Attachment to the substrate by soft coral fragments: desmocyte development, structure, and function

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Abstract. Pieces cut from colonies of the soft coral *Dendronephthya hemprichi* exhibited rapid and effective attachment to hard surfaces. Attachment involved development of root-like processes (RLPs), which appeared at the basal part of the fragment 4 days after its removal from the colony. The fine structural changes and cascade of cellular events occurring in the RLP before and after attachment were studied using SEM, TEM, and LM. The epidermis of the RLPs is actively involved in the attachment process and several distinct phases are documented: appearance of numerous oval vesicles, extrusion of these vesicles resulting in the formation of an outer layer composed of extracellular organic matrix and organellar debris, which functions as an adhesive device leading to initial attachment. The latter phase was followed by the formation of desmocytes, which develop in the RLP epidermis and function as anchoring devices, mediating the firm attachment of the fragment to the substrate. This is the first evidence among anthozoans that desmocytes play an active role in anchoring tissue to substrate and thus extends the range of functions exhibited by desmocytes among anthozoans.

Additional key words: Octocorallia, *Dendronephthya hemprichi*, extracellular organic matrix

Sessile marine invertebrates often encounter highly dynamic forces such as currents and waves. The benthic organisms of coral reefs, among others, while dependent on the water flow for nutrition, gas transport, and waste clearing, are threatened by it if flow intensity is too strong (Koehl 1984). Firmness of attachment to the substrate is thus crucial for their development and survival (Koehl 1984; Ward 1995). Stony corals, which are the major reef builders, begin to precipitate calcium carbonate immediately on settling of the planulae. This initial calcification serves as the basis for deposition of the adult skeleton, which is attached to the reef substrate (Vandermeulen & Watabe 1973). Soft-bodied octocorals mostly lack a solid skeleton. Although they are commonly found on coral reefs (Bayer 1973), their mode of attachment to hard substrate is still unknown.

The transition from pelagic to sessile phase in cnidarian larvae occurs through settlement. Two kinds of ectodermal cells have been reported to take part in this process, gland cells and nematocytes (Chia & Bickell 1978). In planulae of the sea pen *Ptilosarcus gurneyi*, 2 types of gland cells were described and assumed to participate in settlement, but found to be absent in the polyp stage (Chia & Crawford 1977). Vandermuelen (1975) found 4 morphologically distinct secretory cell types in the aboral epidermis of planulae of the stony coral *Pocillopora damicornis,* but only one of them was detected in a 6-h settled polyp. Abelson et al. (1994) described mucus threads secreted by the aboral end of planulae of the soft corals *Litophyton arboreum* and *Dendronephthya hemprichi,* which enable them to settle on high points in the substrate under high-flow regimes. Discharge of nematocytes as a means of larval adhesion to different types of substrates was revealed in a few members of the class Hydrozoa, including planulae of *Hydractinia echinata* and *Proboscidactyla flavicirrata* and actinulae of *Tubularia larynx* (Chia & Bickell 1978).

Desmocytes, found in certain cnidarians and first described by Bourne (1899), are uniquely structured anchoring cells modified for attachment of soft tissue to skeletal surfaces (references in Muscatine et al. 1997). Ectodermal in origin, they are characterized by apical and proximal extensions, which are modified for their function. Attachment to the substrate by desmocytes was described in the pedal disc of scyphistomae of the scyphozoan *Aurelia aurita* (see Chapman 1969). These

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cells contain bundles of tonofibrillae, also termed "rivets," which retain their anchoring function even after the cell dies. One end of such a rivet is bound to the mesogleal fibrillae, and the other end is embedded in the cuticle adherent to the substrate. Desmocytes found in Octocorallia such as *Leptogorgia virgulata* (see Tidball 1982) and *Cornularia cornucopiae* (see Benke & Hündgen 1984) take active part in binding soft tissues onto endoskeletal structures.

In stony corals, desmoidal processes were described in newly settled larval stages of *P. damicornis* and were assumed to be an extracellular product (see Vandermuelen 1975). The fine structure and function of desmocytes was comprehensively studied in the stony coral *Stylophora pistillata* (see Muscatine et al. 1997). In the case of these hexacorallians, it was suggested that desmocytes anchored the calicoblastic epithelium to the coral exoskeleton. In the hydrozoan *Cordylophora caspia,* desmocytes mediate support of the soft coenosarc by linking the mesoglea to the rigid tube of the perisarc (Marcum & Diehl 1978). In all the examples mentioned above, desmocytes have the same function, i.e., attachment of soft tissues to skeletal elements, with the exception of the "rivets" described for *A. aurita* by Chapman (1969), who showed that they serve in attachment to the substrate.

The azooxanthellate alcyonacean *Dendronephthya hemprichi* KLUNZINGER 1877 of the family Nephtheidae is a branching soft coral, highly abundant in flow-exposed reef habitats of the northern Red Sea (Fabricius et al. 1995). It propagates by fragmental fission, resulting in autotomy of small fragments, possessing root-like processes (RLPs) for attachment to the substrate (Dahan & Benayahu 1997). In a recent study involving transplantation of cuttings of *D. hemprichi,* aimed at rehabilitation of denuded reefs in Eilat (Red Sea), we found that the transplants attached quickly and effectively to hard substrates (Barneah 1999). Attachment involved rapid development of similar RLPs, which appeared at the basal part of the transplant as soon as 4 days after its removal from the colony. Intrigued by these findings, in the present study we investigated the fine structural changes and the cascade of cellular events occurring in the RLPs before and after attachment. We demonstrate here that desmocytes found in the RLPs function as anchoring cells, mediating their attachment to the reef substrate.

Methods

Branches (5–10 cm in length) of *Dendronephthya hemprichi* were removed with scissors from colonies inhabiting artificial reefs in the vicinity of the Interuniversity Institute in Eilat. They were transferred to the laboratory and placed in aerated aquaria with running seawater. After 12 h of acclimatization, the fragments were either transferred to an aquarium with hard substrate (granite stones or stony coral skeletons) or kept hung in an aquarium, preventing any contact with substrate. The latter treatment enabled us to monitor the RLP development and ability to attach after a prolonged period of substrate deprivation. It also allowed us to check whether the course of cellular events changed following such a manipulation.

For scanning electron microscopy (SEM), 12 fragments were fixed in 4% glutaraldehyde in seawater at 12 h intervals until 96 h post-cutting. After decalcification for 40 min in a solution of formic acid and trisodium citrate (Benayahu & Loya 1983), the basal part of each fragment was removed, dehydrated through a graded ethanol series, and then critical point dried with liquid CO_2. The preparations were coated with gold and examined with a JEOL JSM 840A scanning electron microscope at 25kv.

For transmission electron microscopy (TEM), fragments (5 each) 5 and 20 days post-cutting and still not attached to the substrate, were fixed in 2.5% glutaraldehyde in seawater and decalcified as described above. Fragments 12 days post-cutting, which had attached to pieces of stony corals' calcitic skeletons, were fixed and then decalcified as described above. All samples were rinsed in buffer phosphate, stained with 1% OsO_4, dehydrated through a graded ethanol series, and embedded in Epon. Sections were cut with a diamond knife, stained with lead citrate, and viewed with a JEOL 1200 EX transmission electron microscope.

The layer of material external to the RLP epithelium observed with SEM and TEM was characterized with two staining techniques: Masson's Trichrome (Gurr 1962), which differentiates between connective tissue, mucus (green), and cytoplasmic elements (red), and Alcian Blue-Periodic Acid Schiff (AB-PAS), which differentiates between acid mucopolysaccharides (blue stain) and neutral polysaccharides (red stain) (Gurr 1962). Fragments, 5 days post-cutting with RLPs were fixed in 4% formaldehyde in seawater for 24 h, rinsed in fresh water, and transferred into 70% alcohol. These samples were decalcified as described above. The fragments were embedded in paraffin, and serial sections 8 μm thick were made for light microscopy (LM).

Results

Immediately after a fragment was cut from a colony of *Dendronephthya hemprichi,* a view of the basal part clearly revealed the inner water canals (Fig. 1A). Epithelium gradually began to cover the surface of the
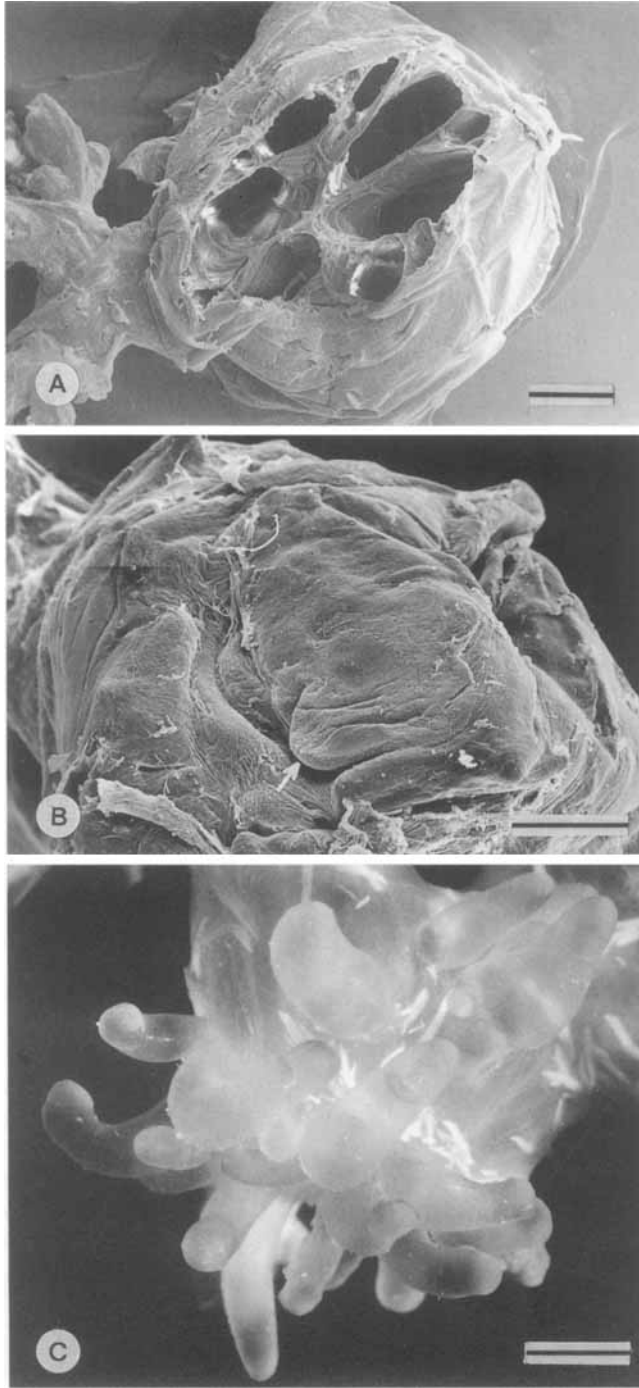
Fig. 1. Healing of the basal part of a fragment. **A.** Basal area immediately after cutting. The base is exposed; inner water canals can be seen. SEM. Scale bar, 1 mm. **B.** Healed basal area 4 days post-cutting with initiation of an RLP (white arrow). SEM. Scale bar, 0.5 mm. **C.** Photomicrograph of basal area covered with RLPs 6 days post-cutting. Scale bar, 5 mm.

cut, and after 4 days the cut surface was completely healed, and the presence of a small RLP could be seen (Fig. 1B). Within the next 2 days numerous RLPs covered the basal part of the fragment (Fig. 1C).

At 5 days after amputation, the apices of the epidermal cells of an RLP appeared in SEM as hemispherical structures, 4–6 μm in diameter (Fig. 2A). In TEM, numerous oval vesicles, 1–1.3 μm in cross section were observed in the apical cytoplasm of the RLP epidermal cells (Fig. 2B). The vesicles contained an electron-opaque core. On the same RLP, some of the epidermal cell apices were coated with an outer layer (Fig. 2C,D) composed of an extracellular organic matrix and cellular debris (Fig. 2D).

The mesoglea stained turquoise with Masson's Trichrome whereas the epidermis stained dark red and the outer layer was mauve (Fig. 3A). With AB-PAS, the mesoglea stained blue, the epidermis stained red, and the outer layer was mauve (Fig. 3A). The Alcian blue positive material in the mesoglea comprised non-sulfated mucopolysaccharides, or weakly sulfated acid mucopolysaccharides. The PAS positive reaction in the epidermis and the outer layer could indicate the presence of neutral mucopolysaccharides (Spicer 1963; Sheehan & Hrapchak 1973) and/or polysaccharides (Gurr 1962).

By 20 days after amputation, the epidermis of the unattached RLPs showed structural changes associated with continuous secretion of the oval vesicles mentioned above. At this stage two distinct surface morphologies of the outer layer could be distinguished (Fig. 4A,B). Away from the tip, the outer layer was amorphous (Fig. 4A), while closer to the tip it was enclosed in numerous thin-walled projections (Fig. 4B). In both cases the outer layer covered the surface microvilli and was composed of extracellular organic matrix with organellar debris. The gaps observed along the edges of the epidermal cells (Fig. 4A,B) suggest that the vesicles were extruded and that their contents had accumulated on the epidermis, forming the outer layer. Numerous multivesicular bodies (MVBs) were present in the epidermal cells (Fig. 4A,B).

After preliminary attachment of the RLPs to the substrate, the epidermis underwent structural changes and desmocytes appeared (Fig. 5A). Each desmocyte was composed characteristically of a cell body and tenons (Fig. 5A). Use of the term "tenon" here follows the terminology adopted by Muscatine et al. (1997). The tenons were perpendicular to the outer surface, and lined most contact area between the epidermal cell and the outer substrate (Fig. 5A). A thin osmophilic lining was present at the margins of the extracellular organic matrix, in contact with the substrate (Fig. 5A). Each tenon (2–2.5 μm high and 0.35
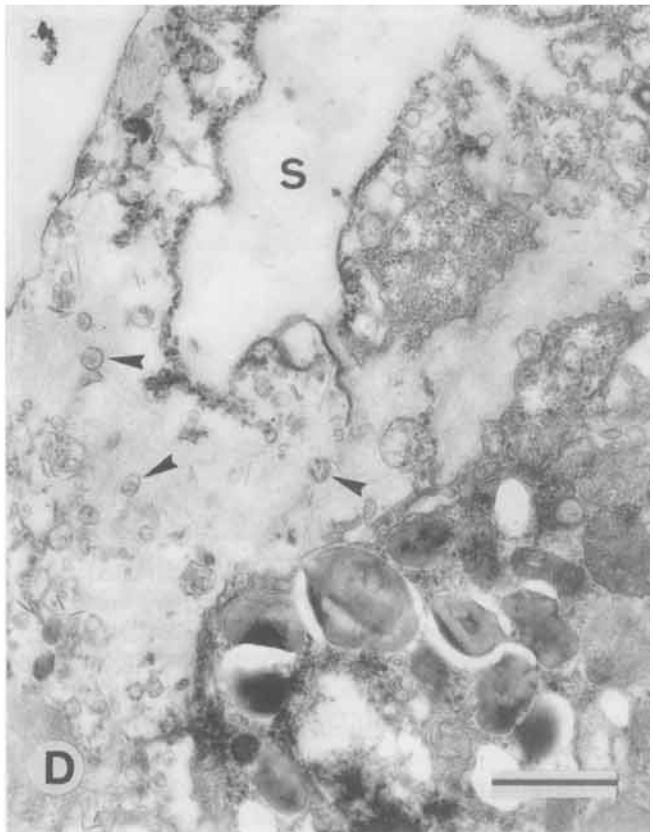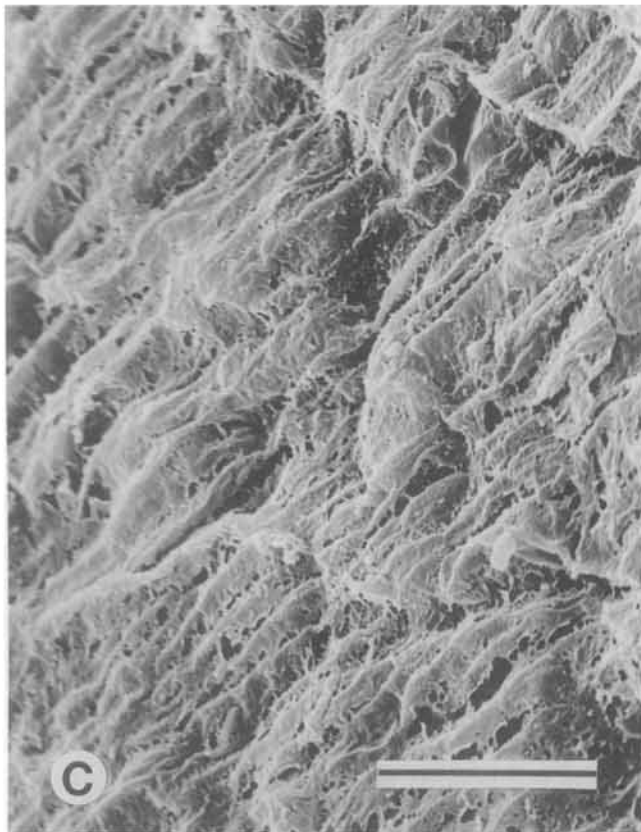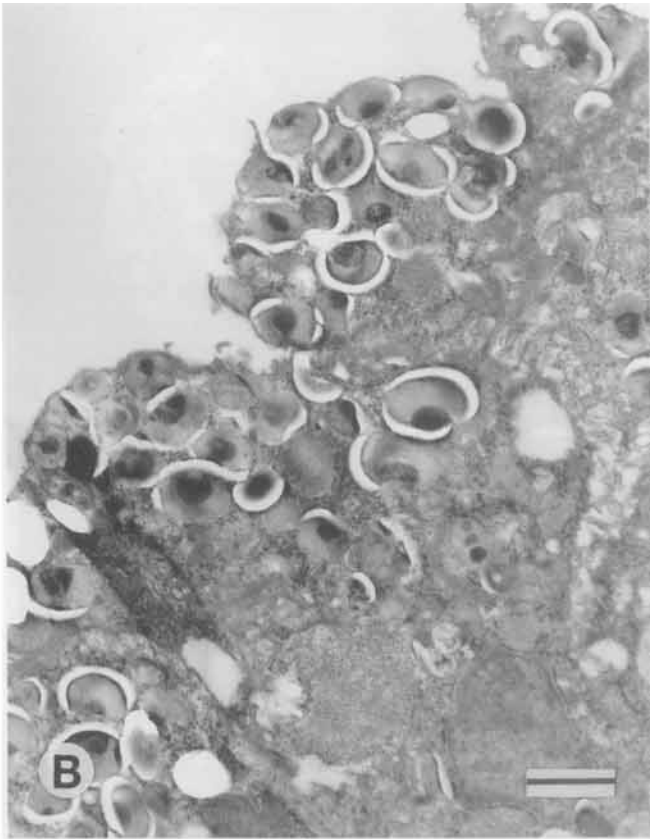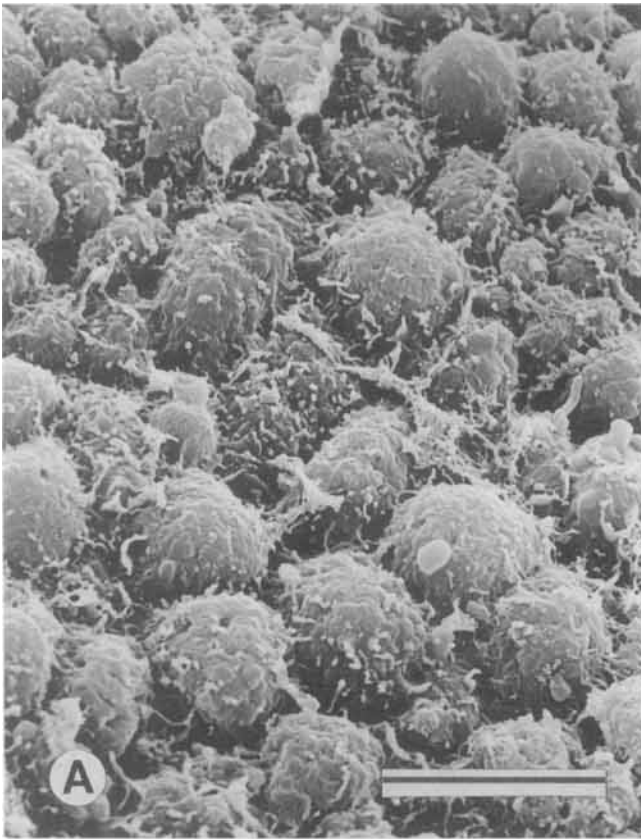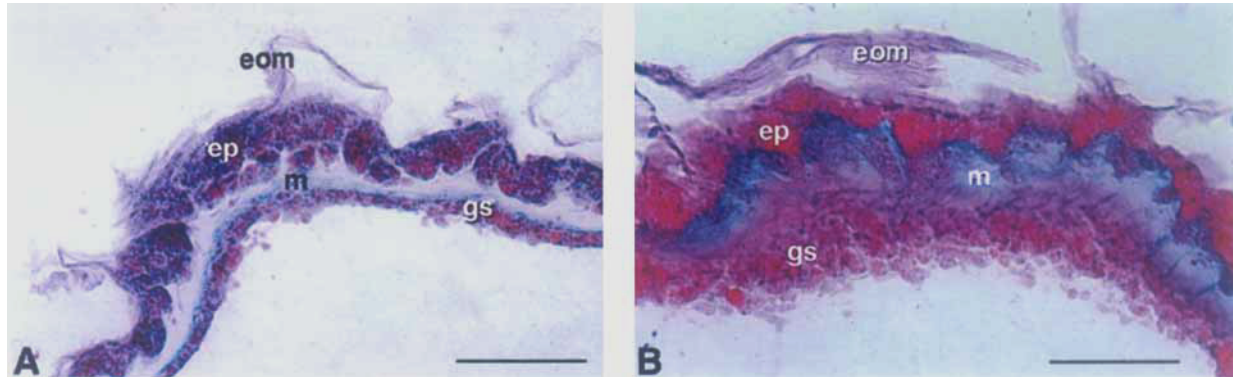
Fig. 3. An RLP in cross section. Extracellular organic matrix (eom), epidermis (ep), mesoglea (m), gastrodermis (gs). Scale bars, 60 μm. **A.** Masson's trichrome staining. **B.** Alcian Blue-Periodic Acid Schiff staining.

μm wide) was embedded in mesoglea. Its distal end, facing the substrate, was anchored in the extracellular organic matrix, while its basal part, facing the meso-glea, was occasionally wider (Fig. 5B). The cell body of each desmocyte contained various organelles such as the Golgi apparatus, endoplasmic reticulum, multi-vesicular body (MVB), mitochondria, and numerous vacuoles (Fig. 5A,C–E). The MVB, 1.5 μm in diameter (Fig. 5D), contained minute vesicles, 0.12 μm in diameter, and its presence further implies intense cellular activity (see Discussion). Plications of the plasma membrane observed at the distal part of the desmocyte cell-body (Fig. 5E) are probably developing tenons (see Tidball 1982 and Discussion below).

Discussion

Fragments removed from a colony of *Dendrone-phthya hemprichi* attached to the substrate by means of numerous root-like processes (RLPs). The epidermis of the RLP played a major role in the attachment process, which involved a cascade of cellular events beginning as soon as the fragment was cut from the colony. Closure of the exposed basal area of a fragment and initiation of RLP development occurred after 4 days. By day 5, the apices of RLP epidermal cells appeared as hemispherical structures, enclosing clusters of oval vesicles. SEM and TEM revealed the presence of an extracellular organic matrix covering the RLP with cellular debris trapped inside. These findings suggest that extrusion of the oval vesicles takes place

concurrent with cell-membrane rupture. It should be stated that the mechanisms of release of membrane-bound cell products are diverse, and few studies have addressed this subject, especially in invertebrates (Deyrup-Olsen & Luchtel 1998).

The outer layer covering the RLP surface enabled initial attachment to substrate. This layer appeared either amorphous or arranged in thin-walled projections. The latter morphology was observed in fragments that were isolated from any substrate for 20 days. This observation suggests that in the absence of substrate, such projections increase the surface area of the RLP, facilitating attachment when a substrate is encountered.

In several cnidarians, epidermal cells synthesize extracellular matrix. Chapman (1969) concluded that the cuticle in the base of scyphistomae of *Aurelia aurita* was secreted from glandular cells containing a cuticular precursor. Vandermeulen (1975), who investigated structural changes of calicoblastic cells in settled planulae of the stony coral *Pocillopora damicornis*, described membrane-bound vesicles, which may represent sites of organic matrix synthesis. This matrix was produced prior to skeletogenesis. Johnston (1979) found such a matrix in the interface between coral tissue and the calcitic skeleton of this coral and suggested that its biosynthesis occurred in small vesicles inside vacuoles. In the stony coral *Fungia fungites*, Yama-shiro & Samata (1996) found an organic matrix secreted by the calicoblastic epithelium during the course

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Fig. 2. Epidermal cellular features of an unattached RLP 5 days post-cutting. **A.** Hemispherical apices of epidermal cells. SEM. Scale bar, 10 μm. **B.** Clusters of oval vesicles within the apices of the epidermal cells. TEM. Scale bar, 1 μm. **C.** Epidermal cells coated by an outer layer. SEM. Scale bar, 10 μm. **D.** Outer layer composed of extracellular organic matrix with cellular debris (arrowheads). The space (s) is due to decalcification of calcareous material within the matrix. TEM. Scale bar, 1 μm.
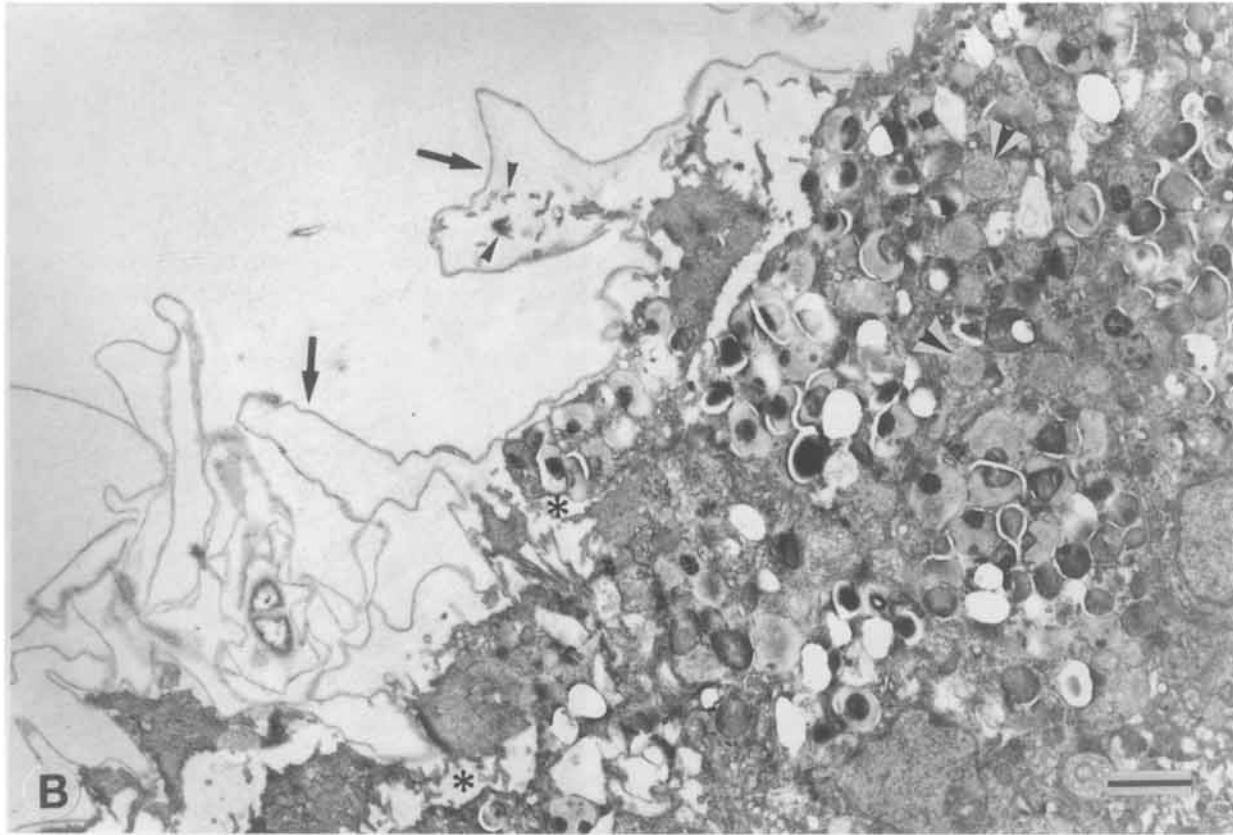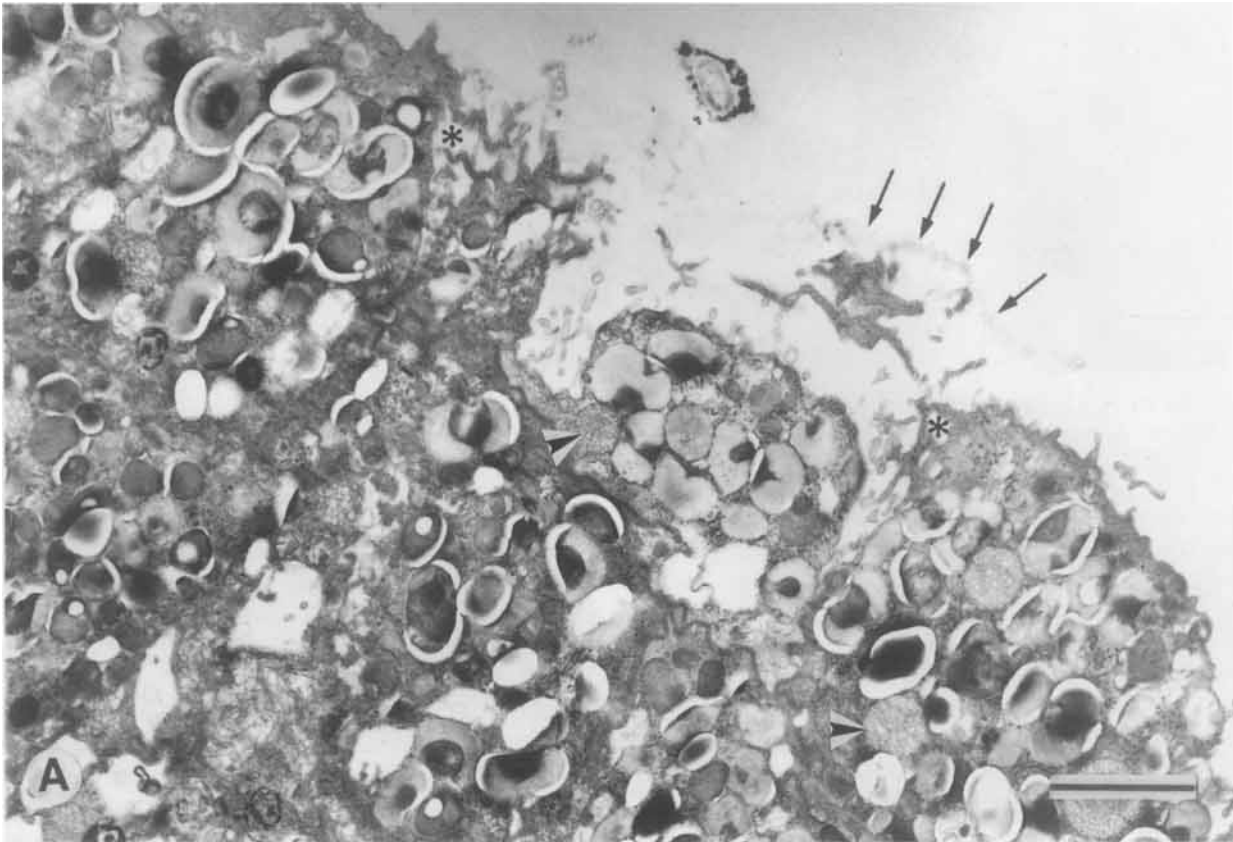
Table 1. Functions of desmocytes among cnidarians.

Organism	Type of anchoring	Reference
Hydrozoa		
Cordylophora caspia	Coenosarc to perisarc	Marcum & Diehl 1978
Scyphozoa		
Aurelia aurita	Scyphistoma to substrate	Chapman 1969
Cassiopea andromeda	Bud to substrate	Hofmann & Honegger 1990
Anthozoa (Hexacorallia)		
Pocillopora damicornis	Tissue to skeleton	Vandermeulen 1975
Stylophora pistillata	Tissue to skeleton	Muscatine et al. 1997
Anthozoa (Octocorallia)		
Heliopora sp.	Tissue to internal skeleton	Bourne 1899
Leptogorgia virgulata	Tissue to internal skeleton	Tidball 1982
Cornularia cornucopiae	Tissue to internal skeleton	Benke & Hündgen 1984
Dendronephthya hemprichi	Tissue to substrate	This paper

of disc detachment from the stalk of juvenile polyps. Similarly, we suggest that the oval vesicles residing in RLP epidermis of *D. hemprichi* are the sites of organic matrix biosynthesis. Our histochemical assays detected the presence of neutral mucopolysaccharides and/or polysacharides in the epidermal cells as well as in the organic matrix. Organic matrices produced by several cnidarians contained chitin, a linear polysaccharide, as one of their components (Wainwright 1962; Chapman 1969; Dunn & Liberman 1983). Presence of chitin in the matrix produced by *D. hemprichi* is yet to be confirmed.

Initial attachment elicited rapid cellular changes in the RLPs' epidermis, leading to the development of highly specialized anchoring cells, the desmocytes. At this stage, oval vesicles in the RLP epidermal cells were rare and areas of plicated plasma membrane appeared. Therefore, it seems that the epidermal cells that first produced the extracellular organic matrix serving for primary adhesion later transformed into desmocytes. A similar scenario was described in the gorgonian *Leptogorgia virgulata*, whose desmocytes developed from skeletogenic cells undergoing morphological changes, during which the plasma membrane displayed multiple folds and a reduction in the number of cellular organelles (Tidball 1982). As attachment of fragments usually occurs within 10 days post-cutting (Barneah 1999), the epidermal cells evidently undergo rapid changes during a short period. An indicator of such changes is the presence of nu-

merous multivesicular bodies (MVBs, Figs. 4A, 6). As far as we know, this is the first evidence of MVBs in cnidarians. In a study dealing with intracellular trafficking of glycosphingolipids (components of the plasma membrane) in vertebrates, MVBs functioned in a degradation pathway leading to final breakdown of the glycosphingolipids (Sandhoff & Klein 1994). Thus, the presence of MVBs in our study may be related to degradation processes occurring in the RLP epidermal cells in the course of morphological transformation.

To date, desmocytes in anthozoans have been shown to anchor tissue to skeleton. Our study shows that these cells also function in attachment to substrate. The desmocytes of *D. hemprichi* are morphologically similar to those described in *Stylophora pistillata* (Muscatine et al. 1997), and functionally similar to the rivet-forming cells found in the pedal disc of the scyphistomae of *A. aurita* (see Chapman 1969). According to Chapman (1969), the rivet-forming cells die after final development, and rivets are cell remnants that continue to retain their function.

Despite variation in function of desmocytes among various taxa (Table 1), they all share a common feature, i.e., association with an organic matrix. For example, in the scyphistomae of *A. aurita*, the rivets are in contact with the cuticle (Chapman 1969). Vandermuelen (1975) suggested that mesogleal processes in primary polyps of *P. damicornis* did not anchor directly to the skeleton, but to an organic matrix lining the gap between the coral tissue and its skeleton. Mus-

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Fig. 4. Epidermis of an unattached RLP, 20 days post-cutting. TEM. Gaps (asterisks) at the edge of the epidermis imply position of extruded vesicles. Multivesicular bodies (black and white arrowheads) can be seen. **A.** An amorphous outer layer (arrows) covers the epidermal microvilli. Scale bar, 2 μm. **B.** The outer layer is enclosed in thin-walled projections (arrows) containing extracellular organic matrix and organellar debris (arrowheads). Scale bar, 1 μm.
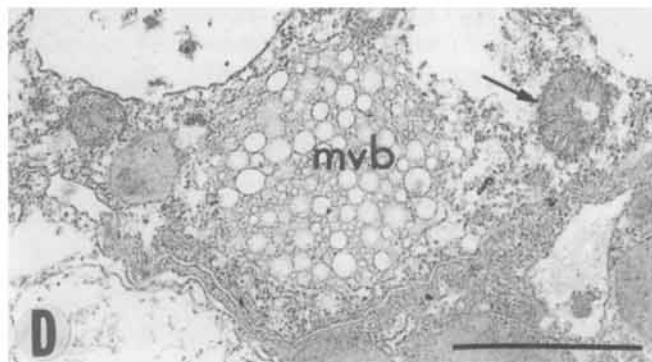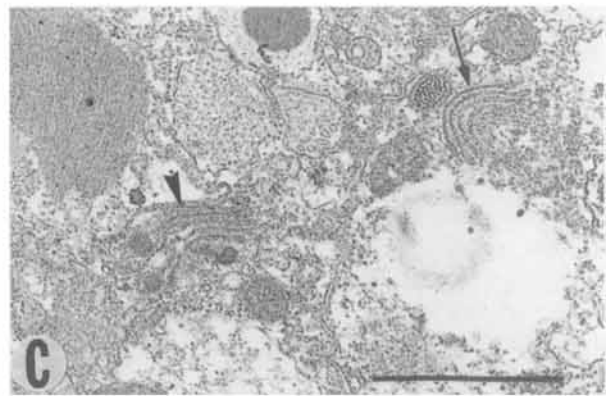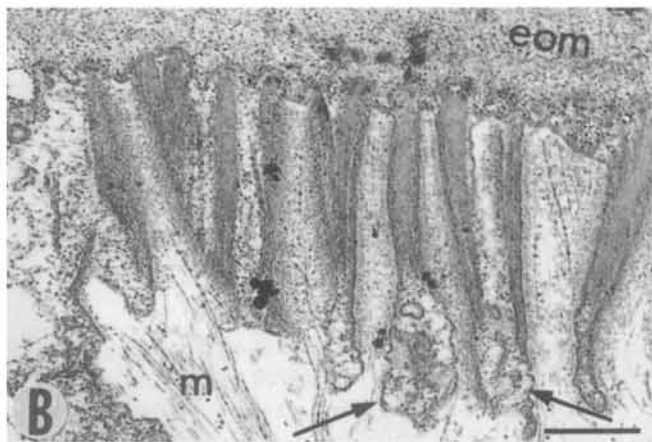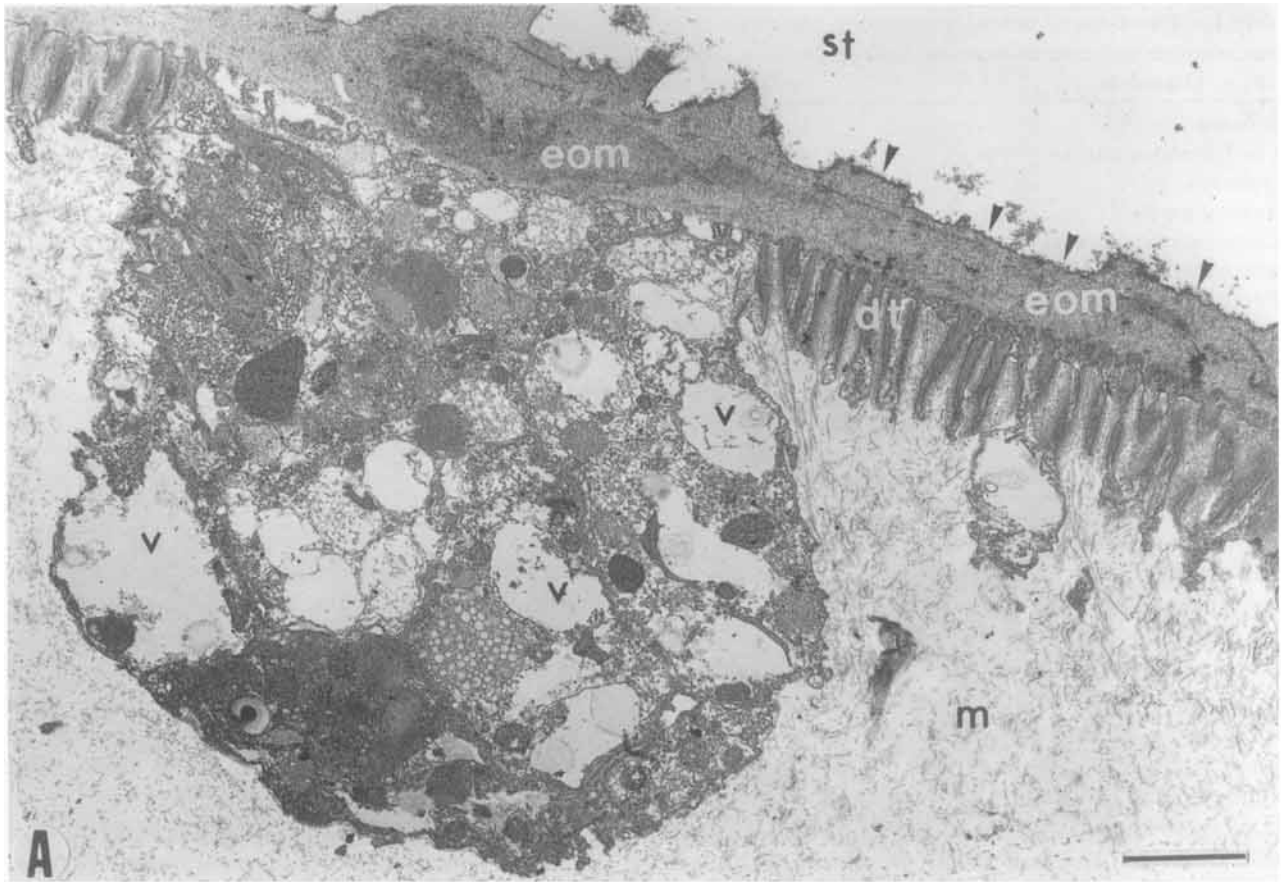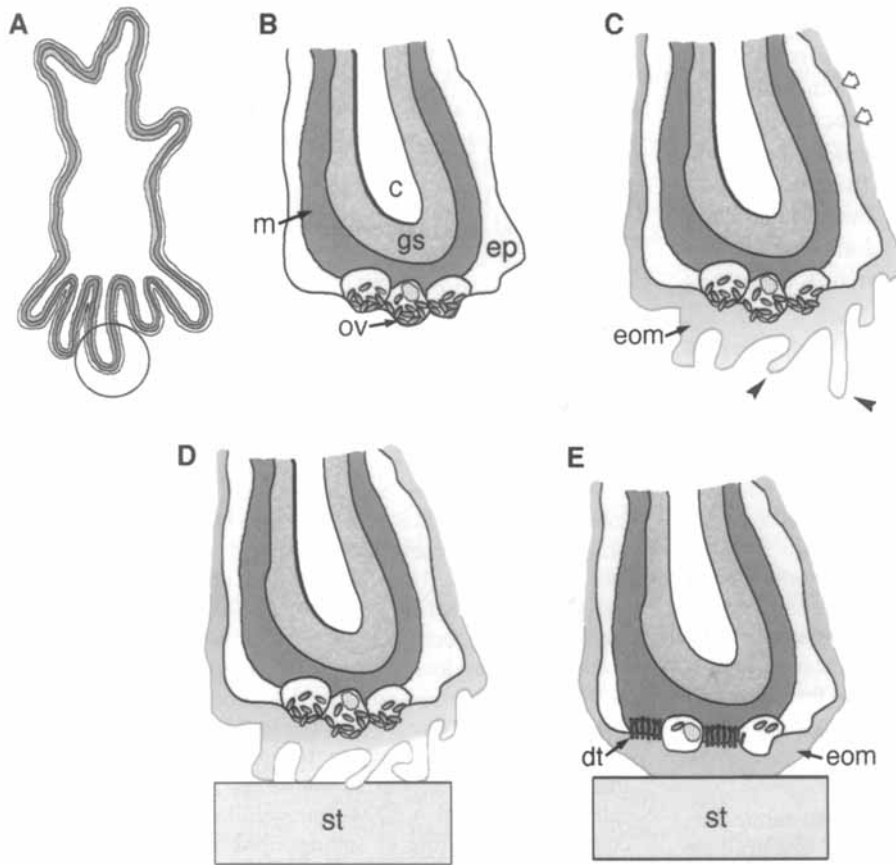
Fig. 6. Diagram of ultrastructural events in RLPs of a fragment during attachment to the substrate. **A.** Fragment in longitudinal section with RLPs at its base. Circled area is illustrated in B–E. **B.** Presence of numerous oval vesicles (ov) within epidermal cells. Epidermis (ep), mesoglea (m), gastrodermis (gs), and water canal (c). **C.** Extrusion of vesicular content forms an extracellular organic matrix (eom) coating the RLP epidermis. This layer is either amorphous (hollow arrows) or composed of thin-walled projections (arrowheads). **D.** Initial attachment to substrate (st) occurs by the aid of extracellular organic matrix. **E.** Development of desmocytes. Desmocyte tenons (dt) arranged perpendicular to the substrate with an extracellular organic matrix (eom) interface.

catine et al. (1997) described in *S. pistillata* two layers of organic material found between the desmocyte cell membrane and the coral skeleton. In each example, the organic matrix interfaces between the desmocytes and a hard surface. In *D. hemprichi,* the distal parts of the tenons facing the substrate are embedded in an extracellular organic matrix (Fig. 5A). Therefore, *D. hemprichi* is the first anthozoan in which desmocytes associated with an extracellular organic matrix are shown to serve as an adhesive device, mediating attachment to a substrate, rather than to a skeletal element produced by the organism.

Figure 6 summarizes the major cellular events leading to attachment of a fragment of *D. hemprichi,* based on our ultrastructural findings. The process begins as soon as the fragment (Fig. 6A) is removed from the colony. The recovery of the cut surface is accompanied by the appearance of numerous oval vesicles within the epidermal cells (Fig. 6B). Extrusion of these vesicles creates an outer layer (Fig. 6C) composed of extracellular organic matrix and organellar debris. This layer functions as an adhesive device, leading to initial attachment in both of its morphologies, either amorphous or arranged in thin-walled projections (Fig. 6D). Desmocyte development follows the initial attachment. Tenons, anchored in the extracellular organic matrix lining the substrate, function as miniature rivets, consequently increasing the grip of the RLP to the substrate (Fig. 6E).

The cascade of events that results in the attachment of fragments of *D. hemprichi* via RLPs to the substrate is elicited as a response to fragment removal. The de-

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Fig. 5. Desmocyte from an attached RLP 12 days post-cutting. TEM. **A.** Desmocyte cell-body with numerous vacuoles (v) and tenons (dt) embedded in mesoglea (m) along the contact area with the substrate (st). An osmophilic lining (arrowheads) encloses the extracellular organic matrix (eom), which interfaces the desmocyte and the substrate. Scale bar, 2 μm. **B–E.** Enlarged images of desmocyte organelles: **B.** Tenons embedded in mesoglea (m), their heads sunk into extracellular organic matrix (eom); their basal parts are wider (arrows). Scale bar, 0.5 μm. **C.** Golgi apparatus (arrow) and endoplasmic reticulum (arrowhead). Scale bar, 0.2 μm. **D.** Multivesicular body (mvb) with numerous minute vesicles and mitochondrion (arrow). Scale bar, 0.5 μm. **E.** Plications of the plasma membrane (arrows) possibly developing tenons. Scale bar, 0.5 μm.

velopment of numerous RLPs in combination with organic matrix production and desmocyte formation in the epidermis enables firm and rapid attachment. It is highly probable that the same scenario occurs in RLPs of naturally autotomized fragments of *D. hemprichi* (Dahan & Benayahu 1997). Whether this mechanism is widespread among other cnidarian taxa that, in the course of their life cycle, face the challenge of anchoring soft tissue to the substrate remains to be examined.

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