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The Chromosomes of Birds during Meiosis

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Abstract
The cytological analysis of meiotic chromosomes is an exceptional tool to approach complex processes such as synapsis and recombination during the division. Chromosome studies of meiosis have been especially valuable in birds, where naturally occurring mutants or experimental knockout animals are not available to fully investigate the basic mechanisms of major meiotic events. This review highlights the main contributions of synaptonemal complex and lampbrush chromosome research to the current knowledge of avian meiosis, with special emphasis on the organization of chromosomes during prophase I, the impact of chromosome rearrangements during meiosis, and distinctive features of the ZW pair.

Meiosis is the specialized form of cell division in which the diploid chromosome set of meiocytes is reduced by half in order to produce haploid gametes. Meiotic chromosomes have been studied for many years because of the central role of meiosis in life but also because they provide a means to analyze synapsis and recombination which take place during the first cell division. Most of the recent advances concerning the structural and functional basis of meiosis in vertebrates were achieved through the analysis of knock-out mice for many different proteins related to the meiotic process [Scherthan, 2003; Morelli and Cohen, 2005]. For birds, the production of genome-edited species is technically challenging, and only recently has it been possible to produce genetically modified chickens with certain efficiency [Oishi et al., 2016]. The knowledge of biochemical and genetic aspects of meiosis in birds has thus lagged far behind that of mice and other model organisms and, therefore, the information obtained through cytological observation of meiotic chromosomes becomes more valuable. In the last 40 years, most of the research into avian meiotic chromosomes has been carried out using either synaptonemal complex (SC) spreads or the visualization of lampbrush chromosomes (LBC) during diplotene in females.

Following the discovery of the SC, this evolutionarily conserved structure has become an excellent tool to study the organization of prophase chromosomes as well as specific aspects of meiosis. SC analysis has helped in the study of specific problems, e.g., synapsis, recombination distribution, or meiotic patterns in standard or aberrant karyotypes or in polyploids in many eukaryotic organisms [von Wettstein et al., 1984]. Although to different extents, all these aspects have been approached in bird oocytes and spermatocytes using this methodology.

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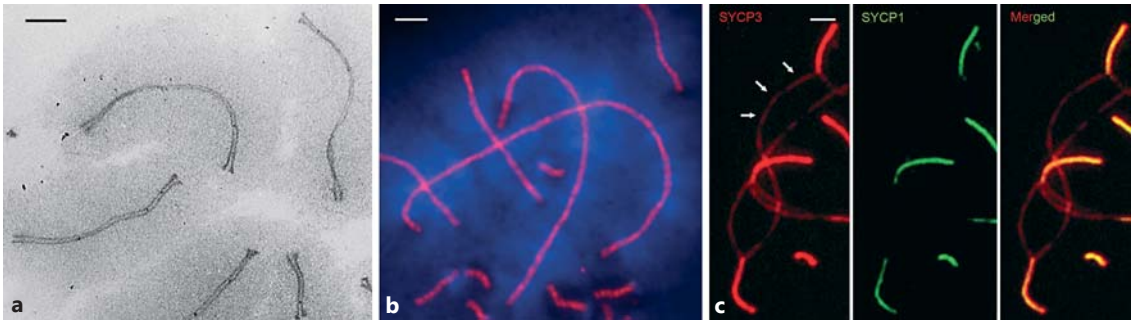

Fig. 1. a, **b** Hypotonically decondensed chromatin extends from the synaptonemal complexes (SCs). **a** Electron micrograph showing a partial view of a silver-stained SC spread. **b** Spread oocyte immunostained with an antibody against the lateral elements of the SC. The chromatin is counterstained with DAPI. **c** Immunostaining with antibodies against SC components raised against the orthologous mouse proteins. Anti-SYCP3 recognizes a protein of the lateral elements and anti-SYCP1 is specific against a component of the transverse fibrils. SYCP1 is present at the synapsed regions, but it is absent from regions that have not reached synapsis (arrows). Scale bars, 1 μm.

Lampbrush studies in combination with molecular cytogenetics have proved to be exceptional tools to integrate chromosomal and genomic studies, to investigate functional features of the ZW pair, and to analyze transcriptional activity at the cytological level in great detail. Different aspects of LBC investigation in birds have been previously reviewed by experts in the field [Mizuno and Macgregor, 1998; Gaginskaya et al., 2009], but they will be highlighted here in the context of particular aspects of avian female meiosis.

This review will focus on the main contributions of cytological investigations to features of avian meiosis such as patterns of crossing over, the impact of karyotype variations on the onset of the meiotic prophase, and a comparative view of the meiotic ZW pair of birds and the XY pair of mammals.

The Organization of Bivalents during the Meiotic Prophase

After chromosome duplication at the premeiotic S phase, the homologous chromosomes consist of 2 co-linear proteinaceous axes from which chromatin loops emerge (Fig. 1a). After completion of alignment and synapsis, the homologs are held by the SC, a tripartite structure consisting of 2 parallel lateral elements, 1 central element, and numerous transverse filaments. The sequential changes of meiotic axes and SCs in birds, along with the synaptic nature of the ZW pair, were first shown in electron microscope observations of microspread oocytes from pre- and post-hatched chickens [Solari, 1977].

The structure of the SC and its meiotic functions are similar in phylogenetically distant species, but molecular characterization showed that not all SC components are equally conserved at the protein sequence level [Yang and Wang, 2009; Fraune et al., 2016]. The conservation of some SC protein components between birds and mammals was apparent from immunocytological analyses showing that antibodies against mammalian SYCP3 and SYCP1 proteins labeled the meiotic axes in avian meiocytes [Pigozzi and Solari, 2005; Calderón and Pigozzi, 2006] (Fig. 1b, c). Subsequently, sequence alignment and expression analyses showed that the 3 major SC proteins SYCP1, SYCP2, and SYCP3 share basic structural features in birds and mammals and are present in chicken gonads at the moment of meiosis [Zheng et al., 2009]. The percentage identity between chicken and mammalian SYCP proteins over the entire alignment indicates different levels of conservation, with the highest similarity for SYCP3 [Zheng et al., 2009]. In addition to specific SC proteins, the cohesin component SMC3 is also present on the meiotic axes of chickens and other birds from leptotene to late diplotene [Pigozzi, 2008; del Priore and Pigozzi, 2012] and is a component of the chromatin axes of lampbrush bivalents in the period of pre-vitellogenesis [Krasikova et al., 2005].

The organization of meiotic chromosomes in the context of the SC leads to the formation of chromatin loops arranged along a structural axis (Fig. 1a). Clear visualization of these loops has been exceptionally achieved in a few species using electron microscopy [Keyl, 1975; Weith and Traut, 1980; Zickler and Kleckner, 1999]. Still, there is sufficient evidence to support the idea that loop sizes

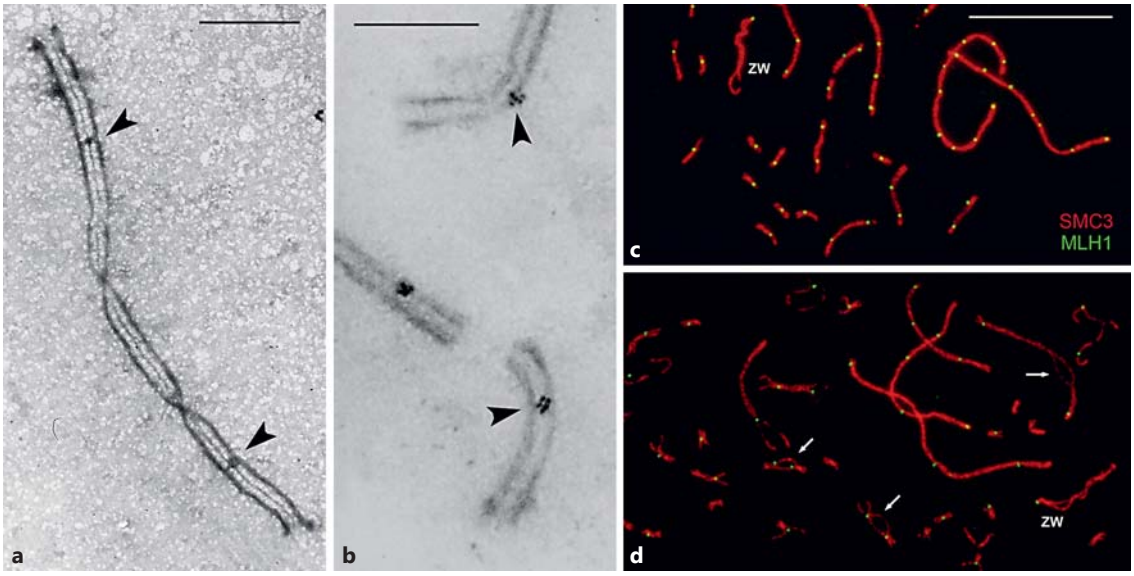

Fig. 2. Crossover markers in pachytene **a** Synaptonemal complex from a spread chicken oocyte stained with phosphotungstic acid (PTA). Two small ellipsoidal recombination nodules (RNs) can be observed (arrowheads). **b** Multipartite RNs in a region of a pigeon oocyte stained with PTA. Scale bars, 1 μm. **c**, **d** MLH1 foci in chicken oocytes. The shortest microbivalents usually have one focus. During early diplotene, foci remain at convergent sites of the lateral elements that start to separate (arrows). A single focus, equivalent to one crossover, is present at the homologous end of the ZW pair. Scale bar, 10 μm. Image in **b** reproduced with permission from Canadian Science Publishing.

vary dramatically among evolutionarily distant organisms but remain constant within a species [reviewed in Zickler and Kleckner, 1999]. Measurements of SC length and DNA content in different vertebrate taxa showed that SC/DNA ratios in birds were approximately twice as high as the SC/DNA ratios of reptiles and mammals [Peterson et al., 1994]. These observations suggest that chromatin loops along pachytene chromosomes are shorter in birds than in other vertebrates and, consequently, that birds build longer SCs than would be expected on the basis of their DNA content. Considering that loop density along meiotic axes is relatively constant [Zickler and Kleckner, 1999; Kleckner, 2006], it is feasible that birds have more numerous shorter loops along meiotic axes than other animals. As a consequence, a larger fraction of DNA will be in contact with meiotic axes in birds than in mammals, a feature that may be related to a higher number of crossovers (CO) per SC unit in birds [Peterson et al., 1994].

Crossover Markers along Avian SCs

Meiotic recombination is the consequence of the repair of programmed DNA double strand breaks (DSBs) generated by the protein Spo11. DSB formation and resection recruit the phosphorylated histone variant γ-H2AX in a process that is specifically correlated with immunostaining of RecA homologs, Rad51 and Dmc1 [reviewed in Hunter et al., 2001]. In agreement with the conserved nature of these mechanisms, γ-H2AX is also present on the asynapsed axes of avian meiocytes during leptotene and zygotene, disappearing after completion of synapsis in pachytene [Schoenmakers et al., 2009]. The repair of any given meiotic DSB is a complex process that ends in CO or non-CO configurations as possible outcomes. In pachytene meiocytes of birds, CO events can be identified along SCs using 2 cytological approaches: the visualization of recombination nodules (RNs) by transmission electron microscopy or by immunodetection with antibodies directed against proteins specifically involved in CO formation (Fig. 2).

RNs represent all the CO events present along pachytene bivalents and are distinct structures on avian SCs, as first shown in chicken oocytes [Rahn and Solari, 1986]. In the chicken and in most birds, RNs are observed as ellipsoidal structures, 120–140 nm in diameter, associated with the central region of SCs in microspread nuclei (Fig. 2a). A particular morphology for RNs was initially described in the domestic pigeon (Fig. 2b), where RNs comprise 1–5 particles surrounded by a grayish interpar-

Table 1. Global crossover rates in birds calculated from recombination nodules (RN) or MLH1-focus counts

Species	Foci/RNs	cM	DNA content, Mb	CO rate, cM/Mb	References
Gallus domesticus	64	3,200	1,222.5	2.6	Pigozzi [2001]
Coturnix japonica	53	2,653	1,320.3	2.0	del Priore and Pigozzi [2015]
Numida meleagris	44	2,219	1,281.2	1.7	Pigozzi, unpubl. data
Anas platyrhynchos	56	2,795	1,408.3	2.0	del Priore and Pigozzi [2016]
Columba livia	63	3,135	1,427.9	2.2	Pigozzi and Solari [1999a, b]
Taeniopygia guttata	46	2,285	1,222.5	1.9	Calderón and Pigozzi [2006]
Lonchura domestica	48	2,415	1,388.7	1.7	Pigozzi, unpubl. data

CO, crossover; RN, recombination nodule.

ticle matrix [Pigozzi and Solari, 1998a]. This multipartite substructure of RNs was evident in other species from 3 different orders [Pigozzi, unpubl. data], indicating an extended feature among birds that might be present in other organisms as well. Detailed analysis in pigeon oocytes and spermatocytes revealed that the number of particles increases from zygotene to late pachytene, probably representing the progressive assembly of the protein machinery present at late steps of crossing over. It remains to be analyzed whether each particle has a unique composition or if they are mixed molecular complexes of the proteins known to be part of RNs [Marcon and Moens, 2003].

Immunodetection of the mismatch repair protein MLH1 along SCs provides an alternative method to count COs along pachytene bivalents of birds (Fig. 2c, d). MLH1 is required for wild-type levels of crossing over during meiosis and is a component of RNs [Marcon and Moens, 2003; Anderson et al., 2014]. Current evidence in yeast and other organisms indicates that MLH1 foci tag the CO events showing chiasmatic interference, while a second type of COs (non-interfering) follows a molecular pathway lacking MLH1 [reviewed in Mezard et al., 2015]. As shown in a number of plants and animals, the proportion of non-interfering COs varies from species to species, accounting for 5–30% of all CO events [de Boer et al., 2006; Falque et al., 2009]. The presence of 2 classes of COs in birds has not yet been tested, but both RNs and MLH1 foci show CO interference in birds [Pigozzi and Solari, 1997; Pigozzi, 2001]. In addition, comparison of the number of MLH1 foci and chiasmata in oocytes of the same species favors the view that MLH1 foci account for most recombination events in birds [Rodionov et al., 1992; Pigozzi, 2001; Rodionov and Chechik, 2002; del Priore and Pigozzi, 2015].

Chiasma maps of the crossing over in oocytes showed that global recombination rates in the chicken were higher than in humans [Rodionov et al., 1992]. The higher levels of recombination in birds than in mammals are also evident from direct counts of RNs or MLH1 foci in 7 species that represent 4 different orders (Table 1). These analyses also indicate that interspecific variations of global recombination rates are less pronounced in birds: the mean recombination rate of the birds studied so far is in the range of 1.7–2.6 cM/Mb (Table 1), while in eutherian mammals it is 0.5–1.1 cM/Mb [Dumont and Payseur, 2008]. Global recombination frequencies estimated from RN or MLH1 mapping in quail, pigeon, and zebra finches indicate that intersex differences are less noticeable in birds than in mammals. It should be pointed out, however, that subtle heterochiasmy was found using high-density linkage maps in chicken, turkey, and the great tit [van Oers et al., 2014 and references therein]. In these species, certain chromosomes have longer maps in males, while other chromosomes have longer maps in females, even though genome-wide recombination levels are similar between the sexes.

As for the intrachromosomal variations in recombination, RN- and MLH1-focus mapping revealed differences in CO distribution along chromosome arms in the largest bivalents. In most cases, metacentric/submetacentric chromosomes show higher amounts of recombination at telomeric regions, a deficiency around the centromere (within 1 μm or less), and a bimodal or a multimodal distribution along the chromosome arms. In contrast, the acrocentric bivalents have pronounced recombination peaks close to the proximal region and the telomere of the long arm [Rahn and Solari, 1986; Pigozzi and Solari, 1999a, b; del Priore and Pigozzi, 2015; Pigozzi and del Priore, 2016]. These observations support the notion that

the primary chromosome structure plays a role in the distribution of crossing over at a coarser level. Extreme accumulation of COs toward opposite ends along macrobivalents was reported among passerine birds [Calderón and Pigozzi, 2006; Pigozzi, 2008; Backström et al., 2010], and the same pattern is observed in 2 unrelated species [Pigozzi, unpubl. data]. It is known that sequence content, epigenetic modifications, along with hot spot density and strength, act to modulate CO levels throughout genomes [reviewed by Mezard et al., 2015]. Recently, high resolution analysis of recombination events in 2 finches demonstrated that, in contrast to mammals, meiotic recombination hotspots appear to be highly conserved over broad periods of evolutionary time [Singhal et al., 2015]. Interestingly, the authors also detected broad scale changes in recombination in certain chromosome regions across the same phylogenetic range for which hotspot conservation is observed. These variations are not explained by chromosomal changes in the analyzed species, so they might respond to variations of hotspot location or strength. More analyses in nondomestic species from different phylogenetic groups are necessary to better characterize the evolutionary processes underlying CO rates and distribution among birds.

The Effects of Abnormal Chromosome Complements on Avian Meiosis

Chromosome anomalies and departures from euploidy in birds have been studied mainly in the chicken. Analyses of experimentally induced chromosome aberrations suggest that gonosome-autosome translocations have moderate consequences on the progress of meiosis in birds. Z-autosome translocations do not significantly affect sperm production in heterozygous cockerels [Dinkel et al., 1979; Blazak and Fechheimer, 1980], and females carrying a reciprocal translocation between the Z and a microchromosome show comparable fertility to normal hens, even though embryo mortality is significantly increased [Telloni et al., 1977]. Electron microscopy of the SCs has shown that Z-autosome translocation carriers form quadrivalents and other configurations with unpaired chromosomes during pachytene in males and females [Solari et al., 1988; Solari, 1989]. In spite of the presence of asynaptic segments in translocation carriers, no evidence of gametogenic failure was reported in these cases [Solari, 1989]. In many species, meiocytes that are defective in homologous recombination or chromosome synapsis are delayed or arrested in the pachytene stage [Roeder and Bailis, 2000; de Rooij and de Boer, 2003]. As previously mentioned, fertility studies indicate that progression beyond pachytene does not seem to be compromised in hens carrying translocations in spite of synaptic defects. These observations along with the existence of a highly error-prone Z-W synapsis in chromosomally normal chickens (see section The ZW Pair during Meiosis) suggests that either a pachytene checkpoint mechanism does not operate during avian oogenesis or that it does exist but the extent of asynapsis in the studied cases is not sufficiently high to trigger it. It should also be pointed out that observations of the fate of oocytes are more limited, because the cells arrest at the end of prophase, and progression to the first meiotic division is subsequent to selection that depends on follicular growth before ovulation. It is possible that oocytes with synaptic defects are subject to elimination at later stages to avoid the formation of unbalanced gametes.

Compared to translocations, alterations of euploidy trigger more drastic consequences in meiosis and fertility. Triploidy occurs in up to 3.3% of chicken embryos and is a known cause of embryo mortality in birds [Forstmeier and Ellegren, 2010]. In domestic chickens, spontaneous triploidy is well documented [de Boer et al., 1984; Bonaminio and Fechheimer, 1993] with viable, but infertile ZZZ and ZZW gonosomal types. Lin et al. [1995] reported that ZZZ birds have irregular meiosis and produce abnormal spermatozoa, but the behavior of chromosomes during meiosis was not described. In triploid ZZW chickens, there is a prevalence of triple synapsis noticeable in the form of microchromosomal triplets. The macrochromosomes, on the other hand, show triple synapsis at some chromosome ends but retain significantly long asynapsed segments in central regions because of the extensive occurrence of interlockings and hookings of the axial elements [Solari et al., 1991]. Competition in sex chromosome pairing results in the predominance of Z-Z synapsis and a univalent W because of the relatively minor homology between the Z and the W chromosome in this species. In ZZW triploids, oocytes start to degenerate 1 week after hatching, and most of the oocytes had disappeared by about 3 weeks of age [Lin et al., 1995]. It was suggested that the univalent state of the W chromosome at the beginning of diplotene could be responsible for this oocyte loss, but the presence of interstitial asynapsis along macrobivalents cannot be ruled out as a possible cause.

In 2 estrildid passerines – the zebra finch and the Bengalese finch – there is a large germline-restricted chromosome that remains univalent in spermatocytes. In both

species, the univalent chromosome is entirely heterochromatic and presumably inactive during the entire meiotic prophase [Pigozzi and Solari, 1998b, 2005; del Priore and Pigozzi, 2014]. From early prophase, the single chromosome presents histone modifications related to chromatin condensation and gene repression, such as histone H3 di- and tri-methylated at Lys9 or HP1β, but it is mostly devoid of the DSB marker γ-H2AX [Goday and Pigozzi, 2010; Schoenmakers et al., 2010; del Priore and Pigozzi, 2014]. These chromatin modifications support the idea that the meiotic silencing of these unique chromosomes occurs before chromosome pairing and is independent of DNA DSBs [Schoenmakers et al., 2010]. In any case, this chromatin inactivation is a distinctive phenomenon, unrelated to mammalian meiotic silencing of unsynapsed chromatin, which occurs comparatively later at the zygotene/pachytene transition, in connection with the timing of unsynapsed configurations [Turner et al., 2005; Burgoyne et al., 2009].

Avian Lampbrush Chromosomes in Cytogenetic and Genomic Research

Chromosomes in the form of lampbrushes are present in the growing oocytes of many animals. Each LBC comprises 2 homologous chromosomes with a characteristic chromomere-loop organization in which loops represent sites of active transcription. Each homolog consists of a linear array of numerous compact chromomeres from which laterally projecting loops arise [reviewed in Callan, 1986]. LBCs of birds were introduced as a model in cytogenetic studies in the mid-1980's [Kropotova and Gaginskaya, 1984; Hutchison, 1987] and have since become effective sources in cytogenetics and genomic research. In the chicken, as well as in other species, macro-LBCs can be individually identified by their length and by the morphological characteristics of marker loops and other cytological markers [Rodionov and Chechik, 2002; Schmid et al., 2005; Daks et al., 2010]. Chiasmata are easily identifiable, so it is possible to estimate map distances between chromosome landmarks along the largest LBCs. The chiasma-based genetic distances on chicken LBC 1 demonstrated an overestimation of experimental distances in the genetic linkage map during the first stages of linkage map construction in the chicken [Rodionov et al., 2002]. Because LBCs are 20–30 times longer than the corresponding mitotic metaphase chromosomes, FISH signals give more precise maps of the location of genes or repetitive elements than mitotic chromosomes [Galkina et al., 2006; Solinhac et al., 2010]. This approach revealed with great detail the boundaries of chromosome rearrangements on certain chicken, quail, and turkey macrochromosomes during karyotype evolution in Galliformes, showing low levels of inter- and intra-chromosomal rearrangements between these species [Galkina et al., 2006; Griffin et al., 2008]. Moreover, a combined approach including microdissection, FISH-probe generation, and sequencing provided a means to assign cytological markers of lampbrush chromosomes to genomic coordinates [Zlotina et al., 2016].

Associated with the LBCs in all birds studied so far, there is a type of transcriptionally inactive structure called protein bodies (PBs), showing a unique molecular composition that turns them into a novel type of intranuclear bodies [Gaginskaya et al., 2009]. PBs lack RNA polymerase II and splicing snRNP, which are typical components of transcriptionally active loops. Instead, they contain proteins of cohesin complexes such as Rad21 and STAG2 and are rich in SYCP3 [Krasikova et al., 2004, 2005]. During the post-lampbrush stage of the germinal vesicle, prior to nuclear envelope breakdown, centromere PBs associated with condensing bivalents fuse to form a karyosphere protein core [Gaginskaya et al., 2009]. At this stage, PBs participate in the 3-dimensional arrangement of chromosomes, leading to congregation of the bivalents in a limited area. Because of this role in chromosome arrangement before cell division and their molecular composition, it was proposed that they may facilitate the correct segregation of parental chromosomes in the large germinal vesicle of birds [Rutkowska and Badyaev, 2008].

Direct evidence of the transcription of protein-coding genes in avian LBCs is lacking. In the chicken, clustered histone genes are inactive at the lampbrush stage [Krasikova et al., 2012], and transcription of ribosomal RNA in the germinal vesicle is repressed, so nucleoli are absent in avian diplotene-stage oocytes [Gaginskaya, 1972]. Moreover, no protein-coding sequences were found to be transcribed from the gene-rich microchromosomes. It should be mentioned, though, that numerous single copy genomic sequences were found to be actively transcribed on the lateral loops of avian LBCs with DNA/RNA hybridization protocols using BAC clones that contain both coding and noncoding sequences [Galkina et al., 2006; Gaginskaya et al., 2009].

The absence of transcription of protein-coding genes contrasts with the active transcription of noncoding sequences. It has been shown that avian LBCs have very long transcription units, with deregulated termination,

Table 2. Avian taxa in which recombination in the ZW pair has been assessed using cytological methods

Order	Species	CO marker	CO location	Reference
Palaeognathae				
Rheiformes	*Rhea americana*	RN	distal 80% of Wq	Pigozzi and Solari [1997]
	Rhea pennata	RN	distal 80% of Wq	Pigozzi and Solari [1999c]
Tinamiformes	*Rhynchotus rufescens*	RN	distal 60% of Wq	Pigozzi and Solari [1999c]
	Eudromia elegans	MLH1 focus	distal 25% of Wq	Pigozzi [2011]
	Nothura maculosa	RN	distal 25% of Wq	Pigozzi and Solari [2005]
	Crypturellus tataupa	MLH1 focus	subterminal, localized	Pigozzi [2011]
Neognathae				
Anseriformes	*Anas platyrhynchos*	RN MLH1 focus	subterminal, ZqWq	Solari and Pigozzi [1993]; del Priore and Pigozzi [2016]
Galliformes	*Gallus domesticus*	RN chiasma LBC MLH1 focus	terminal, ZpWp	Rahn and Solari [1986]; Solovei et al. [1993]
	Coturnix japonica	RN chiasma LBC MLH1 focus	terminal, ZpWp	Solari [1992]; Rodionov and Chechik [2002]
	Meleagris gallopavo	chiasma LBC	terminal	Solovei et al. [1993]
	Numida meleagris	RN	terminal, ZpWp	Solari and Pigozzi [1993]
Columbiformes	*Columba livia*	RN chiasma LBC	subterminal, ZqWq	Pigozzi and Solari [1999b]
Passeriformes	*Fringilla coelebs*	chiasma LBC	terminal	Saifitdinova et al. [2003]
	Taeniopygia guttata	RN MLH1 focus	subterminal, ZpWp	Pigozzi and Solari [1998b]
	Lonchura domestica	MLH1 focus	terminal, ZpWp	del Priore and Pigozzi [2014]
	Passer domesticus	chiasma LBC	terminal	Solovei et al. [1993]

CO, crossover; LBC, lampbrush chromosomes; RN, recombination nodules.

and abundant transcription of noncoding satellite repeats. Each chromomere is composed of numerous miniature loops and a few extended loops consisting of single or multiple transcription units of different lengths and polarities [Kropotova and Gaginskaya, 1984; Hutchison, 1987]. DNA/RNA-transcript in situ hybridization determined that many transcripts correspond to noncoding DNA including telomeric TTAGGG repeat blocks, Z-macrosatellite repeats, pericentromeric satellites, and subtelomeric repeats [Solovei et al., 1994, 1996; Hori et al., 1996; Deryusheva et al., 2007; Kulikova et al., 2016]. It has been proposed that the noncoding RNA product of satellite repeat transcription can hybridize to form long dsRNA molecules. These duplex-forming RNAs sequestered within the oocyte might provide a pool for the production of siRNAs, required for functional regulation during early stages of embryogenesis [Krasikova et al., 2012; reviewed in Gaginskaya et al., 2009].

The ZW Pair during Meiosis

Cytogenetic analyses of avian female meiosis revealed important information about the ZW pair, such as the existence of synapsis, the region over which recombination can occur, and the absence of meiotic sex chromosome inactivation. The synaptic nature and the existence of a recombining region in the ZW pair were first described in pachytene oocytes of chicken [Solari, 1977; Rahn and Solari, 1986]. In the same species, electron microscopy analysis of multivalents carrying 1 of 2 Z-autosome translocations showed that the short arm of the W chromosome synapses with the arm of the Z chromosome opposite to the telomere C-band, shedding light about the position of the pseudoautosomal region (PAR) in this species [Solari et al., 1988]. Since then, the position and extent of the PAR has been characterized at a cytological level from the primitive ratites to the more recent-

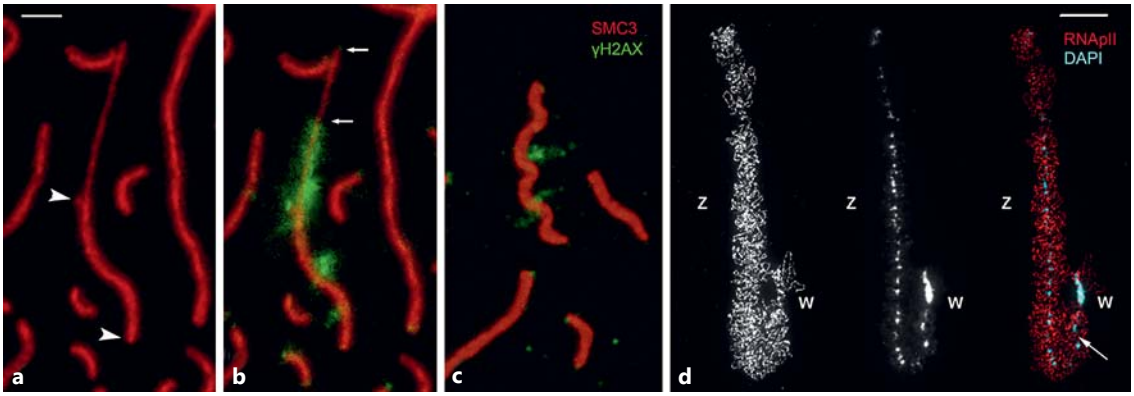

Fig. 3. The ZW pair during pachytene and at the lampbrush stage. **a–c** Pachytene ZW pairs of chicken at pachytene. **a** The Z and W axes are completely synapsed. The arrowheads mark the synaptic segment which has the length of the W chromosome. Scale bar, 1 μm. **b** Double-strand breaks, signaled by the presence of γ-H2AX, predominate at the nonsynapsed region of the Z chromosome. The arrows mark the heterochromatic end of the Z chromosome where the breaks are absent. **c** Absence of chromatin-wide H2AX phosphorylation in the ZW pair. Phosphorylated H2AX only persists at 2 spots that disappear when the ZW pair starts to desynapse. **d** ZW lampbrush sex bivalent of the Japanese quail. The Z and W sex chromosomes form an asymmetrical bivalent with a single chiasma (arrow). Chromosomes are counterstained with DAPI, which shows the blue chromomeres of the Z axis and the condensed W chromosome. In the chicken ZW bivalent, the Z chromosome exhibits a normal lampbrush morphology, whereas the W chromosome is predominantly condensed and packed into several dense chromomeres with only a few lateral loops. The right hand image shows immunodetection of an elongating form of RNA polymerase II with hyperphosphorylated C-terminal domain in the axes of lateral loops. Scale bar, 10 μm. Lampbrush image by courtesy of Alla Krasikova.

ly evolved Neognathae (Table 2). Ratites show the least differentiated Z/W chromosomes, with the PAR comprising about 80% of the long arm of the W chromosome. In contrast, all the neognaths analyzed so far have a single CO, located toward one end of the sex bivalent. A special case is observed among tinamous – the closest living birds to ratites – in which the length of the PAR varies significantly in the 4 species analyzed. In spite of its limited resolution, cytological analysis of the crossing over between the Z and W chromosomes suggested that the small PAR of neognaths could be variable in extent and in sequence content [Pigozzi, 1999]. Extensive genomic comparison of 17 species of birds confirmed this idea, revealing remarkable diversity in the composition of the PARs and in the degree of Z/W differentiation throughout avian phylogeny [Zhou et al., 2014].

The highly heteromorphic ZW pair of most birds shares morphological features and evolutionary mechanisms with the XY pair of eutherian mammals. During meiosis, however, the sex pairs of birds and mammals show morphological and functional differences in the heterogametic sex. During avian oogenesis, the ZW pair reaches a stage of complete synapsis that is mainly heterologous, except at the PAR region. In contrast, the nonhomologous or differential regions of the X and Y chromosomes of mice and other mammals remain largely unsynapsed at prophase I. In addition, the chromatin associated with the ZW pair shows similar condensation to that from autosomal pairs, while the XY pair becomes condensed to form the XY body [reviewed in Solari, 1974]. The XY body is the morphological manifestation of the process of meiotic sex chromosome inactivation (MSCI), which correlates with transcriptional silencing of genes located in the asynapsed segments of the X and Y chromosomes [Turner et al., 2004; Baarends et al., 2005]. In the chicken, a combined analysis of protein markers of MSCI and gene expression during the meiotic prophase could not find evidence of an inactivation of sex-linked loci, and the presence of repressive marks such as H3K9me3 are limited to the W chromosome and the terminal heterochromatin of the Z chromosome [Guioli et al., 2012; Pigozzi unpubl. data]. In contrast to the XY body, chromatin-wide H2AX phosphorylation is absent in the ZW pair, and its presence is compatible with the timing of DSB occurrence and processing (Fig. 3a, b). Altogether these observations support the notion that MSCI is absent in the avian ZW pair.

At the lampbrush phase, the ZW pair of chicken and other birds is observed as a highly asymmetrical bivalent with the typical organization of lateral loops (Fig. 3c).

Only the terminal one-fifth, representing the W chromosome, is relatively thick and condensed and bears only a few pairs of long loops. The elongating form of RNA polymerase II is present in the transcriptionally active loops, similarly to the autosomal bivalents at this stage (Fig. 3c).

It was reported that the Z and W chromosomes fail to achieve synapsis and repair of DSBs in one-fifth of the oocytes in the chicken [Guioli et al., 2012]. This proportion of nonsynaptic Z/W chromosomes is very high compared to male mice, in which X/Y asynapsis is about 4–9% [Rodriguez and Burgoyne, 2000]. The presence of an unsynapsed bivalent and the unrepaired DSBs along the Z chromosome does not seem to affect the meiotic process in these oocytes that nevertheless carried over to diplotene [Guioli et al., 2012]. Further research is necessary to determine whether the high level of asynapsis of the Z/W chromosomes found in this particular chicken stock is a widespread feature in the species and if it is also present in other birds with highly differentiated ZW pairs.

Concluding Remarks

Cytological analysis of avian meiosis has provided substantial information about chromosome structure and function. Among the many aspects that remain to be explored are the changes of chromatin loop organization from pachytene to the lampbrush stage. Birds offer a great model to investigate this issue, because they have small genomes and chromosomes compared to amphibians that also develop lampbrush chromosomes. Another matter that requires further research is the existence of differential quality surveillance mechanisms during pachytene in birds compared to other organisms. Altogether, the comparatively mild effects of asynaptic chromosomes or chromosome segments in translocation heterozygotes, the asynaptic univalent in male finches, and the presence of an elevated number of asynaptic Z and W chromosomes in the chicken without immediate consequences of the progression of meiosis raise questions about the conservation of pachytene checkpoints among vertebrates that are responsible for monitoring crucial prophase events in other organisms. To unveil the fine machinery controlling complex processes such as synapsis and crossing over will require a multiplicity of approaches. The generation of chickens genetically modified for meiotic genes in combination with cytological studies of chromosome dynamics can help unravel the molecular mechanisms underlying these events. The expression levels of Z-linked genes during female prophase I also need a more comprehensive analysis using purified populations of oocytes highly enriched for specific meiotic stages and high throughput methods to measure gene expression. Together with many other aspects of meiosis, these issues will provide plentiful ground for future investigations of meiotic chromosomes in birds.

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Disclosure Statement

The author has no conflicts of interest to declare.

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