CHAMBER FORMATION IN ARCHAIAS ANGULATUS

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ABSTRACT

A knowledge of the process of chamber formation in foraminifera is important for understanding growth and development, and provides evidence about taxonomic relationships. Significant differences in the processes of chamber formation and calcification are known between species with porcellaneous tests and those with calcareous hyaline tests.

For this study, a video camera with a microscope attachment was used to record chamber formation in Archaias angulatus. Detailed study of the recording revealed previously undescribed transitional stages prior to calcification of the test wall. Chamber morphogenesis in Archaias angulatus was also compared to published descriptions of growth in other species of foraminifera.

Chamber formation has been described for relatively few species, and little has been added to our knowledge of the process of chamber formation in calcareous foraminifera since the overview by Hemleben and others (1986). Within the Soritidae, Archaias angulatus has been studied in detail (Marszalek, 1969, 1975), and descriptions of some macroscopic features of chamber formation have been published for Cyclorhabdina compressa (Lutze and Wefer, 1980) and Sorites orbiculus (Kloos, 1981). Previous comparisons of chamber formation in species of the suborders Milioliina, Rotaliina, and Globorotaliina found differences in general morphogenetic processes (Angell, 1980) and also in the system of calcification (Hemleben and others, 1986), but did not examine Archaias angulatus or other soritids.

The macroscopic processes of chamber formation are readily seen with a standard dissecting scope. However, the length of time required for chamber formation can make study of the process difficult. Here I report on the use of a video recording system to study morphologic changes during the process of chamber formation in Archaias angulatus, the transitional stages discovered by using this approach, and how the process of chamber formation in Archaias angulatus compares to that in other species.

METHODS

As part of a study of test strength in some species of larger foraminifera, numerous specimens of Archaias angulatus were collected from shallow water around Bermuda and kept alive in the lab for periods of up to a few days in petri dishes and small culture dishes filled with seawater (Wetmore and Plotnick, 1992). During preparations for the test strength measurements, one individual was found that had just initiated chamber formation. A video camera attached to a zoom stereomicroscope was used to record the process of chamber formation. A few minutes of video were recorded at low and high magnifications at intervals of every 10 minutes for the first hour. This was reduced to once every 30 minutes and then once every hour when the slow rate of change became apparent. The recording process was continued for almost 11 hours, ending when the specimen was accidentally lost. The resulting video recording was studied, and critical segments replayed repeatedly to develop a complete description of the events recorded. Frame capture with Snappy (R) and Zipshot (R) video capture devices was used to provide still images to print for closer examination and for illustrations.

RESULTS

The video recorded the process of chamber formation in sufficient detail to observe the bundling of reticulopodia, large-scale reticulopodial activity, the formation of membrane layers, and the movement of cytoplasm into the new chamber. Small features such as pits in the membrane surface could not be resolved. The following stages of chamber development can be seen in the video. Note the debris particles that provide landmarks for assessing morphologic changes.

1. At the start of the period of observation, a protective cyst had already formed and enclosed the area where the new chamber would form. Within the cyst, the reticulopodia were very closely spaced and appeared to merge into thick bundles. The cyst surface was solid enough to provide a barrier to the movement of various small organisms present in the culture dish. It incorporated very little debris because there was little available. (Pl. 1, fig. 1)

2. The main mass of the reticulopodial network gradually withdrew inwards from the cyst surface. Near the transition from this dense network to a more “normal” network, a thickened region formed and receded with the reticulopodia. The dense reticulopodia extended beyond this thickened region at first. As the reticulopodia retracted, this thickened region became denser and more clearly defined. At the completion of this withdrawal process, the mass of closely-spaced reticulopodia extended just to where the new outer chamber wall would be, with the thickened layer where the new chamber wall would form (Pl. 1, figs. 1–6). The fine morphological differences between the cytoplasmic anlage
and the outer organic membrane, described by Marszalek (1969) based on cytological study, could not be resolved in the video.

3. After the mass of reticulopodia withdrew to the position of the new test wall, a whitish layer began to move out from the old apertural face of the test. This gradually became denser and appeared to form the inner surface of the new test wall, though no details such as pseudopore pits could be resolved (Pl. 1, figs. 4–6; Pl. 2, figs. 1–2).

4. Beginning about four hours after the start of observation, lobes of colored cytoplasm (indicating the presence of symbionts) began to enter the new chamber space. A
clear space remained between this cytoplasm and the whitish layer of stage 3. (Pl. 1, figs. 5–6; Pl. 2, figs. 1–2). Within an hour after the migration of colored cytoplasm into the new chamber, there was a definite increase in the opacity and apparent thickness of the new test wall (Pl. 2, fig. 3), which continued to increase in density for the remainder of the period of observation. (Pl. 2, figs. 3–6). In the final segments recorded before the specimen was lost, the new test wall was still much more translucent than the older portions of the test, and clearly not completely calcified (Pl. 2, figs. 5–6).

**DISCUSSION**

**CHAMBER FORMATION IN ARCHAIAS ANGULATUS**

Previous observations of the macroscopic features of chamber formation have relied on visual observations, still photography and cytological work. The use of video recording in this study revealed gradual transitions that could easily be missed when using more static methods of observation. The gradually increasing thickening at the outer extent of the reticulopodial network, seen during retraction from the protective cyst, was not reported by Marszalek (1969). This transition was noted here because the fast forward, replay, and frame capture capabilities of a video recording made it possible to carefully examine the sequence. The major macroscopic stages of chamber formation in *Archaias angulatus* previously reported by Marszalek (1969) were observed on the video recording. In addition, some transitional features were observed on the video that had not been previously reported. While the specimens for these two studies came from different regions, Bermuda *Archaias angulatus* appear identical to ones from the Florida Keys (Wetmore, unpublished data), and are presumed to be the same species. The six stages that Marszalek described will be discussed in order and compared to what can be seen in the video sequence.

**Stage 1, Cyst Formation**

The “growth cyst” was fully formed and the dense network of reticulopodia was still in contact with it when the video recording was started (Pl. 1, Fig. 1). Just as noted by Marszalek (1969), the cyst formed a barrier to the movement of many smaller organisms.

**Stage 2, Reticulopodial Transformation**

The reticulopodia retract from the cyst once it is complete. They are very closely spaced, uniform in length, and show an increase in the rate of particle movement of 3–5 times that seen during normal activity (Marszalek, 1969). The closely spaced nature of the reticulopodial array is clearly visible in the video, and the process of retraction can be easily followed (Pl. 1, figs. 2–4). Examination of the details of cytoplasmic streaming would require higher magnifications than available on the stereo microscope used for this study.

**Stage 3, Anlage Formation**

Based on visual observations, still photographs, and cytological preparations Marszalek (1969) described the anlage as a mass of vesicular cytoplasm that assumes the general shape of the new chamber, probably serves as a substrate for secretion of the organic membrane, and forms about halfway down the length of the reticulopodia. In the video described here, earlier transitional stages of development can be distinguished. The initial thickening begins out near the growth cyst and then retracts along with the mass of reticulopodia until it reaches the final position for the new test wall (Pl. 1, figs. 1–4). For this stage, the use of video recording resulted in the detection of intermediate steps in the developmental process. However, video by itself would be inadequate to demonstrate the formation of a cytoplasmic anlage prior to secretion of the outer organic membrane.

**Stage 4, Organic Membrane Secretion**

The organic membrane forms within the anlage as a relatively massive structure that is as thick as the calcified chamber wall, and shows the final surface morphology including pseudopores (Marszalek, 1969). The test wall membrane cannot be differentiated in the video, so the process of membrane formation cannot be studied by this means.

**Stage 5, Invasion by Pigmented Cytoplasm**

After formation of the outer membrane, clear cytoplasm enters the new chamber prior to the influx of colored cytoplasm (Marszalek, 1969). This clear cytoplasm cannot be distinguished in the video. This is one limitation of study at these lower magnifications, because the nature of many features cannot be determined, however there is a distinct gap visible between the whitish layer and the colored cytoplasm that enters the chamber later which requires explanation. Either the unidentified whitish layer that moves out from the old apertural face is part of the “clear” cytoplasm of Marszalek (1969), or this whitish layer is an element he did not describe and the clear cytoplasm enters the chamber behind it (see Pl. 2, figs. 4–6). The colored, symbiont-bearing cytoplasm moves into the new chamber before it is obscured by calcification of the outer wall, so the gap between

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**PLATE 1**

Digital frame-capture images from a VHS videotape taken at a range of magnifications. For scale, the width of the most recent chambers is about 50 microns. No scale bars are given because the exact magnifications are not known. The digital display is a timer for the video recorder that registered minutes:seconds:hundredths for the first hour and hours:minutes:seconds:hundredths for the remaining time. 1 Taken shortly after the specimen was discovered. At this early stage of chamber formation the dense reticulopodial network is still in contact with the protective cyst. Note the small particles incorporated into the protective cyst. These provide landmarks for comparing the later images. 2–4 The reticulopodia retract inwards from the protective cyst. A diffuse thickening is visible at their outer edge, which retracts to the position of the new test wall. 4–6 The whitish layer of unknown composition (arrow) moves out from the existing test wall.
Digital frame-capture images from a VHS videotape taken at a range of magnifications, as in Plate 1. Note that after 9:59:59:59 the video timer display reset to 1:00:00:00. 1–4 Lobes of colored, symbiont-bearing cytoplasm (arrows) enter the new chamber and gradually merge while opacity of the new test wall increases, indicating the start of calcification. 5 Backlighting to show the translucent nature of the test wall and the active reticulopodia outside the new chamber. 6 Ten and a half hours after the first observation (P1. 1, fig. 1), the new test wall is still only partially calcified and is surrounded by a layer of cytoplasm.
the white layer and the colored cytoplasm is very obvious. In this case, the video shows more features than reported from visual examination. With only schematic drawings to compare with the video images of this study, it is difficult to determine whether these are real differences or just differences in interpretation.

Stage 6, Calcification

Marszalek (1969) clearly demonstrated that calcification begins after cytoplasm invades the new chamber, based on both macroscopic and cytological observations. While calcification can only be inferred on this video from the gradual increase in opacity of the new chamber wall, the use of cross-polarized light could provide definite evidence of calcification at the magnifications used (for example Angell, 1980).

Comparisons with Other Species

Growth Cyst

The growth cyst is a protective surface which encloses the space inside which the new chamber forms and is external to the new chamber. In Archaia angulatus the reticulopodia form this surface, which is solid enough to provide protection during the initial stages of chamber formation (Marszalek, 1969 and this paper). It is unclear whether a similar feature is formed in any other species of soritids. Published reports of chamber formation in Cyclorbulicina compressa (Lutze and Wefer, 1980) and Sorites orbiculus (Kloos, 1981) do not describe growth cysts, but they may still have been formed. For Cyclorbulicina compressa, Lutze and Wefer (1980) describe the start of the new chamber wall as “a thin, transparent and somewhat milky sheet in the dimensions of the future chamber.” They only observed chamber formation in larger individuals, and it is possible that the individuals they observed had left their growth cyst before calcification of the new chamber, as occurs in the closely related species Archaia angulatus (Marszalek, 1969). Sorites orbiculus creates an enclosed space around the test in which the new chamber forms (Kloos, 1981), but the rigidity of the sediment fringe that surrounds this space was not described. A protective cyst is also formed in Spiroloculina hyalina (Angell, 1980) and in the calcareous hyaline species Rosalina floridana (Angell, 1967) and Heterostegina depressa (Hemleben and others, 1986), but not in the species of planktonic foraminifera that have been studied (Bé and others, 1979; Spero, 1988) or in Calcituba polymorpha (Hemleben and others, 1986).

Anlage Formation

The cytoplasm associated with chamber formation takes dissimilar forms in different species. In Rosalina floridana the anlage is an uninterrupted lobe of distinctive cytoplasm that extends from the final aperture out to the new chamber wall position. This cytoplasm is full of vesicles, mitochondria, and filamentous structures (Angell, 1967). The similar mass of cytoplasm that extends outward from the aperture prior to chamber formation in Heterostegina depressa is also full of vacuoles and mitochondria as well as microtubules and organic particles (Hemleben and others, 1986). In contrast, in Archaia angulatus the anlage cytoplasm forms as a layer within a network of reticulopodia rather than as a lobe extending out from the existing test (Marszalek, 1969). In Orbulina universa, an initial cytoplasmic bulge forms a ring that expands outward until it reaches the position of the new test wall, where a highly vesicular filamentous layer develops that appears analogous to the anlage (Spero, 1988). Bé and others (1979) redefined the anlage as the organic structures directly responsible for the initial calcification of the chamber wall, which in Globorotalia truncatulinoides includes the primary organic membrane (POM) and a cytoplasmic envelope (CE) that forms externally to the POM. This definition differs significantly from the usage of Angell (1967, 1979), who described calcification as occurring within a membrane complex separate from the anlage cytoplasm in Rosalina floridana. In contrast to the above species, which all define the entire shape of the new chamber prior to calcification, Spiroloculina hyalina does not form an overall template prior to calcification of a new chamber wall. In this species, the new test wall starts to form at the old aperture as soon as the cytoplasmic bulge begins to move out. The calcite and organic layers are deposited in a continuous process while the cytoplasmic bulge extends to the final length of the new chamber (Angell, 1980).

Calcification and Organic Membrane Formation

In many species, an organic membrane provides the framework for calcite deposition or crystallization. In Archaia angulatus calcite deposition occurs within the organic membrane (Marszalek, 1969). In Sorites orbiculus the new chamber wall starts as a thin, transparent, deformable membrane that whitens and becomes rigid as it calcifies (Kloos, 1981), and the thin, milky sheet seen in Cyclorbulicina compressa at the start of chamber formation (Lutze and Wefer, 1980) may be a membrane that provides a base for mineralization. In Heterostegina depressa, Globorotalia truncatulinoides and Orbulina universa calcification appears to be initiated on the primary organic membrane (POM) (Bé and others, 1979; Hemleben and others, 1986; Spero, 1988). In contrast, in Spiroloculina hyalina the calcite of the test wall is laid down prior to formation of the inner organic layer (Angell, 1980).

Calcification and Symbiont Location

Calcification of the new test wall in Archaia angulatus begins after the symbiont-containing cytoplasm enters the new chamber (Marszalek, 1969), and can be seen in the video as an increase in opacity of the new test wall that gradually obscures details of the cytoplasm beginning about an hour after migration of the cytoplasm (Pl. 2, figs. 3–6). This expansion of symbiont-containing cytoplasm into the new chamber prior to calcification does not occur in all species with symbionts. In Sorites orbiculus, migration of zoxanthellae-containing cytoplasm occurs after calcification is completed (Kloos, 1981).

Taxonomic Implications

Test wall composition and structure are among the major characteristics that have been used for suprageneric classi-
fication in foraminifera (for example, Haynes, 1981, 1990; Loeblich and Tappan, 1984, 1987). Previously, there appeared to be a relatively simple relationship between the process of chamber formation and test wall structure in foraminifera. Among species with calcareous test walls, porcelaneous tests were known to form of internally produced crystals transported and placed in the test wall while the cytoplasm was extending and forming the wall membrane. In contrast, species with calcareous hyaline tests were known to first develop an anlage within which membrane formation and crystalization occurred (Angell, 1980; Hemleben and others, 1986). No plausible evolutionary transition between these forms was evident, which supported an independent evolutionary origin of calcification in these two groups (Angell, 1980). With the addition of data on Archaias angulatus the apparent divergence between porcelaneous and calcareous hyaline taxa is reduced. Unlike the other porcelaneous taxa studied, Archaias angulatus forms an anlage within which the new test wall is constructed (Marszalek, 1969). This is a potential intermediate form between the other porcelaneous test construction and calcareous hyaline forms. Conceivably a change in the character of the anlage-produced membrane in a porcelaneous test form like Archaias could lead to crystallization occurring on the membrane rather than within the cytoplasm and thus to the evolution of a calcareous hyaline test wall.

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REFERENCES


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