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【Research report】

東方果實蠅(Dacus dorsalis)精子粒腺體衍生物之形成【研究報告】

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Abstract

摘要

本研究係利用超薄切片法，在穿透式電子顯微鏡觀察東方果實蠅(Dacus dorsalis)精子尾部內粒腺體衍生物在精蟲形成過程(Spermeiogenesis)中，發生和形成之變化。 精子內之粒腺體衍生物(Mitochondrial derivatives)係由精母細胞(Spermatocyte)內的粒腺體(Mitochondria)聚合再衍化而成。在精母細胞老熟時期，細胞內許多粒腺體由細胞內各部份聚集於細胞核下方，再漸漸相互癒合，溝通而成一團之構造，稱為Nebenkern. Nebenkern隨著精蟲細胞之變化，而中分為兩部份，再伸長，而漸漸於精子的尾部形成兩條長線形之粒腺體衍生物，位於鞭毛 (Flagellum) 的兩側。粒腺體衍生物的功能為供給能量而使精子活動。

Key words:

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FORMATION OF THE MITOCHONDRIAL DERIVATIVES OF THE SPERMATOZOA IN THE ORIENTAL FRUIT FLY, *DACUS DORSALIS*[1]

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ABSTRACT

The development of the mitochondrial derivatives of the spermatozoa in the oriental fruit fly, *Dacus dorsalis* was observed during spermiogenesis, using electron microscope.

The mitochondrial derivatives are formed from the fusion of many mitochondria in the late stage of the spermatocyte. They fuse each other to become a nebenkern which later divides into two components. These two components extend to form two long mitochondrial derivatives along two sides of the flagellum from the centriole to tip of the tail in the spermatozoa, while the spermatid grows and elongates to transform as the spermatozoa.

INTRODUCTION

The two giant mitochondrial derivatives of the spermatozoa are transformed from numerous mitochondria of the spermatocyte. This transformation has been studied by many researchers of insect spermiogenesis with light microscope (Bowen, 1920, 1922; Johnson, 1933; Pollister, 1930).

Electron microscopic observations of spermiogenesis indicate that the progressive fusion of the numerous mitochondria in the spermatocyte into an interwoven labyrinthian nebenkern mitochondria (Hoage et al, 1968). These rearrangements, configurational changes and the eventual segregation of the spermatocyte mitochondria were investigated by many scholars with different species of insects (Anderson, 1967; Andr'e, 1959, 1962; Bawa, 1964; Hoage, 1968; Payne, 1966; Philips, 1970; Pratt, 1970; Yasuzumi, 1958; Yamazumi et al, 1960).

The present study attempts to investigate the development of the mitochondrial derivatives of the spermatozoa in the oriental fruit fly, *Dacus dorsalis,* with the transmission electron microscope, and to compare the developmental processes with those of other insects. The results provide a helpful information for further study of the effect of the radiation on the spermiogenesis in the same insect.

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MATERIALS AND METHODS

The oriental fruit flies, *Dacus dorsalis* were reared in the laboratory of Institute of Zoology, Academia Sinica, Nankang, Taipei, for more than ten generations. The testes were taken from the newly emerged flies and fixed with 6% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2-7.4 for 3 hours in cold. They were washed in 8% sucrose in 0.1 M cacodylate, and then postfixed with a mixture of 2% osmium tetroxide in 0.1 M cacodylate for 2 hours. After several times of short wash with distilled water, the testes were immersed in 4% uranyl acetate for 2-3 hours, and then, dehydrated rapidly in a graded series of ethanol. They were finally put through propylene oxide and embedded into the Spurr low viscosity medium. The mixture with the specimen was polymerized at 70°C overnight.

The silver or golden color of thin sections were cut with the Porter Blue MT-2 ultramicrotome. All sections were observed with a Hitachi type 11-A electron microscope, and the micrographs were taken at the direct magnifications of 5,000 x to 10,000 x with the acceleration voltage of the electron beam at 50 KV or 75 KV.

OBSERVATIONS

After the telophase II of the late stage of spermatocytes in the oriental fruit fly, *D. dorsalis,* the mitochondria of these cells move to one side instead of scattering around the cells. A few of these mitochondria (M) are shown to be branch-like (Fig. 1).

Figure 2 shows the early spermatids, in which the mitochondria (M) aggregate each other to form a cluster like material in one side of the cell. From the observations of numerous sections, Figure 3 shows that the mitochondria (M) get more closer each other. Each one of the mitochondria appears in various shapes, e.g., round, oval and elongated, whereas some are curved or branched. in Figures 4 and 5, the mitochondria are to be fused each other and to interlock togather as one piece, forming a structure termed nebenkern (Hoage et al., 1968). At the stage shown in Figure 6, the nebenkern divides into two parts and becomes two complete interlocked networks. They are finally going to separate each other.

Electron micrographs show that one of two derivatives of the nebenkern (nm) gradually becomes elongated (Fig. 7). This realignment of the derivatives after separating from each other is related to the growth of the spermatid.

During the early stage of the spermatozoa or the late stage of the spermatids, the differentiation of the spermatozoa occurs. The head and the tail of the spermatozoa have been formed. Two derivatives of the nebenkern become as long as the flagellum (f) within the tail of the spermatozoa (Fig. 8). In this stage as shown in Figure 8, the derivatives of the nebenkern are termed as the mitochondrial derivatives (mit) according to Chapmen (1971). Meanwhile these mitochondrial derivatives appear in irregular segments.

The mitochondrial derivatives become more slender while the spermatozoa grow. The irregular segmentation gradually disappears through the observations under the transmission electron micrographs at the electric beam current of 50 KV, magnification 10,000 x (Figs 10 and 11 mit).

In the transverse section, the mitochonderial derivatives have become aligned in parallel (Fig. 9).

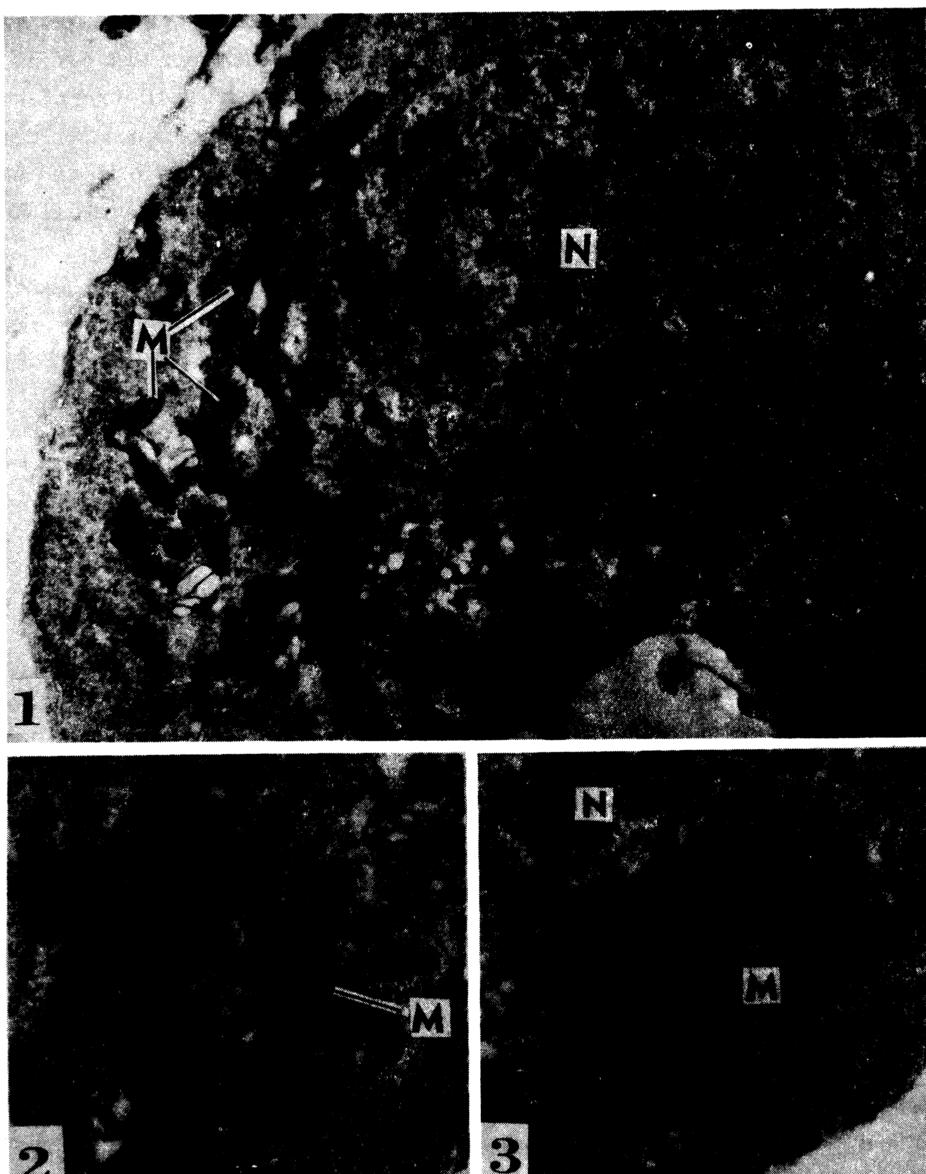
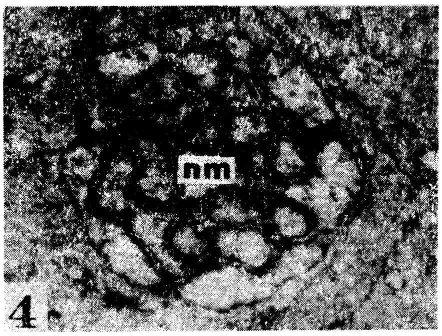
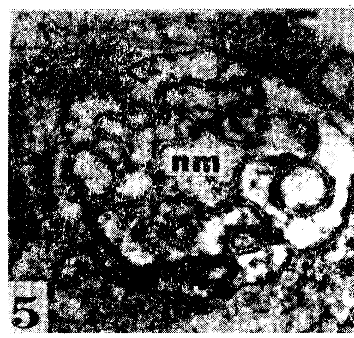


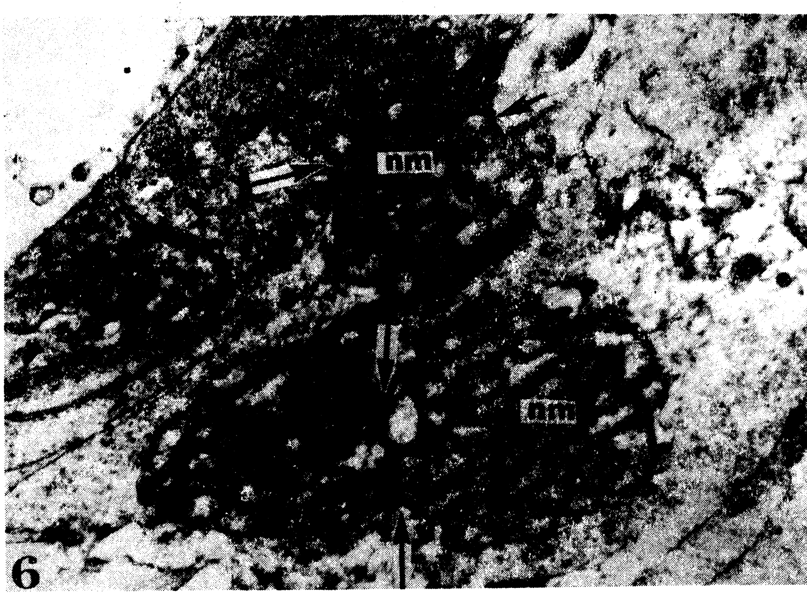
Fig. 1. The spermatocyte of *Dacus dorsalis.* The mitochondria appear branch-like in shape and move to one side of the cell. 15,000 x. M-Mitochondria, N-Nucleus.

Fig. 2. The early spermatid. The mitochondria aggregate each other to form a cluster like material. 15,000 x. M-Mitochondria.

Fig. 3. Same as Figure 2. The mitochondia aggregate each other more closed. 15,000 x. M-Mitochondria, N-Nucleus.







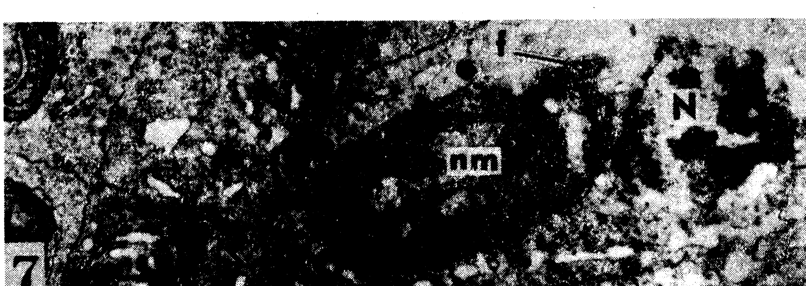


Fig. 4. One part of the spermatid. The mitochondia fuse each other and interlock together as one piece termed the nebenkern. 15,000 x. nm-Nebenkern.

Fig. 5. Same as Figure 4, but more advanced than it, nm-Nebenkern.

Fig. 6. Part of the spermatid. The nebenkern is dividing into two parts. 15,000 x. nm-Neberkern, Arrow-the position of the division.

Fig. 7. The derivative of the nebenkern becoming elongated being related to the growth of the spermatid. 15,000 x. f-Flagellum, N-Nucleus, nm-Nebenkern.

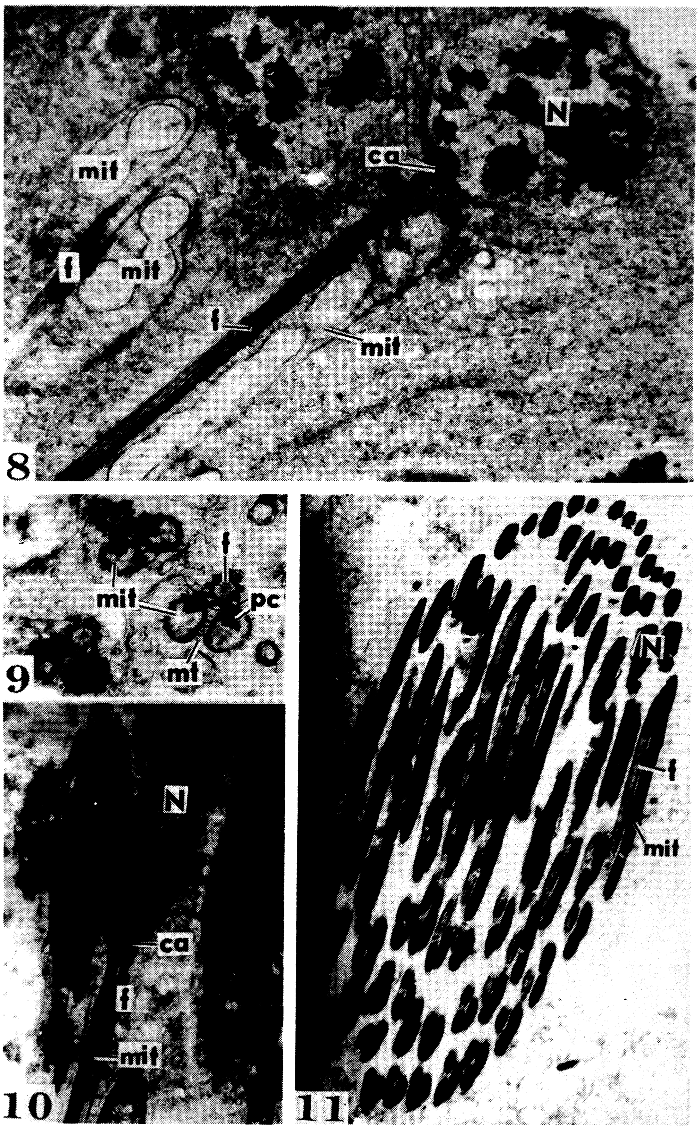


Fig. 8. The early stage of the spermatozoa. The differentiation of the spermatozoa appear. The derivatives of the nebenkern become as long as the flagellum within the tail of the spermatozoa and are termed the mitochondrial derivatives. 15,000 x. ca-Centriole, f-Flagellum mit-Mitochondrial derivatives, N-Nucleus.

Fig. 9. The transverse-section of the spermatozoa showing the structure of the tail. 15,000 x. f-Flagellum, mit-mitochondrial derivatives, mt-microtubules, pc-Paracystalline component.

Fig. 10. The ultrastructure of the spermatozoa in the longitudinal section. 20,000 x. ca-Centriole, f-Flagellum, mit-Mitochondrial derivatives, N-Nucleus.

Fig. 11. The sperm bundle of *Dacus dorsalis*. 20,000 x. f-Flagellum, mit-Mitochondrial derivatives, N-Nucleus.

Within each mitochondrial derivatives (mit), the deposition of a paracrystalline material (pc) in the matrix has commenced. Moreover, several microtubules (mt) are alongside between two mitochondrial derivatives.

DISCUSSION AND CONCLUSION

The mitochondrial derivatives are the organelles whose function is suspected to supply the energy for the movement of the spermatozoa. Although these mitochondria have been lack of cristae, the respiratory enzymes are still retained (Smith, 1968).

Early studies of insect spermatogenesis were precisely ordered steps by which the numerous small mitochondria are transformed into a giant mitochondrial derivative with light microscopy (Bowen, 1922; Johnson, 1931; Pollister, 1930). The results of those investigations were similar each other. After meiosis of the cells, the mitochondria become spherical and cluster together in one area, then they begin a complex series of rearrangement and fusion leading into a large spherical mass, which forms a nebenkern (Bowen, 1920). The nebenkern is initially ring or sphere in shape (Bowen, 1922). Later on, it is separated into two constituents mitochondria. The mitochondria remain closely associated with the flagellum under the nucleus. They become progressively longer and narrower during the spermiogenesis.

With the electron microscopic analysis of spermatogenesis, Andr'e (1959) studied the *Pieris* butterfly, and Yasuzumi et al. (1965) studied the spermatid differentiation in the *Drosophila* and grasshoppers. These results indicated that the mitochondria are agglutinated into a single body; forming an ovoid nebenkern while fusing each other tightly. The nebenkern is viated into two hemisphereshaped halves and increases elongation until they appear as a long tubule.

The development of the mitochondrial derivatives was found in the fire-brat insects, *Thermobia domestica* (Bawa, 1964); the fungus-gnat, *Sciara coprophila* (Phillips, 1966); the drone honey bee, *Apis mellifera* (Hoage et al., 1968) and some Hemipterans, such as *Gelastoceris oculatus* (Payne, 1966) and *Murgantia histrionica* (Pratt, 1970) have similar structure. During spermiogenesis, the mitochondria fuse together and become the mitochondrial nebenkern, which divides into two components nearly equal in diameter. These two components of the mitochondrial nebenkern extend their length as long as that of a spermatozoal tail and are named mitochondrial derivatives. The mitochondrial derivatives in the oriental fruit fly, *D. dorsalis* develop in the same manner as those insects mentioned above.

The paracrystalline components which were first described by Andr'e in 1962, appeared periodically straited in the longitudinal section. They are transformed from the mitochrondrial cristae during the development of the spermatozoa. While the spermatids become elongated, the cristae of the mitochondria gradually change into parallel folds or lamellae to be long axis of the cell. Shortly after, these cristae are realigned only in a small area. This structure is termed as the paracrystalline component (Phillips, 1970).

In most insects, the mitochondrial derivatives of the spermatozoa contain a dense amorphous paracrystalline component (Ross et al., 1969). But in some insects, such as the stilt bug, *Jalysus* sp. and the plant hopper, *Acanalomia* sp., their mitochondrial drivatives of the spermatozoa contain two morphological distinct types of the paracrystalline component (Phillips, 1970). But the present observation reveals that the derivatives in *D. dorsalis* are an àmorphous pattern.

The fine structure of Coccid spematozoan had been studied using electron microscopy (Mosses et al., 1963; Robison, 1965). There is not any structure resembling mitochondria derivatives in the mature spermatozoa of *Parlatoria oleae* (Robison, 1968) and other Coccids (Mosses et al., 1963; Robison, 1968). Doyle (1933) found that the fungus gnats *Sciara* sp. have large spherical mitochondria in the young spermatids, but never form a nebenkern. Their mitochondrial derivatives are formed from the fusion of individual mitochondria along two sides of spermatid flagellum.

In conclusion, the mitochondrial derivatives are developed from the mitochondria in the spermatocyte. The mitochondria fuse together to form a nebenkern in the late stage of the spermatids. The nebenkern divides into two equal parts in the early spermatid. These two parts of the nebenkern elongate gradually, until they become two long tubules located at two sides of the flagellum from the centriole to the tip of the spermatozoa. These processes of the nebenkern development are related to the growth of the spermatozoa. Electron microscopic analyses of spermiogenesis can be done not only in many kinds of insects, but also other animals, such as the toad, *Bufo areneran* (Burges et al., 1965), the plant parasitic Nematode, *Nematoda meloidogyae hapla* (Goldstein et al., 1980). However, some species of the coccid insects are found no mitochondrial derivatives and the fungus gnats, *Sciara* sp. never formed the nebenkern, even though they have mitochondrial derivatives.

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本研究係利用超薄切片法，在穿透式電子顯微鏡觀察東方果實蠅（*Dacus dorsalis*）精子尾部內粒腺體衍生物在精蟲形成過程（Spermeiogenesis）中，發生和形成之變化。

精子內之粒腺體衍生物（Mitochondrial derivatives）係由精母細胞（Spermatocyte）內的粒腺體（Mitochondria）聚合再衍化而成。在精母細胞老熟時期，細胞內許多粒腺體由細胞內各部份聚集於細胞核下方，再漸漸相互癒合，溝通而成一團之構造，稱爲Nebenkern. Nebenkern 隨着精蟲細胞之變化，而中分爲兩部份，再伸長，而漸漸於精子的尾部形成兩條長線形之粒腺體衍生物，位於鞭毛（Flagellum）的兩側。粒腺體衍生物的功能爲供給能量而使精子活動。