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ORIGINAL RESEARCH

A transmission electron microscopy investigation: the membrane complex in spermatogenesis of *Fenneropenaeus chinensis*

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Abstract The transforming characteristics of the membrane complex in spermatogenesis of *Fenneropenaeus chinensis* have been studied by using transmission electron microscopy. Two types of membrane complex have been investigated based on their sources: one originating from nucleus and the other from cytoplasm. The first one, consisted of annular structures, monolayer membrane blebs, and double or multi-lamellar membrane vesicles, emerges in the primary spermatocyte, then diffuses with the nuclear membrane and finally enters the cytoplasm. This type of membrane complex seems to play an important role in the materials transfusion from nucleus to cytoplasm, and it mainly exists inside the primary spermatocyte with some inside the secondary spermatocyte. The latter, originated from cytoplasm, is formed during the anaphase of spermiogenesis. It also exists in mature sperm, locating at both sides of the nucleus under the acrosomal cap. This type of membrane complex mainly comprises rings of convoluted membrane pouches, together with mitochondria, annular lamina bodies, fragments of endoplasmic reticulum, nuclear membrane and some nuclear particles. It releases vesicles and particles into the acrosomal area during the formation of the perforatorium, suggesting a combined function of the endoplasmic reticulum, mitochondria and Golgi's mechanism.

Keywords *Fenneropenaeus chinensis* · Transmission electron microscopy · Transformation characteristics · Ultra-fine structure · Membrane complex

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Introduction

The sperm of decapoda crustacean is structurally different from general zoogenic sperm, and has attracted great research attention. The morphology and structures of such sperms vary from species to species, although they all share the same characteristics, such as without flagella, unmovable and without condensed nucleus. In the past, several research groups have studied the morphology of the sperm and spermatiation, including the membrane complex in spermatogenesis (Moses 1961; Kaye et al. 1961; Langreth 1969; Reger 1970; Arsenault et al. 1979; Arsenault 1984; Haley 1986; Mcknight and Hinsch 1986; Shigekawa and Clark 1986; Du et al. 1988; Medina and Rodrfguez 1992; Medina 1994; Li 1995; Yang et al. 1998; Wang et el. 1999; Kang et al. 2000; Tudge et al. 2001; Zhu et al. 2002;

Medina et el. 2006). However, these results are different from species to species and a systematic study is therefore demanded.

The study of *F. chinensis* sperm has only begun recently. To date, there are a few reports focusing on several important issues, such as the ultra-fine sperm structures (Lin et al. 1991), the morphological variations (Kang et al. 1998; Kang and Wang 2000), endoplasmic reticulum variations (Kang et al. 2000), relationship between ATPase and sperm reproduction (Feng et al. 1995), the insemination ability (Kong et al. 1994), the sperm cryopreservation (Ke and Cai 1996), the cytological variations of the sperm during fertilization (Cai et al. 1997; Zhang et al. 2000; Kang et al. 2001; Liu et al. 2001), and the evaluation of induced triploid shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* cultured under laboratory conditions (Xiang et al. 2006), etc. However, a clear understanding of the membrane complex during the spermatogenesis procedure of *F. chinensis* has yet to be achieved. In this paper, by using transmission electron microscopic technique, we monitored and studied the ultra-fine structural changes of the membrane complex during the spermatogenesis of the *F. chinensis* sperm. We propose that the membrane complex can be classified into two types: one originated from nucleus at the spermatocyte period and the other from cytoplasm during the spermiogenesis.

Methods for processing the germocyte of *F. chinensis*

A live male *F. chinensis* (from Shilihai Prawn Farm in Tanghai, Hebei, China) was dissected quickly in order to obtain the spermary, deferens and testis samples of 0.5 mm^3. The samples were treated with 2.5% glutaraldehyde at room temperature in 0.1 mol L^{-1} sodium cacodylate buffer solution (pH 7.2) for 2 h, then rinsed using 0.1 mol L^{-1} sodium cacodylate buffer solution, followed by treatment with 1% osmium tetroxide in the same buffer solution for 1 h and then rinsed again with 0.1 mol L^{-1} buffer solution. The samples were dehydrated by using alcohol and acetone, then embedded in Epon812 for polymerization at 60 °C for 48 h. An ultra-thin TEM sample (60–90 nm) was obtained by using an ULTRACUT E (701704) cutter, and was placed on 300 mesh Ni TEM grid. The sample was then double stained with uranyl acetate and lead citrate prior to TEM observation (JEM-100SX, operated at 75 kV).

Results

Membrane complex originated from nucleus

During the TEM observation, it is noted that membrane complex, composed of annular structures, monolayer membrane blebs and double or multi-lamellar vesicles, starts to emerge in the primary spermatocyte. At the initial stage, along with the disaggregation of heterochromatin that cohered on inner nuclear membrane, an area occurred which contained a lot of uniform and short fibrous materials with trachychromatic micro-aggregates about 10 nm in diameter. Other areas contain many condensed materials. It is exhibited that monolayer membrane blebs diffused with the fibrous materials to form the double membrane vesicles in which a large quantity of tiny granules, double membrane-wrapped fibrous and/or membrane vesicles formed the multi-layered membrane vesicles (Fig. 1a–c). When a multi-layered membrane vesicle condenses, or double membrane folds and/or circles, the annular structure is created (Fig. 1d and e). Figure 1f shows that the annular structures, the membrane blebs and fibrous vesicles together formed the membrane complex. At this stage, when the nuclear creates the inter-membrane spacing, the membrane complex then diffuses with the inner nuclear membrane and flows into the cytoplasm (Fig. 2a and b).

When the annular structures enter the cytoplasm, they are wrapped by endocytoplasmic reticulum and/or mitochondrium vesicles (Fig. 2c), according to the typical Golgi's mechanism (Fig. 2d), forming the vesicle clumps (Fig. 2e). The primary spermatocyte then splits into secondary spermatocyte where the annular structures are still visible, even though the quantity and volume are both reduced (Fig. 2f). The secondary spermatocyte becomes spermatids after maturation division.

Membrane complex originated from cytoplasm

Membrane complex that originated from the cytoplasm emerges in the spermiogenesis process. The

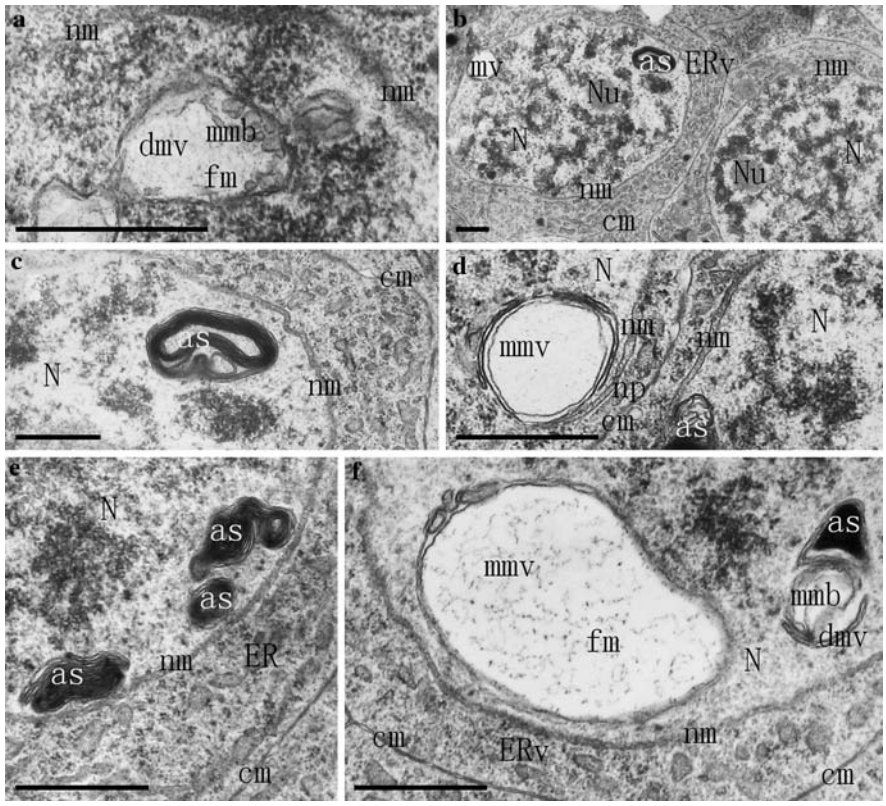

Fig. 1 TEM images (**a**)–(**f**) exhibitting the forming process of membrane complex within the nucleus in primary spermatocyte. The scale bar = 1μm and the letters are defined as: as = annular structure; cm = cytomembrane; dmv = double membrane vesicles; ER = endocytoplasmic reticulum; ERv = endocytoplasmic reticulum vesicles; fm = fibrous materials; mmb = monolayer membrane blebs; mmv = multi-lamellar membrane vesicles; mv = membrane vesicle; N = nucleus; nm = nuclear membrane; np = nuclear pore; and Nu = nucleolus

new spermatid with a spherical nucleus exhibits many endocytoplasmic reticulum vesicles and very few mitochondria vesicles in the cytoplasm. Its double nuclear membrane wraps up the uniformly distributed granular chromatin. Some of the consecutive spermatids share the same cytomembranes (Fig. 3a).

In the prophase of the spermiogenesis, the endocytoplasmic reticulum vesicles that are close to the nucleus in cytoplasm mix together and diffuse into the rough endocytoplasmic reticulum around the nucleus, and the chromatin in the nucleus is agglomerated gradually (Fig. 3b). Along with the polarization of the nucleus, the endocytoplasmic reticulum vesicles, away from the nucleus in the cytoplasm, are assembled to the end opposite to the nucleus and diffused into proacrosomal vacuole where the acrosomal blastema shows a de novo type transformation and grows gradually. When the nucleus shrinks, the chromatin condenses and the rough endocytoplasmic reticulum around the nucleus loosens and appears to be gragmentated in regions (Fig. 3c and d).

In the metaphase of spermiogenesis, the spermatids with sharing cytomembrane separate with each other, and the acrosomal vacuole condenses and shrinks, thus the acrosomal blastema is enlarged. The chromosome is assembled into fine grains, rough endoplasmic reticulum around the nucleus is fragmented, and the nuclear membrane is dissolved gradually, leading to the disappearance of the plasmalemma and organelles (Fig. 4a). At the late stage of metaphase, with cytosome growing downwards, some fragments of rough endocytoplasmic reticulum become smooth endoplasmic reticulum or fold and wrap into a series of tiny vesicles spreading into the tight cytoplasm belt (Fig. 4b).

During the anaphase of spermiogenesis, the acrosome is generated, the chromosome forms flocculent structures, and lamellar cytoplasm wraps up the karyoplasms zone. Under the acrosomal cap in the lateral of nucleus, rings of convoluted membrane punches, mitochondria and annular lamina bodies that wound from endocytoplasmic reticulum, fragments of smooth endocytoplasmic reticulum, nuclear membrane and some nuclear particles constitute the membrane complex that originated from cytoplasm, which constantly releases a lot of vesicles and particulate materials into acrosomal area (Fig. 4c and d).

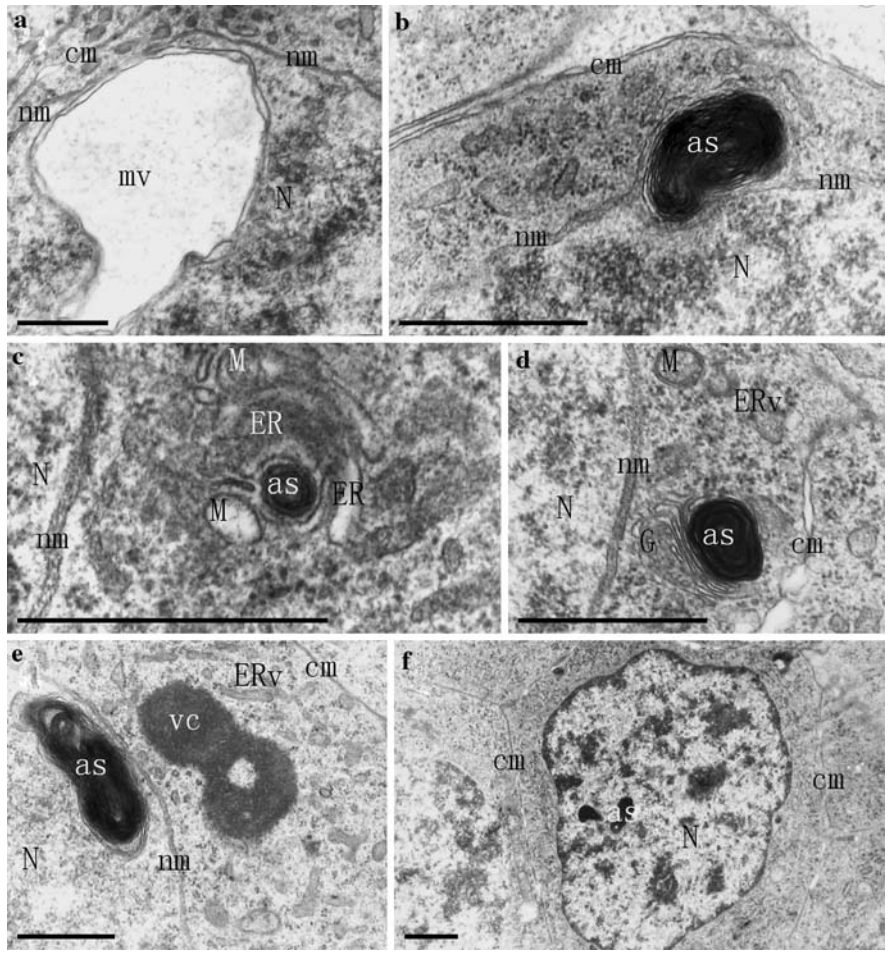

Fig. 2 (**a**) and (**b**) show that membrane complex in the nucleus can shift into the cytoplasm; (**c**)–(**e**) show that the cytoplasmic membrane complex derived from the nucleus combine with endocytoplasmic reticulum and/or mitochondrium vesicles, according to the typical Golgi's mechanism, forming the vesicle clumps; (**f**) shows annular structures in the nucleus of second spermatocyte. The scale bar = 1μm and the letters are defined as: as = annular structure; cm = cytomembrane; ER = endocytoplasmic reticulum; ERv = endocytoplasmic reticulum vesicles; G = Golgi's apparatus; M = mitochondria; mv = membrane vesicle; N = nucleus; nm = nuclear membrane; and vc = vesicle clump

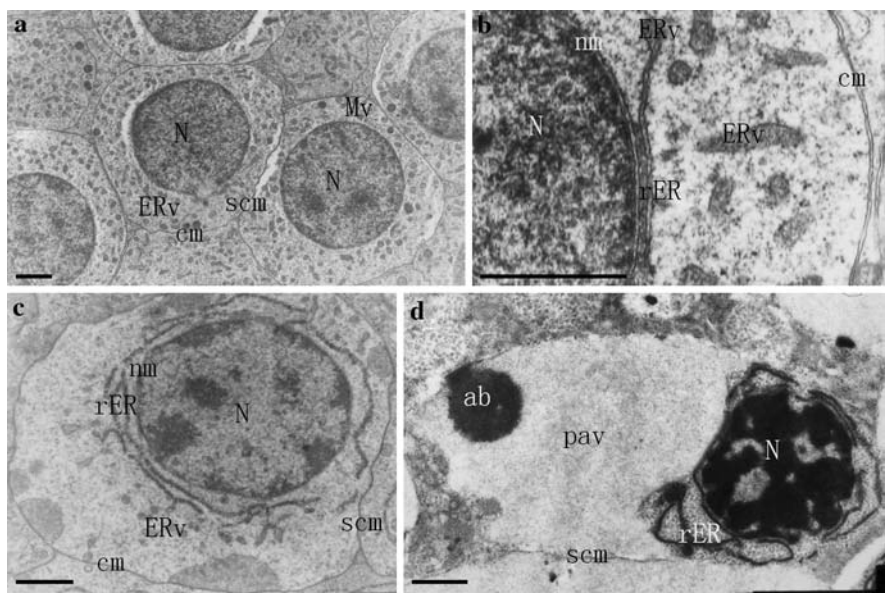

Fig. 3 TEM images (**a**)–(**d**) showing the morphologic transformation of the germ cell from the round spermatid to the prophase in spermiogenesis. The scale bar = 1μm and the letters are defined as: ab = acrosomal blastema; cm = cytomembrane; ERv = endocytoplasmic reticulum vesicles; Mv = mitochondria vesicles; N = nucleus; nm = nuclear membrane; pav = proacrosomal vacuole; rER = rough endocytoplasmic reticulum; and scm = sharing cytomembrane

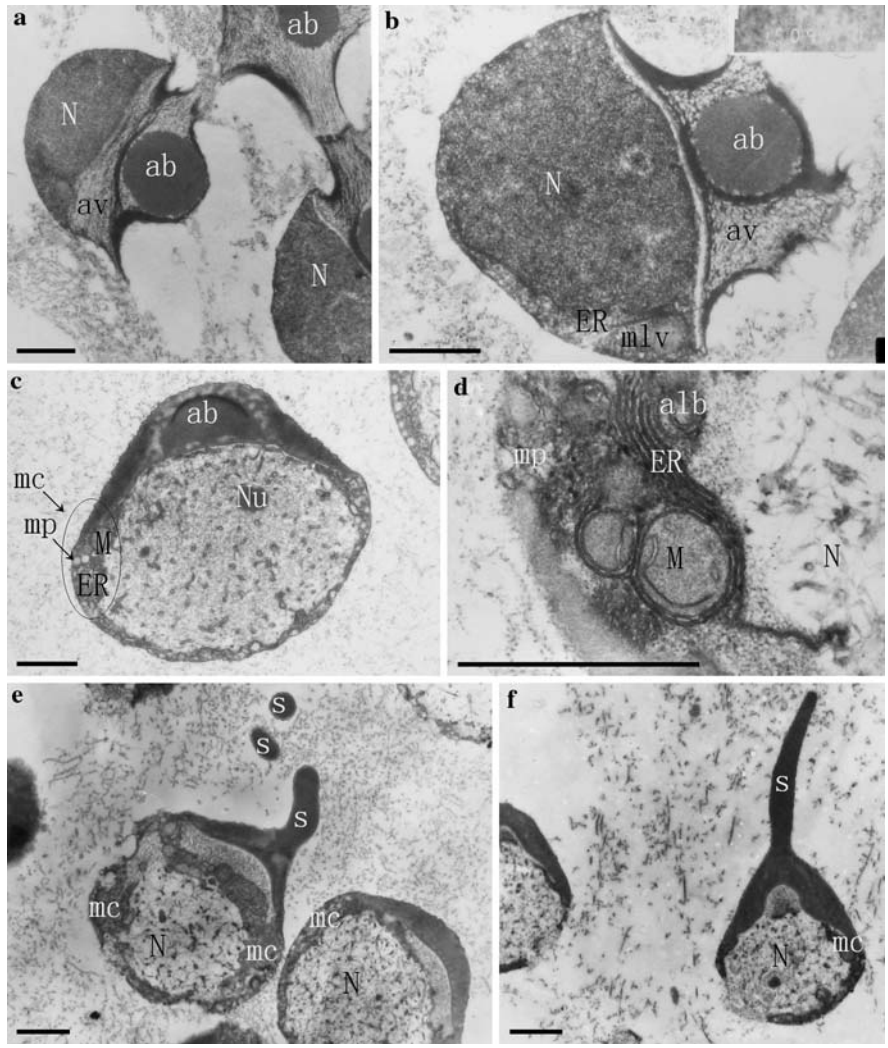

Fig. 4 TEM images of the membrane complex in spermiogenesis (**a**)–(**d**) exhibitting the forming process of the membrane complex derived from cytoplasm; (**e**) and (**f**) show that membrane complex still exists in mature sperm. The sperm of e come from deferens and f from testis. The scale bar = 1μm and the letters are defined as: ab = acrosomal blastema; alb = annular lamina body; av = acrosomal vacuole; ER = endocytoplasmic reticulum; M = mitochondria; mc = membrane complex; mlv = mitochondria-like vesicles; mp = rings of convoluted membrane punches; N = nucleus; Nu = nucleolus; and s = spike

With the formation of the typical spike on an apical body via further differentiation, mature sperms are created. The membrane complex at both sides of the nucleus under the acrosomal cap appears to be unchanged (Fig. 4e and f).

Discussion

There are various types of membrane structure within a cell, e.g. mitochondria membrane, endoplasmic reticulum membrane, Golgi's apparatus and nuclear membrane, etc. These biomembranes are associated with the vital processes involving energy conversion, signal recognition, information transfer and transport of substances, etc. From the investigation of the spermatogenesis of decapoda crustacean, it is noted that membrane complex involving such biomembranes occurs widely in their sperm.

Using TEM to study the ultra-fine structure of membrane complex in spermatogenesis of *F. chinensis*, we have found, for the first time, that a membrane complex exists in the spermatocytal nucleus and it is later shifted into cytoplasm. The membrane complex originating from the nucleus may have played an essential role in materials transportation from nucleus to cytoplasm. The other type of membrane complex originated from cytoplasm shapes at the anaphase and remains in mature sperm. Based on its morphological development, we propose that the latter membrane complex is consisted of endoplasmic reticulum, mitochondria, nuclear

membrane and nuclear materials, etc. This membrane complex may have a combined function of synthesis and secretion, equivalent to the endoplasmic reticulum, mitochondrium and the Golgi's apparatus.

Membrane complex originated from nucleus

Membrane complex originated from nucleus of primary spermatocyte is composed of annular structures, monolayer membrane blebs and double or multi-lamellar membrane vesicles (Fig. 1f). Many trachychromatic granules about 10 nm in diameter that appeared on the fibrous materials in the membrane complex are presumably the RNP and mRNP, etc, and the dense materials around the membrane complex are the condensed chromatin (Fig. 1a and f). The membrane complex penetrates the nuclear membrane and enters the cytoplasm by fusion (Fig. 2a and b).

Metabolism in primary spermatocyte is extremely active. In the nucleus, sufficient amount of materials produced via the transmutation of chromosome and transcription of RNA, etc. enter the cytoplasm as membrane complex, which may be regarded as an effective way of nuclear transport.

The annular structures of the membrane complex can be elaborated by the Golgi's apparatus, combined with endocytoplasmic reticulum and/or mitochondria vesicles (Fig. 2c and d), etc. forming the high contrast special vesicle clumps (Fig. 2e) in the spermatocyte. The vesicle clump is similar to the chromatoid body in the pachytene spermatocyte of rat (Celina et al. 2005), and is considered as a storage material at this stage.

The secondary spermatocyte is less active than the primary spermatocyte during metabolism. The number of membrane complex in the secondary spermatocyte, mainly consisted of annular structures, is gradually reduced (Fig. 2f).

Membrane complex from cytoplasm

Membrane complex originated from cytoplasm occurs during the spermiogenesis of *F. chinensis*, and it is comprised derivative of endoplasmic reticulum, mitochondria and nuclear membrane which is similar to that reported in most species (Langreth 1969; Reger 1970; McKnight and Hinsch 1986; Du et al. 1988; Medina 1994; Li 1995; Tudge et al. 2001; Zhu et al. 2002; Medina et al. 2006). The only difference lies in the typical annular lamina bodies wound from the endocytoplasmic reticulum in the membrane complex of *F. chinensis*. In addition, it can be seen that the nuclear particles flow into the membrane complex. There is no Golgi's apparatus in the membrane complex of *F. chinensis*, and the membrane complexes of *Macrobrachium nipponense* and *Sinopotamon yangtsekiense* dominantly consist of Golgi's apparatus and its derivatives (Yang et al. 1998; Wang et al. 1999). Nuclear membrane consists of only a small component in the present membrane complex, whereas being the dominant phase in the membrane complexes of *Procambarus clarkii* and *Sicyoniain gensis* (Moses 1961; Shigekawa and Clark 1986).

The relationship between membrane complex and endoplasmic reticulum

Membrane complex of *F. chinensis* is mainly composed of rings of convoluted membrane pounches and annular lamina bodies that derived from the rough endocytoplasmic reticulum vesicles during the spermiogenesis, and with the fusion of the vesicles, the ribosome structures are disappeared (Fig. 4c and d), which is identical to that observed by Langreth in crab *C. borealis* (Langreth 1969).

The relationship between membrane complex and mitochondria

During the prophase of spermiogenesis, less circular vesicle-like mitochondria were observed in the cytoplasm (Fig. 3a). In the early stage of the metaphase, the vesicle-like mitochondria disappeared (Fig. 4a), however they emerged as some mitochondria-like vesicles in the cytoplasm belt at the late stage of the metaphase (Fig. 4b). At the anaphase of spermiogenesis, typical mitochondria with rich cristae and double-membrane formed in the membrane complex (Fig. 4c and d). It is apparent that the mitochondria morphology in spermiogenesis is rather diverse. And only the typical mitochondria with rich cristae (Frederick 2002) in the anaphase are a supplier for

the energy metabolizing process, providing ATP for the membrane complex during both material synthesis and material processing.

The relationship between membrane complex and nuclear membrane

At the late stage of metaphase of spermiogenesis, the formation of the nuclear membrane partly occurred (Fig. 4b), whilst in anaphase, the nuclear membrane was disassembled which was subsequently involved in the formation of the membrane complex along with the flocculation of the chromatin. Meanwhile, particulate materials entered into the membrane complex and played a part in this process (Fig. 4c and d).

In Pochon-Masson's early study of *C. maenas*, the author proposed that the membrane complex was derived from nuclear envelope as nuclear pores could often be observed around the membrane complex. The author considered that such nuclear pores were essential for materials transportation during the formation of the membrane complex (Pochon-Masson 1962, 1968). The nuclear pores were not found in the area of membrane complex in this study, however a large number of nuclear particles were indeed observed within the membrane complex. We suggest that the transportation of the nuclear particles at the anaphase of spermiogenesis could result in the noncondensed nucleus in a mature sperm.

Cytochemical changes and biological functions of the membrane complex

At the metaphase stage of spermiogenesis, cytomembrane, nuclear membrane and organelles fused (Fig. 4a and b). The spermatid exchanged materials with the external cellular microenvironment in order to promote the fusion, rearrangement and compositional adjustment, which constitutes the foundation of spermatid palingenesis. During this period, the fragments of the rough endoplasmic reticulum with abundant ribosomes were preserved, implying the active metabolism process regardless the absence of the typical organelles.

Membrane structures such as nuclear membrane and mitochondria etc. emerged again at the late stage of the metaphase of spermiogenesis (Fig. 4b), which imply that the elements forming the membranous structures are still preserved in the phase of fused membrane system. At the right time, membrane structures would emerge again and some of which would form the membrane complex.

Rich ribosome annular lamina bodies within the membrane complex were formed in the spermiogenesis period and a lot of particle materials spread among the membranes. Further, it can be seen that vesicles and granules are discharged from the membrane complex to both sides of the acrosomal areas (Fig. 4c). At this stage, the membrane complex participates were not observed directly in the acrosome area, however the secretion of membrane complex participated throughout the acrosomal formation, implying a combined function of the endoplasmic reticulum, mitochondria and Golgi's apparatus for the membrane complex. This analysis agrees with Kaye's study on crayfish *C. japonicus* in which the authors have proposed the membrane complex or lamellar structure as a special Golgi's apparatus (Kaye et al. 1961).

During spermiogenesis, nuclear chromatin was condensed first (Fig. 3d), then disaggregated to form fine particles (Fig. 4a and b) and became floccules subsequently (Fig. 4c–f). During these processes, materials transfer associated with the re-erection of chromosome occurred. We proposed that one kind of these materials from nucleus to cytoplasm is basic protein, which is similar to Vaughn's viewpoint based on the study of crab *E. analogs* (Vaughn et al. 1969) that basic protein existed in the nucleus of spermiogonium and spermatocyte, but in the nucleus of mature sperm, the basic proteins such as histone or protamine disappeared.

To sum up, we have reported the transformation characteristics of the two types of membrane complex in spermatogenesis of *F. chinensis*, based on TEM investigation. We have found that the one originated from nucleus, which consists of annular structure, monolayer membrane blebs, double or multilamellar membrane vesicles, exists mainly inside the spermatocyte and can migrate from nucleus to cytoplasm; and the other originated from cytoplasm comprises rings of convoluted membrane pouches, mitochondria, annular lamina bodies, fragments of endoplasmic reticulum, nuclear membrane and some nuclear particles. This type of membrane

complex locating at both sides of the nucleus under the acrosomal cap is formed during the anaphase of spermiogenesis and still exists in mature sperm. It releases many vesicles and particles into the acrosomal area during the formation of the perforatorium. We propose that the former membrane complex plays an important role in substance transfusion from nucleus into cytoplasm during the spermatogenesis and the latter type provides a combined function of synthesis and secretion, equivalent to the endoplasmic reticulum, mitochondria and Golgi's mechanism during the formation of the perforatorium.

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