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How brachiopods get covered with nanometric silicon chips†

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The investigation of an early pelagic juvenile of the discinid brachiopod *Discinisca* cf. *tenuis* elucidates the so far enigmatic origin of nanometric silicon chips covering the brachiopod's juvenile shell. The siliceous tablets are products of an intracellular process within specialized cells of the animal's inner mantle epithelium. These specialized cells are arranged in a circumferential row and contain vesicles, which provide 'reaction chambers' osmotically separated from the cytoplasm. Up to 15 tablets per vesicle are released into the cell by vesicle burst, followed by a coordinated extrusion onto the periostracum. In conjunction with the conveyor belt mechanism of periostracum formation, the regime of tablet release accounts for the highly ordered arrangement of siliceous tablets in parts of the shell's surface. The siliceous tablets are discussed as a protective cover against solar radiation, inherited from Palaeozoic linguliform brachiopods.

Keywords: brachiopod; Discinidae; *Discinisca* cf. *tenuis*; development; silicon; ultrastructure

1. INTRODUCTION

Silica plays an important role in the formation of skeletons in the living world, covering a wide range of organisms from plants (e.g. diatoms) to protozoan animals (e.g. radiolarians) to a few metazoan groups, and is also required as a trace element in vertebrate bone development (Carlisle 1981). Within invertebrates siliceous skeletal compounds are only known in sponges and in a small group of brachiopods, the Discinoidea. The Recent discinid brachiopod *Discinisca* cf. *tenuis*, in its early pelagic bivalved developmental stage, is covered with hundreds of nanometric tablets (mean diagonal tablet length: 0.7–2 μm), partly arranged in highly ordered rhombic arrays on the shell surface (figure 1*d*; Williams *et al.* 1998, 2001). The siliceous nature of these tablets was shown using energy dispersive X-rays (EDX) and an electron microprobe on a scanning electron microscope (Williams *et al.* 1998). The tablets showed carbon, silicon and oxygen peaks in the resulting spectra, and the authors inferred that the tablets are composed of silica (SiO_2). A more detailed study using scanning and transmission electron microscopy (SEM and TEM) showed that the juvenile shell of *D.* cf. *tenuis* can be divided into four different regions, namely: (i) the first formed part of the shell irregularly covered with siliceous tablets; (ii) the incrementally growing shell equally covered with siliceous tablets in highly ordered, rhombic arrays; (iii) the lamellar ring consisting of irregular concentric folds of the shell material, which is free of tablets and which separates the so-called mosaic from (iv) the subsequently ongrowing mature shell, which is also free of tablets, but which shows an organic superstructure of concentric ridges (Williams *et al.* 2001). The brachiopods used in the detailed morphological investigation (Williams *et al.* 2001) were (partly) bivalved pelagic juveniles, collected by plankton sampling. Even the smallest specimens of those were too old for the study of the origin of the siliceous tablets. The production of tablets had already ceased in all specimens and the mechanism of tablet production remained unknown. Only a single specimen, recently discovered among the pelagic juveniles of *D.* cf. *tenuis* preserved for TEM studies, was young enough to show the tablet's origin. Here, I give a brief description of the intracellular formation of siliceous tablets by a circumferential row of specialized cells in the mantle epithelium in a very young pelagic juvenile of the discinid brachiopod *D.* cf. *tenuis*.

2. MATERIAL AND METHODS

Pelagic developmental stages of *D.* cf. *tenuis* were collected with a plankton net (mesh size: 200 μm) at Swakopmund, Namibia between February and April 1998. The specimens were fixed at 4 °C for 30 min in 2.5% glutaraldehyde stained with ruthenium red, buffered with 0.1% sodium cacodylate (pH 7.3) and postfixed (4 °C, 40 min) in 1% osmium tetroxide, equally buffered with 0.1% sodium cacodylate solution. TEM specimens were dehydrated in an acetone series and propylene oxide and subsequently embedded in araldite. Ultrathin sections were cut using a Leica Ultracut S microtome. Sections were automatically stained with uranyl acetate and lead citrate in a Leica EM stain and examined in a LEO 912 Omega TEM. For SEM studies, specimens were dehydrated in an acetone series, critical-point dried in a Balzers CPD 030, mounted on aluminium stubs, sputter coated in a Balzers SCD 050 and examined in a LEO VP 1540 SEM. Photographs were taken on negative film (TEM) or as digital images (SEM) and finally arranged as a photographic panel using Adobe Photoshop 6.0 and Adobe Illustrator 10.

3. RESULTS AND DISCUSSION

The investigated specimen has an almost circular, bivalved shell with a diameter of *ca.* 400 μm. The two pairs of larval setae, typical for the hatching stage of *D.* cf. *tenuis* (see Lüter 2001), are already shed and the developing mantle margin has many setal follicles producing a row of short adult setae along the mantle edge on either side of the animal. Additionally, the five pairs of long and curved setae, typical for discinid pelagic juveniles, are present (not seen in figure 1 owing to abrasion during preparation).

In common with adult brachiopods, the investigated specimen has a fully functional but very short periostracal slot in both the ventral and the dorsal mantle (Williams *et al.* 1997). The periostracal slot is an epidermal infolding which determines the border between the inner and the outer mantle epithelium. In the slot, the outer mantle epithelium produces periostracal material, contributing to the outermost, organic layer of the brachiopod shell. In cross-sections of the animal a single tablet-producing cell can be observed at the hinge of the periostracal slot on either side of both the ventral and the dorsal mantle, i.e. a circumferential row of cells releases siliceous tablets onto the

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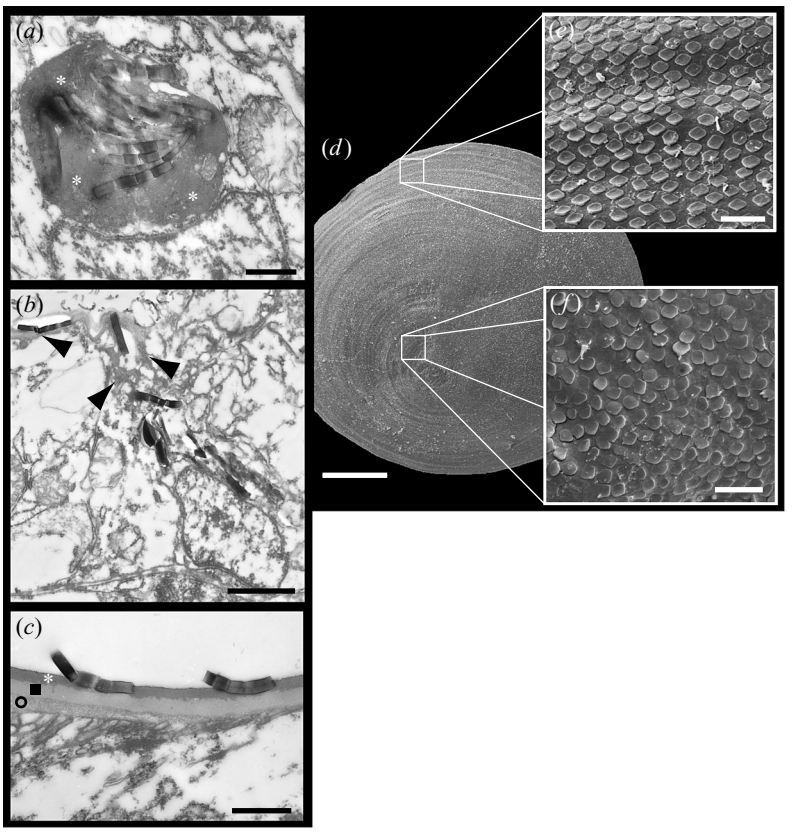

Figure 1. *Discinisca* cf. *tenuis*. Ultrastructure of nanometric siliceous tablets and their building process. (*a*) Cross-section through the vesicle filled with amorphous, organic material (asterisks) and several tablets. (*b*) Release of tablets and amorphous material (arrowheads) onto the outer mantle epithelium. (*c*) Tablets on the dorsal valve underlain by amorphous, organic material from the original vesicle (asterisk), periostracal material produced in the periostracal slot (square), and chitino-phosphatic shell material (circle) released by the underlying outer mantle epithelium. (*d*) Dorsal valve of a young pelagic juvenile with (*e*) ordered rhombic arrays near the margin and (*f*) disordered arrangement of siliceous tablets in the beak region of the dorsal valve. Scale bars, 0.5 μm (*a*,*c*); 1 μm (*b*); 80 μm (*d*); 4 μm (*e*,*f*).

periostracal material in either part of the mantle. Each tablet-producing cell has the usual set of cell organelles. Additionally, a large vesicle containing a set of between 10 and 15 tablets embedded in amorphous organic material can be observed (figure 1*a*). Like sponges, the vesicle is bounded by a membrane and works as an osmotically separated 'reaction chamber' (McGrory & Leadbeater 1981; Harrison & de Vos 1991). After formation of the tablets the vesicle bursts and the tablet-embedding organic material plus tablets are released onto the outer mantle epithelium (figure 1*b*). Through continuous growth of the mantle margin and the periostracum, one tablet after the other is shifted towards the dorsal/ventral side of the shell, comparable with pieces of coal on a conveyor belt. The result of this process is a three-layered shell (figure 1*c*): the outer layer consists of siliceous tablets and amorphous, organic material from the vesicle of the tablet-producing cell, the middle layer is released by the outer mantle epithelium in the periostracal slot and the basal-most layer represents the chitino-phosphatic shell material built by outer mantle epithelial cells with tubular microvilli. The highly ordered and rhombic arrangement of tablets (figure 1*e*) can be explained by tablet release in rather definite time-intervals. The tablet pattern on the shell surface mirrors the pattern of the tablet-producing cells: the distance between two tablets in a concentric row roughly reflects the distance between the midlines of two neighbouring tablet-producing cells. Because the apices of both valves (i.e. the first-formed parts of the shell) are also covered with tablets, tablet production must start as soon as the first shell material is released by the epidermis of the early free-swimming developmental stage. At this early stage of shell formation the periostracal slot is not yet present and the coordinated conveyor belt mechanism has not been switched on. The result of such an early tablet release is an unordered and rather chaotic arrangement of tablets on the shell surface (figure 1*f*). However, after a functional periostracal slot is in place, the release of the tablets must be a highly coordinated process, because: (i) only one tablet leaves the specialized cell at a time (figure 1*b*); (ii) the tablets are obviously picked up by the underlying periostracum and shifted towards the shell's periphery (figure 1*b*); (iii) the observed tablet release from one cell accounts for equal spacing of tablets in a row; and (iv) the highly ordered rhombic arrangement of the tablets (figure 1*e*) can only be explained by simultaneous tablet release by the neighbouring tablet-producing cells sitting in a circumferential row at the hinge of the periostracal slot.

Although the silicon chips are subject to erosion during shell maturation, they leave characteristic imprints on the shell's surface. Imprints possibly caused by tablet-like structures of different chemical composition can be found on juvenile shells of fossilized linguliform brachiopods

from the Palaeozoic (Balinski & Holmer 1999; Williams 2003). Within Linguliformea, Discinidae is the only family where siliceous tablets are observed, with evidence from the Recent discinid species *Discinisca lamellosa* from Chile (Holmer 1989), the deep-sea inhabiting *Pelagodiscus atlanticus* (Williams *et al.* 1998; Balinski & Holmer 1999) and *D.* cf. *tenuis* from Namibia (see previous paragraph). A possible explanation for the paillette cover of the shells of pelagic brachiopod juveniles (resembling thousands of small reflecting discs, like sequins) is that the tablets and tablet-like structures may have served as a protection against solar radiation when the ozone layer was more rarified than today. While swimming in near-surface water, the juvenile brachiopods would have been exposed to UV radiation. Siliceous tablets and tablet-like structures, therefore, may have served as protective reflectors. This hypothesis should be tested in future research, especially because similar siliceous structures are unknown from comparable pelagic juveniles of the Recent linguloid brachiopods *Lingula* and *Glottidia*, which are closely related to *Discinisca* and *Pelagodiscus*. The fossil record of Linguloidea also dates back to the Lower Cambrian. Thus, almost similar developmental stages of species of both superfamilies Linguloidea and Discinoidea may have inhabited the surface waters of Palaeozoic oceans and hence were equally exposed to UV radiation.

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