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The nature of the skeleton and skeletogenic tissues in the Cnidaria

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Abstract

Calcification of zooxanthellate and non-zooxanthellate corals from 2 classes and 3 orders of Cnidaria was investigated using scanning and transmission electron microscopy and light microscopy. The ultrastructure of the skeleton and skeletogenic tissues (the calicoblastic ectoderm) from areas of active and non-active skeleton deposition were investigated. The results show that the fundamental cellular mechanism of calcification is similar in all 3 orders, and that the role of endosymbiotic zooxanthellae may be one that is concerned with the removal of waste products of the calcification process. The results are discussed with respect to the concepts of calcification and its evolution in the Cnidaria.

Introduction

Within Cnidaria the development of a calcareous exoskeleton has occurred in 2 classes (Hydrozoa and Anthozoa). Ancestral soft bodied and skeleton-less lines separated before the Cambrian era to evolve into the present cnidarian orders (Hill & Wells, 1956). Cnidarians that have a calcareous skeleton are commonly referred to as 'corals'. Calcification within the Cnidaria presents 2 particular enigmas: 1) Many cnidarians are associated with endosymbiotic algae, commonly known as zooxanthellae. Zooxanthellae cannot be essential for calcification since corals can calcify without zooxanthellae (Goreau, 1959), and many cnidarians with zooxanthellae do not calcify. However, corals with zooxanthellae calcify faster in the light than in the dark (Vandermeulen *et al.*, 1972) and faster than those corals that lack zooxanthellae (Goreau, 1959). In contrast, sites of maximum zooxanthella numbers and sites of maximum calcification are often physically separated (Pearse & Muscatine, 1971; Oliver, 1984). The fundamental question of how zooxanthellae influence calcification remains unanswered. 2) The degree to which calcification is biologically mediated by the coral tissues, as opposed to a purely chemical process of calcium carbonate precipitation from seawater, is not known.

Despite a wealth of information on skeletal structure, physiological aspects of calcification and the coral tissue/zooxanthellae relationship (see Gladfelter & Kinzie, 1985 for review), there is relatively little information on the ultrastructure of the tissues that deposit the skeleton and the dynamics of skeletal morphogenesis. In addition the cellular components responsible for calcification are unknown.

During this study the skeleton and skeletogenic tissues of zooxanthellate species *Pocillopora damicornis* (L.) (Anthozoa, Scleractinia), *Heliopora coerulea* (Pallas) (Anthozoa, Coenothecalia) and *Millepora dichotoma* (Forskål) (Hydrozoa, Milleporina) and non-zooxanthellate *Caryophyllia*

smithii (Stokes & Broderip) (Anthozoa, Scleractinia) were investigated in order to determine common features and differences in their skeletons and skeletogenic tissues.

Material and methods

Specimens of *C. smithii* were provided by the Marine Biological Association, Plymouth, and were maintained in aquaria prior to preparation for microscopy. Specimens of *P. damicornis*, *H. coerulea*, and *M. dichotoma* were collected at the Phuket Marine Biological Centre, Ko Phuket, Thailand and the tissues and skeleton were fixed and stored in cacodylate buffer for return to the UK. The collection and fixation of all material was carried out at 12.00 h.

To determine sites where maximum calcification was occurring, 5 individuals of *C. smithii* and 5 branches each of *P. damicornis*, *H. coerulea* and *M. dichotoma* were placed into aerated containers of seawater containing 10–15 mg l^{-1} of the vital stain alizarin red-S and stained from 08.00 h until 16.00 h. The skeletons were cleaned of overlying tissues in a 2% sodium hypochlorite solution for 30 min, rinsed in distilled water for 12 h and air dried. The areas of the skeleton which had become stained red, indicating that skeleton deposition had taken place, were noted. After examination, these skeletons were mounted onto scanning electron microscopy (SEM) stubs and coated with gold (Edwards 150B sputter coater). All material was viewed in a JEOL T300 SEM.

The tissues of a minimum of 10 specimens of each coral were prepared for microscopy. Specimens of each coral were anaesthetized in a solution of 1 : 1 0.36 M $MgCl_2 \cdot 6H_2O$ and seawater for 30 min to ensure that the polyps were expanded when fixed. Specimens were fixed in a solution of 3% glutaraldehyde, 0.5% tannic acid and 7.5% sucrose in a 0.1 M sodium cacodylate buffer for 1 h at 24 °C followed by 14 h at 4 °C, then post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at 20 °C. At least 3 specimens of each coral were subsequently demineralized in a 1 : 1 solution of 2% ascorbic acid and 0.3 M sodium chloride for 60 h with renewal of the demineralizing solution every 6–8 h. Mineralized and demineralized material was dehydrated through a graded series of acetone into 100% propylene oxide, then embedded in either Spurr or TAAB resin. Polymerized blocks were sectioned, using a diamond knife, on a Reichert Supercut E ultramicrotome. Sections were taken through areas of active and non-active skeleton deposition as identified by alizarin red-S staining. Sections of 1 μm were stained with toluidine blue for light microscopy, whilst sections of 70–90 nm were mounted onto filmed copper grids, stained in lead citrate and uranyl acetate and viewed in a JEOL 100S transmission electron microscope.

Results

Alizarin red-S staining of the skeleton revealed that during the period of staining, skeletal deposition had occurred predominantly on the tips of vertical skeletal elements: the skeletal spines of *P. damicornis*; the theca and septa of *C. smithii*; skeletal spines at the junction of tube walls of *H. coerulea*; and the tips of vertically orientated skeletal bars of *M. dichotoma*. In addition, alizarin staining was confined to the branch apices of *Pocillopora*, *Heliopora* and *Millepora*.

The skeletal surface of all 4 corals from all areas of the skeleton had a nodular appearance commonly described as fasciculate (Fig. 1). Each fasciculus had a nodular surface morphology, each nodule composed of a conglomerate of small rounded crystals (Fig. 1A, C, E & G). Fractured skeletal surfaces revealed that each fasciculus was composed of a bundle of crystalline needles, each needle appearing on the fascicular surface as a nodule (Fig. 1B, D, F & H). Each crystalline needle consisted of linear series of small crystals. The individual crystals making up the crystalline needles of *P. damicornis* and *H. coerulea* were lath-like in shape, and those of *C. smithii* and *M. dichotoma* were rounded.

Attention was focussed upon the calicoblastic ectoderm from areas of active and non-active

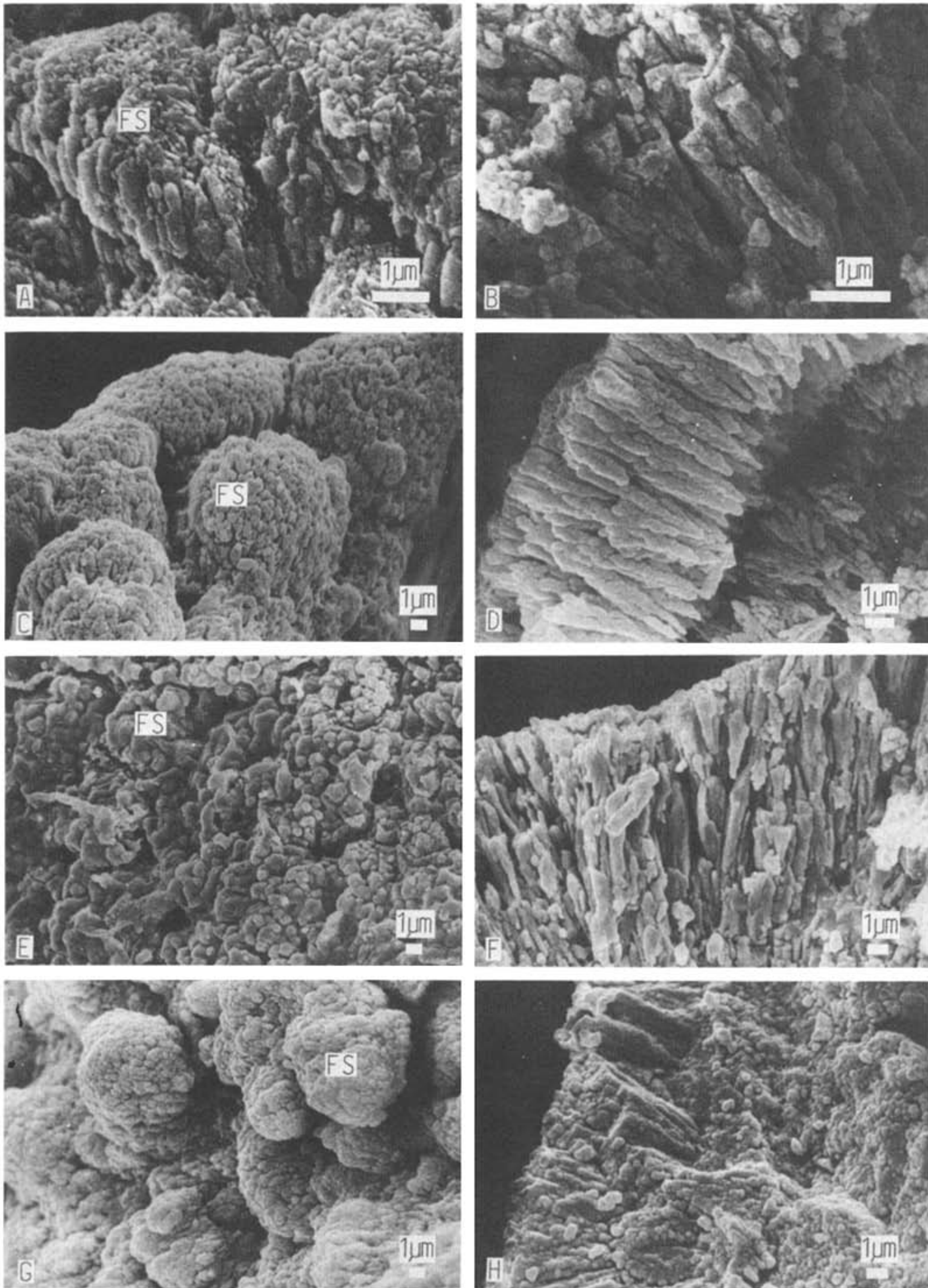

Fig. 1. The fasciculate skeletal surface of: A) *Pocillopora damicornis*; C) *Caryophyllia smithii*; E) *Heliopora coerulea*; G) *Millepora dichotoma*. The surface of each fasciculus is nodular, each nodule a conglomerate of individual crystals. Fractured fasciculi of: B) *P. damicornis*; D) *C. smithii*; F) *H. coerulea*; H) *M. dichotoma* showing the arrangement of individual crystals into linear series forming needles. Note lath-like appearance of individual crystals in B & F and rounded shape of individual crystals in D & H. (FS = fasciculus).

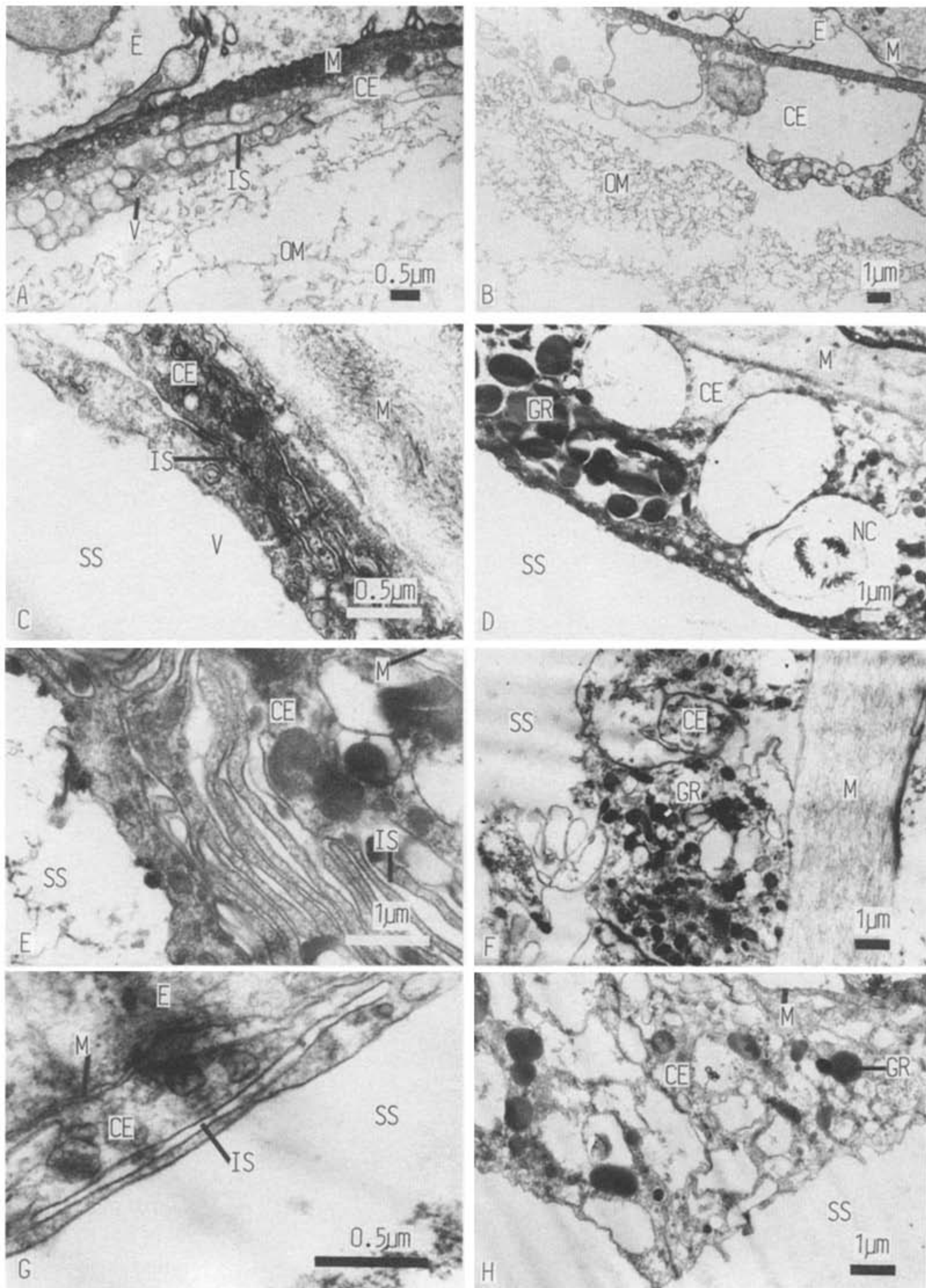

Fig. 2. The calicoblastic ectoderm. A) & B) *Pocillopora damicornis*; C) & D) *Caryophyllia smithii*; E) & F) *Heliopora coerulea*; G) & H) *Millepora dichotoma*. A, C, E & G show the calicoblastic ectoderm overlying areas of active skeleton deposition; and B, D, F & H that overlying areas of non-active skeleton deposition. (CE = calicoblastic ectoderm; E = endoderm; GR = granule; IS = intercellular space; M = mesogloea; NC = nematocyst; OM = organic matrix; SS = skeletal space; V = vesicle).

skeleton deposition because this layer has been described as responsible for skeleton deposition (Johnston, 1980). Calicoblastic ectoderm of all 4 corals overlying areas of the skeleton where deposition was taking place (Fig. 2A, C, E & G) was characterized by being comparatively thin with highly interdigitated lateral cell borders; having prominent intercellular spaces that ran from the mesogloea to the distal border of the calicoblastic ectoderm; containing few intracellular organelles excepting small membrane-bound vesicles; and having an ill-defined mesogloea/calicoblastic ectoderm border.

In all 4 corals the calicoblastic ectoderm overlying areas where deposition of the skeleton was not taking place (Fig. 2B, D, F & H) was characterized by being comparatively thick without interdigitated lateral cell borders; not having prominent intercellular channels; containing large dense membrane-bound granules of the type usually associated with waste products and/or storage (Brown, 1982); and having a well defined mesogloea/calicoblastic ectoderm border. In addition, except for *P. damicornis*, nematocysts were often present in non-calcifying calicoblastic ectoderm.

Discussion

Previous work on skeletogenesis has focussed on scleractinian corals, which are currently the major reef-builders. This study is a first attempt to characterize its cellular processes also in other corals. Models of calcification in corals range from physico-chemical processes of mineral deposition, skeletal structure being determined by the interactions of competitive crystal growth (Barnes, 1970; Gladfelter, 1982), to biological processes where the tissues govern calcification (Johnston, 1980). In the former, biological input was assumed to be restricted to the provision of a supersaturated mineralizing fluid between the skeleton and tissues, and the creation of 'mineralizing' cavities, which would produce a species-specific skeletal architecture.

In support of the second model, it is suggested that a primary role of the calicoblastic ectoderm may be to supply calcium ions to the space between tissues and skeleton, as calcium ions are found predominantly within intercellular spaces (Le Tissier, 1987). Such a role for calcifying epithelia is postulated in other calcifying systems (Simkiss, 1983). Other evidence suggests that the calicoblastic ectoderm has a further role. Barnes (1972) suggested that as skeletal structures extend, a 'conveyor belt system' moves calicoblastic ectoderm cells from the apex of skeletal features, where skeleton deposition is occurring, to proximal areas where no skeleton deposition is taking place. The present study shows that calicoblastic ectoderm cells undergo changes that suggest functional changes from a calcifying to a non-calcifying role. This means that cellular processes of calcification are a facultative feature of the calicoblastic ectoderm, coral tissues being able to determine where and when calcification takes place. The differences between calicoblastic ectoderm overlying areas of active and non-active skeleton deposition, and between these and other types of ectoderm, suggest that the specialized calicoblastic ectoderm becomes modified in order to mineralize. Its specialization is seen in the formation of prominent intercellular channels, and the presence of small membrane-bound vesicles of the type which Johnston (1980) described: he suggested they could form organic matrix as well as contain mineralizing ions. A structural organic matrix has been identified in all 4 corals studied here (Le Tissier, unpubl.). In non-calcifying calicoblastic ectoderm, intracellular bodies of a type associated with waste products/storage are present suggesting that it may be assimilating by-products of calcification. The present study supports the concept that calcification in corals is a biologically mediated process.

From the ultrastructure of the calicoblastic ectoderm overlying areas of active skeletal deposition in the species studied here, taxonomically unrelated corals appear to share the same cellular processes of calcification whether or not zooxanthellae are present. The morphology of skeletal crystals of *P. damicornis* and *H. coerulea* differs from those of *C. smithii* and *M. dichotoma*,

however, and the non-calcifying calicoblastic ectoderm of *P. damicornis* differs from that of the other corals studied. This suggests that these corals differ in the degree of biological regulation necessary for calcification to occur; and in the interactions of zooxanthellae with calcification. Gladfelter (1982) has proposed that rounded crystals reflect a high degree of biological regulation of calcium carbonate precipitation, and lath-shaped crystals a comparatively low degree. This would suggest that biological regulation of calcification occurs to a greater degree in *Caryophyllia smithii* and *Millepora dichotoma* than in *Pocillopora damicornis* and *Heliopora coerulea*.

Most calcification studies centre upon the effect of zooxanthellae (Gladfelter & Kinzie, 1985), as their photosynthesis has been considered juxtaposed to calcification (Pearse & Muscatine, 1971). Goreau (1959), however, has suggested that zooxanthellae enhance calcification indirectly by removing substances that would inhibit calcification or by stimulating the metabolism of host tissues (see Johnston, 1980). Comparison of *C. smithii* and *P. damicornis* supports such concepts: in *Caryophyllia* the presence of waste granules and organelles in non-calcifying calicoblastic ectoderm, as well as of rounded crystals in the skeleton, suggests that calcification in this coral requires a greater degree of regulation than in *Pocillopora*. Again, compared to *Pocillopora* the non-calcifying calicoblastic ectoderm of *Millepora dichotoma* and of *Heliopora coerulea*, and the rounded crystals of the skeleton of *Millepora* suggest similarly that although these two corals are zooxanthellate, they too provide evidence of a higher degree of biological regulation being required before precipitation of calcium carbonate can occur. Although as noted the skeletal crystals of *Heliopora*, in common with *Pocillopora*, indicate a comparatively low degree of biological regulation of calcification, its non-calcifying ectoderm, in common with that of *Millepora*, indicates the opposite. These results indicate that the nature of the symbiotic interactions between zooxanthellae and host tissue can vary with respect to calcification, possibly reflecting independent evolution of symbiosis within cnidarian groups at different times (Goreau, 1961).

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