

CORAL FORMATION

Biological control of aragonite formation in stony corals

Stanislas Von Euw,^{1*} Qihong Zhang,² Viacheslav Manichev,^{3,4} Nagarajan Murali,³ Juliane Gross,^{5,6} Leonard C. Feldman,^{4,7} Torgny Gustafsson,^{4,7} Carol Flach,² Richard Mendelsohn,² Paul G. Falkowski^{1,3,5,6,7*}

Little is known about how stony corals build their calcareous skeletons. There are two prevailing hypotheses: that it is a physicochemically dominated process and that it is a biologically mediated one. Using a combination of ultrahigh-resolution three-dimensional imaging and two-dimensional solid-state nuclear magnetic resonance (NMR) spectroscopy, we show that mineral deposition is biologically driven. Randomly arranged, amorphous nanoparticles are initially deposited in microenvironments enriched in organic material; they then aggregate and form ordered aragonitic structures through crystal growth by particle attachment. Our NMR results are consistent with heterogeneous nucleation of the solid mineral phase driven by coral acid-rich proteins. Such a mechanism suggests that stony corals may be able to sustain calcification even under lower pH conditions that do not favor the inorganic precipitation of aragonite.

The process by which stony corals deposit their calcium carbonate skeleton in the form of aragonite has been extensively discussed for decades without the emergence of a clear consensus (1). There are two prevailing hypotheses: Geochemists generally advocate for a physicochemically dominated process (2, 3) based on complex metabolic controls of calcifying fluid chemistry, whereas biologists argue for a biologically controlled process (4–7) in which the skeletal organic matrix (SOM) secreted by the animal plays the most important roles (8). The first hypothesis contends that an increase (compared with seawater) in pH and the concentrations of calcium and dissolved inorganic carbon species at the calcification site (9–11) generates metastable conditions suitable for the nucleation of the mineral phase. The second hypothesis supposes a template-induced nucleation of the mineral phase mediated by the SOM (12, 13) and, in particular, the acid-rich proteins (14, 15).

Here we examine which of these two hypotheses is valid for stony corals. To this end, we used the well-studied, ubiquitous Indo-Pacific stony coral *Stylophora pistillata* as a model for inves-

tigating the coral biomineralization process. We applied a materials science approach that combines Raman imaging and spectroscopy, scanning helium ion microscopy (SHIM), and solid-state nuclear magnetic resonance (NMR) spectroscopy. This approach reveals the crystallization pathway of aragonite in corals and provides unprecedented insights into the relation between

the mineral phase and the SOM across different spatial scales.

Features common to all stony corals

Scanning electron microscope (SEM) images of the skeleton of *S. pistillata* reveal juxtaposed cuplike structures (i.e., calices) whose calcareous walls constitute the corallites (Fig. 1A). A representative image of a single corallite shows dark line structures observable along the different micromorphological elements of the skeleton: the columella, the septa, and the theca (Fig. 1B). These structures, which appear to be enriched in organic molecules, are called centers of calcification (COCs) (16) and correspond to the initial sites of calcification. Higher-resolution images obtained using polarized light microscopy (PLM) and electron backscatter diffraction (EBSD) give direct evidence that acicular aragonite crystals are oriented outward from the COCs with homogeneous crystallographic orientations (Fig. 1, C and D). The crystals form densely packed ordered structures called skeletal fibers. These images suggest that the skeletal fibers arise from the organic matter-rich environments that constitute the COCs, which supports the hypothesis that the SOM is critical for initiating the deposition of the mineral phase (4, 5).

Spatial distribution of the SOM and orientation of the skeletal fibers

We investigated the relation between the SOM and the mineral phase by applying confocal Raman

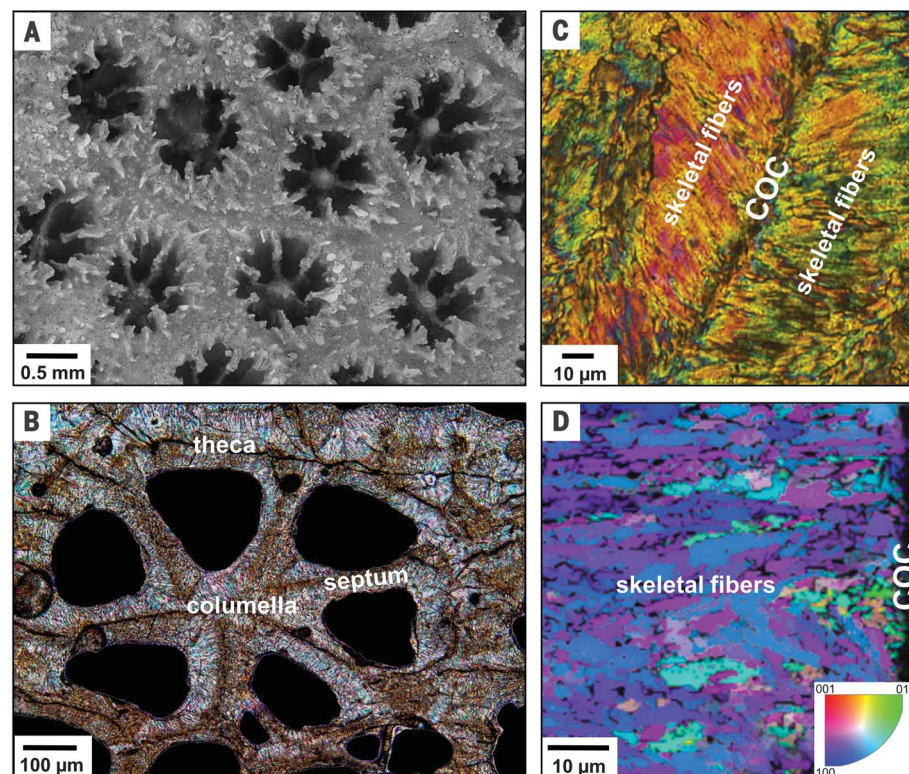


Fig. 1. Morphological skeletal features common to all stony corals. (A) Combination of SEM images showing the intact surface of a skeletal branch. (B and C) PLM micrographs of a single corallite and a trabecula, respectively. (D) EBSD inverse pole figure orientation map of a trabecula.

¹Environmental Biophysics and Molecular Ecology Program, Department of Marine and Coastal Sciences, Rutgers University, 71 Dudley Road, New Brunswick, NJ 08901, USA.

²Department of Chemistry, Rutgers University, 73 Warren Street, Newark, NJ 07102, USA. ³Department of Chemistry and Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854, USA. ⁴Institute of Advanced Materials, Devices, and Nanotechnology, Rutgers University, 607 Taylor Road, Piscataway, NJ 08854, USA. ⁵Department of Earth and Planetary Sciences, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854, USA. ⁶Department of Earth and Planetary Sciences, The American Museum of Natural History, Central Park West, 79th Street, New York, NY 10024, USA. ⁷Department of Physics and Astronomy, Rutgers University, 136 Frelinghuysen Road, Piscataway, NJ 08854, USA.

*Corresponding author. Email: voneuw@marine.rutgers.edu (S.V.E.); falko@marine.rutgers.edu (P.G.F.)

microscopy (CRM), which allows for determination of both the orientation of the skeletal fibers (Fig. 2, A1, A2, B1, and B2) and the spatial distribution of the SOM (Fig. 2, A3 and B3) (17). Two trabeculae are observable, in which skeletal fibers radiate from relatively small areas displaying high fluorescence intensity that correspond to COCs. The concentric organic-rich layers around the COCs, which are barely visible in Fig. 2, A3 and B3 (thickness, $\sim 1\ \mu\text{m}$), are part of the incremental growth lines of the mineral phase within the skeletal fibers (5).

Chemical heterogeneity in the mineral phase

Raman spectroscopy profiles were recorded across the two trabeculae (Fig. 2, C and D). These profiles were recorded twice to both detect the signal from the SOM (orange curves) and structurally characterize the mineral phase (blue curves). The former curves were obtained by integrating the signal of the sp^3CHx stretching modes in the region of 2850 to $3000\ \text{cm}^{-1}$ (fig. S1); the latter were obtained by tracing the full width at half maximum (FWHM) of the aragonite band at

$1087\ \text{cm}^{-1}$ (ascribed to the ν_1 symmetric stretching mode of the CO_3^{2-} units). The presence of organic material concentrated in the COCs was clear, and a broadening of the aragonite band was found in the COCs, in which the FWHM deviation reaches up to $\sim 10\%$. This broadening is diagnostic of chemical heterogeneity in the mineral phase and suggests the presence of “immature” aragonite particles, which are spatially closely related to the SOM in the COCs.

Relation between the initial mineral deposits and the SOM

To examine the role of the SOM concentrated in the COCs, we applied SHIM, which provides ultrahigh-resolution three-dimensional (3D) images with excellent depth of field (fig. S2). A COC was identified, given the direction of the radiating skeletal fibers (fig. S2A). The organic material is observable in the form of a $\sim 3\text{-}\mu\text{m}$ -thick fiber perpendicular to the plane in which the aragonite crystals grow. Four successive magnification micrographs reveal that the “immature” aragonite evidenced by Raman spectroscopy is in the form of nanosized particles intercalated in the organic fiber surface (fig. S2, B to E). These observations suggest that the SOM concentrated in the COCs forms a solid organic substrate on which the solid mineral phase is nucleated.

Detection of highly disordered environments in coral skeleton

The inference of a heterogeneous nucleation event logically gives rise to atomic-scale spatial proximity between specific bioorganic molecules and the mineral phase exposed to the mineral/organic interface (18). We applied solid-state NMR spectroscopy for identifying the bioorganic molecules that are bound to the mineral particles and, therefore, possibly responsible for the nucleation of the mineral phase. To examine these structures, we used a combination of the ^1H - ^{13}C cross-polarization (CP) and single-pulse ^{13}C NMR techniques (19). The former consists of a CP magnetization transfer from ^1H nuclei to nearby ^{13}C nuclei and enables the detection of carbon species localized in hydrogen-rich chemical environments—namely, the SOM and the interfacial carbonates (i.e., those exposed to the mineral/organic interface). The latter detects all of the ^{13}C spins present in the skeleton but only exposes the carbonates constituting the internal structure of the aragonite crystals (i.e., the bulk of the carbonates), which are the major carbon components.

We applied these two techniques to a live coral branch with its polyps that was totally hydrated during the analysis, as well as to a skeletal branch that was dried and powdered (fig. S3). The spectra from the bulk of the carbonates display a single resonance; this resonance is symmetric and centered at $\delta^{13}\text{C} = 171.0$ parts per million (ppm), which is characteristic of crystalline aragonitic environments (19). In contrast, the spectra from the interfacial carbonates display a much broader resonance that is asymmetric and whose maximum intensity is slightly lower ($\delta^{13}\text{C} = 170.7$ ppm). The spectral broadening strongly

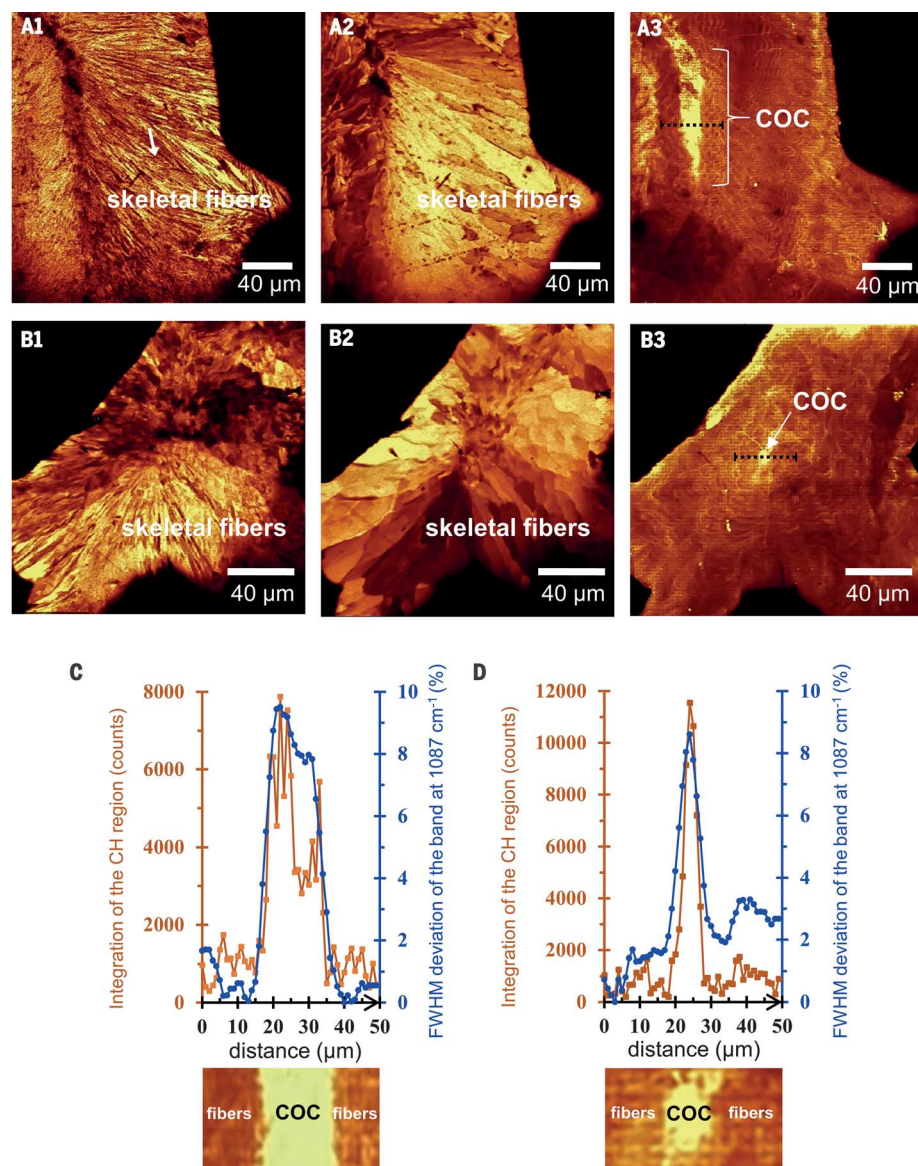


Fig. 2. Structural and chemical characterization of the COCs. (A and B) CRM maps, showing representative sections in the longitudinal (A) and transverse (B) planes along a skeletal branch. The three CRM maps for each section were generated on the basis of the relative intensity distribution of (i) two characteristic Raman bands of aragonite—namely, the translational lattice mode at $152\ \text{cm}^{-1}$ (A1 and B1) and the ν_1 symmetric stretching mode of the CO_3^{2-} units at $1087\ \text{cm}^{-1}$ (A2 and B2)—and (ii) the background signal in the range of 2400 to $2700\ \text{cm}^{-1}$, which is related to the fluorescence caused by the presence of organic compounds (A3 and B3). (C and D) 50-mm-long Raman spectroscopy profiles recorded across the trabeculae exposed in (A) and (B), respectively [dotted black lines in (A3) and (B3)]. CH, carbon-hydrogen bond.

implies that the interfacial carbonates form highly disordered, amorphous environments a few nanometers thick (20) that coat the aragonite crystals. The experiments conducted on living coral demonstrate that these highly disordered environments are not artifacts caused by drying.

Detection of highly disordered environments in synthetic aragonites

We prepared two different protein-free, synthetic aragonite samples in vitro following the protocol described by Wang *et al.* (21). One was precipitated from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O} + {}^{13}\text{C}$ -urea (called aragonite-1), whereas the second was precipitated from seawater + ${}^{13}\text{C}$ -urea (called aragonite-2). X-ray diffraction observations show that aragonite is the only crystalline phase (fig. S4). We applied the same NMR approach as before, from which we demonstrated the presence of analogous highly disordered environments (fig. S5). These observations confirm that the highly disordered environments detected in coral skeleton originate from inorganic carbonates.

Detection of HCO_3^- ions in the interfacial regions

To further characterize the chemical environments of these highly disordered inorganic carbonates exposed to the mineral/organic interface, we used the 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ heteronuclear correlation (HetCor) NMR technique (Fig. 3). This technique is a 2D version of the ${}^1\text{H}$ - ${}^{13}\text{C}$ CP experiment and reveals both the ${}^1\text{H}$ and ${}^{13}\text{C}$ chemical environments of hydrogen-bearing species. It can determine the spatial proximities among the various hydrogen and carbon species in the SOM and interfacial regions. These spatial proximities are detected as correlation peaks in the 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ HetCor NMR spectra, in which the carbon environments [displayed along the horizontal (F2) dimension] are correlated with their respective hydrogen environments [displayed along the vertical (F1) dimension]. Three 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ HetCor NMR experiments were recorded, using three different CP times (t_{CP}): 1, 4, and 8 ms. The normalized 1D ${}^1\text{H}$ and ${}^{13}\text{C}$ projections of the F1 and F2 dimensions, respectively, are shown in Fig. 3, A and B. Evidence of skeletal proteins and sugars is observable in the form of (i) several ${}^{13}\text{C}$ resonances of the proteins' side chains in the aliphatic carbon region ($\delta^{13}\text{C} = 10$ to 65 ppm) and the aromatic carbon region ($\delta^{13}\text{C} = 130$ ppm); (ii) an intense ${}^{13}\text{C}$ resonance from amide, carbonyl, and carboxylate groups that belong to proteins ($\delta^{13}\text{C} = 172$ to 180 ppm; black asterisks in Fig. 3A); and (iii) a broad ${}^{13}\text{C}$ resonance centered at $\delta^{13}\text{C} = 73$ ppm from sugar ring carbons.

This method is a powerful spectral editing tool for enhancing (short CP time, $t_{\text{CP}} = 1$ ms) or suppressing (long CP time, $t_{\text{CP}} = 8$ ms) the signal from the SOM. The corresponding 1D ${}^1\text{H}$ projections (Fig. 3B) were used to evaluate the nature of the hydrogen species bound to the SOM [aliphatic hydrogens and H_2O detected at $\delta^1\text{H} = 1.2$ and 5.2 ppm, respectively (observed at $t_{\text{CP}} = 1$ ms)] and the hydrogen species present in the interfacial regions [H_2O and HCO_3^- detected at

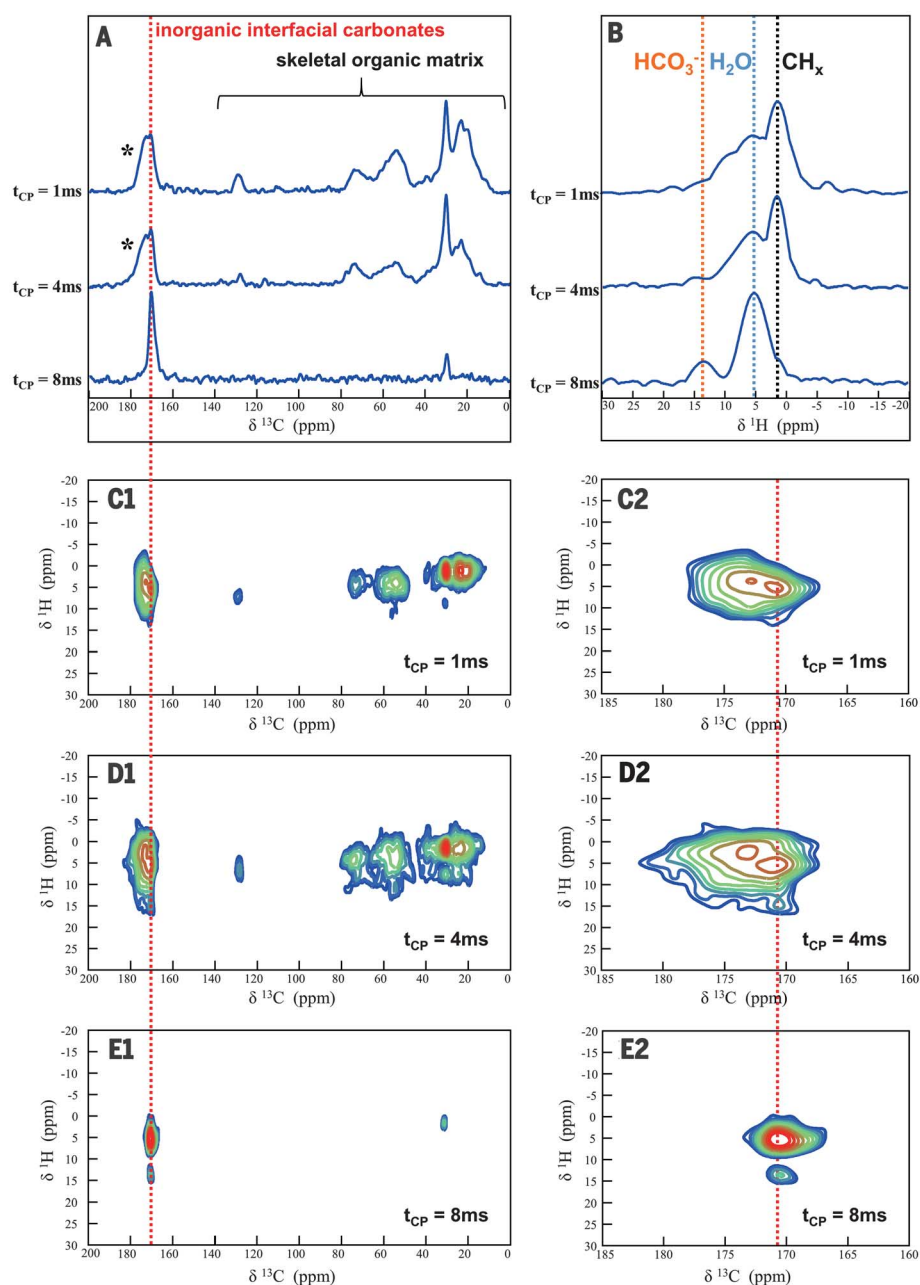


Fig. 3. Spatial correlations between hydrogen and carbon species. (A and B) Normalized 1D ${}^{13}\text{C}$ and ${}^1\text{H}$ projections of the F1 and F2 dimensions, respectively, extracted from the 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ HetCor NMR spectra displayed in (C1, D1, and E1). The spectra were recorded with three different CP times (t_{CP}): 1 (C1), 4 (D1), and 8 (E1) ms. (C2, D2, and E2) Enlargements of the interfacial carbonate regions observable at 170.70 ppm in the ${}^{13}\text{C}$ dimension (red dotted lines), extracted from (C1), (D1), and (E1), respectively. Signal intensity increases from blue to red.

$\delta^1\text{H} = 5.2$ and 14.2 ppm, respectively (observed at $t_{\text{CP}} = 8$ ms)]. These indirect observations were confirmed by the different 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ HetCor NMR spectra (Fig. 3, C1, D1, and E1), from which the interfacial regions have been enlarged in Fig. 3, C2, D2, and E2. In the spectrum recorded with $t_{\text{CP}} = 8$ ms, the presence of two correlation peaks observable at 170.70 ppm in the F2 (${}^{13}\text{C}$) dimension demonstrates the existence of at least two different inorganic carbonate species in the in-

terfacial regions. The two peaks can respectively be attributed to CO_3^{2-} ions near H_2O (observable at $\delta^1\text{H} = 5.2$ ppm) and HCO_3^- ions (observable at $\delta^1\text{H} = 14.2$ ppm), according to their chemical shifts in the F1 (${}^1\text{H}$) dimension.

Detection of HCO_3^- ions in synthetic aragonite

The 2D ${}^1\text{H}$ - ${}^{13}\text{C}$ HetCor NMR technique was also applied to the two protein-free synthetic aragonites

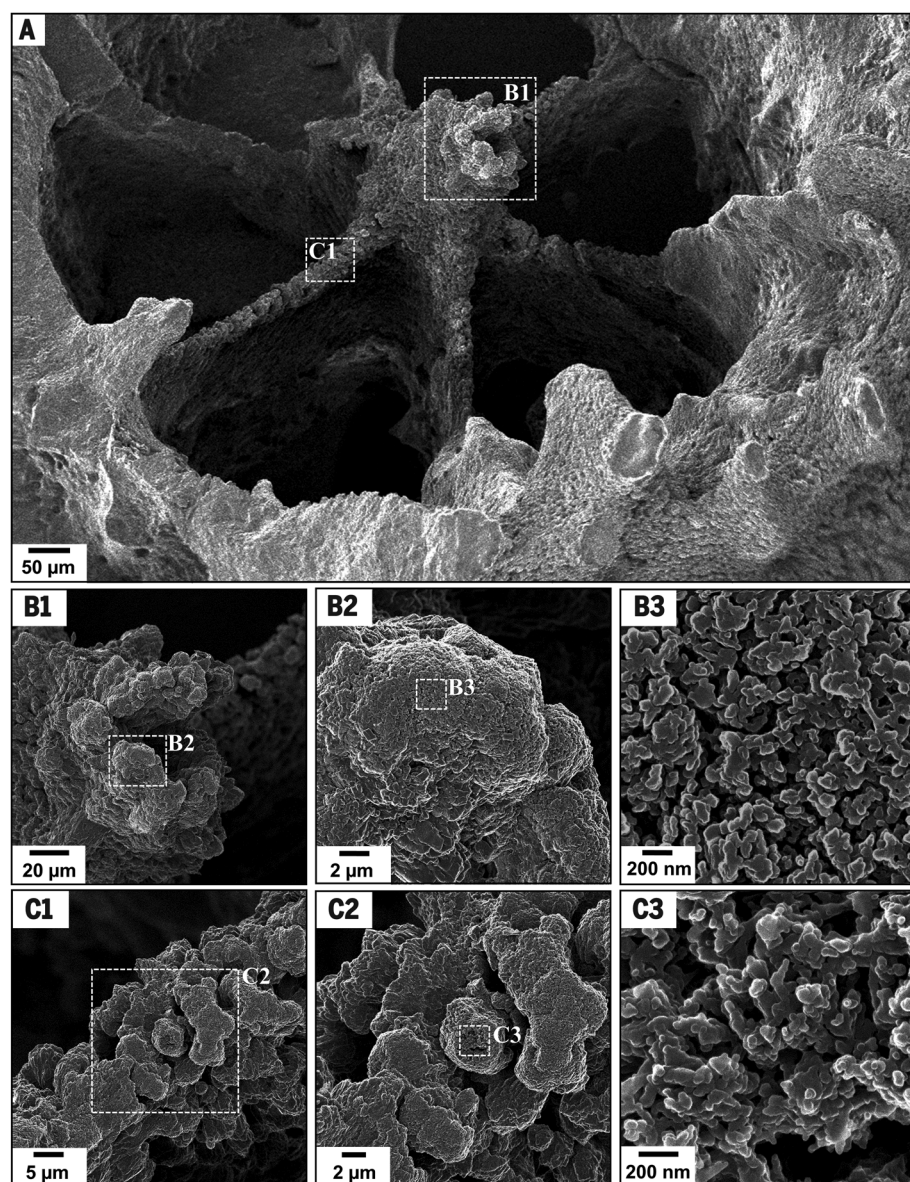


Fig. 4. Initial mineral deposits at the mineralizing front. Shown are SHIM micrographs obtained on the intact surface (unpolished, not etched) of a skeletal branch. The region in (A) is a single corallite, in which a growing columella and six growing septa are observable. (B1 to B3) and (C1 to C3) are successive magnifications that progressively reveal the morphology and size of the mineral particles at the top of the growing columella and on the edge of one of the growing septa, respectively.

(fig. S6). A ^1H signal centered around 1.2 ppm was detected in the highly disordered environments of both aragonite-1 and aragonite-2, potentially resulting from hydroxyl ions (22). Moreover, there is evidence of hydrogen-carbonate ions in highly disordered environments in aragonite-2. These results respectively confirm and suggest the presence of HCO_3^- and OH^- ions in the mineral phase associated with the interfacial regions of coral skeleton.

Examination of the mineral/organic interface

The interfacial HCO_3^- ions were used as a starting point for probing the presence of neighboring

bioorganic molecules. The spectrum with an intermediate CP time ($t_{\text{cp}} = 4$ ms) allows the simultaneous detection of the various ^{13}C signals from the SOM and the ^1H signal from the HCO_3^- ions. The F2 slice taken at the hydrogen-carbonate ion position in F1 (fig. S7) reveals the ^{13}C chemical environments in the interfacial regions. This F2 slice exhibits the expected ^{13}C resonance of the interfacial carbonates, as well as the ^{13}C resonance from the amide, carbonyl, and carboxylate groups belonging to proteins. These results suggest a close spatial proximity, not exceeding a few angstroms, between some skeletal proteins and the mineral phase in coral skeleton.

Given the nature of the interactions involved in the CP magnetization transfer (i.e., heteronuclear ^1H - ^{13}C dipolar couplings), these proteins might be strongly bound to and/or even trapped within the highly disordered calcium carbonate environments that coat the aragonite crystals.

Presence of an amorphous precursor phase

We explored the nature of the initial mineral deposits at the mineralizing front. SHIM observations were obtained from an intact corallite, both on the top of a growing columella and on the edge of a growing septum (Fig. 4). The highest-magnification micrographs show spherical nano-sized particles with a diameter of about 40 to 50 nm. Further, confocal Raman spectroscopy was applied, in which the laser was directed to the top of a growing columella from an intact skeletal branch (fig. S8A). The ν_1 band at 1087 cm^{-1} is broader and is shifted toward lower wave numbers than for well-crystallized aragonite (obtained from skeletal fibers exposed by CRM; white arrow in Fig. 2A1). These features are typical of the presence of amorphous material and have been observed for the stable biogenic amorphous calcium carbonate (ACC) in sternal deposits of the crustacean *Porcellio scaber* (23). Solid-state NMR spectroscopy was also applied, for which the surface of a skeletal branch was first mechanically collected; then, this surface fraction and the underlying fraction of the skeletal branch (called the core fraction) were analyzed separately. The ^{13}C resonance from the surface fraction (FWHM = 1.45 ppm) is wider than the ^{13}C resonance from the core fraction (FWHM = 1.29 ppm) (fig. S8B). This wider ^{13}C chemical shift distribution in the surface region is consistent with the presence of a small fraction of amorphous material. These interrelated investigations strongly suggest that ACC is a precursor phase for coral aragonite. On the basis of our observations and those of others (24), we assume that the “immature” aragonite particles evidenced in the COCs correspond to ACC particles that are not fully converted into aragonite.

Crystal growth process of coral aragonite

The transformation from the amorphous precursor phase to the aragonite crystals was explored by using SHIM to analyze the trabecula units (fig. S9). A COC and the adjoining skeletal fibers are visible in fig. S9, A and B. Three images of successively increasing magnification are shown of the COC (fig. S9, C1 to C3) and of the adjoining skeletal fibers (fig. S9, D1 to D3). The COC is composed of randomly arranged, densely packed, spherical nanosized particles with a diameter of about 40 to 50 nm (interpreted as initially deposited ACC nanoparticles), whereas the adjoining skeletal fibers are composed of micro-sized acicular aragonite crystals with homogeneous crystallographic orientations (fig. S10). Further, the highest-magnification micrograph shows that the surface of the aragonite crystals is highly textured; there are spherical

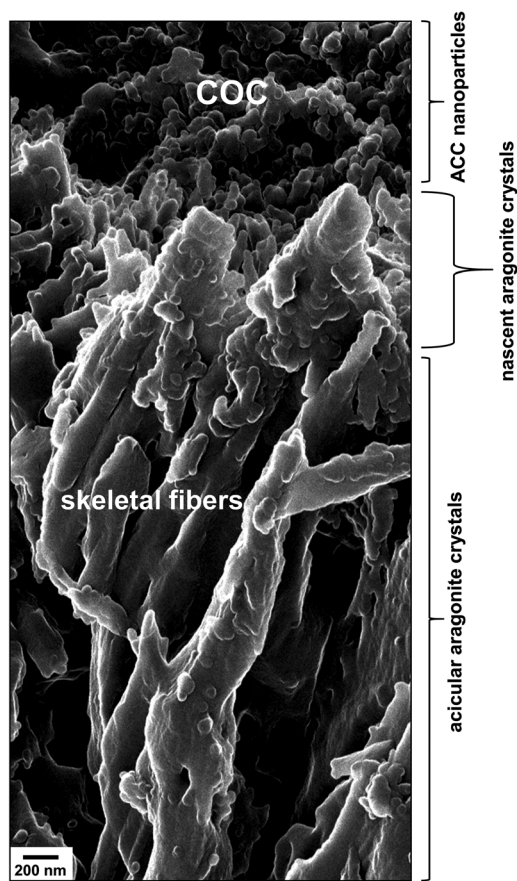


Fig. 5. The crystal growth process.

SHIM micrograph from a restricted area of a trabecula composed of skeletal fibers (bottom region) that arise from the COC (top region). These observations were obtained from the broken, unpolished, etched surface of a skeletal branch that was transversely sectioned.

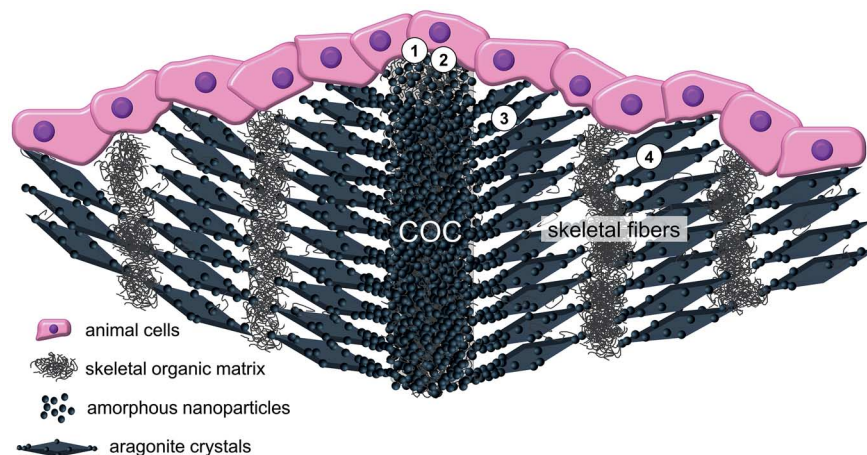


Fig. 6. Working model of coral biomineralization. Step 1, secretion of the SOM by the animal cells. Step 2, deposition of magnesium-rich ACC nanoparticles mediated by the SOM. Steps 1 and 2 might happen simultaneously. Step 3, growth of acicular aragonite crystals by attachment of amorphous precursor nanoparticles. Step 4, formation of the skeletal fibers through the “layered model” (38) of skeletal growth.

nanoparticles that were also observed by transmission electron microscopy (fig. S11).

The edge of a COC, where the adjoining skeletal fibers start to form, was also closely examined (Fig. 5). This section reveals a clear spatiotemporal evolution of the crystal growth process, showing (i) initially deposited ACC nanoparticles in the

COC (top); (ii) highly textured nascent aragonite crystals next to the ACC nanoparticles; and (iii) less textured and more “mature,” acicular aragonite crystals on the bottom. All of these observations support the hypothesis that ACC nanoparticles are initially deposited in the COCs; they further aggregate and serve as building

blocks for constructing aragonite crystal structures through a crystal growth process referred to as crystallization by particle attachment (25).

Chemical composition of the initial mineral deposits

Cathodoluminescence microscopy (CLM), back-scattered electron imaging, and wavelength-dispersive x-ray spectroscopy (WDXS) mapping were coupled in an electron microprobe (fig. S12). CLM reveals the localization of the COCs and enables WDXS maps to show variations in the chemical composition across the trabeculae. The elongated white regions of the CLM image correspond to COCs (fig. S12A and Fig. 2A3), which coincide with magnesium-rich regions, according to the WDXS map (fig. S12B). Further, a SHIM micrograph confirms the presence of spherical nanoparticles in a COC exposed by CLM and CRM (fig. S12C). These observations and measurements support that the initial mineral deposits consist of magnesium-rich ACC nanoparticles. This confirms previous observations in corals (26, 27) and supports the hypothesis that magnesium stabilizes ACC (28).

Conclusions

Our results elucidate the basic steps of the mineral deposition process in stony corals (Fig. 6). The process is initiated by the formation of a transient disordered precursor phase, which is probably in the form of ACC nanoparticles. This is not specific to stony corals; analogous ACC precursor phases have been observed in other marine organisms, including in the aragonitic nacre layer of abalone shells (24), the calcitic spicules of sea urchin larvae (29), and the spines of sea urchins (30). Our measurements indicate that the lifetime of these ACC nanoparticles is likely to be prolonged because they are rich in magnesium (28). Whether the initial mineral deposits are formed by the cells inside vesicles (31) or extracellularly at the calcifying interface between the calciblastic ectodermal cells and the skeleton (6, 7) remains unclear. However, our results not only reveal that ACC precursor nanoparticles are deposited in microenvironments that are enriched in SOM secreted by the animal (i.e., the COCs), but also show that an organic substrate, in the form of fibers, appears to serve as a nucleation site. Further, our results suggest the presence of skeletal proteins strongly bound to and/or trapped within the highly disordered calcium carbonate environments that coat the aragonite crystals. These features are consistent with a heterogeneous nucleation of the solid mineral phase catalyzed by coral acid-rich proteins (12), which are present in the COCs (32) and can precipitate aragonite directly from seawater (15). As more and more ACC nanoparticles are formed, they migrate from the COCs, lose magnesium, and grow to become acicular aragonite crystals by attachment of amorphous precursor nanoparticles (25).

Last, our results strongly suggest that the ability of corals to calcify is biologically controlled

and thus relatively robust. As such, the biological reaction is far from thermodynamic equilibrium, and, hence, biomineralization in stony corals is not simply related to physicochemical parameters such as the equilibrium saturation state of carbonate ions or the bulk pH of seawater (33). This conclusion is further supported by the fossil record of scleractinian corals. These organisms survived the Paleocene-Eocene Thermal Maximum, which was associated with a very large increase in atmospheric carbon dioxide (34). Indeed, scleractinian corals radiated in the Eocene (35). Corals undoubtedly will be endangered in coming decades and centuries by thermal stress, eutrophication, and decreasing ocean pH (36, 37). However, the results presented here and elsewhere (15) suggest that they may retain greater metabolic capability to form aragonite skeletons than commonly supposed.

REFERENCES AND NOTES

1. D. Allemand, E. Tambutté, D. Zoccola, S. Tambutté, in *Coral Reefs: An Ecosystem in Transition* (Springer Netherlands, 2011), pp. 119–150.
2. D. J. Barnes, *Science* **170**, 1305–1308 (1970).
3. B. R. Constantz, *Palaios* **1**, 152 (1986).
4. J. P. Cuif, Y. Dauphin, *Biogeosciences* **2**, 61–73 (2005).
5. J.-P. Cuif, Y. Dauphin, *J. Struct. Biol.* **150**, 319–331 (2005).
6. P. L. Clode, A. T. Marshall, *Protoplasma* **220**, 153–161 (2003).
7. P. L. Clode, A. T. Marshall, *Tissue Cell* **34**, 187–198 (2002).
8. G. Falini, S. Fermani, S. Goffredo, *Semin. Cell Dev. Biol.* **46**, 17–26 (2015).
9. N. Allison, I. Cohen, A. A. Finch, J. Erez, A. W. Tudhope, *Nat. Commun.* **5**, 5741 (2014).
10. A. Venn, E. Tambutté, M. Holcomb, D. Allemand, S. Tambutté, *PLOS ONE* **6**, e20013 (2011).
11. A. T. Marshall, P. L. Clode, R. Russell, K. Prince, R. Stern, *J. Exp. Biol.* **210**, 2453–2463 (2007).
12. J. L. Drake et al., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 3788–3793 (2013).
13. P. Ramos-Silva et al., *Mol. Biol. Evol.* **30**, 2099–2112 (2013).
14. S. Puverel et al., *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **141**, 480–487 (2005).
15. T. Mass et al., *Curr. Biol.* **23**, 1126–1131 (2013).
16. M. M. Ogilvie, *Philos. Trans. R. Soc. B Biol. Sci.* **187**, 83–345 (1896).
17. M. Wall, G. Nehrkke, *Biogeosciences* **9**, 4885–4895 (2012).
18. P. U. P. A. Gilbert, M. Abrecht, B. H. Frazer, *Rev. Mineral. Geochem.* **59**, 157–185 (2005).
19. I. B. Shir, S. Kababya, A. Schmidt, *Isr. J. Chem.* **54**, 74–85 (2014).
20. N. Nassif et al., *Proc. Natl. Acad. Sci. U.S.A.* **102**, 12653–12655 (2005).
21. L. Wang, I. Sondi, E. Matijevic, *J. Colloid Interface Sci.* **218**, 545–553 (1999).
22. P. J. Sideris, U. G. Nielsen, Z. Gan, C. P. Grey, *Science* **321**, 113–117 (2008).
23. U. Wehrmeister et al., *J. Raman Spectrosc.* **42**, 926–935 (2011).
24. R. T. DeVol et al., *J. Am. Chem. Soc.* **137**, 13325–13333 (2015).
25. J. J. De Yoreo et al., *Science* **349**, aaa6760 (2015).
26. A. A. Finch, N. Allison, *Geophys. Res. Lett.* **35**, L08704 (2008).
27. A. Meibom et al., *Geophys. Res. Lett.* **31**, L23306 (2004).
28. E. Loste, R. M. Wilson, R. Seshadri, F. C. Meldrum, *J. Cryst. Growth* **254**, 206–218 (2003).
29. E. Beniash, J. Aizenberg, L. Addadi, S. Weiner, *Proc. Biol. Sci.* **264**, 461–465 (1997).
30. Y. Politi, T. Arad, E. Klein, S. Weiner, L. Addadi, *Science* **306**, 1161–1164 (2004).
31. R. L. Hayes, N. I. Goreau, *Biol. Bull.* **152**, 26–40 (1977).
32. T. Mass, J. L. Drake, E. C. Peters, W. Jiang, P. G. Falkowski, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12728–12733 (2014).
33. J. A. Kleypas, C. Langdon, in *Coastal and Estuarine Studies*, vol. 61, J. T. Phinney, O. Hoegh-Guldberg, J. Kleypas, W. Skirving, A. Strong, Eds. (American Geophysical Union, 2006), pp. 73–110.
34. D. E. Penman, B. Hönisch, R. E. Zeebe, E. Thomas, J. C. Zachos, *Paleoceanography* **29**, 357–369 (2014).
35. G. D. Stanley Jr., *Earth Sci. Rev.* **60**, 195–225 (2003).
36. O. Hoegh-Guldberg et al., *Science* **318**, 1737–1742 (2007).
37. T. P. Hughes et al., *Science* **301**, 929–933 (2003).
38. J. Stolarski, *Acta Palaeontol. Pol.* **48**, 497–530 (2003).

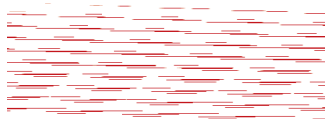
ACKNOWLEDGMENTS

We thank K. Wyman for daily assistance in the laboratory. We thank F. Natale, P. Mukherjee, and T. Erme for technical support. S.V.E. thanks T. Azais and J. Drake for insightful discussions. Our research was supported by NSF grant EF 1416785 to P.G.F. The helium ion microscope was supported by NSF grant DMR 1126468. The source data for the two-dimensional solid-state NMR and Raman spectra will be publically available at <http://ebme.marine.rutgers.edu/research/related-projects/>.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6341/933/suppl/DC1
Materials and Methods
Figs. S1 to S12

20 December 2016; accepted 10 May 2017
10.1126/science.aam6371



Biological control of aragonite formation in stony corals
Stanislas Von Euw, Qihong Zhang, Viacheslav Manichev,
Nagarajan Murali, Juliane Gross, Leonard C. Feldman, Torgny
Gustafsson, Carol Flach, Richard Mendelsohn and Paul G.
Falkowski (June 1, 2017)
Science **356** (6341), 933-938. [doi: 10.1126/science.aam6371]

Editor's Summary

Building coral skeletons

Among other things, corals are threatened by ocean acidification and warming. Being able to project the magnitude of these threats requires an understanding of how corals form their carbonate skeletons. Von Euw *et al.* combined ultrahigh-resolution three-dimensional imaging and two-dimensional solid-state nuclear magnetic resonance spectroscopy to study coral skeletons. They found that mineral precipitation in corals is a biologically controlled process mediated by organic molecules, rather than an abiotic one that depends only on physico-chemical conditions. This has important implications for the health of corals in our warmer, higher-CO₂ future.

Science, this issue p. 933

This copy is for your personal, non-commercial use only.

Article Tools Visit the online version of this article to access the personalization and article tools:
<http://science.sciencemag.org/content/356/6341/933>

Permissions Obtain information about reproducing this article:
<http://www.sciencemag.org/about/permissions.dtl>

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2016 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.