Protist, Vol. 167, 303–318, August 2016
http://www.elsevier.de/protis
Published online date 7 June 2016

Protist

ORIGINAL PAPER

Fine-structural Observations on Siliceous Scale Production and Shell Assembly in the Testate Amoeba *Paulinella chromatophora*

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Submitted December 15, 2015; Accepted May 28, 2016
Monitoring Editor: B. S. C. Leadbeater

The fine structure of shell formation was observed in *P. chromatophora*. Scales were formed one by one in silica deposition vesicles (SDVs) that were supported by an array of microtubules, which are probably involved in determining the shape and size of scales. The timing of silicic acid transport into an SDV was shown to be at an early stage of scale production because silicon was detected within SDVs containing immature scales. During the shell construction process, vesicles containing two types of dense materials were observed. One type of vesicle contains lower-density material and is located at the front edge of the branched, thick pseudopodium, extending from the maternal shell to the newly formed shell. The other type of vesicle, which contains higher-density material, was also observed in the thick pseudopodium. It appears that microtubules are involved in the shell construction process.

Key words: *Paulinella chromatophora*; testate amoebae; siliceous scale production; silica deposition vesicle; shell construction; ultrastructure.

Introduction

Silicon is one of the most abundant elements on earth, and many organisms produce siliceous external coverings and endoskeletons. This process of siliceous structure production is called silica biomineralization, and organisms performing silica biomineralization show a scattered distribution across various eukaryotic lineages (Preisig 1994; Simpson and Volcani 1981). For example, gramineous plants deposit silica to produce so-called plant opals in intercellular and intracellular spaces (Yoshida et al. 1959); sponges produce siliceous spicules in the vesicle of a specialized cell called a

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Abbreviations: SDV, silica deposition vesicle; MTOC, microtubule-organizing center; EDX-STEM, Energy Dispersive X-ray Spectroscopy with Scanning Transmission Electron Microscopy; LDMC, lower-density material containing; HDMC, higher-density material containing.

http://dx.doi.org/10.1016/j.protis.2016.05.002
1434-4610/

sclerocyte (Garrone et al. 1981); and diatoms produce siliceous cell coverings called frustules. These frustules are formed within membrane-bounded spaces called silica deposition vesicles (SDVs) (Drum and Pankratz 1964). The site at which the first SDV appears in the initial step of frustule formation is called the "pattern center." It is close to the microtubule-organizing center (MTOC) and its position differs among species (Schmid and Volcani 1983). The SDV is elongated by the fusion of small vesicles, and silica deposition starts from the pattern center (Chiappino and Volcani 1977). Based on morphological observation and inhibitor experiments, it has been suggested that microtubules and actin filaments are major elements in the control of silica morphogenesis (Cohn et al. 1989; Schmid 1980; Tesson and Hildebrand 2010a, b). Therefore, the microtubules and actin filaments must be involved in the formation of frustules. The biochemistry and molecular biology of the siliceous structures in diatoms have also been well studied, as typified by the discovery of silaffins (Kröger et al. 1999).

The supergroup Rhizaria is one of the major siliceous structure-producing eukaryotic lineages. Within the Rhizaria, most members of Thaumatomonadidae produce siliceous scales to cover their cells (Karpov 1990, 1993, 2000; Karpov and Zhukov 1987; Moestrup 1982; Ota et al. 2012; Scoble and Cavalier-Smith, 2014; Swale and Belcher 1974, 1975); the members of Ebriacea have endoskeletons composed of several branched siliceous rods (Hargraves 2002); the radiolarians also have siliceous endoskeletons (Anderson 1976a,b,c; Gamble 1909); and the euglyphids produce pot-shaped shells composed of many siliceous scales (Ogden and Hedley 1980). However, the silica biomineralization and morphogenesis of siliceous structures in rhizarians have not been well studied compared to those of stramenopiles (diatoms and chrysophytes).

Recently, it has been suggested that the rhizarian clade and the stramenopile clade, which includes diatoms, form a monophyletic group (Adl et al. 2012). This raises the possibility that the capability for silica biomineralization might have been acquired by a common ancestor of the Rhizaria and stramenopile lineages. Therefore, it is important to know more about the silica biomineralization and morphogenesis of siliceous shells in rhizarian organisms to understand the origin and evolution of silica biomineralization.

Euglyphids are filose testate amoebae belonging to the Rhizaria that live in oval siliceous shells and creep around on the substrate with filose pseudopodia that protrude from an aperture at one end of the shell. The shell is composed of many small siliceous scales that are produced inside the cell. The shells and scales vary in shape and size by species and are used as important taxonomic characters (Ogden and Hedley 1980). The siliceous shell formation of euglyphids is divided into two stages: the production of siliceous scales inside the cell and the construction of a new shell for one daughter cell with these scales before cell division (Anderson 1994; Hoogenraad 1927; Lauterborn 1895; Netzel 1972; Ogden 1979; Ogden and Hedley 1980). Immature siliceous scales have been observed using electron microscopy in the cells of several euglyphids, *Assulina muscorum*, *Euglypha rotunda*, *Trinema lineare*, and *Paulinella chromatophora*, and it has been suggested that the scales are formed in SDVs (Anderson 1994; Anderson and Cowling 1994; Hedley and Ogden 1973; Hedley and Ogden 1974a,b; Kies 1974; Netzel 1972). However, there has been no direct evidence for the localization of silicon within SDVs containing immature scales. In *A. muscorum* and *P. chromatophora*, it has also been suggested that the mature scales in SDVs move towards the aperture (anterior) side (Anderson and Cowling 1994; Kies 1974). However, the detailed scale maturation process and timing of silicic acid import into SDVs are still unknown.

Time-lapse video microscopy and scanning electron microscopy of the shell construction stage showed that in *E. rotunda*, the scales, which were made and stored within the cell, moved towards the aperture (anterior) side and were secreted from the aperture. They were then held around it by a cytoplasmic bud, which was part of the cell that was emerging from the aperture (Netzel 1972). The scales for the aperture of the new shell were positioned first and other scales were placed around the cytoplasmic bud as it grew to become one of the daughter cells (Netzel 1972). From previous ultrastructural observations in *E. acanthophora* and *E. strigosa*, microtubules were thought to be involved in shell construction as rails for the transportation of vesicles containing a cement-like substance (Hedley and Ogden 1974a). However, the detailed processes of scale production and shell construction in the euglyphids are still unclear, especially at the ultrastructural level. This information is important for understanding the mechanisms of silica biomineralization and shell construction in rhizarian testate amoebae. Because good culture methods for rhizarian testate amoebae have been unavailable, study of the shell formation process has been limited.