Reference: *Biol. Bull.* **200:** 20–32. (February 2001)

Metamorphosis of Coeloblastula Performed by Multipotential Larval Flagellated Cells in the Calcareous Sponge *Leucosolenia laxa*

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Abstract. The calcareous sponge *Leucosolenia laxa* releases free-swimming hollow larvae called coeloblastulae that are the characteristic larvae of the subclass Calcinea. Although the coeloblastula is a major type of sponge larva, our knowledge about its development is scanty. Detailed electron microscopic studies on the metamorphosis of the coeloblastula revealed that the larva consists of four types of cells: flagellated cells, bottle cells, vesicular cells, and free cells in a central cavity. The flagellated cells, the principal cell type of the larva, are arranged in a pseudostratified layer around a large central cavity. The larval flagellated cells characteristically have glutinous granules that are used as internal markers during metamorphosis. After a free-swimming period the larva settles on the substratum, and settlement apparently triggers the initiation of metamorphosis. The larval flagellated cells soon lose their flagellum and begin the process of dedifferentiation. Then the larva becomes a mass of dedifferentiated cells in which many autophagosomes are found. Within 18 h after settlement, the cells at the surface of the cell mass differentiate to pinacocytes. The cells beneath the pinacoderm differentiate to scleroblasts that form triradiate spicules. Finally, the cells of the inner cell mass differentiate to choanocytes and are arranged in a choanoderm that surrounds a newly formed large gastral cavity. We found glutinous granules in these three principal cell types of juvenile sponges, thus indicating the multipotency of the flagellated cells of the coeloblastula.

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Introduction

All major sponge groups emerged on the earth in the late Precambrian, about 580 million years ago (Li *et al.*, 1998). Thereafter they have changed in different ways and adapted the structure of their larvae and process of metamorphosis to survive through the subsequent geologic eras. As a result of adaptation, sponge development is greatly diversified among higher taxonomic groups (Brusca and Brusca, 1990). Most sponges are viviparous, but some are oviparous. Some viviparous sponges release crawling larvae, but most release swimming larvae, of which there are a number of types. An amphiblastula or coeloblastula is produced by calcareous sponges; a parenchymella is the larva of the majority of demosponges, but several other larval types are known; and a trichimella is exclusive to hexactinellid sponges. The modes of metamorphosis of these larvae are also quite variable (Simpson, 1984). In spite of such variations, recent molecular evidence shows that all sponges are of monophyletic origin (Müller, 1997).

Because all adult sponges are sessile animals, their free-swimming larval period is a critical one for habitat selection. After the free-swimming period, larvae settle on the substratum and initiate metamorphosis. The layer of flagellated cells is a larval organ for swimming, therefore those cells disappear soon after settlement. Because the sponge larvae are generally minute and their nutritive materials are limited, it seems very disadvantageous to discard the flagellated cells during metamorphosis. The developmental fate of these cells in the parenchymellae of demosponges has long been debated. One view holds that the flagellated cells are transformed into the choanocytes of juvenile sponges (Borojevic and Levi, 1965; Boury-Esnault, 1976; Amano and Hori, 1994, 1996), but the opposing view contends that

they are lost by exfoliation or phagocytosis during metamorphosis (Bergquist and Glasgow 1986; Misevic *et al.*, 1990; Kaye and Reiswig, 1991). The flagellated cells in the coeloblastulae of calcareous sponges cannot, however, be discarded, because these cells make up the greater part of the larva.

The coeloblastulae of calcareous sponges have been studied very rarely, and we know little about the process of their metamorphosis (Minchin, 1896; Tuzet, 1947; Borojevic, 1969). In this study we will show the fine structure of the coeloblastula, metamorphosing larva, and juvenile sponge of *Leucosolenia laxa* in clear electron micrographs. After settlement, the larval flagellated cells dedifferentiate into a simple cell mass on the substratum; thereafter they differentiate again into the three principal cell types of a juvenile sponge. We discuss the multipotency of the larval flagellated cells of *L. laxa* by comparing them with the developmental potency of the larval flagellated cells of other sponges.

Materials and Methods

Sponges and larvae

In early September, specimens of *Leucosolenia laxa* were collected from rafts in Mutsu bay in northern Japan. *L. laxa* is one of the most common calcareous sponges in this region. Immediately after collection, the sponges were placed in containers with seawater, brought to the laboratory of Asamushi Marine Biological station within 1 h, and kept in running seawater.

To collect larvae, in the early morning sponges were placed in glassware with seawater. Under the natural illumination, about one-tenth of them released larvae, beginning soon after dawn and ceasing by noon. So the larval release of this calcareous sponge is probably controlled by light cycles, as it is in certain demosponges (Amano, 1986, 1988; Maldonado and Young, 1996). The larvae, which began swimming immediately upon release, were placed in petri dishes with filtered seawater; the seawater was changed daily. The larvae and juveniles can be reared for a week under these conditions.

Electron microscopy

Released larvae swam around mainly under the water surface, and after a swimming period, settled on the glass surface or beneath the air-water interface. Free larvae were picked up on a platinum loop and placed into fixative. Settled larvae were collected from beneath the air-water interface in the same way. This procedure minimized the mechanical stress of the larvae during collection.

It is quite difficult to preserve the ultrastructure of the larvae of this species satisfactorily. After trying a number of fixatives, we found the following formula to be the best: 1% paraformaldehyde, 7.5% glutaraldehyde, and 14% sucrose in 0.1 *M* cacodylate buffer, pH 7.4. Larvae or juvenile sponges were fixed in the ice-cold fixative for 1 h. Then the samples were rinsed twice in the same buffer and postfixed in 1% osmium tetroxide in 0.1 *M* cacodylate buffer, pH 7.4, for 1 h. They were dehydrated through a graded ethanol series, cleared in propylene oxide, and embedded in Spurr epoxy resin (Spurr, 1969). Semithin sections were stained with toluidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate, then examined and photographed under a Hitachi H-500 electron microscope.

Results

Free-swimming larva

The calcareous sponge *Leucosolenia laxa* releases free-swimming coeloblastulae through the osculum with the excurrent water. The free larvae were fixed soon after release and on the next day. We examined larvae collected after different periods of swimming but found no morphological difference; thus we show only the images of the free larvae fixed soon after release. The coeloblastula is a hollow spheroid consisting of a layer of flagellated cells around a large central cavity (Fig. 1). This larva, which is about 90 μm in length and about 50 μm in width, is entirely ciliated—including its posterior pole. The coeloblastula contains three additional types of cells: bottle cells, vacuolar cells, and free cells in the central cavity. Figure 1 also shows the polarity of the larva. Its anterior pole consists exclusively of flagellated cells, and its posterior pole includes several vacuolar cells.

Flagellated cells. Figure 2 shows the flagellated cells of the coeloblastula of *L. laxa*. These cells are organized in a pseudostratified cell layer that is about 15-μm thick and

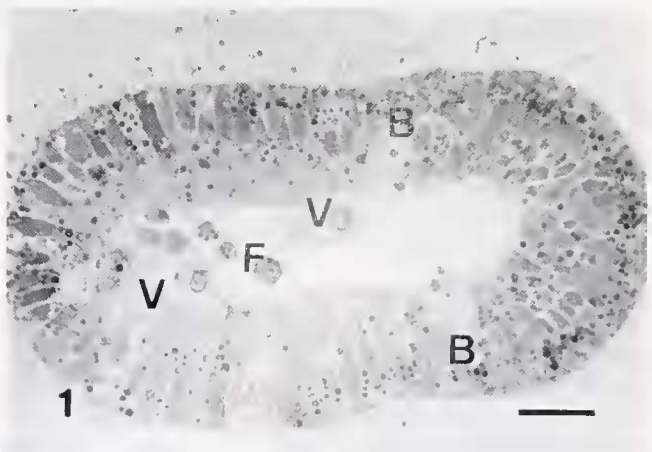


Figure 1. Coeloblastula of *Leucosolenia laxa*. The pseudostratified layer of flagellated cells surrounds a large central cavity. B: bottle cell, F: free cell in the central cavity, V: vacuolar cell. Scale bar = 10 μm.

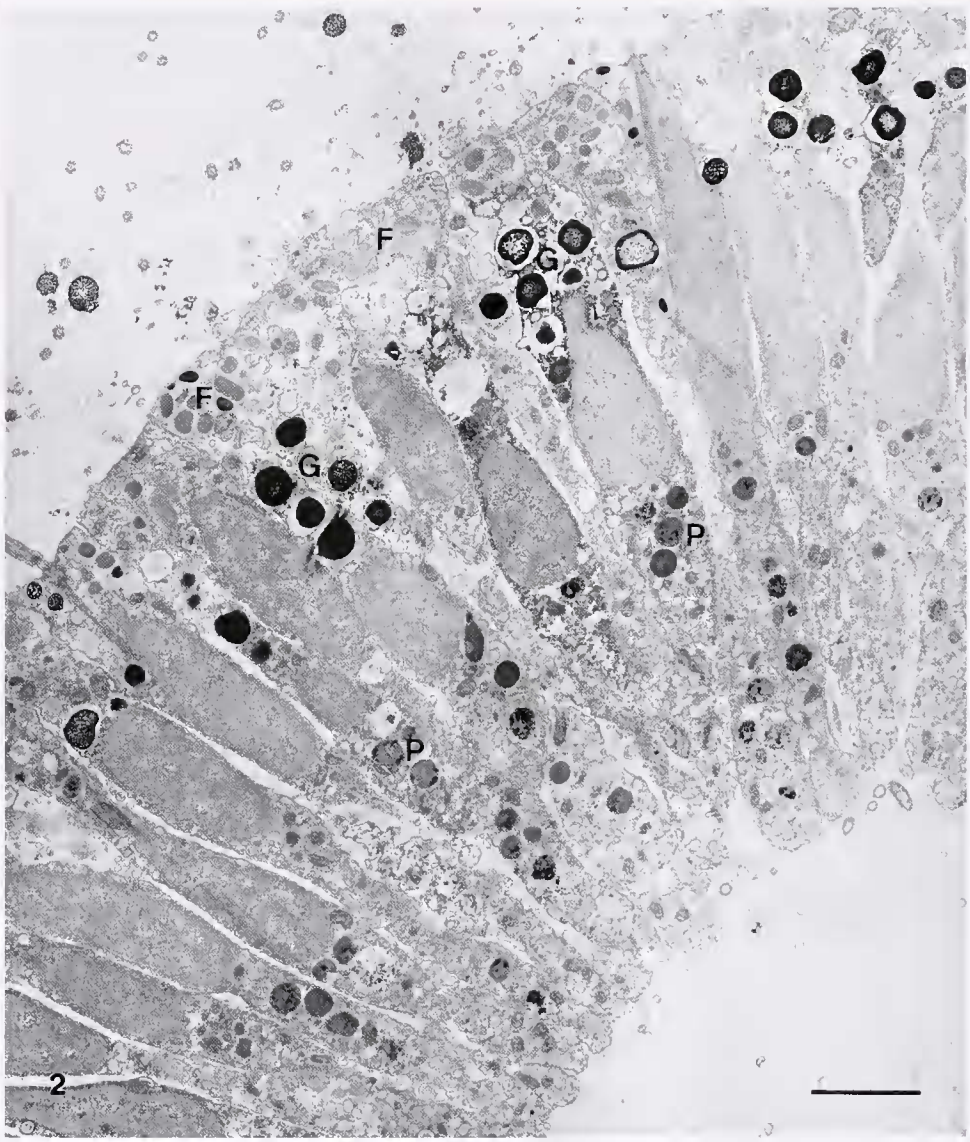


Figure 2. The pseudostratified layer of flagellated cells in the coeloblastula of *Leucosolenia laxa*. F: fibrous granule in the outermost cytoplasm, G: glutinous granule in the apical cytoplasm, P: phagosome-like granule in the basal cytoplasm. Scale bar = 2 μm.

lacks a basal lamina. One flagellum emerges from the outer surface of the cell. There are glutinous granules in the apical cytoplasm and fibrous granules in the outermost cytoplasm. An elongated nucleus, about 5 × 2 μm, has a nucleolus and heterochromatin masses. The basal cytoplasm contains phagosome-like granules, and the innermost region of the cell is filled with many small (about 0.25 μm) vesicles and a few lipid droplets (Fig. 3). There are mitochondria in both the apical cytoplasm and the basal cytoplasm.

Figures 4 and 5 show magnified images of the apical region of flagellated cells. It is apparent that one flagellum emerges from the flat outer surface of the cell. The most proximal portion of the flagellum is somewhat electron-dense. From a basal body, two rootlets extend downward to the sides of a nucleus. The rootlets are striated, with a distance of about 40 nm between the midpoints of two neighboring striae. There is a basal foot running parallel to the outer surface. The Golgi apparatus is always located vertically along the rootlet. Only in the apical region are the neighboring flagellated cells united, by simple apposition (Figs. 4 and 5); in the deeper region, there is a wide space between the cells (Fig. 2).

We found a consistent arrangement and orientation in the apical region of larval flagellated cells. One side of the outermost portion of the cell protrudes toward a neighbor cell, and the protrusion is regularly directed to the anterior end of the larva (Figs. 4 and 5). A basal foot consistently extends in the opposite direction—that is, to the posterior

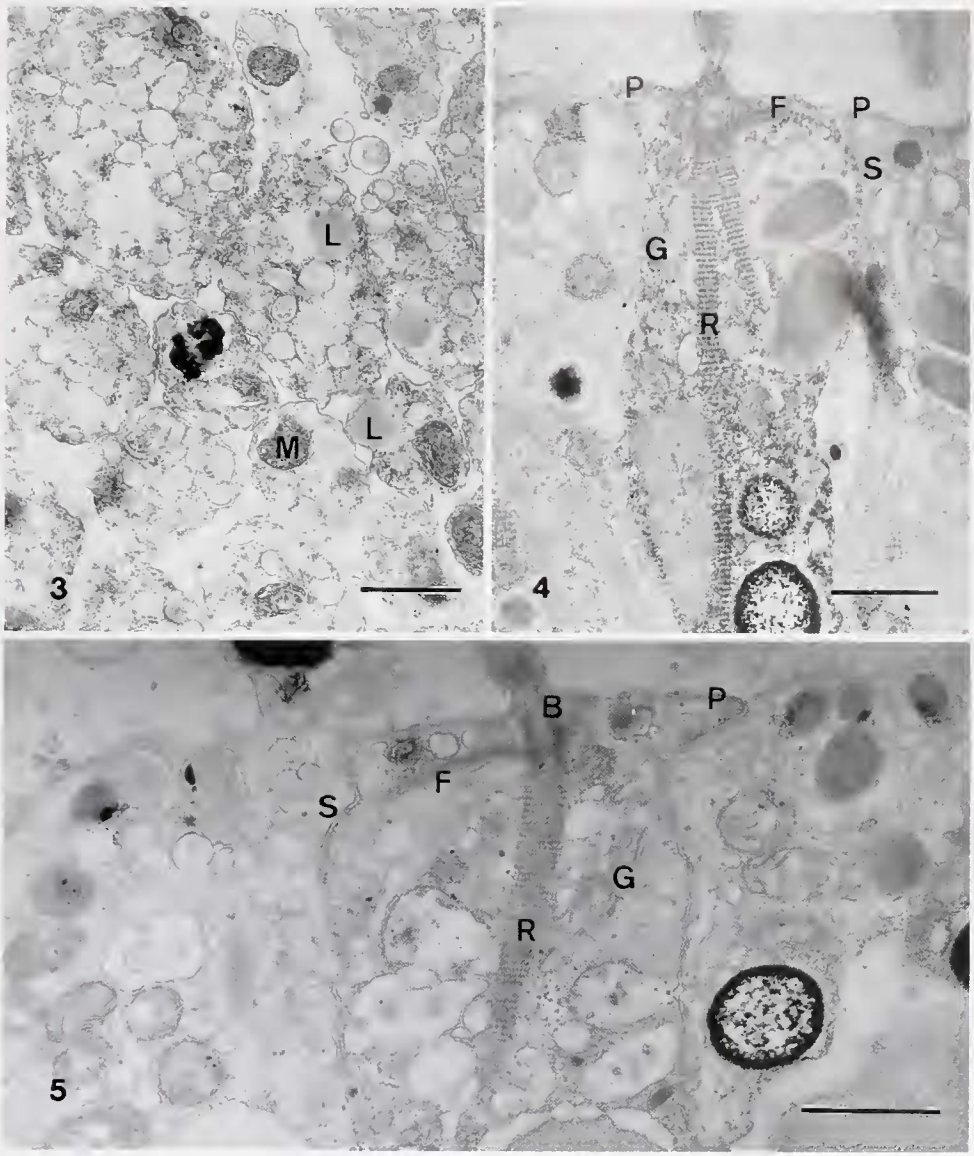


Figure 3. The innermost region of flagellated cells in the coeloblastula of *Leucosolenia laxa*. L: lipid droplet, M: mitochondrion. Scale bar = 1 μm.

Figure 4. Apical region of flagellated cells in the coeloblastula of *Leucosolenia laxa* showing two basal rootlets (R) and a basal foot (F). G: Golgi apparatus, P: protrusion of the outermost portion of flagellated cells, S: simple apposition. Scale bar = 1 μm.

Figure 5. Flagellar basal apparatus of *Leucosolenia laxa*. B: basal body, F: basal foot, G: Golgi apparatus, P: protrusion of the outermost portion of flagellated cells, R: basal rootlet, S: simple apposition. Scale bar = 1 μm.

end of the larva. The Golgi apparatus is always located anteriorly to the rootlet (Fig. 4). These precise spatial arrangements seem to be essential for aligning the direction of an effective stroke.

Figure 6 shows glutinous granules of about 1-μm diameter in the apical cytoplasm of flagellated cells. Their surface layer is very electron-dense, and the inner part is made of the loose network of a fine granular substance. The name glutinous granules was chosen because they are made of a very sticky substance. When paraformaldehyde was omitted from the fixative, some of these granules were discharged from the cell, and their content was released into the water. The released content was found to be glutinous because it adhered to flagella and made them very sticky. The sticky flagella became crooked and tangled with each other in the fixative. Figure 7 shows fibrous granules of about 1.0 $\times$ 0.5 μm. They are yolk granules filled with a fibrous substance

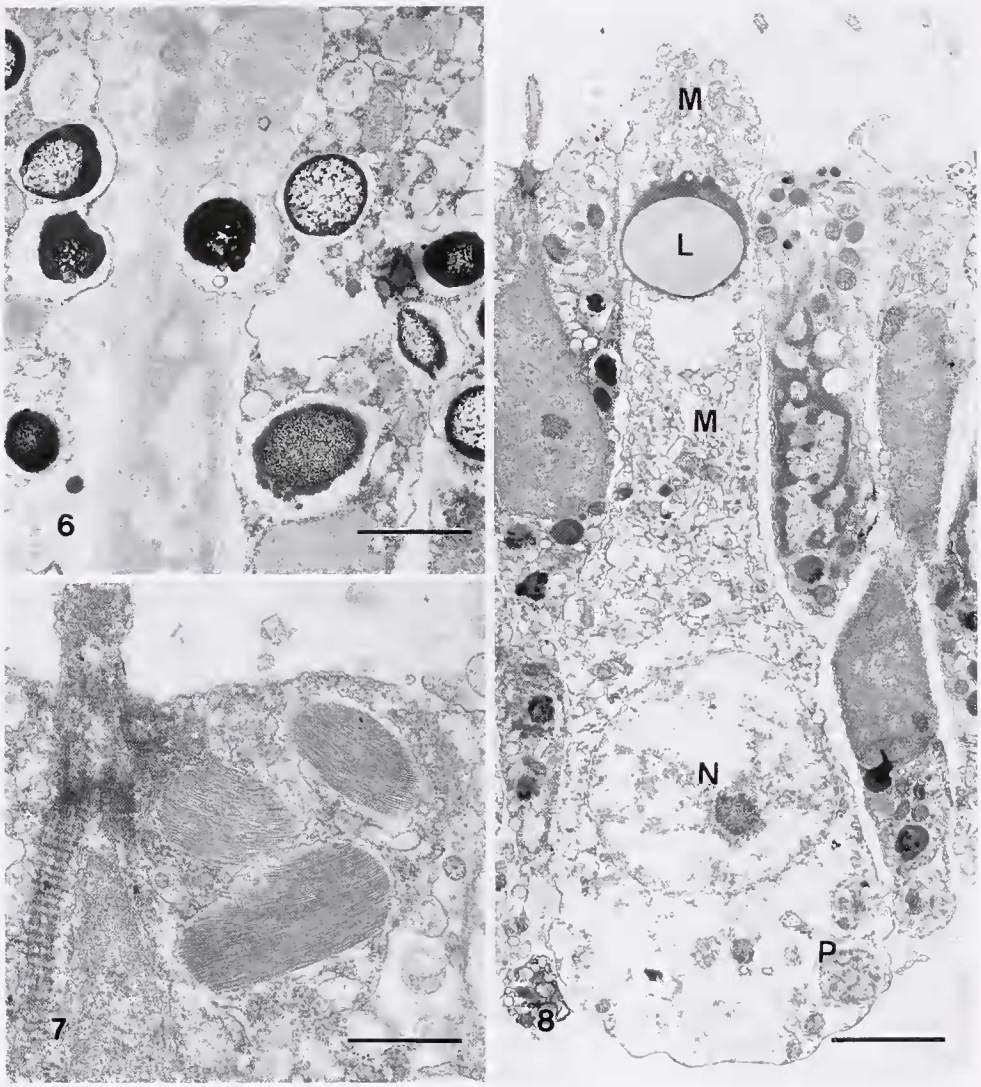


Figure 6. Glutinous granules in the apical cytoplasm of flagellated cells in the coeloblastula of *Leucosolenia laxa*. Scale bar = 1 μm.

Figure 7. Fibrous granules in the outermost cytoplasm of flagellated cells in the coeloblastula of *Leucosolenia laxa*. Scale bar = 0.5 μm.

Figure 8. A bottle cell between flagellated cells in the coeloblastula of *Leucosolenia laxa*. L: lipid droplet, M: membranous structure, N: nucleus, P: phagosome-like granule. Scale bar = 2 μm.

that runs parallel with the long axis. These granules are located in the outermost cytoplasm of flagellated cells (Fig. 2).

Bottle cells. Bottle cells are large (about 18 × 6 μm) cells that are located between flagellated cells but have no flagellum themselves (Fig. 8). There are several bottle cells in a larva. These cells are longer than flagellated cells and protrude outside and into the central cavity. Their nucleolated nucleus is usually located in the basal region. The apical and middle cytoplasm are filled with membranous structures, and the cell shown in Figure 8 has a large lipid droplet. In the basal cytoplasm there are phagosome-like granules, some of them have digested their content.

Vacuolar cells. These cells, having an irregular form, are filled with large vesicles (Fig. 9). Their nucleolated nucleus has a large number of heterochromatin masses. There are about 10 vacuolar cells in a larva, most of them concentrated in the posterior region (Fig. 1). In most cases, a part of the cell is inserted between flagellated cells, but the greater part protrudes into a central cavity.

Free cells in a central cavity. There are a few free cells in a central cavity (Fig. 1). These cells are small, with scanty cytoplasm (Fig. 10). They have an undeveloped Golgi ap-

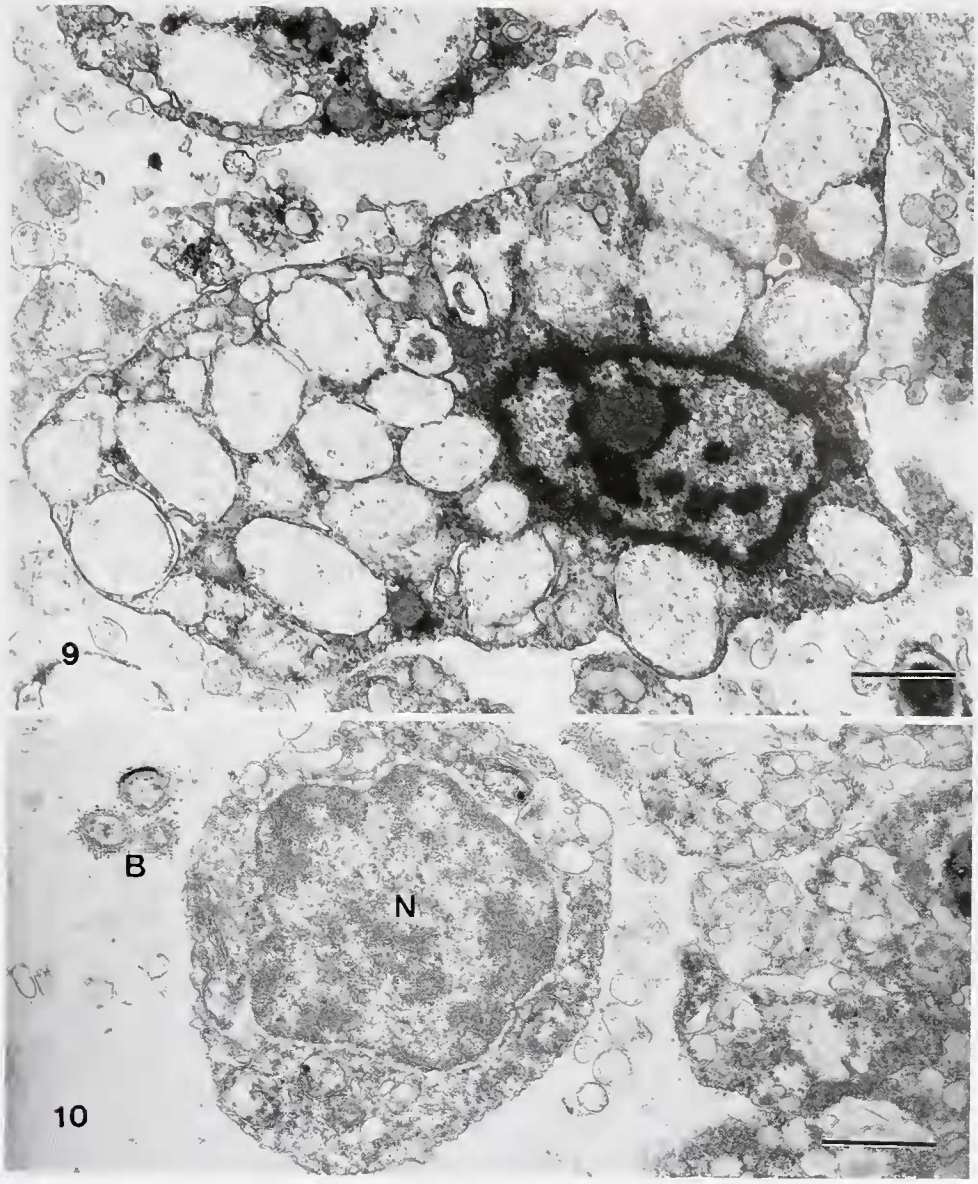


Figure 9. A vesicular cell in the coeloblastula of *Leucosolenia laxa.* Scale bar = 1 μm.
Figure 10. A free cell in the central cavity of the coeloblastula of *Leucosolenia laxa.* B: symbiotic bacteria, N: nucleus. Scale bar = 1 μm.

paratus and a small number of mitochondria. Figure 10 also shows symbiotic bacteria in the central cavity.

Metamorphosis

The coeloblastulae of *L. laxa* begin to settle after a free-swimming period of about 36 h or more, and the settling larvae soon spread out on the substratum. Figure 11 shows a larva that has rounded up about 18 h after settlement. The larva of this stage consists of a peripheral layer of pinacocytes and an inner cell mass (Fig. 12). About 36 h after settlement, choanocytes are differentiated in the inner cell mass and formation of the choanocyte chamber begins. Figure 13 shows a juvenile sponge about 72 h after settlement. It has a large choanocyte chamber, or gastral cavity, with an osculum. Its body wall is very thin and transparent, hence the characteristic triradiate spicules are clearly visible.

Pinacocytes. Soon after settlement, larvae lose their flagella and become a mass of dedifferentiated cells. The cells at the surface become flattened and cover the inner cell mass. Figure 14 shows a part of a settled larva consisting of a pinacoderm and an inner cell mass. It is apparent that both cell types have glutinous granules, thus showing their origin. The pinacocytes have already begun to flatten but are

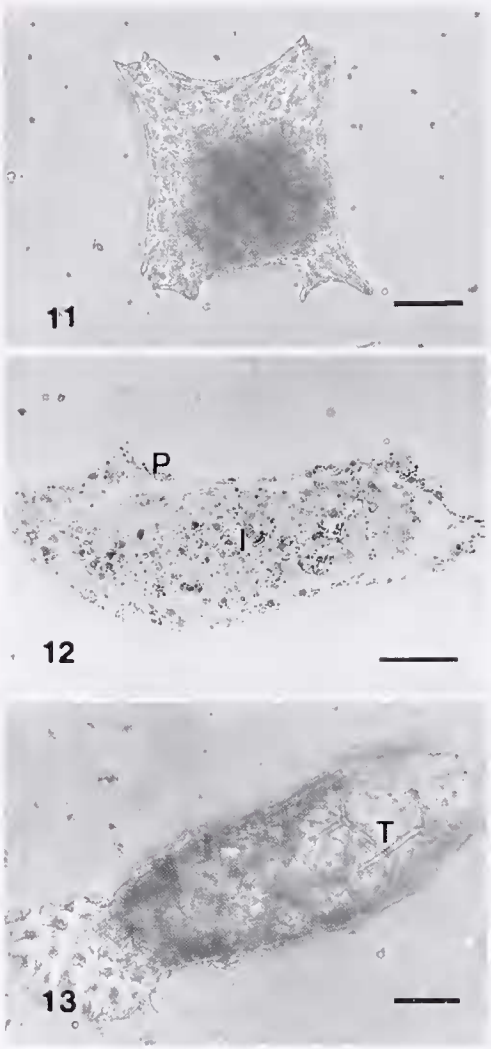


Figure 11. A live metamorphosing larva of *Leucosolenia laxa* about 18 h after settlement. Scale bar = 50 μm.

Figure 12. Section of the metamorphosing larva of *Leucosolenia laxa* about 18 h after settlement. I: inner cell mass, P: pinacoderm. Scale bar = 50 μm.

Figure 13. A live juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. The basal end of this sponge spreads and attaches to the substratum. T: triradiate spicule. Scale bar = 50 μm.

not yet very thin. The cells of an inner cell mass are in the midst of dedifferentiation, and they usually have large autophagosomes in which we found glutinous granules (Fig. 15) and basal rootlets under digestion (Fig. 16). As metamorphosis progresses the pinacocytes become thinner except at their nuclear region; the gap or space between the pinacocytes becomes wider (Fig. 17); and a number of small holes open, piercing through the thin cytoplasm of the pinacocytes (Fig. 18).

Choanocytes. Choanocytes derive from the cells of an inner cell mass. Figure 17 shows immature choanocytes that have been arranged in a cell layer but as yet have no flagellum and no collar. They have glutinous granules and autophagosomes in the cytoplasm.

Figure 18 shows the choanoderm of a juvenile sponge. Choanocytes are flattened, but their central part projects into a gastral cavity. From the center of the projection one flagellum emerges, and from the margin of the projection a row of microvilli, or a collar, emerges. One collar consists of a row of 30–45 microvilli. The nucleolated nucleus, with scattered heterochromatin masses, is always located away from the center of the cell. In the cytoplasm are mitochondria and autophagosomes whose contents have been almost entirely digested.

Although the structure of the choanocyte flagellum is similar to that of larval flagellated cells, the flagellar basal apparatus is quite different. Figure 19 shows that the basal body of choanocytes lacks rootlets and a foot. A Golgi apparatus is located adjacent to the basal body, but perpendicular to it. On the cell membrane is a fuzzy coat encircled by a collar (Fig. 20).

Scleroblasts. Cells beneath a pinacoderm differentiate to scleroblasts in the mesohyl. Figure 21 shows a scleroblast with a large space that had been occupied by a spicule. The fine calcareous spicules, formed in the space surrounded by a membrane, were dissolved in the fixative. We found a small amount of spicule remnants in the space, but no axial filament. These cells have a nucleolated nucleus and glutinous granules in the cytoplasm.

Scleroblasts characteristically have multivesicular bodies. Figure 22 shows that the multivesicular body fuses with a membrane around a spicule and opens into a space for a spicule. The small vesicles of the multivesicular bodies probably contain calcium for spicule formation. Evidence that triradiates are formed by multiple cells is seen in Figure 22, where the spicule is surrounded by two (or more) cells. Although spicules are formed in a space surrounded by a membrane, finished spicules are surrounded by discontinuous cytoplasm or by spongin microfilaments only (Fig. 23).

Vacuolar cells and others. The mesohyl of metamorphosing larvae contains vacuolar cells (Fig. 24), but bottle cells and free cells in a central cavity are never found in the settled larvae. Spongin formation begins soon after the appearance of the mesohyl, about 24 h after settlement. The spongin microfilaments are scattered rather evenly in the mesohyl, but around the spicules they are concentrated.

Discussion

Cell lineages during metamorphosis

The coeloblastulae of *Leucosolenia laxa* consist of four kinds of cells: flagellated cells, bottle cells, vesicular cells, and free cells in a central cavity. Neither bottle cells nor vesicular cells have been described in the coeloblastulae of other calcinean sponges (Minchin, 1896; Borojevic, 1969). The flagellated cells are the principal cell type of the coe-

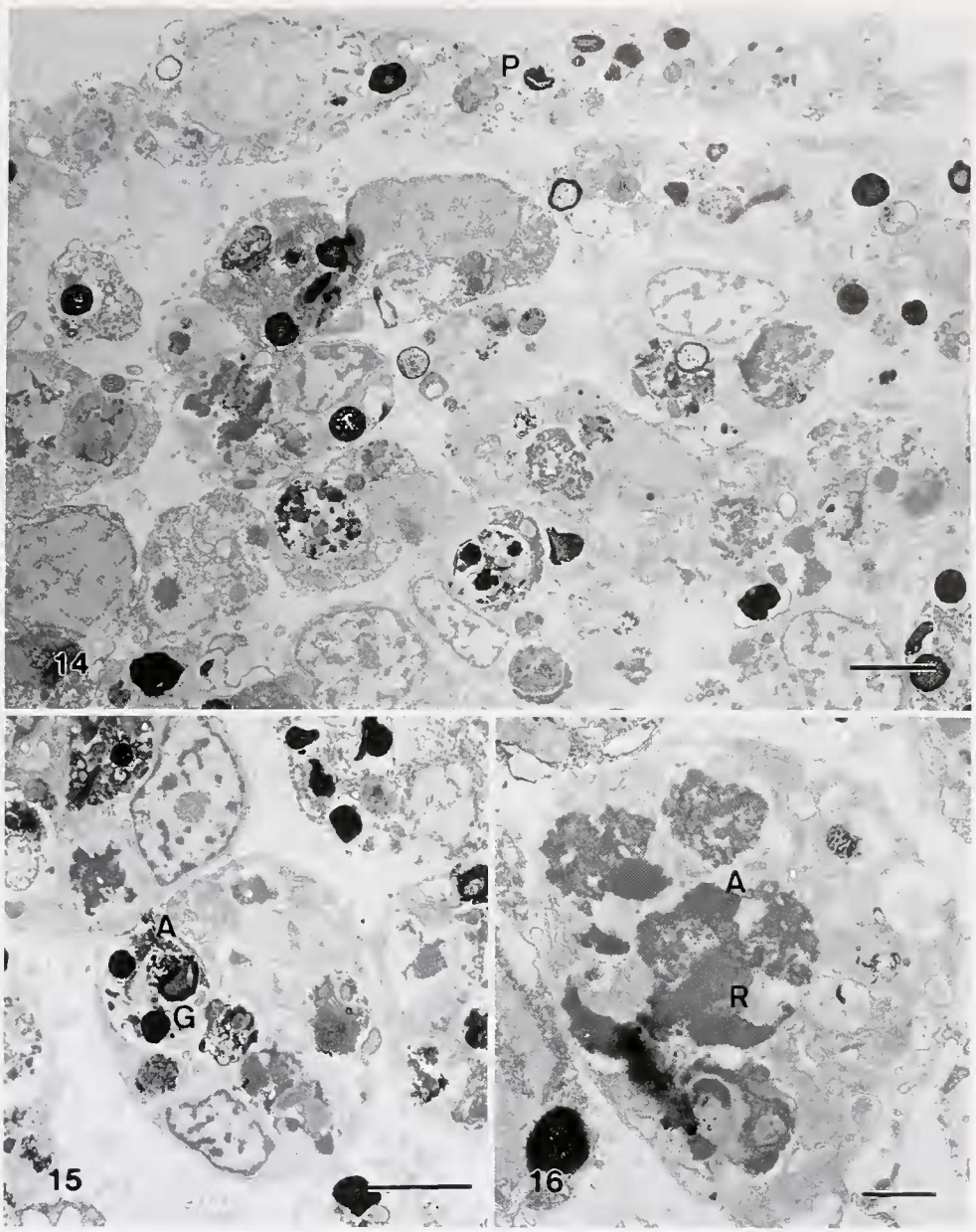


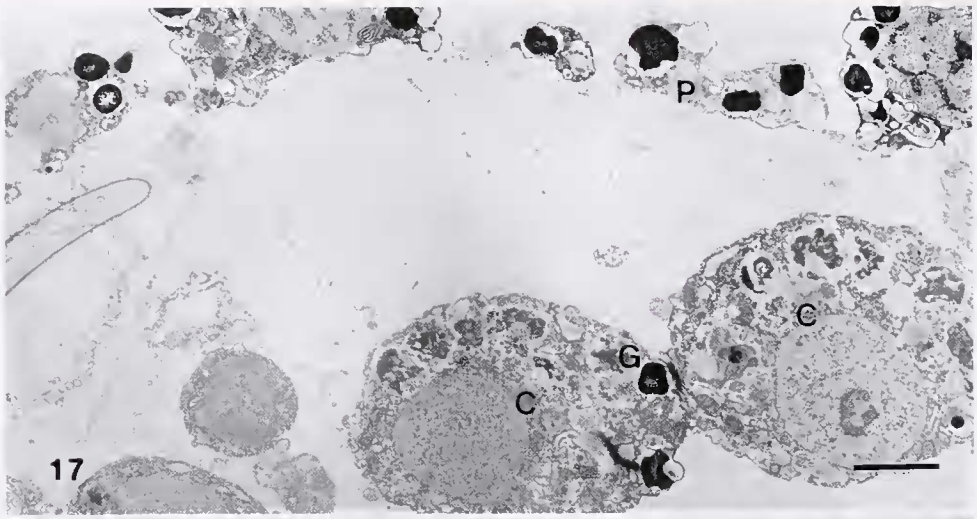
Figure 14. Section of the settled larva of *Leucosolenia laxa*, consisting of a pinacoderm (P) and an inner cell mass about 18 h after settlement. Scale bar = 2 μm.

Figure 15. Autophagosome (A) with glutinous granules (G) in the inner cell mass of the settled larva of *Leucosolenia laxa* about 18 h after settlement. Scale bar = 2 μm.

Figure 16. Autophagosome (A) with a degenerative rootlet (R) in the inner cell mass of the settled larva of *Leucosolenia laxa* about 18 h after settlement. Scale bar = 1 μm.

loblastulae; therefore, we thoroughly studied the fate of these cells during metamorphosis. Examination of a large number of electron micrographs revealed that the flagellated cells become the three principal cell types of juvenile sponges—that is, pinacocytes, scleroblasts, and choanocytes. This conclusion is supported by the fact that these three cell types contain glutinous granules, which only the flagellated cells have in the free-swimming larva. This fact shows clearly that the flagellated cells of the coeloblastulae hold multipotency, although they have been specialized for swimming. Experimental approaches in future research will indisputably demonstrate the developmental potency of the larval cells.

The flagellated cells of coeloblastulae pass through the intermediate stage of dedifferentiation in a settled larva before they differentiate to the cells of a juvenile sponge.



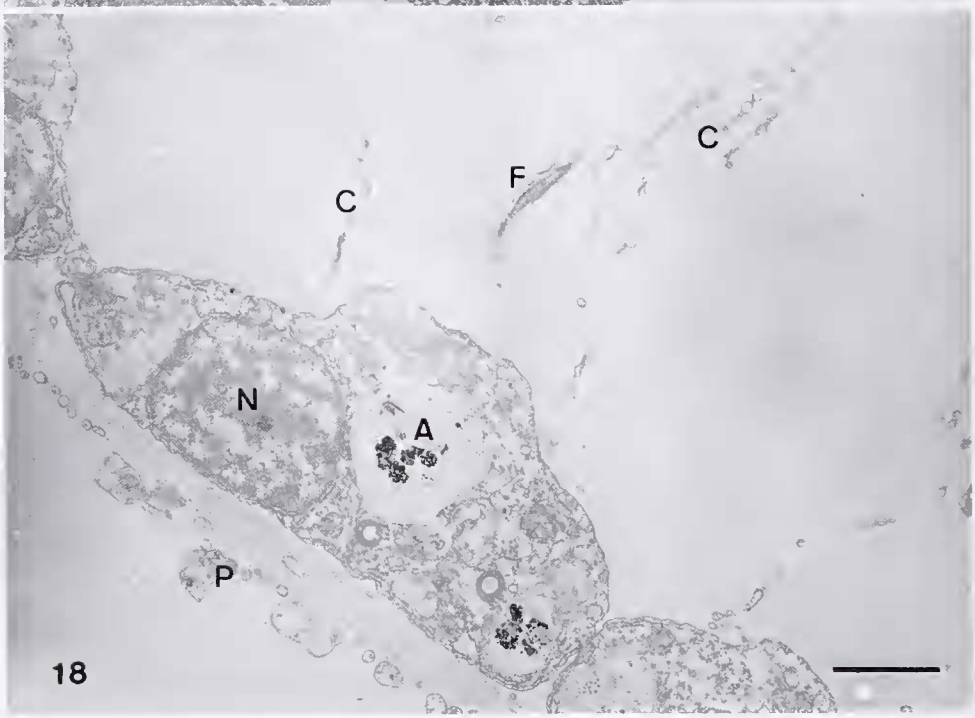


Figure 17. Immature choanocytes (C) of the juvenile sponge of *Leucosolenia lava* about 36 h after settlement. G: glutinous granule, P: pinacoderm. Scale bar = 2 μm.

Figure 18. Choanoderm of the juvenile sponge of *Leucosolenia lava* about 72 h after settlement. A: autophagosome, C: collar, F: flagellum of a choanocyte, N: nucleus, P: pinacoderm. Scale bar = 2 μm.

The flagellated cells are arranged in a pseudostratified layer in a swimming larva. This cell layer is not a true epithelium because—like similar layers in other sponges—it lacks a basal lamina (Gaino *et al.*, 1985; Woollacott and Pinto, 1995). One flagellum emerges from the flat outer surface of the cell. In most sponges, however, larval flagella emerge from a pit at an outer surface (Amano and Hori, 1992, 1994; Woollacott, 1993; Woollacott and Pinto, 1995). After settlement, the flagellated cells soon loose their flagellum and dedifferentiate. The dedifferentiation is apparently triggered by the settlement of the larva. Autophagosomes play a central role in the intracellular processes of dedifferentiation. These autophagosomes are different in origin and structure from the phagosome-like granules in the basal cytoplasm of the larval flagellated cells: the latter were probably formed to take in nutritive materials by phagocytosis during ovogenesis (Fell, 1969); the former are formed *de novo* after settlement and are usually larger than the latter. Glutinous granules, fibrous yolk granules, and basal rootlets in various stages of digestion are observed in the

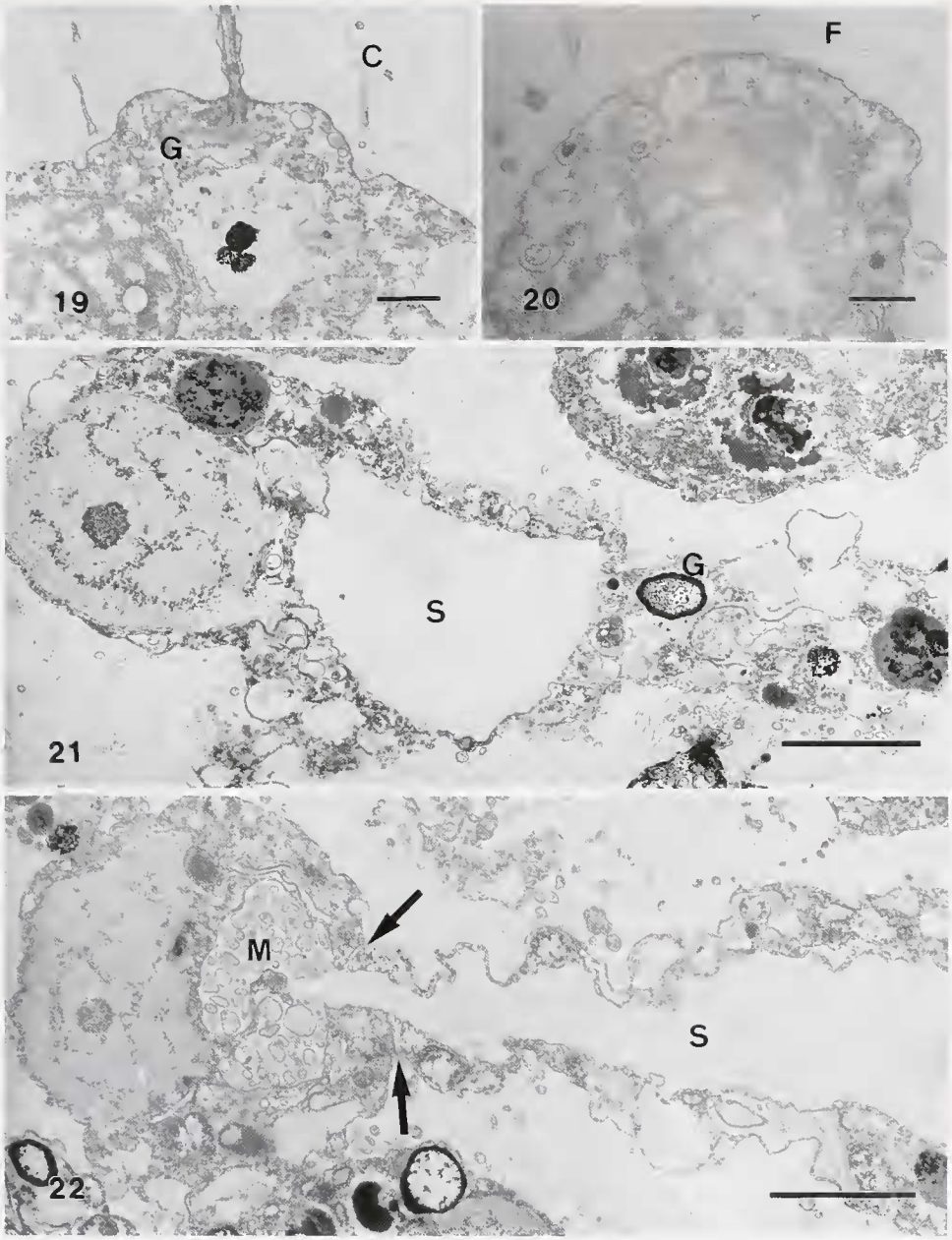


Figure 19. Basal apparatus of a choanocyte in the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. C: collar, G: Golgi apparatus. Scale bar = 1 μm.

Figure 20. Fuzzy coat (F) of a choanocyte in the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. Scale bar = 0.5 μm.

Figure 21. Scleroblast in the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. G: glutinous granule, S: space occupied by a triradiate spicule. Scale bar = 2 μm.

Figure 22. Two scleroblasts forming a triradiate spicule in the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. A multivesicular body (M) opens into the space occupied by a spicule (S). Arrow: cell junction between scleroblasts. Scale bar = 2 μm.

autophagosomes of the inner cell mass of settled larvae. The choanocytes of juvenile sponges contain autophagosomes whose contents have been almost entirely digested.

Bottle cells are described for the first time in this report. These large cells, like the cruciform cells of amphiblastulae (Duboscq and Tuzet, 1937; Amano and Hori, 1992), are located among the flagellated cells. The function of the bottle cells is not known; they apparently do not contribute to the development of the juvenile sponge and disappear during metamorphosis, as do the cruciform cells. Both cell

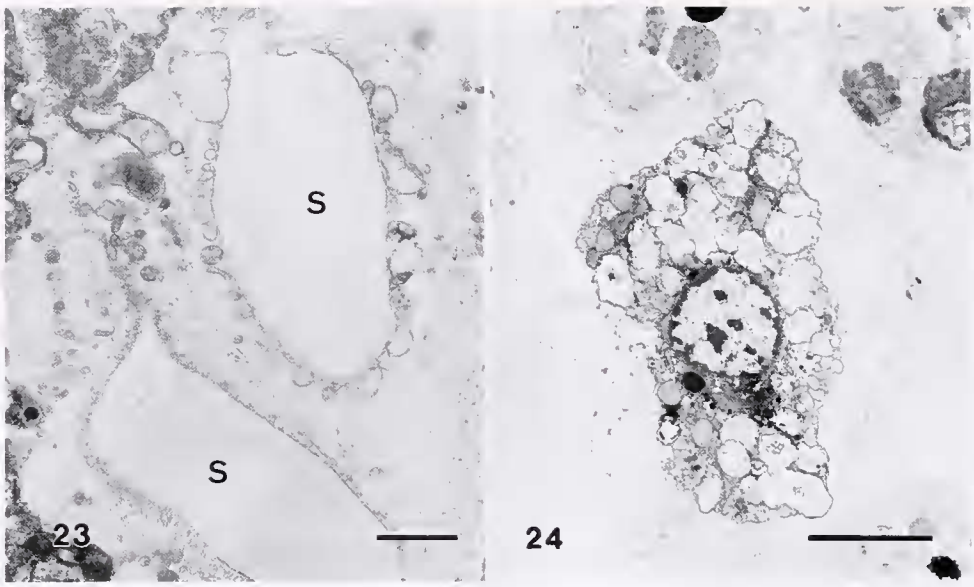


Figure 23. Spicules surrounded by spongin fibers and by discontinuous cytoplasm in the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. S: space occupied by a triradiate spicule. Scale bar = 1 μm.

Figure 24. A vacuolar cell in the mesohyl of the juvenile sponge of *Leucosolenia laxa* about 72 h after settlement. Scale bar = 2 μm.

types may have similar functions, but the bottle cells cannot be named cruciform cells because the former cells are not arranged in each quadrant in a horizontal plane. Vesicular cells in the coeloblastula are also described for the first time. Their function is not known, but we know that they participate in metamorphosis, because they are found in the mesohyl of juvenile sponges. Free cells in a central cavity are probably somatic cells derived from the mother sponge. Similar maternally derived nutritive cells are found in the larvae of other sponges (Warburton, 1961; Gallissian and Vacelet, 1992). These cells disappear during metamorphosis.

Juvenile sponge formation

After settlement, a coeloblastula becomes a mass of dedifferentiated cells on the substratum. The first cells to differentiate are pinacocytes, which are apparently formed from cells at the surface of the cell mass. The pinacocytes are flattened, except at their nuclear region. As a juvenile sponge becomes larger, the cytoplasm of the pinacocytes becomes thinner. We found no ostia in the juvenile sponge; however, after osculum formation, seawater can flow into the gastral cavity through the spaces between the cells and the small holes of the thin cytoplasm.

Scleroblasts are the next cells to differentiate in the cell mass settled on the substratum. Cells beneath the pinacoderm become scleroblasts. These cells form triradiates, a characteristic spicule of calcareous sponges, in the mesohyl of juvenile sponges. The scleroblasts always enclose a large space delimited by a membrane. This space had been occupied by a calcareous spicule that was dissolved in the fixative. Similarly, siliceous spicules are formed in a space surrounded by a membrane called the silicalemma (Simpson and Vaccaro, 1974). An axial filament is essential for siliceous spicule formation (Garrone, 1969; Cha *et al.*, 1999), but the calcareous triradiate is formed without the aid of an axial filament (Ilan *et al.*, 1966; Jones, 1967; Amano and Hori, 1993). Here—as in other calcareous spicules—no axial filament was found in the space formerly occupied by a spicule. The scleroblasts usually have a small number of multivesicular bodies. We showed that the multivesicular bodies fuse with the membrane around the space and open into it. The multivesicular bodies probably supply calcium and membrane components for spicule formation. Similarly, in the metamorphosis of amphiblastulae, a number of small vesicles open into the space for spicule formation (Amano and Hori, 1993). Scleroblasts that form siliceous spicules also have a number of vesicles in the cytoplasm (Simpson and Vaccaro, 1974). Although the spicules are formed within the scleroblasts, the finished spicules free of cells are usually covered by spongin filaments (Jones, 1967; Garrone, 1985).

Lastly, the cells of an inner cell mass differentiate to choanocytes. In the inner cell mass, a space develops *de novo* among the cells and becomes larger as metamorphosis progresses. A layer of immature choanocytes, not yet possessing a flagellum or collar, encircles the large space, which is a gastral cavity. The gastral cavity is different in

origin and structure from the central cavity of coeloblastulae, which is a retained blastocoel (Borojevic, 1969). As the gastral cavity develops, the choanocytes are flattened except at their thick central region. Then one flagellum emerges from the center of the central region and a collar of microvilli develops around the thick central region. The basal body of the flagellum lacks rootlets and a foot; other sponges also lack these structures (Amano and Hori, 1993, 1996). On the cell membrane we found a fuzzy coat encircled by a collar; a similar fuzzy coat is found in demosponges (Bergquist and Green, 1977; Lethias *et al.*, 1983). About 72 h after settlement, an osculum opens at the uppermost part of the metamorph and water flow begins. Thus an asconoid juvenile sponge, an olynthus, is formed.

Coeloblastulae of other calcinean sponges

While the coeloblastulae of *L. laxa* are swimming, the flagellated cells do not migrate into a central cavity and the cells do not lose their flagellum. When the larvae adhere to the substratum for settlement, their flagella still continue active beating. In the coeloblastulae of other calcinean sponges, however, the larval flagellated cells have been reported to migrate into a central cavity while the larvae are swimming (Minchin, 1896; Borojevic, 1969). The reason for this discrepancy is not known. Time to initiate the dedifferentiation of larval flagellated cells may differ among calcinean sponges. A more likely explanation is that bottle cells and vesicular cells were erroneously judged to be the flagellated cells under migration into the central cavity. We showed that the bottle cells and vesicular cells of *L. laxa* protrude extensively into the central cavity. Indeed, Borojevic (1969) noted that larval cells migrate into the blastocoel firstly from the posterior region of the coeloblastula, where vesicular cells are concentrated. If the resolution of larval images is not high enough, it seems to be difficult to know which is the case.

In the calcinean sponge *Clathrina reticulum*, Borojevic (1969) described pigment granules in larval flagellated cells; however, these are apparently glutinous granules. Although the granules in question are indeed very electron-dense, they cannot be pigment granules, because coeloblastulae are colorless. We showed that the granules can be released from the cells and that their content is a sticky substance, so they may participate in larval settlement. We know that some of them are, however, digested intracellularly, because they were found in autophagosomes in the inner cell mass of settled larvae.

Fates of larval flagellated cells

After settlement, flagella and basal apparatus meet different fates of degeneration depending on the types of larvae. In the parenchymellae of demosponges, flagella are withdrawn into the cell and decomposed in the cytoplasm after they are severed at the transitional region between a basal body and the proximal end of an axoneme (Boury-Esnault, 1976; Amano and Hori, 1996). In the coeloblastulae and amphiblastulae of calcareous sponges, however, the axonemes are probably shed soon after settlement (Amano and Hori, 1993). The basal bodies and rootlets, severed from the axoneme, are decomposed in the cytoplasm in the amphiblastulae and parenchymellae (Amano and Hori, 1993, 1996). In the coeloblastula of *L. laxa*, however, they are digested in autophagosomes.

The developmental potency of larval flagellated cells differs greatly among the types of sponge larvae. In the coeloblastula of *L. laxa*, the flagellated cells apparently become all three principal cell types of juvenile sponges. In amphiblastulae, another larval type of calcareous sponges, the flagellated cells become scleroblasts and choanocytes but not pinacocytes (Amano and Hori, 1993). The pinacocytes are derived from larval granular cells. In the parenchymellae of demosponges, the flagellated cells are not multipotential and become only choanocytes (Borojevic, 1966; Amano and Hori, 1996). The parenchymellae usually consist of many more cell types than do coeloblastulae or amphiblastulae, and in such complex larvae, juvenile sponge cells are derived from distinctive larval cells. During the geologic eras after the Vendian age, each type of sponge larva evolved to metamorphose in different ways (Li *et al.*, 1998).

Acknowledgments

We thank Mr. S. Tamura and Mr. M. Washio of the Asamushi Marine Biological Station for help in collecting sponges.

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