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Biomineralization in perforate Foraminifera

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Abstract

In this paper, we review the current understanding of biomineralization in Rotaliid foraminifera. Ideas on the mechanisms responsible for the flux of Ca^{2+} and inorganic carbon from seawater into the test were originally based on light and electron microscopic observations of calcifying foraminifera. From the 1980's onward, tracer experiments, fluorescent microscopy and high-resolution test geochemical analysis have added to existing calcification models. Despite recent insights, no general consensus on the physiological basis of foraminiferal biomineralization exists. Current models include seawater vacuolization, transmembrane ion transport, involvement of organic matrices and/or pH regulation, although the magnitude of these controls remain to be quantified. Disagreement between currently available models may be caused by use of different foraminiferal species as subject for biomineralization experiments and/ or lack of a more systematic approach to study (dis)similarities between taxa. In order to understand foraminiferal controls on element incorporation and isotope fractionation, and thereby improve the value of foraminifera as paleoceanographic proxies, it is necessary to identify key processes in foraminiferal biomineralization and formulate hypotheses regarding the involved physiological pathways to provide directions for future research.

1. Introduction

All foraminifera make tests although a number of different materials are used in their construction. The 'naked' foraminifera produce tests from organic matter, agglutinated foraminifera use sediment grains as building blocks and calcifying foraminifera use constituents dissolved in seawater to secrete calcium carbonate. Formation of $CaCO_3$ tests plays a significant role in ocean biogeochemical cycles and, more importantly, the fossil remains of calcifying foraminifera are widely used to reconstruct past ocean chemistry and environmental conditions. Elemental and isotopic composition of foraminiferal calcite depends on a variety of environmental parameters such as temperature, salinity, pH and ion concentration (McCrea et al., 1950; Epstein et al., 1951; Boyle, 1981; Nürnberg et al., 1996). These physical and chemical variations are the foundation for developing geochemical proxies that quantify environmental changes through time (see Wefer et al., 1999; Zeebe et al., 2008; Katz et al., 2010 for reviews). For example, the magnesium concentration in foraminiferal calcite ($Mg/Ca_{calcite}$) varies primarily with seawater temperature (Nürnberg et al., 1996; Lea et al., 1999; Hönisch et al., 2013) and can be used to reconstruct past sea surface (Hastings et al., 1998; Lea et al., 2000) and deep-water (Lear et al., 2000) temperatures. Reliable application of these proxies requires calibration over a wide range of environmental conditions as well as a thorough understanding of the physiological parameters influencing test formation.

Studies calibrating foraminiferal test composition based on core-tops and controlled growth experiments show that both the chemical and isotopic compositions of these tests are not in equilibrium as defined by inorganic precipitation experiments (Lowenstam and Weiner, 1989; Dove et al., 2003). Microenvironmental controls related to foraminifera physiology have been implicated to explain disequilibrium fractionation in test chemistry (Figure 1). Most foraminiferal species incorporate Mg with one to two orders of magnitude lower concentration compared to non-biologically precipitated calcium carbonate (Bentov and Erez, 2006; Katz, 1973; Bender et al., 1975). The concentration of barium, on the other hand, is ~10 times higher in foraminiferal calcite (Lea and Boyle, 1991; Lea and Spero, 1992) compared to inorganic precipitation results (Pingitore and Eastman, 1984). Additionally, elemental concentrations between foraminiferal species can vary by several orders of magnitude (up to two orders of magnitude for Mg; Bentov and Erez, 2006). The biological controls on element incorporation and isotope fractionation that cause these offsets are often summarized as 'the vital effect' (Urey et al., 1951; Weiner and Dove, 2003).

Figure 1: Minor and trace element composition of foraminiferal (left) and inorganically precipitated (right) calcite precipitated from seawater (middle). Concentrations are qualitative as they differ between foraminiferal species and depend on environmental conditions. Precipitation rates, ionic strength of the medium and presence of organic compounds are also known to affect partition coefficients. All values are in parts per million (ppm) and based on data in Kitano et al. (1975), Ishikawa and Ichikuni (1984), Rimstidt et al. (1998), Marriott et al. (2004), Morse et al. (2007), Tang et al. (2012) He et al. (2013) for inorganically precipitated calcium carbonates, and Lea and Boyle (1991), Rickaby and Elderfield (1999), Segev and Erez (2006), Terakado et al. (2010), Allen et al. (2011) for foraminiferal calcite composition.

Vital effects comprise 1) chemical alterations of the foraminifers' microenvironment due to physiological processes, 2) cellular controls on the composition of the fluid from which calcite is precipitated and 3) controls on nucleation and crystal growth (e.g. by presence of organic templates). Foraminiferal respiration and/or photosynthesis by symbiotic algae alter the foraminiferal microenvironment chemistry and thereby the conditions in which foraminiferal tests mineralize. Because habitat depth differences in the water column (planktonic species) or migration in the sediment and attachment to plant leaves (benthic species) also modify the calcification environment, these ecological factors are sometimes regarded as being part of the vital effect as well (e.g. Schmiedl and Mackensen, 2006). Ecology-based variability in element incorporation, however, can be accounted for when habitat preferences of foraminiferal species are known. Hence, the term "vital effects" should only be used when discussing foraminiferal cellular processes that alter the chemistry of the microenvironment during test mineralization.

To understand the physiological impact on element incorporation and isotope fractionation, the (intra)cellular mechanisms which foraminifera employ to precipitate test $CaCO_3$ must be identified. Biogeochemical mechanisms are involved in regulating concentrations of ions and/or their activity at the site of calcification. Calcification from seawater can be promoted using different mechanisms. Hence, multiple mechanisms have been proposed to explain test calcification, including endocytosis of seawater, transmembrane ion transporters, ion-specific organic templates, production of a privileged space and mitochondrial activity (Spero, 1988; Erez, 2003; Bentov and Erez, 2006; Bentov et al., 2009).

A process-based framework for both inorganic and organismal control of foraminiferal test formation is crucial for the development, calibration and application of geochemical proxies in the geological record. At the same time, a mechanistic understanding of foraminiferal biomineralization will also permit researchers to better interpret data from the fossil record as well as predicting the response of foraminiferal calcification to future environmental changes such as ongoing ocean acidification. Most of the initial observations of chamber formation and calcification in planktonic foraminifera were published during the early period of planktonic foraminifera culturing (e.g. Bé et al., 1977). Highlights of those observations can be found summarized in the seminal text on “Modern Planktonic Foraminifera” (Hemleben et al., 1989). More recently, studying living specimens under controlled conditions (e.g. Kitazato and Bernard, 2014) has further propelled our understanding of foraminiferal growth, reproduction and calcification.

Recent hypotheses on foraminiferal biomineralization are based mainly on experiments with benthic species and although these ideas have to be tested for planktonic species, we will also include the latter group in our discussion. Although a general model for foraminiferal biomineralization is still lacking, and it is not yet clear that a single model fits all groups of foraminifera, details on the underlying mechanisms in different species have accumulated and are described here in the context of previously published biomineralization models.

2. Ions for calcification

2.1 Seawater as the direct source for Ca^{2+} and DIC

Foraminifera calcify by creating a microenvironment supersaturated with respect to $CaCO_3$, while overcoming inhibition by crystallization inhibitors such as Mg^{2+}. Hence, calcification requires a tight control on the concentration and/or ion activity at the site of calcification, commonly referred to as the "delimited" space (Erez, 2003) or "privileged" space. Elevated [Ca^{2+}], [CO_3^{2-}] and/or their ion activities have to be actively maintained in order for calcification to proceed. Simultaneously, the concentrations of crystal growth inhibitors have to be lowered even further. Although CO_3^{2-} needed for calcification may be partially derived from respired CO_2 (Erez, 1978; Grossman, 1987; Ter Kuile and Erez, 1991; Hemleben and Bijma, 1994; Bijma et al., 1999), the majority of the carbon and the Ca^{2+} needed for test formation must be derived from the seawater environment.

Calcification requires equal amounts of Ca^{2+} and CO_3^{2-}. Because seawater Ca^{2+} concentrations are approximately 5 times higher than that of DIC and often >50 times higher than that of CO_3^{2}, foraminifera have to spend more time and/or energy in taking up and concentrating DIC than they have to do for Ca^{2+}. A foraminifer needs to process several times the seawater equivalent of its own cell volume in order to acquire enough Ca^{2+} and inorganic carbon to calcify a new chamber. Although the exact amount needed depends on shape, size and the thickness of the chamber wall (e.g. Brummer et al., 1987), juveniles of some species need 50-100 times their own cell volume to extract the Ca^{2+} required to produce one new chamber (De

Nooijer et al., 2009b). Because seawater [CO_3^{2-}] is significantly lower than [Ca^{2+}], these individuals need the equivalent of ~3,000 times their own volume in order to take up the necessary [CO_3^{2-}] if this anion is used exclusively. However, observations of high pH at the site of calcification (Erez, 2003; De Nooijer et al., 2009a; Bentov et al., 2009) as well as oxygen isotope data from laboratory experiments (Spero et al., 1997; Zeebe, 1999) suggest that foraminifera can convert CO_2 and/or HCO_3^- into the CO_3^{2-} needed for calcification. Evidence that foraminifera concentrate inorganic carbon is also provided by experiments using ^{14}C tracer incorporation kinetics into the skeleton of perforate species (Ter Kuile and Erez 1987, 1988, Ter Kuile et al 1989b). A carbon concentrating mechanism would reduce the volume of seawater necessary for calcification by 50-90% (De Nooijer et al., 2009b). To concentrate the ions needed for calcification, foraminifera must either extract Ca^{2+} and dissolved inorganic carbon (CO_2, HCO_3^- and CO_3^{2-}, or DIC) or take up seawater and subsequently reduce the concentrations and/or activities of all other ions relative to Ca^{2+} and DIC (Figure 2). Removal of protons from (endocytosed) seawater is also a prominent feature in recently developed calcification mechanisms, but will be discussed in a separate section (2.2). In case of the second option, spontaneous nucleation of $CaCO_3$ crystals may be prevented by separation of Ca^{2+} and DIC into different vacuole groups.

Figure 2: Two different mechanisms to concentrate Ca^{2+} and DIC from seawater for calcification: a) Calcium- and bicarbonate-ions are specifically taken up from seawater, or b) the other ions are selectively removed, thereby increasing Ca and DIC concentrations.

Both processes transport ions either directly to the site of calcification or temporarily store these ions. In the case of uptake into some benthic foraminifers, Ca^{2+} and/ or DIC are thought to be present in so-called 'intracellular reservoirs' (also known as ‘pools’; Ter Kuile and Erez, 1988; Erez, 2003). These reservoirs may be seen as temporal storage compartments with high concentrations of ions that are either emptied upon calcification or provide a dynamic cycling of Ca^{2+} and DIC through the cell that is gradually used for calcification. Without an intracellular reservoir, Ca^{2+} and DIC could also be directly transported to the privileged space during calcification (Erez, 2003; Bentov and Erez, 2006). The relative importance of intracellular reservoirs versus direct transport among benthic and planktonic species remains a subject of debate and active research.

2.2 Internal reservoirs

Internal reservoirs may be important for foraminiferal calcification in certain groups. Conceptually speaking, one can envision Ca^{2+} or DIC being derived from internal reservoirs. With seawater as the basis for calcification, carbon reservoirs will have to be approximately 5 times larger than those for Ca^{2+} or have a 5 times faster turnover rate. Evidence suggests that different foraminifer groups employ different strategies. For instance, a time-lag has been observed between uptake and incorporation of labelled inorganic carbon in the large benthic foraminifera *Amphistegina lobifera* suggesting inorganic carbon may be stored in an internal reservoir (Ter Kuile and Erez, 1987; 1988; Ter Kuile and Erez, 1991). In pulse-chase experiments it was observed that ^{14}C was incorporated into the calcite during the chase period in ^{14}C free seawater, implying a large internal reservoir of DIC in the benthic *Amphistegina lobifera* but not in the milliolid *Amphisorus hemprichii* (Ter Kuile et al 1989b). Isotope labelling experiments with the planktonic foraminifer *G. sacculifer* and a number of benthic species using both ^{14}C and ^{45}Ca show that proportionally more labelled ^{45}Ca is incorporated into the shell compared to labelled ^{14}C (Erez, 1978; 1983). For the planktonic species *Orbulina universa* and *Globigerina bulloides*, on the other hand, Bijma et al. (1999) showed that the contribution from an internal carbon pool is insignificant in these species.

To determine whether planktonic foraminifera have an internal Ca-reservoir, Anderson and Faber (1984) grew *G. sacculifer* in artificial seawater spiked with ^{45}Ca. They showed that calcite formed during the first 24 hours contains significantly less ^{45}Ca than that produced in the second 24 hours. These data argue for the existence of an unlabeled intracellular Ca-reservoir that was filled prior to the introduction of the isotopic spike. Using pulse-chase experiments with both a 'hot' incubation period (10-15 days) and 'cold' chase period (10-20 days), Erez (2003) traced the uptake of ^{45}Ca over time in the benthic species *Amphistegina lobifera*, showing that as much as 75% of the Ca^{2+} used during chamber calcification resided in an intracellular reservoir. ^{48}Ca uptake data from experiments using *Orbulina universa*, supported the existence of a Ca-reservoir in a planktonic species, but demonstrated that it was completely flushed of labelled Ca^{2+} within the initial 6 hours of chamber formation and thickening (Lea et al., 1995). These latter observations could indicate that *O. universa* utilizes a small Ca^{2+} reservoir to assist with the initial chamber formation, but that much of the remaining chamber Ca^{2+} is derived from seawater without passing through an internal storage reservoir.

Toyofuku et al. (2008) reported formation of (incomplete) chambers in the benthic *Ammonia beccarii* maintained in seawater devoid of Ca^{2+}. These data clearly support the existence of a Ca^{2+}-reservoir of finite volume in benthic species. If Ca^{2+} and other divalent cations that co-precipitate in the $CaCO_3$ shell are derived from the same internal reservoir, one would expect cation concentrations to reflect Rayleigh fractionation if the reservoir is a closed system. Such a system has been used to partly explain minor and trace element distributions in foraminiferal calcite (Elderfield et al., 1996). However, a model using Rayleigh fractionation relies on a number of assumptions about the internal reservoir regarding its size and initial composition as well as refreshment rate and chamber calcification rate. These unknowns highlight the need to better constrain the size and extent of these reservoirs.

To maintain an intracellular reservoir, a foraminifer needs to sustain a high cation flux rate by continuously vacuolizing, endocytosing and exocytosing large volumes of seawater. Tracing endo- and exocytosis in foraminifera is challenging and has yielded contrasting results. For instance, Bentov et al. (2009) showed that in *Amphistegina lobifera*, seawater is taken up in vacuoles that are subsequently transported to the site of calcification. This implies that seawater, internally modified or not, is directly involved in calcification. De Nooijer et al. (2009b) on the other hand, showed that endocytosis and subsequent exocytosis of seawater in *Ammonia tepida* are not directly related to chamber formation.

2.3 Direct uptake of ions

The ions needed for calcification may be derived from seawater during calcification without storage in an intracellular reservoir (Figure 3). A number of calcification models explicitly or implicitly assume that the ions for calcification are passively transported to the site of calcification through diffusion from the surrounding medium (Wolf-Gladrow et al., 1999; Zeebe et al., 1999). These models are able to explain the impact of photosynthetic symbionts on inorganic carbon chemistry in the vicinity of the foraminifer. Changes in pH and [DIC] due to photosynthesis affect the isotopic composition of the available carbonate (Wolf-Gladrow et al., 1999). However, diffusion of ions to the site of calcification without at least one additional mitigating mechanism, cannot account for the difference between seawater metal composition and Me/Ca ratios in foraminiferal calcite (Figure 1 and references in its caption).

Figure 3: Examples of possible involvement of internal reservoirs versus externally derived ions for calcification. A: Ca^{2+} and DIC are derived from internal reservoirs. B: Ca^{2+} and DIC are transported to the site of calcification without uptake and storage into reservoirs. C: DIC

is taken up directly and Ca^{2+} comes from an internal reservoir. D: Ca^{2+} is taken up during chamber formation and DIC is derived from an intracellular reservoir.

Ca^{2+} and DIC may be actively transported (through transmembrane pumps and/ or channels) to the site of calcification. Although such transport mechanisms are not yet identified in planktonic foraminifera, a number of studies support the existence of this mechanism in benthic species. Using radioactive labeling, Angell (1979) showed that the ions for calcification are taken up *during* chamber formation in the benthic species *Rosalina floridana*. Although this observation does not prove the absence of an internal reservoir *per se*, this observation reduces the turnover rate and/or size of such a reservoir considerably. Similarly, Lea et al. (1995) showed that the intracellular Ca-reservoir in the planktonic foraminifer *O. universa* is very small and/or has a fast turnover rate and does not significantly contribute to the total amount of Ca^{2+} during shell thickening. Results from the benthic *Ammonia* sp. show that intracellular vesicles containing elevated concentrations of Ca^{2+} are involved in chamber formation (Toyofuku et al., 2008), but that their amount within the cell is not sufficient for the production of a new chamber (De Nooijer et al., 2009b). Together, these studies suggest that the majority of the Ca^{2+} utilized for shell calcification is not stored in intracellular reservoirs prior to chamber formation in the species studied. If the internal reservoir refills after chamber formation within a relatively short period of time, it is critical that seawater labeling experiments should start directly after a chamber formation event to avoid underestimation of the true reservoir size. Studies addressing the issue of an intracellular reservoir are summarized in Table 1.

Table 1: Studies discussing internal reservoirs in perforate foraminifera.

3. Intracellular transport

3.1 Transmembrane ion transport

Due to the hydrophobic inner layer of cell membranes, molecules cannot freely move into or out of the cell's interior. Although the majority of ions and molecules diffuse across cell membranes, diffusion constants vary greatly. Small, uncharged molecules (CO_2, O_2, NO) diffuse easily down a concentration gradient whereas large molecules and ions require specialized transmembrane proteins to facilitate or energize membrane transport (Higgins, 1992). These transporter proteins can be divided into channels, carriers and pumps (Figure 4). Carrier proteins undergo substrate binding and transport. They show typical substrate affinities and follow Michaelis-Menten kinetics. Carrier transport is even effective against concentration gradients if a cosubstrate with a respective concentration gradient or charge is involved (secondary active transport). Pumps directly generate this energy for uphill transport from their ATPase activity. Transmembrane channels simply allow facilitated diffusion along electrochemical gradients by creating a selective pore through the cell membrane. For the uptake of inorganic carbon by foraminifera during calcification, a strong pH gradient (high inside; De Nooijer et al., 2009a; Bentov et al., 2009; low outside; Glas et al., 2012) may promote the influx of CO_2 and thus circumvent the need for specialized transmembrane proteins.

Figure 4: selective ion transporters. Ion pumps (left and middle) undergo structural changes that allow passage of ions from and to the binding sites. The example shown here is a simplified Na^+/K^+ exchanger that has specifically binds to Na-ions (blue squares) when in the first configurational state (left). After the structural change, affinity of the Na-binding sites decreases so that the Na-ions are released (middle). At the same time, K-ions (yellow circles)

bind to their binding sites after which the pump returns to state one and releases the K^+ to the cytosol. Ion channels (draw after the KcsA K^+ channel; right) consist usually of a narrow pore allowing certain ions to pass a cell membrane down the electro-chemical gradient. Another feature of some pumps and channels is the relatively large cavity that is created by the transmembrane protein-complex (here present in the cytosol-side of the channel). This can greatly reduce the distance that the ions have to be transported. The type of Ca^{2+}-transporters that are used by foraminifera are unknown, but determining their molecular structure is necessary to 1) know the extent of de-hydration during transport, 2) determine the rate of ion transport and 3) explain the selectivity for Ca^{2+}/ against other ions (e.g. Mg^{2+}) and their fractionation (e.g. Gussone et al., 2003).

3.2 Ca^{2+} transport in foraminifera

In foraminifera, most attention has been directed at ion transporters that might be responsible for the low Mg/Ca at the site of calcification. Logically, this may involve Mg^{2+}-transporters and/ or Ca^{2+} transporters. Because Ca^{2+} acts as a secondary messenger in most eukaryotic cells, cytosolic Ca^{2+} is kept low (< 1µM) by active removal out of the cell or into cytosolic compartments (ER, mitochondria). This makes Ca^{2+}-transporters one of the most ubiquitous and well-studied transmembrane ion transporters. From a variety of cell types, Ca^{2+}-ATPases, Ca^{2+}/H^+ and Ca^{2+}/Na^+ antiporters (e.g. Gonçalves et al. 1998) and Ca^{2+}/phosphate co-transporters (Ambudkar et al., 1984) have been described. Depending on the transporter's structure, ions may pass the membrane either with or without their hydration sphere (Gouaux and MacKinnon, 2005), although (partial) dehydration increases the selectivity greatly (see also Gussone et al., 2003).

The specificity of the transmembrane Ca-transporters varies greatly. For some Ca^{2+}/H^+-antiporters it has been reported that other cations with a small ionic radius (e.g. Zn^{2+}) can be transported in a similar way as Ca^{2+} is transported (Gonçalves et al., 1999). For the same antiporter, the larger Ba^{2+} and Cs^{+} do not substitute for Ca^{2+}. An ion with intermediate size, Sr^{2+} (1.13 Å, compared to 0.99 Å for Ca^{2+}), appears to block the antiport and prevents transport of Ca^{2+} through the membrane. Studies concerning specificity for Ca^{2+} over Mg^{2+} are scarce, but some Ca-ATPases have been reported to have a 10^{3}-10^{5} higher affinity for Ca^{2+} than for Mg^{2+} (Drake et al., 1996; Xiang et al., 2007).

In corals, calcium uptake is directly related to proton pumping (McConnaughey and Whelan, 1997; Sinclair and Risk, 2006). The efflux of H^{+} during calcification (Glas et al., 2012) may therefore help to constrain estimates of calcium pumping rates during calcification. Carbon dioxide uptake and proton efflux are also directly related in cyanobacteria (Ogawa and Kaplan, 1987). Ter Kuile et al. (1989b) suggested that Ca^{2+} is taken up by Ca^{2+}-ATPase and this mechanism was subsequently used by Zeebe and Sanyal (2002) and Zeebe et al. (2008) to show that H^{+} removal is far more energy-efficient than Mg^{2+}-removal during calcification. Such a mechanism would be consistent with a coupling of ion transporters (e.g. Ca^{2+} and H^{+}) during foraminifera calcification.

The amount of Ca^{2+} transported across a membrane depends on 1) transporter density in the membrane, 2) affinity for Ca^{2+} of the transporter and 3) the capacity of the transporter. For example, the Na^{+}/Ca^{2+} exchanger has a low affinity, but high capacity, resulting in transport of up to 5,000 ions per second (Carafoli et al., 2001). Such a transporter is useful when Ca^{2+} is present in high concentrations (e.g. as in seawater) and supply or removal rates of Ca^{2+} have to be high. Cell membrane calcium pumps, on the other hand have a high affinity, but low capacity, making it particularly suitable for transporting Ca^{2+} out of a medium or compartment with a low [Ca^{2+}] (Wang et al., 1992). Finally, transport rates can be affected by the presence of inhibitors, high intracellular [Ca^{2+}] (e.g. Pereira et al., 1993) or shortage of ATP (in case of e.g. Ca^{2+}-ATPase).

3.3 Inorganic carbon transport in foraminifera

Transport of inorganic carbon may be accomplished by bicarbonate-transporters. If seawater or metabolic CO_2 contributes to the inorganic carbon during calcification, diffusion rates across membranes would control the influx of inorganic carbon and thereby influence the rate of calcification. The diffusion rate is determined by the concentration gradient of CO_2, the membrane area over which CO_2 can diffuse, and the solubility of CO_2 in the membrane lipids. The concentration of CO_2 at the site of calcification or in internal reservoirs is determined by pH. Since foraminifera can control the pH in these compartments (Erez, 2003; Bentov et al., 2009; De Nooijer et al., 2009a; Glas et al., 2012), they can produce large CO_2 concentration gradients and hence promote the influx of DIC to the sites of calcification. The flux of ions can also be calculated from calcification rates, which is discussed in section 4.

In case of intracellular storage of ions, calcium and DIC are unlikely to be stored as free ions. Because the cytosol has very low concentrations of free Ca^{2+} and DIC, the cell volume will control the number of ions available for calcification. For the DIC-reservoir (if present) the additional problem is that CO_2 can easily diffuse across cell membranes and subsequent re-equilibration would thus result in net leakage of carbon out of the DIC-reservoir. To overcome this problem, DIC must be sequestered by mechanisms such as elevating the pH in the reservoir. Because there are usually no crystallites visible within the cells of hyaline species, Ca and DIC are likely sequestered together as non-crystalline $CaCO_3$ (i.e. amorphous calcium carbonate or ACC). Such a possibility may have consequences for the minor and trace element composition of the calcite precipitated, since it is known that formation of high-Mg calcite is accompanied by the formation of an amorphous precursor phase (Raz et al., 2000).

Regardless of the process concentrating Ca^{2+} and DIC from seawater, each would produce a supersaturated solution at the site of calcification, with reduced levels of crystal inhibitors that occur naturally in seawater (e.g. Mg^{2+} and PO_4^{2-}). The Ca^{2+} and CO_3^{2-} may form spontaneous $CaCO_3$ crystals, but the specific morphology of foraminiferal chambers show that nucleation and crystal growth is a tightly controlled process.

4. Nucleation of calcification

4.1 Crystal nucleation energy and critical size

Precipitation of a crystal from a solution occurs when free energy of the precipitate is lower than that of the solution. Nucleation of a crystal requires even more energy since ions at the surface of a crystal add to the free energy of the solid phase. This is caused by the fact that ions at the surface of a crystal are not bound on all sides to other ions. The resulting 'interfacial energy' requires the formation of metastable clusters of a critical size to start crystal growth (Figure 5). The interfacial free energy between the cluster and a solution is usually larger than that between the cluster and a solid substrate, resulting in crystal nucleation at solid surfaces rather than within the solution itself (De Yoreo and Vekilov, 2003). If the atomic structure of a substrate matches a particular plane of the nucleating phase (e.g. calcite or aragonite), the interfacial free energy is reduced and nucleation is promoted (De Yoreo and Vekilov, 2003).

In the case of nucleation of $CaCO_3$, presence of negatively charged groups at regular intervals at the site of calcification may be able to bind Ca^{2+} and pre-form a part of the $CaCO_3$ lattice.

Figure 5: relation between free energy changes (Δg) as a function of pre-nucleation sphere (r), where Δg_s is the surface term and Δg_b the bulk term. The sum of Δg_s and Δg_b is the free

energy barrier that can only be overcome by the formation of a nucleation sphere with a critical size (r_c). Biological control over crystal nucleation is often aimed at lowering of this energy barrier and can be achieved by increasing the concentrations of the solutes or the presence of an organic template.

4.2 Organic templates and nucleation of $CaCO_3$ in foraminifera

During biomineralization in foraminifera calcium carbonate nucleates at the site of calcification, likely involving an organic template. In all Rotaliid foraminifera, chamber formation starts with delineation of a finite environment that encompasses an inner chamber volume from the surrounding medium (Angell, 1979; Bé et al., 1979; Hemleben et al., 1986; Spero, 1988; Wetmore, 1999). Cytoplasmic activity by formation of a dense pseudopodial network transports vacuoles, mitochondria and organic particles to a defined zone in which the so-called Organic Primary Envelope, Primary Organic Lining, Anlage or Primary Organic Membrane (POM) is formed (e.g. Banner et al., 1973; Hemleben et al., 1977; Spero, 1988; not to be confused with inner and outer organic linings, nor with the outer protective envelope or cytoplasmic envelope: see section 4). The term POM is often used but may be confusing (Erez, 2003) since these organic templates are not technically membranes. Therefore, we recommend following the suggestion of Erez (2003) to rename the POM as the Primary Organic Sheet (POS). In a number of benthic species, the POS consists of unbranched polysaccharides such as glycosaminoglycans (Hottinger and Dreher, 1974; Langer, 1992). Proteins are also present in the organic lining of foraminifera, sometimes forming different classes based on their amino acid composition (Robbins and Brew, 1990). King and Hare (1972) showed that amino acids make up 0.02-0.04% of the weight of the calcite and that composition among planktonic species varies greatly. Interestingly, the largest compositional difference coincides with the planktonic foraminifera spinose/ non-spinose divide (King and

Hare, 1972), but differences in amino acid composition are also manifest at lower taxonomic levels (Robbins and Healy-Willliams, 1991).

The organic matrix of the benthic *Heterostegina depressa* is shown to contain an EDTA-soluble and -insoluble fraction (Weiner and Erez, 1984). The insoluble fraction contains over-sulphated glycosaminoglycans and a small portion of non-polar proteins, forming the inner organic lining. The soluble fraction contains a number of proteins containing amino acids with acidic residues. Polar groups in both fractions may be involved in biomineralization since they may bind Ca^{2+} ions and thereby overcome the free energy barrier (Figure 5). If such groups are regularly spaced, they may help nucleation further by placing the Ca^{2+} ions in a regular grid with just enough space for the CO_3^{2-} ions to fit in between them. To test this hypothesis, the tertiary structures of the biomolecules (e.g. proteins and saccharides) that are involved in $CaCO_3$ nucleation need to be analyzed.

The presence of polysaccharides and proteins has led to the hypothesis that the POS has two functions in the process of calcification. The carbohydrates may form a structure determining the overall shape of the new chamber. The proteins associated with the polysaccharides, on the other hand, form the 'active' part of the POS by providing charged sites for nucleation of $CaCO_3$ (Towe and Cifelli, 1967). Since the chemical composition of the POS varies between species (Banner et al., 1973), its role in nucleation of calcium carbonate may differ between foraminiferal species (Bé et al., 1979; Hemleben et al., 1986; Spero, 1988; Wetmore, 1999). In some benthic species, the POS coincides with the location of the pores prior to calcification (Wetmore, 1999), suggesting that there are structural differences in the POS within a single chamber that determine where calcite does and does not nucleate. In planktonic species such as *Globorotalia truncatulinoides* and *G. hirsuta*, calcification begins in small nucleation zones at finite locations across the POS, where calcite forms centers of crystal growth that interlock to form the initial calcified chamber (Towe and Cifelli, 1967; Angell, 1979; Bé et al., 1979;

Hemleben et al., 1986). A similar pattern has been observed in *Orbulina universa*, where small islands of calcite form on the POS, followed by calcite island fusion to produce the spherical chamber (Spero, 1988).

Nucleation (and subsequent crystal growth) is also determined by the physico-chemical conditions at the site of calcification. These conditions are only partly known in benthic species (e.g. Erez, 2003; Bentov and Erez, 2005) and have only been modeled in planktonic species (Zeebe et al., 1999; Zeebe and Sanyal, 2002). The volume between the crystal surface and the shielding cytoplasmic envelope or pseudopodial network is extremely small, limiting interpretation from light microscopic observations. However, TEM images of initial calcification in *Orbulina universa* and other planktonic species suggests the privileged space between rhizopodia and calcifying surfaces may be <10 nm (Bé et al 1979; Spero 1988). Little is known about the chemical composition of the fluid from which $CaCO_3$ nucleates, but high concentrations of Ca^{2+} and CO_3^{2-} need to be actively maintained, while the [Mg^{2+}] needs to be reduced to satisfy observations and ensure calcification (Zeebe and Sanyal, 2002). Elevated pH at the site of calcification would promote the conversion of CO_2 and HCO_3^- to CO_3^{2-}, thereby enhancing $CaCO_3$ nucleation and growth. Elevated concentrations of Mg^{2+} around the POS in *Pulleniatina obliquiloculata* (Kunioka et al., 2006) may indicate that in this species, the composition of the calcifying fluid is different during the first stage of chamber formation, possibly due to a different rate or efficiency of the process that locally reduces [Mg^{2+}] vs [Ca^{2+}]. The participation of a small volume of seawater at the beginning of chamber formation may explain the elevated Mg in the first calcite precipitated, although this pattern does not hold for other planktonic species (e.g. such as *Orbulina universa*; Eggins et al., 2004) where the lowest Mg/Ca ratios are associated with the intrashell zone that corresponds to the POS. The above observations of inter species differences in chamber wall elemental composition underscore the need to unravel the mechanisms controlling test calcification.

5. Chamber growth

After initial crystal nucleation, calcification proceeds by addition of calcite on both sides of the POS. Additional layers of $CaCO_3$ are added on top of pre-existing chamber calcite during each chamber formation event in perforate foraminifera (Reiss, 1957; 1960; Bé and Hemleben, 1970; Erez, 2003). Together, the primary and secondary layers of calcite are termed 'lamellar' calcite (Erez, 2003). Most observations on calcification are based on the first stage of chamber formation in which a thin-walled chamber is produced within 1-3 hours (Spero, 1988). Subsequent thickening of the chamber wall proceeds during the next 24-48 hours until a new chamber is formed. Thickening of earlier formed chambers occurs by addition of a calcite layer with each new chamber formation event (e.g. Bentov and Erez 2005, Nehrke et al., 2013). Future studies will need to show whether the timing of the start and end of chamber formation and thickening of previously formed chambers are coincidental, or whether thickening is a continuous process.

Future biomineralization research should also take into account the possibility that cellular controls on calcification may vary over time and location across the foraminifera shell. An example of the potential complexity and diversity of calcification within one specimen is provided by Bentov and Erez (2005). Their research demonstrated that the benthic *Amphistegina lobifera* recovering individuals produce at least three types of calcium carbonate: elongated, intracellular birefringent granules with a high magnesium and phosphorus content, extracellular microspheres with a high Mg concentration and extracellular spherulites with a low Mg content. These spherulites represent the lamellar calcite while the microspherulites represent the initial presipitation over the POS in *A. lobifera.*

During chamber formation, ions could be supplied to the site of calcification (SOC) from internal reservoirs (Figure 3, Table 1) or by transport from the surrounding seawater. The latter can be accomplished by transmembrane ion transporters (section 2), by direct exchange of the calcifying fluid with seawater and/ or by diffusion from ambient seawater. The inner and outer surfaces of newly formed chambers of the benthic *Heterostegina depressa* are covered by thin layer of cytoplasm (Spindler, 1978), suggesting the SOC may be separated from the surrounding medium. In a number of studies (Angell, 1979; Bé et al., 1979), a fan-like arrangement of the pseudopodial network is observed in a zone outside the site of calcification. Although the relation between this arrangement and calcification remains to be investigated, it is likely to play a role in biomineralization since this dense network is not observed between chamber formation events. Also in the planktonic species *G. hirsuta* and *G. truncatulinoides*, calcification proceeds adjacent to a cytoplasmatic envelope (or outer protective envelope) that may play a role in maintaining SOC integrity and shape, and promoting initial calcification (Bé et al., 1979). In the benthic *Ammonia* sp., a pH gradient of >2 pH units is observed across several μm distance and is maintained for hours between the site of calcification (De Nooijer et al., 2009a) and the specimen's microenvironment (Glas et al., 2012). These observations suggest that in *Ammonia* sp., the SOC is separated from the outside environment. Spero (1988) on the other hand, presented transmission electron micrographs that showed the site of calcification in *O. universa* is not shielded by a continuous membrane. Nehrke et al. (2013) recently suggested that the site of calcification in *Ammonia aomoriensis* is largely closed from the surrounding medium, but that a small percentage of the fluid at the SOC is derived from leakage of the cell membranes separating it from the outside medium, explaining observed Mg/Ca for the species studied.

The extent to which the site of calcification is open or closed, in combination with the presence or absence of intracellular ion reservoirs, is an important unknown in understanding foraminiferal calcification (Figure 6). For example, a site of calcification that is physically separated from the surrounding seawater, together with the absence of intracellular ion reservoirs, prescribes the need for transmembrane ion transporters (e.g. Ca^{2+}-APTase; section II) that selectively pump ions from seawater to the SOC. A SOC that is open, on the other hand, will experience relatively high concentrations of Mg and require an active Mg^{2+}-removal mechanism.

Figure 6: summary of the most important parts of the calcification mechanism in foraminifera, including Ca-ion transport, active Mg-removal and contribution from internal reservoirs. See text for description of the individual processes.

Potential ion transport pathways to the site of calcification can be constrained from calcification rates during chamber formation. It is important to distinguish between the overall growth rate of a foraminifer and calcite precipitation rate during biomineralization. The difference between these processes results from the episodic nature of growth (chamber addition) in foraminifera. Some planktonic species have been reported to increase the weight of their shell by 13-15% a day (*G. sacculifer*; Erez, 1983), but this may vary with environmental conditions (Ter Kuile and Erez, 1984 and references therein). Secondly, chamber addition rates vary over a foraminifer's lifetime, decreasing as the individual ages (Ter Kuile and Erez, 1984). Calcite precipitation rates during chamber addition, on the other hand, are much higher and vary between 0.4-0.9 µg/h in the planktonic foraminifer *G. sacculifer* (Anderson and Faber, 1984), 0.06-0.32 µg/h in *O. universa* (Lea et al., 1995) and ~10 µg/h in the benthic *A. tepida* (De Nooijer et al., 2009b). Since such rates are rarely quantified, it is difficult to generalize these values to other species or other conditions. Moreover, calcite precipitation rates can be variable between day and night calcification periods (Erez, 1983; Spero, 1988; Lea et al., 1995). Since incorporation of some elements may depend on precipitation rate (e.g. DePaolo, 2011), it is necessary to quantify these rates across a diurnal time frame when chamber formation is occurring in order to assess the kinetics of element incorporation and thereby proxy-relationships.

Mitochondrial activity may play an important role at the site of calcification and thereby affect trace element incorporation. Besides providing energy, mitochondria pump cytosolic Ca^{2+} and Mg^{2+}, and therefore modulate the cell's [Ca^{2+}] and [Mg^{2+}] (Carafoli et al., 2001). This may be particularly important during calcification when the concentration of these ions increases locally. Spero (1988) shows that calcification in *O. universa* around the POS is associated with pseudopodia containing mitochondria, and hence possibly modulate [Mg^{2+}] at the SOC. Similar results can be found in Bé et al (1979) for *Globorotalia truncatulinoides*. Bentov et al (2009) discuss the possible role of mitochondria in producing metabolic CO_2 that eventually accumulate in the alkaline vacuoles as DIC.

Photosynthesis by symbionts may also affect calcification rates. The relative concentrations of DIC species are influenced by symbiont photosynthesis and CO_2-uptake during the day (or release in the dark) and the resulting diurnal differences in microenvironment pH (Jørgensen et al., 1985; Rink et al., 1998; Köhler-Rink and Kühl, 2000; 2005), thereby influencing uptake and availability of inorganic carbon species. In some large benthic foraminifera (Wetmore, 1999), the symbionts are positioned near the POS prior to calcification, suggesting that their activity could enhance calcification. Elimination of symbionts in *G. sacculifer* resulted in reduced chamber formation rates and early gametogenesis or death of the foraminifera (Bé et al., 1982). Reseeding the aposymbiotic foraminifera with symbionts from donor specimens produced individuals that continued to add chambers and mature at a normal rate. These data suggest that symbiont photosynthesis is critical to both nutrition and chamber calcification. Elevated light intensity promotes growth in *G. sacculifer* (Caron et al., 1982) but not in the benthic foraminifera *Amphistegina lobifera* in which both photosynthesis and calcification are optimal at relatively low light intensities that are found at 20-30 m water depth (Erez 1978, Ter Kuile and Erez, 1984).

Ter Kuile et al. (1989a), on the other hand, suggested that symbionts and foraminifera compete for inorganic carbon. Erez (1983) and Ter Kuile et al. (1989b) showed that inhibition of photosynthesis in both planktonic and benthic species by the photosystem II inhibitor DCMU, does not affect calcification rates and suggested that it is not photosynthesis itself, but rather light which directly promotes calcification. Finally, Ter Kuile et al (1989a) have shown that there is competition for CO_2 between the symbionts and their host in the benthic foraminiferan *A. lobifera*. Clearly, the relationship between symbioses and foraminifera calcification requires additional study.

Pore formation provides important information on foraminiferal biomineralization. In species producing macropores, we observe a pore plate that is continuous with the POS and separates the cytoplasm from the outside medium (Hemleben et al., 1977). In benthic, symbiont-bearing species, symbionts can be found in close proximity to the pores (e.g. Lee and Anderson, 1991) suggesting that respiratory gases such as CO_2 and O_2 may be able to diffuse through the pore plates. In symbiont-barren species, diffusion of gases between cytoplasm and environment could be enhanced by the permeability of a pore plate. Some have suggested that dissolved organic matter may be taken up through the pores in the benthic *Patellina* (Berthold, 1976). In *G. sacculifer*, pseudopodia appear to penetrate through the pore plates (Anderson and Bé, 1976). Pores in the benthic species *Patellina corrugata* have been reported to exist from the beginning of chamber formation (Berthold, 1976) and pores are observed in the *O. universa* sphere once initial calcification has locked in the spherical morphology of the chamber

(Spero, 1988). Some species of planktonic foraminifera have micro- instead of macropores (often in species with secondary apertures; *Globigerinata glutinata*, *Candeina nitida*), ranging from 0.3-0.7 μm (Brummer and Kroon, 1988). These micropores do not appear to have a pore plate, and their function, formation and morphology is less well understood than those for macropores.

6. Overgrowth and encrusting

The primary and secondary layers of calcite in perforate foraminifera are together referred to as 'ontogenetic' or 'lamellar' calcite (Erez, 2003). Additional $CaCO_3$ can be present as ornamentations (pustules, spines, ridges, tooth plates, etc.) or as layers of calcite covering the whole test (crust or gametogenic (GAM) calcite). Whereas ornamentation is present throughout the entire life cycle of a foraminifer (Hemleben, 1975), GAM calcite is exclusive to planktonic foraminifera and is added after the last chamber is formed and just prior to meiotic division of the nucleus and gametogenesis.

In some planktonic species, a calcite crust can be formed after formation of the final chamber (Bé and Ericson, 1963; Bé and Lott, 1964; Bé, 1965; Bé and Hemleben, 1970; Olsson, 1976). The morphology of this calcite is markedly different from that of either ontogenetic or GAM calcite and its element and isotopic composition can differ from that of the ontogenetic calcite because it forms under different environmental conditions of temperature and/or salinity. For instance, crust Mg/Ca is generally lower than that of ontogenetic calcite in *Globorotalia truncatulinoides* (Duckworth, 1977) and *Neogloboquadrina dutertrei* (Jonkers et al., 2012). These lower element concentrations are partly a consequence of conditions deeper in the water column (i.e. lower temperature), but it should be noticed that the observed partitioning for Mg indicates that crust calcification is a biologically controlled process. Interestingly, Nürnberg et al. (1996) found that crusts formed in culture can have a higher Mg/Ca than the ontogenetic calcite.

In a number of species such as *G. sacculifer*, gametogenesis is preceded by the production of a layer of calcite covering spine holes and the terrace-like structures of inter-pore rims (Towe and Cifelli, 1967; Bé, 1980; Hemleben et al., 1985; Brummer et al., 1987). This GAM calcite veneer gives the foraminifera a smooth appearance by covering the rough topography of the shell surface and it has been suggested that it is enriched in some trace elements compared to the ontogenetic calcite (Hathorne et al., 2009). Whether this observation holds for all foraminifera forming GAM calcite, however, remains to be investigated.

From the perspective of biomineralization, variability in the types of $CaCO_3$ that are formed may indicate that foraminifera do not have one single way to produce shell calcite. Rather, the physiological tools to achieve calcite precipitation as discussed in sections 2 and 4, are likely used in different combinations by different species of foraminifera. Moreover, the variability in calcite within single specimens suggests a degree of flexibility of these physiological tools even within single species. Identification of seawater vacuolization, transmembrane ion transport, nucleation promoting organic templates, etc. across species and their contribution to calcification within a foraminifer's life time are critical aspects of foraminiferal biology and keys to understanding foraminiferal biomineralization from a mechanistic perspective.

7. Future directions

A complete mechanistic description of foraminiferal biomineralization and chamber formation does not yet exist. Hence, the biological and environmental interplay that controls the element composition and isotope fractionation of chamber calcite is only partly understood. Literature on foraminiferal calcification is both qualitative and quantitative but on occasion, contradictory. This leaves us with a number of outstanding questions that need to be addressed in order to move this area of foraminifera biology forward. These include:

1. Which foraminiferal species use vacuolized seawater as the primary source for calcification and which use transmembrane transport of Ca^{2+} and DIC during calcification? The investigation into the transport of ions to the site of calcification may be solved by answering a number of more practical questions, including:
   - What is the relation between transmembrane transport and vaculization on the one hand, and production of intracellular calcium and/ or carbon reservoirs on the other hand?
   - What is the biochemical basis of these processes? Which transmembrane transporters are involved (e.g. Ca-ATPases, proton-Ca^{2+} antiporters)? By which mechanism is inorganic carbon concentrated (e.g. involvement of Carbonic Anhydrase)?
   - When characterized, can these (transport) mechanisms explain observed element incorporation and isotopes fractionations. If yes, can these mechanisms explain foraminiferal chemistry for (all) these elements and isotopes *at the same time*?
   - Is there a general difference between planktonic and benthic species in production of vacuolized seawater, internal reservoirs and/or direct ion transport?
   - Do foraminifera employ both mechanisms to calcify and if yes, what is the balance between these two pathways?
2. What is the tertiary structure of the organic matrix/ matrices (e.g. POS, organic linings) involved in biomineralization? Which compounds help to lower the free energy barrier, thereby promoting calcite nucleation? When identified, do these organic compounds have an impact on the partition coefficient of elements and fractionation of isotopes at the first stage of chamber formation?

3. To what extent is the site of calcification in contact with surrounding seawater? If seawater directly contributes (part of) the ions for calcification, can this source explain observed fractionation factors and partition coefficients?
4. What is the role of mitochondria in calcification? Do mitochondria (help to) regulate the Mg/Ca at the site of calcification?

Finally, a more detailed understanding of foraminiferal biominiralization will also allow researchers to compare calcification strategies accross marine calcifiers. Compared to foraminifera, biomineralization in corals (Al-Horani et al., 2003; Sinclair and Risk, 2006; Venn et al., 2013), coccolithophores (Marsh, 2003; Taylor et al., 2011; Ziveri et al., 2012; Bach et al., 2013), gastropods (e.g. Nehrke et al., 2011) and bivalves (Nudelman et al., 2006; Nehrke et al., 2012; Shi et al., 2013) are understood in greater detail. Identification of differences and similarities between these marine calcifying taxa will allow studying (convergent) evolutionairy patterns, help to understand differences in their response to (future) environmental perturbations and facilitate comparison of paleoceanographic information obtained across taxa.

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References

Al-Horani, F.A., Al-Moghrabi, S.M., De Beer, D., 2003. Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. J. Exp. Mar. Biol. Ecol. 288, 1-15.

Allen, K.A., Hönisch, B., Eggins, S.M., Yu, J., Spero, H.J., Elderfield, H., 2011. Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina universa*. Earth Planet. Sci. Lett. 309, 291-301.

Ambudkar, S.V., Zlotnick, G.W., Rosen, B.P., 1984. Calcium efflux from *Escherichia coli*. Evidence for two systems. Journal of Biol. Chem. 259, 6142-6146.

Anderson, O.R., Bé, A.W.H., 1976. The ultrastructure of a planktonic foraminifer, *Globigerinoides sacculifer* (Brady), and its symbiotic dinoflagellates. J. Foramin. Res. 6, 1-21.

Anderson, O.R., Faber, W.W. Jr, 1984. An estimation of calcium carbonate deposition rate in a planktic foraminifer *Globigerinoides sacculifer* using ^{45}Ca as a tracer: a recommended procedure for improved accuracy. J. Foramin. Res. 14, 303-308.

Angell, R.W., 1979. Calcification during chamber development in *Rosalina floridana*. J. Foramin. Res. 9, 341-353.

Bach, L.T., Mackinder, L.C.M., Schultz, K.G., Wheeler, G., Schroeder, D.C., Brownlee, C., Riebesell, U., 2013. Dissecting the impact of CO_2 and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliania huxleyi*. New Phytol. 199, 121-134.

Banner, F.T., Sheehan, R., Williams, E., 1973. The organic skeletons of rotaline foraminifera: A review. J. Foramin. Res. 3, 30-42.

Bé, A.W.H., Ericson, D.B., 1963. Aspects of calcification in planktonic foraminifera (Sarcodina). New York Acad. Sci., Ann., vol. 109, art. 1, pp. 65-81.

Bé, A.W.H., Lott, L., 1964. Shell growth and structure of planktonic foraminifera. Science 145, 823-824.

Bé, A.W.H., 1965. The influence of depth on shell growth in *Globigerinoides sacculifer* (Brady). Micropaleontology 11, 81-97.

Bé, A.W.H., 1980. Gametogenic calcification in a spinose planktonic foraminifer, *Globigerinoides sacculifer* (Brady). Mar. Micropaleontol. 5, 283-310.

Bé, A.W.H., Hemleben, C., 1970. Calcification in a living planktonic foraminifer, Globigerinoides sacculifer (Brady). N. Jb. Geol. Paläont. Abh. 134, 221-234.

Bé, A.W.H., Hemleben, C., Anderson, O.R., Spindler, M., Hacunda, J., Tunitivate-Choy, S., 1977. Laboratory and field observation of living planktonic foraminifera. Micropaleontology 23, 155-179.

Bé, A.W.H., Hemleben, C., Anderson, O.R., Spindler, M., 1979. Chamber formation in planktic foraminifera. Micropaleontology 25, 294-307.

Bé, A.W.H., Spero, H.J., Anderson, O.R., 1982. Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer *Globigerinoides sacculifer*. Mar. Biol, 70, 73-86.

Bender, M.L., Lorens, R.B., Williams, D.F., 1975. Sodium, magnesium and strontium in the tests of planktonic foraminifera. Micropaleontology 21, 448-459.

Bentov, S., Erez, J., 2005. Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells. Geology 33, 841-844.

Bentov, S., Erez, J., 2006. Impact of biomineralization process on the Mg content of foraminiferal shells: A biological perspective. Geochem. Geophy. Geosy. 7, doi:10.1029/2005GC001015.

Bentov, S., Brownlee, C., Erez, J., 2009. The role of seawater endocytosis in the biomineralization process in calcareous foraminifera. P. Natl. Acad. Sci. USA 106, 21500-21504.

Berthold, W.U., 1976. Ultrastructure and function of wall perforations in *Patellina corrugate* Williamson, Foraminifera. J. Foramin. Res. 6, 22-29.

Bijma, J., Spero, H.J., Lea, D.W., 1999. Reassessing foraminiferal stable isotope geochemistry: Impact of the oceanic carbonate system (experimental results). Use of Proxies in Paleoceanography: Examples from the South Atlantic. G. Fischer and G. Wefer. Berlin, Heidelberg, Springer-Verlag: 489-512.

Boyle, E., 1981. Cadmium, zinc, copper, and barium in foraminiferal tests. Earth Planet. Sci. Lett. 53, 11-35.

Brummer, G.J., Hemleben, C., Spindler, M., 1987. Ontogeny of extant spinose planktonic foraminifera (Globigerinidae): a concept exemplified by *Globigerinoides sacculifer* (Brady) and *G. ruber* (D'Orbigny). Mar. Micropaleontol. 12, 357-381.

Brummer, G.J.A., Kroon, D., 1988. Planktonic foraminifers as tracers of ocean-climate history. Free University Press, Amsterdam, 346 pp.

Carafoli, E., Santella, L., Branca, D., Brini, M., 2001. Generation, control, and processing of cellular calcium signals. Crit. Rev. Biochem. Mol. 36, 107-260.

Caron, D.A., Bé, A.W.H., Anderson, O.R., 1982. Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. J. Mar. Biol. Assoc. UK 62, 435-451.

De Nooijer, L.J., Toyofuku, T., Kitazato, H., 2009a. Foraminifera promote calcification by elevating their intracellular pH. P. Natl. Acad. Sci. USA 106, 15374-15378.

De Nooijer, L.J., Langer, G., Nehrke, G., Bijma, J., 2009b. Physiological controls on the seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*. Biogeosciences 6, 2669-2675.

De Yoreo, J.J., Velikov, P.G., 2003. Principles of crystal nucleation and growth. Rev. Mineral. Geochem. 54, 57-94.

DePaolo, D.J., 2011. Surface kinetic model for isotopic and trace element fractionation during precipitation of calcite from aqueous solution. Geochim. Cosmochim. Ac. 75, 1039-1056.

Dissard, D., Nehrke, G., Reichart, G.J., Bijma, J., 2010. Impact of seawater $p\mathrm{CO}_2$ on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminiferal calcite: results from culturing experiments with *Ammonia tepida*. Biogeosciences 7, 81-93.

Dove, P.M., De Yoreo, J.J., Weiner, S., 2003. Biomineralization: Reviews in Mineralogy and Geochemistry 54, Mineralogical Society of America. Washington DC, 381 pp.

Drake, S.K., Lee, K.L., Falke, J.J., 1996. Tuning the equilibrium ion affinity and selectivity of the EF-Hand calcium binding motif: Substitutions at the gateway position. Biochem 35, 6697-6705.

Duckworth, D.L., 1977. Magnesium concentration in the tests of the planktonic foraminifer *Globorotalia truncatulinoides*. J. Foramin. Res. 7, 304-312.

Dueñas-Bohórquez, A., Raitzsch, M., De Nooijer, L.J., Reichart, G.J., 2010. Independent impacts of calcium and carbonate ion concentration on Mg and Sr incorporation in cultured benthic foraminifera. Mar. Micropaleontol, 81, 122-130.

Eggins, S.M., Sadekov, A., 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 Planet. Sci. Lett. 225, 411-419.

Elderfield, H., Bertram, C.J., Erez, J., 1996. A biomineralization model for the incorporation of trace elements into foraminiferal calcium carbonate. Earth Planet. Sci. Lett. 142, 409-423.

Epstein, S., Buchsbaum, R., Lowenstam, H., Urey, H.C., 1951. Carbonate-water isotopic temperature scale. Bull. Geol. Soc. Am. 62, 417-426.

Erez, J., 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273, 199-202.

Erez, J., 1983. Calcification rates, photosynthesis and light in planktonic foraminifera. In: P. Westbroek, E.W. de Jong (eds), Biomineralization and biological metal accumulation. Reidel Publishing Company. 307-312.

Erez, J., 2003. The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. Rev. Mineral. Geochem. 54, 115-149.

Glas, M., Langer, G., Keul, N., 2012. Calcification acidifies the microenvironment of a benthic foraminifer (*Ammonia* sp.). J. Exp. Mar. Biol. Ecol. 424-425, 53-58.

Gouaux, E., MicKinnon, R., 2005. Principles of selective ion transport in channels and pumps. Science 310, 1461-1465.

Gonçalves, P.P., Meireles, S.M., Gravato, C., Vale, M.G.P., 1998. Ca^{2+}-H^{+}-antiport activity in synaptic vesicles isolated from sheep brain cortex. Neurosci. Lett. 247, 87-90.

Gonçalves, P.P., Meireles, S.M., Neves, P., Vales, M.G.P., 1999. Ion selectivity of the Ca^{2+}/H^{+} antiport in synaptic vesicles of sheep brain cortex. Mol. Brain Res. 67, 283-291.

Grossman, E.L., 1987. Stable isotopes in modern benthic foraminifera: A study of vital effect. J. Foramin. Res. 17, 48-61.

Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H.J., Lea, D.W., Bijma, J., Nägler, T.F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}Ca$) in inorganic aragonite and cultured planktonic foraminifera. Geochim. Cosmochim. Ac. 67, 1375-1382.

Hamilton, C.P., Spero, H.J., Bijma, J., Lea, D.W., 2008. Geochemical investigation of gametogenic calcite addition in the planktonic foraminifera *Orbulina universa*. Mar. Micropaleontol. 68, 256-267.

Hastings, D.W., Russell, A.D., Emerson, S.R., 1998. Foraminiferal magnesium in Globigerinoides sacculifer as a paleotemperature proxy. Paleoceanography 13, 161-169.

Hathorne, E.C., James, R.H., Lampitt, R.S., 2009. Environmental versus biomineralization controls on the intratest variation in the trace element composition of the planktonic foraminifera *G. inflata* and *G. scitula*. Paleoceanography 24, doi:10.1029/2009PA001742.

Hemleben, C., 1975. Spine and pustule relationships in some recent planktonic foraminifers. Micropaleontology 21, 334-341.

Hemleben, C., Bé, A.W.H., Anderson, O.R., Tuntivate, S., 1977. Test morphology, organic layers and chamber formation in the planktonic foraminifer *Globorotalia menardii* (d'Orbigny). J. Foramin. Res. 7, 1-25.

Hemleben, C., Spindler, M., Breitinger, I., Deuser, WG., 1985. Field and laboratory studies on the ontogeny and ecology of some Globorotaliid species from the Sargasso Sea off Bermuda. J. Foramin. Res. 15, 254-272.

Hemleben, C., Anderson, O.R., Berthold, W., Spindler, M., 1986. Calcification and chamber formation in Foraminifera - a brief overview. In: Leadbeater BS, Riding R (eds), The systematics association, special volume 30, 237-249.

Hemleben, C., M. Spindler, Anderson, O.R., 1989. Modern planktonic foraminifera. New York, Springer, 363 pp.

Hemleben, C., Mühlen, D., Olsson, R.K., Berggren, W.A., 1991. Surface texture and first occurrence of spines in planktonic foraminifera from the early Tertiary. Geologisches Jahrbuch A 128, 117-146.

Hemleben, C., Bijma, J., 1994. Foraminiferal population dynamics and stable carbon isotopes. NATO ASI series 117, 145-166.

Higgins, C.F., 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8, 67-113.

Hönisch, B., Allen, K.A., Lea, D.W., Spero, H.J., Eggins, S.M., Arbuszewski, J., deMenocal, P., Rosenthal, Y., Russell, A.D., Elderfield, H., 2013. The influence of salinity on Mg/Ca in planktic foraminifers - Evidence from cultures, core-top sediments and complementary $\delta^{18}O$. Geochim. Cosmochim. Acta 121, 196-213.

Hottinger, L., Dreher, D., 1974. Differentiation of protoplasm in Nummulitidae (Foraminifera) from Elat, Red Sea. Mar. Biol. 25, 41-61.

Ishikawa, M., Ichikuni, M., 1984. Uptake of sodium and potassium by calcite. Chem. Geol. 42, 137-146.

Jørgensen, B.B., Erez, J., Revsbech, N.P., Cohen, Y., 1985. Symbiotic photosynthesis in a planktonic foraminiferan *Globigerinoides sacculifer* (Brady), studied with microelectrodes. Limnol. Oceonogr. 30, 1253-1267.

Katz, A., 1973. The interaction of magnesium with calcite during crystal growth at 25-90ºC and one atmosphere. Geochim. Cosmochim. Ac. 37, 1563-1586.

Katz, M.E., Cramer, B.S., Franzese, A., Hönisch, B., Miller, K.G., Rosenthal, Y., Wright, J.D., 2010. Traditional and Emerging geochemical proxies in foraminifera. J. Foramin. Res. 40, 165-192.

King, K., Hare, P.E., 1972. Amino acid composition of the test as a taxonomic character of living and fossil planktonic foraminifera. Micropaleonontology 18, 285-293.

Kitano, Y., Okumura, M., Idogaki, M., 1975. Incorporation of sodium, chloride and sulfate with calcium carbonate. Geochem. J. 9, 75-84.

Kitazato, H., Bernhard J.M., 2014.Approaches to study living foraminifera: collection, maintainance and experimentation. Springer, 227 pp.

Köhler-Rink, S., Kühl, M., 2000. Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera. I The physico-chemical microenvironment of *Marginopora vertebralis*, *Amphistegina lobifera* and *Amphisorus hemprichii*. Mar. Biol. 137, 473-486.

Köhler-Rink, S., Kühl, M., 2005. The chemical microenvironment of the symbiotic planktonic foraminifer *Orbulina universa*. Mar. Biol. Res. 1, 68-78.

Kozdon, R., Ushikubo, T., Kita, N.T., Spicuzza, M., Valley, J.W., 2009. Intratest oxygen isotope variability in the planktonic foraminifer *N. pachyderma*: real vs. apparent vital effects by ion microprobe. Chem. Geol. 258, 327–337.

Kunioka, D., Shirai, K., Takahata, N., Sano, Y., Toyofuku, T., Ujiie, Y., 2006. Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata* test by using a NanoSIMS: Implication for the vital effect mechanism. Geochem. Geophy. Geosy. 7, doi: 10.1029/2006GC001280.

Langer, M.R., 1992. Biosynthesis of glycosaminoglycans in foraminifera: A review. Marine Micropaleontology 19, 245-255.

Lea, D.W., Boyle, E.A., 1991. Barium in planktonic foraminifera. Geochim. Cosmochim. Ac. 55, 3321-3331.

Lea, D.W., Spero, H.J., 1992. Experimental determination of barium uptake in shells of the planktonic foraminifera *Orbulina universa* at 22ºC. Geochim. Cosmochim. Ac. 56, 2673-2680.

Lea, D.W., Martin, P.A., Chan, D.A., Spero, H.J., 1995. Calcium uptake and calcification ratae in planktonic foraminifer *Orbulina universa*. J. Foramin. Res. 25, 14-23.

Lea, D.W., Mashoitta, T.A., Spero, H.J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. Geochim. Cosmochim. Ac. 63, 2369-2379.

Lea, D.W., Pak, D.K., Spero, H.J., 2000. Climate impact of late Quaternary equatorial Pacific sea surface temperature variations. Science 289, 1719-1724.

Lear, C.H., Elderfield, H., Wilson, P.A., 2000. Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca ratios in benthic foraminiferal calcite. Science 287, 269-272.

Lee, J.J., Anderson, O.R. (eds), 1991. Biology of Foraminifera, Academic Press, London, 368 pp.

Lehninger, A.L., Rossi, C.S., Greenawalt, J.W., 1963. Respiration-dependent accumulation of inorganic phosphate and Ca^{2+} by rat liver mitochondria. Biochem. Biophy. Res. Comm. 10, 444-448.

Lowenstam, H.A., Weiner, S., 1989. Biomineralization. Oxford University Press. pp xxx.

Mann, S., 1988. Molecular recognition in biomineralization. Nature 332, 119-124.

Marriott, C.S., Henderson, G.M., Belshaw, N.S., Tudhope, A.W., 2004. Temperature dependence of $\delta^{7}Li$, $\delta^{44}Ca$ and Li/Ca during growth of calcium carbonate. Earth Planet. Sci. Lett. 222, 615-624.

Marsh, M.E., 2003. Regulation of $CaCO_3$ formation in coccolithophores. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 236,743-754.

McConnaughey, T.A., Whelan, J.F., 1997. Calcification generates protons for nutrient and bicarbonate uptake. Earth-Sci. Rev. 42, 95-117.

McCrea, J.M., 1950. On the isotopic chemistry of carbonates and a paleotemperature scale. J. Chem. Phys. 18, 849-857.

Morse, J.W., Arvidson, R.S., Lüttge, A., 2007. Calcium carbonate formation and dissolution. Chem. Rev. 107, 342-381.

Morse, J.W., Bender, M.L., 1990. Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems. Chem. Geol. 82, 265-277.

Mucci, A., 1987. Influence of temperature on the composition of magnesian calcite overgrowths precipitated from seawater. Geochim. Cosmochim. Ac. 51, 1977-1984.

Nehrke, G., Nouet, J., 2011. Confocal Raman microscope mapping as a tool to describe different mineral phases at high spatial resolution within marine biogenic carbonates: a case study on *Nerita undata* (Gastropoda, Neritopsina). Biogeosciences 8, 3761-3769.

Nehrke, G., Poigner, H., Wilhelms-Dick, D., Brey, T., Abele, D., 2012. Coexistence of three calcium carbonate polymorphs in the shell of the Antarctic clam *Laternula elliptica*. Geochem. Geophys. Geosys. 13, Q05014.

Nehrke, G., Keul, N., Langer, G., De Nooijer,. L.J., Bijma, J., 2013. A new model for biomineralization and trace-element signatures of Foraminifera tests. Biogeosciences 10, 6759-6767.

Nudelman, F., Gotliv, B.A., Addadi, L., Weiner, S., 2006. Molusk shell formation: mapping the distribution of organic matrix components underlying a single aragonitic tablet in nacre. J. Struc. Biol. 153, 176-178.

Nürnberg, D., Bijma, J., Hemleben, C., 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperature. Geochim. Cosmochim. Ac. 60, 803-814.

Ogawa, T., Kaplan, A., 1987. The stoichiometry between CO_2 and H^+ fluxes involved in the transport of inorganic carbon in cyanobacteria. Plant Physiol. 83, 888-891.

Olsson, R.K., 1976. Wall structure, topography and crust of *Globigerina pachyderma* (Ehrenberg). Progress in Micropaleontology: 1-9.

Pereira, A.C., Samellas, D., Tiffert, T., Lew, V.L., 1993. Inhibition of the calcium pump by high cytosolic Ca^{2+} in intact human red blood cells. J. Physiol. 461, 63-73.

Pingitore, N.E., Eastman, M.P., 1984. The experimental partitioning of Ba^{2+} into calcite. Chem. Geol. 45, 113-120.

Raz, S., Weiner, S., Addadi, L., 2000. Formation of high-magnesian calcites via an amorphous precursor phase: possible biological implications. Adv. Mater. 12, 38-42.

Reiss, Z., 1957. The Bilamellidea, nov. superfam., and remarks on Creteceous globorotaliids. Cushman Foundation Foramin. Res. Contr. 8, 127-145.

Reiss, Z., 1960. Structure of so-called *Eponides* and some other Rotaliiform Foraminifera. Ministry of Development Geological Survey 29, 1-29.

Rickaby, R.E.M., Elderfield, H., 1999. Planktonic foraminiferal Cd/Ca: paleonutrients or paleotemperature? Paleoceanography 14, 293-303.

Rimstidt, D.J., Balog, A., Webb, J., 1998. Distribution of trace elements between carbonate minerals and aqueous solutions. Geochim. Cosmochim. Ac. 62, 1851–1863.

Rink, S., Kühl, M., Bijma, J., Spero, H.J., 1998. Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. Mar. Biol. 131, 583-595.

Robbins, L.L., Brew, K., 1990. Proteins from the organic matrix of core-top and fossil planktonic foraminifera. Geochim. Cosmochim. Ac. 54, 2285-2292.

Robbins, L.L., Healy-Williams, N.H., 1991. Toward a classification of planktonic foraminifera based on biochemical, geochemical, and morphological criteria. J. Foramin. Res. 21, 159-167.

Saito, T., Thompson, P.R., Breger, D., 1976. Skeletal ultra-microstructure of some elongate-chambered planktonic Foraminifera and related species. In: Takayanagi Y, Saito T (eds), Selected papers in honor of Prof. Kiyoshi Asano. Progress in Micropaleontology Special Publication, 278-304.

Schmiedl, G., Mackensen, A., 2006. Multispecies stable isotopes of benthic foraminifers reveal past changes of organic matter decomposition and deep water oxygenation in the Arabian Sea. Paleoceanography 21, PA4213.

Segev, E., Erez, J., 2006. Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera. Geochem. Geophy. Geosy. 7: Q02P09.

Shi, Y., Yu, C., Gu, Z., Zhan, X., Wang, Y., Wang, A., 2013. Characterization of the pearl oyster (*Pinctada martensii*) mantle transcriptome unravels biomineralization genes. Mar. Biotechnol. 15, 175-187.

Sinclair, D., Risk, M., 2006. A numerical model of trace element coprecipitation in a physicochemical calcification system: Application to coral biomineralization and trace element vital effects. Geochim. Cosmochim. Ac. 70, 3855-3868.

Spero, H.J., 1988. Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. Mar. Biol. 99, 9-20.

Spero, H.J., Bijma, J., Lea, D.W., Bemis, B.E., 1997. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. Nature 390, 497-500.

Spindler, M., 1978. The development of the organic lining in *Heterostegina depressa* (Nummulitidae: Foraminifera). J. Foramin. Res. 8, 258-261.

Tang, J., Niedermayr, A., Köhler, S.J., Böhm, F., Kisakürek, B., Eisenhauer, A., Dietzel, M., 2012. Sr^{2+}/Ca^{2+} and $^{44}Ca/^{40}Ca$ fractionation during inorganic calcite formation: III. Impact of salinity/ ionic strength. Geochim. Cosmochim. Ac. 77, 432-443.

Taylor, A.R., Chrachri, A., Wheeler, G., Goddard, H., Brownlee, C., 2011. A voltage-gated H+ channel underlying pH homeostasis in calcifying coccolithophores. PLoS Biol. 9: e1001085.

Ter Kuile, B., Erez, J., 1984. In situ growth rate experiments on the symbiont-bearing foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*. J. Foramin. Res. 14, 262-276.

Ter Kuile, B., Erez, J., 1987. Uptake of inorganic carbon and internal carbon cycling in symbiont-bearing benthonic foraminifera. Mar. Biol. 94, 499-509.

Ter Kuile, B., Erez, J., 1988. The size and function of the internal inorganic carbon pool of the foraminifer *Amphistegina lobifera*. Mar. Biol. 99, 481-487.

Ter Kuile, B., Erez, J., Padan, E., 1989a. Competition for inorganic carbon between photosynthesis and calcification in the symbiont-bearing foraminifer *Amphistegina lobifera*. Mar. Biol. 103, 253-259.

Ter Kuile, B., Erez, J., Padan, E., 1989b. Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera. Mar. Biol. 103, 241-251.

Ter Kuile, B., Erez, J., 1991. Carbon budgets for two species of benthonic symbiont-bearing foraminifera. Biol. Bull. 180, 489-495.

Terakado, Y., Ofuka, Y., Tada, N., 2010. Rare earth elements, Sr, Ba, Fe, and major cation concentrations in some living foraminiferal tests collected from Iriomote Island, Japan: An exploration for trace element behavior during biogenic calcium carbonate formation. Geochem. J. 44, 315-322.

Towe, K.M., Cifelli, R., 1967. Wall ultrastructure in the calcareous Foraminifera: crystallographic aspects and a model for calcification. J. Paleontol. 41, 742-762.

Toyofuku, T., De Nooijer, L.J., Yamamoto, H., Kitazato, H., 2008. Real-time visualization of calcium ion activity in shallow benthic foraminiferal cells using the fluorescent indicator Fluo-3 AM. Geochem. Geophy. Geosy. 9, Q05005, doi: 10.1029/2007GC001772.

Urey, H.C., Lowenstam, H.A., Epstein, S., McKinney, C.R., 1951. Measurement of paleotemperatures and temperatures of the upper Cretaceous of England, Denmark, and the Southeastern United States. Bull. Geol. Soc. Am. 62, 399-416.

Venn, A.A., Tambutté, E., Holcomb, M., Laurent, J., Allemand, D., Tambuté, S., 2013. Impact of seawater acidification on pH at the site of tissue-skeleton interface and calcification in reef corals. P. Natl. Acad. Sci. USA 110, 1634-1639.

Vetter, L., Kozdon, R., Mora, C.I., Eggins, S.M., Valley, J.W., Hönisch, B., Spero, H.J., 2013. Micron-scale intrashell oxygen isotope variation in cultured planktic foraminifers. Geochim. Cosmochim. Ac. 107, 1-12.

Wang, K.K.W., Villalobo, A., Roufogalis, B.D., 1992. The plasma membrane calcium pump: a multiregulated tranporter. Trends Cell Biol. 2, 46-52.

Weiner, S., Dove, P.M., 2003. An overview of biomineralization processes and the problem of the vital effect. Rev. Mineral. Geochem. 54, 1-29.

Weiner, S., Erez, J., 1984. Organic matrix of the shell of the foraminifer, *Heterostegina depressa.* J. Foramin. Res. 14, 206-212.

Wetmore, K., 1999. Chamber formation in *Archaias ungulatus*. J. Foramin. Res. 29, 69-74.

Wilson, J.E., Chin, A., 1991. Chelation of divalent cations by ATP, studied by titration calorimetry. Anal. Biochem. 193, 16-19.

Wolf-Gladrow, D., Bijma, J., Zeebe, R.E., 1999. Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Mar. Chem. 64, 181-198.

Zeebe, R.E., 1999. An explanation of the effect of seawater carbonate concentration on foraminiferal oxygen isotopes. Geochim. Cosmochim. Ac. 63, 2001-2007.

Zeebe, R.E., Bijma, J., Wolf-Gladrow, D.A., 1999. A diffusion-reaction model of carbon isotope fractionation in foraminifera. Mar. Chem. 64, 199-227.

Zeebe, R.E., Sanyal, A., 2002. Comparison of two potential strategies of planktonic foraminifera for house building: Mg^{2+} or H^+ removal? Geochim. Cosmochim. Ac. 66, 159-169.

Zeebe, R.E., Bijma, J., Hönisch, B., Sanyal, A., Spero, H.J., Wolf-Gladrow, D.A., 2008. Vital effects and beyond: a modeling perspective on developing palaeoceanographical proxy relationships in foraminifera. Geol. Soc., London Special Publications 303, 45-58.

Ziveri, P., Thoms, S., Probert, I., Geisen, M., Langer, G., 2012. A universal carbonate ion effect on stable oxygen isotope ratios in unicellular planktonic calcifying organisms. Biogeosciences 9, 1025-1032.

Biomineralization in perforate Foraminifera

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Abstract

In this paper, we review the current understanding of biomineralization in Rotaliid foraminifera. Ideas on the mechanisms responsible for the flux of Ca^{2+} and inorganic carbon from seawater into the test were originally based on light and electron microscopic observations of calcifying foraminifera. From the 1980's onward, tracer experiments, fluorescent microscopy and high-resolution test geochemical analysis have added to existing calcification models. Despite recent insights, no general consensus on the physiological basis of foraminiferal biomineralization exists. Current models include seawater vacuolization, transmembrane ion transport, involvement of organic matrices and/or pH regulation, although the magnitude of these controls remain to be quantified. Disagreement between currently available models may be caused by use of different foraminiferal species as subject for biomineralization experiments and/ or lack of a more systematic approach to study (dis)similarities between taxa. In order to understand foraminiferal controls on element incorporation and isotope fractionation, and thereby improve the value of foraminifera as paleoceanographic proxies, it is necessary to identify key processes in foraminiferal biomineralization and formulate hypotheses regarding the involved physiological pathways to provide directions for future research.

1. Introduction

All foraminifera make tests although a number of different materials are used in their construction. The 'naked' foraminifera produce tests from organic matter, agglutinated foraminifera use sediment grains as building blocks and calcifying foraminifera use constituents dissolved in seawater to secrete calcium carbonate. Formation of $CaCO_3$ tests plays a significant role in ocean biogeochemical cycles and, more importantly, the fossil remains of calcifying foraminifera are widely used to reconstruct past ocean chemistry and environmental conditions. Elemental and isotopic composition of foraminiferal calcite depends on a variety of environmental parameters such as temperature, salinity, pH and ion concentration (McCrea et al., 1950; Epstein et al., 1951; Boyle, 1981; Nürnberg et al., 1996). These physical and chemical variations are the foundation for developing geochemical proxies that quantify environmental changes through time (see Wefer et al., 1999; Zeebe et al., 2008; Katz et al., 2010 for reviews). For example, the magnesium concentration in foraminiferal calcite ($Mg/Ca_{calcite}$) varies primarily with seawater temperature (Nürnberg et al., 1996; Lea et al., 1999; Hönisch et al., 2013) and can be used to reconstruct past sea surface (Hastings et al., 1998; Lea et al., 2000) and deep-water (Lear et al., 2000) temperatures. Reliable application of these proxies requires calibration over a wide range of environmental conditions as well as a thorough understanding of the physiological parameters influencing test formation.

Studies calibrating foraminiferal test composition based on core-tops and controlled growth experiments show that both the chemical and isotopic compositions of these tests are not in equilibrium as defined by inorganic precipitation experiments (Lowenstam and Weiner, 1989; Dove et al., 2003). Microenvironmental controls related to foraminifera physiology have been implicated to explain disequilibrium fractionation in test chemistry (Figure 1). Most foraminiferal species incorporate Mg with one to two orders of magnitude lower concentration compared to non-biologically precipitated calcium carbonate (Bentov and Erez, 2006; Katz, 1973; Bender et al., 1975). The concentration of barium, on the other hand, is ~10 times higher in foraminiferal calcite (Lea and Boyle, 1991; Lea and Spero, 1992) compared to inorganic precipitation results (Pingitore and Eastman, 1984). Additionally, elemental concentrations between foraminiferal species can vary by several orders of magnitude (up to two orders of magnitude for Mg; Bentov and Erez, 2006). The biological controls on element incorporation and isotope fractionation that cause these offsets are often summarized as 'the vital effect' (Urey et al., 1951; Weiner and Dove, 2003).

Figure 1: Minor and trace element composition of foraminiferal (left) and inorganically precipitated (right) calcite precipitated from seawater (middle). Concentrations are qualitative as they differ between foraminiferal species and depend on environmental conditions. Precipitation rates, ionic strength of the medium and presence of organic compounds are also known to affect partition coefficients. All values are in parts per million (ppm) and based on data in Kitano et al. (1975), Ishikawa and Ichikuni (1984), Rimstidt et al. (1998), Marriott et al. (2004), Morse et al. (2007), Tang et al. (2012) He et al. (2013) for inorganically precipitated calcium carbonates, and Lea and Boyle (1991), Rickaby and Elderfield (1999), Segev and Erez (2006), Terakado et al. (2010), Allen et al. (2011) for foraminiferal calcite composition.

Vital effects comprise 1) chemical alterations of the foraminifers' microenvironment due to physiological processes, 2) cellular controls on the composition of the fluid from which calcite is precipitated and 3) controls on nucleation and crystal growth (e.g. by presence of organic templates). Foraminiferal respiration and/or photosynthesis by symbiotic algae alter the foraminiferal microenvironment chemistry and thereby the conditions in which foraminiferal tests mineralize. Because habitat depth differences in the water column (planktonic species) or migration in the sediment and attachment to plant leaves (benthic species) also modify the calcification environment, these ecological factors are sometimes regarded as being part of the vital effect as well (e.g. Schmiedl and Mackensen, 2006). Ecology-based variability in element incorporation, however, can be accounted for when habitat preferences of foraminiferal species are known. Hence, the term "vital effects" should only be used when discussing foraminiferal cellular processes that alter the chemistry of the microenvironment during test mineralization.

To understand the physiological impact on element incorporation and isotope fractionation, the (intra)cellular mechanisms which foraminifera employ to precipitate test $CaCO_3$ must be identified. Biogeochemical mechanisms are involved in regulating concentrations of ions and/or their activity at the site of calcification. Calcification from seawater can be promoted using different mechanisms. Hence, multiple mechanisms have been proposed to explain test calcification, including endocytosis of seawater, transmembrane ion transporters, ion-specific organic templates, production of a privileged space and mitochondrial activity (Spero, 1988; Erez, 2003; Bentov and Erez, 2006; Bentov et al., 2009).

A process-based framework for both inorganic and organismal control of foraminiferal test formation is crucial for the development, calibration and application of geochemical proxies in the geological record. At the same time, a mechanistic understanding of foraminiferal biomineralization will also permit researchers to better interpret data from the fossil record as well as predicting the response of foraminiferal calcification to future environmental changes such as ongoing ocean acidification. Most of the initial observations of chamber formation and calcification in planktonic foraminifera were published during the early period of planktonic foraminifera culturing (e.g. Bé et al., 1977). Highlights of those observations can be found summarized in the seminal text on “Modern Planktonic Foraminifera” (Hemleben et al., 1989). More recently, studying living specimens under controlled conditions (e.g. Kitazato and Bernard, 2014) has further propelled our understanding of foraminiferal growth, reproduction and calcification.

Recent hypotheses on foraminiferal biomineralization are based mainly on experiments with benthic species and although these ideas have to be tested for planktonic species, we will also include the latter group in our discussion. Although a general model for foraminiferal biomineralization is still lacking, and it is not yet clear that a single model fits all groups of foraminifera, details on the underlying mechanisms in different species have accumulated and are described here in the context of previously published biomineralization models.

2. Ions for calcification

2.1 Seawater as the direct source for Ca^{2+} and DIC

Foraminifera calcify by creating a microenvironment supersaturated with respect to $CaCO_3$, while overcoming inhibition by crystallization inhibitors such as Mg^{2+}. Hence, calcification requires a tight control on the concentration and/or ion activity at the site of calcification, commonly referred to as the "delimited" space (Erez, 2003) or "privileged" space. Elevated $[Ca^{2+}]$, $[CO_3^{2-}]$ and/or their ion activities have to be actively maintained in order for calcification to proceed. Simultaneously, the concentrations of crystal growth inhibitors have to be lowered even further. Although CO_3^{2-} needed for calcification may be partially derived from respired CO_2 (Erez, 1978; Grossman, 1987; Ter Kuile and Erez, 1991; Hemleben and Bijma, 1994; Bijma et al., 1999), the majority of the carbon and the Ca^{2+} needed for test formation must be derived from the seawater environment.

Calcification requires equal amounts of Ca^{2+} and CO_3^{2-}. Because seawater Ca^{2+} concentrations are approximately 5 times higher than that of DIC and often >50 times higher than that of CO_3^{2}, foraminifera have to spend more time and/or energy in taking up and concentrating DIC than they have to do for Ca^{2+}. A foraminifer needs to process several times the seawater equivalent of its own cell volume in order to acquire enough Ca^{2+} and inorganic carbon to calcify a new chamber. Although the exact amount needed depends on shape, size and the thickness of the chamber wall (e.g. Brummer et al., 1987), juveniles of some species need 50-100 times their own cell volume to extract the Ca^{2+} required to produce one new chamber (De

Nooijer et al., 2009b). Because seawater [CO_3^{2-}] is significantly lower than [Ca^{2+}], these individuals need the equivalent of ~3,000 times their own volume in order to take up the necessary [CO_3^{2-}] if this anion is used exclusively. However, observations of high pH at the site of calcification (Erez, 2003; De Nooijer et al., 2009a; Bentov et al., 2009) as well as oxygen isotope data from laboratory experiments (Spero et al., 1997; Zeebe, 1999) suggest that foraminifera can convert CO_2 and/or HCO_3^- into the CO_3^{2-} needed for calcification. Evidence that foraminifera concentrate inorganic carbon is also provided by experiments using ^{14}C tracer incorporation kinetics into the skeleton of perforate species (Ter Kuile and Erez 1987, 1988, Ter Kuile et al 1989b). A carbon concentrating mechanism would reduce the volume of seawater necessary for calcification by 50-90% (De Nooijer et al., 2009b). To concentrate the ions needed for calcification, foraminifera must either extract Ca^{2+} and dissolved inorganic carbon (CO_2, HCO_3^- and CO_3^{2-}, or DIC) or take up seawater and subsequently reduce the concentrations and/or activities of all other ions relative to Ca^{2+} and DIC (Figure 2). Removal of protons from (endocytosed) seawater is also a prominent feature in recently developed calcification mechanisms, but will be discussed in a separate section (2.2). In case of the second option, spontaneous nucleation of $CaCO_3$ crystals may be prevented by separation of Ca^{2+} and DIC into different vacuole groups.

Figure 2: Two different mechanisms to concentrate Ca^{2+} and DIC from seawater for calcification: a) Calcium- and bicarbonate-ions are specifically taken up from seawater, or b) the other ions are selectively removed, thereby increasing Ca and DIC concentrations.

Both processes transport ions either directly to the site of calcification or temporarily store these ions. In the case of uptake into some benthic foraminifers, Ca^{2+} and/ or DIC are thought to be present in so-called 'intracellular reservoirs' (also known as ‘pools’; Ter Kuile and Erez, 1988; Erez, 2003). These reservoirs may be seen as temporal storage compartments with high concentrations of ions that are either emptied upon calcification or provide a dynamic cycling of Ca^{2+} and DIC through the cell that is gradually used for calcification. Without an intracellular reservoir, Ca^{2+} and DIC could also be directly transported to the privileged space during calcification (Erez, 2003; Bentov and Erez, 2006). The relative importance of intracellular reservoirs versus direct transport among benthic and planktonic species remains a subject of debate and active research.

2.2 Internal reservoirs

Internal reservoirs may be important for foraminiferal calcification in certain groups. Conceptually speaking, one can envision Ca^{2+} or DIC being derived from internal reservoirs. With seawater as the basis for calcification, carbon reservoirs will have to be approximately 5 times larger than those for Ca^{2+} or have a 5 times faster turnover rate. Evidence suggests that different foraminifer groups employ different strategies. For instance, a time-lag has been observed between uptake and incorporation of labelled inorganic carbon in the large benthic foraminifera *Amphistegina lobifera* suggesting inorganic carbon may be stored in an internal reservoir (Ter Kuile and Erez, 1987; 1988; Ter Kuile and Erez, 1991). In pulse-chase experiments it was observed that ^{14}C was incorporated into the calcite during the chase period in ^{14}C free seawater, implying a large internal reservoir of DIC in the benthic *Amphistegina lobifera* but not in the milliolid *Amphisorus hemprichii* (Ter Kuile et al 1989b). Isotope labelling experiments with the planktonic foraminifer *G. sacculifer* and a number of benthic species using both ^{14}C and ^{45}Ca show that proportionally more labelled ^{45}Ca is incorporated into the shell compared to labelled ^{14}C (Erez, 1978; 1983). For the planktonic species *Orbulina universa* and *Globigerina bulloides*, on the other hand, Bijma et al. (1999) showed that the contribution from an internal carbon pool is insignificant in these species.

To determine whether planktonic foraminifera have an internal Ca-reservoir, Anderson and Faber (1984) grew *G. sacculifer* in artificial seawater spiked with ^{45}Ca. They showed that calcite formed during the first 24 hours contains significantly less ^{45}Ca than that produced in the second 24 hours. These data argue for the existence of an unlabeled intracellular Ca-reservoir that was filled prior to the introduction of the isotopic spike. Using pulse-chase experiments with both a 'hot' incubation period (10-15 days) and 'cold' chase period (10-20 days), Erez (2003) traced the uptake of ^{45}Ca over time in the benthic species *Amphistegina lobifera*, showing that as much as 75% of the Ca^{2+} used during chamber calcification resided in an intracellular reservoir. ^{48}Ca uptake data from experiments using *Orbulina universa*, supported the existence of a Ca-reservoir in a planktonic species, but demonstrated that it was completely flushed of labelled Ca^{2+} within the initial 6 hours of chamber formation and thickening (Lea et al., 1995). These latter observations could indicate that *O. universa* utilizes a small Ca^{2+} reservoir to assist with the initial chamber formation, but that much of the remaining chamber Ca^{2+} is derived from seawater without passing through an internal storage reservoir.

Toyofuku et al. (2008) reported formation of (incomplete) chambers in the benthic *Ammonia beccarii* maintained in seawater devoid of Ca^{2+}. These data clearly support the existence of a Ca^{2+}-reservoir of finite volume in benthic species. If Ca^{2+} and other divalent cations that co-precipitate in the $CaCO_3$ shell are derived from the same internal reservoir, one would expect cation concentrations to reflect Rayleigh fractionation if the reservoir is a closed system. Such a system has been used to partly explain minor and trace element distributions in foraminiferal calcite (Elderfield et al., 1996). However, a model using Rayleigh fractionation relies on a number of assumptions about the internal reservoir regarding its size and initial composition as well as refreshment rate and chamber calcification rate. These unknowns highlight the need to better constrain the size and extent of these reservoirs.

To maintain an intracellular reservoir, a foraminifer needs to sustain a high cation flux rate by continuously vacuolizing, endocytosing and exocytosing large volumes of seawater. Tracing endo- and exocytosis in foraminifera is challenging and has yielded contrasting results. For instance, Bentov et al. (2009) showed that in *Amphistegina lobifera*, seawater is taken up in vacuoles that are subsequently transported to the site of calcification. This implies that seawater, internally modified or not, is directly involved in calcification. De Nooijer et al. (2009b) on the other hand, showed that endocytosis and subsequent exocytosis of seawater in *Ammonia tepida* are not directly related to chamber formation.

2.3 Direct uptake of ions

The ions needed for calcification may be derived from seawater during calcification without storage in an intracellular reservoir (Figure 3). A number of calcification models explicitly or implicitly assume that the ions for calcification are passively transported to the site of calcification through diffusion from the surrounding medium (Wolf-Gladrow et al., 1999; Zeebe et al., 1999). These models are able to explain the impact of photosynthetic symbionts on inorganic carbon chemistry in the vicinity of the foraminifer. Changes in pH and [DIC] due to photosynthesis affect the isotopic composition of the available carbonate (Wolf-Gladrow et al., 1999). However, diffusion of ions to the site of calcification without at least one additional mitigating mechanism, cannot account for the difference between seawater metal composition and Me/Ca ratios in foraminiferal calcite (Figure 1 and references in its caption).

Figure 3: Examples of possible involvement of internal reservoirs versus externally derived ions for calcification. A: Ca^{2+} and DIC are derived from internal reservoirs. B: Ca^{2+} and DIC are transported to the site of calcification without uptake and storage into reservoirs. C: DIC

is taken up directly and Ca^{2+} comes from an internal reservoir. D: Ca^{2+} is taken up during chamber formation and DIC is derived from an intracellular reservoir.

Ca^{2+} and DIC may be actively transported (through transmembrane pumps and/ or channels) to the site of calcification. Although such transport mechanisms are not yet identified in planktonic foraminifera, a number of studies support the existence of this mechanism in benthic species. Using radioactive labeling, Angell (1979) showed that the ions for calcification are taken up *during* chamber formation in the benthic species *Rosalina floridana*. Although this observation does not prove the absence of an internal reservoir *per se*, this observation reduces the turnover rate and/or size of such a reservoir considerably. Similarly, Lea et al. (1995) showed that the intracellular Ca-reservoir in the planktonic foraminifer *O. universa* is very small and/or has a fast turnover rate and does not significantly contribute to the total amount of Ca^{2+} during shell thickening. Results from the benthic *Ammonia* sp. show that intracellular vesicles containing elevated concentrations of Ca^{2+} are involved in chamber formation (Toyofuku et al., 2008), but that their amount within the cell is not sufficient for the production of a new chamber (De Nooijer et al., 2009b). Together, these studies suggest that the majority of the Ca^{2+} utilized for shell calcification is not stored in intracellular reservoirs prior to chamber formation in the species studied. If the internal reservoir refills after chamber formation within a relatively short period of time, it is critical that seawater labeling experiments should start directly after a chamber formation event to avoid underestimation of the true reservoir size. Studies addressing the issue of an intracellular reservoir are summarized in Table 1.

Table 1: Studies discussing internal reservoirs in perforate foraminifera.

3. Intracellular transport

3.1 Transmembrane ion transport

Due to the hydrophobic inner layer of cell membranes, molecules cannot freely move into or out of the cell's interior. Although the majority of ions and molecules diffuse across cell membranes, diffusion constants vary greatly. Small, uncharged molecules (CO_2, O_2, NO) diffuse easily down a concentration gradient whereas large molecules and ions require specialized transmembrane proteins to facilitate or energize membrane transport (Higgins, 1992). These transporter proteins can be divided into channels, carriers and pumps (Figure 4). Carrier proteins undergo substrate binding and transport. They show typical substrate affinities and follow Michaelis-Menten kinetics. Carrier transport is even effective against concentration gradients if a cosubstrate with a respective concentration gradient or charge is involved (secondary active transport). Pumps directly generate this energy for uphill transport from their ATPase activity. Transmembrane channels simply allow facilitated diffusion along electrochemical gradients by creating a selective pore through the cell membrane. For the uptake of inorganic carbon by foraminifera during calcification, a strong pH gradient (high inside; De Nooijer et al., 2009a; Bentov et al., 2009; low outside; Glas et al., 2012) may promote the influx of CO_2 and thus circumvent the need for specialized transmembrane proteins.

Figure 4: selective ion transporters. Ion pumps (left and middle) undergo structural changes that allow passage of ions from and to the binding sites. The example shown here is a simplified Na^+/K^+ exchanger that has specifically binds to Na-ions (blue squares) when in the first configurational state (left). After the structural change, affinity of the Na-binding sites decreases so that the Na-ions are released (middle). At the same time, K-ions (yellow circles)

bind to their binding sites after which the pump returns to state one and releases the K^+ to the cytosol. Ion channels (draw after the KcsA K^+ channel; right) consist usually of a narrow pore allowing certain ions to pass a cell membrane down the electro-chemical gradient. Another feature of some pumps and channels is the relatively large cavity that is created by the transmembrane protein-complex (here present in the cytosol-side of the channel). This can greatly reduce the distance that the ions have to be transported. The type of Ca^{2+}-transporters that are used by foraminifera are unknown, but determining their molecular structure is necessary to 1) know the extent of de-hydration during transport, 2) determine the rate of ion transport and 3) explain the selectivity for Ca^{2+}/ against other ions (e.g. Mg^{2+}) and their fractionation (e.g. Gussone et al., 2003).

3.2 Ca^{2+} transport in foraminifera

In foraminifera, most attention has been directed at ion transporters that might be responsible for the low Mg/Ca at the site of calcification. Logically, this may involve Mg^{2+}-transporters and/ or Ca^{2+} transporters. Because Ca^{2+} acts as a secondary messenger in most eukaryotic cells, cytosolic Ca^{2+} is kept low (< 1µM) by active removal out of the cell or into cytosolic compartments (ER, mitochondria). This makes Ca^{2+}-transporters one of the most ubiquitous and well-studied transmembrane ion transporters. From a variety of cell types, Ca^{2+}-ATPases, Ca^{2+}/H^+ and Ca^{2+}/Na^+ antiporters (e.g. Gonçalves et al. 1998) and Ca^{2+}/phosphate co-transporters (Ambudkar et al., 1984) have been described. Depending on the transporter's structure, ions may pass the membrane either with or without their hydration sphere (Gouaux and MacKinnon, 2005), although (partial) dehydration increases the selectivity greatly (see also Gussone et al., 2003).

The specificity of the transmembrane Ca-transporters varies greatly. For some Ca^{2+}/H^+-antiporters it has been reported that other cations with a small ionic radius (e.g. Zn^{2+}) can be transported in a similar way as Ca^{2+} is transported (Gonçalves et al., 1999). For the same antiporter, the larger Ba^{2+} and Cs^{+} do not substitute for Ca^{2+}. An ion with intermediate size, Sr^{2+} (1.13 Å, compared to 0.99 Å for Ca^{2+}), appears to block the antiport and prevents transport of Ca^{2+} through the membrane. Studies concerning specificity for Ca^{2+} over Mg^{2+} are scarce, but some Ca-ATPases have been reported to have a 10^{3}-10^{5} higher affinity for Ca^{2+} than for Mg^{2+} (Drake et al., 1996; Xiang et al., 2007).

In corals, calcium uptake is directly related to proton pumping (McConnaughey and Whelan, 1997; Sinclair and Risk, 2006). The efflux of H^{+} during calcification (Glas et al., 2012) may therefore help to constrain estimates of calcium pumping rates during calcification. Carbon dioxide uptake and proton efflux are also directly related in cyanobacteria (Ogawa and Kaplan, 1987). Ter Kuile et al. (1989b) suggested that Ca^{2+} is taken up by Ca^{2+}-ATPase and this mechanism was subsequently used by Zeebe and Sanyal (2002) and Zeebe et al. (2008) to show that H^{+} removal is far more energy-efficient than Mg^{2+}-removal during calcification. Such a mechanism would be consistent with a coupling of ion transporters (e.g. Ca^{2+} and H^{+}) during foraminifera calcification.

The amount of Ca^{2+} transported across a membrane depends on 1) transporter density in the membrane, 2) affinity for Ca^{2+} of the transporter and 3) the capacity of the transporter. For example, the Na^{+}/Ca^{2+} exchanger has a low affinity, but high capacity, resulting in transport of up to 5,000 ions per second (Carafoli et al., 2001). Such a transporter is useful when Ca^{2+} is present in high concentrations (e.g. as in seawater) and supply or removal rates of Ca^{2+} have to be high. Cell membrane calcium pumps, on the other hand have a high affinity, but low capacity, making it particularly suitable for transporting Ca^{2+} out of a medium or compartment with a low [Ca^{2+}] (Wang et al., 1992). Finally, transport rates can be affected by the presence of inhibitors, high intracellular [Ca^{2+}] (e.g. Pereira et al., 1993) or shortage of ATP (in case of e.g. Ca^{2+}-ATPase).

3.3 Inorganic carbon transport in foraminifera

Transport of inorganic carbon may be accomplished by bicarbonate-transporters. If seawater or metabolic CO_2 contributes to the inorganic carbon during calcification, diffusion rates across membranes would control the influx of inorganic carbon and thereby influence the rate of calcification. The diffusion rate is determined by the concentration gradient of CO_2, the membrane area over which CO_2 can diffuse, and the solubility of CO_2 in the membrane lipids. The concentration of CO_2 at the site of calcification or in internal reservoirs is determined by pH. Since foraminifera can control the pH in these compartments (Erez, 2003; Bentov et al., 2009; De Nooijer et al., 2009a; Glas et al., 2012), they can produce large CO_2 concentration gradients and hence promote the influx of DIC to the sites of calcification. The flux of ions can also be calculated from calcification rates, which is discussed in section 4.

In case of intracellular storage of ions, calcium and DIC are unlikely to be stored as free ions. Because the cytosol has very low concentrations of free Ca^{2+} and DIC, the cell volume will control the number of ions available for calcification. For the DIC-reservoir (if present) the additional problem is that CO_2 can easily diffuse across cell membranes and subsequent re-equilibration would thus result in net leakage of carbon out of the DIC-reservoir. To overcome this problem, DIC must be sequestered by mechanisms such as elevating the pH in the reservoir. Because there are usually no crystallites visible within the cells of hyaline species, Ca and DIC are likely sequestered together as non-crystalline $CaCO_3$ (i.e. amorphous calcium carbonate or ACC). Such a possibility may have consequences for the minor and trace element composition of the calcite precipitated, since it is known that formation of high-Mg calcite is accompanied by the formation of an amorphous precursor phase (Raz et al., 2000).

Regardless of the process concentrating Ca^{2+} and DIC from seawater, each would produce a supersaturated solution at the site of calcification, with reduced levels of crystal inhibitors that occur naturally in seawater (e.g. Mg^{2+} and PO_4^{2-}). The Ca^{2+} and CO_3^{2-} may form spontaneous $CaCO_3$ crystals, but the specific morphology of foraminiferal chambers show that nucleation and crystal growth is a tightly controlled process.

4. Nucleation of calcification

4.1 Crystal nucleation energy and critical size

Precipitation of a crystal from a solution occurs when free energy of the precipitate is lower than that of the solution. Nucleation of a crystal requires even more energy since ions at the surface of a crystal add to the free energy of the solid phase. This is caused by the fact that ions at the surface of a crystal are not bound on all sides to other ions. The resulting 'interfacial energy' requires the formation of metastable clusters of a critical size to start crystal growth (Figure 5). The interfacial free energy between the cluster and a solution is usually larger than that between the cluster and a solid substrate, resulting in crystal nucleation at solid surfaces rather than within the solution itself (De Yoreo and Vekilov, 2003). If the atomic structure of a substrate matches a particular plane of the nucleating phase (e.g. calcite or aragonite), the interfacial free energy is reduced and nucleation is promoted (De Yoreo and Vekilov, 2003).

In the case of nucleation of $CaCO_3$, presence of negatively charged groups at regular intervals at the site of calcification may be able to bind Ca^{2+} and pre-form a part of the $CaCO_3$ lattice.

Figure 5: relation between free energy changes (Δg) as a function of pre-nucleation sphere (r), where Δg_s is the surface term and Δg_b the bulk term. The sum of Δg_s and Δg_b is the free

energy barrier that can only be overcome by the formation of a nucleation sphere with a critical size (r_c). Biological control over crystal nucleation is often aimed at lowering of this energy barrier and can be achieved by increasing the concentrations of the solutes or the presence of an organic template.

4.2 Organic templates and nucleation of $CaCO_3$ in foraminifera

During biomineralization in foraminifera calcium carbonate nucleates at the site of calcification, likely involving an organic template. In all Rotaliid foraminifera, chamber formation starts with delineation of a finite environment that encompasses an inner chamber volume from the surrounding medium (Angell, 1979; Bé et al., 1979; Hemleben et al., 1986; Spero, 1988; Wetmore, 1999). Cytoplasmic activity by formation of a dense pseudopodial network transports vacuoles, mitochondria and organic particles to a defined zone in which the so-called Organic Primary Envelope, Primary Organic Lining, Anlage or Primary Organic Membrane (POM) is formed (e.g. Banner et al., 1973; Hemleben et al., 1977; Spero, 1988; not to be confused with inner and outer organic linings, nor with the outer protective envelope or cytoplasmic envelope: see section 4). The term POM is often used but may be confusing (Erez, 2003) since these organic templates are not technically membranes. Therefore, we recommend following the suggestion of Erez (2003) to rename the POM as the Primary Organic Sheet (POS). In a number of benthic species, the POS consists of unbranched polysaccharides such as glycosaminoglycans (Hottinger and Dreher, 1974; Langer, 1992). Proteins are also present in the organic lining of foraminifera, sometimes forming different classes based on their amino acid composition (Robbins and Brew, 1990). King and Hare (1972) showed that amino acids make up 0.02-0.04% of the weight of the calcite and that composition among planktonic species varies greatly. Interestingly, the largest compositional difference coincides with the planktonic foraminifera spinose/ non-spinose divide (King and

Hare, 1972), but differences in amino acid composition are also manifest at lower taxonomic levels (Robbins and Healy-Willliams, 1991).

The organic matrix of the benthic *Heterostegina depressa* is shown to contain an EDTA-soluble and -insoluble fraction (Weiner and Erez, 1984). The insoluble fraction contains over-sulphated glycosaminoglycans and a small portion of non-polar proteins, forming the inner organic lining. The soluble fraction contains a number of proteins containing amino acids with acidic residues. Polar groups in both fractions may be involved in biomineralization since they may bind Ca^{2+} ions and thereby overcome the free energy barrier (Figure 5). If such groups are regularly spaced, they may help nucleation further by placing the Ca^{2+} ions in a regular grid with just enough space for the CO_3^{2-} ions to fit in between them. To test this hypothesis, the tertiary structures of the biomolecules (e.g. proteins and saccharides) that are involved in $CaCO_3$ nucleation need to be analyzed.

The presence of polysaccharides and proteins has led to the hypothesis that the POS has two functions in the process of calcification. The carbohydrates may form a structure determining the overall shape of the new chamber. The proteins associated with the polysaccharides, on the other hand, form the 'active' part of the POS by providing charged sites for nucleation of $CaCO_3$ (Towe and Cifelli, 1967). Since the chemical composition of the POS varies between species (Banner et al., 1973), its role in nucleation of calcium carbonate may differ between foraminiferal species (Bé et al., 1979; Hemleben et al., 1986; Spero, 1988; Wetmore, 1999). In some benthic species, the POS coincides with the location of the pores prior to calcification (Wetmore, 1999), suggesting that there are structural differences in the POS within a single chamber that determine where calcite does and does not nucleate. In planktonic species such as *Globorotalia truncatulinoides* and *G. hirsuta*, calcification begins in small nucleation zones at finite locations across the POS, where calcite forms centers of crystal growth that interlock to form the initial calcified chamber (Towe and Cifelli, 1967; Angell, 1979; Bé et al., 1979;

Hemleben et al., 1986). A similar pattern has been observed in *Orbulina universa*, where small islands of calcite form on the POS, followed by calcite island fusion to produce the spherical chamber (Spero, 1988).

Nucleation (and subsequent crystal growth) is also determined by the physico-chemical conditions at the site of calcification. These conditions are only partly known in benthic species (e.g. Erez, 2003; Bentov and Erez, 2005) and have only been modeled in planktonic species (Zeebe et al., 1999; Zeebe and Sanyal, 2002). The volume between the crystal surface and the shielding cytoplasmic envelope or pseudopodial network is extremely small, limiting interpretation from light microscopic observations. However, TEM images of initial calcification in *Orbulina universa* and other planktonic species suggests the privileged space between rhizopodia and calcifying surfaces may be <10 nm (Bé et al 1979; Spero 1988). Little is known about the chemical composition of the fluid from which $CaCO_3$ nucleates, but high concentrations of Ca^{2+} and CO_3^{2-} need to be actively maintained, while the $[Mg^{2+}]$ needs to be reduced to satisfy observations and ensure calcification (Zeebe and Sanyal, 2002). Elevated pH at the site of calcification would promote the conversion of CO_2 and HCO_3^- to CO_3^{2-}, thereby enhancing $CaCO_3$ nucleation and growth. Elevated concentrations of Mg^{2+} around the POS in *Pulleniatina obliquiloculata* (Kunioka et al., 2006) may indicate that in this species, the composition of the calcifying fluid is different during the first stage of chamber formation, possibly due to a different rate or efficiency of the process that locally reduces $[Mg^{2+}]$ vs $[Ca^{2+}]$. The participation of a small volume of seawater at the beginning of chamber formation may explain the elevated Mg in the first calcite precipitated, although this pattern does not hold for other planktonic species (e.g. such as *Orbulina universa*; Eggins et al., 2004) where the lowest Mg/Ca ratios are associated with the intrashell zone that corresponds to the POS. The above observations of inter species differences in chamber wall elemental composition underscore the need to unravel the mechanisms controlling test calcification.

5. Chamber growth

After initial crystal nucleation, calcification proceeds by addition of calcite on both sides of the POS. Additional layers of $CaCO_3$ are added on top of pre-existing chamber calcite during each chamber formation event in perforate foraminifera (Reiss, 1957; 1960; Bé and Hemleben, 1970; Erez, 2003). Together, the primary and secondary layers of calcite are termed 'lamellar' calcite (Erez, 2003). Most observations on calcification are based on the first stage of chamber formation in which a thin-walled chamber is produced within 1-3 hours (Spero, 1988). Subsequent thickening of the chamber wall proceeds during the next 24-48 hours until a new chamber is formed. Thickening of earlier formed chambers occurs by addition of a calcite layer with each new chamber formation event (e.g. Bentov and Erez 2005, Nehrke et al., 2013). Future studies will need to show whether the timing of the start and end of chamber formation and thickening of previously formed chambers are coincidental, or whether thickening is a continuous process.

Future biomineralization research should also take into account the possibility that cellular controls on calcification may vary over time and location across the foraminifera shell. An example of the potential complexity and diversity of calcification within one specimen is provided by Bentov and Erez (2005). Their research demonstrated that the benthic *Amphistegina lobifera* recovering individuals produce at least three types of calcium carbonate: elongated, intracellular birefringent granules with a high magnesium and phosphorus content, extracellular microspheres with a high Mg concentration and extracellular spherulites with a low Mg content. These spherulites represent the lamellar calcite while the microspherulites represent the initial presipitation over the POS in *A. lobifera.*

During chamber formation, ions could be supplied to the site of calcification (SOC) from internal reservoirs (Figure 3, Table 1) or by transport from the surrounding seawater. The latter can be accomplished by transmembrane ion transporters (section 2), by direct exchange of the calcifying fluid with seawater and/ or by diffusion from ambient seawater. The inner and outer surfaces of newly formed chambers of the benthic *Heterostegina depressa* are covered by thin layer of cytoplasm (Spindler, 1978), suggesting the SOC may be separated from the surrounding medium. In a number of studies (Angell, 1979; Bé et al., 1979), a fan-like arrangement of the pseudopodial network is observed in a zone outside the site of calcification. Although the relation between this arrangement and calcification remains to be investigated, it is likely to play a role in biomineralization since this dense network is not observed between chamber formation events. Also in the planktonic species *G. hirsuta* and *G. truncatulinoides*, calcification proceeds adjacent to a cytoplasmatic envelope (or outer protective envelope) that may play a role in maintaining SOC integrity and shape, and promoting initial calcification (Bé et al., 1979). In the benthic *Ammonia* sp., a pH gradient of >2 pH units is observed across several μm distance and is maintained for hours between the site of calcification (De Nooijer et al., 2009a) and the specimen's microenvironment (Glas et al., 2012). These observations suggest that in *Ammonia* sp., the SOC is separated from the outside environment. Spero (1988) on the other hand, presented transmission electron micrographs that showed the site of calcification in *O. universa* is not shielded by a continuous membrane. Nehrke et al. (2013) recently suggested that the site of calcification in *Ammonia aomoriensis* is largely closed from the surrounding medium, but that a small percentage of the fluid at the SOC is derived from leakage of the cell membranes separating it from the outside medium, explaining observed Mg/Ca for the species studied.

The extent to which the site of calcification is open or closed, in combination with the presence or absence of intracellular ion reservoirs, is an important unknown in understanding foraminiferal calcification (Figure 6). For example, a site of calcification that is physically separated from the surrounding seawater, together with the absence of intracellular ion reservoirs, prescribes the need for transmembrane ion transporters (e.g. Ca^{2+}-APTase; section II) that selectively pump ions from seawater to the SOC. A SOC that is open, on the other hand, will experience relatively high concentrations of Mg and require an active Mg^{2+}-removal mechanism.

Figure 6: summary of the most important parts of the calcification mechanism in foraminifera, including Ca-ion transport, active Mg-removal and contribution from internal reservoirs. See text for description of the individual processes.

Potential ion transport pathways to the site of calcification can be constrained from calcification rates during chamber formation. It is important to distinguish between the overall growth rate of a foraminifer and calcite precipitation rate during biomineralization. The difference between these processes results from the episodic nature of growth (chamber addition) in foraminifera. Some planktonic species have been reported to increase the weight of their shell by 13-15% a day (*G. sacculifer*; Erez, 1983), but this may vary with environmental conditions (Ter Kuile and Erez, 1984 and references therein). Secondly, chamber addition rates vary over a foraminifer's lifetime, decreasing as the individual ages (Ter Kuile and Erez, 1984). Calcite precipitation rates during chamber addition, on the other hand, are much higher and vary between 0.4-0.9 µg/h in the planktonic foraminifer *G. sacculifer* (Anderson and Faber, 1984), 0.06-0.32 µg/h in *O. universa* (Lea et al., 1995) and ~10 µg/h in the benthic *A. tepida* (De Nooijer et al., 2009b). Since such rates are rarely quantified, it is difficult to generalize these values to other species or other conditions. Moreover, calcite precipitation rates can be variable between day and night calcification periods (Erez, 1983; Spero, 1988; Lea et al., 1995). Since incorporation of some elements may depend on precipitation rate (e.g. DePaolo, 2011), it is necessary to quantify these rates across a diurnal time frame when chamber formation is occurring in order to assess the kinetics of element incorporation and thereby proxy-relationships.

Mitochondrial activity may play an important role at the site of calcification and thereby affect trace element incorporation. Besides providing energy, mitochondria pump cytosolic Ca^{2+} and Mg^{2+}, and therefore modulate the cell's [Ca^{2+}] and [Mg^{2+}] (Carafoli et al., 2001). This may be particularly important during calcification when the concentration of these ions increases locally. Spero (1988) shows that calcification in *O. universa* around the POS is associated with pseudopodia containing mitochondria, and hence possibly modulate [Mg^{2+}] at the SOC. Similar results can be found in Bé et al (1979) for *Globorotalia truncatulinoides*. Bentov et al (2009) discuss the possible role of mitochondria in producing metabolic CO_2 that eventually accumulate in the alkaline vacuoles as DIC.

Photosynthesis by symbionts may also affect calcification rates. The relative concentrations of DIC species are influenced by symbiont photosynthesis and CO_2-uptake during the day (or release in the dark) and the resulting diurnal differences in microenvironment pH (Jørgensen et al., 1985; Rink et al., 1998; Köhler-Rink and Kühl, 2000; 2005), thereby influencing uptake and availability of inorganic carbon species. In some large benthic foraminifera (Wetmore, 1999), the symbionts are positioned near the POS prior to calcification, suggesting that their activity could enhance calcification. Elimination of symbionts in *G. sacculifer* resulted in reduced chamber formation rates and early gametogenesis or death of the foraminifera (Bé et al., 1982). Reseeding the aposymbiotic foraminifera with symbionts from donor specimens produced individuals that continued to add chambers and mature at a normal rate. These data suggest that symbiont photosynthesis is critical to both nutrition and chamber calcification. Elevated light intensity promotes growth in *G. sacculifer* (Caron et al., 1982) but not in the benthic foraminifera *Amphistegina lobifera* in which both photosynthesis and calcification are optimal at relatively low light intensities that are found at 20-30 m water depth (Erez 1978, Ter Kuile and Erez, 1984).

Ter Kuile et al. (1989a), on the other hand, suggested that symbionts and foraminifera compete for inorganic carbon. Erez (1983) and Ter Kuile et al. (1989b) showed that inhibition of photosynthesis in both planktonic and benthic species by the photosystem II inhibitor DCMU, does not affect calcification rates and suggested that it is not photosynthesis itself, but rather light which directly promotes calcification. Finally, Ter Kuile et al (1989a) have shown that there is competition for CO_2 between the symbionts and their host in the benthic foraminiferan *A. lobifera*. Clearly, the relationship between symbioses and foraminifera calcification requires additional study.

Pore formation provides important information on foraminiferal biomineralization. In species producing macropores, we observe a pore plate that is continuous with the POS and separates the cytoplasm from the outside medium (Hemleben et al., 1977). In benthic, symbiont-bearing species, symbionts can be found in close proximity to the pores (e.g. Lee and Anderson, 1991) suggesting that respiratory gases such as CO_2 and O_2 may be able to diffuse through the pore plates. In symbiont-barren species, diffusion of gases between cytoplasm and environment could be enhanced by the permeability of a pore plate. Some have suggested that dissolved organic matter may be taken up through the pores in the benthic *Patellina* (Berthold, 1976). In *G. sacculifer*, pseudopodia appear to penetrate through the pore plates (Anderson and Bé, 1976). Pores in the benthic species *Patellina corrugata* have been reported to exist from the beginning of chamber formation (Berthold, 1976) and pores are observed in the *O. universa* sphere once initial calcification has locked in the spherical morphology of the chamber

(Spero, 1988). Some species of planktonic foraminifera have micro- instead of macropores (often in species with secondary apertures; *Globigerinata glutinata*, *Candeina nitida*), ranging from 0.3-0.7 μm (Brummer and Kroon, 1988). These micropores do not appear to have a pore plate, and their function, formation and morphology is less well understood than those for macropores.

6. Overgrowth and encrusting

The primary and secondary layers of calcite in perforate foraminifera are together referred to as 'ontogenetic' or 'lamellar' calcite (Erez, 2003). Additional $CaCO_3$ can be present as ornamentations (pustules, spines, ridges, tooth plates, etc.) or as layers of calcite covering the whole test (crust or gametogenic (GAM) calcite). Whereas ornamentation is present throughout the entire life cycle of a foraminifer (Hemleben, 1975), GAM calcite is exclusive to planktonic foraminifera and is added after the last chamber is formed and just prior to meiotic division of the nucleus and gametogenesis.

In some planktonic species, a calcite crust can be formed after formation of the final chamber (Bé and Ericson, 1963; Bé and Lott, 1964; Bé, 1965; Bé and Hemleben, 1970; Olsson, 1976). The morphology of this calcite is markedly different from that of either ontogenetic or GAM calcite and its element and isotopic composition can differ from that of the ontogenetic calcite because it forms under different environmental conditions of temperature and/or salinity. For instance, crust Mg/Ca is generally lower than that of ontogenetic calcite in *Globorotalia truncatulinoides* (Duckworth, 1977) and *Neogloboquadrina dutertrei* (Jonkers et al., 2012). These lower element concentrations are partly a consequence of conditions deeper in the water column (i.e. lower temperature), but it should be noticed that the observed partitioning for Mg indicates that crust calcification is a biologically controlled process. Interestingly, Nürnberg et al. (1996) found that crusts formed in culture can have a higher Mg/Ca than the ontogenetic calcite.

In a number of species such as *G. sacculifer*, gametogenesis is preceded by the production of a layer of calcite covering spine holes and the terrace-like structures of inter-pore rims (Towe and Cifelli, 1967; Bé, 1980; Hemleben et al., 1985; Brummer et al., 1987). This GAM calcite veneer gives the foraminifera a smooth appearance by covering the rough topography of the shell surface and it has been suggested that it is enriched in some trace elements compared to the ontogenetic calcite (Hathorne et al., 2009). Whether this observation holds for all foraminifera forming GAM calcite, however, remains to be investigated.

From the perspective of biomineralization, variability in the types of $CaCO_3$ that are formed may indicate that foraminifera do not have one single way to produce shell calcite. Rather, the physiological tools to achieve calcite precipitation as discussed in sections 2 and 4, are likely used in different combinations by different species of foraminifera. Moreover, the variability in calcite within single specimens suggests a degree of flexibility of these physiological tools even within single species. Identification of seawater vacuolization, transmembrane ion transport, nucleation promoting organic templates, etc. across species and their contribution to calcification within a foraminifer's life time are critical aspects of foraminiferal biology and keys to understanding foraminiferal biomineralization from a mechanistic perspective.

7. Future directions

A complete mechanistic description of foraminiferal biomineralization and chamber formation does not yet exist. Hence, the biological and environmental interplay that controls the element composition and isotope fractionation of chamber calcite is only partly understood. Literature on foraminiferal calcification is both qualitative and quantitative but on occasion, contradictory. This leaves us with a number of outstanding questions that need to be addressed in order to move this area of foraminifera biology forward. These include:

1. Which foraminiferal species use vacuolized seawater as the primary source for calcification and which use transmembrane transport of Ca^{2+} and DIC during calcification? The investigation into the transport of ions to the site of calcification may be solved by answering a number of more practical questions, including:
   - What is the relation between transmembrane transport and vaculization on the one hand, and production of intracellular calcium and/ or carbon reservoirs on the other hand?
   - What is the biochemical basis of these processes? Which transmembrane transporters are involved (e.g. Ca-ATPases, proton-Ca^{2+} antiporters)? By which mechanism is inorganic carbon concentrated (e.g. involvement of Carbonic Anhydrase)?
   - When characterized, can these (transport) mechanisms explain observed element incorporation and isotopes fractionations. If yes, can these mechanisms explain foraminiferal chemistry for (all) these elements and isotopes *at the same time*?
   - Is there a general difference between planktonic and benthic species in production of vacuolized seawater, internal reservoirs and/or direct ion transport?
   - Do foraminifera employ both mechanisms to calcify and if yes, what is the balance between these two pathways?
2. What is the tertiary structure of the organic matrix/ matrices (e.g. POS, organic linings) involved in biomineralization? Which compounds help to lower the free energy barrier, thereby promoting calcite nucleation? When identified, do these organic compounds have an impact on the partition coefficient of elements and fractionation of isotopes at the first stage of chamber formation?

3. To what extent is the site of calcification in contact with surrounding seawater? If seawater directly contributes (part of) the ions for calcification, can this source explain observed fractionation factors and partition coefficients?
4. What is the role of mitochondria in calcification? Do mitochondria (help to) regulate the Mg/Ca at the site of calcification?

Finally, a more detailed understanding of foraminiferal biominiralization will also allow researchers to compare calcification strategies accross marine calcifiers. Compared to foraminifera, biomineralization in corals (Al-Horani et al., 2003; Sinclair and Risk, 2006; Venn et al., 2013), coccolithophores (Marsh, 2003; Taylor et al., 2011; Ziveri et al., 2012; Bach et al., 2013), gastropods (e.g. Nehrke et al., 2011) and bivalves (Nudelman et al., 2006; Nehrke et al., 2012; Shi et al., 2013) are understood in greater detail. Identification of differences and similarities between these marine calcifying taxa will allow studying (convergent) evolutionairy patterns, help to understand differences in their response to (future) environmental perturbations and facilitate comparison of paleoceanographic information obtained across taxa.

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References

Al-Horani, F.A., Al-Moghrabi, S.M., De Beer, D., 2003. Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. J. Exp. Mar. Biol. Ecol. 288, 1-15.

Allen, K.A., Hönisch, B., Eggins, S.M., Yu, J., Spero, H.J., Elderfield, H., 2011. Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina universa*. Earth Planet. Sci. Lett. 309, 291-301.

Ambudkar, S.V., Zlotnick, G.W., Rosen, B.P., 1984. Calcium efflux from *Escherichia coli*. Evidence for two systems. Journal of Biol. Chem. 259, 6142-6146.

Anderson, O.R., Bé, A.W.H., 1976. The ultrastructure of a planktonic foraminifer, *Globigerinoides sacculifer* (Brady), and its symbiotic dinoflagellates. J. Foramin. Res. 6, 1-21.

Anderson, O.R., Faber, W.W. Jr, 1984. An estimation of calcium carbonate deposition rate in a planktic foraminifer *Globigerinoides sacculifer* using ^{45}Ca as a tracer: a recommended procedure for improved accuracy. J. Foramin. Res. 14, 303-308.

Angell, R.W., 1979. Calcification during chamber development in *Rosalina floridana*. J. Foramin. Res. 9, 341-353.

Bach, L.T., Mackinder, L.C.M., Schultz, K.G., Wheeler, G., Schroeder, D.C., Brownlee, C., Riebesell, U., 2013. Dissecting the impact of CO_2 and pH on the mechanisms of photosynthesis and calcification in the coccolithophore *Emiliania huxleyi*. New Phytol. 199, 121-134.

Banner, F.T., Sheehan, R., Williams, E., 1973. The organic skeletons of rotaline foraminifera: A review. J. Foramin. Res. 3, 30-42.

Bé, A.W.H., Ericson, D.B., 1963. Aspects of calcification in planktonic foraminifera (Sarcodina). New York Acad. Sci., Ann., vol. 109, art. 1, pp. 65-81.

Bé, A.W.H., Lott, L., 1964. Shell growth and structure of planktonic foraminifera. Science 145, 823-824.

Bé, A.W.H., 1965. The influence of depth on shell growth in *Globigerinoides sacculifer* (Brady). Micropaleontology 11, 81-97.

Bé, A.W.H., 1980. Gametogenic calcification in a spinose planktonic foraminifer, *Globigerinoides sacculifer* (Brady). Mar. Micropaleontol. 5, 283-310.

Bé, A.W.H., Hemleben, C., 1970. Calcification in a living planktonic foraminifer, Globigerinoides sacculifer (Brady). N. Jb. Geol. Paläont. Abh. 134, 221-234.

Bé, A.W.H., Hemleben, C., Anderson, O.R., Spindler, M., Hacunda, J., Tunitivate-Choy, S., 1977. Laboratory and field observation of living planktonic foraminifera. Micropaleontology 23, 155-179.

Bé, A.W.H., Hemleben, C., Anderson, O.R., Spindler, M., 1979. Chamber formation in planktic foraminifera. Micropaleontology 25, 294-307.

Bé, A.W.H., Spero, H.J., Anderson, O.R., 1982. Effects of symbiont elimination and reinfection on the life processes of the planktonic foraminifer *Globigerinoides sacculifer*. Mar. Biol, 70, 73-86.

Bender, M.L., Lorens, R.B., Williams, D.F., 1975. Sodium, magnesium and strontium in the tests of planktonic foraminifera. Micropaleontology 21, 448-459.

Bentov, S., Erez, J., 2005. Novel observations on biomineralization processes in foraminifera and implications for Mg/Ca ratio in the shells. Geology 33, 841-844.

Bentov, S., Erez, J., 2006. Impact of biomineralization process on the Mg content of foraminiferal shells: A biological perspective. Geochem. Geophy. Geosy. 7, doi:10.1029/2005GC001015.

Bentov, S., Brownlee, C., Erez, J., 2009. The role of seawater endocytosis in the biomineralization process in calcareous foraminifera. P. Natl. Acad. Sci. USA 106, 21500-21504.

Berthold, W.U., 1976. Ultrastructure and function of wall perforations in *Patellina corrugate* Williamson, Foraminifera. J. Foramin. Res. 6, 22-29.

Bijma, J., Spero, H.J., Lea, D.W., 1999. Reassessing foraminiferal stable isotope geochemistry: Impact of the oceanic carbonate system (experimental results). Use of Proxies in Paleoceanography: Examples from the South Atlantic. G. Fischer and G. Wefer. Berlin, Heidelberg, Springer-Verlag: 489-512.

Boyle, E., 1981. Cadmium, zinc, copper, and barium in foraminiferal tests. Earth Planet. Sci. Lett. 53, 11-35.

Brummer, G.J., Hemleben, C., Spindler, M., 1987. Ontogeny of extant spinose planktonic foraminifera (Globigerinidae): a concept exemplified by *Globigerinoides sacculifer* (Brady) and *G. ruber* (D'Orbigny). Mar. Micropaleontol. 12, 357-381.

Brummer, G.J.A., Kroon, D., 1988. Planktonic foraminifers as tracers of ocean-climate history. Free University Press, Amsterdam, 346 pp.

Carafoli, E., Santella, L., Branca, D., Brini, M., 2001. Generation, control, and processing of cellular calcium signals. Crit. Rev. Biochem. Mol. 36, 107-260.

Caron, D.A., Bé, A.W.H., Anderson, O.R., 1982. Effects of variations in light intensity on life processes of the planktonic foraminifer *Globigerinoides sacculifer* in laboratory culture. J. Mar. Biol. Assoc. UK 62, 435-451.

De Nooijer, L.J., Toyofuku, T., Kitazato, H., 2009a. Foraminifera promote calcification by elevating their intracellular pH. P. Natl. Acad. Sci. USA 106, 15374-15378.

De Nooijer, L.J., Langer, G., Nehrke, G., Bijma, J., 2009b. Physiological controls on the seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*. Biogeosciences 6, 2669-2675.

De Yoreo, J.J., Velikov, P.G., 2003. Principles of crystal nucleation and growth. Rev. Mineral. Geochem. 54, 57-94.

DePaolo, D.J., 2011. Surface kinetic model for isotopic and trace element fractionation during precipitation of calcite from aqueous solution. Geochim. Cosmochim. Ac. 75, 1039-1056.

Dissard, D., Nehrke, G., Reichart, G.J., Bijma, J., 2010. Impact of seawater $p\mathrm{CO_2}$ on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminiferal calcite: results from culturing experiments with *Ammonia tepida*. Biogeosciences 7, 81-93.

Dove, P.M., De Yoreo, J.J., Weiner, S., 2003. Biomineralization: Reviews in Mineralogy and Geochemistry 54, Mineralogical Society of America. Washington DC, 381 pp.

Drake, S.K., Lee, K.L., Falke, J.J., 1996. Tuning the equilibrium ion affinity and selectivity of the EF-Hand calcium binding motif: Substitutions at the gateway position. Biochem 35, 6697-6705.

Duckworth, D.L., 1977. Magnesium concentration in the tests of the planktonic foraminifer *Globorotalia truncatulinoides*. J. Foramin. Res. 7, 304-312.

Dueñas-Bohórquez, A., Raitzsch, M., De Nooijer, L.J., Reichart, G.J., 2010. Independent impacts of calcium and carbonate ion concentration on Mg and Sr incorporation in cultured benthic foraminifera. Mar. Micropaleontol, 81, 122-130.

Eggins, S.M., Sadekov, A., 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 Planet. Sci. Lett. 225, 411-419.

Elderfield, H., Bertram, C.J., Erez, J., 1996. A biomineralization model for the incorporation of trace elements into foraminiferal calcium carbonate. Earth Planet. Sci. Lett. 142, 409-423.

Epstein, S., Buchsbaum, R., Lowenstam, H., Urey, H.C., 1951. Carbonate-water isotopic temperature scale. Bull. Geol. Soc. Am. 62, 417-426.

Erez, J., 1978. Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273, 199-202.

Erez, J., 1983. Calcification rates, photosynthesis and light in planktonic foraminifera. In: P. Westbroek, E.W. de Jong (eds), Biomineralization and biological metal accumulation. Reidel Publishing Company. 307-312.

Erez, J., 2003. The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. Rev. Mineral. Geochem. 54, 115-149.

Glas, M., Langer, G., Keul, N., 2012. Calcification acidifies the microenvironment of a benthic foraminifer (*Ammonia* sp.). J. Exp. Mar. Biol. Ecol. 424-425, 53-58.

Gouaux, E., MicKinnon, R., 2005. Principles of selective ion transport in channels and pumps. Science 310, 1461-1465.

Gonçalves, P.P., Meireles, S.M., Gravato, C., Vale, M.G.P., 1998. Ca^{2+}-H^{+}-antiport activity in synaptic vesicles isolated from sheep brain cortex. Neurosci. Lett. 247, 87-90.

Gonçalves, P.P., Meireles, S.M., Neves, P., Vales, M.G.P., 1999. Ion selectivity of the Ca^{2+}/H^{+} antiport in synaptic vesicles of sheep brain cortex. Mol. Brain Res. 67, 283-291.

Grossman, E.L., 1987. Stable isotopes in modern benthic foraminifera: A study of vital effect. J. Foramin. Res. 17, 48-61.

Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H.J., Lea, D.W., Bijma, J., Nägler, T.F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}Ca$) in inorganic aragonite and cultured planktonic foraminifera. Geochim. Cosmochim. Ac. 67, 1375-1382.

Hamilton, C.P., Spero, H.J., Bijma, J., Lea, D.W., 2008. Geochemical investigation of gametogenic calcite addition in the planktonic foraminifera *Orbulina universa*. Mar. Micropaleontol. 68, 256-267.

Hastings, D.W., Russell, A.D., Emerson, S.R., 1998. Foraminiferal magnesium in Globigerinoides sacculifer as a paleotemperature proxy. Paleoceanography 13, 161-169.

Hathorne, E.C., James, R.H., Lampitt, R.S., 2009. Environmental versus biomineralization controls on the intratest variation in the trace element composition of the planktonic foraminifera *G. inflata* and *G. scitula*. Paleoceanography 24, doi:10.1029/2009PA001742.

Hemleben, C., 1975. Spine and pustule relationships in some recent planktonic foraminifers. Micropaleontology 21, 334-341.

Hemleben, C., Bé, A.W.H., Anderson, O.R., Tuntivate, S., 1977. Test morphology, organic layers and chamber formation in the planktonic foraminifer *Globorotalia menardii* (d'Orbigny). J. Foramin. Res. 7, 1-25.

Hemleben, C., Spindler, M., Breitinger, I., Deuser, WG., 1985. Field and laboratory studies on the ontogeny and ecology of some Globorotaliid species from the Sargasso Sea off Bermuda. J. Foramin. Res. 15, 254-272.

Hemleben, C., Anderson, O.R., Berthold, W., Spindler, M., 1986. Calcification and chamber formation in Foraminifera - a brief overview. In: Leadbeater BS, Riding R (eds), The systematics association, special volume 30, 237-249.

Hemleben, C., M. Spindler, Anderson, O.R., 1989. Modern planktonic foraminifera. New York, Springer, 363 pp.

Hemleben, C., Mühlen, D., Olsson, R.K., Berggren, W.A., 1991. Surface texture and first occurrence of spines in planktonic foraminifera from the early Tertiary. Geologisches Jahrbuch A 128, 117-146.

Hemleben, C., Bijma, J., 1994. Foraminiferal population dynamics and stable carbon isotopes. NATO ASI series 117, 145-166.

Higgins, C.F., 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8, 67-113.

Hönisch, B., Allen, K.A., Lea, D.W., Spero, H.J., Eggins, S.M., Arbuszewski, J., deMenocal, P., Rosenthal, Y., Russell, A.D., Elderfield, H., 2013. The influence of salinity on Mg/Ca in planktic foraminifers - Evidence from cultures, core-top sediments and complementary $\delta^{18}O$. Geochim. Cosmochim. Acta 121, 196-213.

Hottinger, L., Dreher, D., 1974. Differentiation of protoplasm in Nummulitidae (Foraminifera) from Elat, Red Sea. Mar. Biol. 25, 41-61.

Ishikawa, M., Ichikuni, M., 1984. Uptake of sodium and potassium by calcite. Chem. Geol. 42, 137-146.

Jørgensen, B.B., Erez, J., Revsbech, N.P., Cohen, Y., 1985. Symbiotic photosynthesis in a planktonic foraminiferan *Globigerinoides sacculifer* (Brady), studied with microelectrodes. Limnol. Oceonogr. 30, 1253-1267.

Katz, A., 1973. The interaction of magnesium with calcite during crystal growth at 25-90ºC and one atmosphere. Geochim. Cosmochim. Ac. 37, 1563-1586.

Katz, M.E., Cramer, B.S., Franzese, A., Hönisch, B., Miller, K.G., Rosenthal, Y., Wright, J.D., 2010. Traditional and Emerging geochemical proxies in foraminifera. J. Foramin. Res. 40, 165-192.

King, K., Hare, P.E., 1972. Amino acid composition of the test as a taxonomic character of living and fossil planktonic foraminifera. Micropaleonontology 18, 285-293.

Kitano, Y., Okumura, M., Idogaki, M., 1975. Incorporation of sodium, chloride and sulfate with calcium carbonate. Geochem. J. 9, 75-84.

Kitazato, H., Bernhard J.M., 2014.Approaches to study living foraminifera: collection, maintainance and experimentation. Springer, 227 pp.

Köhler-Rink, S., Kühl, M., 2000. Microsensor studies of photosynthesis and respiration in larger symbiotic foraminifera. I The physico-chemical microenvironment of *Marginopora vertebralis*, *Amphistegina lobifera* and *Amphisorus hemprichii*. Mar. Biol. 137, 473-486.

Köhler-Rink, S., Kühl, M., 2005. The chemical microenvironment of the symbiotic planktonic foraminifer *Orbulina universa*. Mar. Biol. Res. 1, 68-78.

Kozdon, R., Ushikubo, T., Kita, N.T., Spicuzza, M., Valley, J.W., 2009. Intratest oxygen isotope variability in the planktonic foraminifer *N. pachyderma*: real vs. apparent vital effects by ion microprobe. Chem. Geol. 258, 327–337.

Kunioka, D., Shirai, K., Takahata, N., Sano, Y., Toyofuku, T., Ujiie, Y., 2006. Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata* test by using a NanoSIMS: Implication for the vital effect mechanism. Geochem. Geophy. Geosy. 7, doi: 10.1029/2006GC001280.

Langer, M.R., 1992. Biosynthesis of glycosaminoglycans in foraminifera: A review. Marine Micropaleontology 19, 245-255.

Lea, D.W., Boyle, E.A., 1991. Barium in planktonic foraminifera. Geochim. Cosmochim. Ac. 55, 3321-3331.

Lea, D.W., Spero, H.J., 1992. Experimental determination of barium uptake in shells of the planktonic foraminifera *Orbulina universa* at 22ºC. Geochim. Cosmochim. Ac. 56, 2673-2680.

Lea, D.W., Martin, P.A., Chan, D.A., Spero, H.J., 1995. Calcium uptake and calcification ratae in planktonic foraminifer *Orbulina universa*. J. Foramin. Res. 25, 14-23.

Lea, D.W., Mashoitta, T.A., Spero, H.J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. Geochim. Cosmochim. Ac. 63, 2369-2379.

Lea, D.W., Pak, D.K., Spero, H.J., 2000. Climate impact of late Quaternary equatorial Pacific sea surface temperature variations. Science 289, 1719-1724.

Lear, C.H., Elderfield, H., Wilson, P.A., 2000. Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca ratios in benthic foraminiferal calcite. Science 287, 269-272.

Lee, J.J., Anderson, O.R. (eds), 1991. Biology of Foraminifera, Academic Press, London, 368 pp.

Lehninger, A.L., Rossi, C.S., Greenawalt, J.W., 1963. Respiration-dependent accumulation of inorganic phosphate and Ca^{2+} by rat liver mitochondria. Biochem. Biophy. Res. Comm. 10, 444-448.

Lowenstam, H.A., Weiner, S., 1989. Biomineralization. Oxford University Press. pp xxx.

Mann, S., 1988. Molecular recognition in biomineralization. Nature 332, 119-124.

Marriott, C.S., Henderson, G.M., Belshaw, N.S., Tudhope, A.W., 2004. Temperature dependence of $\delta^{7}Li$, $\delta^{44}Ca$ and Li/Ca during growth of calcium carbonate. Earth Planet. Sci. Lett. 222, 615-624.

Marsh, M.E., 2003. Regulation of $CaCO_3$ formation in coccolithophores. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 236,743-754.

McConnaughey, T.A., Whelan, J.F., 1997. Calcification generates protons for nutrient and bicarbonate uptake. Earth-Sci. Rev. 42, 95-117.

McCrea, J.M., 1950. On the isotopic chemistry of carbonates and a paleotemperature scale. J. Chem. Phys. 18, 849-857.

Morse, J.W., Arvidson, R.S., Lüttge, A., 2007. Calcium carbonate formation and dissolution. Chem. Rev. 107, 342-381.

Morse, J.W., Bender, M.L., 1990. Partition coefficients in calcite: Examination of factors influencing the validity of experimental results and their application to natural systems. Chem. Geol. 82, 265-277.

Mucci, A., 1987. Influence of temperature on the composition of magnesian calcite overgrowths precipitated from seawater. Geochim. Cosmochim. Ac. 51, 1977-1984.

Nehrke, G., Nouet, J., 2011. Confocal Raman microscope mapping as a tool to describe different mineral phases at high spatial resolution within marine biogenic carbonates: a case study on *Nerita undata* (Gastropoda, Neritopsina). Biogeosciences 8, 3761-3769.

Nehrke, G., Poigner, H., Wilhelms-Dick, D., Brey, T., Abele, D., 2012. Coexistence of three calcium carbonate polymorphs in the shell of the Antarctic clam *Laternula elliptica*. Geochem. Geophys. Geosys. 13, Q05014.

Nehrke, G., Keul, N., Langer, G., De Nooijer,. L.J., Bijma, J., 2013. A new model for biomineralization and trace-element signatures of Foraminifera tests. Biogeosciences 10, 6759-6767.

Nudelman, F., Gotliv, B.A., Addadi, L., Weiner, S., 2006. Molusk shell formation: mapping the distribution of organic matrix components underlying a single aragonitic tablet in nacre. J. Struc. Biol. 153, 176-178.

Nürnberg, D., Bijma, J., Hemleben, C., 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperature. Geochim. Cosmochim. Ac. 60, 803-814.

Ogawa, T., Kaplan, A., 1987. The stoichiometry between CO_2 and H^+ fluxes involved in the transport of inorganic carbon in cyanobacteria. Plant Physiol. 83, 888-891.

Olsson, R.K., 1976. Wall structure, topography and crust of *Globigerina pachyderma* (Ehrenberg). Progress in Micropaleontology: 1-9.

Pereira, A.C., Samellas, D., Tiffert, T., Lew, V.L., 1993. Inhibition of the calcium pump by high cytosolic Ca^{2+} in intact human red blood cells. J. Physiol. 461, 63-73.

Pingitore, N.E., Eastman, M.P., 1984. The experimental partitioning of Ba^{2+} into calcite. Chem. Geol. 45, 113-120.

Raz, S., Weiner, S., Addadi, L., 2000. Formation of high-magnesian calcites via an amorphous precursor phase: possible biological implications. Adv. Mater. 12, 38-42.

Reiss, Z., 1957. The Bilamellidea, nov. superfam., and remarks on Creteceous globorotaliids. Cushman Foundation Foramin. Res. Contr. 8, 127-145.

Reiss, Z., 1960. Structure of so-called *Eponides* and some other Rotaliiform Foraminifera. Ministry of Development Geological Survey 29, 1-29.

Rickaby, R.E.M., Elderfield, H., 1999. Planktonic foraminiferal Cd/Ca: paleonutrients or paleotemperature? Paleoceanography 14, 293-303.

Rimstidt, D.J., Balog, A., Webb, J., 1998. Distribution of trace elements between carbonate minerals and aqueous solutions. Geochim. Cosmochim. Ac. 62, 1851–1863.

Rink, S., Kühl, M., Bijma, J., Spero, H.J., 1998. Microsensor studies of photosynthesis and respiration in the symbiotic foraminifer *Orbulina universa*. Mar. Biol. 131, 583-595.

Robbins, L.L., Brew, K., 1990. Proteins from the organic matrix of core-top and fossil planktonic foraminifera. Geochim. Cosmochim. Ac. 54, 2285-2292.

Robbins, L.L., Healy-Williams, N.H., 1991. Toward a classification of planktonic foraminifera based on biochemical, geochemical, and morphological criteria. J. Foramin. Res. 21, 159-167.

Saito, T., Thompson, P.R., Breger, D., 1976. Skeletal ultra-microstructure of some elongate-chambered planktonic Foraminifera and related species. In: Takayanagi Y, Saito T (eds), Selected papers in honor of Prof. Kiyoshi Asano. Progress in Micropaleontology Special Publication, 278-304.

Schmiedl, G., Mackensen, A., 2006. Multispecies stable isotopes of benthic foraminifers reveal past changes of organic matter decomposition and deep water oxygenation in the Arabian Sea. Paleoceanography 21, PA4213.

Segev, E., Erez, J., 2006. Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera. Geochem. Geophy. Geosy. 7: Q02P09.

Shi, Y., Yu, C., Gu, Z., Zhan, X., Wang, Y., Wang, A., 2013. Characterization of the pearl oyster (*Pinctada martensii*) mantle transcriptome unravels biomineralization genes. Mar. Biotechnol. 15, 175-187.

Sinclair, D., Risk, M., 2006. A numerical model of trace element coprecipitation in a physicochemical calcification system: Application to coral biomineralization and trace element vital effects. Geochim. Cosmochim. Ac. 70, 3855-3868.

Spero, H.J., 1988. Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. Mar. Biol. 99, 9-20.

Spero, H.J., Bijma, J., Lea, D.W., Bemis, B.E., 1997. Effect of seawater carbonate concentration on foraminiferal carbon and oxygen isotopes. Nature 390, 497-500.

Spindler, M., 1978. The development of the organic lining in *Heterostegina depressa* (Nummulitidae: Foraminifera). J. Foramin. Res. 8, 258-261.

Tang, J., Niedermayr, A., Köhler, S.J., Böhm, F., Kisakürek, B., Eisenhauer, A., Dietzel, M., 2012. Sr^{2+}/Ca^{2+} and $^{44}Ca/^{40}Ca$ fractionation during inorganic calcite formation: III. Impact of salinity/ ionic strength. Geochim. Cosmochim. Ac. 77, 432-443.

Taylor, A.R., Chrachri, A., Wheeler, G., Goddard, H., Brownlee, C., 2011. A voltage-gated H+ channel underlying pH homeostasis in calcifying coccolithophores. PLoS Biol. 9: e1001085.

Ter Kuile, B., Erez, J., 1984. In situ growth rate experiments on the symbiont-bearing foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*. J. Foramin. Res. 14, 262-276.

Ter Kuile, B., Erez, J., 1987. Uptake of inorganic carbon and internal carbon cycling in symbiont-bearing benthonic foraminifera. Mar. Biol. 94, 499-509.

Ter Kuile, B., Erez, J., 1988. The size and function of the internal inorganic carbon pool of the foraminifer *Amphistegina lobifera*. Mar. Biol. 99, 481-487.

Ter Kuile, B., Erez, J., Padan, E., 1989a. Competition for inorganic carbon between photosynthesis and calcification in the symbiont-bearing foraminifer *Amphistegina lobifera*. Mar. Biol. 103, 253-259.

Ter Kuile, B., Erez, J., Padan, E., 1989b. Mechanisms for the uptake of inorganic carbon by two species of symbiont-bearing foraminifera. Mar. Biol. 103, 241-251.

Ter Kuile, B., Erez, J., 1991. Carbon budgets for two species of benthonic symbiont-bearing foraminifera. Biol. Bull. 180, 489-495.

Terakado, Y., Ofuka, Y., Tada, N., 2010. Rare earth elements, Sr, Ba, Fe, and major cation concentrations in some living foraminiferal tests collected from Iriomote Island, Japan: An exploration for trace element behavior during biogenic calcium carbonate formation. Geochem. J. 44, 315-322.

Towe, K.M., Cifelli, R., 1967. Wall ultrastructure in the calcareous Foraminifera: crystallographic aspects and a model for calcification. J. Paleontol. 41, 742-762.

Toyofuku, T., De Nooijer, L.J., Yamamoto, H., Kitazato, H., 2008. Real-time visualization of calcium ion activity in shallow benthic foraminiferal cells using the fluorescent indicator Fluo-3 AM. Geochem. Geophy. Geosy. 9, Q05005, doi: 10.1029/2007GC001772.

Urey, H.C., Lowenstam, H.A., Epstein, S., McKinney, C.R., 1951. Measurement of paleotemperatures and temperatures of the upper Cretaceous of England, Denmark, and the Southeastern United States. Bull. Geol. Soc. Am. 62, 399-416.

Venn, A.A., Tambutté, E., Holcomb, M., Laurent, J., Allemand, D., Tambuté, S., 2013. Impact of seawater acidification on pH at the site of tissue-skeleton interface and calcification in reef corals. P. Natl. Acad. Sci. USA 110, 1634-1639.

Vetter, L., Kozdon, R., Mora, C.I., Eggins, S.M., Valley, J.W., Hönisch, B., Spero, H.J., 2013. Micron-scale intrashell oxygen isotope variation in cultured planktic foraminifers. Geochim. Cosmochim. Ac. 107, 1-12.

Wang, K.K.W., Villalobo, A., Roufogalis, B.D., 1992. The plasma membrane calcium pump: a multiregulated tranporter. Trends Cell Biol. 2, 46-52.

Weiner, S., Dove, P.M., 2003. An overview of biomineralization processes and the problem of the vital effect. Rev. Mineral. Geochem. 54, 1-29.

Weiner, S., Erez, J., 1984. Organic matrix of the shell of the foraminifer, *Heterostegina depressa.* J. Foramin. Res. 14, 206-212.

Wetmore, K., 1999. Chamber formation in *Archaias ungulatus*. J. Foramin. Res. 29, 69-74.

Wilson, J.E., Chin, A., 1991. Chelation of divalent cations by ATP, studied by titration calorimetry. Anal. Biochem. 193, 16-19.

Wolf-Gladrow, D., Bijma, J., Zeebe, R.E., 1999. Model simulation of the carbonate system in the microenvironment of symbiont bearing foraminifera. Mar. Chem. 64, 181-198.

Zeebe, R.E., 1999. An explanation of the effect of seawater carbonate concentration on foraminiferal oxygen isotopes. Geochim. Cosmochim. Ac. 63, 2001-2007.

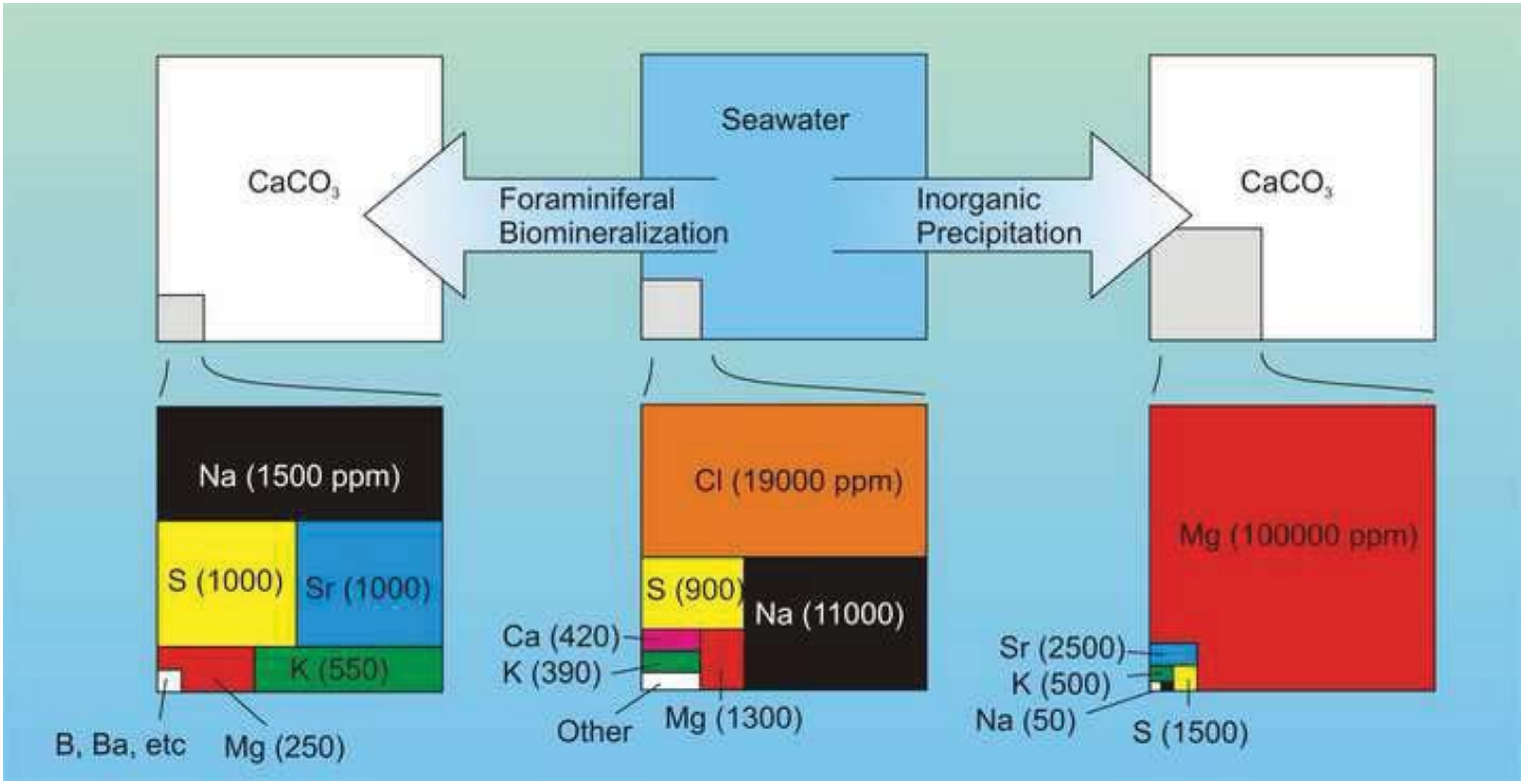
Zeebe, R.E., Bijma, J., Wolf-Gladrow, D.A., 1999. A diffusion-reaction model of carbon isotope fractionation in foraminifera. Mar. Chem. 64, 199-227.

Zeebe, R.E., Sanyal, A., 2002. Comparison of two potential strategies of planktonic foraminifera for house building: Mg^{2+} or H^+ removal? Geochim. Cosmochim. Ac. 66, 159-169.

Zeebe, R.E., Bijma, J., Hönisch, B., Sanyal, A., Spero, H.J., Wolf-Gladrow, D.A., 2008. Vital effects and beyond: a modeling perspective on developing palaeoceanographical proxy relationships in foraminifera. Geol. Soc., London Special Publications 303, 45-58.

Ziveri, P., Thoms, S., Probert, I., Geisen, M., Langer, G., 2012. A universal carbonate ion effect on stable oxygen isotope ratios in unicellular planktonic calcifying organisms. Biogeosciences 9, 1025-1032.

Figure 1



CaCO$_3$
Seawater
CaCO$_3$
Foraminiferal Biomineralization
Inorganic Precipitation
Na (1500 ppm)
S (1000)
Sr (1000)
K (550)
B, Ba, etc
Mg (250)
Cl (19000 ppm)
S (900)
Na (11000)
Ca (420)
K (390)
Other
Mg (1300)
Mg (100000 ppm)
Sr (2500)
K (500)
Na (50)
S (1500)

Figure 2
Click here to download high resolution image

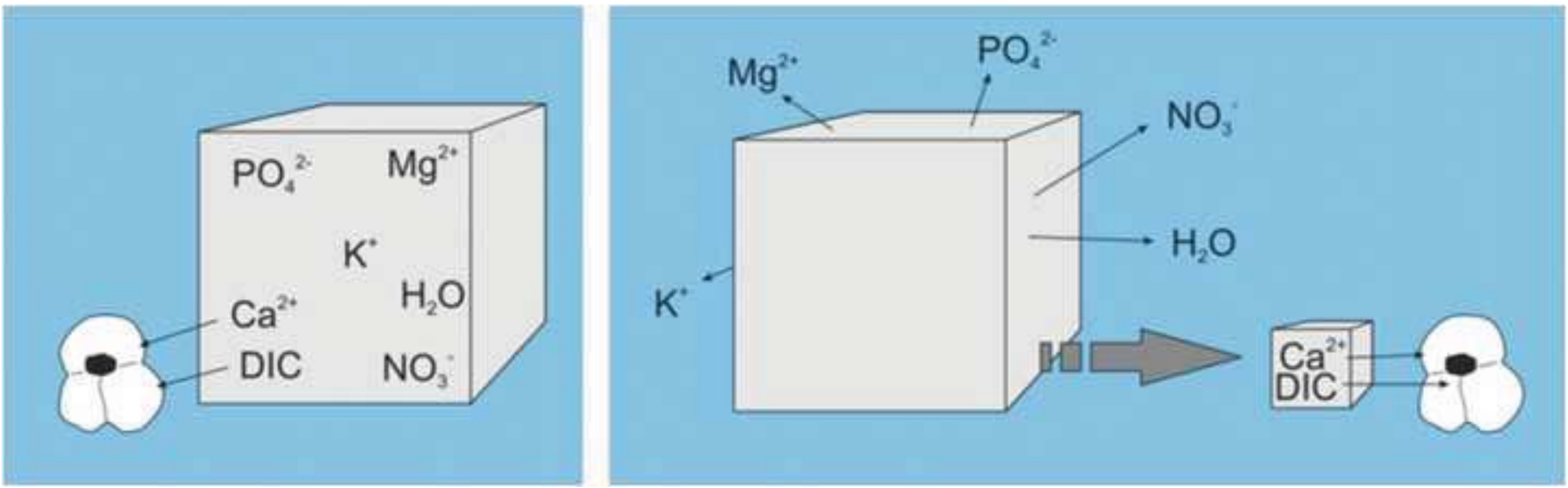


Figure 3

Click here to download high resolution image

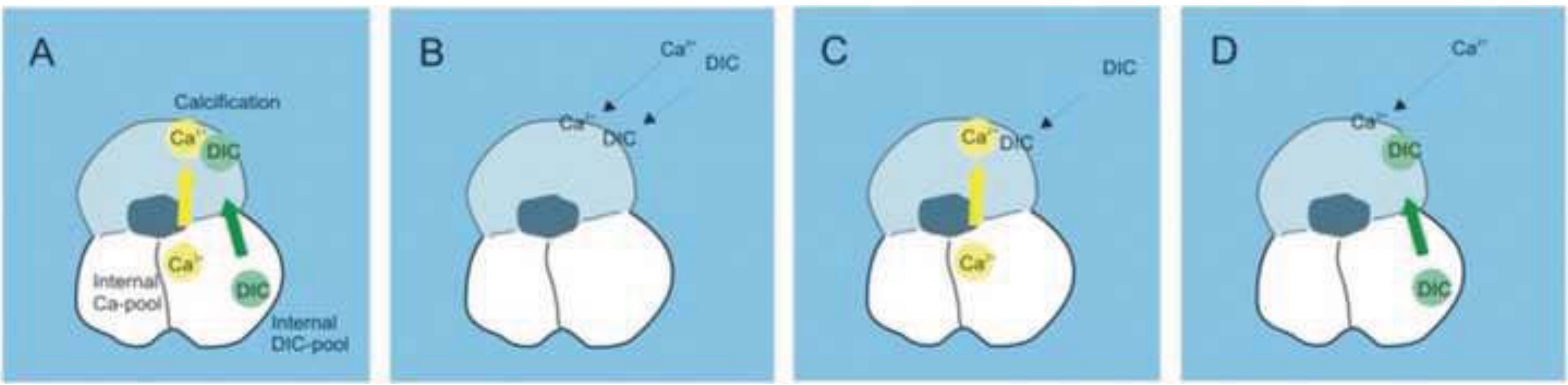


Figure 4

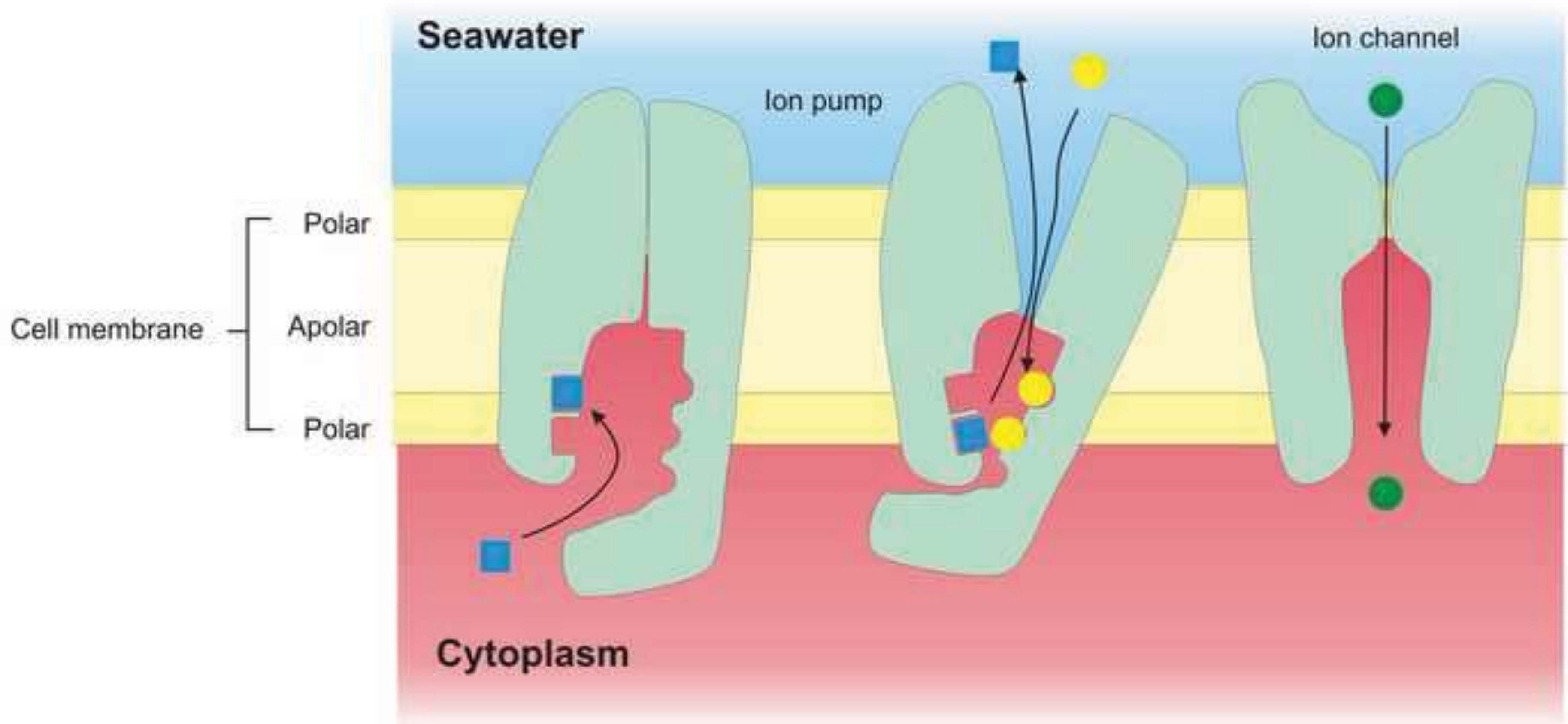
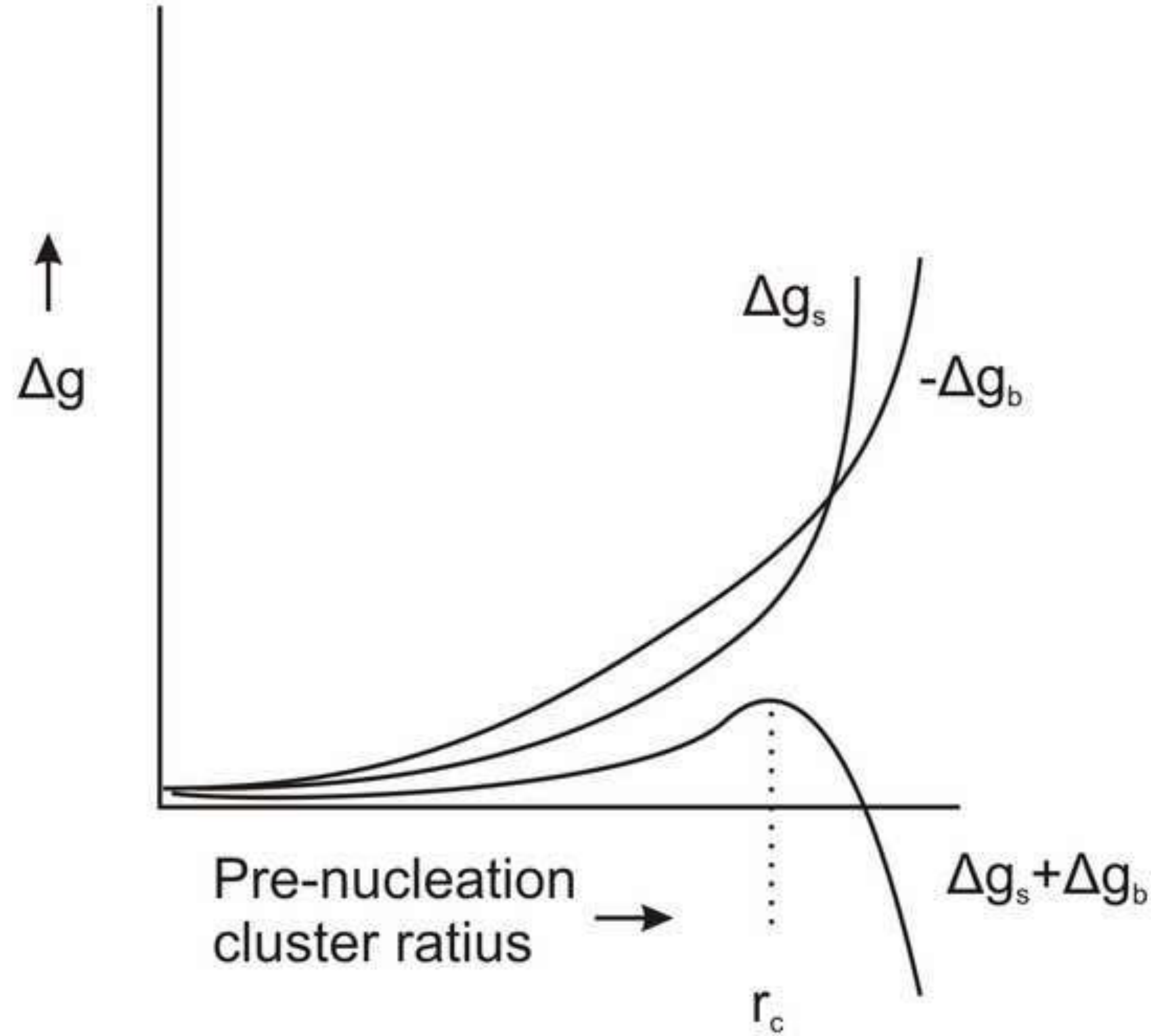

Seawater
Ion pump
Ion channel
Cell membrane
Polar
Apolar
Polar
Cytoplasm

Figure 5



Δg
Δg_s
$-\Delta g_b$
Pre-nucleation
cluster ratius
r_c
$\Delta g_s + \Delta g_b$

Figure 6

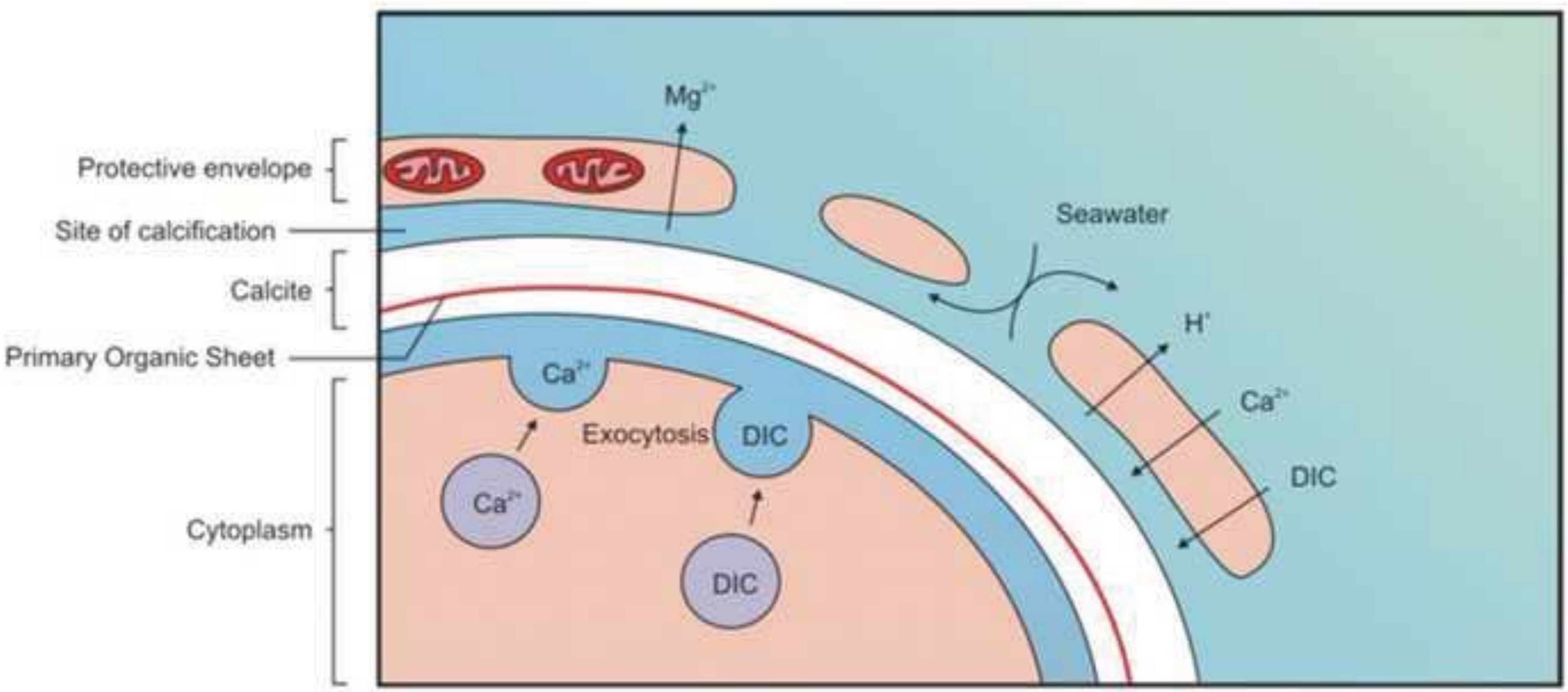

Protective envelope
Site of calcification
Calcite
Primary Organic Sheet
Cytoplasm
Mg^{2+}
Seawater
H^{+}
Ca^{2+}
DIC
Ca^{2+}
Exocytosis
DIC
Ca^{2+}
DIC

Table 1: Studies discussing internal reservoirs in perforate foraminifera.

	Ca^{2+} reservoir	DIC reservoir
Large volume reservoirs	Anderson and Faber (1984) Erez (2003) Toyofuku et al. (2008)	Ter Kuile and Erez (1987; 1988; 1989b; 1991) Erez (1978; 1982) Bentov et al. (2009)
No or small volume reservoirs	Angell (1979) Lea et al. (1995) Nehrke et al. (accepted)	Angell (1979)