THE ULTRASTRUCTURE OF LARVAE FROM THE MARINE SPONGE *HALICHONDRIA MOOREI* BERGQUIST (PORIFERA, DEMOSPONGIAE)

by

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Résumé

Les larves de l'éponge marine *Halichondria moorei* Bergquist ont été étudiées au moyen des techniques ultrastructurales, à la fois dans l'éponge adulte et, *in situ*, après leur fixation à un substrat artificiel. L'auteur constate que les larves fixées au moment de la métamorphose possèdent une glycocalyce complexe et unique, tapissant les cellules sur leur surface supérieure. Cette structure unique, qu'on a appelé le manteau larvaire de l'éponge ne se présente ni chez l'adulte ni sur les larves non fixées. L'ultrastructure et le mode possible de synthèse de ce manteau larvaire sont discutés et on note sa ressemblance par certains aspects avec le manteau superficiel frisotté de l'amibe. En examinant les fonctions de ce manteau larvaire, il n'a pas été possible de prouver son rôle dans l'ingestion et on doit ainsi admettre comme un postulat que la fonction principale de cette formation serait d'apporter une protection et un appui aux larves fixées au cours de la métamorphose.

L'examen de l'adhérence des larves d'éponges à un substrat artificiel permet de suggérer que le mécanisme de fixation ressemble en partie à l'adhérence de nombreuses cellules en culture à leur substrat. Cette hypothèse est corroborée par l'absence, chez ces larves d'éponges, des glandes spécialisées du cément qui entrent en jeu, on le sait, dans la fixation au substrat chez les autres Invertébrés marins. L'auteur émet l'hypothèse que la fixation au substrat chez ces larves d'éponges exige deux séries d'actions réciproques : la première entre le manteau cellulaire et un tapis sécrété par la larve; la seconde entre le tapis et le substrat. On voit ainsi que le tapis s'interpose entre les cellules fondamentales des larves et le substrat auquel elles sont fixées.

Introduction

Although the reproductive processes of some sponge species have been adequately described, precise information concerning many aspects of reproduction and development within the Phylum Porifera is still outstanding. This paper examines the ultrastructural changes occurring during settlement and metamorphosis of larvae from *Halichondria moorei*, a common intertidal sponge in the Waitemata Harbour, New Zealand (Bergquist, 1961). *H. moorei* was chosen for study because some aspects of its reproduction have been the

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subject of an earlier report (Bergquist and Sinclair, 1968) and the formation and development of larvae within this species are considered to be typical of the Sub-class Ceractinomorpha. Settlement of *H. moorei* larvae occurs within 60 hours of release by attachment at the anterior pole and a functional canal system exists after 72 hours (Bergquist et al, 1970).

The larvae of *H. moorei* are particularly suitable for ultrastructural studies because of their relatively large size (mean overall length, 1.4mm) and because they are known to lack spicules in the early stages of development (Bergquist and Sinclair, 1968).

Materials and methods

Ultrastructural observations were performed on incubating larvae within the adult sponge, and after their removal by maceration of the sponge tissue. Fresh samples were obtained and fixed in 3 percent glutaraldehyde in sodium cacodylate buffer (O.1M, pH 7.3, 1100 m0sM), rinsed in buffer, post-fixed in buffered 1 percent 0s04, dehydrated in graded acetone, and mounted in Epon 812. Staining was achieved either *en bloc* with ruthenium red 1500 ppm (Luft, 1971) or on thin sections by double staining with uranyl acetate and lead citrate (Venable and Coggeshall, 1965). Thin sections were examined in a Philips EM200 electron-microscope and thick sections from Epon-embedded material were stained with methylene blue and examined in the light-microscope.

Examination after settlement was performed on larvae which had been allowed to attach to hardened Epon 812 lining the bottoms of shallow walled Petri-dishes. After an attachment period of 3-5 days, the larvae were fixed and embedded *in situ* using the techniques described above. After embedding, the Epon was removed from the Petri-dishes and suitable sized blocks for sectioning were prepared by sawing and trimming. Staining was carried out using the two methods described above. All techniques were carried out at room temperature (approximately 20°C).

RESULTS

Reproduction appeared to be by asexual means as archaeocytes were seen to form small, loose clusters within the sponge. Such clusters subsequently increased in size and became surrounded by a spongin sheath. After a period of compaction, the cells enclosed within the spongin sheaths differentiated to form the ciliated larvae. Ultrastructural observations at this stage of development immediately preceding release from the adult confirmed the presence of an external ciliated cell layer (Plate I, 1).

After settlement, the external ciliated cells were found to be replaced in the dorsal region by a pinacoderm composed of a

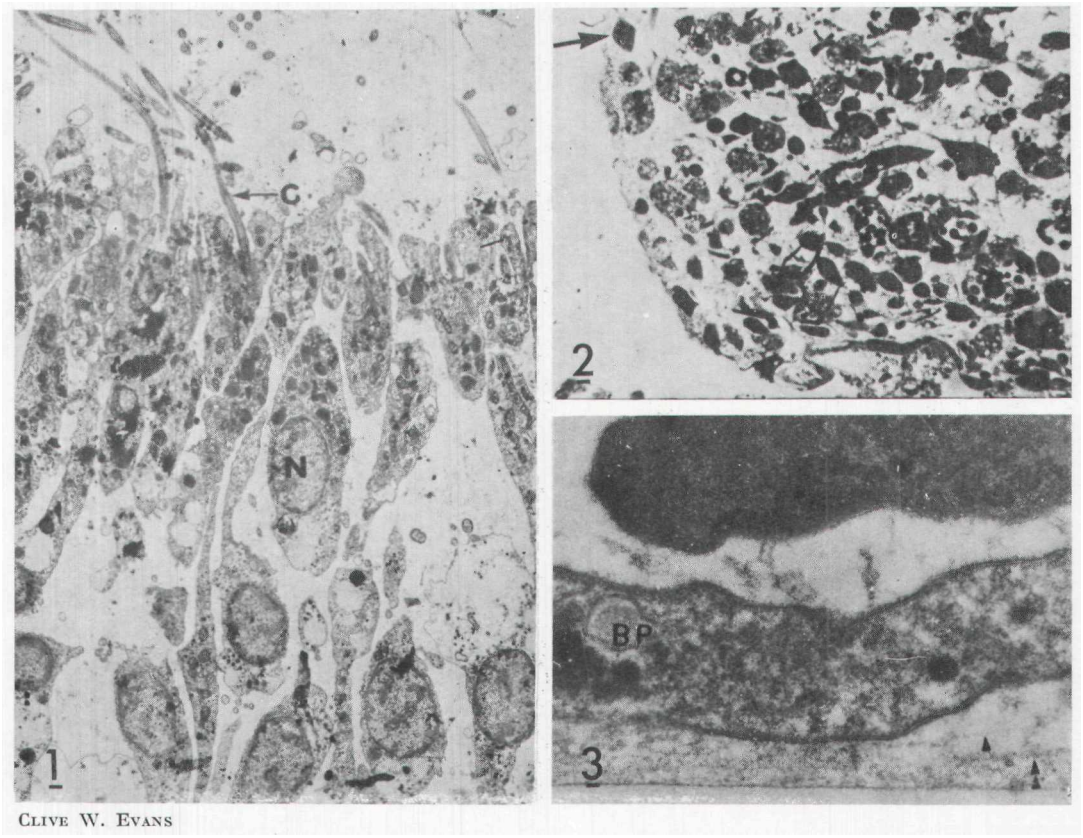

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PLATE I

1 :Ultrastructural observations confirm the presence of a limiting ciliated cell layer (c) on unsettled larvae removed from the body of the adult sponge. N, nucleus. X6200.

2: Light-micrograph of a larva attached to the basal Epon. Several T-pinacocytes are visible near the surface (arrow) and the change in shape to the flattened B-pinacocytes is obvious in the basal attachment region. X200.

3: The groundmat is clearly seen mediating between the basal-pinacocyte (BP) and the Epon substrate. The small and large fibrous components of the ground-mat are just discernible (single and double arrowheads respectively). X52,000.

single layer of distinctly shaped cells, the T-pinacocytes (Plates I,2 ; II,4). In the basal region, ciliated cells were replaced by a layer of flattened nucleolate cells referred to as the basal or B-pinacocytes (Plates I,3 ; II, 1 and 2). Contact with the artificial substrate appeared to be made in localized areas or sole-plates of the B-pinacocytes (Plates I,3 ; II,2). Each soleplate, however, did not adhere directly to the basal Epon since a groundmat was found to mediate between the cells and the substrate. The 1100Å thick groundmat overlaid particles of sand and debris which would have settled out from suspension before attachment of the larva, and was composed of collagen fibrils (diam. 200Å) within an amorphous substance (Plate II, 1 and 2). There was some evidence of finer filaments (Diam. 80Å) within the amorphous substance of the groundmat, but generally these were confined to an area surrounding the larva (Plate II,3). In regions where the larva had lifted from the substrate ruthenium red penetration was enhanced and a thickened cell coat (450Å) was obvious. Most cells of the larva, however, were surrounded with a ruthenium red-positive cell coat typically 200Å thick.

The external surface of the T-pinacocytes was found to contain a distinctive cell coat (the larval coat) in the early stages after settlement (Plate III, 1 and 4). After ruthenium red staining, the ultra structure of the larval coat could be interpreted as a four component system comprising a granular mucoid basal region (the basal substance) overlaid by a flat fibrous structure (the basal interconnecting strand) to which are attached filamentous lollipops, themselves interconnected by an additional mucous strand (the interconnecting strand). The mucoid basal substance was clearly distinct from the typical acid-mucopolysaccharide (AMP) cell coat which it overlaid (Plate III,4).

Further ultrastructural examination of settled larvae revealed that the cells were often separated by a well defined 150-200Å cell gap in the junctional region (Plate III,1). Ruthenium red penetrated these junctional regions and demonstrated the presence of the ubiquitous cell coat and occasional clumps or strands of mucous material stretching between the opposed cells. There is no available evidence at the moment to describe these junctional structures in terms which imply a similarity to any of the known cell junctions typical of other organisms.

DISCUSSION

The technique involving polymerized Epon as an artificial substrate for the *in situ* preparation of settled larvae generally provided satisfactory results, but two minor difficulties were apparent. Occasionally, the larvae lifted from the substrate during preparation (Plate II,1) and care had to be taken during the fixation and dehydration steps. The main difficulty was associated with softening

of the basal Epon which made sectioning problematical, but this could be overcome by employing a very hard Epon as the substrate and then using a much softer Epon for the covering layer. By a process of trial and error compression of the basal Epon during sectioning could be avoided and the standard of sections thus greatly improved.

Little information is available at present on the mechanism of attachment of the sponge larva to its substrate. It is clear from ultrastructural observations of the larva itself, however, that attachment does not involve secretions from specialized gland cells such as have already been demonstrated in other marine invertebrates (Nott, 1973) since no such structures were obvious. The absence of specialized glands and ducts for the secretion of an adhesive cement implies that surface adhesion in sponge larvae is a property of the B-pinacocytes overlying the substrate and of the secretions from this cell type. In other words, larva-substrate adhesion may in effect be considered analogous to cell- substrate adhesion.

It is apparent from the electron-micrographs that adhesion to the substrate is mediated by a groundmat which is collagenous in part and which is secreted by the sponge. Since the AMP cell coat is apparent on all cell types including the B-pinacocytes, then it seems likely that adhesion to the substrate involves two possibly different phenomena: adhesion of the cell coat to the groundmat and adhesion of the groundmat to the substrate. The presence of collagen fibrils within the groundmat is an interesting observation and it seems likely that they may serve to strengthen this structure and to distribute shear forces occurring at the sole-plates or adhesive sites. It is tempting to speculate on the nature of the groundmat and to consider its relationship to the aggregation promoting factor demonstrated in many sponge cells (Henkart et al, 1973) and indeed future studies will be carried out in this direction.

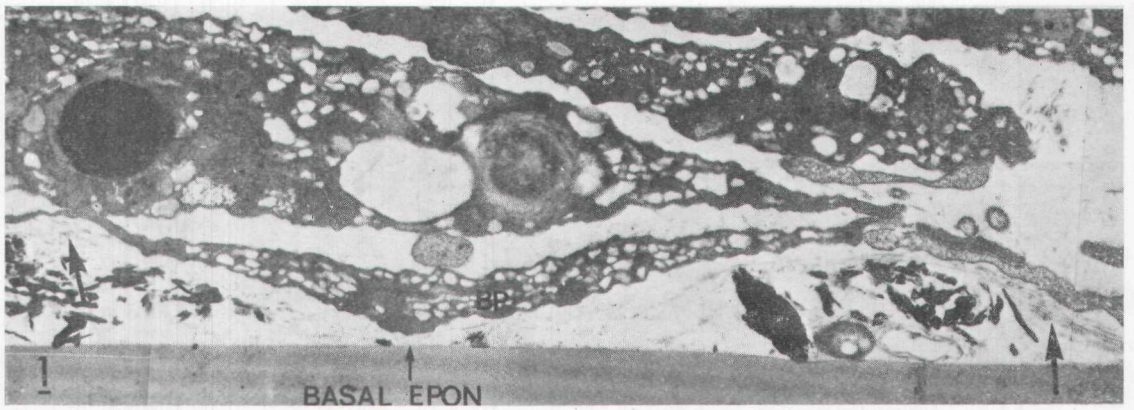
Nevertheless, the involvement of a groundmat or micro-exudate has been postulated in the attachment of cultured cells to their substrates (Terry and Culp, 1974) and more recently a model has been provided for the involvement of a groundmat in cell-substrate adhesion (Culp, 1974). Although studies on the attachment of sponge

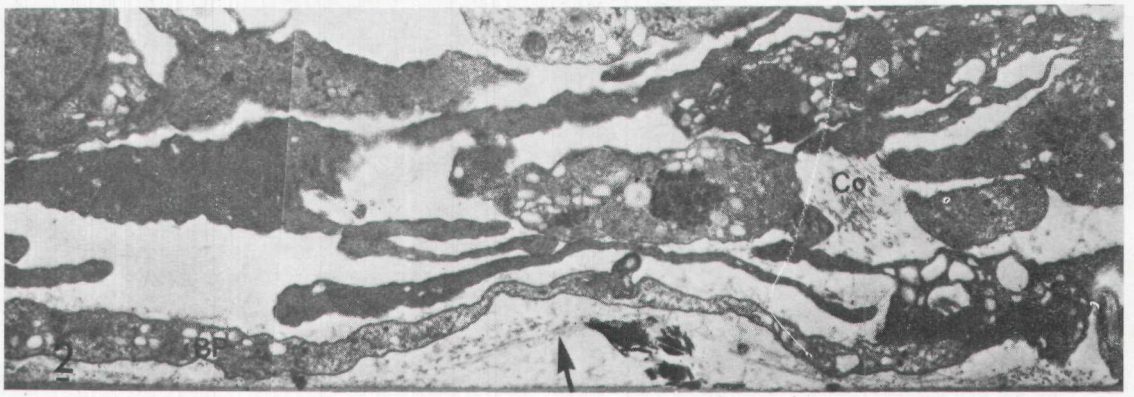
PLATE II

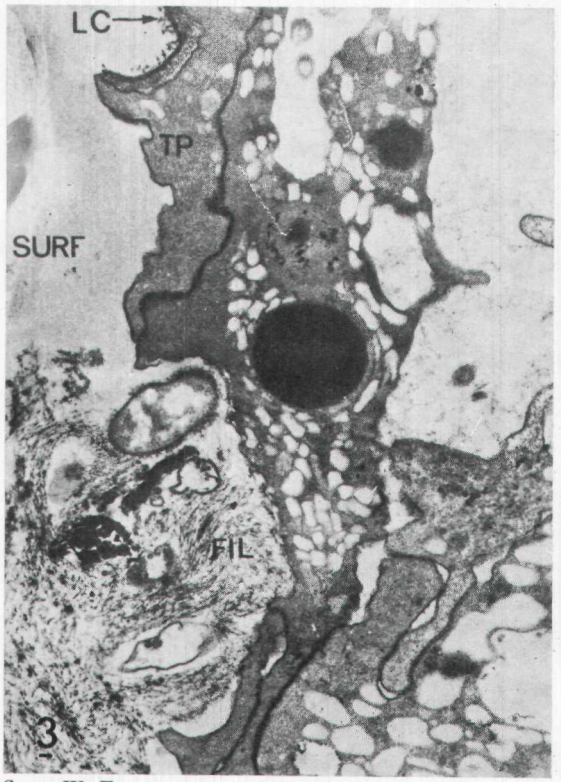
1 and 2: Two areas from a montage of the basal contact region. Contact with the artificial substrate is mediated by the basal groundmat and occurs in localized regions or sole-plates in preference to the entire basal area of the cell. In some regions, penetration and uptake of ruthenium red appears to have been limited (small arrow). The large fibrous component of the groundmat (large arrows), which is believed to represent spongin A or collagen (Co) is obvious especially in those regions where the groundmat rises off the substrate to overlay sand particles. X 12,400.

3: At the edge of a settled larva the filamentous zone (FIL) is particularly obvious. The larval coat (LC) is restricted to the T-pinacocytes (TP) at the surface (SURF). X 14,800.

4: A characteristic T-pinacocyte at the side of a settled larva. The larval coat is obvious at the surface to the left of the photograph, and a lysosome (LYS) is apparent within the cytoplasm. No material was found in observed lysosomes which might have suggested that the larval coat had been ingested by the cell. N, nucleus. X 14,300.







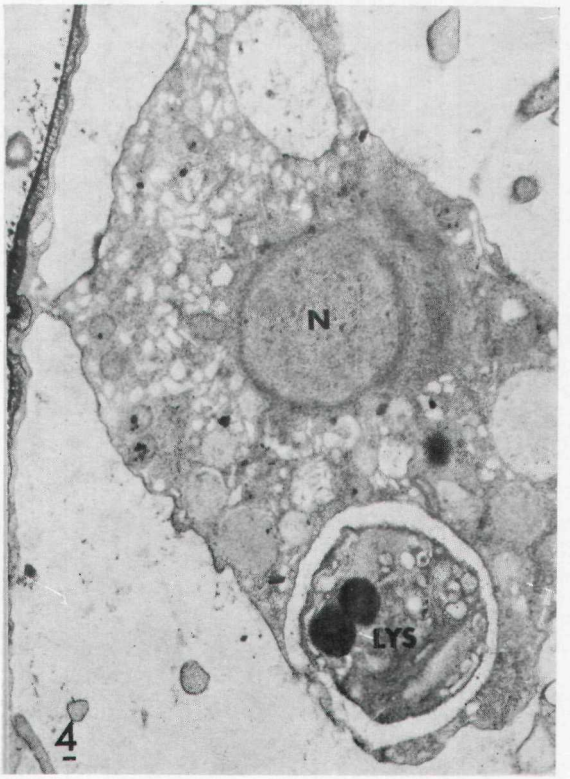


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PLATE II

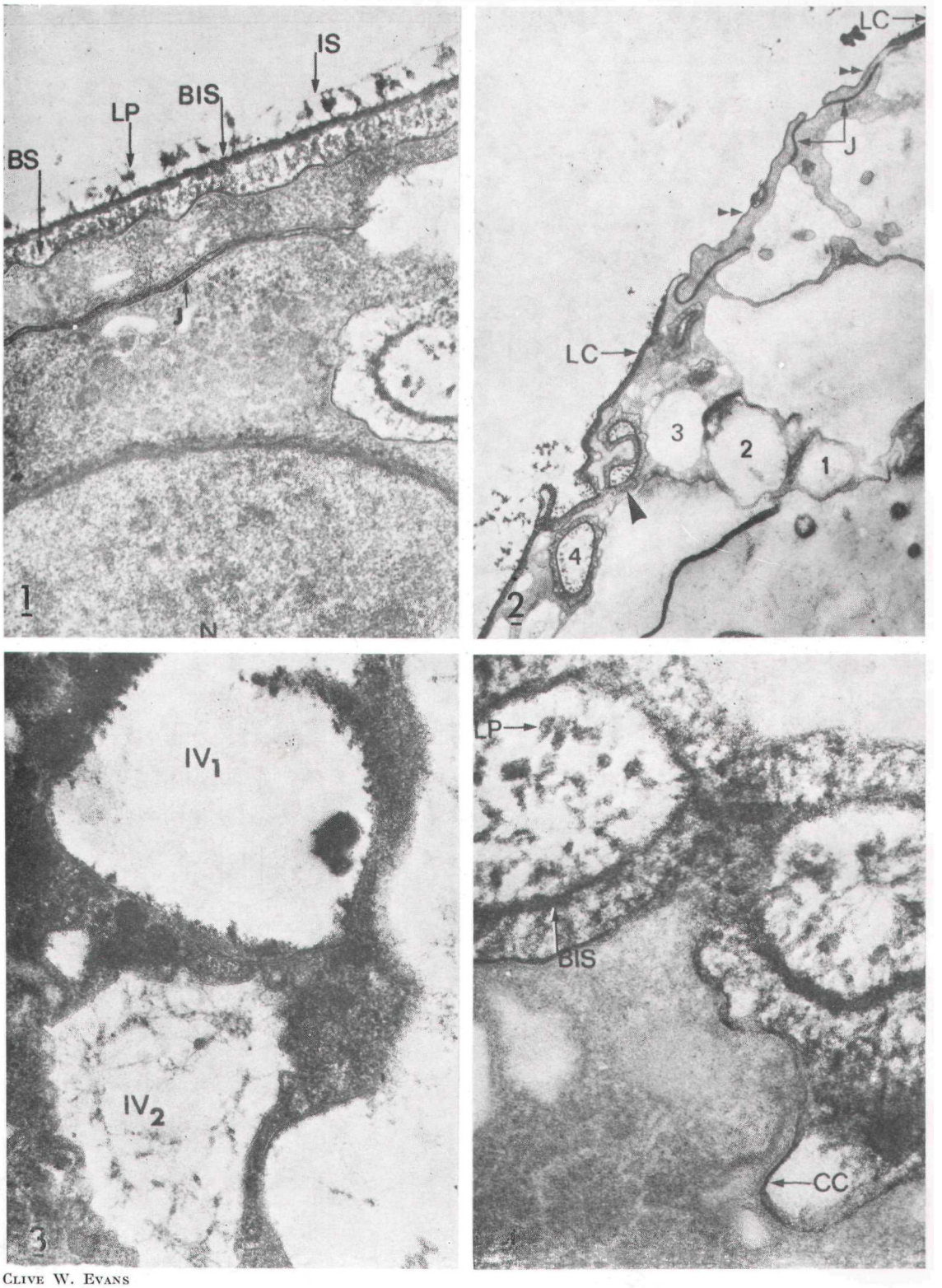

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Plate III

larvae are still in the initial stages, it is apparent from the electron-micrographs that substrate adhesion in these organisms involves more than just the cell coat as was originally suggested for cultured cells (Poste and Greenham, 1970).

In their study of settled larvae from *Mycale contarenii,* Borojevic and Lévi (1967) reported on the presence of filaments finer than collagen within the attachment region. Fine filaments (diam. 80Å) represented only a very minor component of the groundmat in *H. moorei,* whereas they were the major component of the fibrous zone which completely surrounded the settled larva (Planche III,1). The precise relationship between the fine filaments of the larvae of these two sponge species remains uncertain, and at present no suggestions can be made as to what role the fibrous zone in *H. moorei* larvae may serve.

The larval coat is clearly a unique and complex structure, yet it bears many similarities to the fuzzy coat described on the surface membranes of various amoebae (Hausmann, 1975; Revel and Ito, 1967). The fuzz of the amoeba surface coat is composed of numerous knobbed filaments (not unlike the lollipop structres described in the sponge larval coat) which overlay an amorphous layer possible analogous to the basal substance. There are, however, no structures equivalent to the two interconnecting strands of the larval coat within the amoeba fuzzy layer.

The surface coat of the amoeba apparently behaves very much like a biological cation exchange resin, and it is believed to be involved in concentrating positively charged material at the surface prior to ingestion by phagocytosis or pinocytosis (reviewed in Revel and Ito, 1967). Although occasional lysosomes were evident in the T-pinacocytes indicating that this cell type is at least capable of ingesting material, no material was found entrapped in cell surface invaginations which would be expected if feeding was to take place by phagocytosis for example. It is possible that the lysosomes contain material phagocytosed from within the larva and there is thus no

PLATE III

1 : The unique structure of the larval coat is clearly revealed in this electromicrograph. The interconnecting strand (IS) extends between the filamentous lollipops (LP) which arise from the basal interconnecting strand (BIS). The BIS overlies the granular basal substance (BS) which is clearly distinct from the darker AMP cell coat. A cell junction (J) is visible between two pinacocytes. X 49,400.

2: It is suggested that the larval coat (LC) forms within intracellular vesicles (1-3) of the T-pinacocytes as they progress towards the surface where they subsequently fuse (large arrowhead). The difference in the intensity of staining between these vesicles and surface indentations (4) suggests they are truly intracellular in nature since ruthenium red penetrates cell membranes poorly. Ruthenium red is seen to concentrate in the cell junctions (J), and some areas are apparent where the larval coat is lacking (double arrowheads). It is possible that these may be regions of new cell growth. X 14,300.

3: Two intracellular vesicles (IV) located within a T-pinacocyte. In IV_2, the development of the larval coat is becoming obvious. X 49,400.

4: Two intracellular vesicles appear to have just made contact with the cell surface. At these magnifications the fibrous nature of the basal interconnecting strand (BIS) is just discernible despite the intensitv of staining of associated mucosubstances. CC, cell coat; LP, lollipop. X 129,000.

need to implicate a role for the larval coat in feeding. Indeed the absence of particles trapped to the surface of the larva and the early development of a functional canal system argue against the need for such a method to obtain food. Since the larval coat is lost within 5 days after attachment and because it has not been found on the adult sponge nor on unsettled larvae, then it is reasonable to suggest that it may confer some degree of protection and support to the larva during metamorphosis.

The larval coat is interpreted as being secreted by the underlying T-pinacocytes since electron-micrographs of the edges of settled sponge larvae revealed that it was localized to this cell type (Plate II,3). Infoldings of the T-pinacocyte cell surface were evident occasionally, and usally appeared as "vesicles" in thin sections (Plate III,2: stage 4). These "vesicles" actually representing surface indentations were always accessible to ruthenium red staining *en bloc* and displayed the typical larval coat. The intracellular vesicles, on the other hand, were not accessible to ruthenium red presumably because the large size and high positive charge of the ruthenium red molecule renders penetration of the cell membrane difficult. In such vesicles, ruthenium red provided only minimal ultrastructural differentiation and thus certain vesicles lying deep within the T-pinacocytes were interpreted as reliably representing intracellular stages in the formation of the larval coat (Plate II,2: stages 1-3; II,3). Occasionally regions were found within the larvae which suggested, when examined in cross-section, that the contents of the vesicles had just been discharges at the surface (Plate III,4). It is therefore proposed that the larval coat is formed from the organization of diffuse mucoid material secreted within intracellular vesicles of the T-pinacocytes. The precise mechanism by which the unique ultrastructure is achieved and whether the intracellular vesicles are derived directly from the Golgi apparatus or not are some problems which remain outstanding, but studies in progress are aimed at confirming and extending the present proposals so that some insight can be gained on such problems.

Summary

Larvae from the marine sponge *Halichondria moorei* Bergquist have been examined by ultrastructural techniques both within the adult sponge and *in situ* after attachment to an artificial substrate. Settled larvae undergoing metamorphosis were found to possess a complex and unique glycocalyx lining the cells of their upper surface. This unique structure, which has been referred to as the sponge larval coat, was not present on adult sponges nor was it found on unsettled larvae. The ultrastructure and possible mode of synthesis of the larval coat have been discussed, and its similarity in some respects to the amoeba fuzzy surface coat has been noted. In examining the possible functions of the larval coat, no evidence could be obtained to suggest a role in ingestion and thus it is postulated that the main functions of this structure may be to confer protection and support to the settled larvae during metamorphosis.

Adhesion of sponge larvae to an artificial substrate has also been examined and it is suggested that the mechanism of attachment bears some similarity to the adhesion of many cultured cells to their substrates. This hypothesis is supported by the absence in sponge larvae of specialized cement glands which are known to be involved in substrate attachment in other marine invertebrates. It is postulated that substrate attachment in sponge larvae involves two sets of interactions: the first is between the cell coat and a groundmat secreted by the

larvae and the second involves the attachment of the groundmat to the substrate. The groundmat is thus seen to mediate between the basal cells of the larvae and the substrate to which the larvae are attached.

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