Chromosome Res (2013) 21:753–763
DOI 10.1007/s10577-013-9379-3

Dissociation of the X chromosome from the synaptonemal complex in the XY body of the rodent *Galea musteloides*

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Received: 24 June 2013 / Revised: 26 August 2013 / Accepted: 29 August 2013 / Published online: 17 September 2013

Abstract The XY body from spermatocytes of the rodent *Galea musteloides* shows progressive changes of the synaptonemal complex (SC) axes and the X-chromatin during pachynema. There is a gross thickening of the X-axis and the formation of a large X chromosome loop at mid and late pachytene stages. The SC proteins synaptonemal complex protein 3 (SYCP3), synaptonemal complex protein 1, and synaptonemal complex central element protein 3 and the proteins breast cancer 1, MutL homolog 1 (MLH1), and radiation-repair 51 (related to meiotic processes), the cohesin structural maintenance of chromosome 3, the centromeric protein (with CREST antibody), and the silenced chromatin (with phosphorylated (139ph) H2A histone family, member X (γ-H2AX) antibody) were analyzed in this XY body. The thick X-axis, including the interstitial loop, becomes formed by four to six laminae showing a cross-striation with a periodicity of about 20 nm. The whole length of the gross X-axis shows no significant changes during pachynema, but the interstitial chromatin of the X chromosome and the X centromere are included in the large loop, and it becomes separated from the SC. A conventional SC formed by the Y-axis, a central region and a thin lateral element originally corresponding to the X-axis, remains undisturbed up to the end of pachynema. A single MLH1 focus develops either at the distal or the proximal region of the loop end attached to the conventional SC. The chromatin surrounding the thickened axis is labeled with γ-H2AX. It is shown that most of the SYCP3 protein associated with the X chromosome loop is not involved in the SC maintenance, but it is located with the cohesin axis separated from the SC proper.

Keywords XY body · Synaptonemal complexes · SYCP3 · MLH1 · MSCI · γ-H2AX

Responsible editor: Walther Traut

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Abbreviations

ATR	Ataxia telangiectasia and Rad3 related
BRCA1	Breast cancer 1
CR	Central region
CREST	Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia
DNA	Deoxyribonucleic acid
EM	Electron microscopy

IFI	Indirect immunofluorescence
LE	Lateral element
MLH1	MutL homolog 1
MSCI	Meiotic sex chromosome inactivation
MSUC	Meiotic silencing of unsynapsed chromatin
PAR	Pseudoautosomal region
PBS	Phosphate-buffered saline
PTA	Phosphotungstic acid
RAD51	Radiation-repair 51
SC	Synaptonemal complex
SMC3	Structural maintenance of chromosome 3
SYCP1	Synaptonemal complex protein 1
SYCP3	Synaptonemal complex protein 3
SYCE3	Synaptonemal complex central element protein 3
γ-H2AX	Phosphorylated (Ser139) histone 2, A.X

Introduction

The different structural components of the synaptonemal complex (SC) at pachynema are known to show variations along pachytene substages; the most frequent of these variations being the thickening and branching of the lateral elements (LEs) corresponding to the differential regions of the X and Y chromosomes (Solari 1970, 1974; Moses 1977; Solari 1980; del Mazo and Gil-Alberdi 1986; Solari et al 1993; Solari and Rey Valzacchi 1997; Echeverría et al. 2003; Solari and Rahn 2005; Page et al. 2006).

Looping of the LEs is a much more rare event, and it has been seen only in samples in which inversions or duplications of autosomal segments are present in these autosomes (Moses et al 1982; Bojko 1990; Solari et al. 1991; Sciurano et al. 2012) and in mice carrying combined translocations (de Boer and Jong 1989); however, these autosomal loops are present only at early pachynema and they disappear at later pachytene substages, assumedly by "synaptic adjustment" (Henzel et al 2011; Burgoyne et al 2009). In the present paper, we show the development of a large, permanent loop in the X-axis of the XY body of the rodent *Galea musteloides*, which differs sharply in its behavior, structure, and protein composition compared with the transient loops mentioned above. Moreover, the presence of this permanent loop allows the analysis of the behavior of its corresponding chromatin. Unexpectedly, it shows the location of the whole X-chromatin segment throughout the loop, posing new questions about the relationships between the X-axis, which contains the major part of the protein of the LEs of SCs, synaptonemal complex protein 3 (SYCP3), and the corresponding chromatin and the deoxyribonucleic acid (DNA) sequences expected to be associated with the SYCP3 protein. Furthermore, the presence of partial synapsis of the meiotic X and Y chromosomes is corroborated by the presence of a "conventional" stretch of synaptonemal complex, as is commonly seen in mammals (Solari 1974), but with special features as regards the localization of crossover events (see "Results").

The presence of these exceptional features in all spermatocytes of the specimens analyzed from *G. musteloides* may not be restricted to this South American species. A study of the XY body of the guinea pig *Cavia porcellus* (Echeverría et al. 2003) which is a caviomorph rodent related to *G. musteloides* shows several features similar to the one studied here. Thus, we propose that the present conclusions allow new prospects for research on the XY body of mammals.

Materials and methods

Two adult males of the common yellow-toothed cavy, *G. musteloides*, were collected at different locations in Mendoza (Argentina) as part of the project on the basic molecular mechanisms of meiosis in humans and other mammals (see "Acknowledgments") in collaboration with Prof. JC Cavicchia (IHEM, Mendoza, Argentina). All institutional and national guidelines for the care and use of animals were followed. The testicular tissue from both animals was removed and subdivided in smaller pieces to perform optical and electron microscopy (EM) and fluorescence immunolocalization of meiotic proteins. Two pieces of tissue were processed for semithin (0.5 μm thick) and thin (0.08 μm thick) sections to analyze the seminiferous epithelium in detail with the light microscope and the ultrastructure of the XY body in spermatocyte nuclei by EM, respectively. Electron micrographs were obtained with a Zeiss EM 109T, equipped with a digital camera Gatan171 ES1000W, at the LANAIS' service (CONICET). Another piece of tissue was used to perform spermatocyte spreads for synaptonemal complexes as described by Sciurano and Solari (2013). Some slides were stained either with 4 % phosphotungstic acid in ethanol or silver nitrate, and others

were kept at −70 °C until used for immunofluorescence microscopy. For immunolocalization of meiotic proteins (except for phosphorylated (Ser139) histone 2, AX (γ-H2AX)), a citrate buffer epitope retrieval was performed in 0.01 M sodium citrate buffer (pH 6) before the blocking step (see Sciurano and Solari 2013). The following primary antibodies were incubated at 4 °C: a mouse anti-SC protein synaptonemal complex protein 1 (SYCP1) at 1:100 (P.J. Moens and B. Spyropoulus, York University, Toronto, Ontario, Canada); a rabbit anti-SYCP3 at 1:100 (Abcam Ltd., UK); a rabbit anti-synaptonemal complex central element protein 3 (SYCE3) (Prof. M. Alsheimer, University of Würzburg, Germany) at 1:100; a rabbit anti-breast cancer 1 (BRCA1) (C20, Santa Cruz Biotechnology, CA, USA) at 1:10; a mouse anti-MutL homolog 1 (MLH1) at 1:10 (BD Pharmingen, USA); a rabbit anti-structural maintenance of chromosome 3 (SMC3) at 1:500 (Millipore Corp., USA); and human calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (CREST) serum at 1:10 (Laboratorios IFI, Buenos Aires, Argentina). A mouse anti-γ-H2AX antibody (Abcam Ltd, Cambridge, UK) at 1:500 in phosphate-buffered saline (PBS) and a rabbit anti-radiation-repair 51 (RAD51) at 1:30 (Santa Cruz Biotechnology, USA) were incubated at 37 °C. All incubations were performed overnight in a humid chamber. After washing, the corresponding secondary antibodies were used at the specified dilutions in PBS 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). The separate images were superimposed using the program Adobe Photoshop CS (Adobe Systems Inc., USA). The distance of the MLH1 foci from the telomere in the XY pair, the length of each chromosome of the entire set, the length of the loop, and the SC in the XY pair were measured using the freeware computer application MicroMeasure version 3.3 (Reeves 2001).

Results

The analysis of more than 50 electron micrographs of spermatocyte spreads and several hundreds of spermatocyte nuclei by fluorescence immunolocalization of different meiotic proteins shows a special behavior of the XY pair of *G. musteloides* along pachytene stages.

XY body behavior along the substages of pachynema

At the end of zygotene and beginning of pachytene stages, the X and Y chromosomes approach each other from one of their ends and begin to synapse. At these stages, the X and Y chromosome axes are thin and show slightly thick differentiated regions (Fig. 1a). During early pachynema, a long SC is formed between the submetacentric X and Y chromosomes. This long SC covers almost the entire length of the Y-axis and remains until the end of pachynema (Figs. 1 and 2 and Table 1).

With the markers used here, the proteinaceous composition of the X and Y structural cores involves the structural cohesin SMC3, and the SC involves the major component of the axial elements and the transversal filaments of the SC, SYCP3, and SYCP1, respectively, and the central element protein SYCE3 (Fig. 1b, d, f).

During mid and late pachynema, a large loop is formed by the dissociation of the X chromosome axis into two asymmetrical axial elements (Fig. 1g–j): a thick axis formed by multiple strands and a thin lateral element which forms the SC with the Y chromosome (see below).

At very late pachytene stage, the Y-axis also dissociates from the main SC except at the end closer to the nuclear envelope where a short synaptic segment remains visible (Fig. 1k, l).

Loop formation and its evolution during mid and late pachytene stages

From mid pachytene stage, most of the thickened outer part of the X-axis begins to dissociate gradually from the central region of the SC and forms a loop, which grows outwards as pachynema progresses (Fig. 1). This dissociation becomes larger at the interstitial part of the SC, but it does not occur at the end regions of the SC; instead, there are two points of attachment which are consistent with the two alternative recombination regions (see below): one is close to the nuclear envelope and, the other, near the distal part of the SC.

On the other side, an inner, thin X-axis remains in place maintaining the original SC with the Y chromosome, up to very late pachytene stages (Fig. 1g, i, k).

In addition, the thickened axial element contains the larger mass of SYCP3 and the whole SMC3 proteins of

the X chromosome, distinguishing it from the thin lateral element involved in the SC (Fig. 1g–j).

Interestingly, the larger the loop is, the shorter the differential X-axis becomes. This fact suggests that the loop grows while the differential X-axis becomes shorter, without modifying the total length of the X chromosome axis (Table 1 and Fig. 2). Additionally, during mid–late pachynema, the loop contains the X centromere, as shown by CREST labeling (Fig. 1j).

The MLH1 focus is mainly located in the proximal region near the nuclear envelope

To observe the localization of early and late recombination nodules, we looked for the presence of the RAD51 and the MLH1 proteins, respectively (Fig. 3). The recombinase RAD51 is present as multiple foci along the developing chromosomal axes (both from autosomes and sex chromosomes) at leptotene stage. During zygotene and early pachytene stages, RAD51 foci are located on the full-length of single axes and on the synapsed segments. As synapsis progresses, these foci disappear from those regions and remain restricted to the XY pair, particularly, on the differential X chromosome region and the asynaptic loop (Fig. 3a, b). Finally, the RAD51 foci disappear from the XY pair at late pachynema (Fig. 3c).

As mentioned above, there are two points of attachment of the dissociated X-axis and the long SC. These binding sites are consistent with two alternative recombination regions (Fig. 3d–g). Most of the spermatocyte nuclei (45/50) have an MLH1 focus close to the nuclear envelope (0.67±0.18 μm, range=0.5–1.05 μm, see Fig. 3d, f). In a few cases (5/50), there is an alternative MLH1 focus at the region distal from the nuclear envelope (4.15±0.9 μm, range=2.9–5.33 μm, see Fig. 3e, g). This latter focus has never been observed on an XY pair which forms a loop (Fig. 3e); it has always been present when the Y chromosome is almost completely synapsed with a non-dissociated X chromosome.

Multistranded nature of the sex chromosomal axes

As pachynema progresses, the X chromosome axis becomes thicker due to the deposition of fibrillar material that forms four to six laminae (Fig. 4a, b), whereas the Y-chromosome axis remains without any modification until very late pachytene stages (Fig. 1). Towards the mid pachytene stage, the multistranded X-axis is extended from the differential region to the synaptic region of the XY pair and begins to dissociate from the mid-segment of the SC (Fig.1e–j) to form the loop.

At very late pachynema, the multistranded organization of the axes is not only evident in the X chromosome, but also in the Y chromosome (Fig. 1k, l). Both the X and Y thickened axes are formed by SMC3- and SYCP3-positive strands as shown with immunofluorescence (Fig. 1h, j, l).

At the fine structural level, the thin sections of the laminae from the thick X-axis show a transverse striation with a periodicity of about 20 nm (15 periods measured, with a mean per period of 0.46 of the cytoplasmic ribosome diameter) (Fig. 4a).

Localization of the silenced chromatin in the XY body

The analysis of the ultrastructure of the XY body by electron microscopy and fluorescence immunolocalization shows that the XY body in *G. musteloides* is similar to that of other mammals as regards to functional traits. The XY body is an oblong, subnuclear structure, attached to the nuclear envelope, with a differential packing of its chromatin (Fig. 4a, b). At early pachytene stages, the DNA damage repair protein, BRCA1, is localized along the asynaptic differential regions of the X chromosome and that of the Y chromosome (Fig. 4d), whereas the variant histone γ-H2AX is present in the corresponding chromatin. As pachynema progresses, both proteins are also shown on the developing loop (Fig. 4c, e). Furthermore, the BRCA1 and γ-H2AX proteins still remain in the loop at late pachynema. These observations strongly suggest that the mentioned asynaptic regions are transcriptionally silenced (Fig. 4c–e). In contrast, both proteins are absent in the synaptic segments between X and Y chromosomes all along the pachytene stage (Fig. 4c–e).

Discussion

The basic behavior of the XY body in *G. musteloides* agrees with the conventional one

During early zygonema and early pachynema, the axes of the X and Y chromosomes approach to each other, first by one of their ends, and then they form a stretch of SC that is identical to that of autosomal pairs and to those formed at earlier pachytene stages in other

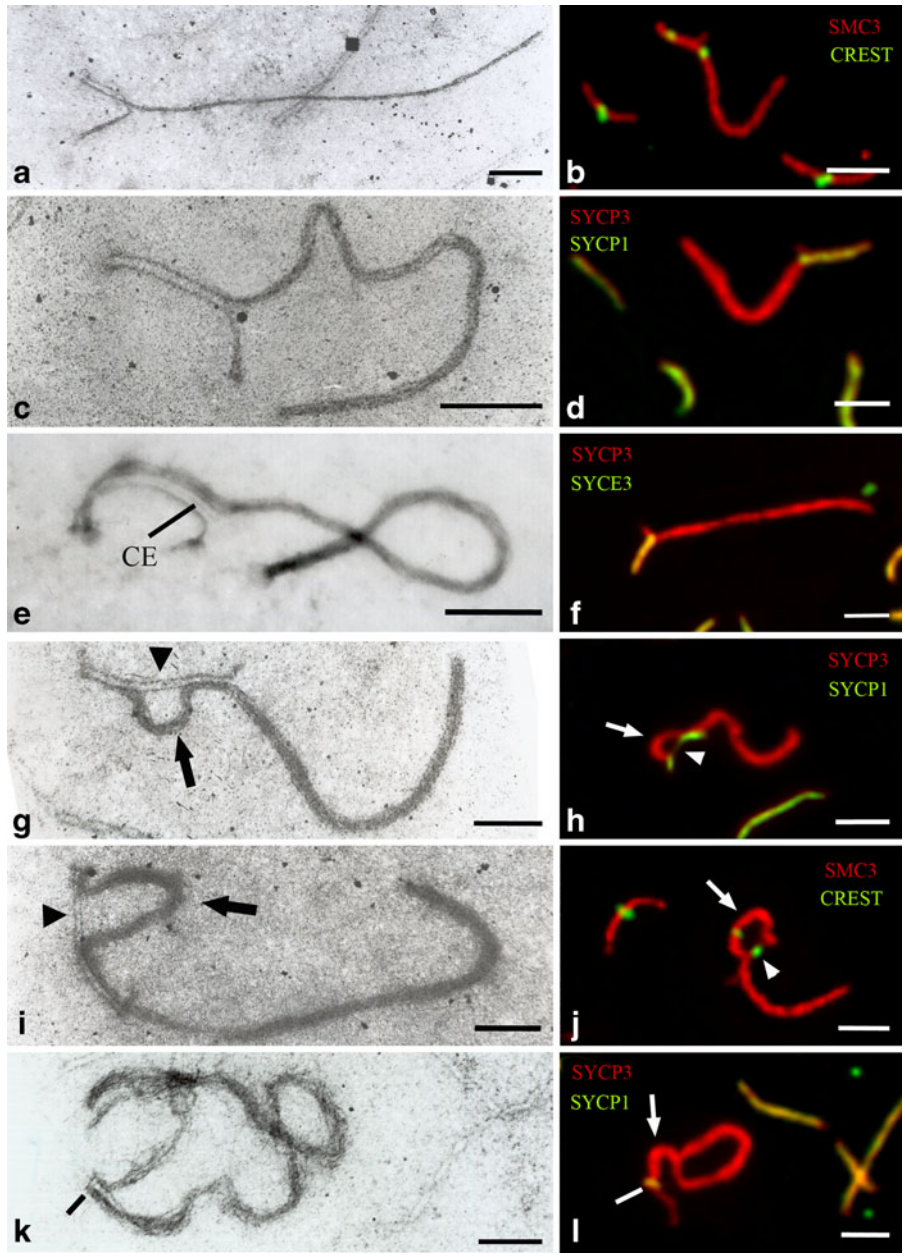


Fig. 1 **a**–**l** Behavior of the XY body in *G. musteloides* from early to late pachytene stages comparing the ultrastructure of the XY pair by EM and the localization of meiotic proteins SYCP3, SYCP1, SYCE3, SMC3, and serum CREST by fluorescence immunostaining. *On the left*, these images show the stepwise formation of an asymmetrical loop (*arrows*), which involves the dissociated X-axis, and the remaining synaptonemal complex segment (*arrowheads*) by EM. *On the right*, the corresponding analysis with indirect immunofluorescence confirms the protein composition of the dissociated SYCP3- and SMC3-positive X-axis and the remaining SC labeled by anti-SYCP1 and anti-SYCE3 antibodies. At very late pachytene stage, the synaptic region between the X and Y chromosome is restricted to a short segment close to the nuclear envelope (*lines*). *CE* central element. *Bars*: EM images, 1.5 μm; IFI images, 4 μm

mammals (see "Results"). Furthermore, at the early pachytene stages, the X and Y chromosomes have a conventional SC that would correspond to the PAR found in the vast majority of mammals (reviewed in Solari 1994). Thus, the peculiar features of this XY body develop after these early stages.

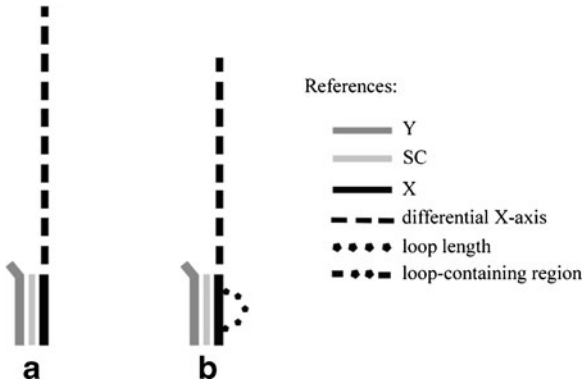


Fig. 2 **a**, **b** Schematic drawings of the XY pair at early (**a**) and late (**b**) pachytene stages. These diagrams at scale correspond to the measurements shown in Table 1

Thickening and delamination of the X-axis

Looping and thickening of autosomal axes (LEs) that are kept in an asynaptic condition during early–mid pachytene stages in meiocytes that carry chromosomal rearrangements are almost always resolved by synaptic adjustment during later pachytene substages. On the other hand, the axes of the meiotic sex chromosomes of mammals have their "differential regions" that show permanent asynapsis, and again, these regions of the X and Y axes show thickenings and other differentiations (see "Introduction")

The present observations in *G. musteloides* show an extreme instance of gross thickening and formation of several laminae from the axis of the X chromosome. As shown in EM sections, there are four to six well-defined laminae that show a regular, cross-striated structure that has a periodicity of 20 nm (see "Results"). Thus, it is suggested that a considerable synthesis of the main protein component, SYCP3, takes place during mid and late pachytene stages in the spermatocytes of *G. musteloides*. The self-interactions of SYCP3 protein molecules in mammals are not sufficiently known. However, relevant information comes from somatic cells transfected with plasmids encoding the full-length SYCP3 protein (Yuan et al 1998), in which the cells develop intranuclear, fibrillar bundles of SYCP3 protein, which have a cross-striated pattern that shows a periodicity of ~20 nm when examined under the electron microscope (Yuan et al 1998), as shown in *G. musteloides* (see "Results"). As the immunolocalizations show that the gross axis contains SYCP3, we postulate that the fibrous mass of the thick X-axis is predominantly—or perhaps exclusively—formed by the protein SYCP3.

Table 1 Comparison of the average length of the X chromosome axis, the synaptonemal complex of the XY pair, and the loop length during early and late pachytene stages

		Early pachynema	Late pachynema
Length of X chr. axis	Mean ± SE (μm)	18.6±3.0	19.2±1.8
	Range (μm)	13.9–27.0	15.4–23.8
	n	30	30
Length of Y chr. axis	Mean ± SE (μm)	4.2±0.5	4.8±0.7
	Range (μm)	3.1–5.5	3.2–7.0
	n	30	30
Length of differential X-axis[a]	Mean ± SE (μm)	**14.8±2.9**	**11.7±2.0**
	Range (μm)	10.4–22.6	6.78–14.23
	n	30	30
SC length	Mean ± SE (μm)	3.8±0.6	3.7±0.6
	Range (μm)	2.6–5.0	2.6–5.0
	n	30	30
Loop length	Mean ± SE (μm)	NA	6.2±1.7
	Range (μm)	NA	4.0–10.0
	n	NA	30
Loop-containing region[b]	Mean ± SE (μm)	3.8±0.6[c]	7.3±1.3
	Range (μm)	2.6–5.0	4.6–10.6
	n	30	30

NA not applicable, *chr.* chromosome

[a] *t* value=4.9; *df*=58; $p=9.1\times10^{-6}$, *highly significant* (bold characters)

[b] *t* value=13.24; *df*=58; $p=3.5\times10^{-19}$, *very highly significant*

[c] At early pachynema, the corresponding loop region is the same as the SC region (because the loop does not exist at this stage)

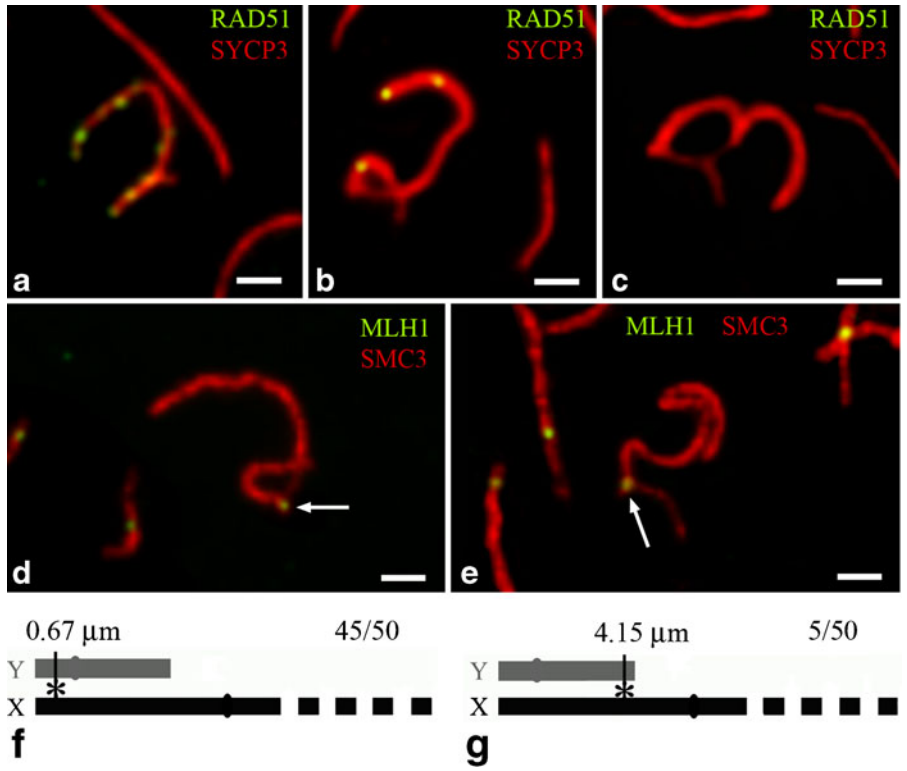


Fig. 3 **a**–**g** Fluorescence immunolocalization of early and late recombination nodules of the XY pair in *G. musteloides*. **a**–**c** As pachynema progresses, the recombinase RAD51 is seen as foci along the differential X-axis as well as in the large synaptic region of the XY pair (**a**) and, later, it is also associated with the loop (**b**) until the late pachytene stage when it disappears from the XY pair (**c**). **d**–**g** Late recombination nodules. Two alternative recombination sites (*arrows*) are seen in the XY pair by immunolocalization of the MLH1 protein: the preferential MLH1 focus is located close to the nuclear envelope (**d**, **f**) and the alternative one is present on the opposite site (**e**, **g**). The latter is never seen in a loop configuration. **f**–**g** The diagrams at scale summarize the average location of the MLH1 focus in each case (*thin black lines with asterisks*): **d**, **f**, preferential location; **e**, **g**, minor location. *Bars*, 3 µm (**a**–**e**)

Formation of the loop by SYCP3 protein

When the loop first appears, it leaves a thin "remnant" LE on the SC stretch. As the loop carries with itself the cohesin SMC3 (and the repair-linked protein BRCA1), it is assumed that the chromatin from both sister chromatids detaches from the stretch of SC and remains connected with the protein components of the central region of the SC only at the sites in which a potential crossover has been signaled (see "Results"). This thin remnant LE is not resolved from the one corresponding to the Y chromosome by light microscopy, and thus, it cannot be determined if it contains SYCP3 by immunofluorescence. However, by using PTA staining—a relatively preferential stain for LEs proteins—this thin remnant LE is clearly seen (see "Results"). The present observations suggest that it is actually a thin lamina containing SYCP3 that remains associated with the central region of the SC, but that has no chromatin associated with it.

This unexpected result may not be at all exceptional. *Polycomplexes*, independent from the chromatin in meiotic cells, have been described in many species (Goldstein 1987). The existence of polycomplexes and pseudocomplexes (de Boer and Jong 1989) suggests that the assembly of SC-like structures is the result of affinities sufficiently large, among the proteins of the central region of the SC (SYCP1, SYCE3 and other) with SYCP3, to form these kinds of structures devoid of associated chromatin (reviewed in Fraune et al 2012). On the other hand, in mice mutant for SYCP3−/−, there is still the formation of "chromosomal cores" containing cohesin, and these "cores" form structures that promote synapsis (Pelttari et al 2001). Thus, it may be concluded

that in the XY body of *G. musteloides*, the chromatin of the loop detaches itself from the remnant LE of the interstitial SC complex, leaving a minimal layer containing SYCP3 protein attached to the SC but devoid of chromatin.

The location of the centromeric chromatin in the loop

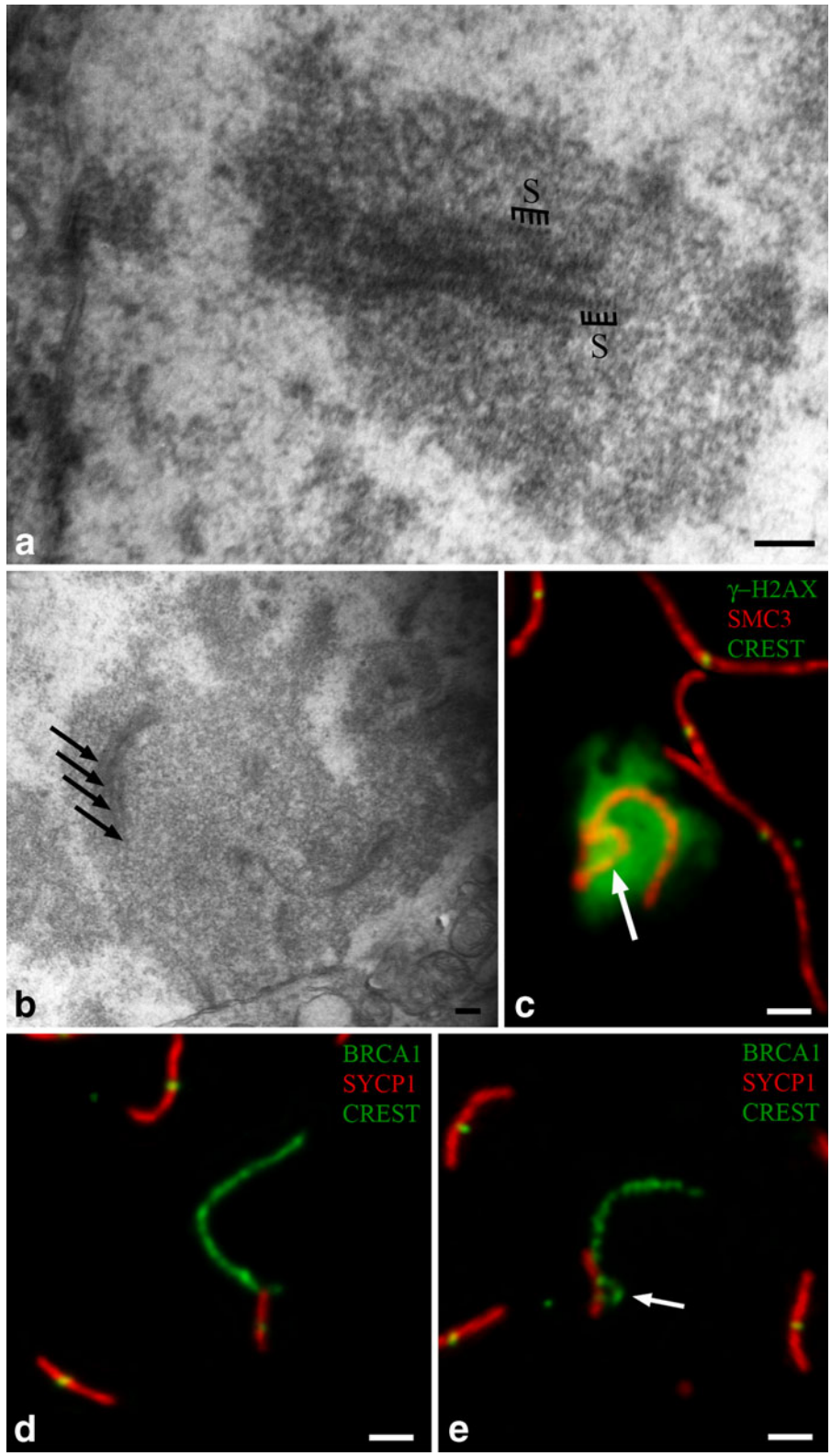
The imaging of the centromeric proteins through the labeling with the CREST antibody (see Fig. 1b versus Fig. 1j in "Results") showed that in most of the spermatocytes at mid–late pachynema, the centromere appears in the loop, at variable locations regarding to the loop ends. Although the loop length increases during the late pachytene substages, the length of the whole X-axis remains without significant variations, and the length of the differential segment of the X-axis becomes shorter (see "Results"). The entrance of this centromere into the loop region does not depend on a shortening of the whole X-axis. The enlarging of the loop at late substages must mean that a larger part of the chromatin of the X chromosome becomes detached from the remnant SC. This expansion of chromatin detachment is variable and it may contain the centromere. This remarkable displacement of the chromatin occurs despite the maintenance of two SC-touching regions at the ends of the loop (see below). When an MLH1 focus is seen in the distal region, the centromere is always outside the synaptic region. This fact means that an effective crossover does not occur farther of the centromere region, that is, in the long arm of the X chromosome, but only at one site, at either the short arm region near the nuclear envelope or at its distal region.

The two alternative locations of a single crossover in the XY pair

In a preliminary report on the fine structure of the XY body of *G. musteloides*, we showed the detachment of the X-axis, the formation of the loop at late pachynema (Solari et al 1993), and the permanence of a short SC segment near the nuclear envelope throughout the late pachytene substage that would be the site of a complete crossover, which keeps the "end-to-end" association between the X and Y chromosomes during diplotene stage and up to metaphase I. Moreover, we described the formation of another recombination nodule called "early recombination nodule" (Carpenter 1987) that could represent a gene conversion event. The present observations on the location of complete crossovers confirm these data.

The fact that the two locations of MLH1 signals (in different cells) are coincident with the two locations in which the gross SYCP3- and SMC3-containing loop ends and associates with the remnant SC strongly suggests that the SYCP3 axis must be linked to the other SC elements to form a crossover event. This suggestion agrees with previous observations on homologous recombination consisting in successive steps that involve (1) an early assembly of recombinases RAD51 and DMC1 on the sites of double strand breaks at leptonema; (2) the assembly of a protein complex containing MSH4, MSH5, RPA, and associated proteins that are seen associated with the central region of the SC at zygotene stage (reviewed in Fraune et al 2012); and (3) the incorporation of MLH1 that marks the definitive crossover event on the central region of the SC (Moens et al 2007). The observations in mutant SYCP3−/− oocytes from mice (Li et al 2011) suggest that SYCP3-containing axes promote interhomologue recombination instead of interchromatid events. Furthermore, the observations showing that the interchromatid cohesion-containing axis needs to be associated to the SC—in order to be able to form crossover events (Revenkova et al. 2004; Murdoch et al 2013)—agree with the present observations on the ends of the SMC3-containing loop located at the crossover locations. Moreover, the central region (CR) of the SC is known to be necessary for crossover completion (for instance, lack of the SYCP1 protein forming the transverse filaments of the CR of the SC inhibits crossovers, de Vries et al 2005).

Fig. 4 **a**–**d** Structure of the SYCP3 laminae and chromatin silencing of the XY body in *G. musteloides*. **a** This electron micrograph of a thin section shows the transverse striations (*S*) of the laminae of the X-axis, having a periodicity of ~20 nm. **b** Electron micrograph showing the differential condensation of the chromatin in the XY body and the multistrandness of the thickened X-axis (*black arrows*). **c** The variant histone of γ-H2AX is located on the chromatin of the differential X-axis as well as in the loop (*white arrow*). **d**, **e** Double immunolocalization of the BRCA1 and SYCP1 proteins at early (**d**) and mid pachytene stages (**e**). *Bars*, 0.2 μm (**a**–**b**); 3 μm (**c**–**e**) ▶


S
S
a
b
γ–H2AX
SMC3
CREST
c
BRCA1
SYCP1
CREST
d
BRCA1
SYCP1
CREST
e

Probably, when the crossover more frequent site (near the nuclear envelope) matures, then an inhibition for another crossover site in the nearby chromatin takes place, as in the already known phenomenon of chiasmatic interference.

"Silencing" of the XY body of *G. musteloides*

In recent years, the fact described originally by Monesi that the chromatin of the XY body seemed to be unable to incorporate tritiated uridine (Monesi 1965) and thus to synthesize RNA has been confirmed and explained in molecular terms (reviewed in Solari 1994; Khalil et al. 2004; Page et al. 2012). Also, the XY body of the guinea pig *C. porcellus* (Echeverría et al. 2003), a rodent of the same family as *G. musteloides*, shows features similar to those presently described. The compact nature of the chromatin in the mammalian XY body agrees with the cited observations (reviewed in Solari 1994). The DNA damage repair protein BRCA1 and the variant histone γ-H2AX have been found associated to non-synapsed chromosomal segments during meiotic prophase, both in the differential regions of the X and Y chromosomes at pachynema (Turner et al 2005; Sciurano et al 2012; Page et al 2012) and at non-synapsed regions in autosomes carrying chromosome rearrangements (Burgoyne et al 2009; Sciurano et al 2012). Thus, the transcriptional silencing in the XY body or MSCI (Turner 2007) seems to be included in a more general phenomenon, the MSUC (Schimenti 2005).

The present observations show that BRCA1 is located along the axis and γ-H2AX in the surrounding chromatin, throughout the differential, non-synaptic axis of the X chromosome of *G. musteloides*, and also in the large loop formed at mid–late pachytene stages by the SYCP3/SMC3-containing axis. Thus, it is suggested that a similar silencing of the corresponding chromatin also occurs in the present case.

The resolving power of our techniques does not allow ascertaining if the chromatin at the attachment points of the loop, where a recombination nodule is formed, has the same features as that at the "free" X-axis. The permanence of these attachment points through late pachynema suggests that, at these locations, there are structural and compositional features different from those of the free axis. In fact, the approachment of the cohesin axis containing SMC3 to the elements of the central region of the SC seems to be needed for the formation of this nodule. Furthermore, the regions nearby the attachment points do not show detachment of the X-axis from the SC, nor have BRCA1 labeling, and it is in those regions where the MLH1 foci are mostly located (see "Results").

Conclusions: the exceptional XY body of *G. musteloides* shows particular aspects of the SC machinery

The present results show some exceptional features of the axial components and the attached chromatin in the XY body of *G. musteloides*. The late development of a loop differs from those which originated from a lack of sequence homology for pairing. The DNA sequences possibly associated with SYCP3 in the lateral elements of SCs are mostly unknown, although preliminary data suggest that these sequences are markedly enriched for a specific family of short interspersed elements (Johnson et al. 2013). As the cohesin axis is independent from the protein SYCP3 (see above) and is needed for recombination, it is suggested that the cohesion-containing SMC3 core is internal to the SYCP3 structures, and that the bases of chromatin loops traverse the SYCP3 of the LE to reach the cohesin core. This core is continuous along the large SYCP3 loop, but it may be loosened at the attachment ends to allow the entrance of a segment from one of the sister chromatids that will be involved in a crossover (a related diagram has been proposed by Hernández-Hernández et al. 2009). New approaches to this matter are needed to have a more definitive scheme of the molecular structure of the synaptonemal complex.

Acknowledgments The able help of Lic C. Deparci is sincerely thanked. We are also very grateful to Prof. M. Alsheimer (University of Würzburg, Germany) for the generous gift of SYCE3 antibody and P.J. Moens† and B. Spyropoulus (York University, Canada) for SYCP1 antibody. We specially thank Prof. Dr. A. Brusco and Eng. Lisandro Anton from LANAIS' service (CONICET). This work was supported by grants from UBACYT 20020100100030 (AJS) and PICT-2010-2718 (RBS).

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