Involvement of the cohesin Rad21 and SCP3 in monopolar attachment of sister kinetochores during mouse meiosis I

María Teresa Parra[1], Alberto Viera[1], Rocío Gómez[1], Jesús Page[1], Ricardo Benavente[2], Juan Luis Santos[3], Julio S. Rufas[1] and José A. Suja[1,*]

[1]Departamento de Biología, Edificio de Biológicas, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[2]Department of Cell and Developmental Biology, Theodor Boveri Institute, University of Würzburg, 97074 Würzburg, Germany
[3]Departamento de Genética, Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain
*Author for correspondence (e-mail: jose.suja@uam.es)

Accepted 20 October 2003
Journal of Cell Science 117, 1221-1234 Published by The Company of Biologists 2004
doi:10.1242/jcs.00947

Summary

SCP3 is a meiosis-specific structural protein appearing at axial elements and lateral elements of the synaptonemal complex. We have analysed the behaviour of SCP3 and the cohesin subunit Rad21 in mouse spermatocytes by means of a squashing technique. Our results demonstrate that both proteins colocalize and are partially released from chromosome arms during late prophase I stages, although they persist at the interchromatid domain of metaphase I bivalents. Thus, Rad21 cannot be considered a 'mitotic'-specific variant, but coexists with Rec8. During late prophase I SCP3 and Rad21 accumulate at centromeres, and together with the chromosomal passenger proteins INCENP and aurora-B kinase, show a complex 'double cornet'-like distribution at the inner domain of metaphase I centromeres beneath the associated sister kinetochores. We have observed that Rad21 and SCP3 are displaced from centromeres during telophase I when sister kinetochores separate, and are not present at metaphase II centromeres. Thus, we hypothesise that Rad21, and the superimposed SCP3 and SCP2, are involved in the monopolar attachment of sister kinetochores during meiosis I, and are not responsible for the maintenance of sister-chromatid centromere cohesion during meiosis II as previously suggested.

Key words: Meiosis, Mouse, Kinetochore, Monopolar attachment, Sister-chromatid cohesion

Introduction

During meiosis two successive cellular divisions take place without an intervening DNA replication step thus resulting in the generation of haploid germ cells. During meiotic prophase I homologues pair, synapse and recombine. These processes are essential to ensure a correct chromosome segregation during meiosis I, and are thought to be mediated by a meiosis-specific structure, the synaptonemal complex (SC). The SC is a proteinaceous structure found between synapsed homologues during the pachytene stage of prophase I (for review, see Zickler and Kleckner, 1999). Ultrastructurally, the SC appears as a tripartite structure composed by two parallel lateral elements (LEs) and a central element. The LEs and the central element are held together by fine fibres called transverse filaments. Axial chromosome structures called axial elements (AEs) appear during leptotene, associated with the two sister chromatids of each homologue, and are the precursors of the LEs found in zygotene and pachytene. During diplotene and diakinesis, homologues desynapse and the SC disassembles with the concomitant separation of LEs and disappearance of the central element.

Two AE/LE components have been characterised in mammals: SC proteins SCP2 and SCP3. The localization of rat/mouse SCP3 (Moens et al., 1987; Lammers et al., 1994), also called COR1 in hamster (Dobson et al., 1994), has been mainly studied in surface-spread rodent spermatocytes. SCP3 first appears at AEs in leptotene spermatocytes, and is found at LEs during pairing and synapsis during zygotene and pachytene, respectively. In diplotene spermatocytes, SCP3 is present at desynapsed LEs and still persists between sister chromatids until the metaphase I/anaphase I transition (Dobson et al., 1994; Moens and Spyropoulos, 1995). SCP2 shows a similar distribution pattern during prophase I (Schalk et al., 1998). Interestingly, it has been reported that SCP3 persists at centromeres up to anaphase II. Consequently, it has been proposed that SCP3 may have a role in sister-chromatid arm cohesion during meiosis I and in centromere cohesion during meiosis II (Moens and Spyropoulos, 1995). It has been demonstrated that SCP3 is essential for both formation and maintenance of AEs/LEs since in its absence no AEs/LEs are found (Yuan et al., 2000; Pelttari et al., 2001).

During meiosis sister-chromatid cohesion is released in two steps (Suja et al., 1992; Miyazaki and Orr-Weaver, 1994; Petronczki et al., 2003). Arm cohesion is lost during the metaphase I/anaphase I transition to allow the segregation of recombined homologues. This reductional segregation is possible since sister kinetochores remain closely associated and then bivalents reach an accurate biorientation. Recent studies have proposed that in budding yeast the monopolar attachment of sister kinetochores is facilitated by the

'monopolin' complex (Toth et al., 2000; Rabitsch et al., 2003), while in fission yeast this is ensured by the cohesin subunit Rec8 (Watanabe and Nurse, 1999; Yokobayashi et al., 2003). Centromere cohesion is released during the metaphase II/anaphase II transition to allow chromatid segregation. It has been demonstrated that arm and centromere cohesion are maintained by a cohesin complex in both mitotic and meiotic chromosomes (for reviews, see Nasmyth, 2001; Nasmyth, 2002). Recently, several subunits of the cohesin complex have been found to be associated with mammalian AEs/LEs (for reviews, see Lee and Orr-Weaver, 2001; Nasmyth, 2001; Petronczki et al., 2003). Thus, it has been reported that the cohesin subunits belonging to the structural maintenance of chromosome proteins SMC1α, SMC1β and SMC3 (Eijpe et al., 2000; Revenkova et al., 2001), and the non-SMC cohesin subunits STAG3 (Pezzi et al., 2000; Prieto et al., 2001; Pelttari et al., 2001), STAG2 and Rad21 (Prieto et al., 2002), and Rec8, a meiosis-specific variant of Rad21 (Eijpe et al., 2003; Lee et al., 2003), are present at AEs/LEs from leptotene up to diplotene. After the SC disassembly, STAG3 remains at the interchromatid domain of metaphase I chromosomes, but is lost during the metaphase I/anaphase I transition (Prieto et al., 2001). Accordingly, it has been proposed that STAG3 is mainly involved in maintaining sister-chromatid arm cohesion. By contrast, it has been reported that SMC1β, SMC3 and Rec8 persist at centromeres from metaphase I up to anaphase II thus regulating sister-chromatid centromere cohesion (Revenkova et al., 2001; Eijpe et al., 2003; Lee et al., 2003).

During recent years it has been suggested that INCENP and aurora-B kinase, two chromosomal passenger proteins present at the inner centromere during mitotic metaphase, and redistributing to the spindle midzone during anaphase, are implicated, not only in coordinating chromosome segregation with cytokinesis, but also in centromere cohesion (Adams et al., 2001; Vagnarelli and Earnshaw, 2001). Interestingly, INCENP distributes abnormally at centromeres in chicken mutant cells for the cohesin subunit Rad21 (Sonoda et al., 2001), and its dynamics is affected in *Drosophila* cells depleted of Rad21 (Vass et al., 2003). Additionally, aurora-B phosphorylates the cohesin subunit Rec8, and therefore promotes the release of sister-chromatid cohesion during both meiotic divisions in *C. elegans* (Rogers et al., 2002). We have recently found in mouse spermatocytes that INCENP and aurora-B colocalize at the inner domain of metaphase I centromeres and at a connecting strand joining sister kinetochores in metaphase II centromeres. Accordingly, we have proposed that these passenger proteins may regulate sister-chromatid centromere cohesion during both meiotic divisions (Parra et al., 2003).

We have studied the subcellular distribution and behaviour of SCP3 in all post-diplotene stages on squashed spermatocytes. To determine the accurate location of SCP3 at metaphase I centromeres we have double immunolabelled SCP3 with CENP-A, a histone H3-like protein present at the inner kinetochore plate of mammalian trilaminar kinetochores, with CENP-E, a kinesin-related microtubule motor protein located at the outer kinetochore plate and fibrous corona, and with INCENP and aurora-B kinase. We also studied the codistribution of SCP3 and the mitotic cohesin Rad21. We discuss the dynamic redistribution of SCP3 and Rad21 during late prophase I stages, and demonstrate that these proteins are not present at metaphase II centromeres. Additionally, we propose that the inner centromere domain, also detected by electron microscopy, maintains the association of sister kinetochores to allow their monopolar attachment, thus allowing an accurate reductional segregation during meiosis I. We suggest that in mammals the mechanisms maintaining the association of sister kinetochores during meiosis I are different from those maintaining centromere cohesion up to anaphase II. In addition, we present for the first time the complex organization of the centromere and of their different domains in metaphase I chromosomes.

Materials and Methods

Immunofluorescence microscopy

Testes from adult male C57BL/6 mice were used for this study. Testes were removed and then fixed for immunofluorescence as described previously (Page et al., 1998). Briefly, testes were fixed in freshly prepared 2% formaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.7 mM KH_2PO_4, pH 7.4) containing 0.1% Triton X-100 (Sigma). After 5 minutes several seminiferous tubules were placed on a slide coated with 1 mg/ml poly-L-lysine (Sigma) with a small drop of fixative and the tubules were gently minced with tweezers. The tubules were subsequently squashed and the coverslip removed after freezing in liquid nitrogen. The slides were then rinsed three times for 5 minutes in PBS and incubated for 45 minutes at room temperature with primary antibody. In double labelling experiments, primary antibodies were incubated simultaneously. Following three washes in PBS, the slides were incubated for 30 minutes at room temperature with a fluorescein isothiocyanate (FITC)-conjugated or Texas Red-conjugated goat anti-rabbit IgG (Jackson) secondary antibody at a 1:150 dilution in PBS, a FITC-conjugated donkey anti-guinea pig IgG (Jackson) secondary antibody at a 1:200 dilution in PBS, or a Texas Red-conjugated goat anti-human IgG (Jackson) secondary antibody at a 1:150 dilution in PBS. The slides were subsequently rinsed in PBS and counterstained for 3 minutes with 5 μg/ml DAPI (4′,6-diamidino-2-phenylindole). After a final rinse in PBS, the slides were mounted with Vectashield (Vector Laboratories). The edges of the coverslips were sealed with nail polish. Observations were performed using an Olympus BH-2 microscope equipped with epifluorescence optics and the images were recorded with an Olympus DP50 digital camera and saved in Adobe Photoshop 6.0.

Antibodies

To detect SCP3 we used either the rabbit polyclonal serum A1 (Lammers et al., 1994) at a 1:400 dilution in PBS, a guinea pig polyclonal serum against a 12-mer peptide corresponding to residues 27-38 of rat SCP3 (Alsheimer and Benavente, 1996) at a 1:100 dilution, or a rabbit polyclonal serum (K921) against human SCP3 at a 1:50 dilution. SCP2 was detected with the rabbit polyclonal serum 493 (Schalk et al., 1998) at a 1:100 dilution. Centromeres were detected with the human autoimmune anticentromere (ACA) serum EK at a 1:100 dilution. For detecting CENP-A we used the rabbit polyclonal serum SS2 raised against a synthetic peptide covering amino acid residues 3-17 of human CENP-A (Valdivia et al., 1998) at a 1:100 dilution. To detect CENP-E we used pAb1.6, a rabbit polyclonal serum that recognises the neck region (amino acids 256-817) of CENP-E (Lombillo et al., 1995; Parra et al., 2002), at a 1:400 dilution. INCENP was detected with the rabbit polyclonal serum 1186 (Eckley et al., 1997), at a 1:100 dilution. To detect aurora-B kinase we employed the mouse monoclonal AIM-1 antibody (Transduction Labs) at a 1:30 dilution. The Polo-like kinase I (PLK1) was detected with a cocktail of mouse monoclonal antibodies (Zymed) used at a 1:20 dilution. The telomeric protein Rap1 was detected with a rabbit polyclonal serum (Scherthan et al., 2000), at a 1:500 dilution. The

cohesin Rad21 was detected with two different rabbit polyclonal sera (Gregson et al., 2001; Prieto et al., 2002), at 1:20 and 1:50 dilutions, respectively.

Electron microscopy

Seminiferous tubules were processed for EM as previously described (Wolf, 1987). Briefly, seminiferous tubules were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) at room temperature for 5 minutes. Then, 3 volumes of 8% tannic acid (Merck) in phosphate buffer were added for 1 hour. Fixed seminiferous tubules were washed in phosphate buffer, and postfixed in 2% osmium tetroxide at room temperature for 1 hour. Finally, fixed tubules were washed in phosphate buffer, dehydrated in an ethanol series and embedded in Epon 812.

Mouse testes were subjected to the osmium tetroxide/p-phenylenediamine (Os-PPD) technique as previously described (Antonio et al., 1996). Seminiferous tubules were fixed in 2% OsO_4 in bidistilled water at room temperature for 45 minutes and rinsed in 6.5% sucrose in distilled water for 30 minutes. During dehydration in ethanol the material was treated with 4% p-phenylenediamine (PPD) (Merck) in 70% ethanol for 2 hours at room temperature. Then, testes were rinsed in 70% ethanol, dehydrated and embedded in Epon 812. Thin sections (0.2-0.5 μm thickness) were obtained in a Reichert-Jung ultramicrotome, transferred to copper/rhodium grids, and observed without posterior contrasting. All observations were made in a Jeol 1010 transmission electron microscope operated at 80 kV.

Results

SCP3 and the cohesin subunit Rad21 are partially released from desynapsed LEs during late diplotene/diakinesis

We used the squashing technique since it unambiguously allows differentiation of all meiotic stages, and does not affect chromosome condensation and positioning in dividing spermatocytes (Suja et al., 1999). The three different anti-SCP3 antibodies we used produced a similar pattern of labelling throughout spermatogenesis. SCP3 first became detectable in leptotene spermatocytes, and appeared at the unpaired and paired AEs during zygotene. During pachytene, the autosomal SCs and the unsynapsed sex AEs were labelled (Fig. 1A), while during early diplotene desynapsed LEs were observed (Fig. 1D). Rad21 colocalized with SCP3 during all prophase I stages (Fig. 1A-F). In late diplotene spermatocytes, some accumulations of SCP3 were detected along some desynapsed LEs, and unsynapsed sex AEs (Fig. 1G,H). Additionally, a few SCP3 agglomerates were observed separated from LEs in the nucleoplasm (Fig. 1G-I). In early diakinesis, an increasing number of SCP3 accumulations were observed along desynapsed LEs that in turn had become thinner (Fig. 1J). In addition, large SCP3 agglomerates were found in the nucleoplasm (Fig. 1J). During late diakinesis, the fluorescence intensity shown by the nucleoplasm increased dramatically so that desynapsed LEs became barely visible (Fig. 1K,L). During this stage, bright and large SCP3 agglomerates were found in the nucleoplasm (Fig. 1K,L), and accumulations of SCP3 were still found on the desynapsed LEs, and unsynapsed sex AEs (inset in Fig. 1L). Additionally, an intense T-shaped SCP3 labelling was found at some centromeres (inset in Fig. 1K). During prometaphase I, the fluorescence intensity found in the nucleoplasm decreased, and large SCP3 agglomerates were clearly discerned (Fig. 1M-O). SCP3 was also observed as wiggly thin lines inside condensing bivalents (Fig. 1M,N). During this stage most centromeres showed an intense T-shaped SCP3 labelling (inset in Fig. 1M). Altogether, these results indicate that once LEs desynapse during diplotene SCP3 and Rad21 are partially released from chromosomes and accumulate at discrete regions along LEs, disperse in the nucleoplasm and concentrate there as large agglomerates.

SCP3 and Rad21 concentrate at centromeres but still remain at the interchromatid domain in metaphase I bivalents

Metaphase I spermatocytes showed large SCP3 agglomerates in the faintly labelled cytoplasm (data not shown). Additionally, SCP3 labelled the surface of contact between sister chromatids, the so-called interchromatid domain, and centromeres in all bivalents (Fig. 2).

A careful examination of the SCP3 labelling in autosomal metaphase I bivalents revealed the presence of a series of small round or slightly elongated patches at the interchromatid domain (Fig. 2A and Fig. 3A-D). This labelling was observed from centromeres to chiasma sites, where it always interrupted, and from chiasma sites towards distal telomeres (Fig. 3B,C). SCP3 also labelled the interchromatid domain in the sex bivalent (Fig. 3E). As a rule, the labelling at the interchromatid domain of the sex bivalent was brighter and more continuous than those observed in autosomal bivalents (compare Fig. 2C and Fig. 3E with Fig. 2A and Fig. 3A-D). Interestingly, the interchromatid domain also appeared labelled in occasionally found sex univalents (Fig. 3F,G). Besides this labelling at the interchromatid domain, a brighter SCP3 labelling was also observed at centromeres (Fig. 3A-D). After comparing the SCP3 fluorescence intensity shown by centromeres during diplotene with that present at prometaphase I and metaphase I ones, it became evident that SCP3 had progressively concentrated at centromeres (compare insets in Fig. 1G and M). Normally, the SCP3 labelling at the centromere of the X chromosome was larger than that found at the centromere of the Y chromosome (Fig. 3E-G). As during prophase I stages, Rad21 colocalized with SCP3 at the interchromatid domain and centromeres of metaphase I sex (Fig. 3H) and autosomal bivalents (Fig. 3I), and sex univalents (Fig. 3J).

SCP3 and SCP2 colocalize with Rad21 and show a complex ‘double cornet’-like arrangement at the inner domain of metaphase I centromeres

A closer examination of the SCP3 labelling at metaphase I centromeres revealed that the signal was T- or Y-shaped (Fig. 3A,E). However, this morphology was only observed when centromeres were viewed from the side (Fig. 4A,C,E,F,H,I). Surprisingly, when centromeres were viewed from the top, two side-by-side associated SCP3 rings were discerned (Fig. 4B,D,G,J). Similar results were observed for the distribution of SCP2 (data not shown). Moreover, Rad21 also showed the same distribution pattern in side and top views (Fig. 4I,J). Thus, SCP3, SCP2 and Rad21 appeared as two rings with a longitudinal projection towards the inner centromere region emerging from the contact zone between the rings. This longitudinal projection was continuous with the series of small patches found at the interchromatid domain. Consequently,

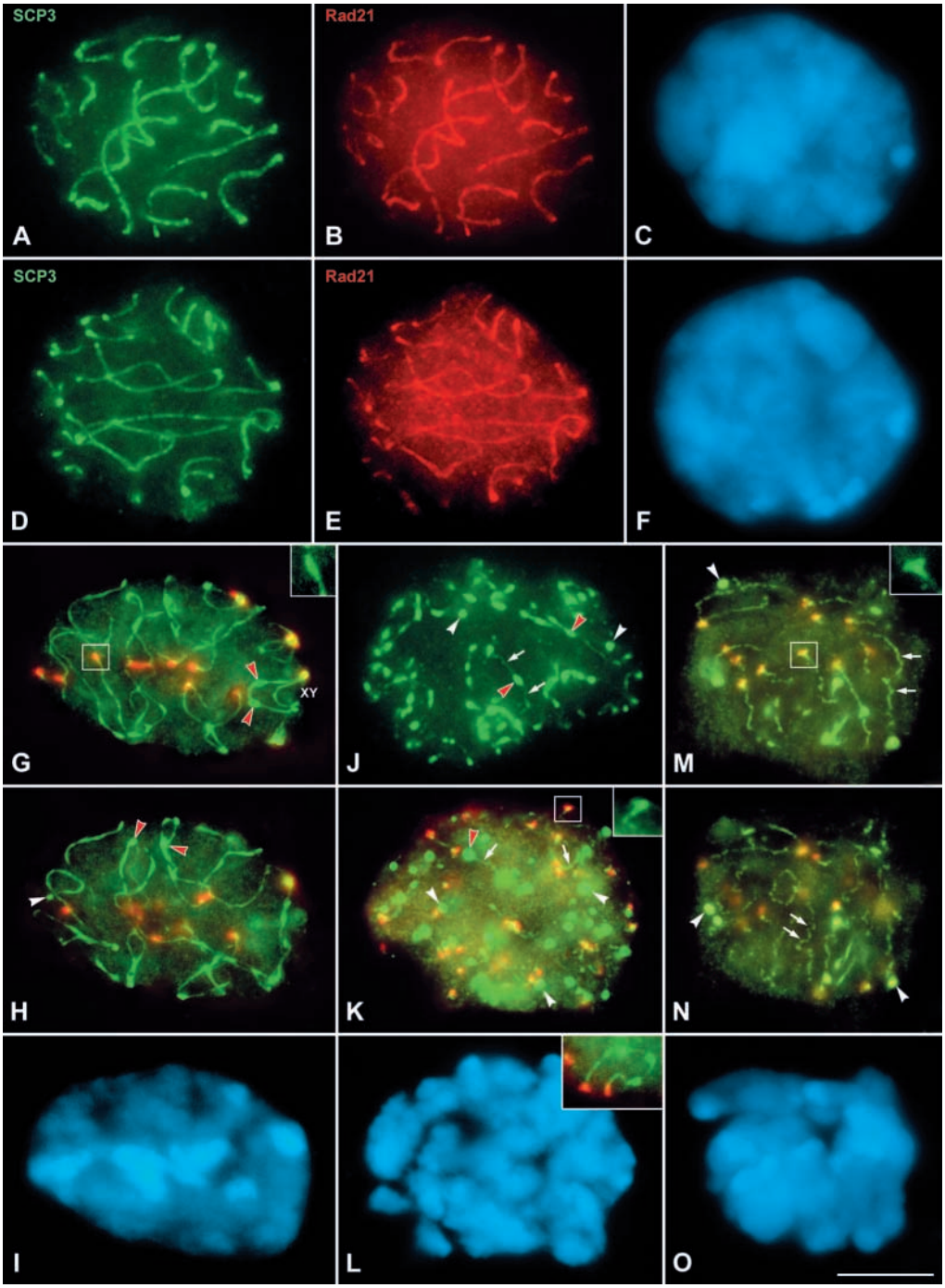

Fig. 1. SCP3 accumulates on the LEs during late diplotene, is partially lost from LEs during diakinesis, but still persists at centromeres and arms in prometaphase I bivalents. SCP3 is labelled in green; Rad21 in red in B,E; centromeres in red in G,H,K,M,N, and DNA in blue.
(A-C) Superimposition of five focal planes throughout a pachytene spermatocyte showing colocalization of SCP3 and Rad21 at SCs.
(D-F) Superimposition of nine focal planes throughout an early diplotene spermatocyte showing colocalization of SCP3 and Rad21 at desynapsed LEs. (G-I) Partial projections from different focal planes throughout a late diplotene nucleus where some accumulations of SCP3 (red arrowheads) are found on some desynapsed LEs. Some small SCP3 agglomerates (white arrowhead in H) are also observed separated from LEs. The sex AEs are indicated (XY). The inset in G is a magnification of the boxed area showing the SCP3 labelling at an homologous centromere.
(J) Superimposition of different focal planes throughout an early diakinesis nucleus where a large number of SCP3 accumulations (red arrowheads) are observed along thin desynapsed LEs (white arrows). Some SCP3 agglomerates (white arrowheads) are detected in the nucleoplasm.
(K,L) Single focal plane throughout a late diakinesis nucleus showing a high SCP3 labelling dispersed in the nucleoplasm where some large SCP3 agglomerates (white arrowheads) are present. An SCP3 accumulation (red arrowhead) is observed in continuity with a thin desynapsed LE (white arrows). The inset in K is a magnification of the boxed area showing the SCP3 labelling at a homologous centromere. The inset in L shows three SCP3 accumulations along unsynapsed sex AEs. (M,N) Two focal planes of a prometaphase I spermatocyte. The nucleoplasm shows a faint labelling and some large SCP3 agglomerates (arrowheads). SCP3 persists at centromeres and appears as wiggly lines (arrows) inside bivalents. The inset in M is a magnification of the boxed area showing the SCP3 labelling at a homologous centromere. Scale bar: 10 μm.

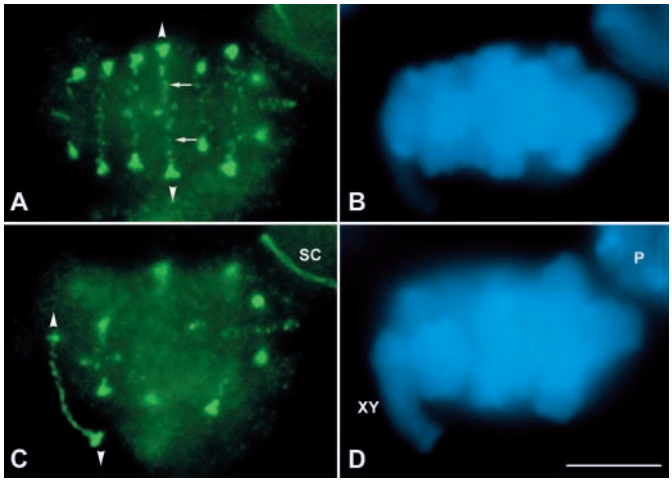

Fig. 2. SCP3 is highly enriched at homologous centromeres (arrowheads), but is still present at the interchromatid domain (arrows) in metaphase I bivalents. SCP3 is labelled in green and DNA in blue. (A,C) Partial projections from different focal planes throughout the same metaphase I spermatocyte. The SCP3 labelling in the sex bivalent (XY) is observed in C. A pachytene (P) SC fragment (SC) is apparent in the upper right corner in C. Scale bar: 10 μm.

SCP3, SCP2 and Rad21 showed an unpredicted complex 'double cornet'-like arrangement at metaphase I centromeres.

SCP3 and SCP2 are located beneath closely associated sister kinetochores at the inner centromere domain colocalizing with INCENP and aurora-B kinase

Since the morphology of SCP3 signals at metaphase I centromeres was unexpected, we determined the accurate localization of SCP3 by colocalizing with different well known centromere/kinetochore proteins. We first performed a double immunolabelling of SCP3 and centromeres with an ACA serum. As a rule, a single ovoid ACA signal was observed at each centromere when they were viewed from the side (Fig. 4A). The superimposition of SCP3 and ACA signals demonstrated that SCP3 was located beneath the ACA signals, although some degree of colocalization was observed (Fig. 4A). Top views of centromeres showed a single ACA signal on which the SCP3 rings were superimposed (Fig. 4B). Since ACA sera recognize several centromere/kinetochore proteins, and in order to precisely determine the location of SCP3, we made a double immunolabelling of SCP3 and CENP-A, an inner kinetochore protein. CENP-A appeared as two closely associated round signals, corresponding to sister kinetochores, at each centromere when they were viewed from the side (Fig. 4C). The superimposed images of SCP3 and CENP-A showed that SCP3 was located beneath the CENP-A signals although partially colocalizing (Fig. 4C). When centromeres were viewed from the top, CENP-A signals appeared encircled by the SCP3 rings (Fig. 4D). We also performed a double immunolabelling of SCP3 with CENP-E, a motor protein located at the outer kinetochore (Parra et al., 2002). When centromeres were viewed from the side, CENP-E appeared as a small plate adjacent to the underlying SCP3 signal (Fig. 4E). We then carried out a double immunolabelling of SCP3 with INCENP. When centromeres were viewed from the side it became apparent that SCP3 and INCENP mostly colocalized, however, the INCENP signal was more diffuse and surrounded the longitudinal projection emerging between the SCP3 rings (Fig. 4F). Although SCP3 and INCENP signals were similar in side view, a single INCENP signal was observed when centromeres were viewed from the top (Fig. 4G). The same results were observed after colocalizing SCP3 with the aurora-B kinase, that in turn colocalized with INCENP (Parra et al., 2003). Altogether, these results show that SCP3 and SCP2 are located at the inner centromere domain beneath the closely associated sister kinetochores, and colocalizing with the chromosomal passenger complex proteins INCENP and aurora-B.

Since neither of the kinetochore proteins we had tested appeared inside the SCP3/SCP2/Rad21 rings, we thought that proximal telomeres could be occupying that position given that mouse chromosomes are telocentric (Kipling et al., 1991; Garagna et al., 2003). To validate such hypothesis we performed a double immunolabelling of SCP3 and the telomeric protein Rap1. We detected two separated spots corresponding to sister proximal telomeric sequences that partially colocalized with SCP3. However, telomeric signals were not located at the same plane as the SCP3 rings viewed from the side (Fig. 4H). Taking into account the relative distribution of kinetochore proteins, SCP3 and Rap1, it is clear that telomeric sequences are located beneath sister kinetochores at the inner centromere domain.

The inner centromere domain is ultrastructurally revealed with the Os-PPD technique

The Os-PPD technique reveals preferentially SCs and nucleoli in mammalian spermatocytes (Esponda and Stockert, 1978). Since the LE proteins SCP3 and SCP2 are retained at the inner domain of metaphase I centromeres, we decided to test whether this cytochemical technique also revealed this domain at ultrastructural level. In sections of metaphase I spermatocytes the condensed chromatin appeared of low contrast but centromeres appeared highly contrasted (Fig. 5). When centromeres were examined at higher magnification, the outer and middle electron-lucent kinetochore plates became evident (arrowheads in Fig. 5B,C). Beneath kinetochores, a highly contrasted region showing a T-like appearance, and reminiscent of the SCP3 labelling observed by immunofluorescence, was detected (Fig. 5B,C). This result demonstrates that the inner centromere domain has a protein/ribonucleoprotein composition different from that in the rest of the centromere and condensed chromatin.

Sister kinetochores are closely associated in metaphase I, slightly separate during anaphase I, and during both stages they interact with microtubules

Recently, it has been suggested that the monopolar orientation of each homologous centromere during metaphase I is due to the close association of sister kinetochores, and that one possibility is that only one of the sisters attaches to kinetochore microtubules (Petronczki et al., 2003). To verify this possibility we analysed serial sections of metaphase I and anaphase I chromosomes by transmission electron microscopy. During

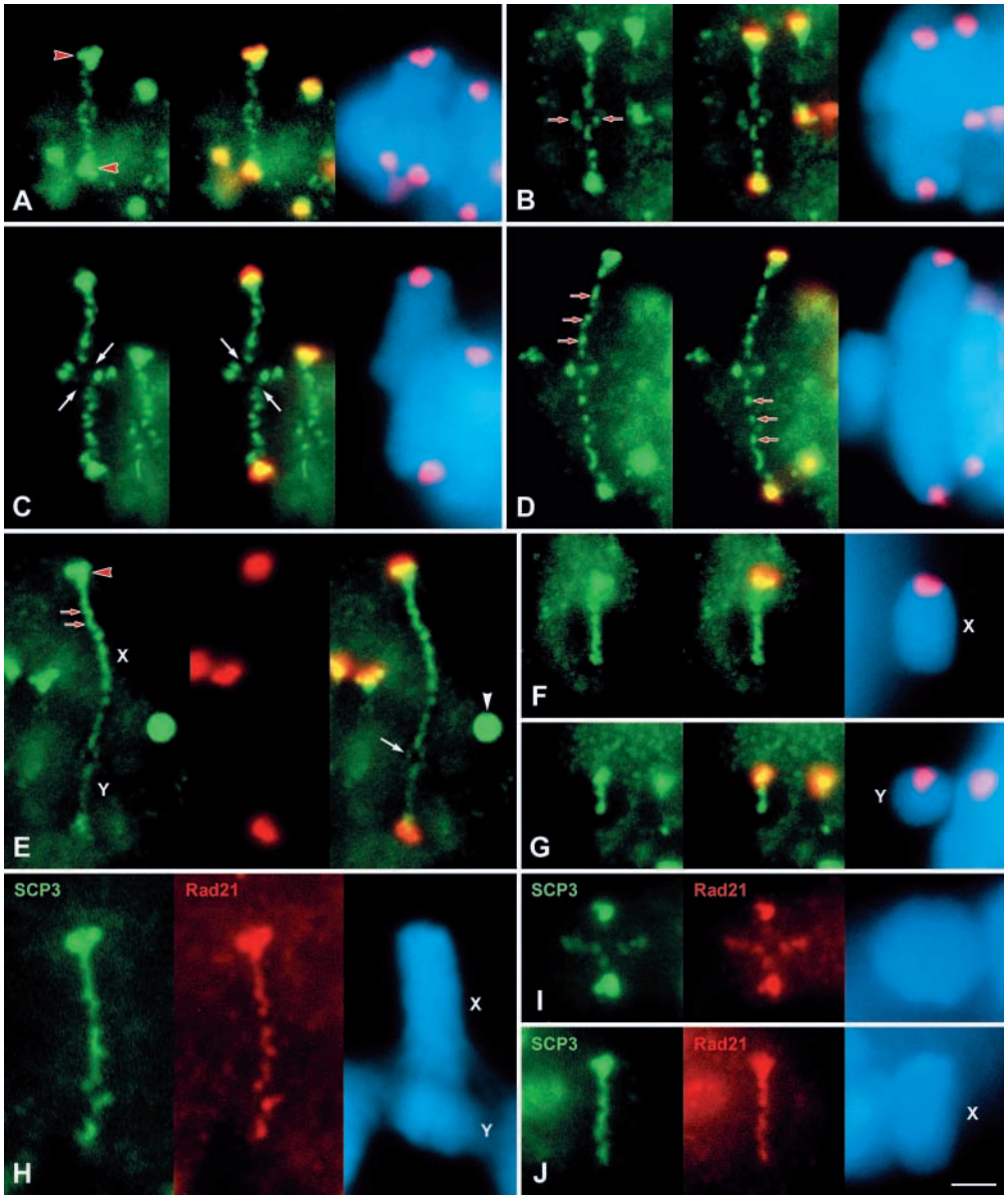

Fig. 3. Selected metaphase I autosomal (A-D,I) and sex (E,H) bivalents, and sex univalents (F,G,J). SCP3 is labelled in green, centromeres in red in A-G, Rad21 in red in H-J, and DNA in blue. In the autosomal bivalents SCP3 appears highly enriched at homologous centromeres (red arrowheads in A). A fainter SCP3 labelling is also found at the interchromatid domain (red arrows in D). Note however that this labelling does not reach the distal chromosome ends (red arrows in B), and that it is interrupted at chiasma sites (white arrows in C). In the sex bivalent the SCP3 labelling at the centromere of the X chromosome (X) (red arrowhead in E) is bigger than that found at the centromere of the Y chromosome (Y). The SCP3 labelling at the interchromatid domain of the sex chromosomes is larger and more continuous (red arrows in E) than that observed in autosomal bivalents independently of whether they appear as a bivalent (E,H) or as univalents (F,G,J). In E the chiasma site in one sex bivalent is indicated by an arrow and a large SCP3 agglomerate (indicated by an arrowhead) lies in the cytoplasm near the sex bivalent. (H,I) Rad21 colocalizes with SCP3 in sex (H) and autosomal (I) bivalents, and for instance in the univalent X chromosome (J). Scale bar: 2 μm.

metaphase I, sister kinetochores could not be determined as discrete entities, but a continuous outer kinetochore plate was observed (Fig. 6). Microtubules interacted all along this kinetochore plate (Fig. 6B-G). By contrast, sister kinetochores were slightly separated during anaphase I, and both interacted with microtubules (Fig. 7).

SCP3 and Rad21 are lost from chromosome arms during the metaphase I/anaphase I transition, and detach from sister kinetochores during telophase I

During anaphase I, SCP3 was not observed at chromosome arms, but still persisted at centromeres (Fig. 8A-C). In addition to this centromere labelling, large SCP3 agglomerates were found in the cytoplasm (Fig. 8A). When anaphase I poles were viewed from the top, two closely associated ACA signals, representing sister kinetochores, were evident at each centromere. In some of them two clear SCP3 rings were observed surrounding the ACA signals (Fig. 8D-F). During telophase I, sister kinetochores were more separated than during anaphase I (compare Fig. 8E and H). Interestingly, in these telophase I chromosomes SCP3 did not appear as rings, but as small elongated bars lying between sister kinetochores (white arrowheads in Fig. 8G-I), or separated from them (blue arrowheads in Fig. 8G-I). Rad21 colocalized with SCP3 at these bars (Fig. 8J-L). Thus, the change of distribution of SCP3 and Rad21 at telophase I centromeres is concomitant with the separation between sister kinetochores.

SCP3 and Rad21 are not present at metaphase II centromeres

Since it has been reported that SCP3 persists at metaphase II centromeres in surface-spread spermatocytes (Moens and Spyropoulos, 1995; Revenkova et al., 2001; Eijpe et al., 2003), we next investigated its distribution in meiosis II squashed

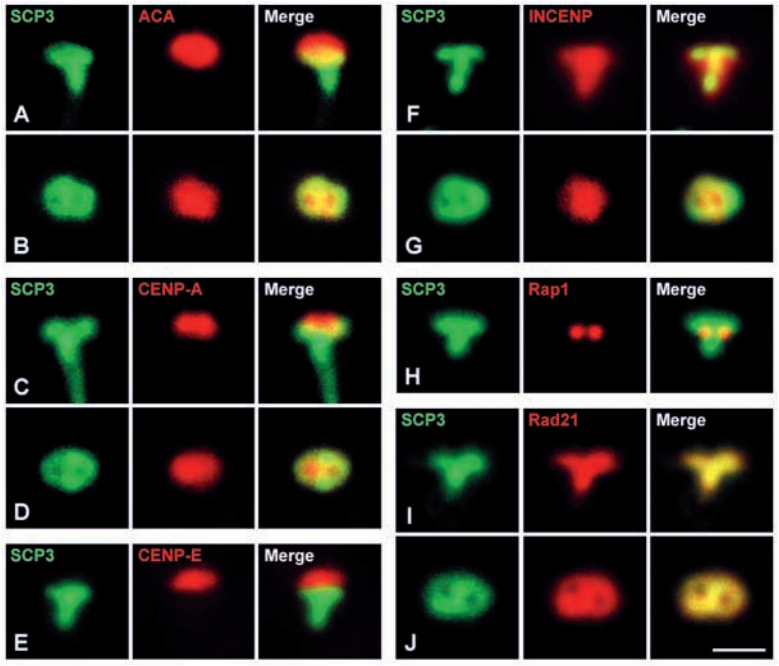

Fig. 4. SCP3 is located beneath associated sister kinetochores at the inner domain of metaphase I centromeres, showing a complex 'double cornet'-like arrangement, and colocalizing with the cohesin Rad21. SCP3 is labelled in green, and centromeres (ACA), CENP-A, CENP-E, INCENP, Rap1 and Rad21 are labelled in red. In merged images colocalization regions appear in yellow. SCP3 appears as a T- or Y-like structure in centromeres viewed from the side (A,C,E,F,H,I), but as two closely associated rings when centromeres are viewed from the top (B,D,G,J). SCP3 appears beneath the ACA and CENP-A signals with which there is some degree of colocalization (A,C). The ACA, and the pair of CENP-A dots representing sister kinetochores, appear surrounded by the SCP3 rings (B,D). CENP-E appears as a single plate separated from the underlying SCP3 signal (E). By contrast, SCP3 colocalizes at the inner centromere domain with INCENP (F) and Rad21 (I). In this sense, Rad21 is the only protein appearing as two closely associated rings colocalizing with SCP3 when centromeres are viewed from the top (J). Sister proximal telomeric sequences, as detected with the anti-Rap1 antibody, are detected as a pair of dots partially colocalizing with the middle region of the T-shaped SCP3 signal (H). Scale bar: 1 μm.

preparations of spermatocytes. In interkinesis nuclei, SCP3 was detected as elongated bars that were separated from pairs of sister kinetochores (Fig. 9A,B). The number of bars diminished in comparison to those found in telophase I reforming nuclei. By contrast, no SCP3 labelling was observed either on the chromosomes or in the cytoplasm in prophase II, and prometaphase II spermatocytes, where unaligned chromosomes were sporadically observed (Fig. 9C,D), and in metaphase II (Fig. 9E-F) and early anaphase II (Fig. 9I,J) spermatocytes. Rad21, like SCP3, was not present in metaphase II chromosomes (Fig. 9K,L). It is interesting to emphasize that in telophase I chromosomes and unaligned prometaphase II chromosomes the distance between sister kinetochores was always smaller than that observed in aligned metaphase II chromosomes (compare Fig. 8H and inset in Fig. 9D with Fig. 9G,H). SCP3 was again observed in telophase II nuclei partially colocalizing with kinetochore signals, and also in early round spermatids (data not shown). However, SCP3 disappeared from elongating spermatids.

Discussion

Release of LE components and Rad21 from chromosome arms during late prophase I stages

We have found that from late diplotene up to metaphase I SCP3 is progressively released, although not completely, from desynapsed LEs. These data, summarized in Fig. 10A, argue that once homologues desynapse, SCP3, and also SCP2, are released from LEs to accumulate at discrete sites along them. Then, this SCP3/SCP2 population may be delivered to the

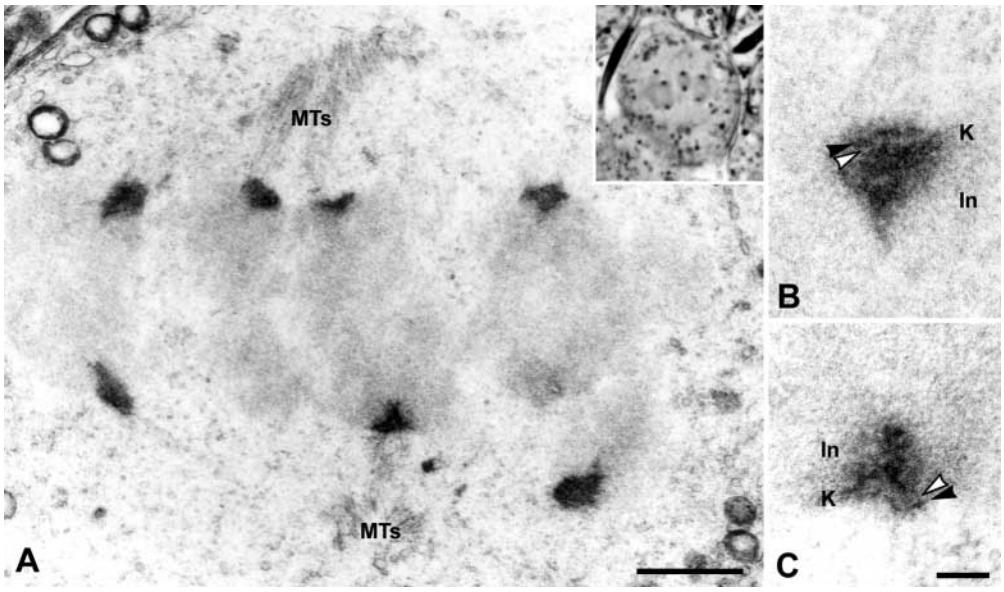

Fig. 5. The ultrastructure of the inner centromere domain is revealed by the Os-PPD technique. (A) Section of a metaphase I spermatocyte. Condensed bivalents show very low contrast, while centromeres show high contrast. Bundles of kinetochore microtubules (MTs) are present. A semithin section of a metaphase I spermatocyte viewed by phase-contrast microscopy is shown in the inset. (B,C) Enlarged centromeres facing opposite poles where the outer (black arrowheads) and middle electron-lucent (white arrowheads) kinetochore plates are visible. Beneath kinetochores (K), a highly contrasted T-shaped region, representing the inner centromere domain (In), is observed. Scale bar: (A) 2.5 μm; (B,C) 0.5 μm.

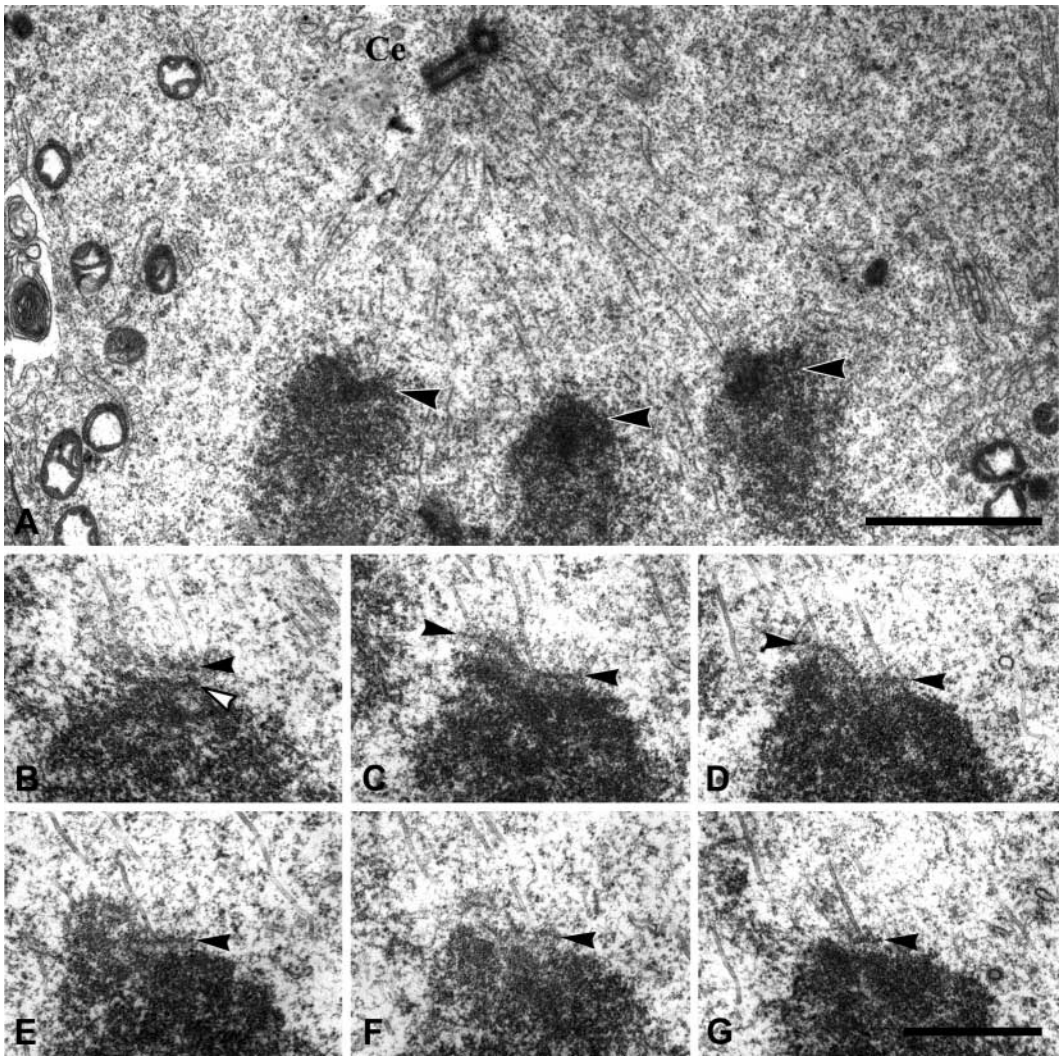

Fig. 6. Sister kinetochores are closely associated during metaphase I. (A) Section of a metaphase I spermatocyte. Centromeres (arrowheads) are more contrasted than the condensed chromatin at the arms. A pair of centrioles (Ce) is visible at a cell pole. (B-G) Six serial sections of a metaphase I centromere. Sister kinetochores are not resolved, but a continuous outer kinetochore plate (black arrowheads), where kinetochore microtubules end, is discernible. The inner kinetochore plate is visible in some sections (white arrowhead in B). Scale bars: (A) 3 μm; (B-G) 600 nm.

nucleoplasm where it self-assembles to generate the large nucleoplasmic agglomerates, which are reminiscent of the previously described polycomplexes (Zickler and Kleckner, 1999). This possibility is supported by the fact that SCP3 self-assembles in the nucleoplasm and cytoplasm when expressed in cultured somatic cells (Yuan et al., 1998). During prometaphase I the release of SCP3/SCP2 from chromosome arms continues, but is not complete, and consequently, discrete patches remain at the interchromatid domain of metaphase I bivalents.

The observation that most SCP3 and SCP2 are released from chromosome arms during late prophase I stages may have an outstanding functional implication. It has been reported that in mitotic chromosomes from metazoans most cohesin complexes dissociate from arms during prophase because of their phosphorylation (Losada et al., 2000; Waizenegger et al., 2000; Warren et al., 2000). Although it was initially proposed that this bulk release of cohesin would be required for proper chromosome condensation (Losada and Hirano, 2001; Nasmyth, 2001; Nasmyth, 2002), it has been recently demonstrated that this release is only necessary for the resolution of sister chromatids (Losada et al., 2002). We think this also applies for mammalian meiosis I chromosomes. Although SCP3 is not a cohesin subunit, we have observed that Rad21 colocalizes with SCP3 throughout male mouse meiosis, and is also partially removed from chromosome arms during late prophase I stages. Interestingly, the meiosis-specific cohesin subunit STAG3 behaves similarly during the same stages (Prieto et al., 2001). These results lead us to propose that during meiosis I the cohesin subunits STAG3 and Rad21, as well as the superimposed LE components SCP3 and SCP2 (Pelttari et al., 2001), are released from chromosome arms during late prophase I stages to permit the resolution of sister chromatids. It has been reported that the aurora-B kinase and the Polo-like kinase act in concert to promote the dissociation of cohesin complexes from arms in mitotic chromosomes (Losada et al., 2002; Sumara et al., 2002). Recently, we have demonstrated that aurora-B does not label LEs (Parra et al., 2003). Similarly, we have found that Polo-like kinase I does not label SCs. Thus, it is tempting to speculate that another kinase may phosphorylate cohesin complexes to promote their partial release from arms during late prophase I stages. However, since these kinases do not localize to chromosome arms during mitotic prophase their participation during meiosis I cannot be ruled out.

The cohesin complexes, associated with the LE

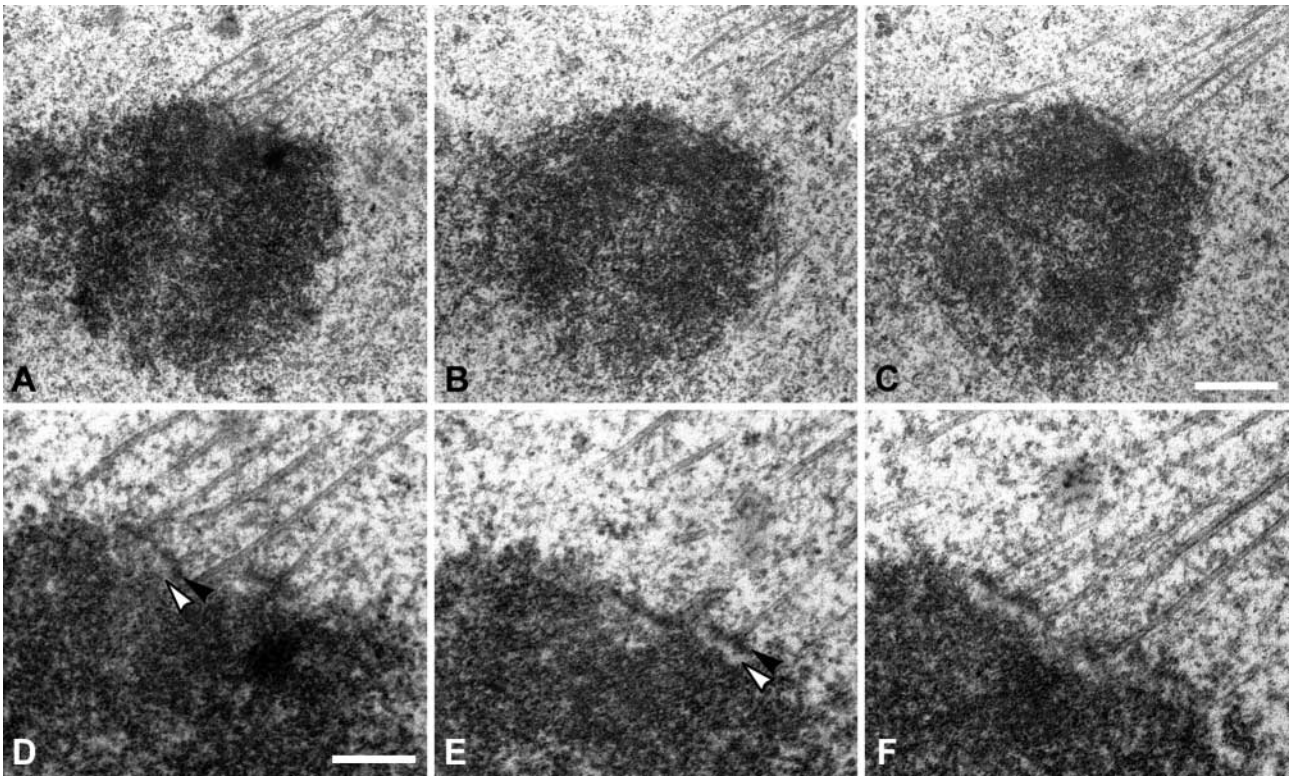

Fig. 7. Sister kinetochores are individualized in anaphase I. (A-C) Three serial sections of an anaphase I chromosome. (D-F) High magnification of kinetochores in A-C. Two outer kinetochore plates, corresponding to sister kinetochores are evident. The outer (black arrowhead) and middle electron-lucent (white arrowhead) kinetochore plates can be seen. Note that both sister kinetochores interact with microtubules. Scale bars: (A-C) 300 nm; (D-F) 150 nm.

components SCP3 and SCP2, persisting at the interchromatid domain of metaphase I bivalents are then sufficient to resist splitting forces applied to homologous centromeres by spindle microtubules with opposite polarity. Thus, the few cohesin complexes, at least those containing Rad21 and STAG3, retained at the interchromatid domain would ensure the essential maintenance of sister-chromatid arm cohesion until the metaphase I/anaphase I transition. Obviously, this possibility raises the interesting question of how these last cohesin complexes retained at the interchromatid domain are protected against their release during late prophase I stages.

Accumulation of Rad21 and LE components at centromeres during late prophase I stages

During pachytene and diplotene an enlarged region of SCP3, and also of SCP2, is detected at both ends of each LE. Since mouse chromosomes are telocentric, centromere regions are found at one of these ends. In late diakinesis and prometaphase I most centromeres show a large and bright T-shaped SCP3 labelling. This labelling is found at all centromeres in metaphase I chromosomes. These findings, together with the observation that SCP3 colocalizes with Rad21 indicate that cohesin complexes containing Rad21 associated with the superimposed LE components SCP3 and SCP2, concentrate and change their distribution at centromeres during diakinesis and prometaphase I (Fig. 10A). This redistribution occurs once centromeres have detached from the nuclear envelope. One possibility accounting for this redistribution is that some Rad21/SCP3/SCP2 complexes that were released from LEs reaccumulate then at centromeres. Alternatively, the complexes that remain bound to LEs may move along them to accumulate at centromeres, or some complexes that were not previously associated with LEs associate then with centromeres.

The inner domain of metaphase I centromeres

Although previous studies have reported the persistence of SCP3 at metaphase I centromeres (Moens and Spyropoulos, 1995; Revenkova et al., 2001), we describe for the first time its three-dimensional ‘double cornet’-like arrangement. Double labelling experiments of SCP3 with the kinetochore proteins CENP-A and CENP-E, located at the inner and outer kinetochore plates, respectively, demonstrate that SCP3 is located below the inner plates of the closely associated sister kinetochores (Fig. 10B) as observed by electron microscopy. SCP3 colocalizes with the chromosomal passenger proteins INCENP and aurora-B kinase (Fig. 10B) (Parra et al., 2003). Since these passenger proteins are located at the inner centromere domain in mitotic and meiotic chromosomes (Parra et al., 2003), SCP3, SCP2 and Rad21, with which they colocalize, are also located at this domain in metaphase I centromeres. We have revealed this domain by using the Os-PPD cytochemical technique by electron microscopy. Interestingly, a similar T-shaped structure called the

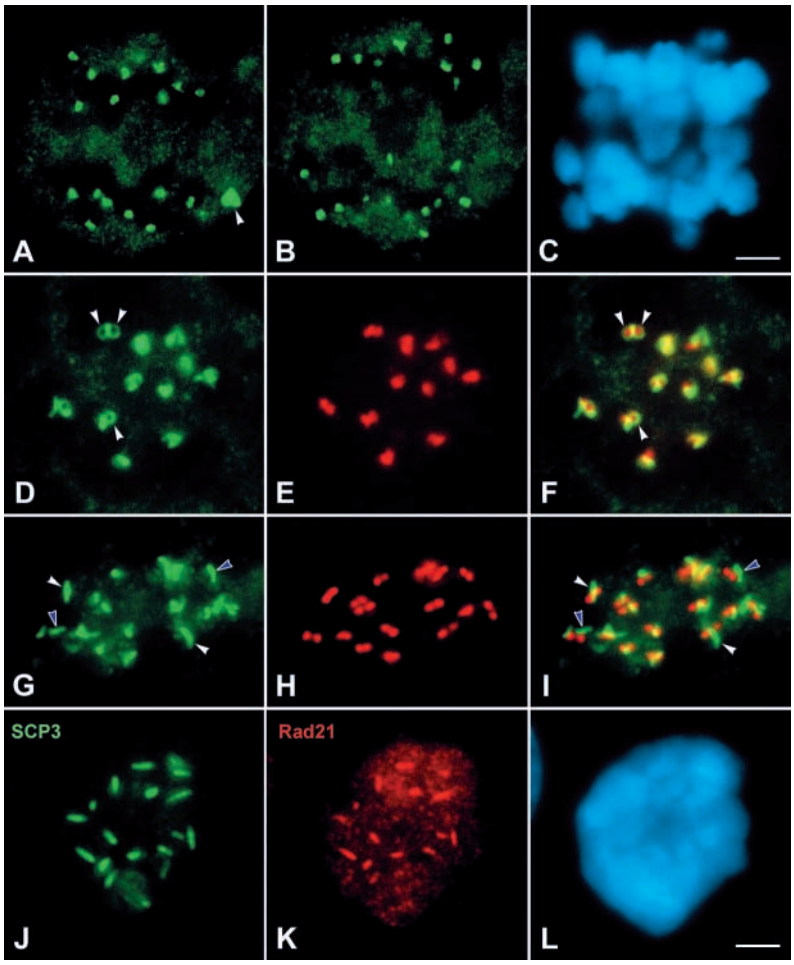

Fig. 8. SCP3 and Rad21 are lost from chromosome arms during the metaphase I/anaphase I transition, but are retained at centromeres until telophase I. SCP3 is labelled in green, centromeres in red in E, F, H, I, Rad21 in red in K, and DNA in blue. (A-C) Partial projections from different focal planes throughout an anaphase I spermatocyte. SCP3 has been lost from chromosome arms but is still highly enriched at homologous centromeres. A large SCP3 agglomerate (arrowhead in A) is found in the cytoplasm. (D-F) Top view of an anaphase I cell pole. SCP3 rings (arrowheads) are observed at some centromeres where the two sister kinetochores lye side by side. (G-I) Partial projection from two focal planes of a telophase I cell pole. SCP3 is usually found as small bars that may appear between the slightly separated sister kinetochores (white arrowheads) or displaced from them (blue arrowheads). (J-L) Single focal plane of a telophase I cell pole. Rad21 and SCP3 colocalize at bars. Scale bars: (A-C) 2.5 μm; (D-L) 5 μm.

'centromeric filament' was previously demonstrated by silver staining in sections of metaphase I centromeres of different vertebrates (Tandler and Solari, 1991; Solari and Tandler, 1991).

The cohesin variants Rad21 and Rec8 coexist during mammalian meiosis I

During budding yeast meiosis the cohesin subunit Scc1/Mcd1/Rad21 is replaced by Rec8, a meiosis-specific variant (Klein et al., 1999). By contrast, in fission yeast both variants, Rad21 and Rec8, are present during meiosis (Watanabe and Nurse, 1999; Yokobayashi et al., 2003). It has been proposed that during mammalian meiosis, Rad21 is replaced by Rec8, as occurs in budding yeast (for reviews, see Nasmyth, 2001; Lee et al., 2002; Lee et al., 2003; Eijpe et al., 2003). However, it has been recently shown that Rad21 also appears during the diplotene stage of male mouse meiosis (Prieto et al., 2002). Moreover, here we demonstrate that Rad21 parallels the distribution of SCP3 from leptotene up to interkinesis. Our visualization of Rad21 with two different specific antibodies that do not cross-react with Rec8, together with results reporting that Rec8 is present during mammalian male meiosis (Eijpe et al., 2003; Lee et al., 2003), would indicate that Rad21 is not replaced by Rec8. Consequently, Rad21 cannot be considered a mitosis-specific variant in mammals. Interestingly, we have found that in metaphase I bivalents, Rad21 is present at the interchromatid domain and appears highly enriched at centromeres, while Rec8 is only detected at the interchromatid domain (J.A.S. et al., unpublished). Thus, both variants coexist at the interchromatid domain, but only Rad21 appears at the inner centromere domain. These data suggest that in mammalian meiosis Rec8 reinforces arm cohesion, and that different cohesin complexes, with either Rad21 or Rec8, coexist at chromosome arms.

Additionally, the previously suspected 'mitotic' cohesin subunit STAG2 also appears during male mouse meiosis (Prieto et al., 2002) as well as the meiosis-specific variant STAG3 (Prieto et al., 2001). A similar situation has been observed for their respective homologues, Psc3 and Rec11, in fission yeast meiosis (Kitajima et al., 2003). Moreover, two variants of SMC1, namely SMC1α and SMC1β, also coexist during mammalian meiosis (Revenkova et al., 2001). Thus, different combinations may generate diverse cohesin complexes regulating differentially sister-chromatid arm and/or centromere cohesion.

Monopolar attachment of sister kinetochores during meiosis I and sister-chromatid centromere cohesion

We have detected that SCP3 and Rad21 have a 'double cornet'-like arrangement at centromeres during metaphase I and

anaphase I. However, these proteins appear as bars that are not located at the inner centromere domain during telophase I and interkinesis. Interestingly, the distance between sister kinetochores increases from metaphase I up to telophase I, as occurs for instance in grasshopper spermatocytes (Suja et al., 1992; Suja et al., 1999). Consequently, the displacement of SCP3 and Rad21 from centromeres is concomitant with the separation of sister kinetochores. This result, together with the observation that SCP3 and Rad21 rings encircle the inner plates of sister kinetochores labelled with CENP-A (Fig. 10C), lead us to propose that Rad21, associated with SCP3 and SCP2, is involved in the maintenance of the association between sister kinetochores, i.e. in sister-kinetochore cohesion, until ana/telophase I. We do not disregard the possibility that other cohesin subunits, for instance Rec8, could also collaborate in this function. Indeed, it has been proposed that Rec8 is required for the monopolar attachment of sister kinetochores in meiosis I in fission yeast (Watanabe and Nurse, 1999; Yokobayashi et al., 2003). Alternatively, proteins similar to budding yeast monopolins (Toth et al., 2000; Rabitsch et al., 2003) could also be involved in regulating sister-kinetochore cohesion.

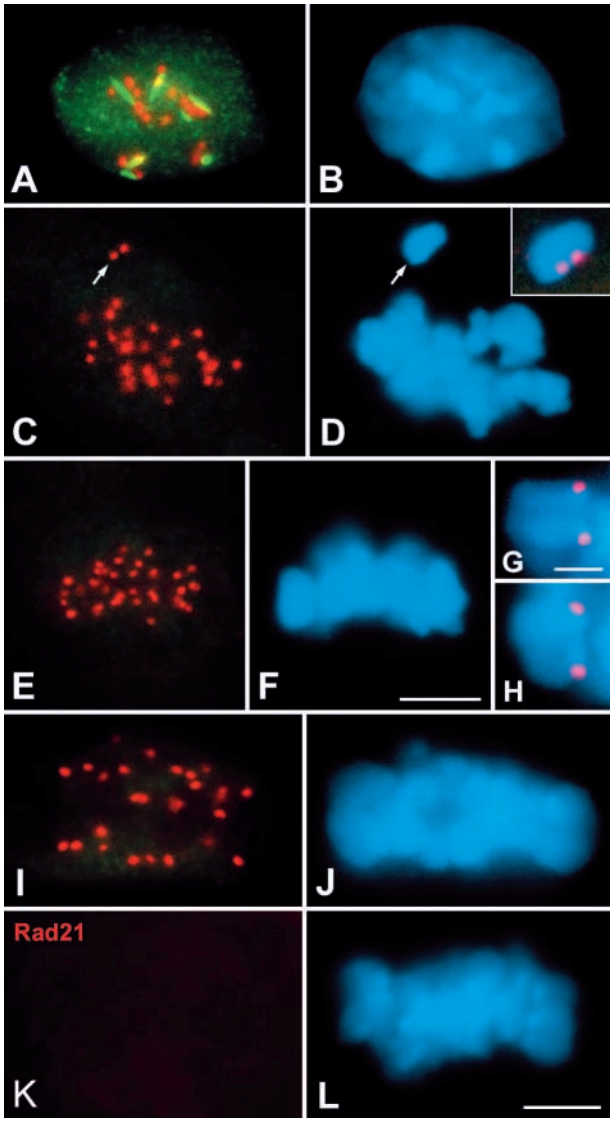

Fig. 9. SCP3 persists at chromocentres during interkinesis, but is not present at centromeres of prometaphase II or metaphase II chromosomes. SCP3 is labelled in green, centromeres in red in A,C,D,E,G,H,I, Rad21 in red in K, and DNA in blue. (A,B) Single focal plane of an interkinesis nucleus where SCP3 is found as elongated bars at chromocentres but displaced from pairs of sister kinetochores. (C,D) Single focal plane throughout a prometaphase II spermatocyte. SCP3 is not present at centromeres. Sister kinetochores are separated at the centromere of an unaligned chromosome (arrow; and see inset). (E,F) Partial projection of two focal planes throughout a metaphase II spermatocyte. SCP3 is not present at centromeres where sister kinetochores are facing opposite poles. (G,H) Selected metaphase II chromosomes. Sister kinetochores occupy opposite locations at the centromere. (I,J) Partial projection of three focal planes throughout an early anaphase II spermatocyte. No SCP3 labelling is found between segregating chromatids. (K,L) Single focal plane of a metaphase II spermatocyte. Rad21 is not present at all. Scale bars: (A-F) 5 μm; (G,H) 2 μm; (I-L) 3 μm.

During metaphase II, sister kinetochores are located back-to-back at the centromere and attach to microtubules emanating from opposite poles. This situation implies that from metaphase I, when sister kinetochores are closely associated, up to metaphase II, when sister kinetochores are individualized, the centromere changes its structure while maintaining cohesion. Our results clearly indicate that this change of centromere structure occurs during the telophase I/metaphase II transition. Given that SCP3 and Rad21 are displaced from centromeres during telophase I, and are not found at prometaphase II and metaphase II centromeres, we hypothesize that Rad21, as well as SCP3 and SCP2, are not involved in the maintenance of centromere cohesion until the metaphase II/anaphase II transition, but in monopolar attachment of sister kinetochores during meiosis I. Moreover, we have found that Smc1, Smc3, Rec8 and STAG3 do not persist at metaphase II centromeres (J.A.S. et al., unpublished data). Consequently, we consider that in mammalian meiosis, unlike the situation in yeasts (Klein et al., 1999; Watanabe and Nurse, 1999), cohesin complexes are not necessary to maintain centromere cohesion until meiosis II.

Our demonstration of the absence of SCP3 from metaphase II centromeres is contradictory with previous results obtained by surface-spreading that proposed its persistence until the metaphase II/anaphase II transition (Dobson et al., 1994; Moens and Spyropoulos, 1995; Revenkova et al., 2001; Eijpe et al., 2003). We think that the apparent discrepancy is due to the kinds of methodologies used. Surface-spreading disturbs the interaction between chromosomes and spindle microtubules, and centromeres do not preserve their native architecture. By contrast, our squashing technique does not affect chromosome condensation and spindle interaction. We believe that cells reported to be in metaphase II by surface-spreading are in fact telophase I cells since SCP3 appears as bars that are frequently displaced from centromeres where sister kinetochores appear individualised. As telophase I and metaphase II chromosomes are almost identical in gross morphology, is not possible to differentiate them when chromatin compaction has been disrupted by surface-spreading.

In summary, our results demonstrate that a significant population of the LE proteins SCP3 and SCP2, associated with the underlying cohesin subunit Rad21, are released from chromosome arms during late prophase I stages. However,

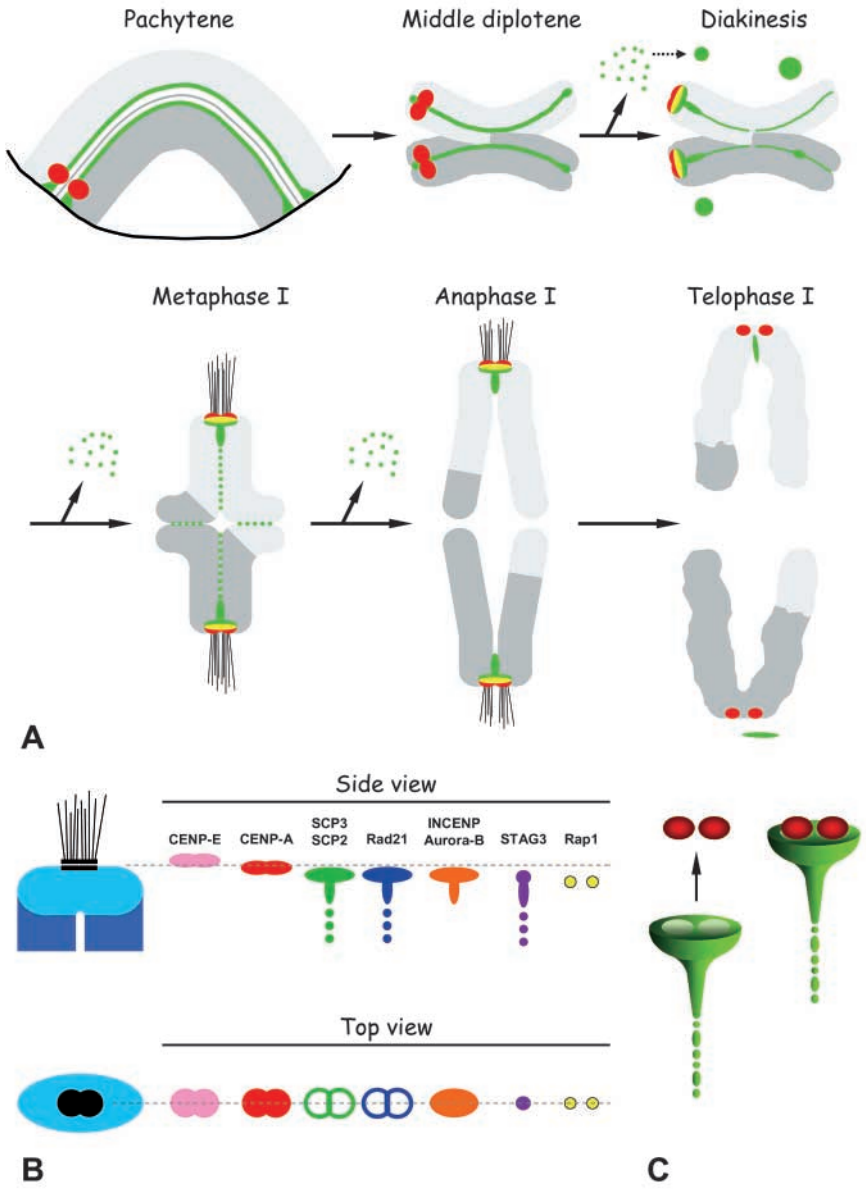

Fig. 10. Schematic representation of the distribution of SCP3 and Rad21 from pachytene up to telophase I (A); their localization and that of other proteins at metaphase I centromeres (B); and their three-dimensional localization relative to sister kinetochores (C). (A) One chromosome is depicted in light grey while its homologue is depicted in darker grey. Centromere labelling is red, and SCP3/Rad21 is green. Regions of overlap are in yellow. From middle diplotene up to metaphase I there is a continuous loss of SCP3/Rad21 from chromosome arms, and an accumulation at centromeres, that in turn change their structure. During diakinesis SCP3/Rad21 also appear as accumulations on desynapsed LEs and as large agglomerates in the nucleoplasm. SCP3 and Rad21 remain at the interchromatid domain of metaphase I bivalents as small patches that are lost during the metaphase I/anaphase I transition. A population of SCP3/Rad21 persist at anaphase I centromeres but is released when sister kinetochores separate during telophase I. (B) A metaphase I centromere. Different proteins are aligned among them in side and top views. Trilaminar sister kinetochores are tightly associated at the surface of the centromere, and associated with microtubules emanating from the same pole. A single dashed line aligned with sister kinetochores has been included to facilitate the analysis of the relative distribution of these proteins. (C) Interpretation showing how sister kinetochores (red) are associated and supported by the 'double-cornet'-shaped inner centromere domain containing SCP3, SCP2, Rad21, INCENP and aurora-B.

cohesin complexes persisting at the interchromatid domain would be sufficient to ensure the maintenance of sister-chromatid arm cohesion until the metaphase I/anaphase I transition. Additionally, Rad21, as well as SCP3 and SCP2, accumulate at the inner centromere domain during late prophase I stages. We propose that these complexes, showing a 'double cornet'-like arrangement, ensure the monopolar attachment of sister kinetochores by joining them, but are not responsible for the maintenance of sister-chromatid centromere cohesion until the metaphase II/anaphase II transition.

We wish to express our sincere thanks to Manuel Valdivia for generously supplying the ACA serum and the anti-CENP-A antibody, Christa Heyting for providing rabbit anti-SCP3 and anti-SCP2 antibodies, Tim Yen for providing the anti-CENP-E antibody, William C. Earnshaw for supplying the anti-INCENP antibody, Titia de Lange for supplying the anti-Rap1 antibody, Kyoko Yokomori for providing the anti-Rad21 antibody, and José Luis Barbero for providing the anti-Rad21 and anti-SCP3 antibodies. This work was supported by grants BMC2002-00043 and BMC2002-01171 from Dirección General de Investigación (Spain). Ricardo Benavente received support from the DFG (grant Be 1168/4-4). María Teresa Parra and Alberto Viera are supported by predoctoral fellowships from Fundación Ramón Areces and UAM, respectively, and Rocío Gómez is supported by a collaboration fellowship from UAM.

References

Adams, R. R., Carmena, M. and Earnshaw, W. C. (2001). Essential roles of *Drosophila* inner centromere protein (INCENP) and Aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J. Cell Biol.* **153**, 865-879.

Alsheimer, M. and Benavente, R. (1996). Change of karyoskeleton during mammalian spermatogenesis: expression pattern of nuclear lamin C2 and its regulation. *Exp. Cell Res.* **228**, 181-188.

Antonio, C., González-García, J. M., Page, J., Suja, J. A., Stockert, J. C. and Rufas, J. S. (1996). The osmium tetroxide/*p*-phenylenediamine procedure reveals the chromatid cores and kinetochores of meiotic chromosomes by light and electron microscopy. *J. Histochem. Cytochem.* **44**, 1279-1288.

Dobson, M. J., Pearlman, R. E., Karaiskakis, A., Spyropoulos, B. and

Moens, P. B. (1994). Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. *J. Cell Sci.* **107**, 2749-2760.
Eckley, D. M., Ainsztein, A. M., Mackay, A. M., Goldberg, I. G. and Earnshaw, W. C. (1997). Chromosomal proteins and cytokinesis: Patterns of cleavage furrow formation and inner centromere protein positioning in mitotic heterokaryons and mid-anaphase cells. *J. Cell Biol.* **136**, 1169-1183.
Eijpe, M., Heyting, C., Gross, B. and Jessberger, R. (2000). Association of mammalian SMC1 and SMC3 proteins with meiotic chromosomes and synaptonemal complexes. *J. Cell Sci.* **113**, 673-682.
Eijpe, M., Offenberg, H., Jessberger, R., Revenkova, E. and Heyting, C. (2003). Meiotic cohesin Rec8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1, and SMC3. *J. Cell Biol.* **160**, 657-670.
Esponda, P. and Stockert, J. C. (1978). Localization of the synaptonemal complex under the light microscope. *Chromosoma* **68**, 83-90.
Garagna, S., Zuccotti, M., Capanna, E. and Redi, C. A. (2003). High-resolution organization of mouse telomeric and pericentromeric DNA. *Cytogenet. Genome Res.* **96**, 125-129.
Gregson, H. C., Schmiesing, J. A., Kim, J.-S., Kobayashi, T., Zhou, S. and Yokomori, K. (2001). A potential role for human cohesin in mitotic spindle aster assembly. *J. Biol. Chem.* **276**, 47575-47582.
Kipling, D., Ackford, H. E., Taylor, B. A. and Cooke, H. J. (1991). Mouse minor satellite DNA genetically maps to the centromere and is physically linked to the proximal telomere. *Genomics* **11**, 235-241.
Kitajima, T. S., Yokobayashi, S., Yamamoto, M. and Watanabe, Y. (2003). Distinct cohesin complexes organize meiotic chromosome domains. *Science* **300**, 1152-1155.
Klein, F., Mahr, P., Gálová, M., Buonomo, S. B. C., Michaelis, C., Nairz, K. and Nasmyth, K. (1999). A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* **98**, 91-103.
Lammers, J. H. M., Offenberg, H. H., van Aalderen, M., Vink, A. C. G., Dietrich, A. J. J. and Heyting, C. (1994). The gene encoding a major component of the lateral elements of synaptonemal complexes of the rat is related to X-linked lymphocyte-regulated genes. *Mol. Cell. Biol.* **14**, 1137-1146.
Lee, J. Y. and Orr-Weaver, T. L. (2001). The molecular basis of sister-chromatid cohesion. *Annu. Rev. Cell Dev. Biol.* **17**, 753-777.
Lee, J., Yokota, T. and Yamashita, M. (2002). Analyses of mRNA expression patterns of cohesin subunits Rad21 and Rec8 in mice: Germ cell-specific expression of *rec8* mRNA in both male and female mice. *Zool. Sci.* **19**, 539-544.
Lee, J., Iwai, T., Yokota, T. and Yamashita, M. (2003). Temporally and spatially selective loss of Rec8 protein from meiotic chromosomes during mammalian meiosis. *J. Cell Sci.* **116**, 2781-2790.
Lombillo, V. A., Nislow, C., Yen, T. J., Gelfand, W. I. and McIntosh, J. R. (1995). Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes in vitro. *J. Cell Biol.* **128**, 107-115.
Losada, A. and Hirano, T. (2001). Shaping the metaphase chromosome: Coordination of cohesion and condensation. *BioEssays* **23**, 924-935.
Losada, A., Yokochi, T., Kobayashi, R. and Hirano, T. (2000). Identification and characterization of SA/Scc3p subunits in the Xenopus and human cohesin complexes. *J. Cell Biol.* **150**, 405-416.
Losada, A., Hirano, M. and Hirano, T. (2002). Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. *Genes Dev.* **16**, 3004-3016.
Miyazaki, W. Y. and Orr-Weaver, T. L. (1994). Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.* **28**, 167-187.
Moens, P. B. and Spyropoulos, B. (1995). Immunocytology of chiasmata and chromosomal disjunction at mouse meiosis. *Chromosoma* **104**, 175-182.
Moens, P. B., Heyting, C., Dietrich, A. J. J., van Raamsdonk, W. and Chen, Q. (1987). Synaptonemal complex antigen localization and conservation. *J. Cell Biol.* **105**, 93-103.
Nasmyth, K. (2001). Disseminating the genome: Joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* **35**, 673-745.
Nasmyth, K. (2002). Segregating sister genomes: The molecular biology of chromosome separation. *Science* **297**, 559-565.
Page, J., Suja, J. A., Santos, J. L. and Rufas, J. S. (1998). Squash procedure for protein immunolocalization in meiotic cells. *Chromosome Res.* **6**, 639-642.
Parra, M. T., Page, J., Yen, T. J., He, D., Valdeolmillos, A., Rufas, J. S. and Suja, J. A. (2002). Expression and behaviour of CENP-E at kinetochores during mouse spermatogenesis. *Chromosoma* **111**, 53-61.
Parra, M. T., Viera, A., Gómez, R., Page, J., Carmena, M., Earnshaw, W. C., Rufas, J. S. and Suja, J. A. (2003). Dynamic relocalization of the chromosomal passenger complex proteins inner centromere protein (INCENP) and aurora-B kinase during male mouse meiosis. *J. Cell Sci.* **116**, 961-974.
Pelttari, J., Hoja, M-R., Yuan, L., Liu, J-G., Brundell, E., Moens, P. B., Santucci-Darmanin, S., Jessberger, R., Barbero, J. L., Heyting, C. and Höög, C. (2001). A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. *Mol. Cell. Biol.* **21**, 5667-5677.
Petronczki, M., Siomos, M. F. and Nasmyth, K. (2003). Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**, 423-440.
Pezzi, N., Prieto, I., Kremer, L., Pérez Jurado, A., Valero, C., del Mazo, J., Martínez-A, C. and Barbero, J. L. (2000). STAG3, a novel gene encoding a protein involved in meiotic chromosome pairing and location of STAG3-related genes flanking Williams-Beuren syndrome deletion. *FASEB J.* **14**, 581-592.
Prieto, I., Suja, J. A., Pezzi, N., Kremer, L., Martínez-A, C., Rufas, J. S. and Barbero, J. L. (2001). Mammalian STAG3 is a cohesin specific to sister chromatid arms during meiosis I. *Nat. Cell Biol.* **3**, 761-766.
Prieto, I., Pezzi, N., Buesa, J. M., Kremer, L., Barthelemy, I., Carreiro, C., Roncal, F., Martínez, A., Gómez, L., Fernández, R., Martínez-A, C. and Barbero, J. L. (2002). STAG2 and Rad21 mammalian mitotic cohesins are implicated in meiosis. *EMBO Rep.* **3**, 543-550.
Rabitsch, K. P., Petronczki, M., Javerzat, J.-P., Genier, S., Chwalla, B., Schleiffer, A., Tanaka, T. U. and Nasmyth, K. (2003). Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. *Dev. Cell* **4**, 535-548.
Revenkova, E., Eijpe, M., Heyting, C., Gross, B. and Jesseberger, R. (2001). Novel meiosis-specific isoform of mammalian SMC1. *Mol. Cell. Biol.* **21**, 6984-6998.
Rogers, E., Bishop, J. D., Waddle, J. A., Schumacher, J. M. and Lin, R. (2002). The aurora kinase AIR-2 functions in the release of chromosome cohesion in *Caenorhabditis elegans* meiosis. *J. Cell Biol.* **157**, 219-229.
Schalk, J. A. C., Dietrich, A. J. J., Vink, A. C. G., Offenberg, H. H., van Aalderen, M. and Heyting, C. (1998). Localization of SCP2 and SCP3 protein molecules within synaptonemal complexes of the rat. *Chromosoma* **107**, 540-548.
Scherthan, H., Jerratsch, M., Li, B., Smith, S., Hultén, M., Lock, T. and de Lange, T. (2000). Mammalian meiotic telomeres: protein composition and redistribution in relation to nuclear pores. *Mol. Biol. Cell* **11**, 4189-4203.
Solari, A. J. and Tandler, C. J. (1991). Presence of a centromeric filament during meiosis. *Genome* **34**, 888-894.
Sonoda, E., Matsusaka, T., Morrison, C., Vagnarelli, P., Hoshi, O., Ushiki, T., Nojima, K., Fukagawa, T., Waizenegger, I. C., Peters, J. M., Earnshaw, W. C. and Takeda, S. (2001). Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. *Dev. Cell* **1**, 759-770.
Suja, J. A., Antonio, C. and Rufas, J. S. (1992). Involvement of chromatid cohesiveness at the centromere and chromosome arms in meiotic chromosome segregation: A cytological approach. *Chromosoma* **101**, 493-501.
Suja, J. A., Antonio, C., Debec, A. and Rufas, J. S. (1999). Phosphorylated proteins are involved in sister-chromatid arm cohesion during meiosis I. *J. Cell Sci.* **112**, 2957-2969.
Sumara, I., Vorlaufer, E., Stukenberg, P. T., Kelm, O., Redermann, N., Nigg, E. A. and Peters, J. M. (2002). The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. *Mol. Cell* **9**, 515-525.
Tandler, C. J. and Solari, A. J. (1991). An 'axis-like' material in the centromeric region of metaphase-I chromosomes from mouse spermatocytes. *Genetica* **84**, 39-49.
Toth, A., Rabitsch, K. P., Galova, M., Schleiffer, A., Buonomo, S. B. and Nasmyth, K. (2000). Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. *Cell* **103**, 1155-1168.
Vagnarelli, P. and Earnshaw, W. C. (2001). INCENP loss from an inactive centromere correlates with the loss of sister chromatid cohesion. *Chromosoma* **110**, 393-401.
Valdivia, M. M., Figueroa, J., Iglesias, C. and Ortiz, M. (1998). A novel centromere monospecific serum to a human autoepitope on the histone H3-like protein CENP-A. *FEBS Lett.* **422**, 5-9.

Vass, S., Cotterill, S., Valdeolmillos, A. M., Barbero, J. L., Lin, E., Warren, W. D. and Heck, M. (2003). Depletion of DRad21/Scc1 in *Drosophila* cells leads to instability of the cohesin complex and disruption of mitotic progression. *Curr. Biol.* **13**, 208-218.

Waizenegger, I. C., Hauf, S., Meinke, A. and Peters, J.-M. (2000). Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* **103**, 399-410.

Warren, W. D., Steffensen, S., Lin, E., Coelho, P., Loupart, M.-L., Cobbe, N., Lee, J. Y., McKay, M. J., Orr-Weaver, T., Heck, M. M. S. and Sunkel, C. E. (2000). The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis. *Curr. Biol.* **10**, 1463-1466.

Watanabe, Y. and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* **400**, 461-464.

Wolf, K. W. (1987). Cytology of Lepidoptera. I. The nuclear area in secondary oocytes of *Ephestia kuehniella* contains remnants of the first division. *Eur. J. Cell Biol.* **43**, 223-229.

Yokobayashi, S., Yamamoto, M. and Watanabe, Y. (2003). Cohesins determine the attachment manner of kinetochores to spindle microtubules at meiosis I in fission yeast. *Mol. Cell. Biol.* **23**, 3965-3973.

Yuan, L., Pelttari, J., Brundell, E., Björkroth, B., Zhao, J., Liu, J-G., Brismar, H., Daneholt, B. and Höög, C. (1998). The synaptonemal complex protein SCP3 can form multistranded, cross-striated fibers in vivo. *J. Cell Biol.* **142**, 331-339.

Yuan, L., Liu, J-G., Zhao, J., Brundell, E., Daneholt, B. and Höög, C. (2000). The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol. Cell* **5**, 73-83.

Zickler, D. and Kleckner, N. (1999). Meiotic chromosomes: Integrating structure and function. *Annu. Rev. Genet.* **33**, 603-754.