

MORPHOGENESIS OF THE AXONEME IN THE SPERMATOCYTE OF *COCCINELLA SEPTEMPUNCTATA* LINNAEUS

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Abstract Using cell whole mount preparation, early morphogenesis and ultrastructure of the axoneme of *Coccinella septempunctata* L. spermatocyte were investigated by transmission electron microscope. During spermatogenesis two pairs of basal body-axoneme complexes originated from centrioles are found in the spermatocyte and they are separated completely from each other at interkinesis. The centriolar adjunct begins to generate while a basal body-axoneme complex is attached to the nuclear envelope of a spermatid nucleus, and it, on the proximal end of a growing axoneme, reaches a maximum before chromatin condensation. The growing axoneme is accompanied by the condensable nucleus elongation. The early axoneme of a basal body-axoneme complex consists nine doublets with only inner and outer dynein arms, no central microtubules.

Key words *Coccinella septempunctata* L., axoneme, spermatocyte, microtubule

1 INTRODUCTION

The axoneme, the main motor structure of flagella and cilia, has been investigated in a variety of eukaryotes (Gibbons 1981, Witman 1990). It is generally believed that one of the functions of the centriole is its capacity to generate a flagellum or a cilium (Wheatley 1982). In insects, the formation of an axoneme in the male reproductive cell is variable. The occurrence of axoneme in some lepidopterans begins in the primary spermatocyte (Friedlander and Wahrman 1971, Lai-Fook 1982, Godula 1985, Wolf and Traut 1987, Wang 1994). In *Locusta migratoria manilensis* however, its first appearance takes place in early spermatid.

This paper is to describe another case that the formation of axoneme occurs initially in the spermatocyte of *Coccinella septempunctata* L.

2 MATERIALS AND METHODS

Pupae and adults of the lady beetle were collected from vegetable and wheat field in the suburb of Beijing and reared with aphids in the laboratory. Cysts were collected from 30-40 follicles of an adult testis. The selected cysts in which the male germ cells were developing synchronously were used as cell whole mount preparation as described before (Wang 1994). Samples were observed by Hitachi H-300 electron microscope.

3 RESULTS

3.1 Emergence of basal body-axoneme complex

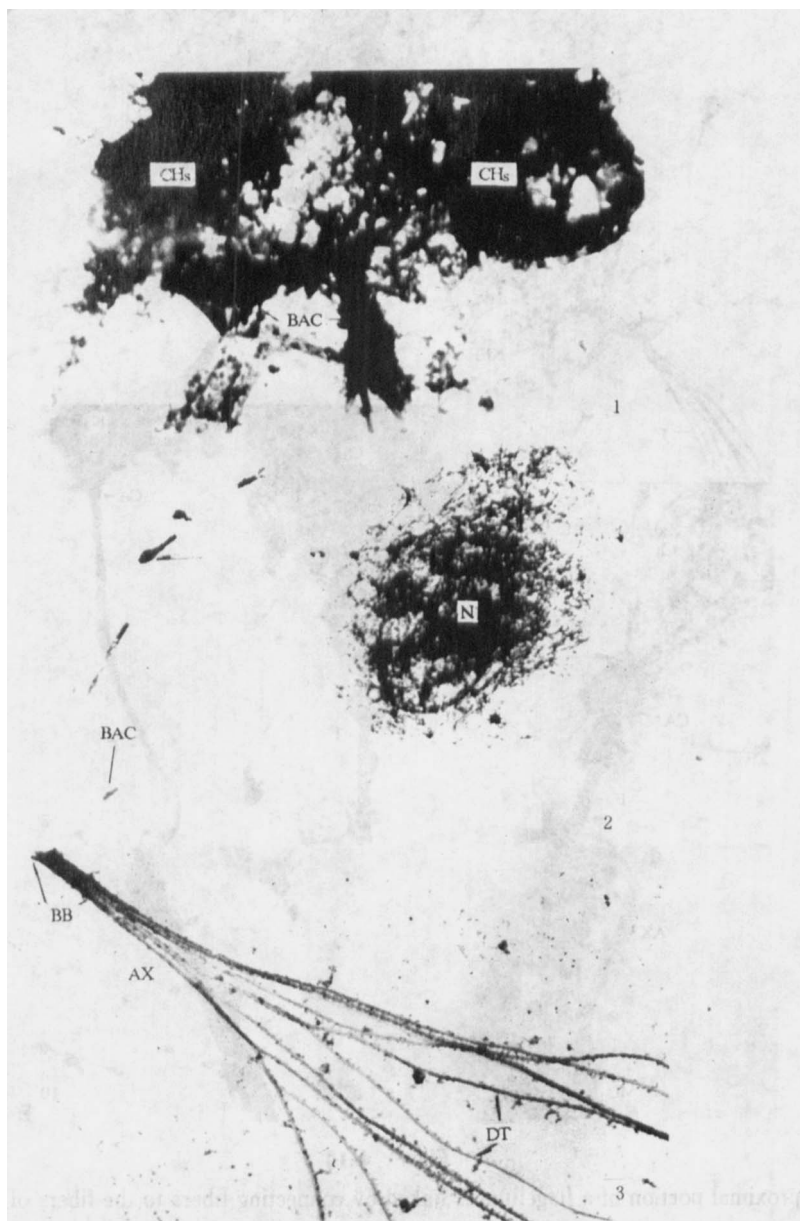
The term basal body-axoneme complex (BAC) is used for the structure, which includes the basal body originated from the centriole and the axoneme extending from it. As description by Nath *et al.* (1951), it is one of the characteristics of meiosis in the spermatocytes of coleopterans that there is a distinct pause between two meiotic phases called resting secondary spermatocyte. Fig. 1 shows two rectangular BACs appearing in the spermatocyte at meiosis and linking each other together with fibers (arrow), and chromatids becoming condensed and losing their distinct outline. The resting secondary spermatocyte nucleus with an interphase-like state at the separate BACs at interkinesis are shown in Fig. 2. During this period the length of BACs is approximately at the range of 1.4-5.0 μm (including basal body about 0.35 μm), and each possesses a distal swelling (Fig. 2 arrow). It is shown in fig. 3 that the axoneme portion of BAC consists of nine microtubular doublets with dynein arms, and the two central microtubules are absent. The nine doublets are easily separated from each other, namely, the A-microtubule of one doublet does not connect to the B-microtubule of adjacent one via links or arms. So a developing axoneme in early stage is incomplete in terms of the microtubule inventory.

3.2 Attachment of BAC and occurrence of centriole adjunct

It is shown in Fig. 4 and 5 that the proximal end of a BAC is connected to the fibers of the nuclear fibrous framework with connecting filaments, and an amorphous deeply stained material begins to accumulate on the proximal portion near to the nucleus. Subsequently, the dense material accumulates to become the centriole adjunct (CA) to which the disperse chromatin fibers are preferentially linked (Fig. 6), and reaches the maximum when the nucleus shrinks (Fig. 7). Accompanying the chromatin condensation and spermatid nucleus reduction in size, the CA appears to be more compact, and the flagellum extends gradually (Fig. 8). Fig. 9 shows the elongating nucleus and mitochondrial derivatives and the growing axoneme are juxtaposed. Fig. 10 displays the anterior portion of a elongating spermatid in which the further condensed nucleus spins around the flagellum. It seems that the elongation of spermatid nucleus is closely associated with the growing flagellum.

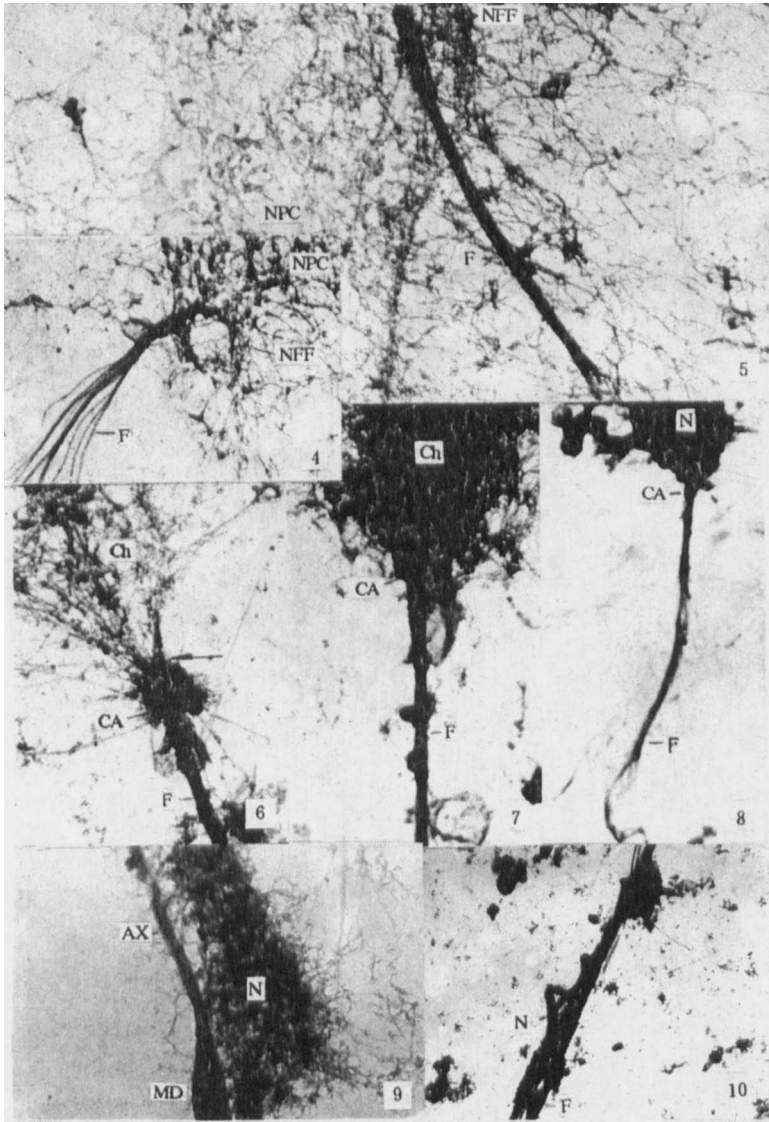
4 DISCUSSION

In spermatogenesis of *Coccinella septempunctata*, the axoneme developed from the basal body occurs in the spermatocyte as in the primary spermatocyte of *Spodoptera lit-*



Figs. 1-3

1. Two orthogonally oriented basal body-axoneme complexes (BAC) in a spermatocyte at anaphase I. The condensed chromosomes (CHs) are delivered to the opposite poles. $\times 18\ 000$. **2.** Four separated basal body-axoneme complexes (BAC) and a typical nucleus with interphase-like chromatin at interkinesis. Arrow points the distal swelling of the complex. $\times 4\ 000$. **3.** A basal body (BB)-axoneme complex shows that the axoneme consists of nine doublets (DT) with dynein arms, but no links between doublets and devoid of central microtubules. $\times 14\ 400$.



Figs. 4-10

4,5. The proximal portion of a flagellum is linked by connecting fibers to the fibers of the nuclear fibrous framework (NFF) with numerous nuclear pore complexes (NPC) and an amorphous material begins to accumulate on it. $\times 6\ 640$, $\times 10\ 000$. **6.** A mature centriole adjunct (CA) with maximum size. Numerous dispersed chromatin fibers (Chs) in the spermatid nucleus are linked to the CA (arrow). $\times 10\ 400$. **7.** Showing the condensing chromatin, a compact centriolar adjunct (CA) and a growing flagellum (F). $\times 10\ 080$. **8.** A compact centriolar adjunct (CA) connects a developing flagellum (F) and the reduced spermatid nucleus (N). $\times 7\ 040$. **9.** Showing the elongating nucleus (N) and mitochondrial derivatives (MD) and the growing axoneme (AX). $\times 5\ 600$. **10.** The anterior portion of an elongating spermatid displaying the nucleus (N) spinning around the flagellum (F). $\times 4\ 800$.

toralis (Godula 1985), *Calpodes ethius* (Lai-Fook 1982), *Ephestia kuehniella* (Wolf and Traut 1987) and *Bombyx mori* (Wang 1994) rather than in the early spermatid of *Locusta migratoria manilensis* (Wang and Zhong 1993).

With regard to flagellum assembly at beginning stage, 250-300 proteins have been estimated to be involved in flagellar formation (Luck 1984). The experimental study by Dentler and Rosenbaum (1977) involving polymerization of brain tubulin to partially disrupted flagella of *Chlamydomonas* indicated that polymerization on to the outer doublet tubules occurs at their distal (+) ends, whereas polymerization on to the central tubules occurs at their proximal (-) ends. It is possible in *C. septempunctata* that a precursor of axoneme, mainly tubulin, for doublets is provided by the distal swelling of BAC. On the other hand, It has been showed that the formation of central microtubules in growing BAC of *C. septempunctata* lags behind that of doublets as the finding in *Ephestia kuehniella* (Wolf and Traut 1987).

The origin and function of a centriole adjunct (CA) are still uncertain. It is found in our laboratory that there are two ways to form the CA. In *C. septempunctata*, the formation of the CA occurs after a BAC is attached to the spermatid nucleus. But in the case of *L. migratoria*, following appearance of the CA on the spermatid nucleus, the proximal centriole as a basal body is anchored on the nuclear envelope and is surrounded by the CA, and the axoneme originates from it (Wang and Zhong 1993). Our results also indicate that it is of cytoplasmic origin instead of nuclear origin (Werner 1965). Concerning the function of the CA, most researchers have always favoured the view that it is a mechanical support in fastening the sperm head and tail together (Phillips 1970). Other function is attributed to the source of nutritional materials for the flagellum assembly. Based on numerous dispersed chromatins being connected to the CA (Fig. 6), it might have another role acting as an organizer to rearrange the chromatin in the nucleus of the differentiating spermatid in addition to the suggested nutritional and mechanical functions.

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七星瓢虫精母细胞内轴丝的形态发生

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应用细胞整装技术研究了七星瓢虫精子轴丝的早期形态发生和超微结构。在精子发生期间,起源于中心粒的两对基体-轴丝复合体出现在精母细胞内,在分裂间期它们彼此完全分离。当基体-轴丝复合体附着于精细胞核的核膜上,中心粒附体开始发生于生长轴丝的近心端,在染色质凝聚前中心粒附体最大。生长着的轴丝伴随着凝聚细胞核伸长。一个早期基体-轴丝复合体的轴丝是由具有内、外动力蛋白臂的9个双微管组成,缺少中央微管。