APPLICATION OF BOVINE HETEROSOME PAINTING PROBES TO ANALYSIS OF THE SEX BIVALENT IN RAMS*

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Abstract

Genetic conservation of heterosomes in Bovidae enabled bovine molecular probes to be used in interspecific hybridizations to identify X and Y chromosomes in the sex bivalent of rams. Observations of the X-Y bivalent focused on the analysis of chromosome pairing during prophase I of meiotic division, and synaptonemal complexes were the subject of observations. On the basis of sex bivalent morphology (identified by silver staining or FISH technique), the frequency of the early-dissociated X-Y bivalent in rams was estimated to be 2.5%, 2.2% and 0.5% (1.7% on average). These results, obtained with the use of FISH technique, are lower than the values reported for animals with a normal 54,XY karyotype, in which the early-dissociated sex bivalent was found for about 3% of primary spermatocytes.

Key words: Bovidae, X-Y bivalent, synaptonemal complexes, FISH

Analysis of genetic conservation in Bovidae showed a syntenic and conservative nature of sex chromosomes in this species, which enabled molecular probes obtained for one species to be used for hybridization with the heterosomes of other species (Revay et al., 2002; Kozubska-Sobocińska et al., 2003). Interspecific hybridizations were applied for metaphase chromosomes (Kozubska-Sobocińska et al., 2005) and to identify heterosomes in spermatozoa (Rejduch et al., 2005; Kozubska-Sobocińska and Rejduch, 2008). This paper describes utilization of bovine probes to analyse the sex bivalent in rams.

In heterogametic animals, heterosomes pairing depends on dimorphism in the sex chromosome pair. Non-homologous regions of heterosomes do not form synaptonemal complexes and lateral elements undergo considerable modifications, while they only pair in pseudoautosomal fragments (PAR-X and PAR-Y) that correspond to homologous regions pairing in autosomes (Page et al., 2006).

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In the case of complete homology of pairing autosomes, synapsis takes place in early pachytene; however, delayed synapsis is typical of X and Y heterosomes in mammals (Page et al., 2006; Czyżewska et al., 2007). Many authors have also pointed to early dissociation of the X-Y bivalent compared to autosomal bivalents (Burgoyne, 1982).

The aim of the present study was to analyse the sex bivalent of rams using bovine heterosome specific probes for interspecific hybridizations.

**Material and methods**

**Experimental material**

Gonad fragments taken from three rams immediately after slaughter were investigated.

**Analytical methods**

**Meiotic chromosome preparations for synaptonemal complexes analyses** (Counce and Meyer, 1973)

Gonad fragments from slaughtered rams were placed in RPMI-1640 culture medium. Several drops of 0.2M saccharose solution (6.8 g of saccharose dissolved in 100 ml of deionized water) were placed on cleaned slides. Slices of testes were minced with a forceps and minimum amounts of minced tissue were put to a drop of saccharose on the glass slide. Slides were dried at 30–37°C (on a heating plate) and fixed with 4% paraformaldehyde in 0.1M saccharose solution (pH 8.5) for 10 min. Slides were washed in 0.4% detergent solution (Foto-flo) at pH 8.5 for 5–8 seconds and dried vertically.

Synaptonemal complex preparations were hybridized following the protocols of Pinkel et al. (1986) and Solinas-Toldo et al. (1995) with commercial molecular probes specific for bovine X and Y heterosomes (Cambio Ltd., Cambridge, UK).

Bivalents were observed under the OPTON-Axiophot fluorescence microscope using triple attenuation filters (DAPI/FITC/Texas Red). The positions of selected synaptonemal complexes with clear fluorescent signals were archived on a computer using LUCIA software.

Preparations intended for further observations under a light microscope were washed with equilibrium buffer to elute DAPI stain and then stained with silver nitrate. The previously registered cells with synaptonemal complexes were searched and again archived after staining with silver nitrate.

**Results**

X-Y bivalents were observed in 660 primary spermatocytes obtained from the gonads of 3 rams.

The applied methods made it possible to determine the percentage of cells characterized by early-dissociated X-Y bivalent. The results are shown in Table 1.
Table 1. Analysis of synaptonemal complexes in 3 rams based on pairing of the X-Y bivalent during prophase I

<table>
<thead>
<tr>
<th>Ram</th>
<th>No. of primary spermatocytes analysed</th>
<th>No. of X-Y bivalents analysed</th>
<th>No. of early-dissociated X-Y bivalents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>220</td>
<td>197</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td>186</td>
<td>4 (2.2)</td>
</tr>
<tr>
<td>3</td>
<td>220</td>
<td>195</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Total</td>
<td>660</td>
<td>578</td>
<td>10 (1.7)</td>
</tr>
</tbody>
</table>

Figure 1. Synaptonemal complexes. Pairing of the sex bivalent in primary spermatocyte of the ram: (a) arrow indicates fully paired X-Y bivalent stained with silver nitrate; (b) fluorescence signals identifying heterosomes in the X-Y bivalent

Figure 2. Early dissociation of the sex bivalent in primary spermatocyte of the ram: (a) arrows indicate X and Y chromosomes stained with silver nitrate; (b) hybridization signals identifying heterosomes after early dissociation of the X-Y bivalent
In the observed 660 primary spermatocytes, regular chromosome pairing in the three rams studied was found for 98.3% bivalents on an average (Figures 1A and 1B). The proportion of cells with early-dissociated X-Y bivalent (Figures 2A and 2B) was 2.5%, 2.2% and 0.5% in three investigated rams.

Discussion

Specific morphology of the X-Y bivalent at different stages of pairing enables several substages of prophase I to be identified (Villagomez, 1993; Słota, 1998; Świtoński and Stranzinger, 1998; Rejduch, 2001).

Detailed analysis of the structure of synaptonemal complexes in farm animals, performed using an electron microscope, showed that the specific X-Y bivalent pairing depends on homology between heterosomes (Świtoński and Stranzinger, 1998; Page et al., 2006; Zickler, 2006).

The specific morphology of the X-Y bivalent at different stages of pairing makes it possible to identify several substages of prophase I: late zygotene – during which autosomes are not paired and X and Y chromosomes begin to pair in terminal sites; early pachytene – when autosomal chromosomes begin to pair and X and Y chromosome pairing continues; mid-pachytene – when all chromosomes (both autosomes and heterosomes) are strictly paired; and late pachytene – when autosome pairing continues and dissociation of the X-Y bivalent begins (Villagomez, 1993).

In early pachytene, all homologue autosomes are fully conjugated (Marec, 1996). Chromosome fragments with nucleolar organizer region are the last to complete synapsis (Rasmussen, 1986). Considerable delay in conjugation as a consequence of incomplete homology is typical for heterosomes (Burgoyne, 1982; Page et al., 2006).

The observations of chromosomes at pachytene stages in boars carrying centromeric heterochromatin polymorphism (heterozygous forms) showed no abnormalities resulting from different lengths of polymorphic homologues (Slota, 1998). Likewise, electron microscope (JEOL-JEM 100C) analysis of primary spermatocytes from a boar carrying centromeric heterochromatin polymorphism in pair no. 18 did not show any differences in length between lateral elements of the bivalents of this pair, although early-dissociated X-Y bivalent was observed (Slota, 1998).

A low degree of early dissociation of the sex bivalent at mid-pachytene stage was found in bulls, carriers of 60,XX/60,XY chimerism (Rejduch et al., 2000; Rejduch, 2001).

A similar study in rams showed that early-dissociated X-Y bivalent was five times more frequent in animals with 54,XX/54,XY karyotype compared to animals with a normal 54,XY karyotype (15% vs. 3% spermatocytes on average) (Rejduch, 2000).

In our experiment, based on genetic conservation of heterosomes in Bovidae (Kozubska-Sobocińska et al., 2003; Rejduch et al., 2005; Kozubska-Sobocińska and Rejduch, 2008), commercial bovine heterosome painting probes were applied to identify sex chromosomes in bivalents obtained from testicular tissue of the rams.

Bovine probes are most often used for interspecific hybridizations in Bovidae – with metaphase chromosomes of sheep and goats (Rejduch et al., 2004; Kozub-
FISH technique in analysis of X-Y bivalent in rams

ska-Sobocińska et al., 2005; Rychlik et al., 2005) and identification of heterosomes in spermatozoa of rams (Rejduch et al., 2005; Kozubska-Sobocińska and Rejduch, 2008).

One example can be using the probe obtained by microdissection of Yp12.1–12.6 chromosome fragment from *Bos indicus* to identify the complementary sequence in the X-Y bivalent at metaphase I in *Bos taurus* and perform comparative hybridization of the appropriate segment on the q arm of the Y heterosome in *Bos taurus* (Goldammer et al., 1996). A probe specific for the Yp12 fragment was also used to identify the Y chromosome in metaphase plates and spermatozoa (Révay et al., 2000). The high conservation of sex chromosomes in *Bovidae* was evidenced by hybridization signals in bull spermatozoa, following the application of probes obtained by sorting of the yak (*Bos grunniiens*) heterosomes (Révay et al., 2002).

In the present study, specific morphology of the X-Y bivalent at different stages of pairing, stained with silver nitrate (Figures 1A and 2A) or identified on the basis of hybridization signals (Figures 1B and 2B), made it possible to determine the frequency of early dissociation of sex bivalent. Differently labelled bovine painting probes applied in FISH technique resulted in distinct hybridization signals identifying, without any doubt, X and Y chromosomes (Figure 2B), whereas these heterosomes on silver stained slides could be recognized unequivocally only after dissociation of X-Y bivalent (Figure 2A).

The analysis of 220 primary spermatocytes in each of the three rams showed that the proportion of early-dissociated X-Y bivalent in different animals was 2.5%, 2.2% and 0.5%. It is worth noting that these results are slightly lower than those given by Rejduch (2000), who reported that in animals with a normal 54,XY karyotype, early dissociation of the sex bivalent occurred in about 3% of spermatocytes.

References


Zastosowanie bydlęcych sond malujących heterosomy w analizach biwalentu płciowego u tryków

STRESZCZENIE

Konserwatyzm genetyczny heterosomów u Bovidae umożliwił wykorzystanie bydlęcych sond molekularnych do hybrydyzacji międzygatunkowych, w celu identyfikacji chromosomów X i Y w biwalencie płciowym u tryków. Obserwacje biwalentu X-Y skoncentrowały się na analizie koniugacji chromosomów podczas profazy I podziału mejotycznego, a przedmiotem obserwacji były kompleksy synaptonemalne. Na podstawie specyficznej morfologii biwalentu płciowego (identyfikowanego barwieniem srebrowym lub techniką FISH) częstość wczesnej dysocjacji biwalentu X-Y u tryków oszacowano na poziomie 2,5%, 2,2% oraz 0,5% (średnio 1,7%). Wyniki te, otrzymane po zastosowaniu techniki FISH, są niższe od wartości podawanej dla zwierząt o prawidłowym kariotypie 54,XY, u których wczesna dysocjacja biwalentu płciowego dotyczy około 3% spermatocytów I rzędu.

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