The Duo-Gland Adhesive System of the Archiannelids Protodrilus and Saccocirrus and the Turbellarian Monocelis

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Summary. The duo-gland adhesive systems in three archiannelids (Protodrilus sp., Saccocirrus sonomacus and S. eroticus) and one turbellarian (Monocelis cincta) were studied by scanning and transmission electron microscopy. Protodrilus attaches to the substrate by the posterior margin of its bilobed and dorso-ventrally flattened pygidium. Saccocirrus also adheres by a bilobed pygidium, but each lobe is ovoid in transverse section, and its median-ventral surface is divided into numerous ridges. Adhesive glands open along the crest of each ridge. Saccocirrus also adheres along bands of adhesive structures that encircle each body segment. Monocelis attaches to the substrate by a crescent shaped area at the posterior margin of the ventral surface. Although the external morphology of the adhesive area is different in each species examined, the basic cellular organization is similar. The adhesive areas contain two types of glands named viscid and releasing. The viscid glands produce granules (0.8–2.0 μm long) that are thought to contain the adhesive that binds the worm to surfaces. The releasing glands secrete granules (0.15–0.2 μm in diameter) that are believed to break the attachment. The releasing granules are identical to those described in other species, whereas the viscid granules have a variety of complex substructures unlike any described previously. The possible homology of the adhesive systems in the archiannelids and those in other taxa with duo-glands is discussed.

A. Introduction

Intertidal animals need to prevent waves from dislodging them from the substrate. Well known means of attachment are the byssal threads of mussels

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(Tamarin, 1975), cement of barnacles (Barnes, 1970), tube feet of echinoderms (Chaet, 1965; Harrison, 1968), pedal discs of cnidarians (Davis, 1973), and the feet of many snails and chitons (Clark, 1964). Many small animals do not adhere strongly to the substrate but seek shelter from the waves in cracks in rocks or in the holdfasts of various plants and animals. Riedl (1971) calculated that water flows through the interstices of a sandy beach at a rate of 0.5 millimeters per second, and Tyler (1976) pointed out that, for most interstitial organisms, this rate is equivalent to one body length per second. Attachment devices are, therefore, necessary to prevent meio-benthic organisms from being swept away from interstitial habitats.

Adhesive structures on many interstitial turbellarians and gastrotrichs contain two types of glands. This was first noted in the gastrotrich Turbanella hyalina by Boaden (1968) who proposed two functional alternatives; either one gland secretes a material that is catalysed by the secretion of the second gland to form the adhesive or, more likely, one gland secretes the adhesive and the second a substance that breaks the attachment. Tyler (1975, 1976, 1977), studying adhesion in turbellarians, gastrotrichs and an archiannelid, developed the latter concept and named it the duo-gland adhesive system. Viscid granules, the larger of the two types of secretory products, were believed to contain the adhesive. The second type of gland discharges smaller granules, called releasing granules. These were thought to break the attachment. Teuchert (1975) has confirmed the presence of two types of secretory cells in the adhesive papilla of the gastrotrich Turbanella cornuta, but evidence for the function of these two secretions remains circumstantial.

This paper describes the duo-gland adhesive systems of three archiannelids and one turbellarian and discusses the possibility that they are homologous.

B. Materials and Methods

Specimens of Sacoccoris somuncus Martin, 1977, and Protodrilus sp. were collected in coarse sand from the mean water level at Shell Beach, 12.9 km north of Bodega Bay, California. Monocelis cinera Karling, 1966, was collected at Point Pinos, Pacific Grove, California, from sediment in high intertidal pools. Specimens of Sacoccoris eroticus Gray, 1969, were provided by Dr. Robert L. Fernald of the Friday Harbor Marine Laboratories, University of Washington. He collected them from the type locality of S. eroticus, which is Orcas Island, Washington, USA.

Worms were extracted from sediment using the magnesium chloride anaesthetization technique (Hulings and Gray, 1971) and stored at 13°C in large (20 cm diameter) glass bowls filled with artificial seawater that was changed once a week. Although food was not added to these cultures, the animals remained in good condition for over six months.

For electron microscopical investigations the animals were fixed for 1 h at room temperature in 2% glutaraldehyde in buffer (0.2 M sodium chloride, 0.2 M sodium cacodylate and 0.04 M calcium chloride). Specimens for transmission electron microscopy (TEM) were dissected into smaller pieces at this stage. All specimens were post-fixed for 1 h in 1% osmium tetroxide in the above buffer at 4°C, then dehydrated in ethanol. Specimens for TEM were embedded in Epon. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate and viewed in an RCA EMU 3. Samples for scanning electron microscopy (SEM) were transferred in vials containing 100% ethanol to a Pelco model H critical point drying apparatus that utilized liquid CO₂. Dried specimens were mounted on stubs with conducting silver paste, coated with 200 Å of gold in a Technics Hummer II and viewed in a Coates and Wetter SEM.
C. Observations

*Protodrilus* sp., *Saccocirrus sonomacus*, *S. eroticus* and *Monocelis cincta*, are small (lengths 10 mm, 25 mm, 20 mm and 8 mm, respectively) interstitial worms that can adhere tenaciously to the substrate with the caudal parts of their bodies. Attachment can be established and terminated quickly. All four species adhere to the substrate at the slightest disturbance, for example, when they are touched with a probe or when their bowl is jarred.

1. *Protodrilus* sp.

In *Protodrilus*, attachment occurs along the posterior margin of the bilobed and dorso-ventrally flattened pygidium (Fig. 1). Longitudinal striations are seen with the light microscope leading 0.3 mm from the posterior margin of each lobe anteriorly to an ovoid and dark staining area. With TEM the ovoid area is seen to contain the nuclear and synthetic part of both viscid and releasing glands. Figure 2 shows the synthetic zone of a viscid gland with rough endoplasmic reticulum, numerous Golgi bodies and granules in various stages of maturation. The posterior part of each cell is drawn into a long, microtubule-lined neck that extends to the posterior tip of a pygidial lobe. These necks give the striated appearance seen with the light microscope. The glands are arranged into two strata, with the releasing glands dorsal to the viscid glands (Fig. 3). Each gland opens to the outside through a chimney formed by microvilli that barely penetrate the cuticle (see insert, Fig. 1).

A secretory granule from the viscid gland is ovoid, membrane-bounded, 1.2 μm long and 0.5 μm in diameter (Fig. 12). In sections its contents are differentiated into light, circular areas separated by a reticulum of darker staining material. Scattered over both areas are patches of flocculent, dense staining material.

Granules from the releasing glands are spheroidal, membrane-bounded, about 0.2 μm in diameter and homogeneously dense (Fig. 13).

2. *Saccocirrus sonomacus* and *S. eroticus*

*Saccocirrus sonomacus* possesses adhesive glands on the pygidial and segmental regions of its body. When a worm is disturbed the pygidium attaches firmly to the substrate and the body curls into a ball. Particles of sediment also attach to the segmental adhesive glands. Removal of the pygidium does not prevent the worm from adhering strongly to the substrate.

*Saccocirrus* has a bilobed pygidium (Fig. 4). Each lobe is oval in transverse section and its median-ventral surface is divided into 7–12 ridges that are perpendicular to the long axis of the lobe. Each ridge is about 0.8 mm wide, and those in the middle are slightly longer than anterior or posterior ones. The nuclear and synthetic part of both viscid and releasing glands lies deep in the epidermis. The secretory granules travel through microtubule-lined necks to the
Fig. 1. SEM (scanning electron micrograph) of two pygidial lobes of *Protoberis* in ventral view. CG end of ventral ciliated groove. ×1700. Insert: SEM of posterior margin of a lobe showing openings of three viscid glands (V) and two releasing glands (arrows). ×9500

Fig. 2. TEM (transmission electron micrograph) showing synthetic region of viscid gland cells in *Protoberis*. ER rough endoplasmic reticulum; G Golgi apparatus; I immature viscid granule; N nucleus; V viscid granule. ×6500

Fig. 3. TEM of transverse section through a pygidial lobe of *Protoberis* showing viscid gland necks ventral to releasing gland necks. R releasing granule; V viscid granule; arrows indicate microtubules. ×16,500
Fig. 4. SEM of pygidal lobes of *Saccocirrus sonomacus* showing ridges (R) on median-ventral surface of each lobe. Necks of viscid and releasing glands open along the crest of each ridge (arrows). × 200

Fig. 5. TEM of longitudinal section through ridge on pygidium of *Saccocirrus sonomacus* showing releasing (R) and viscid (V) granules reaching surface of worm along the crest of ridge. M muscle; S intercellular space. × 3800

Fig. 6. SEM of segmental adhesive glands on one segment of *Saccocirrus sonomacus*. Prior to secretion, tips of viscid gland necks appear as three disks on each mound (arrows). After secretion viscid granules appear as small, white rods (r). In upper left corner of micrograph is debris (X) apparently stuck to adhesive glands. × 3600. Insert: SEM showing location of segmental adhesive glands (A) on larger (L) of two subdivisions of each body segment. × 110
surface of the worm, at the crest of each ridge (Fig. 5). The gland necks wind their way to the surface through large, irregularly shaped spaces, and between muscle and epidermal cells.

The releasing granules are 0.2 μm in diameter, membrane-bounded and typically electron dense (Fig. 15). The viscid granules are spheroidal, 1.5–2.0 μm in diameter, and membrane-bounded (Fig. 14). In sections they contain an electron dense rosette with typically 12 lobes radiating from a less dense core. Granular material between the rosette and the outer membrane is even less electron dense.

Figure 6 (insert) shows the location of adhesive glands on several segments. Each belt, composed of a series of mounds, encircles the entire worm except for a small gap on the midventral surface. Each mound contains two or three gland necks that surround a single releasing gland neck (Figs. 7 and 9). Numerous rod-shaped viscid granules can be seen on the surface of these mounds. The releasing granules in the segmental region (see Figs. 16 and 17) appear the same as those in the pygidium, but the segmental viscid granules differ from those in the pygidium. The segmental viscid granules are cylindrical (with round or flattened tips), 1.5 μm long, 0.8 μm in diameter and membrane-bounded (Figs. 16 and 17). Each granule contains a cylindrical core of numerous 600 Å diameter spheres, surrounded by a lighter staining, granular cylinder. Viscid granules are synthesized in cells containing abundant RER and Golgi bodies. Adjacent to Golgi bodies are small vacuoles filled with lightly staining fibrillar material. These vacuoles become smaller and electron-dense as they mature to form the final secretory granule (Fig. 8).

The viscid and releasing glands in both the pygidial and segmental regions of a second species, *S. eroticus*, were studied. No differences could be discerned between corresponding structures in the two species.

3. *Monocelis cineta*

*Monocelis* is such a rapidly moving flatworm that it is difficult to follow its movements even in a small petri dish. However, it is apparent that the posterior end of the worm bears adhesive glands and that they are used not only when the worm is disturbed but also in turning and stopping.

Fig. 7. TEM of transverse section through body of *Saccocirrus somomacus* showing viscid gland necks on either side of a releasing gland neck. *CT* cuticle; *R* releasing granule; *V* viscid granule. ×12,000

Fig. 8. TEM of section through segmental adhesive gland of *Saccocirrus somomacus* showing synthetic part of viscid gland with two granules in different stages of formation. *CF* condensing granule; *ER* endoplasmic reticulum; *M* mitochondrion; *N* nucleus; *V* mature viscid granule. ×17,000

Fig. 9. TEM of section tangential to body surface of *Saccocirrus somomacus* showing clusters of two or three viscid gland necks surrounding neck of single releasing gland. Gland necks pass between epidermal cells. *N* nucleus of epidermal cell; *R* releasing granule; *V* viscid granule. ×14,000
Fig. 10. SEM of ventral surface of caudal end of Monocelis showing crescent-shaped zone of adhesive papillae (arrows) posterior to ciliated sole. × 500. Insert: Higher magnification (× 26,000) SEM of tip of an adhesive papilla showing microvilli around ovoid viscid granules (V).

Fig. 11. TEM of longitudinal section through adhesive papilla on tail of Monocelis showing viscid (V) and releasing (R) granules in their respective necks. MV microvilli. × 4800. Insert: TEM of transverse section through adhesive papilla showing several viscid gland necks and one releasing gland neck (R). V viscid granule; arrows point to microvilli. × 3200.
The adhesive zone consists of numerous papillae on the ventral surface of the crescent-shaped posterior margin of the worm (Fig. 10). The ventral ciliation ends just anterior to this zone. Each papilla is composed of epidermal cells and the necks of several viscid and releasing glands (Fig. 11). The epidermal cells, termed anchor cells (see Tyler, 1975), have clusters of microvilli that surround the gland necks. In SEM the microvilli appear to surround smooth-surfaced, membrane-bounded bodies, 0.8 μm in diameter (see insert, Fig. 10). These bodies are most likely viscid granules judging from comparable structures seen in TEM where the distinction between viscid and releasing granules is more apparent. The two types of glands intermingle, and the nuclear and synthetic part of these cells lies deep in the body parenchyma.

The viscid granules are ovoid, 0.8 μm long, 0.3 μm in diameter and membrane-bounded. At low magnification they appear homogeneously dense but at higher magnification they are seen to contain tubular structures (Fig. 18). The releasing granules are similar to those described earlier; they are small spheres, 0.15 μm in diameter with a homogeneous content (Fig. 19).

**D. Discussion**

1. **Functional Morphology of the Duo-Gland Adhesive System**

The duo-gland adhesive system as defined by Tyler (1975) has been found in 26 species of Turbellaria, 14 species of Gastrotricha and 4 species of Archiannelida. In these animals it has been suggested that adhesion is established by secretions from single-celled viscid glands (Boaden, 1968; Tyler, 1975, 1976). The secretions, in membrane-bounded vesicles called viscid granules, pass from the cell body through microtubule-lined necks and are released into a collar formed by microvilli from surrounding epidermal cells. The microtubules may participate in movement of the granules from the site of production to the site of secretion (see Allen, 1975; Murphy and Tilney, 1974), and, additionally, they may provide a cytoskeleton preventing the gland necks collapsing (Dickson and Mercer, 1966; Oschman, 1967). The microvillar collar keeps the adhesive material concentrated in one area (Singla, 1977) and transfers the tension of adhesion to the body wall (Tyler, 1977). The attachment is established quickly, and it is strong and long-lasting. Termination of adhesion is effected by secretion of releasing granules. The contents of these granules are proteinaceous, and presumably they cleave the adhesive, a glycoprotein (Tyler, 1975; Martin, unpublished results). Evidence that the larger of the two granules contains the adhesive is circumstantial. The most convincing micrographs show distorted areas that were apparently attached to the substrate at the time of fixation and in which the smaller (releasing) granules were peripheral to the distorted area (see Tyler, 1976).

2. **Viscid and Releasing Granules in Turbellaria and Archiannelida**

In most respects the adhesive and releasing system of *Monocelis* agrees with the construction of other Proseriate flatworms. The necks of the two types of glands
branch near the bodywall of the worm, and the viscid and releasing granules are secreted into a ring of microvilli. Tyler (1975) described the contents of the viscid granules in all the turbellarians he examined as homogeneous. In *Monocelis* described in the present paper, however, these granules have a tubular substructure. The procedure for tissue preparation for EM used by Tyler is different from that used by me, that is, Tyler used a slightly higher concentration of fixatives (both glutaraldehyde and osmium tetroxide) and a 0.1 M phosphate buffer. The effect of such differences on the morphology of the viscid granules is unknown.

Tyler (1975) studied only one archiannelid, *Diurodrilus* (Family Dinophilidae), yet it possesses a duo-gland system seemingly identical to that of turbellarians and gastrotrichs. Of the four remaining archiannelid families, the duo-gland adhesive system is described in representatives of the Protodrilidae and Saeccocirridae in this paper. The Nerillidae appear to lack specialized adhesive structures and, although specimens of Polygordiidae were unavailable for the present study, this family probably possesses a duo-gland adhesive system on the pygidium (see Fraipont, 1887).

In *Diurodrilus* sp., *Protodrilus* sp., *Saccocirrus sonomacus* and *S. eroticus* the releasing granules are approximately the same size (0.1–0.2 μm in diameter) and have homogeneous contents. The morphology of the viscid granules, however, varies in the three genera. In *Saccocirrus* the viscid granules of the pygidium and the segmental adhesive glands are different from each other but are identical to corresponding granules in closely related species.

The archiannelids have been considered to be a phylogenetically distinct group by some authors or a heterogenous grouping of annelids by others (see Hermans, 1969). The variety of viscid granule morphology tends to support the latter opinion. However, information on other archiannelid adhesive systems (especially of the genera *Polygordius* and *Protodriloides*) is needed to reveal the range of adhesive granules present within the group before this characteristic

Fig. 12. TEM of section through viscid granules (V) of *Protodrilus*. M microtubule. ×29,000

Fig. 13. TEM of section through releasing granules (R) of *Protodrilus*. M microtubule. ×27,000

Fig. 14. TEM of section through viscid granules (V) from pygidium of *Saccocirrus sonomacus*. ×12,000

Fig. 15. TEM of section through releasing granules (R) from pygidium of *Saccocirrus sonomacus*. ×12,000

Fig. 16. TEM of longitudinal section through viscid (V) and releasing (R) granules from segmental adhesive glands of *Saccocirrus sonomacus*. CT cuticle. ×12,000

Fig. 17. TEM of transverse section through viscid (V) and releasing (R) granules from segmental adhesive glands of *Saccocirrus sonomacus*. M microtubules. ×12,000

Fig. 18. TEM of section through viscid (V) granules of *Monocelis*. ×18,000

Fig. 19. TEM of section through releasing (R) granules of *Monocelis*. M microtubules. ×27,000
will be useful in discussing the affinities of the various archiannelid genera. It might also be important to compare the type of substrate in which a particular species is found with the type of viscid granules it possesses.

3. Are the Duo-Gland Adhesive Systems of the Turbellaria, Gastrotricha and Archiannelida Homologous?

The duo-gland adhesive systems in representatives of each of these three taxa are very similar. The only basic difference is the presence or absence of a cuticle that may help spread the tension of adhesion over a larger area of the body wall. Archiannelids and gastrotrichs have a cuticle whereas turbellarians lack one.

The viscid granules in the archiannelid (Diurodrilus), gastrotrichs and turbellarians described by Tyler (1975) are all homogeneous, ovoid, membrane-bounded and about 0.2–0.7 μm in diameter. None of the viscid granules described in this paper has such a simple morphology, and each type of granule appears to be genus specific. However, the presence of different adhesive systems in other lower invertebrate taxa (such as Acoela, Nemertea, Nemertodermatida, Lecithoepitheliata, Rotifera and Gnathostomulida—see Tyler, 1975; Crezee and Tyler, 1976; Pedersen, 1965; Sterrer, 1970; Lippens, 1974; Dickson and Mercer, 1966) reveals that the duo-gland system is not the only adhesive system possible, and this fact lends support to the homology of all duo-gland adhesive systems. At present, the evidence for or against homology of the archiannelid, turbellarian and gastrotrich duo-gland adhesive systems seem ambiguous, and the question of whether or not the duo-gland systems in these three taxa are homologous cannot be answered at this time.

4. Are There Other Types of Duo-Gland Adhesive Systems?

Adhesion is such a fundamental biological property that it seems likely that the ability to modify the adhesive bond is widespread. Hermans (personal communication) suggests that adhesive systems utilizing two types of glands may be much more common than currently suspected. In the system presented by Tyler (1975, 1976) and in this paper, one gland appears to produce the adhesive and a second gland produces a releasor. It is also possible that the sticky quality is only achieved by the mixture of two glandular products, but such a theory does not explain the mechanism by which these animals detach so rapidly. Such a possibility that two glandular products combine to form an adhesive has been considered by Boaden (1968) and Tyler (1976), and it is also suggested by the production of a cleansing mucus (that undoubtedly has adhesive qualities) from two types of glands in the whelk Buccinum (Hunt, 1973). A final variation in adhesive systems with two types of glands might involve one type of gland that secretes an adhesive material which is polymerized and thereby made more permanent by the secretion of a second gland type. The byssal threads of mussels (Tamarin, 1975) and the cement of barnacles (Barnes, 1970) appear to function in this way, but obviously this would be unsuitable for the temporary attachment of motile animals.
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