JOURNAL OF MORPHOLOGY 268:518–528 (2007)

Metamorphosis of Cinctoblastula Larvae (Homoscleromorpha, Porifera)

Alexander V. Ereskovsky,[1]* Daria B. Tokina,[2] Chantal Bézac,[3] and Nicole Boury-Esnault[4]

[1]*Department of Embryology, Biological Faculty, St Petersburg State University, St Petersburg 199034, Russia*
[2]*Zoological Institute, Russian Academy of Sciences, St Petersburg 199034, Russia*
[3]*Centre d'Océanologie de Marseille, Station marine d'Endoume, Université de la Méditerranée, UMS-CNRS 2196, 13007 Marseille, France*
[4]*Centre d'Océanologie de Marseille, Station marine d'Endoume, Université de la Méditerranée, UMR-CNRS 6540 DIMAR, rue de la Batterie des Lions, 13007 Marseille, France*

ABSTRACT The metamorphosis of the cinctoblastula of Homoscleromorpha is studied in five species belonging to three genera. The different steps of metamorphosis are similar in all species. The metamorphosis occurs by the invagination and involution of either the anterior epithelium or the posterior epithelium of the larva. During metamorphosis, morphogenetic polymorphism was observed, which has an individual character and does not depend on either external or species specific factors. In the rhagon, the development of the aquiferous system occurs only by epithelial morphogenesis and subsequent differentiation of cells. Mesohylar cells derive from flagellated cells after ingression. The formation of pinacoderm and choanoderm occurs by the differentiation of the larval flagellated epithelium. This is possibly due to the conservation of cell junctions in the external surface of the larval flagellated cells and of the basement membrane in their internal surface. The main difference in homoscleromorph metamorphosis compared with Demospongiae is the persistence of the flagellated epithelium throughout this process and even in the adult since exo- and endopinacoderm remain flagellated. The antero-posterior axis of the larva corresponds to the baso-apical axis of the adult in Homoscleromorpha. J. Morphol. 268:518–528, 2007.

KEY WORDS: metamorphosis; Homoscleromorpha; morphogenesis; ultrastructure; sponges

Metamorphosis is the short-term stage of postembryonic development during which the larva develops into a juvenile. During this period fundamental morphological and physiological changes occur, which involve new organogenesis and organization of the basic body plan. For sessile animals that have free-living larvae, after a period of dispersal, the larvae acquire the ability to settle that initiate metamorphosis (Fell, 1997).

After settlement, the main feature of the metamorphosis of sponge larvae is the acquisition of the poriferan Bauplan, which is mainly represented by the aquiferous system. The first adult structures to be formed de novo are the exopinacoderm and the basopinacoderm which, respectively, isolate the young sponge from the external environment and fix it to the substratum. Later, organization of the choanocyte chambers and the water current channels, the opening of the ostia and osculum as well as the acquisition of the elements of the skeleton occur. The sequence of these events has been described only in few species (Brien and Meewis, 1938; Lévi, 1956; Boury-Esnault, 1976; Evans, 1977; Ilan and Loya, 1990; Kaye and Reiswig, 1991; Amano and Hori, 1993, 1996, 2001; Leys and Degnan, 2002; Gonobobleva and Ereskovsky, 2004).

Homoscleromorpha have recently been considered as a clade that has no clear relationships to all other Demospongiae (Borchiellini et al., 2004). Homoscleromorph species share some morphological, anatomical, cytological, biochemical and embryological characters that are common to eumetazoans and absent in other poriferan clades (Boury-Esnault et al., 1984, 1992, 1995, 2003; Baccetti et al., 1986; Solé-Cava et al., 1992; Boute et al., 1996; Muricy et al., 1996a,b, 1999; Boury-Esnault and Jamieson, 1999; Ereskovsky and Boury-Esnault, 2002).

We have already described early development from egg to coeloblastula stage in five species of *Oscarella* (Ereskovsky and Boury-Esnault, 2002) and embryogenesis from coeloblastula to larva in eight species of Homoscleromorpha (Boury-Esnault et al., 2003). The most distinctive feature of early development in homoscleromorphs is the formation of a coeloblastula from a stereoblastula by "multipolar egression," i.e., progressive migration of the internal cells to the periphery. Penetration of symbiotic bacteria into the embryos of all species investigated, and maternal cells into embryos of *Oscar-*

Contract grant sponsor: INTAS 97-854; Contract grant sponsor: RFBR; Contract grant numbers: 03-04-49773, 06-04-48660.

*Correspondence to: Alexander V. Ereskovsky, Department of Embryology, Biological Faculty, St Petersburg State University, Universitetskaya nab. 7/9, St Petersburg 199034, Russia.
E-mail: aereskovsky@mail.ru

Published online 11 April 2007 in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10506

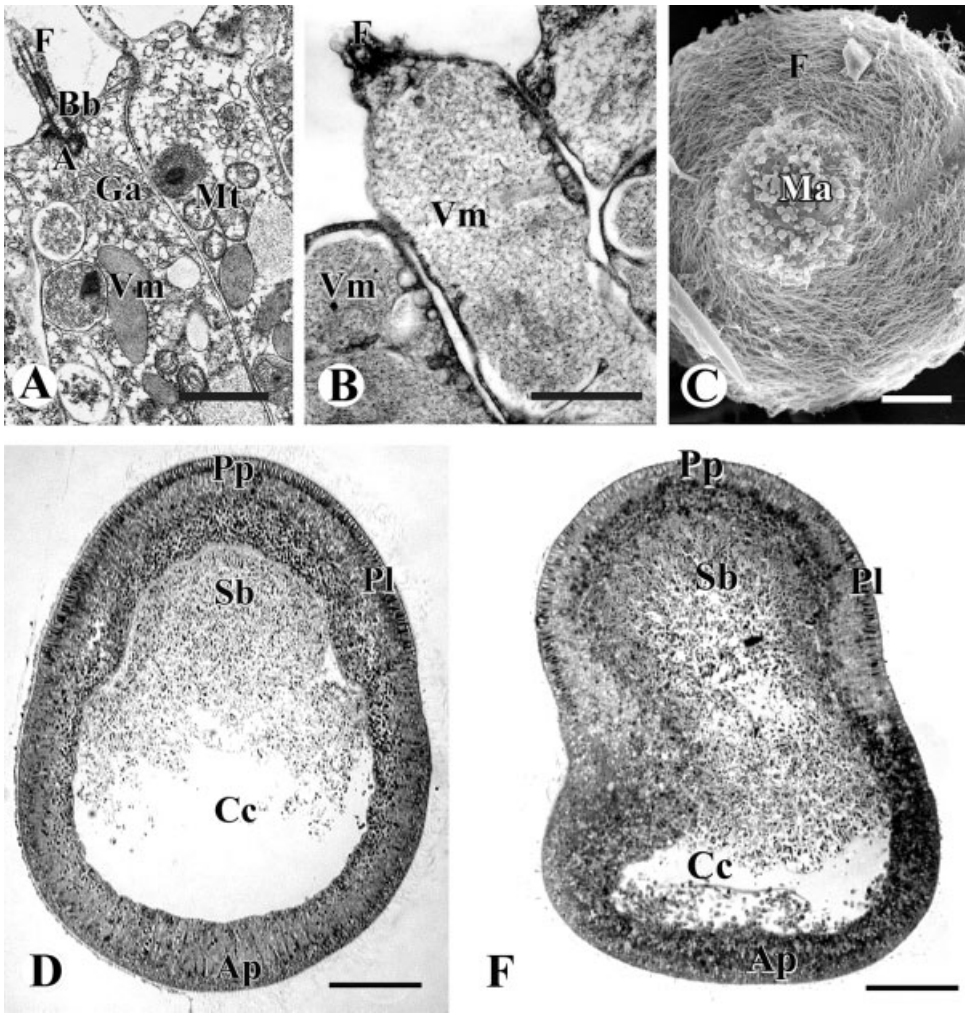

Fig. 1. Larvae of Homoscleromorpha. **A:** Distal parts of flagellated cells of the anterior pole of a free-swimming cinctoblastula larva of *Oscarella tuberculata* (TEM). Scale bar 2 μm. **B:** Distal parts of flagellated cells of the anterior pole of a competent cinctoblastula of *Oscarella tuberculata* (TEM). Scale bar 1 μm. **C:** Mucous area on the apical pole of cinctoblastula of *Oscarella tuberculata* just before the fixation (SEM). Scale bar 25 μm. **D:** Semi-thin section of a free-swimming cinctoblastula of *Corticium candelabrum*. Scale bar 50 μm. **E:** Semi-thin section of a flattened cinctoblastula of *Corticium candelabrum* just before fixation. Scale bar 50 μm. A, accessory centriole; Ap, anterior pole; Bb, basal body of flagellar apparatus; Cc, central cavity; F, flagella; Ga, Golgi apparatus; Ma, mucous area; Mt, mitochondria; Pl, postero-lateral cells with intranuclear paracrystalline inclusions; Pp, posterior pole; Sb, symbiotic bacteria; Vm, vacuoles with mucus.

ella lobularis and *O. imperialis,* has been observed. Symbiotic bacteria and maternal cells migrate from the mesohyl into the space between eggs and follicular cells before follicular closing (Ereskovsky and Boury-Esnault, 2002). Morphogenesis of the larva is similar in all investigated species. The result of embryogenesis is a flagellated larva, the "cinctoblastula" (Boury-Esnault et al., 2003). It is composed of a columnar epithelium of polarized, monoflagellated cells among which are scattered a few nonflagellated ovoid cells. Cinctoblastulae have a distinct antero-posterior polarity that is discernible due to the shape of the larva and the distribution of three flagellated cell types: the cells of the anterior pole, those of the postero-lateral zone which have an intranuclear paracrystalline inclusion, and those of the posterior pole. Zonula adherens-like junctions are observed at the distal parts of the cells. A basement membrane underlying the flagellated cells lines the larval cavity. The single basal rootlet of the flagella is cross striated (Boury-Esnault et al., 2003).

Although several histological studies have described the larva and its metamorphosis in *Oscarella lobularis* (= *tuberculata*) (Barrois, 1876; Heider, 1886; Maas, 1898; Meewis, 1938; Lévi, 1956) and *Plakina monolopha* (Schulze, 1880), only one ultrastructural study described some steps of the metamorphosis of *O. lobularis* (Lévi and Porte, 1962).

We describe here all the phenomena from the settlement to the metamorphosis of the cinctoblastula and the organization of the rhagon in five species of

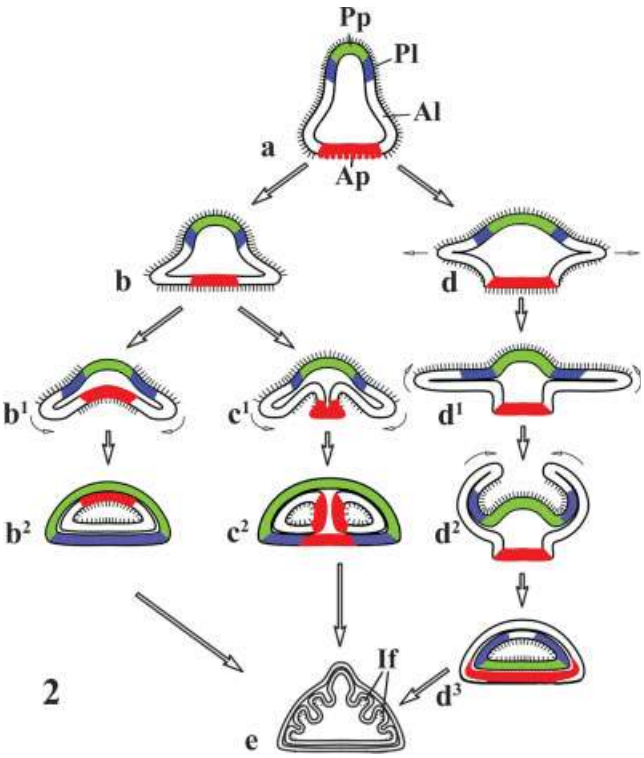

Fig. 2. Diagram of the different paths of formation of the rhagon during the metamorphosis of homoscleromorph larvae. (a) Cinctoblastula; (b–b^2) basal invagination; (c^1,c^2) basal ring-like invagination; (d–d^3) extension and folding up of lateral sides of post-larva; (e) rhagon. Al, antero-lateral cells; Ap, anterior pole cells; If, internal folds of post-larva epithelium; Pl, postero-lateral cells with intranuclear paracrystalline inclusions; Pp, posterior pole cells.

Homoscleromorpha using light, transmission, and scanning electron microscopy (SEM). The presence of an intranuclear paracrystalline inclusion in cells forming a belt around the posterior pole is a natural marker that we have used to monitor the fate of cells during metamorphosis.

MATERIALS AND METHODS

Metamorphosis was investigated in five Mediterranean species of homoscleromorph sponges: *Oscarella tuberculata* (Schmidt, 1868), *O. lobularis* (Schmidt, 1862), *O. microlobata* (Muricy et al., 1996a), *Corticium candelabrum* (Schmidt, 1862), and *Plakina trilopha* (Schulze, 1880).

The sponges were collected by SCUBA diving from July to August 2000, 2001, and August to September 2002 in the western Mediterranean Sea, at depths of 5–25 m.

In the laboratory, larvae were released from maternal sponges by table-lamp illumination following dark adaptation. Larvae were maintained at 18°C. Larvae were transferred to sterile petri dishes containing 30 ml of sea water. Settlement and metamorphosis of the larvae were monitored under a microscope and larvae were fixed at the beginning of substrate attachment and after 3, 12, 24, 72, and 144 h.

For SEM, the fixative used was a 5:1 mixture of 2% OsO_4 in filtered sea water and saturated mercuric chloride (Johnston and Hildemann, 1982). Specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold–palladium, and observed under a Hitachi S570 SEM.

For transmission electron microscopy (TEM), two fixation methods were used: 1) one volume of 2.5% glutaraldehyde, four volumes of 0.4 M cacodylate buffer, and five volumes of seawater (1,120 mOsm) and post-fixation in 2% OsO_4 in seawater (Boury-Esnault et al., 1984), and 2) samples were prefixed in 1% OsO_4 in 0.1 M phosphate buffer for 10 min and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (1,120 mOsm) at room temperature for 1 h. After fixation, samples were washed in 0.1 M phosphate buffer and postfixed in 1% OsO_4 in 0.1 M phosphate buffer for 1 h, dehydrated through a graded ethanol series, and embedded in Araldite (Ereskovsky and Boury-Esnault, 2002). Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Zeiss-1000 TEM.

For the definition of terms concerning general embryology, see Gilbert and Raunio (1997) and Stern (2004). For the definition of terms for sponge morphology and embryology, see Harrison and De Vos (1991), and Boury-Esnault and Rützler (1997).

RESULTS

Competence

Before settlement there is a period of preparation during which larvae become competent for metamor-

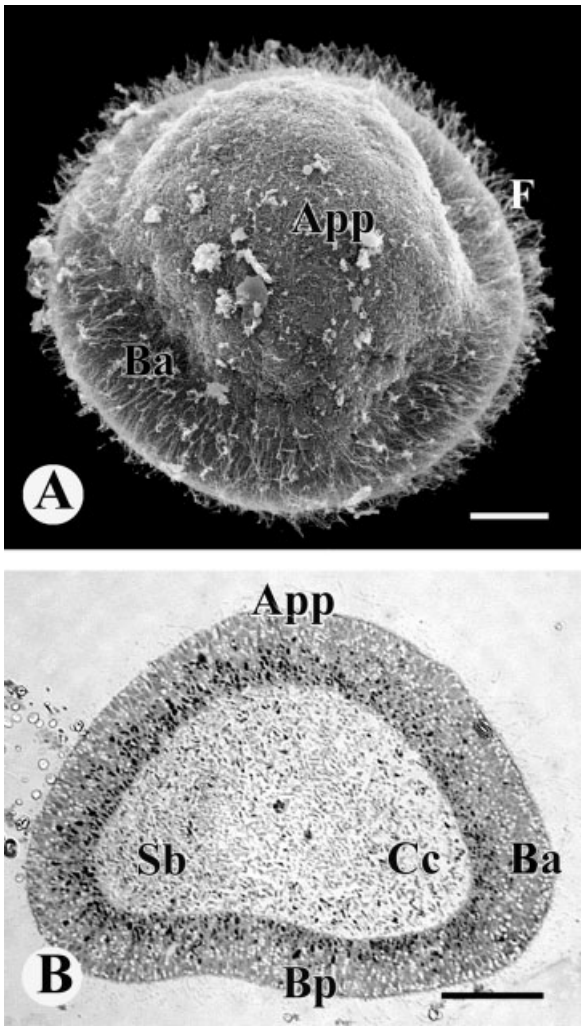

Fig. 3. Post-larva of *Plakina trilopha*, fixed to substratum. **A:** SEM. Scale bar 50 μm. **B:** Semi-thin micrograph. Scale bar 50 μm. App, apical part; Bp, basal part; Cc, central cavity; Ba, border area; F, flagella; Sb, symbiotic bacteria.

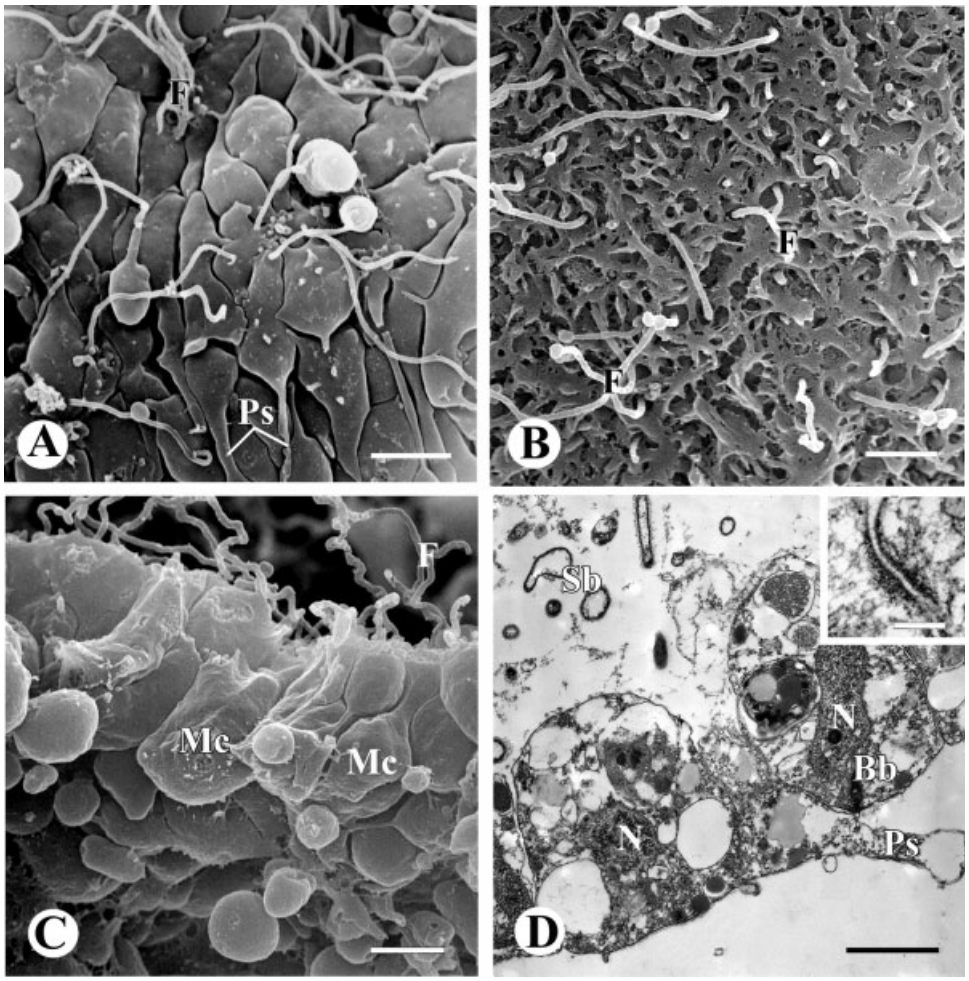

Fig. 4. Apical cells of homoscleromorphs post-larvae. **A:** Superficial side of apical cells of the post-larva of *Oscarella tuberculata* (SEM). Scale bar 3.5 μm. **B:** The surface of apical cells of *Plakina trilopha* post-larva with a complex network of pseudopodia (SEM). Scale bar 4 μm. **C:** Cells of apical part of a post-larva of *Oscarella microlobata* (SEM). Scale bar 3 μm. **D:** Flagellated cells which differentiate into pinacocytes in *Plakina trilopha* (TEM). Scale bar 2 μm. Insert: Detail of a desmosome-like junction. Scale bar 0.25 μm. Bb, flagellar basal body; F, flagella; Mc, cell, beginner migration; N, nucleus; Ps, pseudopodia; Sb, symbiotic bacteria.

phosis. The main changes occur within the cells of the anterior pole. The vacuoles with mucous content merge with each other and migrate close to apical surfaces of the cells (Fig. 1A,B). The mucus is secreted by the vacuoles just as the larvae settle (Fig. 1C). The granule of secreted mucous varies from 1.1 to 4.3 μm in diameter (Fig. 1B). During the same period the basal parts of the cells of the anterior pole and cells of the transition zone begin to split. In competent larvae the cells of the posterior pole show pseudopodial activity. Before attachment, anterior pole of the larva flattens and ovoid larva becomes shorter along an antero-posterior axis (Fig. 1D,E).

Larvae settle 12–48 h after release from the parent sponge. Attachment occurs by the anterior pole. During contact with the substratum, larvae can rotate around the axis clockwise for a short time. Some minutes after fixation, the anterior pole of the larva flattens, and the posterior pole, recognizable by its color, becomes ovoid.

Fixation on Substratum

Larvae that settle take the shape of a bell with a convex posterior pole in *Oscarella microlobata* and *Plakina trilopha* (Figs. 2b,c[1], 3A,B), or a flattened form in *Oscarella tuberculata* (Fig. 2d,d[1]).

At the beginning of metamorphosis, modifications of shape and size of the cells occur in all parts of the post-larvae. In *O. tuberculata* and *Corticium candelabrum* the external surface of the cells of the previous posterior pole becomes flat. They form numerous pseudopodia (Fig. 4A). For *P. trilopha* the external surface of these cells forms a complex network of pseudopodia (Fig. 4B). This may be due to the expansion of the cytoplasmic villi surrounding the flagella of the larvae. Some cells begin to migrate inside the post-larvae and to differentiate into cells of the mesohyl (Figs. 4C, 5A,B). Flagellated cells which differentiate into choanocytes have a prismatic shape, and when they differentiate into pina-

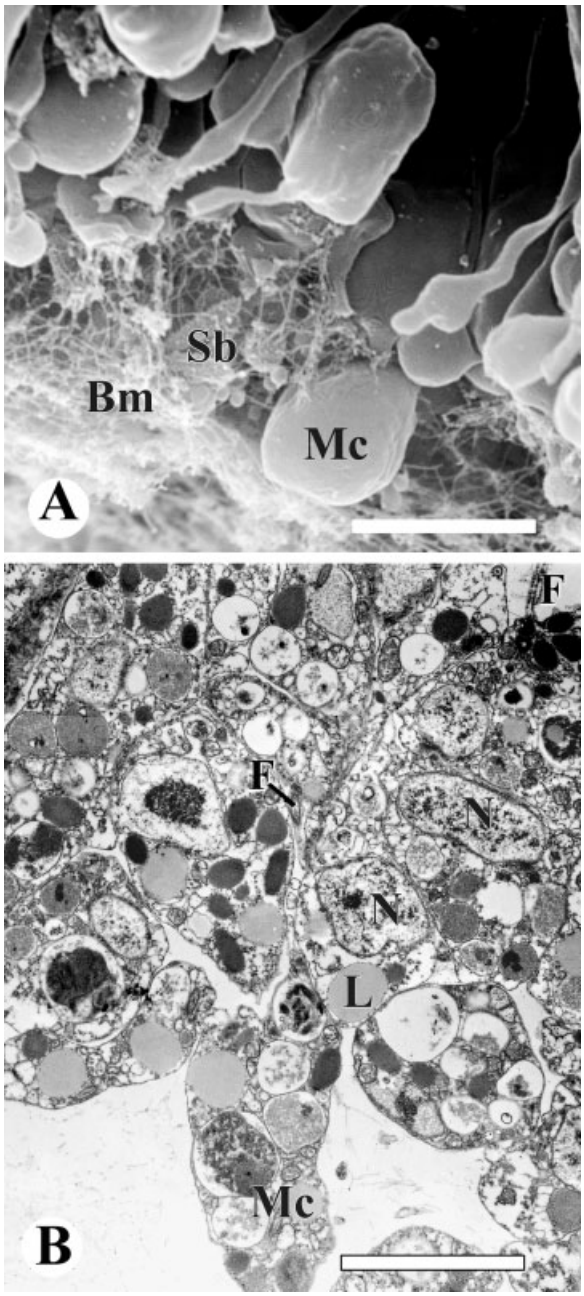

Fig. 5. Larval cells migrating inside of post-larva. **A:** Internal part of post-larva of *Oscarella tuberculata* with the cell migrated inside of larval cavity (SEM). Scale bar 3 μm. **B:** Flagellated cell migrated into central cavity of *Plakina trilopha* post-larva (TEM). Scale bar 3 μm. Bm, basement membrane; F, flagella; L, lipid droplet; Mc, migrated cell; N, nucleus; Sb, symbiotic bacteria.

cocytes they become cuboidal (Fig. 4D), and then flat.

The border between the apical and lateral part of the post-larvae is well visible (Fig. 3A). The flagella of the cells of this zone persist (Fig. 3A). This zone is constituted by two kinds of larval cells: the lateral cells and the cells with intranuclear paracrystalline inclusion, described earlier (Boury-Esnault et al., 2003).

Lateral cells of the post-larva show greater change in size and shape than basal and apical ones; many take on an irregular shape. Others migrate into the larval cavity (Figs. 4C, 5A,B) and the form of these cells becomes prismatic or pear-shaped. The cell junctions become not visible and flagella are resorbed into the cytoplasm. The basement membrane may be present or partially reduced (Fig. 5A,B).

The shape of the basal cells of the post-larva changes from prismatic to cuboidal due to the flattening of the cell (Fig. 6A,B). During this process the apical surface of the post-larva flattens and expands. The cells maintain the specialized junctions (Fig. 4D, insert), and the basement membrane of the larval epithelium (Fig. 5A). The external surface of basal post-larva cells shows numerous pseudopodia which are the sign of high activity. Some cells of this zone differentiate into basopinacocytes, responsible for the fixation of the rhagon to substratum. The external surface of these cells become flat, cytoplasmic villi disappear, and flagella draw into the cytoplasm (Fig. 6C).

Fragments of internal sides from the epithelial cells, containing yolk granules, are found in the cavity under the cells (Fig. 6A,B). These fragments are spherical or oval in shape, with a diameter from 1.2 to 4.2 μm.

The form of the postero-lateral cells of the edge area is variable. They become flat, forming numerous pseudopodia, larval microvilli are reduced but the flagella are present. The edge area mainly consists of larval cells with intranuclear paracrystalline inclusions which gradually disappear (Fig. 6D).

Metamorphosis

The basic morphogenetic event is the invagination and involution of a layer of flagellated cells to the inside of the metamorphosing larva. This occurs by different paths (Figs. 2, 7A–E). The most frequent way is the formation of a cavity by invagination of the basal layer of the post-larva (Figs. 2b–b^2, 7A). Sometimes in *O. tuberculata* the basal layer remains attached in the center and a ring-like cavity is formed (Figs. 2c^1,c^2, 7B). In a third case, the first event is the flattening of the larva, followed by the folding of the marginal zones in the direction of the apical pole of the post-larvae (Figs. 2d–d^3, 7C–E). In this case, the apical flagellated epithelium becomes internal. Secondary involution of flagellated epithelium occurs in some larvae (Fig. 7A,D,E). During this morphogenesis cells from the marginal zones migrate inside the post-larva.

Formation of the Adult Structure

The first adult structure to form is the exopinacoderm (Fig. 8A,B). Soon after settlement the surface cells become flattened and cover the inner cells (Fig. 8A,B). These cells display numerous pseudopo-

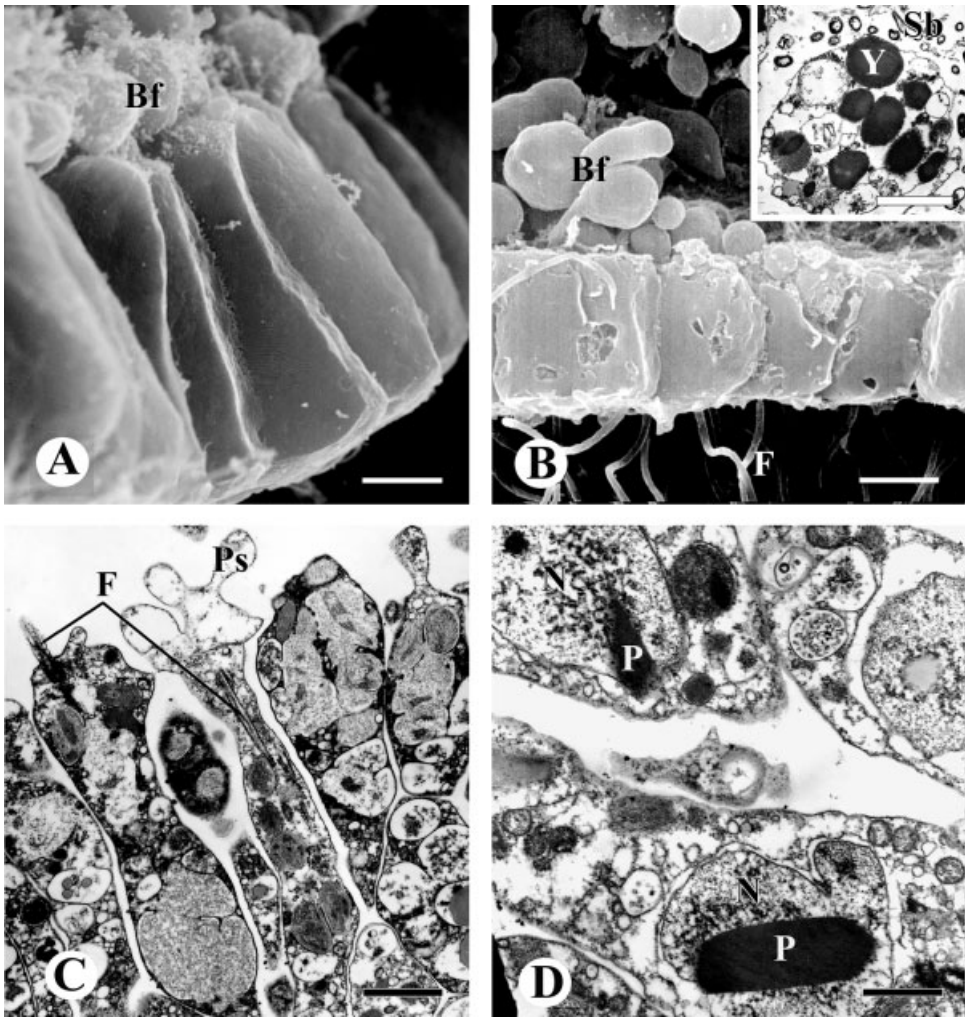

Fig. 6. Post-larva cells differentiation. **A:** Early stage of differentiation of basal cells into basopinacocytes for *Plakina trilopha* (SEM). Scale bar 4 μm. **B:** Intermediary stage of differentiation into basopinacocytes for *Plakina trilopha* (SEM). Scale bar 4 μm. Insert: Basal fragments of cell with the yolk inclusions (TEM). Scale bar 2 μm. **C:** Resorption of flagellum on a former cell of posterior pole of post-larva of *Oscarella tuberculata.* Scale bar 2 μm (TEM). **D:** Gradual disappearance of intranuclear paracrystalline inclusions in the cells of the edge of metamorphosed larva in *Oscarella tuberculata* (TEM). Scale bar 0.5 μm. Bf, basal fragments of cells; F, flagella; N, nucleus; P, paracrystalline inclusion; Ps, pseudopodia; Sb, symbiotic bacteria; Y, yolk inclusions.

dia (Fig. 8B) and retain the flagella. During metamorphosis, the exopinacocytes become thinner except in the nuclear region.

The development of the aquiferous system (choanocyte chambers and canals) begins after exopinacoderm formation (Figs. $2b^2$,c^2,d^3, 8C,D). During the formation of the canals cells become cuboidal, and then flat, which leads to an increase in the surface area of epithelia lining the closed space inside the rhagon. The result is the formation of numerous folds (Fig. 8C). Internal parts of these folds of the flagellated epithelia become choanocyte chambers, whereas adjoining parts of folds become canals (Figs. 2e, 8C,D).

The differentiation of larval cells into adult cells during metamorphosis occurs according to their location. During the first stages of pinacocyte and choanocyte differentiation, larval flagella persist (Figs. 8A,B, 9A–C). But modifications of the basal flagellar apparatus are observed: the flagellar basal rootlet breaks up. Differentiated endopinacocytes are flattened cells, fusiform in perpendicular section (Figs. 8D, 9A). During choanocyte differentiation, the cells are cuboidal or prismatic, the distal part develops microvilli, and the nucleus is generally basal (Fig. 9B,C). The cells that migrate to the larval cavity during metamorphosis show the most significant changes. They completely loose the flagellum and the flagellar basal apparatus, and become amoeboid (Fig. 9D). Later they differentiate into various cells of the mesohyl: archaeocytes, spherulous cells, and sclerocytes (*Plakina* and *Corticium*). The ostia and osculum are formed after the aquiferous system development.

In early stages of metamorphosis, a dense population of symbiotic bacteria is located in the apical part of post-larva. Later these bacteria are uni-

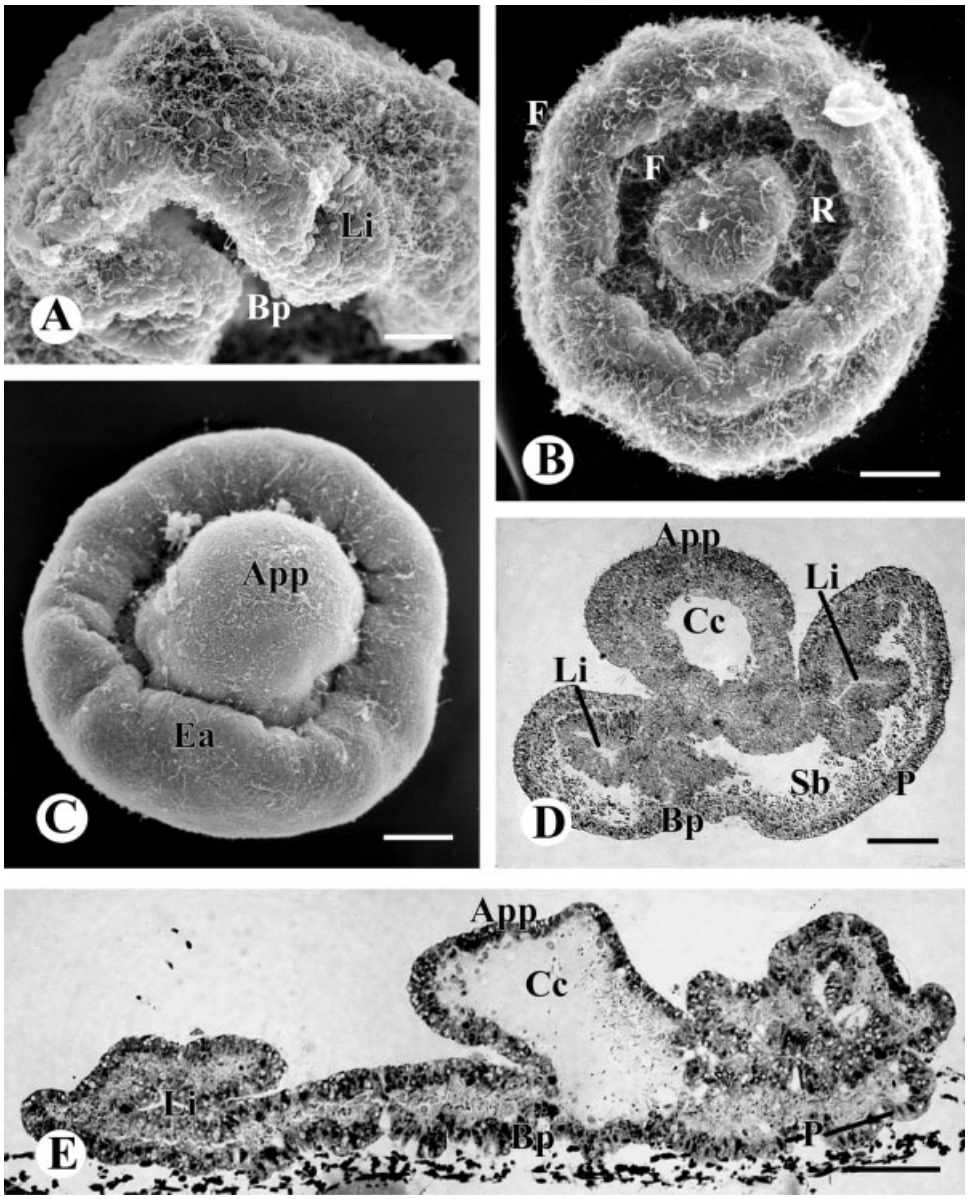

Fig. 7. Morphogenetic events during the metamorphosis. **A:** Dome-shaped invagination of basal epithelium of a post-larva (*Oscarella tuberculata*) (SEM). Scale bar 30 μm. **B:** Ring-like involution of the cells surrounding the central attached part of a post-larva (*Oscarella tuberculata*) (SEM). Scale bar 30 μm. **C:** Extension and involution of lateral-apical flagellated epithelium in *Corticium candelabrum* (SEM). Scale bar 50 μm. **D:** Involution of antero-apical flagellated epithelium in *Plakina trilopha.* Semi-thin section. Scale bar 50 μm. **E:** Involution of antero-apical flagellated epithelium in *Plakina trilopha.* Semi-thin section. Scale bar 25 μm. App, apical part; Bp, basal part; Cc, central cavity; Ea, edge area; F, flagellum; Li, secondary lateral involutions; P, cells with intranuclear paracrystalline inclusions; R, ring-like involution; Sb, symbiotic bacteria.

formly distributed inside the rhagon (Figs. 1E, 3B, 4D, 8D, 9A). During metamorphosis symbiotic bacteria have not been observed to be phagocytosed, and continue to divide. Also during metamorphosis, we did not observe either phagocytosis of cells or cell proliferation.

DISCUSSION

We have shown in previous works that the larval epithelium of homoscleromorphs is a true columnar epithelium, homologous to the eumetazoan epithelium: intercellular junctions join the apical parts of the cells, and a basement membrane underlying the flagellated cell is lining the larval cavity (Boury-Esnault et al., 2003). Here we have demonstrated that the formation of the adult pinacoderm and choanoderm during metamorphosis occurs through the differentiation of the larval epithelium. This cytodifferentiation is similar to that of the eumetazoan epithelium (Gilbert, 2003). The transformation of single-layered epithelia is well known in de-

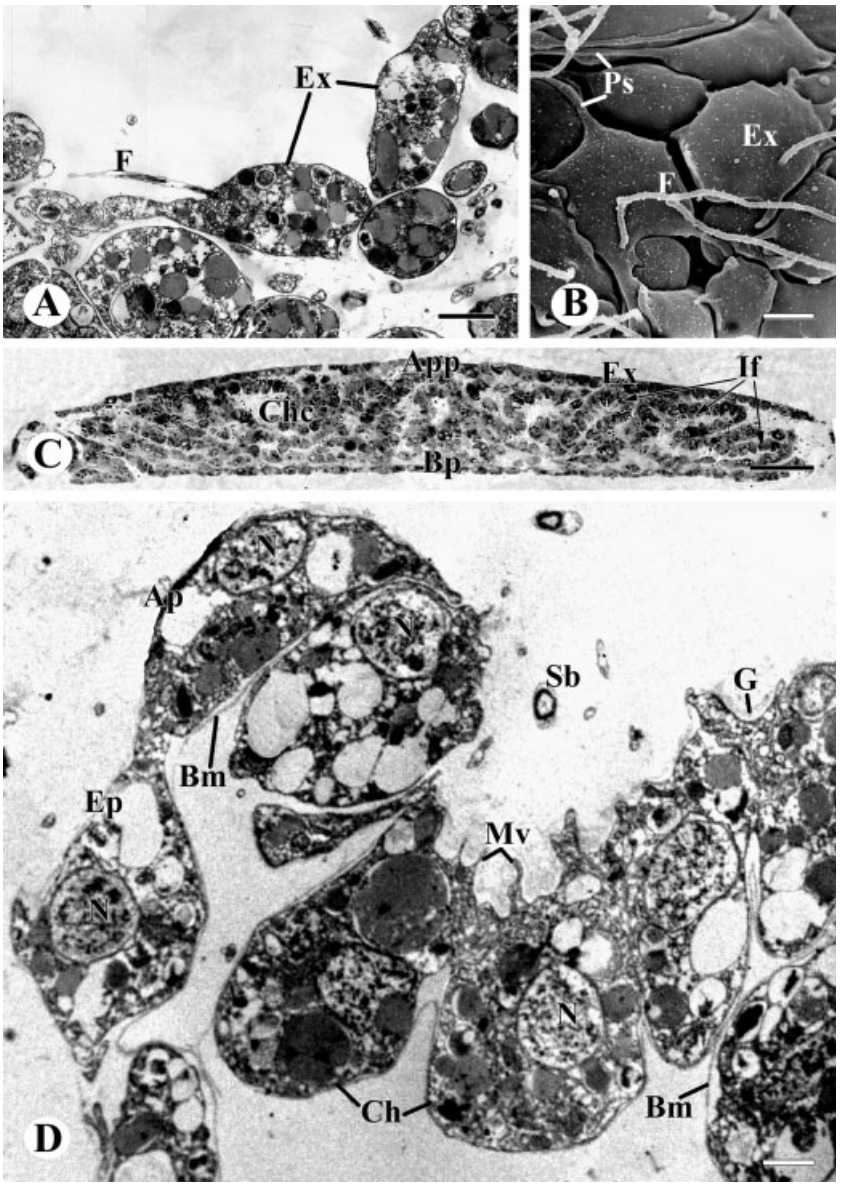

Fig. 8. Pinacoderm and choanocyte chamber formation in *Oscarella tuberculata*. **A,B:** Exopinacoderm formation. Scale bar 2 μm. A: TEM; B: SEM. **C:** Rhagon with developing aquiferous system. Semi-thin section. Scale bar 25 μm. **D:** Endopinacoderm and choanocyte chamber formation in the rhagon (TEM). Scale bar 2.5 μm. Ap, apopylar cell; App, apical part; Bm, basement membrane; Bp, basal part; Ch, choanoblast; Chc, choanocyte chamber; Ep, endopinacoblast; Ex, exopinacoblast; F, flagellum; G, glycocalyx; If, internal folds of post-larva epithelium; Mv, microvilli; N, nucleus; Ps, pseudopodia; Sb, symbiotic bacteria.

velopmental biology during the formation of various hollow organs when an increase of the surface occurs without proliferation (Wolpert, 1998; Gilbert, 2003). We have also shown here that the anteroposterior axis of the larva corresponds to the basoapical axis of the adult in Homoscleromorpha.

In all sponge species studied so far metamorphosis is accompanied by major disorganization of the external layer of larval flagellated cells, and formation of the pinacoderm and choanoderm occurs by new association of separate cells forming epithelial layers (Lévi, 1956; Borojevic and Lévi, 1965; Boury-Esnault, 1976; Bergquist and Green, 1977; Kaye and Reiswig, 1991; Amano and Hori, 1993, 1996, 2001; Ivanova, 1997; Leys and Degnan, 2002). The main difference between homoscleromorph metamorphosis and that occurring in other Demospongiae is the persistence in the former of the larval epithelium throughout this process.

In all homoscleromorph species studied so far, the steps of metamorphosis are similar. Events occurring after cinctoblastula settlement can be subdivided into three stages: 1) the beginning of cell differentiation according to the position of the cells in the post-larva; 2) invagination and involution of larval epithelium, both processes being accompa-

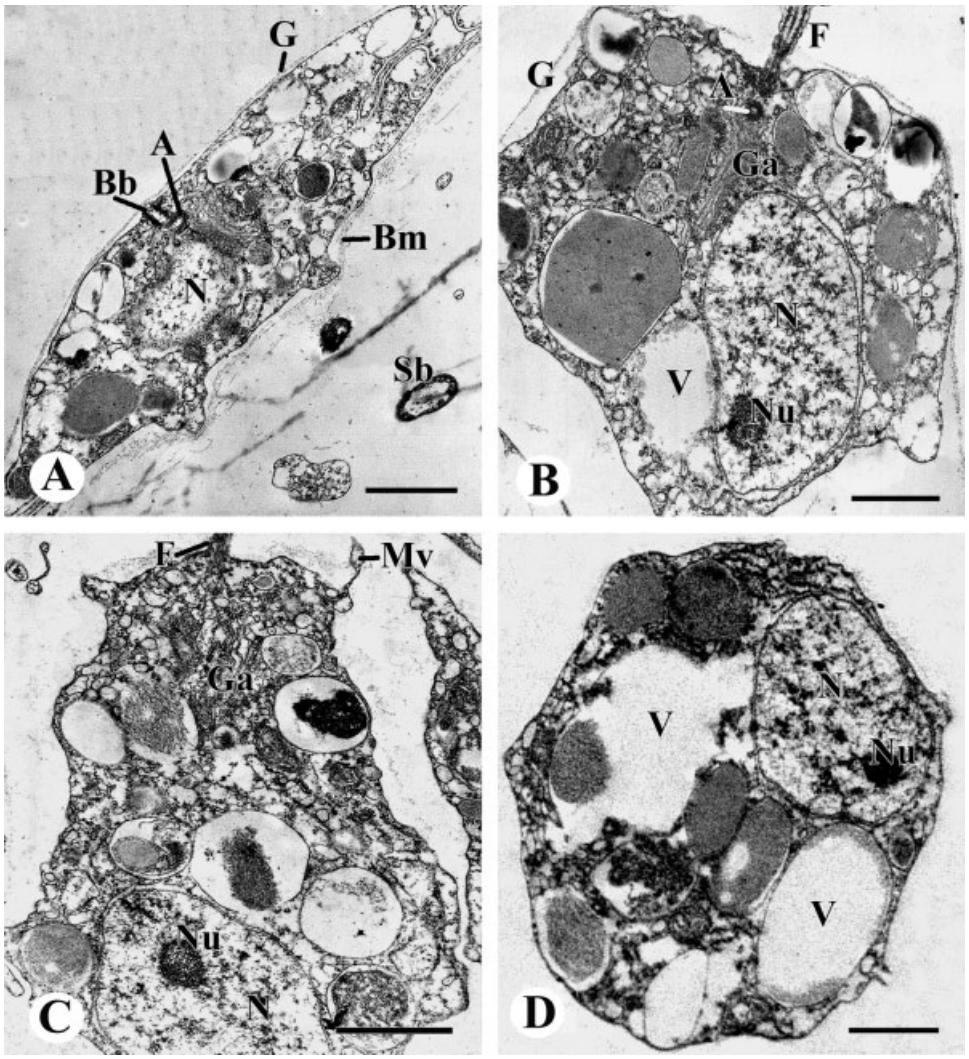

Fig. 9. Adult cell differentiation (TEM) in the rhagon of *Oscarella tuberculata*. **A:** Endopinacocyte differentiation (TEM). Scale bar 2 μm. **B,C:** Differentiation of choanocytes (TEM). Scale bar 1 μm. **D:** Differentiation of mesohylar vacuolar cell (TEM). Scale bar 1 μm. A, accessory centriole; Bb, basal body of flagellar apparatus; Bm, basement membrane; F, flagellum; G, glycocalyx; Ga, Golgi apparatus; Mv, microvilli; N, nucleus; Nu, nucleolus; Sb, symbiotic bacteria; V, vacuole.

nied by ingression of cells from apical part and circumferential belt of the post-larva; 3) development of the aquiferous system.

During metamorphosis of Homoscleromorpha, earlier researchers (Heider, 1886; Maas, 1898; Meewis, 1938) described only the invagination of the basal epithelium with the subsequent involution of marginal sides. Meewis (1938) also described the involution of the apical epithelium at the last stages of aquiferous system development. We have shown that the metamorphosis occurs by the invagination and involution of either the basal epithelium or the apical epithelium (Fig. 2).

The larvae released by the same parent and cultivated under the same conditions have shown morphogenetic polymorphism during metamorphosis. Therefore, this polymorphism has an individual character and does not depend on either external factors or species specific factors for *Oscarella tuberculata, O. microlobata, Plakina trilopha, Corticium candelabrum*. Whichever morphogenetic path is followed during formation of the aquiferous system, the same rhagon is formed. However, this plasticity occurs only during metamorphosis and morphogenesis of the rhagon, and has not been observed during embryogenesis (Ereskovsky and Boury-Esnault, 2002; Boury-Esnault et al., 2003).

We have demonstrated here that during metamorphosis, the fate of cinctoblastula cells depends on their position (Table 1). During the metamorphosis by basal invagination (Heider, 1886; Maas, 1898; Meewis, 1938), antero-lateral cells of larvae play the leading role in formation of the aquiferous system of the rhagon (Fig. 2b–b^2,c–c^2). In this case, posterior cells generate the exopinacoderm and postero-lateral cells, the basopinacoderm. A similar

TABLE 1. The fate of the flagellated cells of the cinctoblastula in different morphogenetic paths followed during metamorphosis and formation of the rhagon

Cells	Morphogenesis		
	Basal invagination	Basal ring	Apical involution
Anterior pole	ch, en	bp, ch, en	bp, ex
Antero-lateral	ch, en	ch, en	bp, en, ex
Postero-lateral with intranuclear inclusions	bp	bp	ch, en
Posterior pole	ex	ex	ch, en

bp, basopinacoderm; ch, choanoderm; en, endopinacoderm; ex, exopinacoderm.

sequence has been described for calcaronean amphiblastulae (Amano and Hori, 1993) and in larvae of *Halisarca dujardini* (Gonobobleva and Ereskovsky, 2004). When apical involution occurs the exopinacoderm differentiates from cells of the lateral zone. In the case of apical involution, antero-lateral cells differentiate into baso- and exopinacoderm, whereas postero-lateral and posterior cells give rise to aquiferous system elements (Fig. 2d–d^3). Mesohylar cells differentiate from cells of antero-lateral and postero-lateral zones. As a general rule, the first structure of the adult sponge formed during metamorphosis is the exopinacoderm (Bergquist and Green, 1977; Amano and Hori, 1993, 2001; Leys and Degnan, 2002; Gonobobleva and Ereskovsky, 2004). In homoscleromorphs the first steps of exopinacoderm development occur when the basal layer invaginates (Fig. 2b,c) or after involution of the apical epithelium (Fig. 2d).

In Demospongiae species studied so far, the basic source of choanocytes is usually either internal cells of the parenchymella (Bergquist and Green, 1977; Misevic and Burger, 1982; Bergquist and Glasgow, 1986; Weissenfels, 1989; Misevic et al., 1990; Kaltenbach et al., 1999) or flagellated cells of the parenchymella (Borojevic and Lévi, 1965; Amano and Hori, 1996; Ivanova, 1997; Leys and Degnan, 2002). During rhagon development of Homoscleromorpha all the structures are formed only by direct transformation of flagellated cells, and therefore flagellated cells are the sole source of choanocytes in Homoscleromorpha.

In previous works (Ereskovsky and Boury-Esnault, 2002; Boury-Esnault et al., 2003), we have shown that embryological and larval ultrastructural characters support the monophyly of Homoscleromorpha. The formation of the coeloblastula by multipolar egression, the morphogenesis and morphology of cinctoblastula larvae, the presence of a basement membrane underlying the larval epithelium, and the characteristics of the basal apparatus of the flagellum are similar in all the homoscleromorph species investigated so far. Analyses of sequences of 18S rRNA cannot support the monophyly of Demospongiae in the traditional sense and this is due to the Homoscleromorpha being considered as a separate branch from the remaining demosponges (Borchiellini et al., 2004). In the present state of phylogenetic resolution, four poriferan clades have been identified: Hexactinellida, Calcispongia, Homoscleromorpha, and Demospongiae *sensu stricto,* i.e., without Homoscleromorpha. The relationships between these four clades and the other eumetazoans have not yet been resolved. However, it is tempting to speculate that synapomorphies for a possible clade Eumetazoan + Homoscleromorpha could be the presence of a basal membrane (Boute et al., 1996; Exposito et al., 2002), the morphogenesis of the epithelia of the adult, and polarity axis conservation between the larva and the adult.

ACKNOWLEDGMENTS

The authors thank Jean Vacelet for helpful discussions, Christian Marschal and Roland Graille for diving assistance, and Michael Paul for improving the English. A. Ereskovsky received a special grant from the French "Ministère de l'Education nationale et de la Recherche" and Federal Scientific Policy of Belgium, intended to promote S&T collaboration with eastern and central Europe.

LITERATURE CITED

Amano S, Hori I. 1993. Metamorphosis of calcareous sponges. II. Cell rearrangement and differentiation in metamorphosis. Invert Reprod Dev 24:13–26.

Amano S, Hori I. 1996. Transdifferentiation of larval flagellated cells to Choanocytes in the metamorphosis of the demosponge *Haliclona permollis.* Biol Bull 190:161–172.

Amano S, Hori I. 2001. Metamorphosis of coeloblastula performed by multipotential larval flagellated cells in the calcareous sponge *Leucosolenia laxa.* Biol Bull 200:20–32.

Baccetti B, Gaino E, Sarà M. 1986. A sponge with acrosome: *Oscarella lobularis*. J Ultrastruct Mol Struct Res 94:195–198.

Barrois CH 1876. Embryologie de quelques éponges de la Manche. Docteur ès Sciences Naturelles Thesis, Faculté des Sciences de Paris.

Bergquist PR, Green CR. 1977. An ultrastructural study of settlement and metamorphosis in sponge larvae. Cah Biol Mar 18:289–302.

Bergquist PR, Glasgow K. 1986. Developmental potential of epithelial cells of ceractinomorph sponge larvae. Exp Cell Biol 45:111–122.

Borchiellini C, Chombard C, Manuel M, Alivon E, Vacelet J, Boury-Esnault N. 2004. Molecular phylogeny of Demospongiae: Implications for classification and scenarios of character evolution. Mol Phyl Evol 32:823–837.

Borojevic R, Lévi C. 1965. Morphogénèse expérimentale d'une Eponge à partir de cellules de la larve nageante dissociée. Z Zellforsch 68:57–69.

Boury-Esnault N. 1976. Ultrastructure de la larve parenchymella d'*Hamigera hamigera* (Schmidt) (Demospongiae, Poecilosclerida). Origine des cellules grises. Cah Biol Mar 17:9–20.

Boury-Esnault N, Rützler K. 1997. Thesaurus of Sponge Morphology. Washington, DC: Smithsonian Press. 55 pp. Smithsonian Contributions to Zoology, Vol. 296.

Boury-Esnault N, Jamieson BGM. 1999. I. Porifera. In: Jamieson BGM, editor. Progress in Male Gamete Biology. New Delhi: Oxford and IBH Publishing Co. pp 1–20.

Boury-Esnault N, De Vos L, Donadey C, Vacelet J. 1984. Comparative study of the choanosome of Porifera. I. The Homoscleromorpha. J Morphol 180:3–17.

Boury-Esnault N, Solé-Cava AM, Thorpe JP. 1992. Genetic and cytological divergence between colour morphs of the Mediterranean sponge *Oscarella lobularis* Schmidt (Porifera, Demospongiae, Oscarellidae). J Nat Hist 26:271–284.

Boury-Esnault N, Muricy G, Gallissian M-F, Vacelet J. 1995. Sponges without skeleton: A new Mediterranean genus of Homoscleromorpha (Porifera, Demospongiae). Ophelia 43:25–43.

Boury-Esnault N, Ereskovsky AV, Bézac C, Tokina DB. 2003. Larval development in Homoscleromorpha (Porifera, Demospongiae). Invert Biol 122:187–202.

Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Noro N, Miyazaki K, Yoshigato K, Garrone R. 1996. Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell 88:37–44.

Brien P, Meewis H. 1938. Contribution à l'étude de l'embryogénèse des Spongillidae. Arch Biol (Bruxelles) 49:178–250.

Ereskovsky AV, Boury-Esnault N. 2002. Cleavage pattern in *Oscarella* species (Porifera, Demospongiae, Homoscleromorpha), transmission of maternal cells and symbiotic bacteria. J Nat Hist 36:1761–1775.

Evans CW. 1977. The ultrastructure of larvae from the marine sponge *Halichondria moorei* (Berquist) (Porifera, Demospongiae). Cah Biol Mar 18:427–433.

Exposito JY, Cluzel C, Garrone R, Lethias C. 2002. Evolution of collagens. Anat Rec 268:302–316.

Fell PE. 1997. The concept of larvae. In: Gilbert SF, Raunio AM, editors. Embryology. Constructing the Organism. Sunderland: Sinauer Associates. pp 21–28.

Gilbert SF. 2003. Developmental Biology. Sunderland: Sinauer Associates. 520 pp.

Gilbert SF, Raunio AM (editors). 1997. Embryology. Constructing the Organism. Sunderland: Sinauer Associates. 537 pp.

Gonobobleva EL, Ereskovsky AV. 2004. Metamorphosis of the larva of *Halisarca dujardini* (Demospongiae, Halisarcida). Bull Inst R Sci Nat Belg Biol 74:101–115.

Harrison FW, De Vos L. 1991. Porifera. In: Harrison FW, Westfall JA, editors. Microscopic Anatomy of Invertebrates. II. Placozoa, Porifera, Cnidaria and Ctenophora. New York: Wiley-Liss. pp 29–89.

Heider K. 1886. Zur Metamorphose der *Oscarella lobularis* O. Schmidt. Arbeit Zoologisch Inst Universität Wien 6:175–236.

Ilan M, Loya Y. 1990. Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. Mar Biol 105:25–31.

Ivanova LV. 1997. New data about morphology and metamorphosis of the spongillid larvae (Porifera, Spongillidae). II. The metamorphosis of the spongillid larvae. In: Ereskovsky AV, Keupp H, Kohring R, editors. Modern Problems of Poriferan Biology. Berlin: Berliner Geowiss. Abh. (E). Vol. 20, pp 73–91.

Johnston IS, Hildemann WH. 1982. Cellular organization in the marine demosponge *Callyspongia diffusa*. Mar Biol 67:1–7.

Kaltenbach JC, Kuhns WJ, Simpson TL, Burger MM. 1999. Intense concanavalin A staining and apoptosis of peripheral flagellated cells in larvae of the marine sponge *Microciona prolifera*: Significance in relation to morphogenesis. Biol Bull 197:271–273.

Kaye HR, Reiswig HM. 1991. Sexual reproduction in four Caribbean commercial sponges. III. Larval behaviour, settlement and metamorphosis. Invert Reprod Dev 19:25–35.

Lévi C. 1956. Étude des Halisarca de Roscoff. Embryologie et systématique des démosponges. Arch Zool Exp Gén 93:1–181.

Lévi C, Porte A. 1962. Etude au microscope électronique de l'éponge *Oscarella lobularis* Schmidt et de sa larve amphiblastula. Cah Biol Mar 3:307–315.

Leys SP, Degnan BM. 2002. Embryogenesis and metamorphosis in a haplosclerid demosponge: Gastrulation and transdifferentiation of larval ciliated cells to choanocytes. Invert Biol 121:171–189.

Maas O. 1898. Die Keimblätter der Spongien und die Metamorphose von *Oscarella* (*Halisarca*). Z Wiss Zool 63:665–679.

Meewis H. 1938. Contribution à l'étude de l'embryogénèse des Myxospongidae: *Halisarca lobularis* (Schmidt). Arch Biol Liège 50:1–66.

Misevic GN, Burger MM. 1982. The molecular basis of species specific cell–cell recognition in marine sponges, and a study on organogenesis during metamorphosis. Prog Clin Biol Res B 85:193–209.

Misevic GN, Schlup V, Burger MM. 1990. Larval metamorphosis of *Microciona prolifera*: Evidence against the reversal of layers. In: Rützler K, editor. New Perspectives in Sponge Biology. Washington, DC: Smithsonian Institution Press. pp 182–187.

Muricy G, Boury-Esnault N, Bézac C, Vacelet J. 1996a. Cytological evidence for cryptic speciation in Mediterranean *Oscarella* species (Porifera, Homoscleromorpha). Can J Zool 74:881–896.

Muricy G, Solé-Cava AM, Thorpe JP, Boury-Esnault N. 1996b. Genetic evidence for extensive cryptic speciation in the subtidal sponge *Plakina trilopha* (Porifera, Demospongiae, Homoscleromorpha). Mar Ecol Prog Series 138:181–187.

Muricy G, Bézac C, Gallissian M-F, Boury-Esnault N. 1999. Anatomy, cytology and symbiotic bacteria of four Mediterranean species of *Plakina* (Demospongiae, Homosclerophorida). J Nat Hist 33:159–176.

Schmidt O. 1862. Die Spongien des Adriatischen Meeres. Leipzig: Wilhelm Engelmann. 88 pp.

Schmidt O. 1868. Die Spongien der Küste von Algier mit Nachträgen zu den Spongien des Adriatischen Meeres (drittes Supplement). Leipzig: Verlag von Wilhelm Engelmann. 42 pp.

Schulze FE. 1880. Untersuchungen über den Bau und die Entwicklung der Spongien. Die Plakiniden. Z Wiss Zool 34: 407–451.

Solé-Cava AM, Boury-Esnault N, Vacelet J, Thorpe JP. 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae, Homoscleromorpha) in the Mediterranean Sea. Mar Biol 113:299–304.

Stern CD (editor). 2004. Gastrulation: From Cells to Embryo. London: University College. 731 pp.

Weissenfels N. 1989. Biologie und Mikroskopische Anatomie der Süβwasserschwämme (Spongillidae). New York: Gustav Fischer, Stuttgart. 110 p.

Wolpert L. 1998. Principles of Development. New York: Oxford University Press. 580 pp.