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FINE STRUCTURAL OBSERVATIONS OF THE APICAL ORGAN IN THE LARVA OF POLYORDIUS (ANNELIDA: POLYCHAETA)

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Abstract

The larva of the archiannelid Polygordius was studied using light microscopy (LM), scanning (SEM) and transmission electron microscopy (TEM). The embryo of Polygordius develops into a large trochophore larva adapted to planktonic swimming and feeding. The young larva is characterized by the presence of an approximately hemispherical episphere including a basal prototroch and a well-developed apical organ which lacks a central ciliary tuft. The conical hypo­sphere is smaller and contains the metatrocho­phore segments. The apical organ of the epi­sphere consists of a large, bulbous mass of cells that project deeply into the spacious blastocoel. Two tentacular knobs and two ciliary aggregations are located on the surface of the apical organ. The entire surface of the episphere is provided with scattered cilia. Two pigmented eyespots or ocelli are embedded in the uppermost region of the apical organ. A second presumed photoreceptor organ, the phaosome, is situated centrally between the two ocelli. The apical organ is covered by a thick cuticle penetrated by numerous microvilli. Ectodermal cells peripheral to the apical organ contain numerous vacuoles of flocculent, lightly staining material. Mucus cells are also present in this region of the episphere. An apical intraepidermal nerve plexus is located in the basal most region of the apical organ. Axons occur in the apical and basal regions of the apical organ. Morphological evidence supports the suggestion that the apical organ functions in chemoreception and/ or mechanoreception related to substrate selection, metamorphosis and other planktonic behaviors.

KEY WORDS: apical organ, fine structure, Polygordius, larva

Introduction

The family Polygordiidae has previously been classified with other interstitial annelids; Saccocirridae, Protodrilidae, Nerillidae, Dinophilidae, and Diuodrilidae forming the heterogeneous taxon Archiannelida. The question of whether the archiannelids are primitive or secondarily simplified has been much discussed. Hermans (1969) suggested that the archiannelids should form an order within class Polychaeta. Jouin (1971) concluded that the five archiannelid families have few affinities with one another, or with any definitive polychaete family. Westheide (1985) recently proposed that the Polygordiidae should be raised in rank and considered a separate order, Poligordiida, within Polychaeta.

Characteristic external features of Polygordius include the presence of two short prostomial tentacles and the absence of parapodia and setae. Segmentation is internal, and the elongated body is covered by a cuticle giving the animal a distinct nematode-like appearance. Some Polygordius species reach a length of about 80 mm and a diameter of about 1 mm.

The primitive type of spermatozoon in Polygordius indicates that fertilization is external (Fränzen 1956, 1977). The fertilized egg develops into a large, planktotrophic trochophore. The trochophore is known to have a rather long planktotrophic existence of several weeks to a month.

The unusual metamorphosis of the Polygordius larva was studied earlier by Woltereck (1902, 1905) and more recently by Jägersten (1972). Large parts of the larval body are cast off at metamorphosis. The metatrochophore segments in the hyposphere elongate and come in direct contact with the apical organ. The apical organ forms the prostomium and brain in the juvenile worm. As in numerous other marine invertebrate larval forms, the apical organ in

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the larva of Polygordius is suggested to function as a sensory organ and it is believed to be important in activities related to locomotion, feeding, settlement site selection and metamorphosis.

Previous ultrastructural studies of the Polygordius larva are few. Brandenburger and Eakin (1981) studied the ocelli of Polygordius of appendiculatus. They found the photoreceptor organelles to be arrays of microvilli, and thus similar to photoreceptors found in several other annelids (Eakin et al. 1977). Fransen (1980) studied the ultrastructural organization of the coelum in a metamorphosed planktonic Polygordius larva.

In this paper, the morphological and ultrastructural features of the apical organ in the larva of Polygordius are examined and described. These observations are compared with the findings of similar ultrastructural studies on the apical organ of several other marine invertebrate larvae. The paper provides additional confirmation of the suggested sensory nature of the apical organ and the role it plays during planktonic life, settlement and metamorphosis.

Materials and Methods

The archiannelid Polygordius appendiculatus is known to occur at 18 meters in shell-sand sediments (amphioxus-sand) in the Gullmar fjord region. It is most probable that the larval material reported on in this paper is P. appendiculatus, but since accurate identification to the species level is uncertain for these planktonic larval forms, we designate the material hereafter as Polygordius. The Polygordius larvae were obtained from surface waters using a hand-held plankton net. They were collected during August and September from the pier of Klubbans Biological Station, on the Gullmar fjord, Swedish west coast.

Larvae were fixed for transmission electron microscopy (TEM), scanning electron microscopy (SEM) and light microscopy (LM) in cold, (5°C) 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4). Following glutaraldehyde fixation the larvae were rinsed in cold, 0.1M sodium cacodylate buffer and postfixed for one hour in 1.0% osmium tetroxide using the same buffer. Material for TEM and LM was block stained with uranyl acetate in 70% ethanol. After dehydration with ethanol the material was processed through propylene oxide into Epon. Sections at a thickness of 1-5 µm were cut with a glass knife for LM. TEM sections were stained with uranyl acetate for 20 minutes and lead citrate for two minutes. Micrographs were obtained using a JEOL JEM-100B transmission electron microscope operated at 60 kV. LM sections were stained with methylene blue and photographed with Kodak Panatomic-X film using a Leitz compound phase-contrast microscope.

Material for SEM was fixed as described for TEM. After fixation larvae were dehydrated through a graded series of ethanol, transferred to Freon TF and critical point dried from instrument quality carbon dioxide. The larvae were mounted on double-stick tape and Au/Pd coated with a JEOL JFC-1100 ion sputter unit. The material was examined and photographed with Kodak Plus-X pan professional film using a JEOL JSM-35 scanning electron microscope at 8-15 kV.

Results

General Larval Morphology

The general external features of the morphology of the Polygordius larva are depicted in Fig 1. The episphere is approximately hemispherical. The hyposphere is smaller and conical. The most prominent external structures are the apical organ and the prototroch. The entire epidermis is covered by a thin hyaline cuticle which is penetrated by scattered cilia in the episphere. The living larva is transparent and ciliary movements in the gut and blastocoel are easily observed.

Larvae are approximately 650-750 µm in length and usually about 550-650 µm in width. The prototroch cilia are relatively short. As seen from above (Fig 2), the apical organ has an oval shape and measures 150 µm by 120 µm. There is a distinct border between the apical organ and the regular epidermis of the episphere. The apical organ is often observed to protrude above the surrounding epidermal surface (Figs 1 and 3). Two tentacular knobs are situated medially, with a spacing of about 30 µm. They will become the tentacles of the adult. Two distinct aggregations of cilia and one smaller patch are located at the margin of the apical organ (Fig 2). Other cilia are sparsely scattered over the surface of the apical organ. There is no prominent apical tuft.

During early developmental stages the blastocoel contains few cells. With further development the young larva becomes a typical trochophore with mouth, gut, anus, and prototroch (Fig 4). In older larval segmentation appears at the posterior pole, near the anus. The segments of the metatrochophore are folded within the non-segmented areas of the larva (Fig 4). During development of the metatrochophore mesodermally derived cells come in contact with the basal and lateral portions of the apical organ. At the 18 segment stage of development the blastocoel is nearly filled with the developing juvenile structures.

Apical Organ

The apical organ is a large, bulbous structure positioned in the uppermost region of the episphere. Internally the apical organ protrudes deeply into the blastocoel (Figs 4 and 5). In Polygordius it consists of at least five distinct cell types or structures; ciliary aggrega-

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Fig 3: SEM of an early metamorphosed larva; the metatrochophore with apical organ (ao), episphere (e), prototroch (p), hyposphere (h) and metatrochophore segments (ms). Bar = 200 µm.

Fig 4: Schematic diagram of Polygordius larva and apical organ. Internal structures are visible through the transparent epidermis in living larvae. Shows phaosome (ph), ocelli (o), ciliary aggregations (ca), nerve plexus (np) of the apical organ (ao), and blastocoel (b), prototroch (p), metatrochophore segments (ms), mouth (mo), gut (gu), and anus (a) of the larva.

Fig 5: LM photomicrograph of apical organ (ao) with epidermis (ep), capsular cells (cap), ocellus (o), and apical nerve plexus (np). Bar = 30 µm.
tions, larval eyes (ocelli), a phaosome photoreceptor, capsular cells and apical nerve plexus.

Each of the two aggregations of cilia has a length of 20-25 µm and a width of 7-10 µm (Fig 2). Cilia which are believed to be sensory emerge from three to four rows of narrow cells. The cilia are rather short (8-8 µm). An accurate description of the ciliary microtubular arrangement of the cilia was not possible with the serial sections available. The majority of ciliary aggregation cells are multiciliated epidermal cells joined apically by zonulae adherentes and septate junctions. Their nuclei are situated basally. The cytoplasm of each cell contains several mitochondria and a basal Golgi complex. There are numerous microvilli and cytoplasmic folds between the cilia of these cells (Fig 6). Cells surrounding the multiciliated cells lack cilia but each has a long (2 µm) microvillous border and a distinct glycoconalix. Microvilli penetrate the thick cuticle and often have distal swellings. The cells are rich in secretory granules and Golgi complexes. They have a rather irregular columnar shape and the large nucleus has an irregular outline.

Two simple ocelli or larval eyespots are embedded in the uppermost region of the apical organ. Each ocellus consists of two cup-shaped pigment cells and a sensory cell with an elaborate array of microvilli (Fig 7). The ocelli of Polychordius were described earlier by Brandenburger and Eakin (1981).

Situated centrally in the apical organ and between the two ocelli are a number of structures that closely resemble phaosome photoreceptor cells. The morphology of these cells suggest that they represent a second type of photoreceptor in the larva. The approximate location of the phaosome in relation to other structures in the apical organ is shown in Fig 4. The entire phaosome measures about 10 µm in diameter and is slightly smaller than each larval ocellus. The phaosome cells are deeply embedded within the apical organ and no direct contact with the most apically placed cells was observed. The phaosome cells contain numerous intertwining microvilli and cilia that project into the vacuolar-like structure. In the phaosome there appears to be no regular pattern in the distribution or arrangement of the microvilli. The microvilli are situated in a homogeneous, electron-transparent matrix. Cilia that are presumed to be sensory protrude into the vacuole and microvillar mass (Fig 8). The cilia emerge from typical basal bodies closely apposed to the cell membrane. The microtubular configuration of the cilia was not observed. There is no close structural connection between the microvilli and the cilia. The nuclei of the phaosome cells are relatively large and the chromatin is usually unevenly distributed. The cytoplasm of the cells is often filled with a variety of vesicles, submicrovillar cisternae and Golgi bodies. The dispersed mitochondria are small and round.

A distinct nerve process consisting of at least five axons is situated at the base of the phaosome juxtaposed to the ciliary rootlets (Fig 8). The nerve process is 3 µm in diameter and is believed to extend to the apical nerve plexus of the apical organ. Cells of the nerve process contain large mitochondria and vesicles of varying size and density.

The epidermis and cuticle of the episphere is distinctly different in structure from that observed at the surface of the apical organ (Fig 9). The epidermis consists of a simple cuboidal epithelium. The majority of cells contain large electron-lucent vacuoles distally. A cuticle with microvilli and cell processes penetrating a somewhat dense matrix similar to that observed in the apical organ is common in this region. The epidermal episphere also includes prominent mucus cells containing electron-dense flocculent material (Fig 9); they often released material to the surface.

A thin basal lamina and mesenchyme cells line the apical organ (Fig 10). Large capsular cells are common especially in the region of the apical nerve plexus. Capsular cells are believed to be a type of support cell and were found throughout and surrounding the apical organ. The large cells are 8-10 µm in diameter and are often wedge-shaped (Fig 10). They have a large granular nucleus and very little observable cytoplasmic material. The intraepithelial apical nerve plexus is an aggregation of axons located at the base of the apical organ (Fig 4). The axons of the plexus have no distinct directional orientation and are observed as a tortuous mass. The axons are thin (0.5 µm) and contain mitochondria and...
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Numerous vesicles of varying size and electron density (Fig 11). Their ultrastructure suggests a neurosecretory and/or transmitting function. The apical nerve plexus may be a developmental center for the outgrowth of juvenile and adult nerves.

Prototroch

The prototroch exists of two rows of large ciliated cells. Prototrochal cilia are approximately 30 µm long. The ciliary axoneme has the conventional 9+2 configuration of microtubuli. The prototrochal cilia emerge from basal bodies that are provided with long striated rootlets. The striated rootlets project deeply into the cell and are oriented mainly perpendicular to the cell surface. Numerous mitochondria are located between the ciliary rootlets of the prototroch cells (Fig 12).

Discussions

The apical organ in the Polygordius larva is a composite sensory organ that contains a number of differing cell types and structures. Some of these structures, e.g., the two ocelli, and in earlier developmental stages the apical tuft, are believed to be transitory larval structures which regress early or are shed at metamorphosis. Otherwise, the major structures of the apical organ, unlike those of many other marine invertebrate larvae, are retained in the proctodium of the juvenile worm after settlement and metamorphosis.

In the young Polygordius larva the apical organ is well equipped with photoreceptor structures: two ocelli and a centrally located phaosome. It is probable that ocelli aid in guidance and are sensitive to phototaxis and other sensory stimuli during the planktonic swimming phase of the larva. Two epidermal nerves provide a means by which photo-, chemical and/or physical sensations perceived at the apical organ could be transmitted to the prototroch and other larval effectors. The apical organ of the Polygordius larva is well suited for the perception of stimuli and is presumed to play an important role in the selection of a suitable substrate at settlement and metamorphosis. The adult worm is very specific in its requirements of habitat with regard to sediment consistency and sediment depth. Other possible physical and chemical factors such as bacterial films, presence of conspecific adults and adult prey as reported for a number of other marine invertebrate larvae (Scheltema 1974, Burke 1983) may also be important.

A conspicuous structure of the apical organ in many pelagic invertebrate larvae is an apical ciliary tuft. The apical tuft has been designated as a sensory structure with a mechanosensory and/or chemosensory function (Chia and Koss 1979, Lacalli 1981). Ciliary structures (usually termed apical tuft, apical bristles, or apical sense organs) commonly observed on the apical organ of most trochophores have often been assigned a sensory function (Bonar 1978). Although the apical tuft is commonly observed, its precise function remains obscure.

Hyman (1951) considered the apical tuft to be a primitive characteristic because of its presence in pilidium and veliger larvae. In the pilidium larva the apical tuft can be up to 110 µm long and is suggested to function in control of swimming movements and in mechanoreception (Cantell 1969, Cantell et al. 1982). Polychaete trochophores tend to lack an apical tuft. Some polychaete trochophore larvae do, however, have apical cilia. Observations on the behavior of the trochophore of Spirobilaris polycerus revealed no obvious function for the apical tuft and in the metatrochophore stage the tuft was absent or very much reduced (Lacalli 1981). Lacalli also stated that the apical tuft in polychaetes regresses or is suppressed and is often replaced by secondary apical structures in many species. In fully developed Polygordius trochophores a long distinct ciliary tuft is lacking. However, in earlier developmental stages an apical tuft is present (Woltereck 1902, 1905).

The two tentacular knobs and the two aggregations of short cilia may have a sensory function. The two tentacular knobs lengthen at metamorphosis, and become characteristic structures of the proctodium of the adult worm. The two ciliary aggregations at the margin of the apical organ are presumed to be sensory and consist of multiciliated cells each containing up to four cilia. However, a definite interpretation of the function of ciliary cells is difficult from ultrastructural and morphological observations alone. For example, Horridge (1965) described ciliary modifications from various sensory organs and suggests that it is not possible to determine chemo- and/or mechanosensory based only on ciliary morphology. However, several characteristics of the apical tuft (position, projections to the apical nerve plexus and multiciliation) of the cells of the ciliary aggregations in the apical organ of Polygordius are similar to sensory cells described for other invertebrate larvae (Bonar 1978, Chia and Koss 1979, Lacalli 1981, Chia and Koss 1984, Lacalli 1984). Ciliary aggregations of centrally located stiff cilia, similar in external appearance and position to those observed in the apical organ of Polygordius occur in the trochophore of Harmothoe. These ciliated cells have the normal 9+2 microtubuli arrangement and have long tapering projections which extend towards the neurite mass, and are suggested to be sensory (Holborow et al. 1969, Holborow 1971). A survey of invertebrate and vertebrate sensory structures reveals that numerous cells with cilia or modified cilia are employed at the site of stimulus reception (Moran and Rawley 1983).

It is commonly agreed that when possible electrophysiological and behavioral studies, in conjunction with ultrastructural observations are needed to clearly elucidate possible sensory function in the superficial sensory organs of invertebrate larvae. However, small larval size
Phaosomes are common structures in oligochaetes (Röhlich et al. 1970) and leeches, and are considered to be sensory (Röhlich and Török 1964). They have also been described from pogonophorans (Nörrevang 1974) and from the larva of a spionid polychaete (Nilonen 1980). In the leeches (Hirudinea), the phaosome cells contain a microvillous rhabdome extending into an intracellular vacuole (Clark 1967) with no observable connection between the vesicle and the external cell membrane. The scattered photoreceptor cells in the epidermis of the earthworm Lumbricus have a similar intracellular vacuole with microvilli than found in the sensory cells of the ocelli. The presence of presumed photoreceptor cells of the phaosome type containing both microvilli and cilia illustrates the variety of possible photoreceptor structures in annelids.

Capsular cells are conspicuous cellular components of the apical organ in Polygordius. These cells were often observed to encapsulate and surround the entire apical organ. The large capsular cells are believed to have a supportive function in the apical organ and were most commonly observed surrounding the apical nerve plexus. Cells similar in structure and possible function were observed by Lacalli (1981) in the trochophore of the polychaete Spirornbranchus polycerus. Lacalli did not rule out the possibility that capsular cells may contribute axonal extensions to the nerve plexus of S. polycerus. This seems unlikely in the apical organ of Polygordius.

The apical nerve plexus occupies a major basal portion in the apical organ of Polygordius. This region is tightly filled with numerous slender axons. It is possible that axons or neuronal processes from the multiciliated cells of the ciliary aggregations or other apically situated cells converge in this region, although this could not be observed in serial section. No cell bodies were observed in the apical nerve plexus. Neuronal aggregations from basal extensions of apical organ cells, similar to the apical nerve plexus, are not uncommon in invertebrate larvae (Holborow et al. 1969, Holborow 1971, Marsden and Lacalli 1978, Chia and Koss 1979, Lacalli 1981, 1982, 1984, Pranzén and Sensenbaugh 1983, Sensenbaugh, unpublished observations). These axonal aggregations have been designated larval brain, cerebral commissure, and nerve plexus.

The slender neuronal processes or axons of the apical nerve plexus are often characterized by neurotubuli (microtubuli) and numerous membrane bound vesicles of various sizes and densities. In the polyclad Mueller's larva of Psuedoceros canadensis there are two types and sizes of vesicles: small clear vesicles, and larger dense-cored vesicles with a supportive matrix. Both types of vesicles were common in the central nervous system. Similar vesicles have been observed in axons and other sensory cells and structures in invertebrate larvae (Stricker and Reed 1981, Sensenbaugh, unpublished observations).

Metamorphosis from larva to juvenile in Polygordius involves numerous drastic morphogenetic alterations in larval morphology. The apical organ becomes the most important part of the head region and remains after metamorphosis. By the contraction of apically located

Fig 12: TEM of prototroch showing cilia (c), mitochondria (m) of the prototroch cell. A pigment cell containing two pigment granules (pg) is located directly above the prototroch cells; va = vacuoles. Bar = 5 μm.
muscles, the apical organ and segmented parts of the hyposphere fuse. The prototroch, episphere and unsegmented anterior part of the hyposphere are shed from between the apical organ and the segments. Shed larval structures including the prototroch and pigment cells are ingested by the young worm after metamorphosis (Jägersten 1972).

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