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Morphology of the Symbiosis Between *Corculum cardissa* (Mollusca: Bivalvia) and *Symbiodinium corculorum* (Dinophyceae)

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Abstract. Light and transmission electron microscopy of tissues of the symbiotic clam *Corculum cardissa* (L) showed that a symbiotic dinoflagellate, *Symbiodinium corculorum* (Trench), is found predominantly in the mantle and the gills. The data suggest that in *C. cardissa* the algae are located in a zooxanthellal tubular system that is associated with the hemocoel and is similar to that seen in tridacnine ("giant") clams. The algae occur within the lumen of the tertiary tubules and are thus separated from the hemolymph by a tissue that is one cell layer thick. Under a light microscope the tertiary tubules appear as rows of symbionts originating from the digestive diverticulum, presumably branching from the primary tubules that are also seen in symbiotic tridacnine clams. This morphological arrangement is discussed with regard to the ontogeny and the evolution of the tubular system within symbiotic bivalves.

Introduction

Several species of marine bivalves in the family Cardiidae harbor symbiotic dinoflagellates that belong to the genus *Symbiodinium*. These bivalves include all of the species in the subfamily Tridacninae, including the well-known genera of larger clams, *Tridacna* and *Hippopus*, as well as less well-known genera of much smaller clams in the subfamily Fraginae, such as *Corculum* and *Fragum* (Kawaguti, 1950, 1983; Schneider, 1998). For many years, the symbiotic algae in tridacnines were depicted as being located in the hemal spaces, whence they were culled by wandering amoebocytes and digested in the digestive gland; the indigestible remains were thought to reside in the kidneys (*e.g.*, Yonge, 1936, 1953, 1975, 1980; Goreau *et al.*, 1973). However, a system of tubules, arising from one of the diverticular ducts of the stomach of *Tridacna* and ramifying through much of the clam and containing the symbionts, was described by K. Mansour (1946a, b), but forgotten. Finally, 46 years later, the "zooxanthellal tubular system" was redescribed by Norton *et al.* (1992), who proposed that the primary, secondary, and blind-ended tertiary tubes of the tubular system do not connect with the hemocoel; therefore the algae are not found in the hemolymph compartment (see Fitt, 1993, for a review).

In the heart cockle, *Corculum cardissa*, as in the giant tridacnine clams, symbiotic dinoflagellates are located in the mantle tissue; but unlike the tridacnine, *C. cardissa* has many algae located in the gills as well (Kawaguti, 1968). Early electron microscopic images, in both instances, were interpreted as indicating that the algae are within the hemal system (Kawaguti, 1966, 1968). This interpretation was consistent with the author's observations that blood cells are apparently in contact with the algae in *C. cardissa* and *Tridacna* (Kawaguti, 1966, 1968).

The occurrence of symbiotic algae in a tubular system in *Tridacna* (Mansour, 1946a, b; Norton *et al.*, 1992) raises the question of whether a similar zooxanthellal tubular system also occurs in the cockle *Corculum cardissa*, or any other related species (*i.e.*, *Fragum* spp.). The goal of this study was to document evidence of a tubular system in *C. cardissa*, and to determine whether the tubules would penetrate the gill tissue, a conceptually difficult morphology. In the current study, ultrastructural observations indicate that a tubular system also exists in

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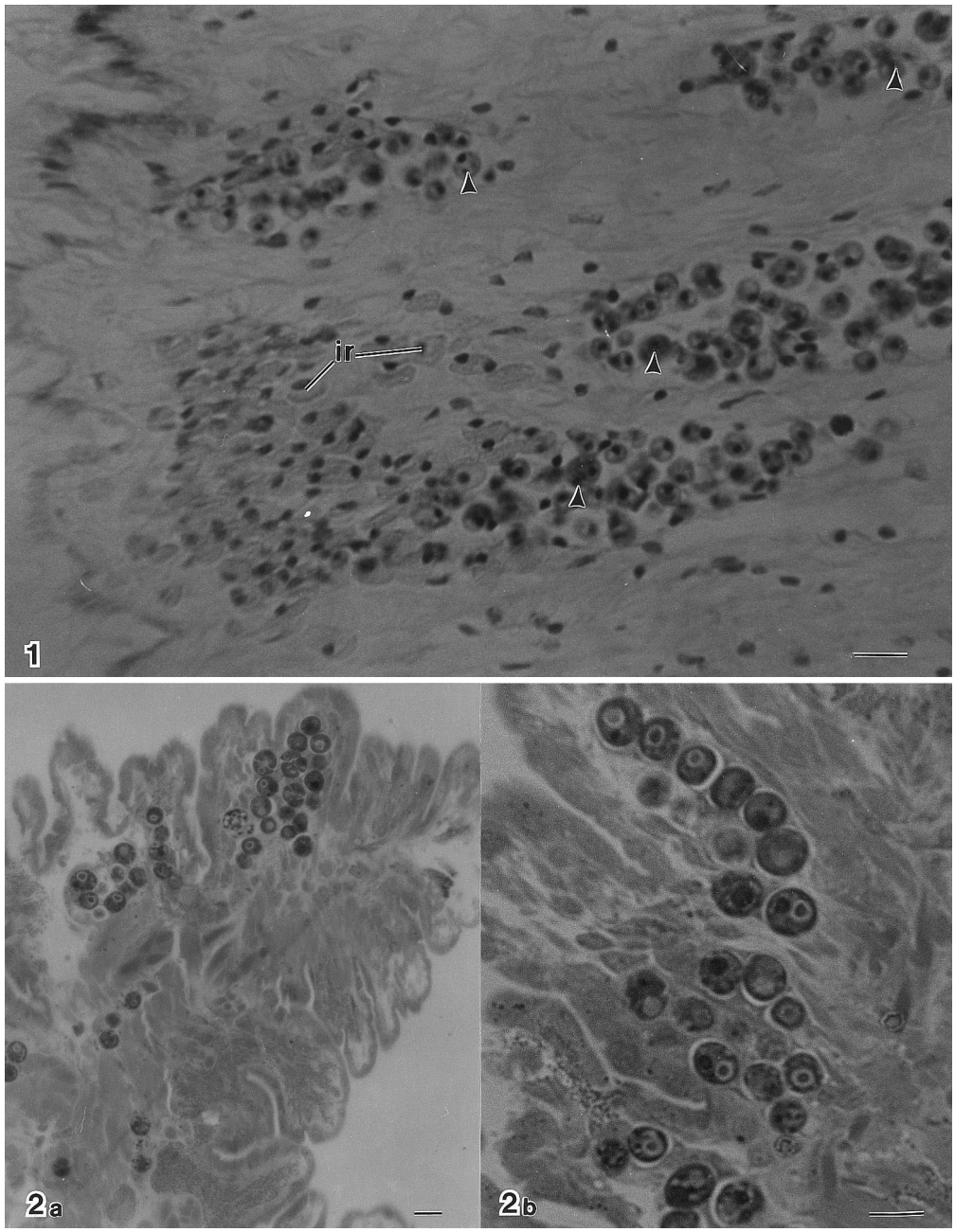

Figure 1. Light micrograph of a paraffin-embedded section through the mantle tissue of *Tridacna maxima* showing rows of symbiotic algae (arrowheads) in tertiary tubules. Iridophores (ir), animal cells with crystalline proteins that refract light, are found in the mantles of all species of clams containing symbiotic algae. Scale bar, 20 μm.

Figure 2. Light micrograph of a paraffin-embedded section through the gill tissue of *Corculum cardissa* showing rows of symbiotic algae in tertiary tubules. (a) Overview; scale bar = 12 μm. (b) Higher magnification, scale bar = 10 μm.

Corculum; that the symbiotic dinoflagellates occur within the lumina of the tubes, which themselves are located within the hemocoel; and that the algae within the tertiary tubules are separated from the hemolymph by a tissue that is mostly only one cell layer thick.

Materials and Methods

Corculum cardissa (Linne) was collected from the sandy reef flat at about 0.5 m depth in Belau (Palau), Western Caroline Islands. Animals were fixed in 6% glutaraldehyde,

postfixed in 3% osmium tetraoxide, dehydrated, and embedded in Spurr's medium as previously described (Trench *et al.*, 1981). The tissues of *Tridacna* spp. were fixed, embedded, and observed as described in Trench *et al.* (1981). Thick sections (1 μm) were prepared for examination by light microscopy on an LKM Ultratome V. These were photographed with an Olympus Vanox microscope and a PM-10 camera. Ultrathin sections for electron microscopic examination were prepared on an RMC-6000 ultramicrotome, stained with uranyl acetate and lead citrate in the standard manner, and observed and photographed with a Philips 400 transmission electron microscope (TEM).

Results and Discussion

Light microscopic examination of *Corculum cardissa* revealed that, similar to observations made on symbionts living in mantle tissues of *Tridacna* (Fig. 1; Mansour, 1946a; Fitt and Trench, 1981; Norton *et al.*, 1992; Norton and Jones, 1992), algal cells are arranged in rows in both the mantle (*e.g.*, Kawaguti, 1968; Figs. 1, 2) and the gills (Fig. 2). However light microscope observations could not resolve the tertiary tubule structure in either genus of clam.

Electron microscopic examination of the gills of *Corculum* (Fig. 3) shows that the algae are indeed juxtaposed to animal blood cells. Kawaguti's (1968) early descriptions from *C. cardissa* note that algae are sometimes accompanied by "wandering cells," but he includes no figures. In contrast, TEM pictures of symbionts in *Tridacna crocea* and *T. maxima* clearly show nearby animal cells (Kawaguti, 1966; Fitt and Trench, 1981). Algal symbionts in *C. cardissa* are not in direct contact with the animal's blood cells, but are separated from the hemolymph and the blood cells

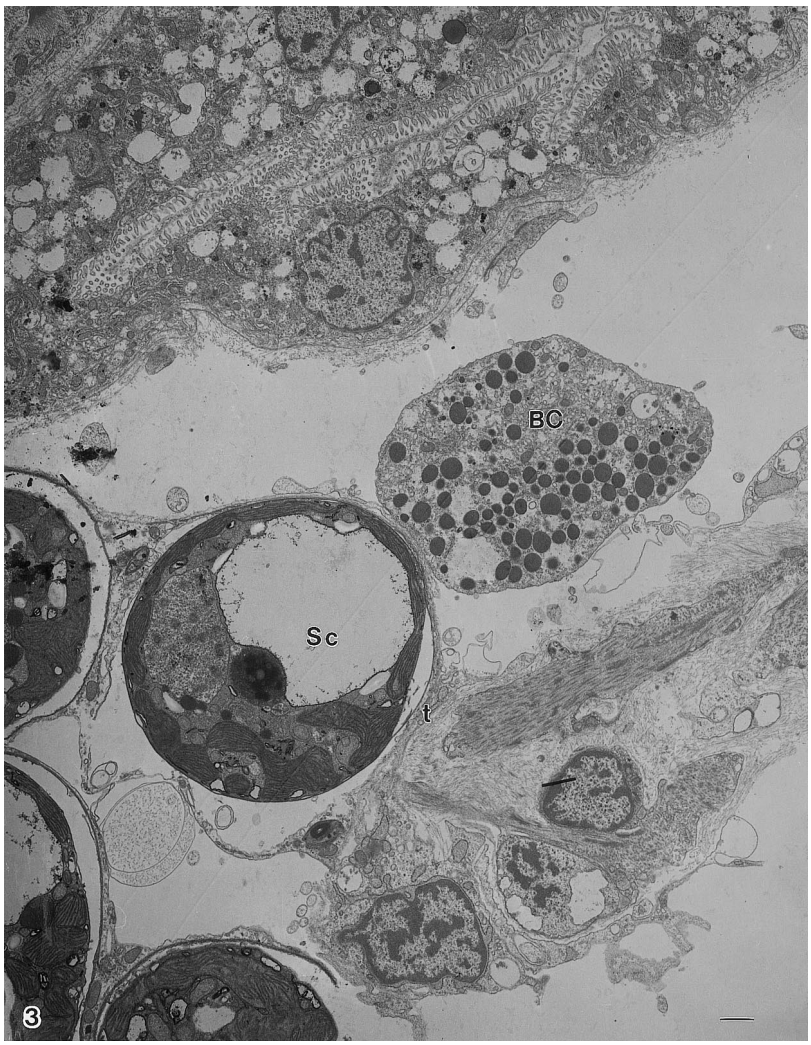

Figure 3. Transmission electron micrograph of a portion of the gill of *Corculum cardissa* showing a portion of the tertiary tubule (t), and a blood cell (BC) close to cells of the alga *Symbiodinium corculorum* (Sc) in the tubule. Scale bar, 1 μm.

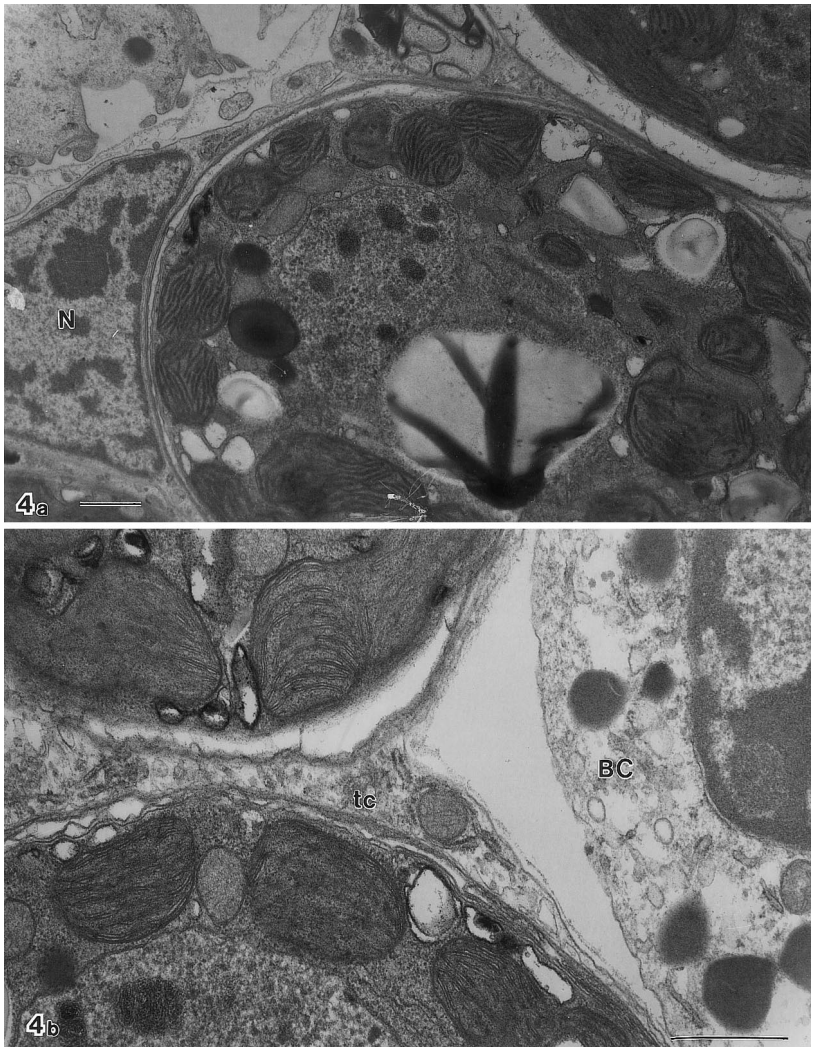

Figure 4. Transmission electron micrograph of the relation between the symbiotic algae, the tubule cells, and the blood cells in gill tissue of *Corculum cardissa*. (a) An algal cell closely appressed to a tubule cell. The algal cell wall is juxtaposed to the tubule cell plasmalemma, which can be followed around the enclosed cell nucleus, which it encloses. (b) Two algal cells in adjacent tertiary tubules, separated by the cytoplasm of the two tubule cells (tc). A blood cell is close by (bc). Scale bars, 1 μm.

by the cells of the tubules, which at the tertiary level are about one cell layer thick (Figs. 3–5). Evidence for this comes from closer examination of high-magnification TEM images of the structural relations between the algae, the cells of the tubules, and the blood cells (Fig. 4). First, algae in the lumina of the tubules are often pressed against the inner plasmalemma of the tubular cells; when nuclei of the appressed tubular cells are apparent (as in Fig. 4a), this could lead to the interpretation that the algae are intracellular (*e.g.*, Kawaguti, 1968). Second, the algae are clearly separated from the molluscan blood cells by the cells composing the tubules (Fig. 4b). Overlapping cell processes form the tertiary tubules (Fig. 5). Two or more unseparated adjacent symbionts (Fig. 6) also indicate that the algae are in tubules and not living intracellularly within host cells.

In bivalves that harbor symbiotic dinoflagellates, the structure of the tubular system, in which the tubules arise from the digestive system and are contiguous with it, suggests that the morphological and functional relation between host and symbionts in bivalves is directly analogous to that found in symbiotic cnidarians (Fitt, 1993). In both cases, the algae enter the digestive system *via* the mouth. In bivalves, symbiotic dinoflagellates enter *via* the mouth, and exit *via* the anus (Ricard and Salvat, 1977; Trench *et al.*, 1981; Maruyama and Heslinga, 1997); their entire residence in the clam is in association with the digestive system. In contrast, symbionts in cnidarians enter and exit *via* the mouth and eventually take up residence inside of host digestive cells. The location of the symbionts in bivalves and cnidarians is also analogous with respect to metabolite flux between host

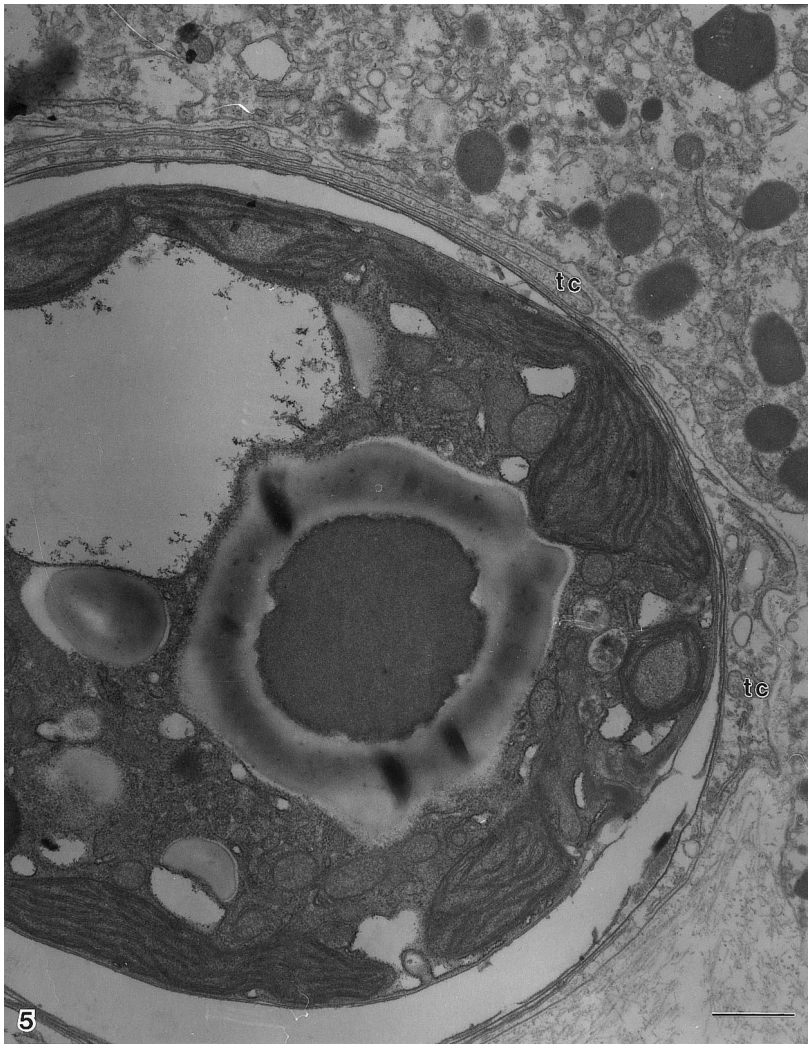

Figure 5. Transmission electron micrograph of an alga in the gill of *Corculum cardissa* surrounded by overlapping processes of tertiary tubule cells (tc). Scale bar, 1 μm.

and symbiont (Fitt *et al.*, 1985). In bivalves, where the algae are intercellular, they are separated from the hemolymph (circulating nutrients) by the proximal and distal plasmalemma of the tubule cells (Fig. 7). Hence, nutrient exchange between the algae and the hemolymph of bivalves may potentially be regulated by the tubule cells. In cnidarians, where the algae are intracellular, the symbionts are separated from their nutrient source, the gastrovascular system, by two membranes, the host cell plasmalemma and the symbiosome membrane.

The presence of the tubular system in symbiotic bivalves is also significant from ontogenetic and evolutionary perspectives. Studies of algal symbioses in tridacnines (Fitt and Trench, 1981) clearly show that the tubules develop only in the presence of dinoflagellate symbionts; the algae in the tubules are observed as "rows extending from the region of the stomach and digestive gland toward the developing siphonal tissue" (Fitt *et al.*, 1981). Juvenile clams that were allowed to develop in the absence of *Symbiodinium* did not show evidence of this feature. From the report of Norton *et al.* (1995), it is also apparent that, when the algae are lost from *Tridacna* during events of thermal "stress," the tubules atrophy. Whether the tubules are reformed should the symbiosis recover, or whether lack of recovery of bleached clams is the result of the inability of the tubular system to regenerate, is unknown.

For marine symbioses, only two other instances have been recorded in which the symbionts appear to play a significant role in the ontogenetic event in the host. One example is the process of strobilation in symbiotic scyphozoans such as *Mastigias* (Sugiura, 1964) and *Cassiopeia* (Colley and Trench, 1985); these jellyfish produce ephyrae only in the presence of *Symbiodinium*. Another example is the influence that *Vibrio fischeri*, a symbiotic luminous bacteria, has on morphogenesis of certain parts of the light organ in the squid *Eprymna scolopes* (Claes and Dunlap,

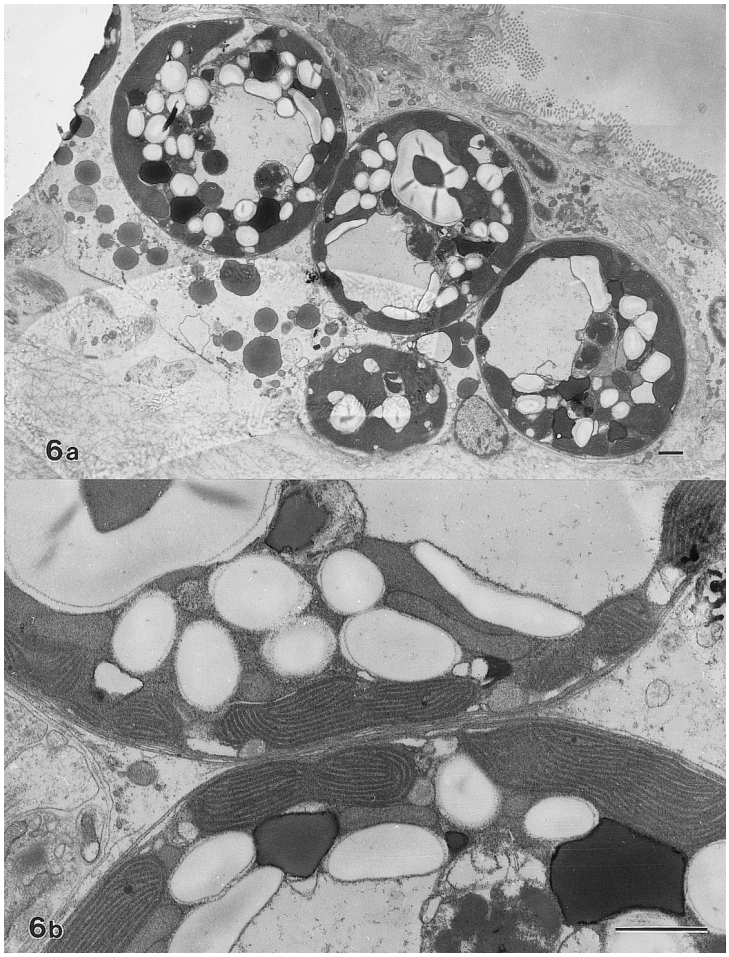

Figure 6. Symbiotic algae in tertiary tubules in the gills of *Corculum cardissa*, showing (a) several symbionts in a tubule and (b) high magnification of the two cells in 6a. Scale bar = 1 μm.

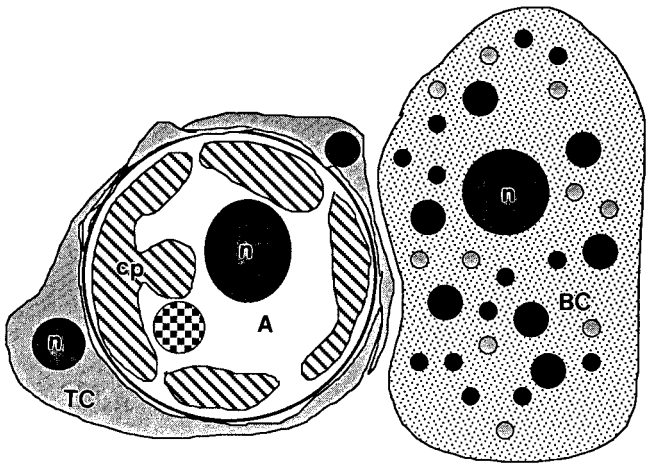

Figure 7. Schematic representation of the relation between the alga (A), the tubule cells of the tertiary tubules (TC), and the hemolymph containing blood cells (bc). n, nucleus; cp, chloroplast.

2000). In neither example are the "signals" that elicit the developmental response in the host known. In the case of the dinoflagellate associations, the exopolysaccharides exuded by *Symbiontinium* (Markell *et al.*, 1992; Markell and Trench, 1993) may be a source of the signals. For instance, in the process of root nodulation in leguminous plants, the initiation of root hair curling and infection thread formation are dependent on chemical signals from the bacterial symbionts (Brewin, 1991).

A system of tubules originating in the stomach and ramifying through the hemolymph is uncommon in bivalve molluscs and appears to be directly related to symbiosis with dinoflagellates. As far as is known, no nonsymbiotic bivalves demonstrate this feature. In addition to the tridacnine clams and *Corculum cardissa* described here, the bivalve *Fragum fragum* has also been reported to harbor symbiotic dinoflagellates (Kawaguti, 1983), and recent TEM images (Kempf, unpubl.) show morphological features similar to those presented here for *C. cardissa*. This finding supports the interpretation

that all algal symbionts in molluscs occur in tubule extensions of the digestive system. Cladistic analyses based on morphological characters (Schneider, 1992, 1998) and phylogenetic relationships based on analysis of small subunit ribosomal RNA gene sequences (Maruyama *et al.*, 1998) both indicate that the known bivalves with symbiotic dinoflagellates are closely related, all belonging to Cardiidae. In addition, the available molecular genetic evidence (NcNally *et al.*, 1994) reveals that the symbiotic algae associated with *Corculum* and *Tridacna* are also very closely related (LaJeunesse, 2000), but not identical. We also suppose that freshwater bivalves, such as *Anodonta*, that are symbiotic with the green alga *Chlorella* sp. (Pardy, 1980) may demonstrate a tubular structure in which to house the algae, as these symbionts probably also enter their hosts through the digestive system.

The only other molluscan group that shows an analogous morphology is the opisthobranch gastropods: some saccoglossan opisthobranchs temporarily harbor derived chloroplasts from feeding (Trench, 1975), and eolid nudibranchs often maintain dinoflagellates for a short time after feeding on symbiotic cnidarians (Kempf, 1984). Most significantly in relation to symbiotic bivalves, in eolidacean nudibranchs "branches of the posterior aorta . . . accompany the branches of the midgut gland (digestive diverticulum) into the cerata . . ." (Hyman, 1967, p. 477), suggesting development of tubules in conjunction with development of blood vessels, or *vice versa*.

We speculate that bivalves, like their gastropod relatives, possess a suite of genes that encode the expression of the tubular system, but these genes are expressed only after activation by some "signal" produced by dinoflagellate symbionts as they enter the host digestive tract. These situations would be analogous to the production of various galls in plants following infection by bacteria, fungi, insects, or other parasitic plants (Bidwell, 1979), all of which produce chemical signals.

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