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The XY Body of the Cat (*Felis catus*): Structural Differentiations and Protein Immunolocalization

Roberta B. Sciurano[a, b] Geraldine De Luca[a] I. Mónica Rahn[a, b]
Alberto J. Solari[a, b]

[a] 2da. U.A. Biología Celular, Histología, Embriología y Genética, Facultad de Medicina, Universidad de Buenos Aires, and [b] CONICET, Buenos Aires, Argentina

Keywords
Cats · Meiosis · Protein markers · Synaptonemal complex · XY body

Abstract
The heteromorphic X and Y chromosomes behave in a special way in mammalian spermatocytes; they form the XY body and synapse only partially. The aim of this article was to study the origin and the role of the special differentiations in the XY pair of the domestic cat during pachytene by analyzing its fine structural characteristics and the immunolocalization of the main meiotic proteins SYCP3, SYCP1, SYCE3, SMC3, γ-H2AX, BRCA1, H3K27me3, and MLH1. The cat XY body shows particularly striking structures: an extreme degree of axial fibrillation in late pachynema and a special location of SYCP3-containing fibrils, bridging different regions of the main X axis, as well as one bridge at the inner end of the pairing region that colocalizes with the single mandatory MLH1 focus. There are sequential changes, first bullous expansions, then subdivision into fibrils, all involving axial thickening. The chromatin of the XY body presents the usual features of meiotic sex chromosome inactivation. An analysis of the XY body of many eutherians and metatherians suggests that axial thickenings are primitive features. The sequential changes in the mass and location of SYCP3-containing fibers vary among the clades because of specific processes of axial assembly/disassembly occurring in different species.

During the first meiotic prophase, a supramolecular structure that holds the homologous chromosomes together is formed: the synaptonemal complex (SC) [Moses, 1956, 1968; reviewed in Yang and Wang, 2009]. The general structure of the SC is highly conserved in almost all sexually reproducing organisms, yeasts [Dresser and Giroux, 1988; reviewed in Loidl, 2003], plants [reviewed in Hamant et al., 2006], and metazoans [reviewed in Fraune et al., 2016]. However, the amino acid sequences of the involved proteins are highly divergent [reviewed in Cahoon and Hawley, 2016]. At the ultrastructural level, the SC is a large, zipper-like scaffold, basically composed

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Roberta B. Sciurano, PhD, MSc
2da. U.A. Biología Celular, Histología, Embriología y Genética
Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Piso 10
Buenos Aires C1121ABG (Argentina)
E-Mail roberta_sciur@hotmail.com

Table 1. XY-pair configurations in pachytene spermatocyte spreads: pearl-necklace differentiations without/with SC formation, thickened axes and SC formation, and connected (by a bridge), separated, and tangled XY axes

XY pair	Type I	Type II	Type III	Type IV	Type V	Type VI	Total
Absolute number, *n*	2	11	21	83	33	79	229
Frequency, %	0.9	4.8	9.2	36.2	14.4	34	100

of 2 lateral elements (LEs) that run parallel along the maternal and paternal chromosomal axes, a central element, and transverse filaments that span from the central element to each lateral element. The assembly and disassembly of the SC are stepwise processes associated with the progression of the first meiotic prophase. Following the induction of programmed double-strand breaks and resection through the SPO11 protein complex in leptonema [Mahadevaiah et al., 2001], which label the initiation of recombination processes, the assembly of the SC begins. The progression of SC assembly is closely associated with the dynamic maturation of recombination nodules during prophase I [Moens et al., 2007].

The X and Y chromosomes behave in a special way during the first meiotic prophase in mammalian spermatocytes [reviewed in Solari, 1994 and in Handel, 2004]. The heteromorphic X and Y chromosomes generally synapse only partially [Solari, 1974] at the pseudoautosomal region [Burgoyne, 1982] leaving 2 unpaired differential regions. Moreover, both chromosomes form a well-known subnuclear structure characterized by the special compaction of its chromatin fibers, the XY body [Solari, 1974; reviewed in Handel, 2004]. The structural components of the SC of the XY pair undergo stepwise changes along pachytene substages in mammals. Thickening and splitting of the unpaired regions of the X and Y axes are the most frequent variations [Solari, 1970, 1974; Moses, 1977; del Mazo and Gil-Alberdi, 1986; Solari et al., 1993; Echeverría et al., 2003; Page et al., 2006a; Sciurano et al., 2006, 2012, 2013]. Furthermore, those differentiations allow the staging of pachynema in 5 different substages in human spermatocytes [Solari, 1980].

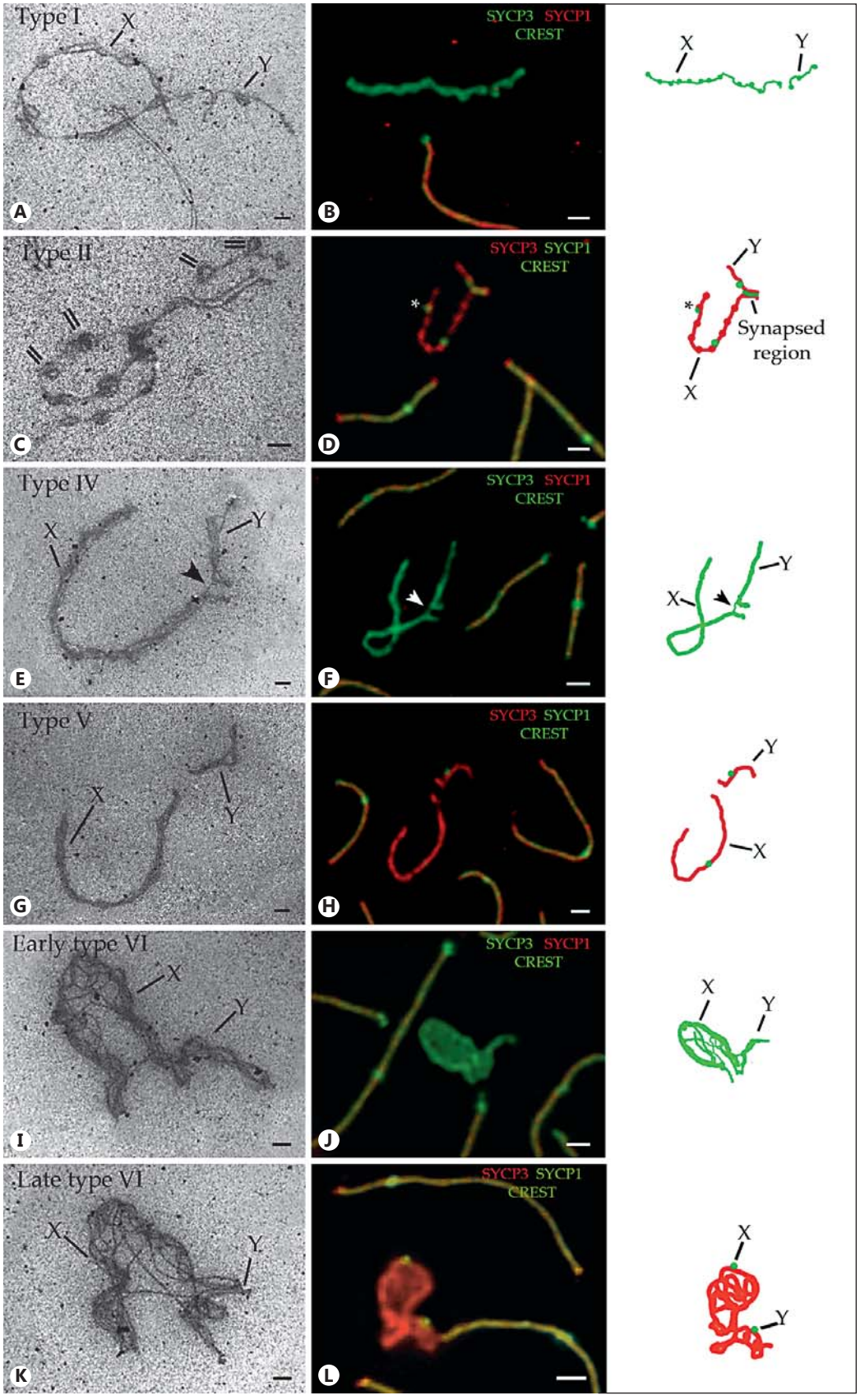
In cats, Gillies and Cowan [1985] observed that the XY pair exhibits a variety of changes in spermatocyte spreads. However, the authors did not describe the different substages of pachynema and did not establish a definite sequence of changes in these peculiar sex chromosomes during the first meiotic prophase.

The aim of the present work was to analyze the structural and functional variations of the XY pair in the domestic cat that differ from other mammals and pose new questions. Additionally, the location of several structural meiotic proteins in this supramolecular structure is discussed.

Materials and Methods

Testes from 4 adult European shorthair cats and 1 Siamese cat were surgically removed by veterinarians during normal castration operations at the Instituto de Zoonosis "Luis Pasteur" (Buenos Aires, Argentina). For each animal, 2 pieces of tissue were processed for semi-thin (0.5 μm) and thin (0.08 μm) sections to analyze the seminiferous epithelium in detail by light microscopy and the ultrastructure of the XY body in spermatocyte nuclei by electron microscopy (EM), respectively. Another piece of tissue was used to perform spermatocyte spreads for SCs as described by Sciurano and Solari [2014]. Some slides were stained with 4% phosphotungstic acid in ethanol or silver nitrate, and others were kept at −70°C until use for immunofluorescence techniques. To obtain electron micrographs, a Zeiss EM 109T equipped with a digital camera Gatan171 ES1000W from the LANAIS' service (CONICET) was used.

For immunolocalization of meiotic proteins (except for γ-H2AX and H3K27me3), an epitope retrieval was performed in 0.01 M sodium citrate buffer (pH 6) before the blocking step [Sciurano and Solari, 2014]. The following primary antibodies were incubated at 4°C: rabbit anti-SYCP1 at a dilution of 1:100 (Abcam Ltd., Cambridge, UK) in PBS; rabbit anti-SYCP3 at 1:100 (Abcam Ltd.); rabbit anti-SYCE3 at 1:200 (Prof. M. Alsheimer, University of Würzburg, Germany); rabbit anti-SMC3 at 1:200 (Millipore Corp., USA); mouse anti-MLH1 at 1:10 (BD Pharmingen, USA); CREST serum at 1:300 (Laboratorios IFI, Buenos Aires, Argentina); and rabbit anti-BRCA1 (C20, Santa Cruz Biotechnology, CA, USA) at 1:10. Mouse anti-γ-H2AX antibody (Abcam Ltd.) at 1:400 and rabbit anti-H3K27me3 at 1:100 (Abcam Ltd.) were incubated at 37°C. All incubations were performed overnight in a humid chamber. After washing, the corresponding secondary antibodies were incubated at



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(For legend see next page.)

Table 2. Data on the X and Y axes and the SC in spermatocytes of the cat

	X chromosome axis[a]		Y chromosome axis[a]		Total SC length[b], μm	Distance from Y telomere[c], μm	
	total length, μm	CI	total length, μm	CI		SYCP3-bridge	MLH1 focus
Mean	10.4	0.45	3.2	0.44	1.4	0.9	0.8
SD	2	0.03	0.5	0.03	0.3	0.2	0.1
Range	7.3–14.1	0.38–0.50	2.6–4.3	0.38–0.49	1.0–1.9	0.5–1.1	0.5–1.0

CI, centromeric index; SC, synaptonemal complex. [a] $n = 12$. [b] In early pachytene stages (Type-II/III XY pair). [c] $n = 30$.

the specified dilutions for 2 h. Slides were examined using a Leica DM microscope (Leica Microsystems, Wetzlar, Germany) and photographed with a Leica DFC 300 FX digital camera (Cambridge, UK).

Single images were processed using the program Adobe Photoshop CS (Adobe Systems Inc., USA). The distance of the MLH1 foci and that of the bridge in the pairing region from the telomere of the XY pair, the length of each sex chromosome, and the SC length in the XY pair were measured using the freeware computer application MicroMeasure version 3.3 [Reeves, 2001].

Results

Synaptic Behavior of the XY Pair during Pachytene Substages

The ultrastructural analysis of 45 spread nuclei of pachytene spermatocytes by EM and the observation of several hundreds of primary spermatocytes by fluorescence immunolocalization of meiotic protein markers revealed that the XY pair undergoes striking changes in morphology as well as protein localization during the pachytene stage (Table 1). These changes were identical in samples from all the studied animals. At the beginning of this stage, the X and Y chromosomes come close to each other from one of their ends (Type-I XY pair; Fig. 1A, B; Table 1) and begin to synapse (Type-II XY pair; Fig. 1C, D; Table 1). At those substages, the X and Y axes show a series of bullous expansions – resembling a pearl-necklace – which are labeled by SYCP3 (Fig. 1A–D). Those differentiations begin as separately located knots on the unpaired regions, and then span all along the sex chromosome axes resulting in the formation of multistranded, thick XY axes (Fig. 1E–L). The synaptic region of the cat XY pair, labeled by SYCP1 and SYCE3 (Fig. 1C, D, 3C–E), reaches almost half of the Y chromosome length (Table 2). The multistrandedness becomes more remarkable as pachynema progresses. At mid-pachytene, the unpaired regions of the X and Y cores are subdivided in many thin SYCP3-positive fibrils (Type-III–IV XY pairs; Fig. 1E, F, 3B; Table 1). Many of those fibrils begin to stretch as bridges from one site of the X axis to the other site giving rise to a hairpin configuration (Fig. 1I, J). Surprisingly, one of those fibrils is always present at an average distance of 0.9 ± 0.2 μm from the telomeric region of the Y chromosome (Table 2). Moreover, that particular bridge-forming fibril is consistently located in the same region in which immunolocalization techniques show the presence of an MLH1 focus in the pairing region (details in the next section). At later pachytene stages, the X and Y chromosomes behave in 2 different ways: (1) in 14.4% of the pachytene nuclei, both chromosomes are completely separated, and thus lack SYCP1 and SYCE3 protein markers (Type-V XY pair; Fig. 1G, H, 3H; Table 1); (2) the joining of the X and Y axes is so remarkable that it keeps both chromosomes together in 34% of the observed pachytene nuclei (Type-VI XY pair; Fig. 1K, L; Table 1).

Fig. 1. A–L Parallelism between ultrastructural (**A**, **C**, **E**, **G**, **I**, **K**) and proteinaceous (**B**, **D**, **F**, **H**, **J**, **L**) variations of the differential axes of the XY pair along pachytene substages. The respective drawings are shown on the right. **C** Double lines indicate bullous expansions. **D** Occasionally SYCP1 clumps appear on the thickened X axis (asterisk). **E**, **F** A particular bridge-forming fibril is found in the Type-IV XY pair (arrowhead). Scale bars, 0.5 μm for electron micrographs and 1 μm for immunofluorescence images.

Colocalization of the Mandatory Recombination Focus and One SYCP3-Positive Bridge in Mid-Pachytene Spermatocytes

The simultaneous fluorescence immunolocalization of MLH1 (late recombination marker), CREST serum (centromeres), and SYCP3 proteins in pachytene spermatocytes reveals that there is a single recombination focus in the pairing region of domestic cats (Fig. 2A–C). The mean location of this MLH1-positive focus in mid-pachytene nuclei is 0.8 ± 0.1 μm (range 0.5–1 μm) from the telomere of the Y chromosome nearest to the pairing region (Table 2).

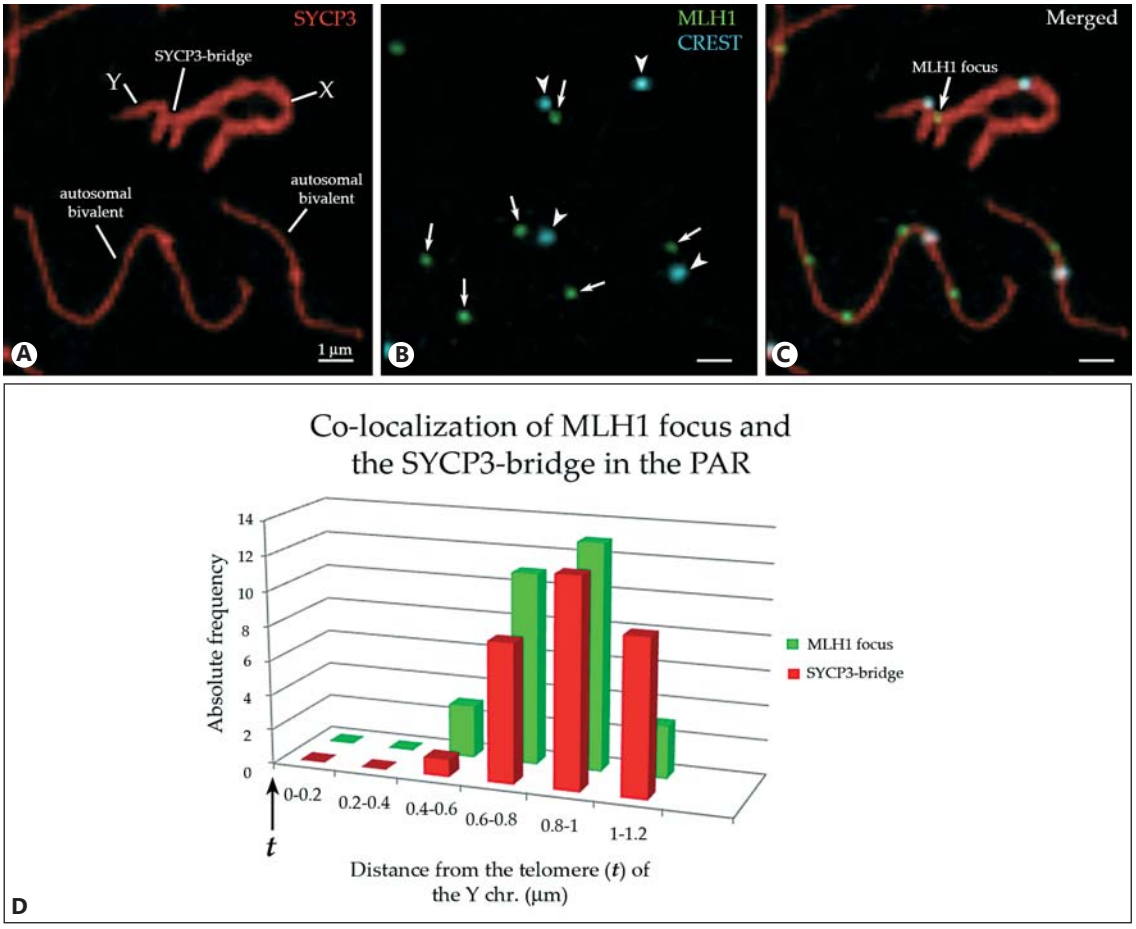

Fig. 2. A–C Simultaneous fluorescence immunostaining of SYCP3 (lateral elements, red), MLH1 (late recombination, green), and CREST (centromeres, blue) showing the colocalization of the SYCP3-bridge and the MLH1 focus in the pairing region of the X and Y chromosomes in cats. **D** Histogram depicting the location of the SYCP3-bridge and MLH1 focus. Scale bars, 1 µm.

Remarkably, the localization of the aforementioned SYCP3-bridge in the pairing region overlaps the location of the single MLH1 focus present where the X and Y axes converge (Fig. 2A–C). The colocalization of both structures, the MLH1 focus and the SYCP3-bridge along the Y axis, is shown in Figure 2D.

Thin Sections Observed with EM and Semi-Thin Sections for Light Microscopy

Semi-thin sections showed that the seminiferous epithelium is normal, without unexpected signals of apoptosis or necrosis. The seminiferous tubules have the usual phases of spermatogenesis.

The XY body is a condensed intranuclear, oval body, attached to the nuclear envelope at one of its sides. Its chromatin fibers display a homogenous inter-fiber distance which differs from that of autosomal chromatin (Fig. 3A, B, B′). Inside the XY body, there are sections of filamentous structures of different widths (Fig. 3A, B), and some of them show branchings which are thinner than the major filaments (Fig. 3B).

Most spermatocyte nuclei show sections of SCs in areas offside of the XY body. The SCs show LEs that are devoid of fibrils or gross multistrandness (Fig. 3B′).

Presence of Remodeling and Silencing Protein Markers on the Differential Chromatin in the XY Body of the Cat

The X and Y chromosomes form a clearly visible chromatin domain within the spermatocyte nuclei of the analyzed cats. Light microscopy and EM, as well as fluorescence immunolocalization, reveal the typical morpho-

functional characteristics of the XY body in mammals. This XY body in cats is observed as a condensed chromatin territory, attached to the nuclear envelope, with a differential packing of its chromatin compared to the autosomal chromatin as observed in thin sections (see above). At early pachytene stages, the DNA damage repair protein, BRCA1, is localized as clumps along the unpaired differential regions of the X and Y chromosomes (Fig. 3C). As pachynema progresses and those pearl-necklace differentiations become transformed into multistranded axes, BRCA1 protein extends along each strand (Fig. 3E), including those strands that connect or bridge one side of the X chromosome with the other, or those that connect the prematurely desynapsed X and Y chromosomes at mid/late-pachytene stage (Fig. 3G). Furthermore, the variant histone γ-H2AX, as well as the trimethylated histone H3K27, are present in the differential chromatin of the X and Y chromosomes all along the pachytene stage (Fig. 3D, F, H). In contrast, the aforementioned proteins are absent in the synaptic region of the XY pair (Fig. 3C–E).

These observations are similar to those made on the typical meiotic sex chromosome inactivation (MSCI) process.

Discussion

Protein Nature of the Multistranded, Thick Differentiations in Unpaired XY Axes

The splitting of the unpaired X axis in the pachytene stage seems not to be rare in mammals. It has been reported in marsupials [Solari and Bianchi, 1975; Franco et al., 2007], in armadillos [Sciurano et al., 2006, 2012], and in rodents [del Mazo and Gil-Alberdi, 1986; Koykul and Basrur, 1995; Echeverría et al., 2003; Sciurano et al., 2013]. However, the functional significance of these splittings remains largely unknown. On the other hand, the XY pair of other species, like the sand rat and the Mongolian gerbil [Solari and Ashley, 1977; Franco, 2012] displays very thin axes that neither become split nor thicken in later stages. Taken together, these observations suggest that the amount of SYCP3 able to attach to the sex chromosome axes (or the hypothetical receptors of this protein) – either in the sex-chromosome DNA or in intermediate proteins – is differentially regulated in different species. There is strong evidence that axes are located in the central region of the sex chromosomes, as particularly well shown in the Armenian hamster [Solari, 1974; Pathak et al., 1979]. However, there are no strong evidences on

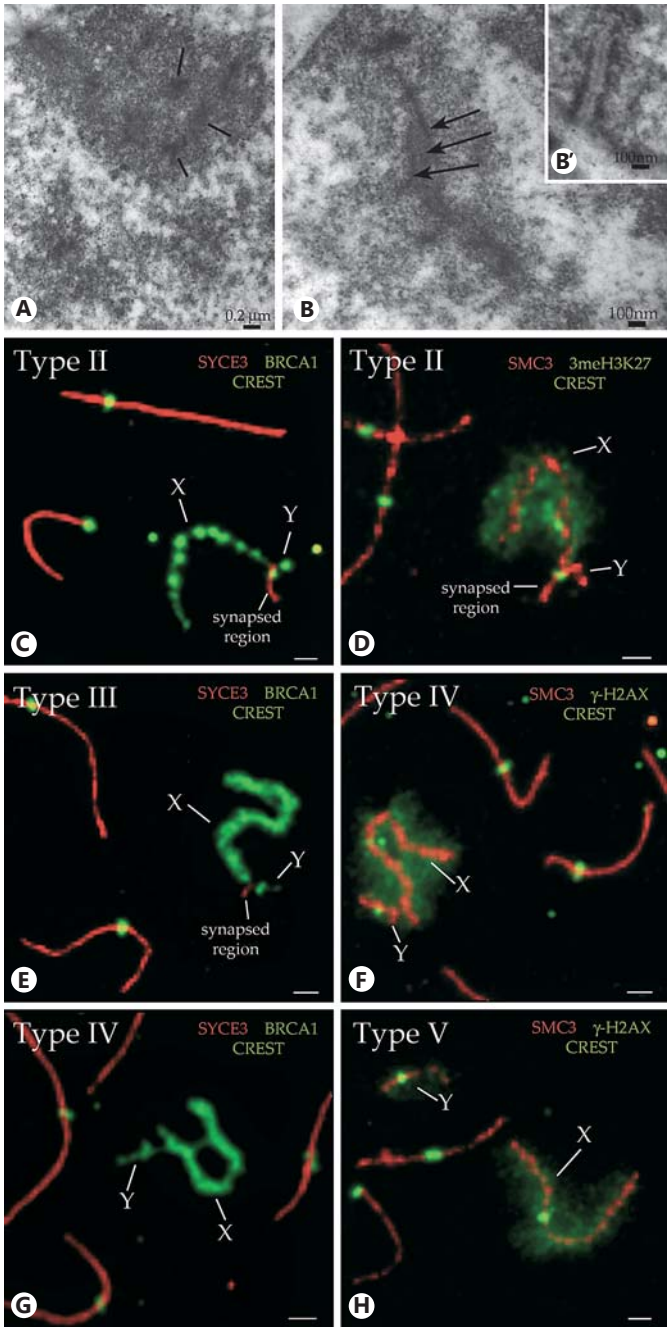

Fig. 3. A–H Chromatin remodeling and transcriptional silencing in the XY body of the domestic cat. **A**, **B** Fine structure of the XY body showing the uniform compaction of chromatin fibers and the multistrandedness of unpaired axes in transversal sections (**A**, lines) as well as in longitudinal sections (**B**, arrows). **B′** Neither of those typical ultrastructural characteristics is observed in autosomes. **C**, **E**, **G** Simultaneous immunolocalization of BRCA1 and SYCE3 (central element) distinguishes the synaptic (paired) and the unpaired, pearl-necklace-like, and thickened differentiations of the XY axes in cats. **D**, **F**, **H** The 2 transcriptional silencing markers, γ-H2AX and H3K27me3, are present on the differential chromatin, whereas the structural maintenance of chromosomes, SMC3, decorates the X and Y cores. **C–H** Scale bars, 1 µm.

the spatial relationships between SYCP3 and other axial proteins as cohesins. Thus, this question merits more research.

The actual existence of multiple, SYCP3-containing fibers in late pachytene, which form tangles and bridges, is shown by their appearance in both spread and sectioned spermatocytes, which are prepared by very different techniques (see Methods). Sectioned material shows the absence of necrosis or other pathological conditions that might explain these fibers as artifacts due to distorted structures in sick cells. Furthermore, the presence of such multiple and apparently disordered SYCP3-containing fibers has been reported in other species, such as armadillos [Sciurano et al., 2012] and the rodent *Galea musteloides* [Sciurano et al., 2013]. Moreover, morphological evidence for the existence of these multiple, tangled fibers has been reported in other species like the mink [Koykul and Basrur, 1995] and even in an earlier study of meiotic sex chromosomes of the cat [Gillies and Cowan, 1985]. Thus, the existence of these multiple fibers in the sex chromosomes at late pachytene stages seems to be out of doubt.

As shown in Results, during the mid/late stages of pachynema in the cat, the SYCP3-containing fibers have lost any spatial relationship with the core or center of the sex chromosomes. The multiplicity of the paths of these fibers argues against any relationship with that core, but also against any relationship with the interchromatid space. Then, as mentioned above, the relationship between these fibers and the cohesins remains an important subject to be investigated.

The pearl-necklace differentiations at early pachytene stages in the cat and the expansions of these knots result in the formation of multistranded, thick XY axes as pachynema advances (see Results). In the X and Y axes of the cat, the number of split fibers as well as their narrowness are very different from the substructures of the autosomal axes (first described by Woollam and Ford [1964] and then by other authors).

In other species, the laminar organization with split X and Y axes [Sciurano et al., 2013] is similar to the self-assembled, multistranded fibers observed in cultured somatic cells which ectopically expressed SYCP3 protein [Baier et al., 2007]. Those fibers consist of a large number of closely associated, thin strands of 5–10 nm in diameter, and display a transversal striation with a periodicity of 20 nm [Yuan et al., 1998; Baier et al., 2007]. More recently, crystallographic studies showed that human SYCP3 molecules form a highly elongated helical tetramer of 20 nm in length, which is arranged in 4 alternating-antiparallel SYCP3 molecules, such that each end of the tetramer contains 2 N-termini and 2 C-termini. According to these observations, N-terminal sequences can bind double-stranded DNA, enabling SYCP3 to link distant sites along the sister chromatid [Syrjänen et al., 2014], although the DNA sequences apparently associated with SYCP3 in the LEs of SCs are not yet well determined. Preliminary studies have suggested that interspersed repetitive elements play a role in linking DNA to the axial elements of the mammalian SC [Pearlman et al., 1992; Hernández-Hernández et al., 2008; Johnson et al., 2013].

Thus, it can be assumed that the main structural organization of the X/Y axes is formed by individually identifiable threads, which contain at least SYCP3 protein. However, the presence of other structural proteins cannot be excluded. In rat spermatocyte nuclei, Schalk et al. [1998] observed bridges between the LEs that are labeled by an anti-SYCP2 antibody using immune-gold techniques.

In addition, protein SYCP1 (and also SYCE3) is often present in variable and small patches, located as clumps at the thickened, differential X axis in cat spermatocytes (see Results). The latter structural localization has been previously reported in other mammalian species in normal conditions (in marsupials [Page et al., 2006b; Franco et al., 2007]), in null-mutant mouse models having mutations of cohesin-complex genes, in which SYCP1 is illegitimately found between sister-chromatid axes in univalent chromosomes [reviewed in Biswas et al., 2016], and in non-meiotic cells when transfected with SYCP1 (and SYCE3) constructs [Öllinger et al., 2005; Hernández-Hernández et al., 2016]. On the basis of these observations, we suggest that the presence of SYCP1 foci at the bulged, split regions of the X axis containing SYCP3 is not related to the assembly of a functional SC, but instead to the affinity of specific domains of both proteins that associate under the steric, proper conditions. In addition, it must be remarked that – contrary to homologous regions of the X and Y chromosomes – none of the presently reported primary spermatocytes show MLH1 foci or recombination nodules in the regions having these thickened X axes.

The mechanisms of the synapsis/desynapsis process in the XY body of the cat seem to differ from the usual behavior in other mammalian species [Sciurano et al., unpublished]. The XY body undergoes a premature desynapsis at mid/late-pachytene as a consequence of the disassembly of the SC at the pairing region. The mechanism of premature desynapsis of the XY pair, as seen in the natu-

ral model of the cat, might be related to the phosphorylation of specific SC components; this modification might induce a structural supramolecular modification that causes the components of the central space (SYCP1, SYCE3, among others) to lose contact with the axial cores, as it has already been suggested by other authors [Tarsounas et al., 1999; Fukuda et al., 2012].

It must be noted that the multistranded, thick differentiations of the unpaired XY axes do not correspond to the heterologous self-synapsis previously observed by other authors on sex-reversal mouse models or in X0 female mice. The fine structure and the pattern of protein markers of self-synapsed axes are completely different from those that show splittings as in the present case. In the sex-reversed condition [Chandley and Speed, 1987; Mahadevaiah et al., 1988, 1993], the most frequent conformation of pachytene spermatocytes (as well as oocytes) shows separate X and Y axes, in which the Y and/or X axes displayed a hairpin configuration [reviewed in Tease and Cattanach, 1989; Mahadevaiah et al., 1993]. Moreover, a central element of the SC stretched all along the thin, self-paired axes was observed when stained with phosphotungstic acid in XY,*Sxr* male mice [Chandley and Speed, 1987]. On the other hand, the immunolocalization of protein markers associated with transcriptional silencing and chromatin remodeling showed that those hairpin configurations are BRCA1, ATR, γ-H2AX, and ubi-H2A negative in the X0 mouse model [Baarends et al., 2005; Turner et al., 2005]. Thus, these hairpins are different to the transcriptionally silenced XY body, which is positive for the mentioned protein markers [reviewed in Turner, 2007]. In addition, it has been suggested that self-synapsis of the X and Y univalents would allow the avoidance of meiotic silencing of unsynapsed chromatin and, consequently, meiocyte degeneration and death [reviewed in Turner, 2007]. In contrast to those observations, the present analysis of the sex chromosomes in cats revealed: (1) a variable pattern of SYCP1 clumps on the thick X axis; (2) a typical mammalian XY body with the conventional MSCI protein markers.

The Association between the Specifically Located SYCP3-Bridge and the Single Recombination Focus of the XY Pair in Cats

One mandatory recombination event, labeled by MLH1 protein, is localized in the pairing region of the XY pair in cats, confirming previous results [Borodin et al., 2007]. As mentioned before, the thick X axis frequently adopts a hairpin configuration with bridges that connect one stretch of the X axis to another axial region. Remarkably, one of those bridges, the SYCP3-positive bridge, is extended through the central space between the X and Y axes, and is observed in 36.2% of the pachytene spermatocyte nuclei. The central space at those sites is devoid of SYCP1 and SYCE3 protein markers, meaning that no canonical synapsis occurs. Instead, the SYCP3-positive bridge is always localized at the same place in the pairing region (0.9 ± 0.2 μm), and this location is identical to that of the single MLH1 focus (0.8 ± 0.1 μm). At the site of this SYCP3-bridge, the convergent X and Y axes are several micrometers apart (1 ± 0.2 μm, range 0.7–1.3), and the space between them is bridged by DAPI-positive chromatin (see www.karger.com/doi/10.1159/000479569 online suppl. Fig. 1), which likely corresponds to the exchanged non-sister chromatids. We suggest that eventually the use of FISH techniques for specific chromosomal segments could demonstrate that the chromatin of the non-sister chromatids is still attached to the SYCP3-bridge at this stage. The presence of DAPI-positive material in this specific place supports the interpretation that the SYCP3-containing bridge helps to avoid the premature desynapsis (and the consequent aneuploidy) of sex chromosomes, reinforcing the function of the mandatory crossing-over.

The localization of special SYCP3-containing structures connecting the X and Y chromosome axes during the pachytene and later stages of first meiosis has already been observed in other mammals (in marsupials [Page et al., 2003; Franco et al., 2007]; in gerbils [de la Fuente et al., 2007; Franco, 2012]). In marsupials, the lack of a homologous region in the X and Y chromosomes [Hore et al., 2007] results in the abolition of a typical SC and a regular chiasma between them. Instead, there is a ring-like structure of dense material, the dense plate [Solari and Bianchi, 1975], which accumulates on the inner side of the nuclear envelope and is labeled by SYCP3 [Page et al., 2003; Franco et al., 2007]. It has been suggested that the formation of the dense plate in marsupials could be a mechanism to keep the unpaired X and Y chromosomes together during a sufficient time until their dissociation at anaphase I [Solari and Bianchi, 1975]. Another example is observed in pachytene spermatocytes of some gerbils where the most usual association between the X and Y chromosomes is "telosynapsis" without formation of any SC [Solari and Ashley, 1977]. In *Meriones unguiculatus*, there is a remnant SYCP3-positive accumulation, devoid of chromatin, between the ends of those telosynaptic sex chromosomes from diakinesis to telophase I [de la Fuente et al., 2007; Franco, 2012].

The present observations on a SYCP3-bridge at the pairing region of the XY body may be another instance of the role of SYCP3 as a reassuring structure for proper disjunction of the sex chromosomes, which in cats may reinforce the presence of an early-disjoining crossover between the sex chromosomes.

The Conserved MSCI Process in the Domestic Cat

Some post-translational modifications of histones play an important role in gene silencing by modifying the higher-order chromatin structure and, thus, chromatin accessibility [reviewed in Crichton et al., 2014 and Zhang et al., 2016]. The mammalian XY body [Solari, 1974; reviewed in Handel, 2004] reflects the occurrence of a transcriptional silencing process (MSCI) [reviewed in Turner, 2007]. Three protein markers associated with transcriptional silencing and chromatin remodeling were evaluated on the cat XY body in the present work: the DNA damage repair protein BRCA1, the phosphorylated histone γ-H2AX, and the trimethylated histone H3K27. As shown in Results, the unpaired X and Y axes are labeled with BRCA1 when pearl-necklace formations appear at early pachynema and then again along the premature desynapsed axes at late pachynema. As pachytene progresses, the unpaired axes of the X and Y chromosomes become split and thickened and evidence individual BRCA1-positive threads, in contrast to the synapsed region where BRCA1 is absent. On the other hand, the unpaired chromatin is labeled by γ-H2AX and H3K27me3 all along pachytene stages, in the separated axes of sex chromosomes at early pachytene stages as well as in the conjoined XY body at later pachytene stages. The presence of those chromatin remodeling/silencing markers revealed that the organization of the unpaired chromatin differs from the synapsed chromatin of the pairing region in the XY body, not only at the ultrastructural level but also at a functional level.

These observations suggest that the transcriptional silencing and chromatin remodeling of the XY body are conserved processes in domestic cats, as well as in other mammals.

Conclusions

1. The transcriptional silencing and chromatin remodeling of the XY body are basic and conserved mechanisms among mammals; however, striking variations in the structure of the unpaired axes and their behavior during meiotic prophase are observed in cats (see Results) as well as in other species [Sciurano, in preparation]. The extensive display of their component fibrils is species-specific and may be the result of early disassembly of the axes and loss of the early axis in the core position.
2. The finding of a colocalization of the single MLH1-containing focus and a SYCP3-containing bridge overlapping a DAPI-marked track, suggests that a recombining region in this XY pair is constrained in its location to the inner end of the pairing region.
3. The fibrillation of the axes into multiple SYCP3-containing strands might be related to a release of the known ability of SYCP3 protein to associate with DNA that is triggered at mid/late pachytene, when core proteins lose their location at the axis of the sex chromosomes, and SYCP3 protein associates with other DNA stretches. A deeper analysis of these variations and their functional aspects is needed for a proper understanding of these processes.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Normal castration operations performed by veterinarians at the Instituto de Zoonosis "Luis Pasteur" (Buenos Aires, Argentina) follow all institutional and national guidelines for the care of animals.

Disclosure Statement

The authors have no conflicts of interest to declare.

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