids during the karyotype evolution (reviewed in Iannuzzi et al., 2009).

From an evolutionary point of view, the presence of the reported rearrangement can influence the activity of some genetic factors. For example, it has been shown that a chromosomal break event, close to the *SCNN1B* gene in pig, is responsible for a limited ability to taste NaCl (Groenen et al., 2012). Another example of how a chromosomal inversion can affect the activity of a gene was given by observing the genetic mechanism that leads to the formation of the phenotype called the tobiano white-spotting pattern in horse. This phenotype is associated with a chromosomal inversion on equine chromosome 3 that jeopardizes the action of the *KIT* gene (Brooks et al., 2007). Finally, chromosomal inversion can lead to reproductive disorders in the same species (Morin et al., 2017) and reproductive barriers between species because it negatively affects pairing and synapsis in meiosis (Noor et al., 2001).

Bioinformatics analysis showed that near the break points of the inverted fragment, there are no protein or ncRNA genes.

Considering the proximal EBP (23,799,700-23,829,100 bp), the closest transcribed genetic element is located 35 kb upstream the EBP in tail-to-tail orientation. This gene is homologous to HSA C15orf40, and the presence of several cattle EST (i.e., DV893393) demonstrates that it is actively transcribed in different tissues.

Considering the distal EBP (25,529,400-25,530,100), two genes could be involved in the EBP: MORF4L1 (located at 121 kb downstream, tail-to-tail orientation) and BTBD1 (11 kb upstream, but head-to-tail orientation). Both are expressed in several tissues and no mutation is known to date. Therefore, it is not possible to predict the effect of a possible alteration of their activity.

In conclusion, our cytogenetic analysis confirmed for the first time regarding autosomes that there are other differences between bovine and sheep karyotype, in addition to the already known centric fusions. Furthermore, we demonstrated how the combination of a bioinformatics approach to a physical mapping by FISH analysis results is a valid tool for the identification of currently unknown rearrangements between related species. Finally, it is important to highlight that in an age of massive and high-throughput sequencing, the FISH technique still remains an important tool to test the accuracy of genome assemblies and a further confirmation of genomic alterations, identified alongside other methodologies.

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Figure Legends

Figure 1

1a: Graphic representation of the correspondence between BTA21 21.5-27.0 Mb and OAR18 20.0-26.0 Mb; BES were used as probes; the unexpected inverted genome region is evidenced in the grey box. **1b**: List of the BACs used as probes in the bioinformatics analysis and their position on BTA21 and OAR18 chromosomes; the inverted region is evidenced in gray. **1c, g, i**: FISH experiments on cattle metaphases. **1d, h, l**: FISH on sheep metaphases. **1e**: FISH on goat metaphases. **1f**: FISH on water buffalo metaphases. The BACs shown in red were

marked with Cy3 whereas the BACs shown in green were labeled and detected with Biotin-FITC. Some sheep metaphases were obtained from fibroblast cultures.

Figure 2

2a: Graphic representation of the position of the BACs that delimit the EBPs on bovine chromosome 21 and the correspondences on sheep chromosome 18. In the middle of the representation of the two chromosomes, the presumed position of the two EBPs (proximal and distal EBP) identified by the bioinformatics analysis is reported. **2b**: Graphic representation of the two 6F18 and 319I16 BACs used to visually highlight their separation in sheep following the event of break and inversion. The genomic regions including the proximal[1] and distal[2] EBPs obtained from the bioinformatics analysis are indicated. The genomic regions including the proximal[3] and distal[4] EBPs at molecular level obtained from the BLAT analysis are indicated.