Chromosoma (Berl.) 70, 375–384 (1979)

CHROMOSOMA

Ultrastructural Studies of Late Meiotic Prophase Nuclei of Spermatocytes in *Ascaris suum*

S.C. Kundu[1], and Yu.F. Bogdanov

Institute of Molecular Biology, Academy of Sciences of USSR, 32, Vavilov Str., Moscow 117312, U.S.S.R.;[1] present address (also for reprint requests): Division of Life Sciences, Jawaharlal Nehru University Centre, Imphal – 795003, India

Abstract. Post pachytene stages of meiotic prophase in males of *Ascaris suum* have been analyzed with the electron microscope. No synaptonemal-like polycomplexes (PCs) have been observed in the nucleoplasm or cytoplasm during the period from pachytene to diakinesis. From Serially sectioned diplotene nuclei it was found that the bivalents are located near the periphery of the nuclei, the central part of the nuclei being vacant. Each nucleus contains one nucleolus. Up to 1 μm long stretches of unpaired lateral elements (LEs) are found in some of the diplotene bivalents. These LEs are morphologically similar to unpaired LEs in early zygotene nuclei. Partial 3-dimensional reconstruction of two nuclei shows that the bivalents contain some small stretches of synaptonemal complex (SC) up to 1.9 μm long. Some bivalents at diakinesis show remnants of SCs. At this stage chromosomes are fibrous, condensed, attached to the nuclear envelope and mostly with a rounded profile in cross section. The synchronous development of the spermatocytes and small bivalents at diplotene in *A. suum* make this system a good object for the study of localization of SC remnants.

Introduction

Recent investigations on spermatocytes and oocytes of *Ascaris lumbricoides* var. suum (*A. suum*) by Bogdanov (1977) and Fiil et al. (1977) have revealed that synaptonemal-like polycomplexes (PCs) are found in the cytoplasm of the meiocytes at leptotene. This situation differs from that observed in the meiosis of other organisms (see Rasmussen, 1975) in which PCs are found within the nuclei after the pachytene stage as a result of the disintegration of regular SCs. For this reason formation of PCs in *A. suum* during the leptotene stage was regarded as precocious. Some tentative explanations about the way

0009-5915/79/0070/0375/$02.00

in which the PCs are precociously formed in the cytoplasm, and their relation with the regular SC formation during the zygotene stage have been provided earlier (Bogdanov, 1977). Fiil et al. (1977) have found that in some female animals synaptonemal-like PCs reappear in the cytoplasm at the late pachytene and at the early diffuse stages but the diplotene and diakinetic stages were not studied by them.

A search was made to inquire whether or not PCs are regularly found in *A. suum* during these periods of meiosis. Meiotic prophase I in spermatocytes proceeds synchronously in *A. suum* (Bogdanov, 1977) and this system provides thus a good possibility for investigating the fate of SCs during the diplotene and diakinesis.

Materials and Methods

Ascaris suum males were dissected and fixed according to Bogdanov (1977). Consecutive sections of about 800 Å thickness of the nuclei at diplotene and diakinesis were cut on a Porter-Blum MT 11 microtome. The serial sections were stained in a saturated, aqueous, uranyl acetate solution, poststained in lead citrate and examined with a Philips EM 200 or JEM 7. The male reproductive tract is a coiled, single reflexed tube which when extended measures 110–130 cm. The meiosis occupies about 75 to 80 cm of the tube. Early diffuse stage starts at about 30 cm, diplotene at about 61 cm and diakinesis at about 67 cm from the anterior part of the testis. In each piece of the testis synchrony of stages is observed up to the end of diakinesis. The more rapid stages of the meiotic divisions after diakinesis, e.g. metaphase I and anaphase I (Fig. 1 d) can be found within the same testis piece of 0.5 cm length.

Results

Light Microscopy

Previous ultrastructural description of meiotic nuclei in *A. suum* males was limited to the early diffuse stage of meiotic prophase I (Bogdanov, 1977). This stage (Fig. 1 a) is observed immediately after the pachytene stage; it occupies more length than pachytene. Synchronously growing pachytene spermatocytes occupy about 9 cm, whereas the diffuse stage occupies approximately 35 cm of the testis tube. After the diffuse stage spermatocytes enter into the diplotene stage (Fig. 1 b) which is present in about 6 cm of the testis tube. The distinct bivalents become visible at this stage in contrast to the rather dispersed chromatin threads which are characteristic of the preceding diffuse stage (see also Fig. 2 h of Bogdanov, 1977). Sometimes chiasmata can be seen in these diplotene bivalents (Fig. 1 b). Two types of chromatin organization are visible within each bivalent when fine adjustment of the light microscope is used: condensed and diffuse (Fig. 1 b). From 17 to 20 chromatin bodies are seen within the diakinetic nuclei (Fig. 1 c). This result corresponds to the observations of Goldstein and Moens (1976) that in *A. suum* there are 12 bivalents and 5 heterochromatic bodies. In some cases extra chromatin bodies look like partners of the bivalents. It seems that they are separated or repulsed from each other during the squashing procedure. Counting of chiasmata in our material was not possible.

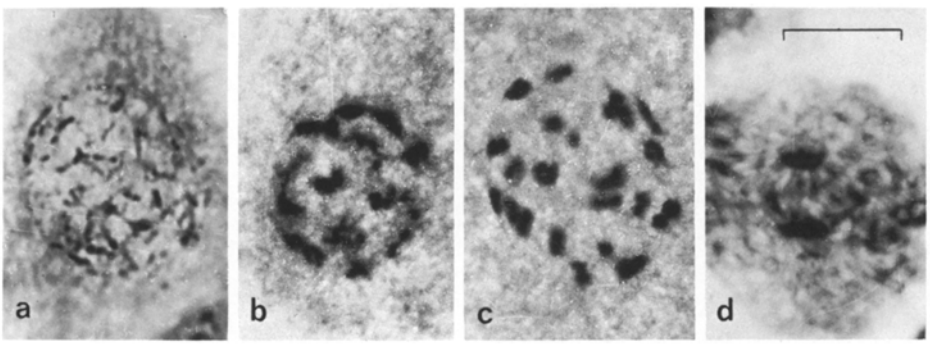

Fig. 1a–d. Light microscopic photographs of meiotic stages of spermatocytes in *Ascaris suum*. ×1,560. Bar equals 10 µm. **a** Diffuse stage (squash prepared from the 40th cm portion of the testis tube starting from the anterior part of the testis). **b** Diplotene (66th cm portion of the testis tube). **c** Diakinesis (69th cm portion). **d** Anaphase I (72nd cm portion)

Ultrastructure of Chromosomes and SCs at Diplotene

The general observation in this study was that we found no PCs in the nucleoplasm or cytoplasm between the diffuse stage and diakinesis. It means that in *A. suum* males PCs are characteristic features of the leptotene and zygotene stages only. Our conclusion is based on observations of the late meiotic prophase in four males (two from Bogdanov, 1977 and two in this work).

Serial sections were prepared to study diplotene nuclei. Portions of two nuclei comprising 38 and 41 serial sections were reconstructed from nuclei spanning about 85 serial sections each. Partial reconstructions of these two nuclei are shown in Fig. 2a and b. Additionally small series of 8 to 17 sections taken from other nuclei have been observed. At this stage the chromosomes are located at the periphery of the nuclei; the central part of all the observed nuclei is vacant except for the nucleolus (Fig. 2a). The chromosomes are not very condensed at this stage. One bivalent is attached to the nucleolus. This corresponds to the observations of Goldstein and Moens (1976). In the mid diffuse and early diplotene nuclei the ring-like structures are often observed in the nucleoplasm and/or attached to the nucleolus. Three dimensional reconstruction of the diplotene nucleus indicates that these rings originate from the nucleolus (Fig. 2a). In some sections axial cores, similar to unpaired lateral elements (LEs) of SCs at zygotene (see Bogdanov, 1977), are visible (Fig. 3a and b). In other sections there are no signs of cores. Three-dimensional reconstruction shows that some chromosomes have at least some stretch of LE. The longest stretch of LE is about 1 µm in length (Fig. 2). Short stretches of SC are found within reconstructed bivalents. The length of SC stretches is usually less than 0.5 µm but in some cases it is found that the length can be as high as 1.9 µm when calculated according to Gillies (1972). In some bivalents two stretches of SC are found in a linear order (bivalent II in Fig. 2a; bivalents II and IV in Fig. 2b). The distance between two linearly arranged SC stretches is about 0.43 µm in bivalent II of (Fig. 2a) and about 0.75 µm

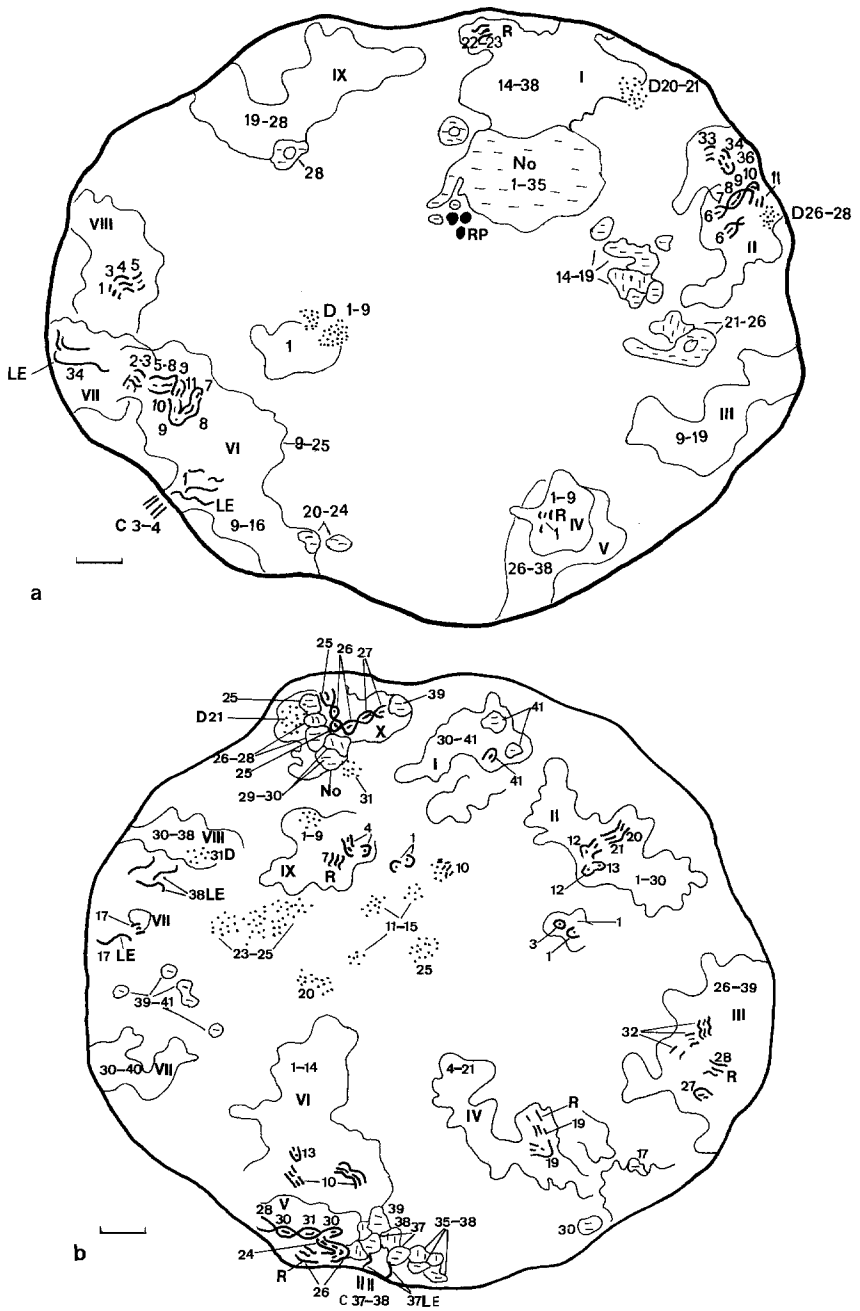

Fig. 2a and b. Partial 3-D reconstruction of two diplotene nuclei; **a** 38 consecutive sections; **b** 41 consecutive sections. All the SC stretches, SC remnants (*R*), unpaired lateral elements (*LE*) of SC, nucleolus (*NO*) or nucleolar material which is not often distinguishable from heterochromatin (dashes), RNP-products (*RP*), dot-like structures (*D*), centriole (*C*) and bivalent area (single line) with Roman numbers have been mapped. Numbers refer to section numbers. ×11,500[1]

[1] Bars in the figures represent 0.5 μm, if not otherwise stated

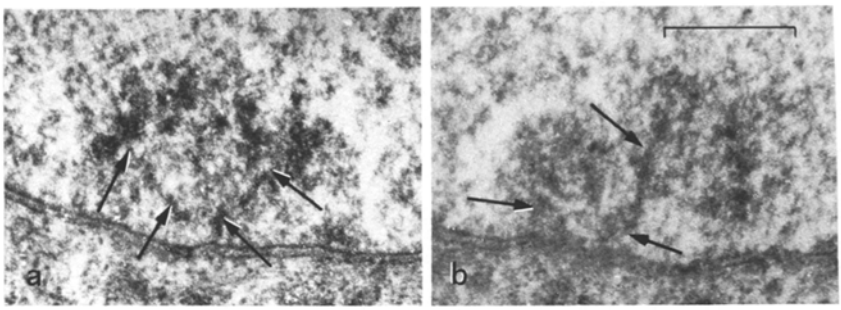

Fig. 3a and b. Two consecutive sections through unpaired lateral element (arrows) shown in Figure 2a. Section numbers 33 (**a**) and 34 (**b**). × 36,500

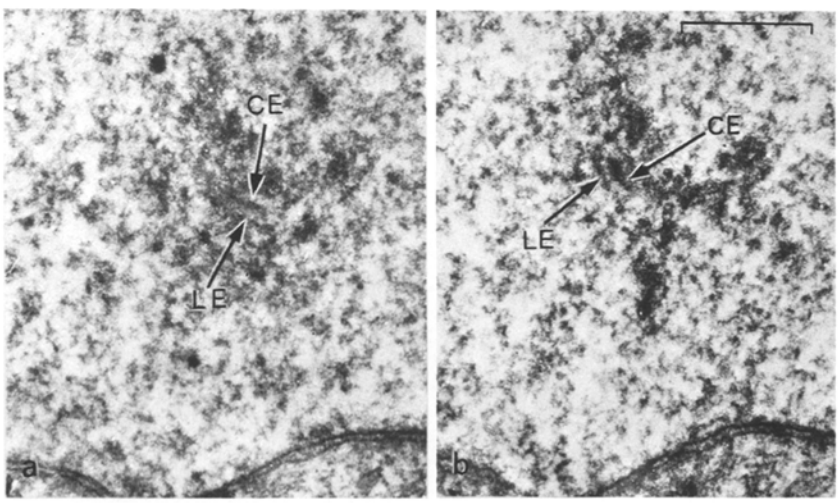

Fig. 4a and b. Two consecutive sections through the short stretch of SC of a bivalent VI shown in Figure 2a. Central element (*CE*). Lateral element (*LE*). × 36,500

in bivalent II of (Fig. 2b). In bivalents III, VI and IX (Fig. 2b) it is seen that the SC stretches are not arranged in a linear order within the bivalent. It seems that such disordered localization of SC stretches is due to the contraction of bivalents during diplotene. Morphologically SC stretches at diplotene (Figs. 4 and 6) are usually different from those of typical zygotene and pachytene SCs. In the short stretches of SC observed during the diplotene (and preceding diffuse stage) the central element (CE) is often thicker than lateral element (Fig. 6). Sometimes the thickness of the CEs of diplotene SC stretches is equal to the thickness of nodules found in pachytene SC (compare Fig. 6 to Fig. 24 of Bogdanov, 1977). Similar thickened CEs in the short stretches of SC have been observed in the diffuse nuclei of the *A. suum* female (Bogdanov and Kundu, unpublished results). According to Carpenter (1975), "recombination nodules" of SCs in *Drosophila* correspond to the places of crossing over. One can speculate that during the diplotene stage in *Ascaris* short stretches of SC are preserved only

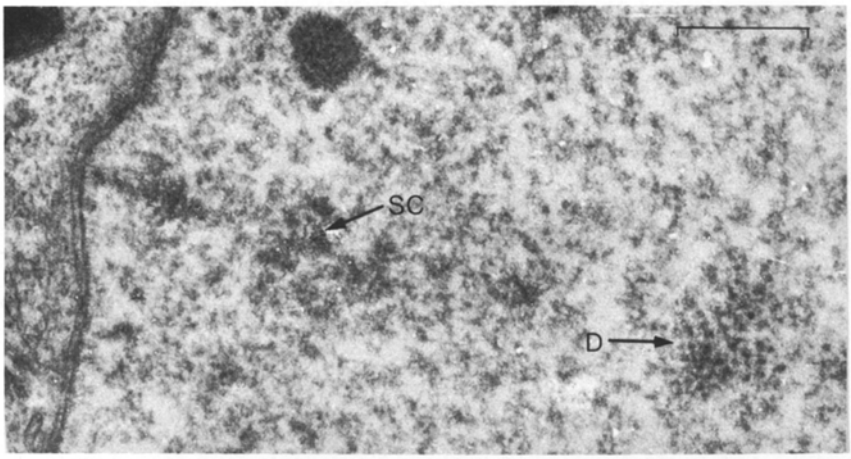

Fig. 5. Section number 3 of Figure 2a shows the stretch of SC (arrow) which belongs to the same SC as in Figure 4, dot-like structures (*D*). ×36,500

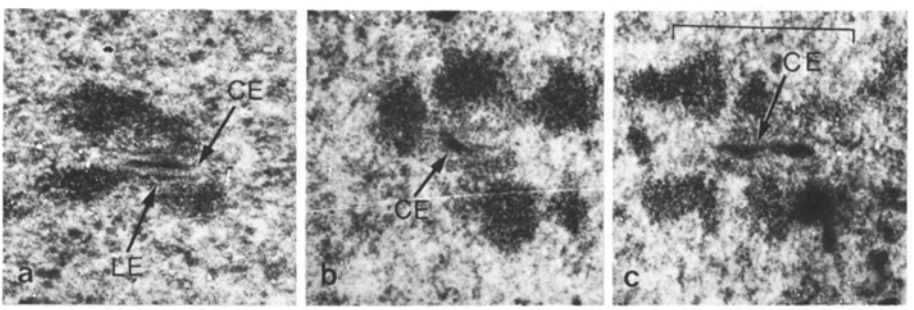

Fig. 6a–c. Sections through short stretches of SCs from 3 different diffuse nuclei. Figures show thick and prominent central elements (*CE*). These stretches of SCs are always found within the compact and electron dense chromatin. ×48,000

at the site of chiasmata where "recombination nodules" are located. This situation is similar to the observation of Zickler (1977) in *Sordaria*, where most stretches of SC remaining at diplotene contain a nodule. The remnants of the SCs are also visible in some of the bivalents in the 3-D reconstructions. In some sections of diplotene nuclei small groups of dense dot-like structures are visible (Fig. 5). These dots are seen in up to 4 to 9 sections consecutively. It has been identified from transverse sections that these dots are short fibres of about 100 Å in diameter (Fig. 5). The 3-D reconstruction shows that these groups of fibres usually are located close to the telomeres of the chromosome (See Figs. 2 and 5). These dot-like structures are also seen in the diffuse nuclei of the Ascaris female. No definite conclusion can be drawn about the nature of these fibres or dots, but Perov et al. (1976) interpret similar ultrastructures within the puffs of polytene chromosomes of *Chironomus thummi* as transcriptional products.

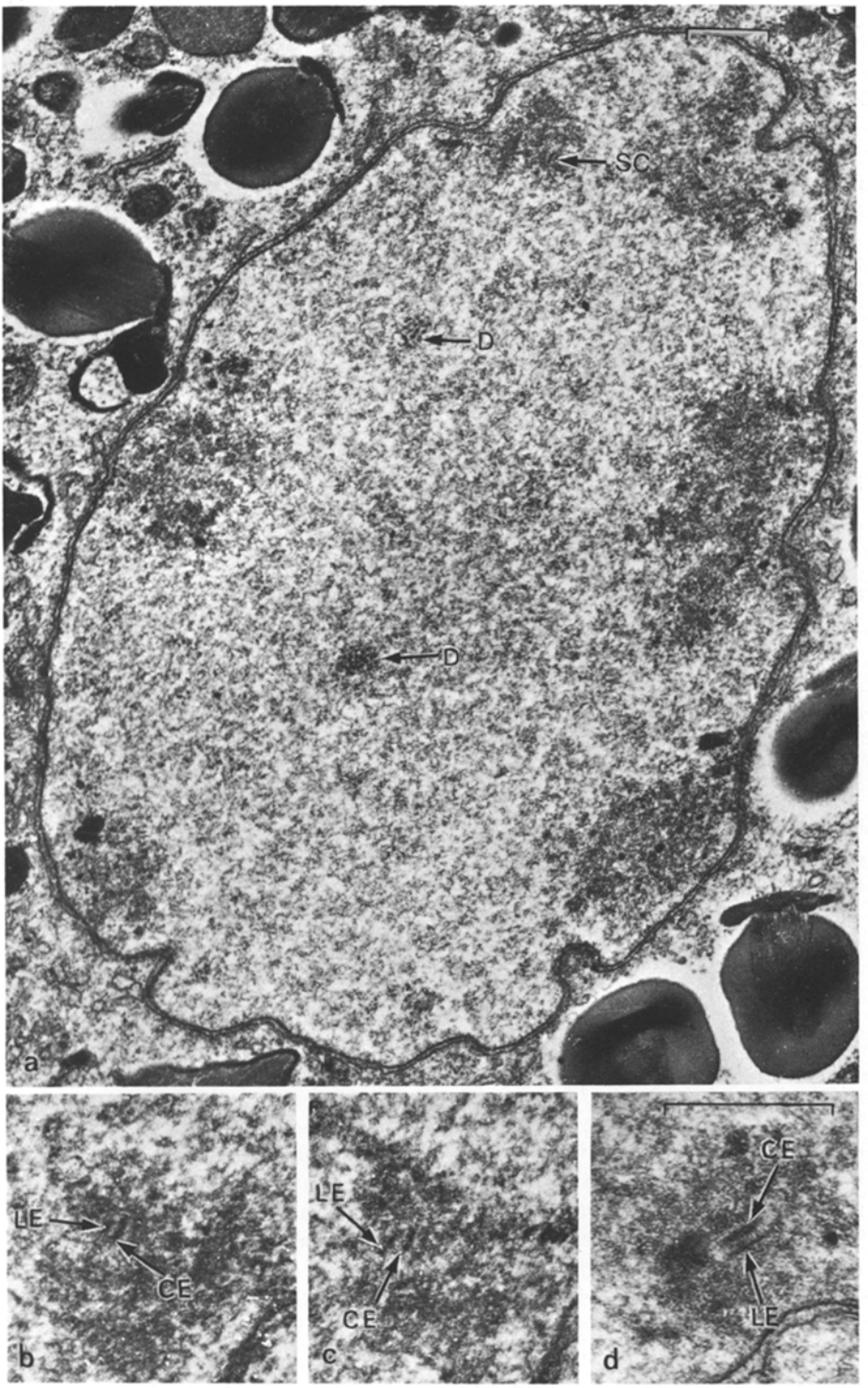

Fig. 7a–d. **a** Whole section of an early diakinetic nucleus. Bivalents located near the periphery of the nuclear envelope. Small stretch of SC within a bivalent (arrow). Dot-like structure (*D*). **b** Higher magnification of the SC stretch of **a** shows central element (*CE*) more prominent than lateral element (*LE*). **c** Higher magnification of the same SC stretch in the third section of the series. Central element (*CE*). Lateral element (*LE*). **d** SC stretch from another early diakinetic nucleus. Figure shows thicker central element (*CE*) than lateral element (*LE*). Chromatin is fibrous. **a** × 23,000, **b–d** × 48,000

Ultrastructure of Chromosomes and SCs at Diakinesis

During diakinesis (Fig. 1c) the bivalents are highly contracted and arranged around the perimetre of the nucleus. They are attached to the nuclear envelope (Fig. 7a). Bivalents at diakinesis show a homogenous fibrous appearance with either one small fibrillar zone in the centre or none. In some bivalents short stretches of SC are visible (Fig. 7b). The dotlike structures are also found in the nuclei at this stage (Fig. 7a). The reduced nucleolus is rarely found as an aggregate within the nucleus; in most cases the nucleolar material is dispersed as drops. The nuclear envelope is periodically invaginated at late diakinesis.

Discussion

We have not observed the reappearance of SCs at the diffuse stage, diplotene and diakinesis in the male. We assume that there is only one round of PC formation in the male, i.e. before synapsis. The PCs which were observed in the male during pachytene by Fiil et al. (1977) may be the remnants of cytoplasmic PCs formed during the presynaptic period.

The notion that the short stretches of SCs in the diplotene nucleus in different organisms which have been studied so far, are localized at the potential sites of chiasmata, is widely spread in the literature (see von Wettstein, 1977; Westergaard and von Wettstein, 1972; Gillies, 1975a). Gillies (1975b) found 5.6 short stretches of SC per bivalent in *Zea mays* microsporocytes at early diplotene when the length of the bivalents is about 40% of pachytene length. The number of these short stretches is about double the chiasma freequency of 2.7 per bivalent reported by Darlington (1934) for the late diplotene in *Zea mays*. Thus only half of the SC stretches may serve as sites of chiasmata in this plant. Our Fig. 1b and especially Mutafova's (1975) Fig. 1c show that in the spermatocytes and oocytes of *A. suum* there are from 1 to 4 chiasma per bivalent. The partially 3-D reconstructed diplotene nuclei of *A. suum* demonstrate that each bivalent does not contain more than four separate SC stretches as seen for example in bivalent III of (Fig. 2b). Three separate SC stretches are found in bivalent VI of (Fig. 2b). The other bivalents (or heterochromatic bodies) do contain 0 to 2 (usually 1) SC stretches. Thus, we might conclude that the number of SC stretches in diplotene bivalents does not exceed the number of chiasmata. We also found that the diplotene chromosomes of *A. suum* male retain rather long stretches of unpaired LEs of SCs (up to 1 μm in length). The morphology of these LEs resemble that of the unpaired LEs of the early zygotene stage. The conclusion which could be drawn from this observation is that the pairing process of LEs is reversible. Lateral elements enter and leave SCs as units of the SC architecture, and the central element (CE) is formed after matching of LEs in this worm. Similar assembly of LEs with delayed CE appearance was observed during precocious formation of cytoplasmic polycomplexes in *A. suum* males and females (Bogdanov, 1977; Fiil et al, 1977).

Persistence of typical SC within bivalents at diakinesis is unusual. In many cases reported SCs disappear from the bivalents during diplotene with notable exceptions in human spermatocytes (Holm and Rasmussen, 1977) and in *Ascaris* spermatocytes (Goldstein, 1977), where small SC stretches or their remnants are retained in the bivalents up to early metaphase I and diakinesis respectively. Such retainment has also been reported for the sex chromosomes of the spider *Lycosa malitiosa* (Benavente and Wettstein, 1977). However, it must be noted that the degree of chromatin condensation of diakinetic chromosomes in *A. suum* is rather less than in the chromosomes of other organisms (see for example, Moens, 1968). The less dense condition of *A. suum* diplotene and diakinetis chromosomes may account for the persistance of SC stretches.

The small size of *A. suum* diplotene and diakinetic chromosomes, a relatively small number of bivalents (n = 12) and synchronous development of meiosis make spermatocytes of *A. suum* a convenient object for further 3-D reconstruction studies of the nuclei at late meiotic prophase stages.

Acknowledgements. We are grateful to Professor Peter B. Moens for his hospitality to one of us (SCK) during a stay in his laboratory in Toronto and for providing the facilities for serial sectioning. We wish to thank Dr. A.S. Tikhonenko and Professor Yu.S. Chentsov for the use of their electron microscopes and other facilities of their laboratories. The technical assistance of Miss Lika Zuleva is also acknowledged. We also wish to thank Professor S.K. Sen and Professor D. von Wettstein for critical reading of the manuscript. SCK is the recipient of a USSR Government post-doctoral fellowship.

References

Benavente, R., Wettstein, R.: An ultrastructural cytogenetic study on the evolution of sex chromosomes during the spermatogenesis of Lycosa malitiosa (Arachnida). Chromosoma (Berl.) **64**, 255–277 (1977)

Bogdanov, Yu.F.: Formation of cytoplasmic synaptonemal-like polycomplexes at leptotene and normal synaptonemal complexes at zygotene in Ascaris suum male meiosis. Chromosoma (Berl.) **61**, 1–21 (1977)

Carpenter, A.T.C.: Electron microscopy of meiosis in Drosophila melarogaster females: II: The recombination nodule – a recombination-associated structure at pachytene? Proc. nat. Acad. Sci. (Wash.) **72**, 3186–3189 (1975)

Darlington, C.D.: The origin and behavior of chiasmata. VII. Zea mays. Z. indukt. Abstamm.- u. Vererb.L. **67**, 96–114 (1934)

Fiil, A., Goldstein, P., Moens, P.B.: Precocious formation of synaptonemal-like polycomplexes and their subsequent fate in female Ascaris lumbricoides var. suum. Chromosoma (Berl.) **65**, 21–36 (1977)

Gillies, C.B.: Reconstruction of the Neurospora crassa pachytene karyotype from serial sections of synaptinemal complexes. Chromosoma (Berl.) **36**, 119–130 (1972)

Gillies, C.B.: Synaptinemal complex and chromosome structure. Ann. Rev. Genet. **9**, 91–110 (1975a)

Gillies, C.B.: An ultrastructural analysis of chromosomal pairing in maize. C.R.Trav. Lab. Carlsberg **40**, 135–161 (1975b)

Goldstein, P.: Chromosomes of Ascaris lumbricoides var. suum. Ph.D. Thesis, York University, Toronto 1977

Goldstein, P., Moens, P.B.: Karyotype analysis of Ascaris lumbriciodes var. suum. Chromosoma (Berl.) **58**, 101–111 (1976)

Holm, P.B., Rasmussen, S.W.: Three-dimensional reconstructions of meiotic chromosomes in human spermatocytes. In: Chromosomes today **6**, 83–93 (1978)

Moens, P.B.: The structure and function of the synaptonemal complex in Lilium longiflorum sporocytes. Chromosoma (Berl.) **23**, 418–451 (1968)
Mutafova, T.: Morphology and behaviour of sex chromosomes during meiosis in Ascaris suum. Z. Parasitenk. **46**, 291–295 (1975)
Perov, N.A., Kiknadze, I.I., Chentsov, Yu.S.: The puff ultrastructure in polytene chromosomes of Chironomus thummi. Tsitologia (Russ.) **18**, 840–846 (1976)
Rasmussen, S.W.: Synaptonemal polycomplexes in Drosophila melanogaster. Chromosoma (Berl.) **49**, 321–331 (1975)
Wettstein von, D.: The assembly of the synaptonemal complex. Phil. Trans. B **277**, 235–243 (1977)
Westergaard, M., Wettstein von, D.: The synaptinemal complex Ann. Rev. Genet. **6**, 71–110 (1972)
Zickler, D.: Development of the synaptonemal complex and the "recombination nodules" during meiotic prophase in the seven bivalents of the fungus *Sordaria macrospora* Anersw. Chromosoma (Berl.) **61**, 289–316 (1977)

Received June 19–August 7, 1978 / Accepted August 7, 1978 by D. von Wettstein
Ready for press September 16, 1978

Note Added in Proof

It is preferable to term the thickened parts of the central elements of the SC in *Ascaris suum* as recombination nodules (RN) as all other authors do when describing similar structures in other organisms. At the XIV. International Congress of Genetics (Moscow August 19–31, 1978), C.B. Gillies, and P.B. Holm & S.W. Rasmussen reported further evidence supporting the idea about an intimate relation between RNs and crossing-over in *Neurospora* and human, respectively (to be published in the Proceedings of the Congress and elsewhere). Our observations concerning the presence of RNs in most stretches of SC in diplotene-diakinesis and an approximate coincidence between number of retained SC stretches and number of chiasmata per bivalent in *A. suum* are in agreement with their results.