*Gazi University Journal of Science*
*GU J Sci*
*24(2):187-192 (2011)*

GUJS
*www.gujs.org*

# The Ultrastructure of the Synaptonemal Complexes in *Galleria mellonella* (Lepidoptera) Spermatocytes

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*Received: 30.04.2010 Accepted:16.05.2010*

**ABSTRACT**

Fine structure of synaptonemal complexes (SCs) was investigated in primary spermatocytes of *Galleria mellonella* testes. In longitudinal sections, the SCs were discriminated as linear or curved rods in the prophase nuclei of the first meiotic division. These rods were attached to the inner surface of the nuclear envelope by their one end. Two electron dense axial or lateral elements were clearly distinguished in fine structure of the SCs. A central element was formed by parallel bands with a lesser electron density than the lateral elements. The diameters of the central and the lateral elements were 37 nm and 48.7 nm, respectively. The central element is embedded within the electron lucent central region. Fine transverse filaments crossing the central region between the lateral and central elements were seen. The width of this area was measured 40.4 nm. Taken together, total diameter of the SCs in the testes of *G.mellonella* reaches to 218 nm.

**Key words:** *Synaptonemal complex, Testis, Galleria mellonella, Insect, Electron microscopy*

## 1. INTRODUCTION

The first data about synaptonemal complexes (SCs) were obtained from the studies of Moses and Fawcett, in 1956 separately [1]. They described SCs as the special structures supporting the homologous chromosomes in pachytene stage of the prophase. The structure and composition of SCs were reviewed recently [2, 3].

SCs are meiosis-specific protein structures that located in nuclei [4, 5] and appear between homologous chromosome pairs longitudinally during first meiotic prophase [4, 6, 8]. SCs play a crucial role in chromosome pairing [5, 9-14], crossover [1], recombination [5], and prevention of sister-chromatid pairing [15-16] and are affected by diseases [9] and mutations [17]. SCs are particularly critical structures for determination of the reason of infertility or semi-reproductivity in human and economically important farm animals [18-20].

In this study, ultrastructural morphology of SCs in the testis of *Galleria mellonella* larvae was described. Since some of the meiotic abnormalities can only be detected by investigation of their morphological changes, determination of fine structures of SCs has a great importance.

## 2. EXPERIMENTAL

*Galleria mellonella* L. (Lepidoptera: Pyralidae) was grown up at 30±1°C and 40-60% humidity in dark condition. *G.mellonella* was fed with synthetic food in petri dish [21, 22]. The testes from three days old larvae were used for investigation.

For electron microscopic investigation, dissected testes were fixed in Karnovsky [23] fixative and then postfixed with 1% $OsO_4$ [24]. The materials were embedded in Epon 812. The thin sections were stained with uranyl acetate and lead citrate [25] and then examined in Jeol 100C transmission electron microscope (TEM).

The measurements were performed on the best ten chosen micrographs of the SCs using a special counting lens.

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## 3. RESULTS

Young germ cells including primary-secondary spermatogonia and primary spermatocytes are found in the testes of three days old larvae of *G.mellonella*. Primary spermatocytes were clearly discriminated by their characteristic big nuclei, plenty of free polysomes and a few number of short rough endoplasmic reticulum channels during the TEM investigation. SCs were distinguished as linear or curved fibrous structures (Figures 1, 2). They were attached to the inner surface of the nuclear envelope by their one end in the nuclei of the middle prophase I cells.

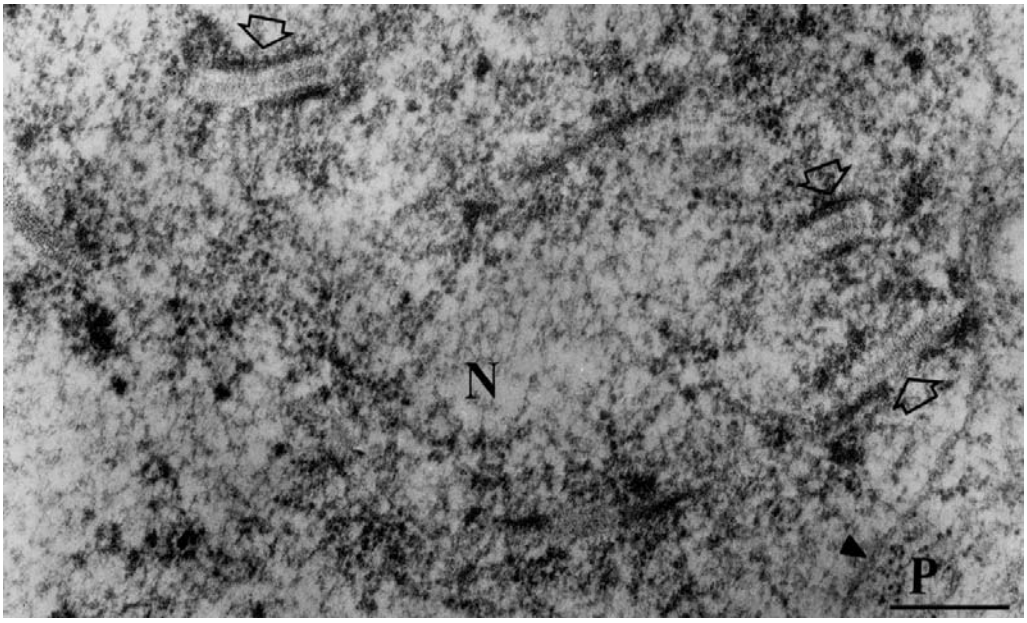


Figure 1. Synaptonemal complexes (⇒) in primary spermatocyte nuclei at prophase stage of meiosis. ►, nuclear envelope; P, free polyribosomes (scale bar: 0.5 μm).

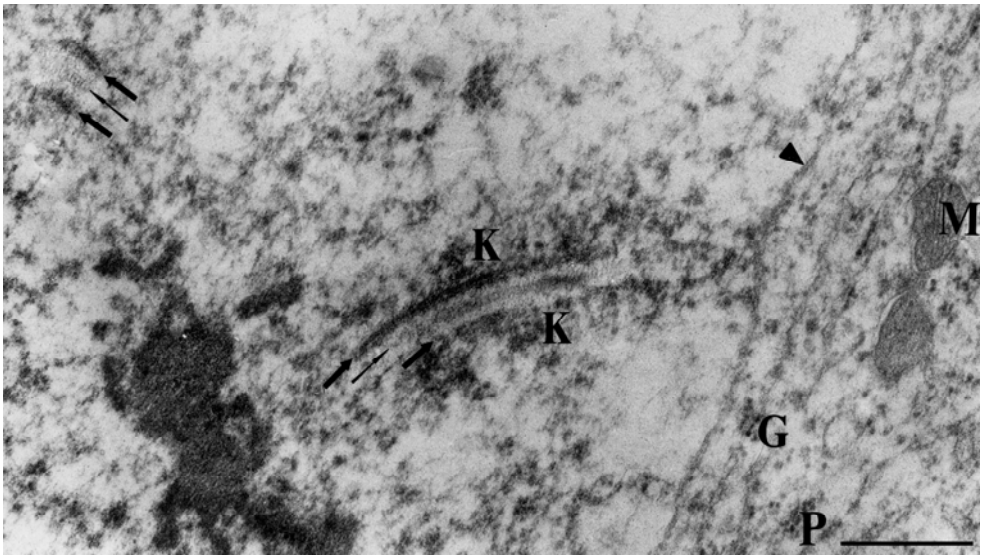


Figure 2. Central (→) and lateral (➔) elements of synaptonemal complexes. K, chromosome; ►, nuclear envelope P, free polysomes; G, rough endoplasmic reticulum; M, mitochondrium (scale bar: 0.5 μm).

SCs consist of an electron lucent central region (CR) and two electron dense lateral or axial elements (LEs) on both sides the CR. There is an electron dense central element (CE) at the middle part of the CR. CE consists of the bonds which are lower than lateral elements in electron density. Its diameter was measured as 37 nm (Figures 2, 3). The electron lucent area between the CE and LEs is crossed by very thin transverse filaments (TFs). Its diameter was measured as 40.4 nm. These structures form the central region of the SCs with 118 nm in width (Figure 3). Two different layers according to their electron density were distinguished in the lateral elements. The inner layer with low electron density and the outer layer with high electron density were

measured as 25.2 nm and 23.5 nm in diameter, respectively. Hence, total width of the LEs and whole SCs became as 48.7 nm and 215.2 nm, respectively (Figure 3). Characteristically electron dense chromatic fibers very close to the outer layer of the SCs were distinguised. The abundance of the SCs was plenty in the middle of the prophase. Their numbers reduce at the end of the prophase and completely disappear in the metaphase stage.

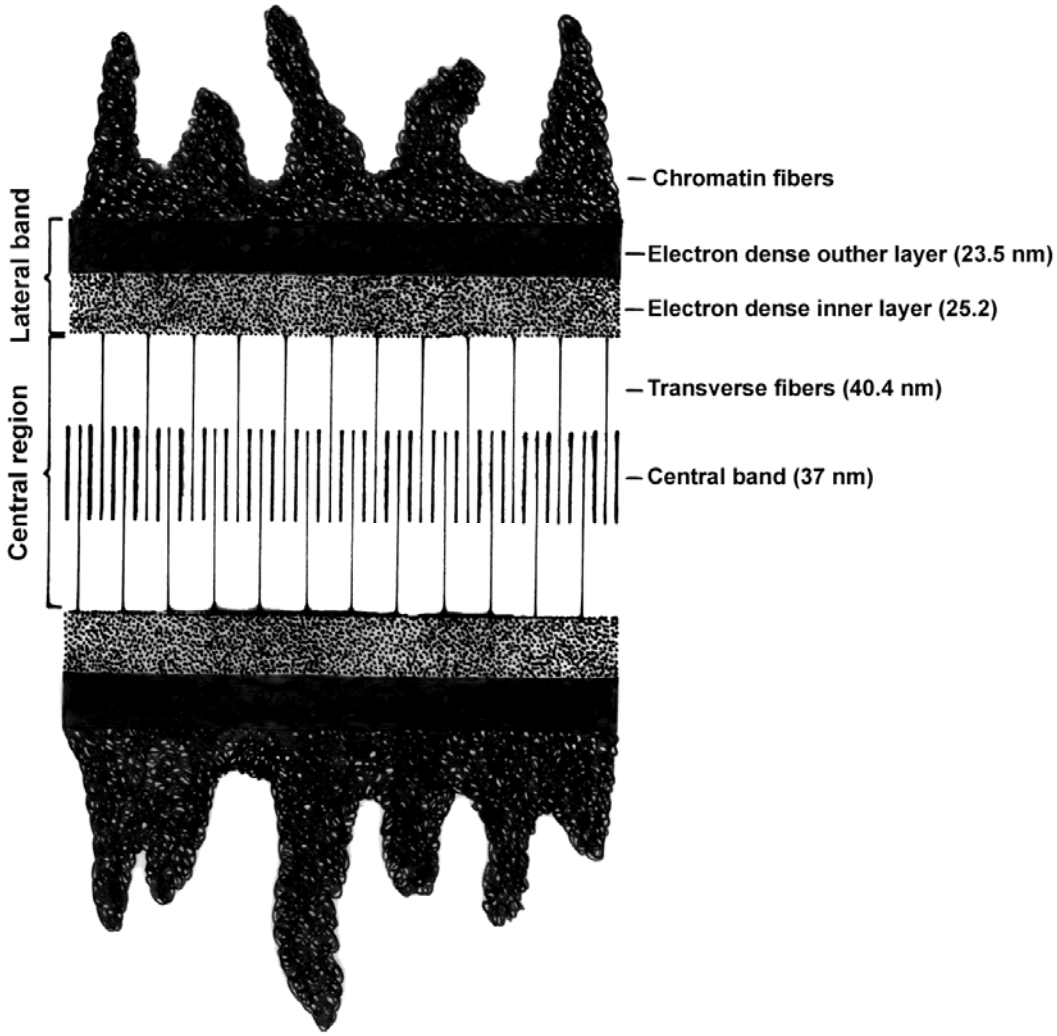


Figure 3. Schematic demonstration of synaptonemal complexes and average diameters of the synaptonemal complex elements

## 4. DISCUSSION

The SCs are specific structures of meiosis [4, 5] and therefore can only be observed in oocytes and spermatocytes. The differences in their morphology, length and diameter of the SCs were distinguished depending on to the species and the sexes [26]. The most conspicuous difference appears in the central region between male and female sexes. For example, the width of the CR in *Bombyx mori* was 70-80 nm [1, 27] and 100-120 nm in oocytes and spermatocytes, respectively [28]. The radial elements of SCs found in the ovaries of *G.mellonella* [29] couldn't be detected in spermatocytes of the same insect in the present study. On the other hand, the morphology of the TEs of SCs in *G.mellonella* was very similar to those found in two insect species, *Drosophila melanogaster* and *Blaps cribrosa* [30]. When compared the diameters of the several parts of the SCs among the investigated insect species [1, 31] it appears that the diameters were found slightly higher in *G.mellonella*.

The significance of SCs is related with chromose-pairing (synapsis) [5, 9-14]. In leptonema stage of prophase I, each chromosome forms one lateral element and starts to close contact with nuclear envelope by one end of the chromosome [1, 32]. It is still unclear that why the chromosome ends attach to the nuclear envelope and what is the significance this process [33]. Probably, at the starting-point for the formation of specific pairing between homologous, the chromosomes are stabilized on nuclear envelope and then SCs support pairing of the correct chromosome partners. Demonstration of a large amount nuclear protein lamin C2 in the nuclear envelope explained by their particular

role in the formation of the linkage between the nuclear envelope and the SCs.

The formation of the SCs before occurance of homologous recombination is indispensable fort he normal crossover [1]. The recombination between homologous chromosomes doesn't occur in *Drosophila* males, and also SCs don't appear in C(3) G17 mutated females [34]. On the other hand, it was shown that the trigger of recombination is functionally dependent on the formation of the homologous chromosome pairings (SC formation) in yeasts [15]. All these studies implicate the importance of the SCs in proper and correct crossover and recombination. However, the formations of SCs don't mean that the crossover is going to occur. For example, *Bombyx mori* females don't perform crossover although the formation of SCs during meiosis [27]. It was demonstrated that SCs formed in case of null mutants (mei-W68 and mei-P22) of *Drosophila* with meiotic crossover and recombinant disfunction [35]. Therefore, it is thought that the SC doesn't trigger crossover but prepares pre-conditions for crossover and is indispensable [1, 4, 10]. Another role of SCs is to prevent the sister-chromatide pairing. Thus, the formation of homologous chromosome pairs is supported [15, 16] and abnormal pairings are prevented.

SCs are affected from diseases [9] and mutations [8, 17]. Any abnormality in SCs such as disfunction or existence of SCP1 (a SC protein) leads to an increase in male infertility [9]. In the existence of SCP3, one of the lateral element proteins, infertility occurs by depending on the increase of apoptotic cells in meiotic prophase stage [8]. Thus, SC abnormalities are observed in most of the infertile or less reproductive males. These abnormalities include irregular [36], fragmented [37, 38] or damaged [39] SCs and its related abnormal chromosome pairings or distributions such as trivalent, hexavalent [19, 40], asinapsis [17, 38, 41] or desinapsis [42], non-homologous pairings [41, 43, 44] or lacks in pairings [39, 45]. The abnormalities generally appear at pachytene stage when SCs become the clearest [41, 44, 46]. From all those reasons, determination of the SC abnormalities makes easy the detection of infertility or semi-reproductivity [18, 20]. Analysis of SC abnormalities give more reliable results compare with analysis on testis size and shape and semen structure [47].

As a result, SCs are meiosis specific fibrous structures. SCs appear in meiotic prophase and disappear at the end of the same stage. Investigation of SCs is a particularly critical to determine the reason of infertility or semi-reproductivity in human and economically important farm animals. To reveal the fertility or sterility of hybrid farm animals and also useful and harmful insects [45, 48], the effect of the several genotoxic substances [49, 50] and translocations [20, 51] on fertility investigations of SCs structures reliable studies because, they are affected from diseases and mutations. So, electron microscopic analyses of structural defects of SCs [52] are very high importance in connection with this purpose.

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