THE APICAL STRUCTURE IN *PEROPHORA ANNECTENS* (TUNICATE) SPERMATOZOA: FINE STRUCTURE, DIFFERENTIATION AND POSSIBLE ROLE IN FERTILIZATION*

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SUMMARY

The apical structure in *Perophora annectens* spermatozoa is approximately 4 μm in length and it is helically coiled. Its major component is a striated structure, which may be analogous to a perforatorium. The plasmalemma enclosing the anterior quarter of the apical structure is covered by extracellular materials, the anterior ornaments.

During spermiogenesis, the apical structure is first recognized as a small blister of the plasmalemma at the apex of the young spermatid. It develops into a conical protrusion and then into a finger-like process (approximately 1 μm in length). This process is transformed into an elongated process (approximately 4 μm in length) with electron-dense material in its core. Finally, the elongated process is helically coiled to form an apical structure in which electron-dense material forms dense striations.

Vesicles (50–70 nm in diameter), presumably derived from the Golgi apparatus, have been recognized in the blisters of younger spermatids, and can be followed through to the finger-like process. In the finger-like process these vesicles are transformed into smaller vesicles (20–30 nm in diameter), which probably fuse with the anterior plasmalemma of the finger-like process. This suggests that chorion lysin(s) is associated with the anterior membrane enclosing the apical structure in these spermatozoa.

INTRODUCTION

In spite of a relatively large number of descriptive and experimental studies on ascidian development, little is known about the events associated with fertilization (Berrill, 1975). The ascidian egg is enclosed in a non-cellular chorion, which is, in turn, covered by a layer of vacuolated follicle cells. Moreover, a layer of test cells surrounds the egg surface. The follicle cells are thought to be involved in sperm attraction (Miller, 1975), and/or egg flotation (Lambert & Lambert, 1978). The chorion, which appears as a thick network of interwoven microfibrils (DeSantis, Jamunno & Rosati, 1980), presents a barrier to the successful penetration of the spermatozoa. Such an elaborate set of egg envelopes leads to the supposition that ascidian spermatozoa must utilize lysins in order to make contact with the egg.

In a previous paper (Fukumoto, 1981), I described an apical structure at the tip of *Perophora formosana* spermatozoa. The present paper describes the fine structure and

differentiation of the apical structure in *P. annectens* spermatozoa. Some speculations are also presented on the role of the apical structure in fertilization.

MATERIALS AND METHODS

The compound ascidian, *Perophora annectens* (Phlebobranchia, Enterogona) was collected during the summer of 1981 at San Juan Island, Washington, U.S.A.

The specimens for both whole-mount and thin-section electron microscopy were prepared according to the method previously reported (Fukumoto, 1981). Electron micrographs were taken with a Philips EM300 or a Hitachi H-300 electron microscope, operated at 60 kV and 75 kV, respectively.

OBSERVATIONS

The spermatozoon of *P. annectens* is approximately 70 μm long. It has an elongated head and a tail with a tapered end (Fig. 1A). It lacks a midpiece. The head is extremely elongated and approximately 30 μm in length. At the apex of the head there is a corkscrew-shaped structure about 4 μm in length, which has been designated as the apical structure (Fig. 1B).

Fine structure of the apical structure

An appropriate longitudinal section through the apex of the spermatozoon reveals that the apical structure is composed of electron-dense material, which appears to have aggregated periodically to form a striated structure (*ss*) and a helical string (*hs*) that runs longitudinally in the apical structure along its helical ridge (Fig. 2A, B, C). In an appropriate transverse section through the apical structure the striated structure appears as a round mass (Fig. 3A, B). Each band in the striated structure is approximately 7 nm thick and is arranged perpendicular to the long axis of the apical structure. The striated structure lacks a limiting membrane (Fig. 4). Extracellular materials, which are termed the anterior ornaments (*ao*), coat the anterior quarter

Fig. 1. A. Whole mount of a fully differentiated spermatozoon from the sperm duct. At the apex of the head an apical structure can be observed (arrow). B. Enlargement of the apical structure, which is helically coiled. *h*, head; *t*, tail. A, ×1500; B, ×37 500.

Fig. 2. Longitudinal section through the apical structure. An anterior ornament coats the apex of the plasmalemma. A, B and C correspond to the posterior, the middle and the anterior region of the apical structure, respectively. *ao*, anterior ornament; *fl*, fluff; *hs*, helical string; *n*, nucleus; *ss*, striated structure. ×90 000.

Fig. 3. Transverse section through the apical structure. The striated structure appears as a round mass. A and B correspond to the posterior and the anterior regions of the apical structure, respectively. *ao*, anterior ornament; *hs*, helical string; *ss*, striated structure. ×90 000.

Fig. 4. Enlargement of a part of the apical structure. Note the absence of a limiting membrane around the striated structure. The striations are obvious (arrows). ×250 000.

Fig. 5. Transverse section through the anterior region of the head. Filamentous structures can be observed as solid dots exclusively in the matrix of the mitochondria. *m*, mitochondrion; *n*, nucleus. ×60 000.
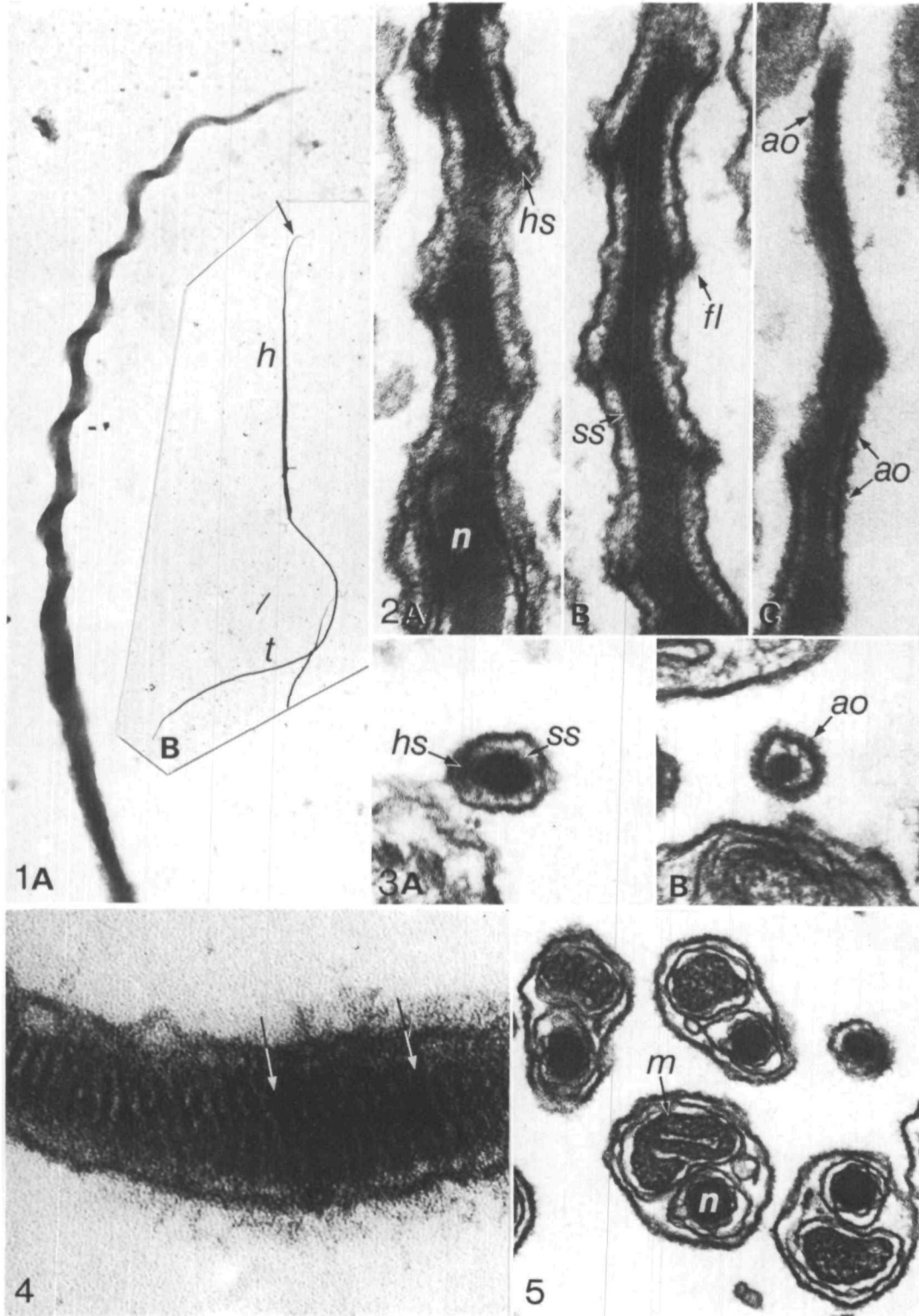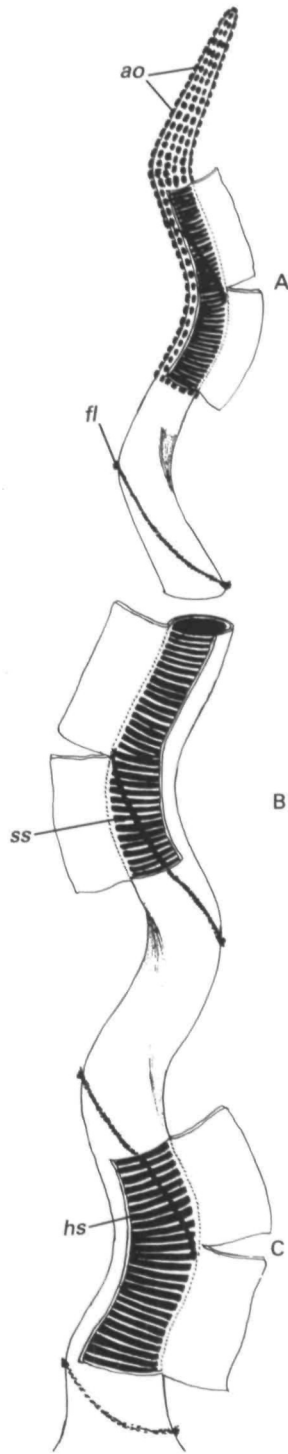
Figs 1–5

Fig. 6

(approximately 1 μm in length) of the plasmalemma enclosing the apical structure (Fig. 2c). In a cross-section through the head just beneath the apical structure the mitochondrion is applied laterally to the nucleus. Filamentous structures that appear as solid dots are found exclusively in the mitochondrial matrix (Fig. 5) as found previously in *P. formosana* (Fukumoto, 1981). A schematic illustration of the apical structure in *P. annectens* is shown in Fig. 6.

Differentiation of the apical structure

The testis of *P. annectens* consists of several lobes in which germ cells develop synchronously, forming many clusters. During the course of spermiogenesis, the chromatin strands in the spermatid nucleus coil up in a definite pattern, becoming denser as the nucleus elongates. The course of spermiogenesis can be divided morphologically into five stages, from stage 1 (early spermatid) to stage 5 (mature spermatozoon).

As shown in Fig. 7, the spermatids at stage 1 have a spherical nucleus, a single motochondrion and a well-developed Golgi apparatus. At the apex of the spermatid, the plasmalemma is thicker (Fig. 7, arrow). Many Golgi vesicles of various sizes, which contain moderately electron-dense material, are observed in the vicinity of the Golgi apparatus (Fig. 8). In an appropriate longitudinal section through the apex of the spermatids at stage 1, the plasmalemma expands outward to form a small blister in which vesicles are observed just inside the plasmalemma (Fig. 9A, B, c). They are 50–70 nm in diameter and filled with moderately electron-dense material. The plasmalemma of the blister thickens slightly (about 10 nm in thickness) and is covered by a fuzzy extracellular material. An electron-dense material, the 'dense plate' (Cotelli, DeSantis & Monroy, 1980) is found adjacent to the anterior end of the nucleus where the inner and outer nuclear membranes approach each other (Fig. 9B, c).

In the stage 2 spermatid, which contains a slightly elongated nucleus and a round mitochondrion, the blister observed at the apex in younger spermatids develops further into a conical protrusion (Fig. 10A). Vesicles can also be recognized in the protrusion (Fig. 10B). Fuzzy extracellular material is observed covering the outside of the plasmalemma of the protrusion.

Fig. 6. Schematic illustration of the apical structure in *P. annectens*. The plasmalemma enclosing the anterior quarter of the apical structure is decorated by the anterior ornaments (*ao*). A helical string (*hs*) can be observed running inside along the ridge of the helix. Fluff (*fl*) is present on the plasmalemma just outside the region corresponding to the helical string. A, B and C are views inside the anterior, middle and posterior regions, respectively. *ao*, anterior ornament; *fl*, fluff; *hs*, helical string; *ss*, striated structure.

Fig. 7. Stage 1 spermatids. Note the spherical nucleus, a round mitochondrion and a well-developed Golgi apparatus. A small blister can be observed (arrow) at the apex. *g*, Golgi apparatus; *m*, mitochondrion; *n*, nucleus. ×14 000.

Fig. 8. Enlargement of the Golgi apparatus in a stage 1 spermatid. Note the Golgi vesicles of various sizes. *gv*, Golgi vesicle; *n*, nucleus. ×60 000.

Fig. 9. Longitudinal section through three spermatids showing the probable sequence of blister development (A, B, c) at stage 1. Vesicles can be observed in the blister (thin arrows). One of them is in close contact with the plasmalemma (thick arrow). *dp*, dense plate; *fu*, fuzzy material; *n*, nucleus. ×90 000.
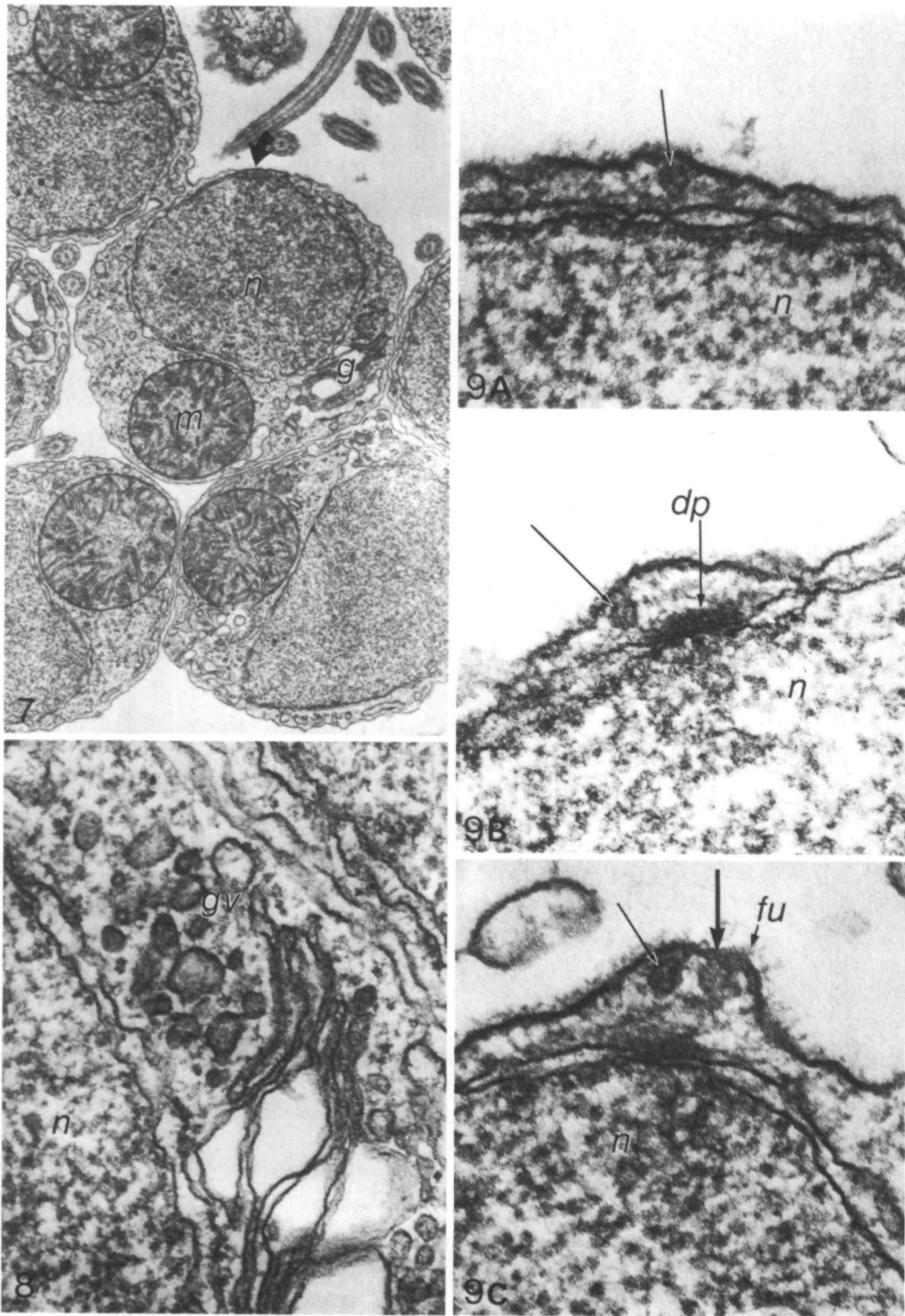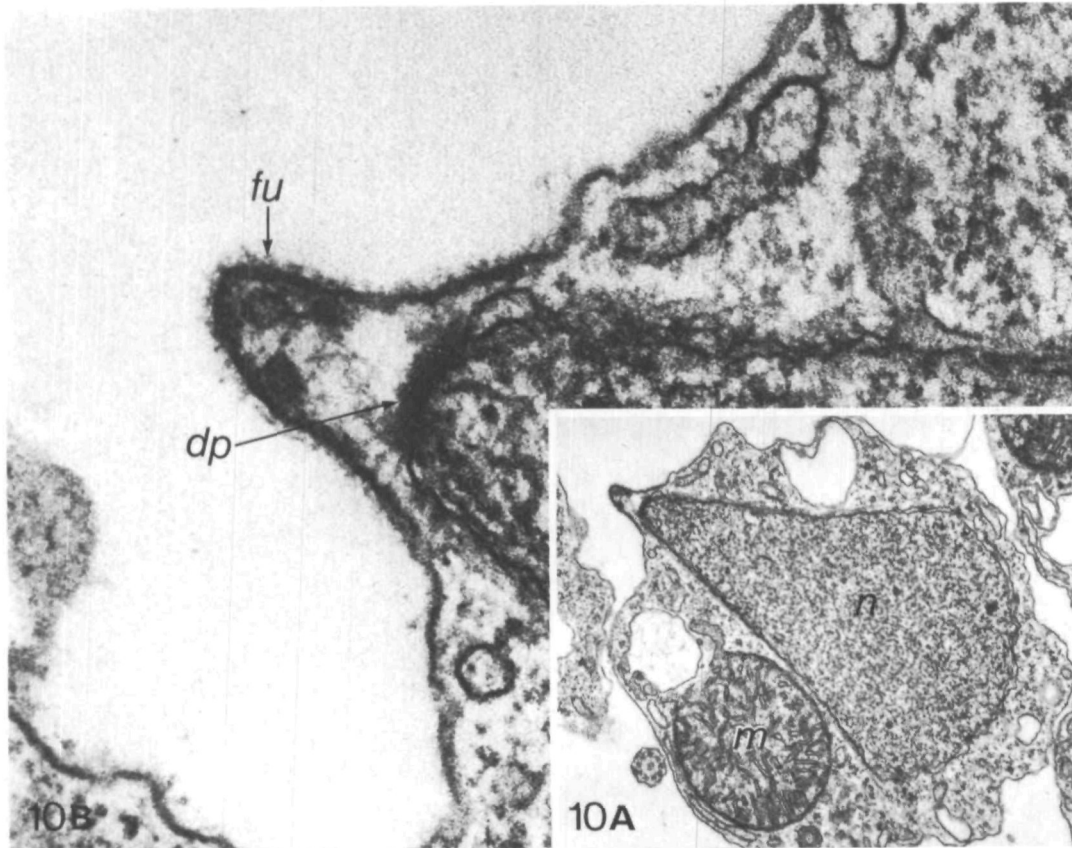
Fig. 10. longitudinal section through a stage 2 spermatid. Note the conical protrusion at the apex. Vesicles containing moderately electron-dense material can be observed. *dp*, dense plate; *fu*, fuzzy material; *m*, mitochondrion; *n*, nucleus. A, ×14000; B, ×90000.

In the stage 3 spermatid the nucleus is elongating, and the chromatin strands are arranged parallel to one another along the longitudinal axis of the nucleus (Fig. 11A). The mitochondrion is spherical. A well-developed Golgi apparatus is observed in the cytoplasm. At the apex the conical protrusion has developed into a finger-like process (approx. 1 μm in length), in which vesicles can be seen (Fig. 11B, C). The plasmalemma of the process is decorated on its external surface by a fuzzy extracellular material, which displays some regularity in structure, with a repeating period of approximately 25 nm.

It is of particular interest that the vesicles in the process appear to transform into smaller vesicles approximately 20–30 nm in diameter (Figs 12, 13A, B, C), some of which make close contact with the anterior plasmalemma (Fig. 13A, B, C, D, E, F, arrows).

In the stage 4 spermatid, the chromatin strands in the nucleus are almost fully coiled up to form a dense elongated nucleus, along which the mitochondrion elongates (Fig. 14A). The finger-like process at stage 3 extends to form an elongated process (approx. 4 μm in length) with an electron-dense core (Fig. 14B). The anterior quarter

M. Fukumoto
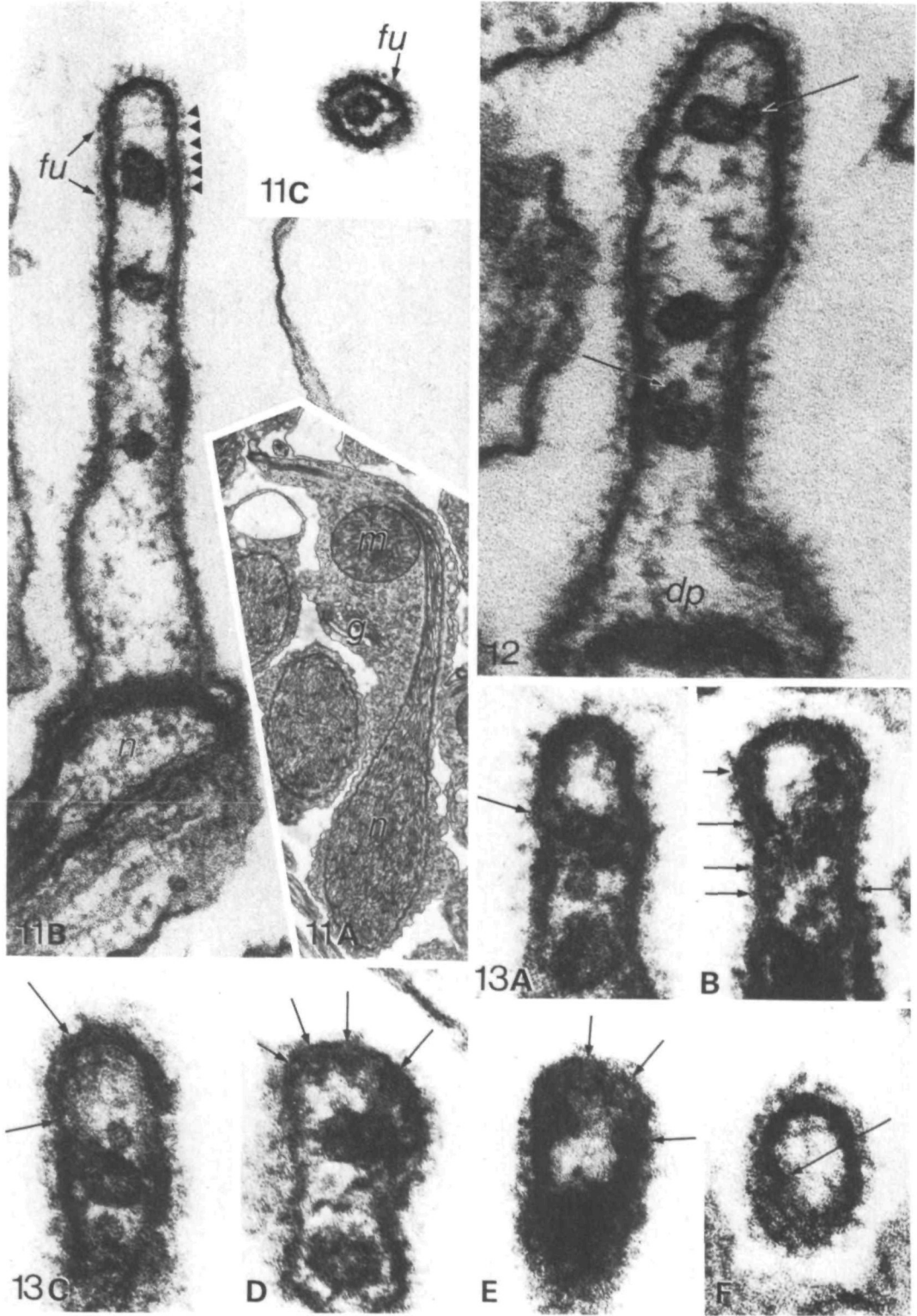
Figs 11–13

(approx. 1 μm long) of the elongated process, which corresponds to the finger-like process at stage 3, is decorated outside with the anterior ornaments (Figs 15, 16). The vesicles that were observed in the finger-like process at stage 3 have disappeared in the elongated process (Fig. 16A). At the apex of the nucleus, the dense plate can be recognized (Fig. 16c). In the cytoplasm, free ribosomes and polysomes are prominent (Fig. 16B). The differentiation of the apical structure in *P. annectens* is schematically illustrated and summarized in Fig. 17.

DISCUSSION

Except for the presence of the apical structure, the spermatozoon of *P. annectens* has the same structural features characteristic of the ascidian spermatozoa that have been described (Franzen, 1976; Fukumoto, 1979; Cloney & Abbott, 1980). The *P. annectens* spermatozoon has a specialized structure designated as an apical structure at its anterior tip, as has been described in *P. formosana* spermatozoa (Fukumoto, 1981). Although the apical structure in *P. formosana* is a finger-like process, approximately 2 μm long, that of *P. annectens* is longer and has a more specialized structure. It is approximately 4 μm in length and is helically coiled to form a corkscrew-shaped structure with a repeating distance of approximately 0·3 μm (Fig. 1B). The plasmalemma enclosing the anterior quarter of the apical structure is covered with anterior ornaments, which are arranged in fairly regular order (Figs 2c, 16A).

Fig. 11. A. Longitudinal section through a stage 3 spermatid. B. Longitudinal section through a finger-like process in which vesicles are observed. Note regular arrangement of fuzzy extracellular materials (arrowheads). C. Transverse section through the process in which vesicle is observed. *fu*, fuzzy material; *g*, Golgi apparatus; *m*, mitochondrion; *n*, nucleus. A, ×10000; B, c, ×90000.

Fig. 12. Longitudinal section through a finger-like process. Note the vesicles being transformed into smaller vesicles (arrows). *dp*, dense plate. ×150000.

Fig. 13. A–E. Longitudinal sections through the anterior region of a finger-like process. The vesicles appear to be transformed into smaller vesicles, some of which are in close contact with the plasmalemma (arrows). F. Transverse section through the anterior region of a finger-like process. A small vesicle presumably fusing with the plasmalemma (arrow). ×150000.

Fig. 14. A. Longitudinal section through the anterior region of a stage 4 spermatid. Note the almost fully condensed nucleus and the elongating mitochondrion. B. Longitudinal section through an elongated process in which electron-dense material has been deposited. *edm*, electron-dense material; *m*, mitochondrion; *n*, nucleus. A, ×15000; B, ×40000.

Fig. 15. Longitudinal section through an elongated process that is beginning to take on a helical configuration. The plasmalemma of the anterior quarter is covered by the anterior ornaments which correspond to the fuzzy material in younger stages. *ao*, anterior ornament; *edm*, electron-dense material. ×40000.

Fig. 16. A. Longitudinal section through the anterior region of the elongated process in which electron-dense material has accumulated. Note absence of the vesicles in the process. B. Section showing cytoplasm of a stage 4 spermatid. Note the ribosomes and polysomes (arrows). C. Longitudinal section through the posterior region of the apical structure. The dense plate (*dp*) is present. *ao*, anterior ornament; *edm*, electron-dense material; *n*, nucleus. A, c, ×90000; B, ×60000.
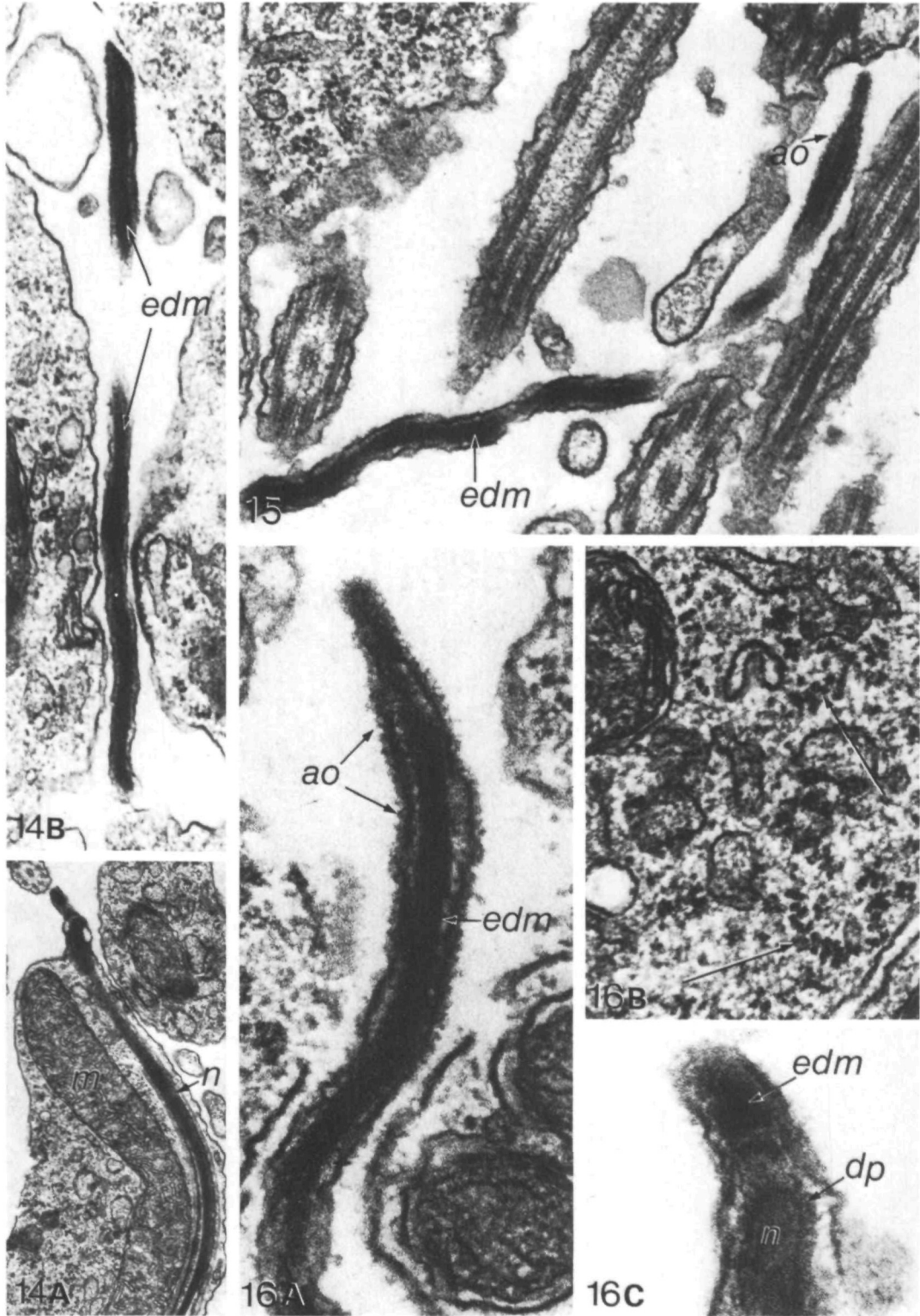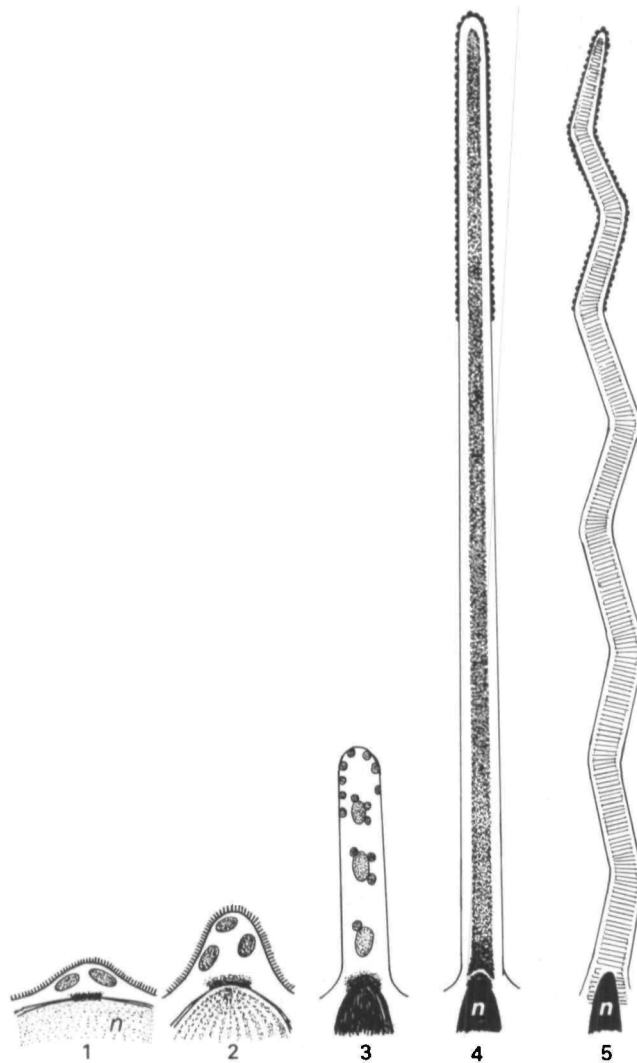
Figs 14–16. For legend see p. 183.

Fig. 17. Schematic illustration showing the differentiation of the apical structure in *P. annectens*. In younger spermatids, the plasmalemma at the apex expands to form a small blister (stage 1), which is covered with fuzzy extracellular material. The blister develops further through a conical protrusion (stage 2) into a finger-like process (stage 3), which is also decorated by a fuzzy extracellular material on its whole surface. This process increases in length to become an elongated process with electron-dense material in its core (stage 4). Finally, the elongated process is helically coiled to form a structure (apical structure) in which electron-dense material becomes aggregated periodically to form a striated structure (stage 5). The anterior quarter (approx. 1 μm in length) of both the elongated process (stage 4) and the apical structure (stage 5) corresponds to the finger-like process at stage 3. Vesicles (presumably pro-acrosomal vesicles) have been recognized in younger stages from stages 1–3. In the finger-like process at stage 3, these vesicles appear to transform into smaller vesicles, which ultimately fuse with the anterior plasmalemma of the finger-like process.

Apparently these ornaments have differentiated from fuzzy extracellular materials that are observed covering the outer surface of the small blister (stage 1), the conical protrusion (stage 2) and the finger-like process (stage 3) in early spermatids.

The major component in the apical structure is a striated structure, with striations approximately 7 nm thick that stack perpendicular to the long axis of the apical structure (Fig. 4). It has been reported that striated structures are present in the acrosomes of some other animals (Furieri, 1970; Longo & Anderson, 1970; Friend & Fawcett, 1974; Reger, Itaya & Fitzgerald, 1979); these structures may reflect a crystalline alignment of enzymes responsible for the dissolution of the egg envelopes at fertilization (Stambaugh & Buckley, 1969; Friend & Fawcett, 1974; Reger, Itaya & Fitzgerald, 1979). However, the striated structure described here lacks the limiting membrane (acrosomal membrane) that is characteristic of all the acrosomes previously reported in a variety of animal species. Because of its localization in the apex of the spermatozoon, the striated structure in *P. annectens* may be analogous to a perforatorium, previously reported in some groups of animals (Baccetti, Bigliardi & Burrini, 1980) rather than to an acrosome.

No precursors of the striated structure have been detected in earlier stages (stages 1, 2 and 3). The presence of many free ribosomes and polysomes at stage 4 (Fig. 16B) suggests the possibility that an electron-opaque material is synthesized in the cytoplasm and deposited to form the striated structure in the elongated-process at stage 4.

Vesicles have been recognized in the blister of younger spermatids (stage 1), which can be detected in the finger-like process in more advanced spermatids (stage 3). They are 50–70 nm in diameter and filled with moderately electron-dense material. These vesicles presumably originate from the Golgi apparatus and may correspond to the vesicles referred to as the putative acrosome in *Ascidia callosa* (Cloney & Abbott, 1980), as acrosomal vesicles in *Ciona intestinalis* (Cotelli, DeSantis & Monroy, 1980), and as vesicles in *Perophora formosana* (Fukumoto, 1981). A finding of particular interest is that the vesicles in the present material appear to become transformed into smaller vesicles, some of which make contact with the plasmalemma in the anterior region of the finger-like process at stage 3. This suggests that the smaller vesicles might fuse with the plasmalemma enclosing the anterior region of the apical structure.

In ascidians, the egg is enclosed by a non-cellular chorion, which presents a barrier to the successful penetration of the spermatozoa. The ascidian spermatozoa might utilize chorion lysins in order to make a hole in the chorion. Indeed, it has been suggested that in the solitary ascidians, *C. intestinalis* and *Halocynthia roretzi*, spermatozoa utilize proteases as lysins for digesting the chorion (Woollacott, 1977; Hoshi, Numakunai & Sawada, 1980).

In the present material, the membrane at the apex of the spermatozoa, which covers the anterior region of the apical structure, might have became highly specialized for fertilization: the contents of the smaller vesicles, which might be acrosome-related materials such as lysins, are incorporated into the anterior plasmalemma enclosing the apical structure in *P. annectens* spermatozoa prior to the completion of spermiogenesis.

The fragmentation of a vesicle into smaller ones is an unusual and interesting phenomenon that has not been previously described.

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