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Identification and Structure of Lampbrush Sex Bivalents Prior to and After the Reproductive Period of the European Domestic Goose *Anser anser*

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Lampbrush chromosomes (LBCs) present in bird oocytes are a new model in cytogenetics with particular significance for bird chromosome analysis. The fact that female birds are heterogametic makes it possible to observe both sex chromosomes in the form of decondensed structures typical of lampbrush chromosomes. A change in transcription activity associated with physiological processes in geese prior to and after the reproductive season is reflected in chromosome morphology. Lampbrush chromosomes obtained after the reproductive period have reduced side loops, sites of intensive transcriptional activity. However, noticeable characteristics in the chromosomes include inactive chromomeres. Chiasmata, PBs, large side loops (ML) and telomeric loops (T, TLL, and GLL) are structures that undergo degradation latest after the termination of reproduction, and as a result, constitute the basis of identification of individual bivalents in different periods of the cell's transcriptional activity.

Key words: *Anser anser* goose, meiosis, lampbrush chromosomes (LBCs), lampbrush sex bivalent.

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Apart from a large diploid number and the presence of microchromosomes, heterogamety in females, connected with the presence of the ZW chromosomes, is a typical karyotype characteristic in birds. The bird W chromosome, similarly to the mammalian Y chromosome, in most species is often completely heterochromatic and is one of the smallest chromosomes in the karyotype.

Lampbrush chromosomes (LBCs) permit the analysis of the structure of both sex chromosomes and provide information on the expression of genes present in the lampbrushes. LBCs are greatly elongated diplotene bivalents found in the growing oocytes of all animals except mammals, some insects and some reptiles. The lampbrush form of even the smallest of chromosomes is easily visible and shows useful linear differentiation. It is particularly applicable in the case of the W chromosome which only in birds can be observed in the form of a large sex bivalent.

As a tool, lampbrush chromosomes were introduced into poultry cytogenetics by KROPOTOVA and GAGINSKAYA (1984), and by HUTCHISON (1987). The former authors support a thesis that

chromosomes provide valuable information on bird gene expression and are irreplaceable in cytogenetic research on animals with small genomes in which a large number of small-sized mitotic chromosomes makes it impossible for scientists to carry out microchromosome analysis. In a report on the genome and karyotype of *Gallus domesticus*, LBCs have been recognized as a new model in avian cytogenetics (RODIONOV *et al.* 2005).

Lampbrush chromosomes found in different species are characterised by very similar structure, and they perform the same function. Utilisation of morphological similarity of amphibian and avian lampbrush chromosomes proved to be especially significant in overcoming stagnation in the analysis of avian karyotypes and genomes, particularly when the comparative analysis of avian and amphibian LBCs was applied (GAGINSKAYA *et al.* 1993). Structural similarity of LBCs is particularly visible in birds. Lampbrush chromosomes of domesticated birds are characterized by a similar location of chiasmata and marker loops. Basically they differ only in size and this may result from varying sizes of genomes of individual species (GREGORY 2002; RODIONOV & CHECHIK 2002).

The ZW bivalent, the structure of which is species-dependent, is an exception. Moreover, even within a single bird species, different forms of lampbrush sex bivalents have been observed (SOLOVEI *et al.* 1993; MIZUNO & MACGREGOR 1998; RODIONOV *et al.* 2005). The ZW bivalent is most difficult to identify in the entire LBC set of a bird. It resembles a markedly asymmetric single chromosome which along its length is of predominantly typical lampbrush structure. The W chromosome is almost completely heterochromatic and often assumes a characteristic bar-like shape in the distal part of the Z chromosome. The Z and W chromosomes are connected by one chiasm in proximity to the telomere (MIZUNO & MACGREGOR 1998).

Most analyses of lampbrush chromosomes pertain to gallinaceous birds, *Gallus domesticus* in particular. This provided the basis for the present research with the aim of identifying and describing the morphology of lampbrush sex bivalents prior to and after the reproductive period of the European domestic goose, *Anser anser*.

Material and Methods

Chromosomes in the form of lampbrushes were prepared by manual isolation from oocytes with diameters of 1.5–3.5 mm from sexually mature geese (10 before the reproductive period and 10 after the reproductive period) by applying a standard procedure (MACGREGOR & VARLEY 1988) modified for bird oocytes (SOLOVEI *et al.* 1992; RODIONOV & CHECHIK 2002). Preparations were stained with Coomassie Blue R 250. The number of LBCs analysed from each individual ranged from 5 to 10. Bivalent identification was carried out on the basis of marker structures typical of lampbrush sex bivalents (sex LBCs) described on

bird lampbrush chromosomes (SOLOVEI *et al.* 1993; MIZUNO & MACGREGOR 1998; RODIONOV *et al.* 2005). The following marker structures were observed on the lampbrush sex bivalents identified: TBLs – telomeric bow-like loops, MLs – marker loops, DBLs – distal boundary loops, PBLs – proximal boundary loops, GLLs – giant lumpy loops, CH – chiasmata, PBs – protein bodies.

The preparations obtained were analysed under an Olympus BX 50 light microscope and were filed by means of an analogue Panasonic video camera. A detailed computer analysis was carried out by applying the Multiscan image analysing system, the Karyotype software, and graphic software compatible with the system.

Bivalents before the reproductive period were denoted by the small letter (a), bivalents after the reproductive period were denoted by the small letter (b). Marker structures of the bivalents were numbered with consecutive numbers.

Results

The ZW bivalent in birds is in the shape of an asymmetric univalent. One of the telomeres, the shape of which resembles a compact head, is treated as a condensed W microchromosome (HUTCHISON 1987; MIZUNO & MACGREGOR 1998). The Z chromosome, like the autosomes, is characterised by an arrangement of looped and non-looped regions typical of LBCs.

Three different lampbrush sex bivalents obtained from geese prior to their laying period (ZWa) are shown in Figure 1, whereas Figure 2 presents two different forms of lampbrush sex bivalents obtained from geese after reproduction (ZWb). Only well visible marker structures have

Fig. 1. Structure of lampbrush sex bivalents prior to the reproductive period. Arrows indicate marker structures of bivalents. Bar = 10 μ m.

Fig. 2. Structure of lampbrush sex bivalents after the reproductive period. Arrows indicate marker structures of bivalents. Bar = 10 μ m.

been indicated on individual bivalents: a1, a2 and a3, as well as b1 and b2. The description of all the bivalent marker structures was abandoned because for clarity.

Lampbrush sex bivalents (ZWa)

TBL structures (TBLa1, TBLa2 and TBLa3) associated with the free telomere of the Z chromosome were identified on three bivalents ZW_a. A proteinaceous structure (PBA2-1) hides the TBL loop on bivalent a2. GLL structures (GLLa1, GLLa2 and GLLa3) were identified in the proximity of the chiasma linking chromosomes Z and W. There are typical loops easily identifiable even on bivalents which have a compacted loop coat (fig. a3). Along the whole length of chromosome Z there occur centres of marker loops (ML) in strictly defined sites. They are best visible on chromosomes whose structures are less condensed (MLa1-1 – MLa1-3) – figure a1. They also occur in sites on the chromosomes that are characterised by a denser loop structure (MLa2-1 – MLa2-4 and MLa3-1 – MLa3-3), figures a2 and a3, respectively. DBLa3 and PBLa3 structures (fig. a3) are loops flanking the centromere region of the sex bivalent. Two different--

sized protein bodies (PBA3-2 and PBA3-3) are located in their proximity on bivalent a3, and are connected with the chromatin of all three sex bivalents.

The W chromosome structure can be different and depends on the structure of the whole sex LBC. On the a1 bivalent, on which all the marker structures are in the shape of large side loops, the W chromosome occurs in the form of a heterochromatic lump. In contrast, on the a2 and a3 bivalents, on which side loops are withdrawn to the chromosome axis and chromomeres can already be distinguished, the W chromosome is of a bar-like shape and is densely “spotted” with chromomeres. On each sex LBC presented there is one protein body associated with the W chromosome (PB-W).

Lampbrush sex bivalents (ZWb)

TBL structures associated with the free telomere of chromosome Z were identified on two bivalents ZW_b. They consist of large bow-shaped telomeric loops (TBLb1 and TBLb2). Proteinaceous structures in the proximity of the TBL loop were found on bivalents b1 and b2: a small one on bivalent b1 (PBb1-1) and a large one on bivalent b2 (PBb2-1).

On chromosomes Z of bivalents b1 and b2 three centres of marker loops MLb1-1 – MLb1-3 and MLb2-1 – MLb2-3 respectively, were identified. Both bivalent loops MLb1-2 and MLb2-2 are very well preserved and can be easily identified. They occur in the interstitial part of the bivalents and are connected with large protein bodies PBb1-2 and PBb2-2, respectively. Similarly, loops MLb1-3 and MLb2-3 are situated in the proximity of the chiasma linking chromosomes Z and W. GLL structures, as in bivalents a1, a2 and a3, were identified in the proximity of the chiasma linking chromosomes Z and W. In “post-laying” bivalents, GLL structures form very large loops which, in the case of bivalent b2, constitute almost 30% of its length (GLLb2). In turn, loop GLLb1 takes an open form on bivalent b1.

The structure of chromosome W is different and, as in the pre-reproduction bivalents, depends on the structure of the whole sex bivalent. On bivalent b1, loops are withdrawn towards the chromosome axis. Chromosome W has a similar structure. In turn, bivalent b2 of chromosome W has a typical heterochromatin club-like shape.

Discussion

A comparison of the structure of lampbrush sex bivalents obtained from geese prior to and after the reproductive period established that chiasmata, PBs, and marker structures such as large side loops (ML) and telomeric loops (TL and GLL) are structures which are the last to undergo degradation. The different portrayal of lampbrush sex bivalents obtained from geese prior to and after the reproductive period results from changes in transcriptional activity associated with physiological processes and is reflected in the chromosome mor-

phological makeup. LBCs of geese prior to the reproductive period were characterised by large side loops indicating active transcription. Chromosomes obtained from geese after reproduction had small side loops and visible chromomeres, connected with decreased cell transcription activity (GALL 1983; GAGINSKAYA & TSVETKOV 1988; GAGINSKAYA *et al.* 1993; MORGAN 2002).

In geese lampbrush sex bivalents, various forms of the ZW bivalent were observed both prior to and after the reproductive period, in accordance with descriptions for other domestic birds such as chicken, quail, turkey, pigeon (SOLOVEI *et al.* 1990, 1993; MYAKOSHINA & RODIONOV 1994; MIZUNO & MACGREGOR 1998; RODIONOV *et al.* 2005); as well as wild birds including sparrow and chaffinch (SOLOVEI *et al.* 1993; SAIFITDINOVA *et al.* 2003).

Distinctive TBL structures identified on goose sex LBCs prior to the laying period, occurring in the free Z chromosome telomere in other bird species, have been observed only in chickens (SOLOVEI *et al.* 1993; MIZUNO & MACGREGOR 1998). Loops do not occur on sex bivalents of quail, turkey, pigeon, sparrow and chaffinch (SOLOVEI *et al.* 1993; MYAKOSHINA & RODIONOV 1994; MIZUNO & MACGREGOR 1998; SAIFITDINOVA *et al.* 2003).

Large GLL loops constitute another characteristic structure of lampbrush sex bivalents localized in the region of chiasma connecting the Z and W chromosomes. In sex bivalents of geese, the loops are large and easily identifiable but not as clear as the enormous GLLs in turkey, pigeon, sparrow and chaffinch. Such structures have not been found on the quail chromosomes, whereas on chicken lampbrush sex bivalents the GLL structure may take forms ranging from small but considerably condensed loops to very large loops reaching a length

Fig. 3. Various loop forms GLL (according to SOLOVEI *et al.* 1993; modified).

of up to one-third of the whole bivalent length. The loops may have a closed (a typical loop) or an open structure – Figure 3 (SOLOVEI *et al.* 1993; MIZUNO & MACGREGOR 1998; SAIFITDINOVA *et al.* 2003).

Interestingly, the structure of goose lampbrush sex bivalents after the reproductive period shows very well preserved TBL loops on bivalents, as well as ML loops. Just like for pre-laying period bivalents, two markers ML are associated with protein bodies (PBs). In turn, in the proximity of chiasma linking chromosomes Z and W, enormous GGL loops occur on post-laying bivalents. One of the analysed bivalents contained an open structure. GLL loops were also observed on sex bivalents of geese prior to reproduction, however, they were not as well developed. Various forms of GLL loops were observed in chicken, however, they were found only occasionally on chromosomes before the reproductive period (SOLOVEI *et al.* 1993). In turn, enormous GGL loops in the proximity of chiasma linking chromosomes Z and W were typical of lampbrush sex bivalents present in the oocytes of pigeon, chaffinch and sparrow (SOLOVEI *et al.* 1993; MIZUNO & MACGREGOR 1998; SAIFITDINOVA *et al.* 2003).

Marker structures, called MLs, occur on goose lampbrush sex bivalents in different numbers and are of different size. ML structures have also been observed in other bird species. Their differentiation is a result of changes in cell transcriptional activity (GAGINSKAYA & TSVETKOV 1988; MORGAN 2002). No marker loops have been detected within the W chromosome. The loops are typical of quail and pigeon bivalents (SOLOVEI *et al.* 1993; SAIFITDINOVA *et al.* 2003).

An interesting issue is the role and location of protein bodies. On goose sex bivalents, one PB has always been observed connected with the W chromosome, the remaining PBs have been found in various numbers and in different places on the bivalent. Protein bodies have not been observed on chicken, quail and turkey LBCs. By contrast, on pigeon, chaffinch and sparrow, sex bivalents PBs have been found at permanent sites. In the aforementioned species, one PB is always connected with the W chromosome. Moreover, in pigeon and chaffinch PBs occur in the centromeric segment of the lampbrush sex bivalent (SOLOVEI *et al.* 1993; SAIFITDINOVA *et al.* 2003; KRASIKOVA *et al.* 2004). A similar arrangement of PBs has been found on goose sex LBCs (Fig. 1a3).

Structures called protein bodies only occur in cells associated with LBCs. They are perfectly circular but differ in size and are linked with the chromosome axis of every LBC at regular intervals in the heterochromatin region (SOLOVEI *et al.* 1996; MORGAN 2002). With respect to morphology, PBs resemble Cajal bodies (CBs) which are associated with amphibian LBCs. However, immunocyto-

chemical studies have revealed that PBs do not contain p80 coilin which is found in CBs. The distinctive composition of PBs indicates that they perform a completely different function than CBs. They may be involved in coordinating the spatial arrangement of chromosomes. Localisation of PBs is often connected with repeat sequences surrounding a centromere. Investigations of the potential role of proteins associated with centromeres and centromeric heterochromatin in biogenesis, as well as location of PBs, is a new field in studies on the structure of lampbrush chromosomes (GALL 2000; MORGAN *et al.* 2000; MORGAN 2002).

In conclusion, the use of lampbrush chromosomes in avian cytogenetics makes it possible to more broadly investigate the structure of microchromosomes, and is a unique chance to analyse the ZW bivalent. Particularly promising is the utilization of LBCs in research on transcriptional activity, gene mapping and cytogenetics.

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