Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells

Shmuel Bentov
Jonathan Erez
Institute of Earth Sciences, The Hebrew University of Jerusalem, Edmond Safra Campus, Givat Ram, Jerusalem 91904, Israel

ABSTRACT

Stable isotopes and trace elements in foraminifera shells are important for determining the paleochemistry of the ocean, and Mg/Ca ratios and $\delta^{18}O$ values in foraminifera have recently been used to determine simultaneously the temperature and salinity of past oceans. However, large variations between species and significant Mg intra-shell heterogeneity indicate a major role for biological factors in determining the Mg/Ca ratio in foraminifera. Our novel in vivo observations on the biomineralization process of perforate foraminifera show that their calcareous wall is composed of two different calcite types: a thin, high-Mg primary layer, and low-Mg layers that cover the primary layer on both sides. The data may suggest that two biomineralization pathways are employed in the formation of the different calcite types. A new conceptual framework is provided to explain part of the Mg heterogeneity in foraminiferal shells and the variable sensitivity of the Mg/Ca ratio to temperature in different species.

Keywords: foraminifera, biomineralization, Mg/Ca ratio, vital effects, paleotemperature.

INTRODUCTION

Several field and laboratory studies have shown that Mg/Ca in shells of foraminifera can faithfully record paleotemperatures of the oceans (e.g., Nurnberg et al., 1996; Rosenthal et al., 1997; Lea et al., 1999; Lear et al., 2000; Toyofuku et al., 2000). When combined with $\delta^{18}O$ data, these temperature estimates allow for the solution of the well-known paleotemperature equation (Epstein et al., 1953), from which $\delta^{18}O$ of the past ocean and its related salinity can be combined to reconstruct the circulation patterns of the oceans in the past. However, different species have different overall Mg content at the same temperature (Blackmon and Todd, 1959) and they also show different responses to temperature (Lea, 2003). The distribution of Mg in foraminiferal shells is inhomogeneous, often with a clear dichotomy between regions of high and low Mg content (Erez, 2003; Eggins et al., 2004). The Mg content may also display temperature-independent changes during ontogeny, as shown by *Globigerinoides sacculifer* (Nurnberg et al., 1996). These observations suggest that the biomineralization process strongly affects the Mg/Ca ratio of foraminiferal shells.

The shell wall of most calcareous marine foraminifera living today is bilamellar, composed of two layers that are deposited with each newly formed chamber (Reiss, 1957). Transmission electron microscopy studies suggest that these two lamellae initially form on both sides of an organic matrix, termed the primary organic membrane (POM) (Hemleben et al., 1986). The inner lamella forms the interior of the new chamber, and the outer lamella covers the new chamber as well as the entire previous whorl of chambers. However, these earlier studies were unable to make in vivo observations on the cellular processes during calcification, mainly because of the masking effect of the existing shell.

EXPERIMENTS AND OBSERVATIONS

We investigated, in vivo, the biomineralization processes in the bilamellar benthic, symbiont-bearing species *Amphistegina lobifera* from the Gulf of Eilat, Red Sea. When live specimens are incubated in acidified seawater (pH $\leq$ 4), their shells partially dissolve, and within a few days the protoplasm starts budding to form protoplasmic spheres of various sizes (Fig. 1A). When these protoplasmic spheres are transferred to normal seawater, they transform into amoeboids that remain alive and functional for several weeks. The amoeboids contain symbiotic algae, have intensive pseudopodial activity (Fig. 1B), and precipitate $CaCO_3$ on the substrate (Fig. 1C). Their endoplasm (which is totally obscured by the shell in intact foraminifera) frequently contains numerous small, mineralized, light-polarizing granules, 0.5–1.0 μm in size (Fig. 1C). These cytoplasmic granules, which are probably membrane bound (Fig. 1D), cycle with the endoplasm stream, and are readily soluble in HCl (1 mM, 10 min), NaOCl (0.05% 10 min), and distilled water. Scanning electron microscope and electron probe microanalysis (EPMA) show that the granules contain bundles of needle-like crystals composed of Mg, Ca, and P at an approximate ratio of 2:1:3, respectively (Fig. 2A). Similar cytoplasmic granules were observed in embryonic, not yet calcified offspring of *A. lobifera* during its asexual reproduction, and in the cytoplasm of adult individuals that were physically crushed without any chemical treatment. Amoeboids containing granules were also prepared from other benthic species, i.e., *A. lessoni* and *Hetetostigina depressa*, and the planktonic species *G. sacculifer*, *G. ruber*, and *Orbulina universa*. Similar cytoplasmic granules in crustaceans and mollusks have been described (Brown, 1982; Simkiss, 1989), and may be similar to calcium storage organelles named acidocalcisomes that have been described in various protists (Docampo et al., 1995). It is possible that the cytoplasmic granules described here provide temporary calcium storage sites for the initial stages of $CaCO_3$ precipitation. We observed that only amoeboids with cytoplasmic granules start to precipitate $CaCO_3$ spherulites within a few days after the transfer to normal seawater. Radioactive labeling experiments (Bentov, 1997) showed that amoeboids that were incubated with ^{45}Ca incorporated appreciable amounts of the tracer into the cell, probably into the cytoplasmic granules. During the following nonradioactive chase incubation, ^{45}Ca was transferred from the cellular storage sites (possibly the granules) to the newly precipitated $CaCO_3$. These observations may explain ear-

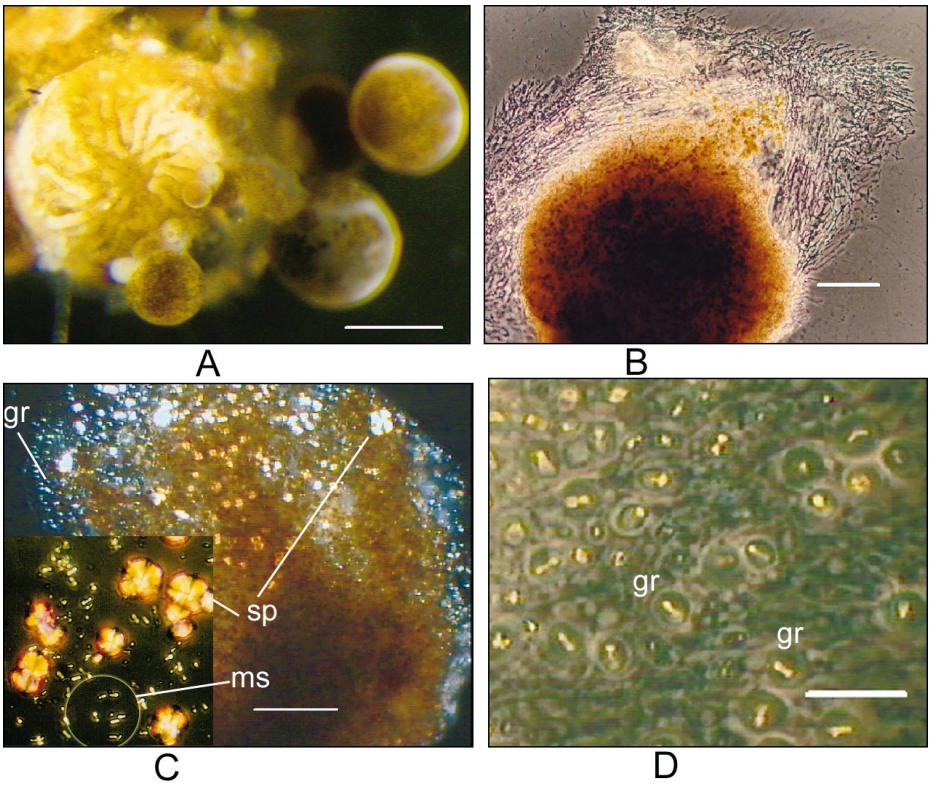

Figure 1. Amoeboids produced from *Amphistegina lobifera* and their biomineralization products. A: *A. lobifera* in acidic seawater, showing partial dissolution of calcareous shell and protoplasm spheres that bud from cell. B: Protoplasm sphere after being transferred to normal seawater produces reticulate pseudopods and acquires amoeboid shape. Amoeboids display two distinct compartments: main central brownish part with symbionts, and peripheral pseudopodial network. C: Polarized light image of amoeboid with cytoplasmic granules (gr) and $CaCO_3$ deposits (enlarged in inset). There are two types of deposits: high-Mg microspheres and bone-shaped deposits (ms) with no extinction cross and usually larger, low-Mg spherulites (sp) with extinction cross. D: Light microscopy of cytoplasmic granules in live cell, showing that each granule is positioned within a vesicle and probably bound by a membrane. Scale bars: 300 μm (A), 80 μm (B), 100 μm (C), 10 μm (D).

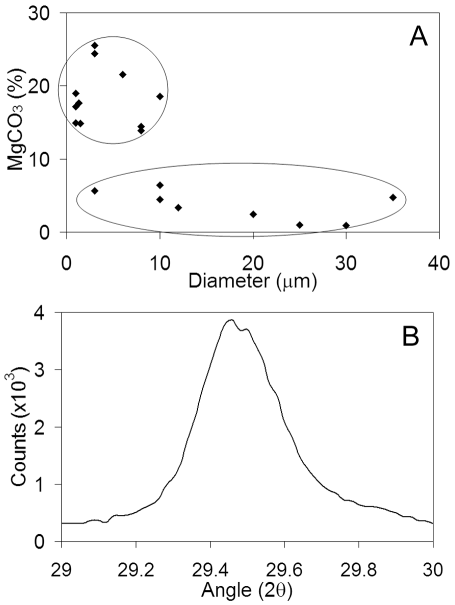

Figure 3. Two types of calcite produced by amoeboids and in natural *Amphistegina lobifera* shells. A: $MgCO_3$ content (mol %) vs. size of $CaCO_3$ precipitated by amoeboids. Microspheres (<10 mμ) that frequently develop into elongated structures are high-Mg calcite, while radial spherulites that frequently develop into larger size are low-Mg calcite. B: X-ray diffraction analysis of powdered *A. lobifera* shows asymmetry that indicates shell is composed of two phases: rare phase is high-Mg calcite, while common phase is low-Mg calcite.

lier reports of the existence of an internal Ca pool, serving for calcification in *G. sacculifer* (Anderson and Faber, 1984).

The amoeboids often move, leaving behind a trail of $CaCO_3$ deposits, which can be divided into two types (Fig.1C, inset): high-Mg microspheres <10 μm (Fig. 2B) and low-Mg spherulites (Fig. 2C) that are usually >10 μm (Fig. 3A). The chemical and morphological differences between the two calcite types that were deposited concurrently may suggest that two calcification mechanisms are involved in their formation. The relations between the discrete deposits of the amoeboids and the normal calcification by intact foraminifera were revealed by further observations of the par-

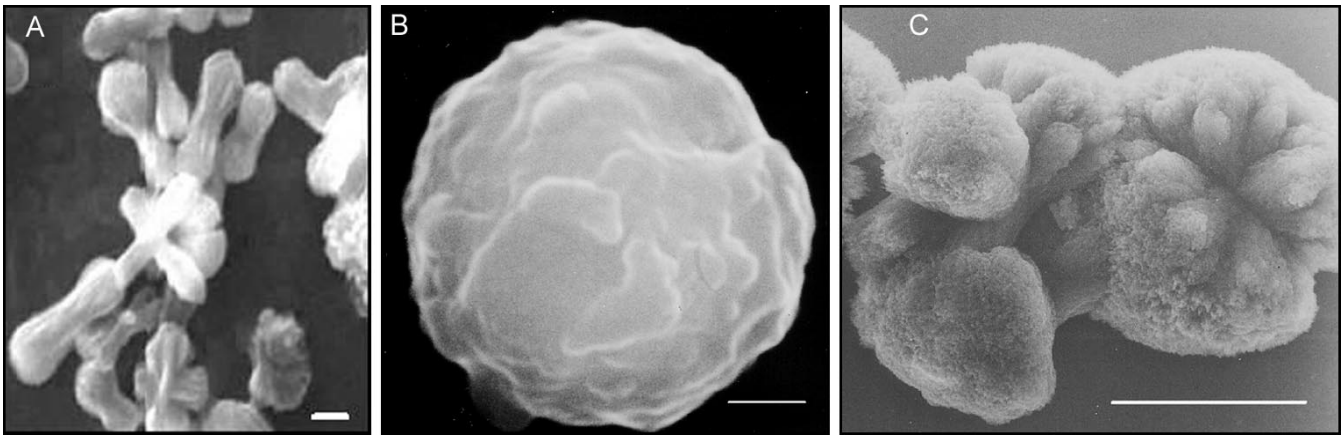

Figure 2. Scanning electron microscope images of intracellular (A) and extracellular (B, C) biominerals, produced by amoeboids (B and C after removal of organic matter). A: Cytoplasmic granules isolated from an amoeboid. Granules are made of bundles of elongated microcrystals composed of P-Mg-Ca in a ratio of 3:2:1. B: Extracellular $CaCO_3$ microsphere with highMg content. Similar microspheres, embedded in organic matrix, form primary wall that defines a newly formed chamber (see Fig. 5). C: Extracellular $CaCO_3$ spherulites with low Mg content. Scale bars: 1 μm (A), 300 nm (B), 10 μm (C).

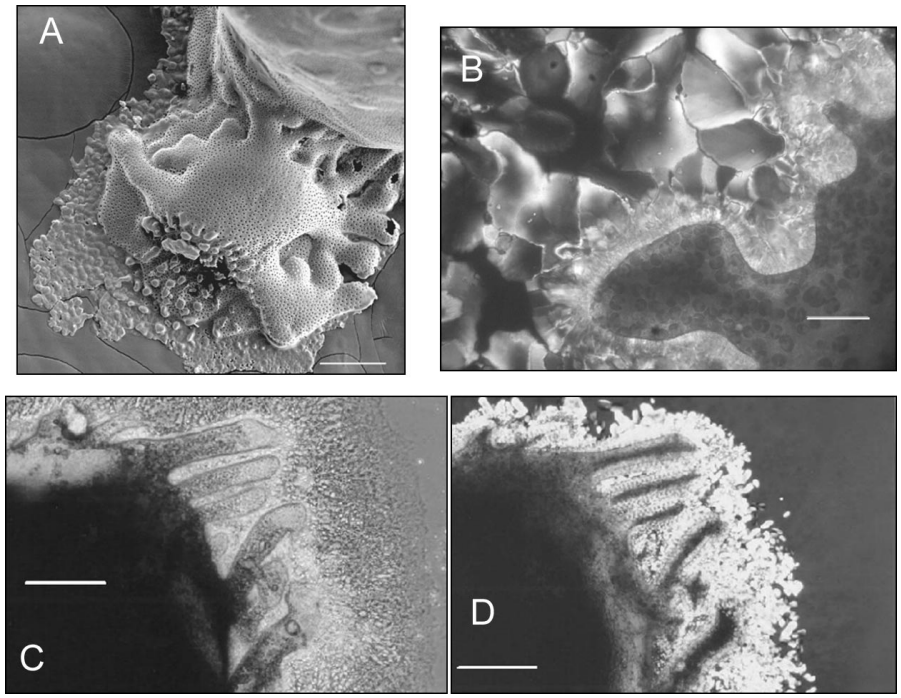

Figure 4. Chamber formation on glass substrate by *Amohistegina lobifera* recalcifying after partial dissolution. A: Scanning electron microscope image of recalcified individual showing new chamber being constructed on glass substrate. In spite of abnormal foundation, chamber maintains its normal features, such as an ornamented aperture, perforation, and typical lobes of *A. lobifera*. In periphery of new chamber, part of secondary outer lamella is deposited on glass substrate; note that this part is not perforated. B: Polarized light image of part of a last chamber and secondary lamella. Secondary crystal units grow laterally, creating interlocking layer. C: Phase contrast image of chamber formation at end of first stage of calcification, showing cytoplasm layer that bounds calcification site and lobes of newly formed chamber, where primary wall already exists. D: Polarized light image a few hours after the onset of secondary calcification, showing layer of secondary $CaCO_3$ that was deposited around a new chamber and filled gaps between lobes. Scale bars: 100 μm (A, C, D), 20 μm (B).

tially dissolved parent individuals. When these specimens are transferred to normal seawater, the exposed intra-shell protoplasm adheres to the glass and the organism starts to build new chambers over the glass substrate (Fig. 4). In intact foraminifera, a new chamber is deposited on top of the older shell, but the recalcifying individual may recognize the glass as its former shell and consequently uses it as a substrate for its new chamber. This false recognition provides a unique opportunity to observe in detail the biomineralization process and its cellular microenvironment in vivo.

Our observations on the recalcified foraminifera show that calcification in these lamellar foraminifera is a two-stage process. In the first stage, the organism deposits numerous high-Mg $CaCO_3$ microspheres (~1–2 μm in diameter) on both sides of the primary organic membrane, forming a thin primary wall with a typical morphologic shape of a normal chamber, including the embryonic pores (Fig. 5). These microspheres are similar both chemically and morphologically to those observed in the amoeboids (Fig. 2B). The second stage of calcification involves the formation of the bilamellar structure by deposition of the inner and outer lamellae on both sides of the primary wall. The outer lamella coats the entire exposed shell, and in the case of recalcified specimens expands to the glass (Fig. 4). During this stage, the organism generates a thin cytoplasmic sheet (Fig. 4C), different from the typical filamentous pseudopodia, which probably create a confined space necessary for biologically controlled calcification. Beneath this cytoplasmic sheet, the secondary crystal units grow laterally, creating an interlocked continuous layer. When the secondary lamella is deposited over perforated shell, it preserves the pore structure, but when it is deposited over the glass, it forms a continuous imperforated layer (Figs. 4A, 4B). This may indicate that the formation of perforated secondary calcite depends on previous perforation of the underlying layer.

EPMA analysis of recalcified individuals shows that their primary wall contains as much as 12 mol% $MgCO_3$, while the secondary layers are made of lower-Mg calcite (to 3 mol% $MgCO_3$). It is therefore possible that the high- and low-Mg calcites deposited by the amoeboids (Fig. 3A) correspond to the primary and secondary calcite of the normal wall, respectively. An X-ray diffraction analysis of powdered whole specimens of *A. lobifera* suggests that the shell is composed of two phases (Fig. 3B). The rare phase is high-Mg calcite that probably represents the primary walls, while the common phase is low-

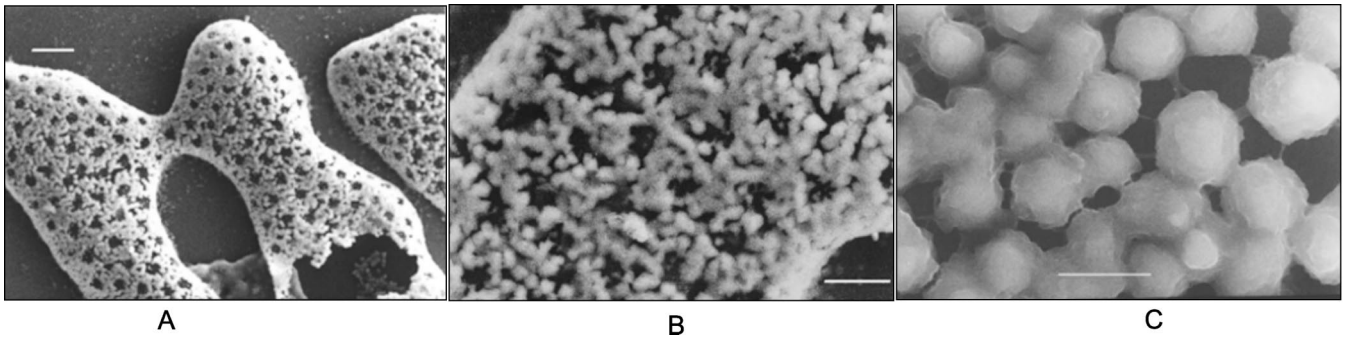

Figure 5. Scaning electron microscope images of primary wall of recalcified individual. A: Primary wall showing typical lobes of chamber margins of *Amphistegina lobifera* that are constructed on glass substrate. At this stage, wall is composed of high-Mg microspheres assembled within organic matrix, and it is extremely fragile. Note that pore positions are already defined. B: Detailed view of primary wall displays globular nature of crystal units that form primary wall. C: Primary wall fragment that retains its organic matrix (not treated with NaOCl). Organic matrix cements microspheres that form primary wall, while it is yet not self-supporting. Scale bars: 10 μm (A), 5 μm (B), 3 μm (C).

Mg calcite representing the secondary layers, which form the bulk of the calcitic shell.

DISCUSSION

The observations presented here are directly relevant for understanding the vital effects on the chemical composition of foraminiferal shells, particularly with respect to Mg/Ca paleothermometry. These vital effects include the large interspecies (Blackmon and Todd, 1959) and intra-shell (e.g. Duckworth, 1977) variability and the deviation of the apparent distribution coefficients from that of inorganic calcites and its variable temperature dependence (reviewed in Lea, 2003). This study demonstrates a temperature-independent variability of Mg/Ca ratio that is inherent to the calcification process, and may represent different biomineralization pathways for the two phases composing the perforate foraminiferal wall. The high-Mg phase that forms the primary wall is associated with the POM and separates the inner and outer lamellae. It is possible that the high-Mg primary wall may acquire Ca (and Mg) from the soluble cytoplasmic granules, which are enriched in Mg. The relative proportions of the two different calcite phases may influence the Mg variability within and between species mentioned here. For example, the low-Mg content of planktonic foraminifera may be related to the extremely thin primary wall, a fact that led in the past to the controversy about the very essence of their bilamellar nature (Reiss, 1957; Reiss and Luz, 1970). This proportion may change with various environmental factors and can also play an important role in determining the apparent temperature dependency of the foraminiferal Mg/Ca ratio. For example, temperature-mediated increase in the proportion of the high-Mg primary calcite would increase the slope of the Mg/Ca vs. temperature and may explain the augmented response of Mg/Ca ratio to temperature, compared to inorganic calcite, that was reported for low-Mg foraminifera (Nurnberg et al., 1996; Rosenthal et al., 1997).

CONCLUSIONS

This study demonstrates a temperature-independent variability of Mg/Ca ratio that is inherent to the biomineralization process of the chamber walls. However, temperature and possibly other environmental factors may change the Mg content within the boundaries set by this biological framework. A better understanding of the sublayers that compose the wall and their biomineralization processes will help improve our understanding of the Mg/Ca ratio recorded in foraminiferal calcite and its use as a reliable and more accurate paleothermometer.

ACKNOWLEDGMENTS

This work is part of S. Bentov's Ph.D. thesis. We thank S. Weiner for discussions and review of the initial manuscript. This work was funded by research grants from the U.S.-Israel Binational Science Foundation and the German Federal Ministry of Education and Research (Red Sea Program), awarded to J. Erez.

REFERENCES CITED

Anderson, O.R., and Faber, W.W.J., 1984, An estimation of calcium carbonate deposition rate in a planktonic foraminifer Globigerinoides sacculifer using ^{45}Ca as a tracer: A recommended procedure for improved accuracy: Journal of Foraminiferal Research, v. 14, p. 303–308.

Bentov, S., 1997, Biomineralization processes in foraminifera [M.S. thesis]: Jerusalem, Hebrew University, 75 p.

Blackmon, P.D., and Todd, R., 1959, Mineralogy of some foraminifera as related to their classification and ecology: Journal of Paleontology, v. 33, p. 1–15.

Brown, B.E., 1982, The form and function of metal containing ''granules'' in invertebrate tissues: Biological Reviews, v. 57, p. 621–667.

Docampo, R., Scott, D.A., Vercesi, A.E., and Moreno, S.N.J., 1995, Intracellular Ca2+ storage in acidocalcisomes of Trypanosoma-Cruzi: Biochemical Journal, v. 310, p. 1005–1012.

Duckworth, D.L., 1977, Magnesium concentration in the tests of the planktonic foraminifer *Globoratalia truncatulinoides*: Journal of Foraminiferal Research, v. 7, p. 304–312.

Eggins, S.M., Sadekov, A., and De Deckker, P., 2004, Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: A complication for seawater thermometry?: Earth and Planetary Science Letters, v. 225, p. 411–419, doi: 10.1016/j.epsl.2004.06.019.

Epstein, S., Buchsbaum, R., Lowenstam, H.A., and Urey, H.C., 1953, Revised carbonate-water isotopic temperature scale: Geological Society of America Bulletin, v. 64, p. 1315–1326.

Erez, J., 2003, The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies: Reviews in Mineralogy and Geochemistry, v. 54, p. 115–149, doi: 10.2113/0540115.

Hemleben, C., Anderson, O.R., Berthold, W., and Spindler, M., 1986, Calcification and chamber formation in Foraminifera—A brief overview, *in* Leadbeater, B.S.C., and Riding, R., eds., Biomineralization in lower plants and animals, Volume 30: New York, Oxford University Press, p. 237–249.

Lea, D.W., 2003, Elemental and isotopic proxies of marine temperatures, *in* Elderfield, H., ed., The oceans and marine geochemistry: Treatise on Geochemistry, Vol. 6: Oxford, Elsevier-Pergamon, p. 365–390.

Lea, D.W., Mashiotta, T.A., and Spero, H.J., 1999, Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing: Geochimica et Cosmochimica Acta, v. 63, p. 2369–2379, doi: 10.1016/S0016-7037(99)00197-0.

Lear, C.H., Elderfield, H., and Wilson, P.A., 2000, Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite: Science, v. 287, p. 269–272, doi: 10.1126/science.287.5451.269.

Nurnberg, D., Bijma, J., and Hemleben, C., 1996, Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures: Geochimica et Cosmochimica Acta, v. 60, p. 803–814, doi: 10.1016/0016-7037(95)00446-7.

Reiss, Z., 1957, The Bilamellidea, nov. superfam. and remarks on Cretaceaous Globorotaliids: Journal of Foraminiferal Research, v. 8, p. 127–145.

Reiss, Z., and Luz, B., 1970, Test formation pattern in planktonic foraminiferids: Revista Espanola de Micropaleontologia, v. 2, p. 85–96.

Rosenthal, Y., Boyle, E.A., and Slowey, N., 1997, Temperature control on the incorporation of magnesium, strontium, fluorine, and cadmium into benthic foraminiferal shells from Little Bahama Bank: Prospects for thermocline paleoceanography: Geochimica et Cosmochimica Acta, v. 61, p. 3633–3643, doi: 10.1016/S0016-7037(97)00181-6.

Simkiss, K., 1989, Structural and analytical studies on metal ion-containing granules, *in* Mann, S., et al., eds., Biomineralization, Chemical and biochemical perspectives: Weinheim, VCH Verlagsgesellschaft, p. 427–460.

Toyofuku, T., Kitazato, H., Kawahata, H., Tsuchiya, M., and Nohara, M., 2000, Evaluation of Mg/Ca thermometry in foraminifera: Comparison of experimental results and measurements in nature: Paleoceanography, v. 15, p. 456–464, doi: 10.1029/1999PA000460.

Manuscript received 19 April 2005
Revised manuscript received 1 July 2005
Manuscript accepted 6 July 2005

Printed in USA