Morphological record of oxygenic photosynthesis in conical stromatolites

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Oxygenic photosynthesis irreversibly changed the Earth’s surface environment by using light energy to split water and evolve molecular oxygen (Eq. 1)

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{O}_2(\text{g}) + \text{CH}_2\text{O}. $$

The timing of the evolution of this metabolism is controversial (1–7), but it must have predated the geochemical record of initial oxygenation of the atmosphere at 2.4 billion years ago (Ga) (8). The sedimentary record of photosynthetic microbial communities is at least 1 billion years older (9) and commonly written in the laminated and lithified sedimentary structures called stromatolites. Conical stromatolites are a distinct morphological group that, to date, lacks plausible abiotic analogs, but the metabolic provenance of their microbial builders remains difficult to establish even in structures much younger than 2.4 Ga.

Modern conical stromatolites are thought to owe their distinct shape to biological processes because thin filamentous cyanobacteria are known to form small cones (10) even in the complete absence of lithification. Light is essential for the growth of cyanobacterial biomass in modern cone-building biofilms (10). The upward migration of phototactic filaments is thought to be another mechanism contributing to the vertical growth of modern cones (10), although it remains unclear whether this vertical growth is due to phototaxis or simply to the enhanced growth at the tips of cones. Once a conical structure forms, models suggest that the fast vertically accreting mat may propagate the shape through successive laminae in the presence of fast lithification (11).

Models assuming an enhanced accumulation of biomass at topographic highs (11) or the vertical orientation of long filaments at the tips of stromatolites (10) can account for the thickening of the laminae in the centers of conical stromatolites (12). Yet, these models cannot account for other common characteristics of the crestal zone, a prominent but poorly understood internal feature defining an entire class of Paleo- and Mesoproterozoic conical stromatolites that formed in quiet zones outside the influence of waves and storms (12) (Fig. L1). The crestal zone often has contorted or discontinuous laminae, some of which appear to have enclosed submillimeter- to millimeter-sized bubbles (13) [Fig. 1 and supporting information (SI) Fig. S1]. Using enrichment cultures of modern cone-forming cyanobacteria from Yellowstone National Park, here, we test the influence of light on the centimeter- to submillimeter-scale external and internal morphology of cyanobacterial cones and link the formation of oxygen-rich bubbles in the tips of modern cyanobacterial cones to the fossil gas bubbles present in the central zone of modern, Proterozoic, and even some Archean conical stromatolites.

**Bubble Formation at the Tips of Modern Conical Structures**

Light is critical both for the growth of photosynthetic cone-forming cyanobacteria (10) and as a determinant of the resulting morphology of biofilms (Fig. S2). Although ridged or flatter structures grow at higher light intensities, low-light conditions enhance the growth of prominent cones with axes inclined in the direction of light (Fig. S2). Higher photosynthetic activity in the tips of these cones (Fig. 2B), in turn, often leads to the formation of oxygen-rich bubbles in our enrichment cultures but not on the sides of cones (i.e., in the zones of highest photosynthetic activity under light-limiting conditions). These bubbles, colonized by a mesh of filamentous cyanobacteria, are generally much smaller than cones and do not confer a conical shape onto the mat. Bubbles are either clearly visible (Fig. 2A) or can be detected in cross-sections of modern lithifying cones as submillimeter- and millimeter-diameter features with nearly-circular cross-sections (i.e., completely enclosed by laminae) (Fig. 2C and Figs. S3 and S4). Bubbles surrounded by a thick, actively photosynthesizing mesh can grow into irregularly shaped blisters, disrupt the laminae (Fig. 2C and Fig. S5) or initiate the growth of millimeter- to centimeter-diameter tubes.

If mechanically and gravitationally stable bubbles are to form, to persist, and to become preserved by fast lithification, they should be smaller than \(\approx 5 \text{ mm} \) in diameter and heavily enmeshed. The recognition of fossil bubbles as features with nearly-spherical cross-section will then be possible if the surrounding microbial mesh is preserved by sufficiently small crystals (i.e., smaller than the average lamina thickness), and if significant recrystallization does not occur. In modern hot springs (14) and in our cultures that contain less than \(\approx 2 \text{ mM} \) inorganic carbon, only bubbles smaller than \(\approx 0.2 \text{ mm} \) are enclosed by a thick mesh (Fig. S3). Larger nearly-spherical bubbles in these environments will be enclosed by a much more porous or discontinuous mesh of filaments and not necessarily preserved or identifiable. Bubbles that form in the centers of very narrow central zones (\(\approx 200 \mu\text{m} \)) would be similarly difficult to preserve and recognize. In deeper waters, at lower photosyn-
thetic rates, or in solutions rich in dissolved inorganic carbon, however, even millimeter-scale photosynthetic bubbles will be extensively covered by a microbial mesh and more likely to be preserved by syngenetic mineralization (Figs. S4, S5, and S6).

**Bubbles in Proterozoic Conical Stromatolites**

Nearly-circular features enclosed by contorted laminae are primarily observed at the tips of some well-preserved Proterozoic conical stromatolites but not on the sides (Fig. 1 and Fig. S1). The analogy with modern laminated cones suggests that these circular features originated as submillimeter- to millimeter-sized gas bubbles. The localization of millimeter-scale contorted laminae in conical stromatolites to the topographic highs implies that those were the spots of active in situ production of gas, whereas the absence of fossil bubbles on the sides of conical stromatolites suggests that the local gas concentration was not sufficient to create bubbles. Areas enclosed by contorted and sometimes disrupted laminae (12) in the crests of conical stromatolites are thus a likely consequence of gas release resulting from higher microbial metabolic activity on the topographic highs (Fig. 1 and Fig. S1). These inferred gas bubbles in coniform stromatolites can occupy anywhere from 0% to ~20% of the central zone (13).

**Discussion**

The presence of cone-forming cyanobacteria can easily explain the growth of bubbles enclosed by primary laminae in the crests of conical stromatolites and the accumulation of sufficient gas to overcome considerable hydrostatic pressure at depth (more than twice the atmospheric pressure at 10 m). On the other hand, light-independent microbial production of gases such as H₂, CH₄, CO₂, CO, and H₂S, N₂O, and N₂ require abundant organic matter or high concentrations of nitrate in the solution, the latter requiring the presence of atmospheric oxygen. Unlike oxygen bubbles that give a circular shape to the laminae formed by primary producers at the top of the structure (Fig. 2 and Fig. S4), fenestrae originating from the decay of unmineralized organic matter in rapidly accreting tips tend to destroy the primary lamination and tend to have irregular shape distributions (e.g., ref. 15). In contrast, the inferred bubbles in conical stromatolites are restricted only to the crests even in the very narrow (millimeter-wide) central zones (Fig. S1), where they do not disrupt the surrounding laminae. The degradation of organic matter by methanogenic microbes can produce bubbles at large depths (16) that, coupled with methanotrophy, can lead to the precipitation of large-scale carbonate structures with central vents. These carbonate structures differ from conical stromatolites because

**Fig. 1.** Center of a conical stromatolite [Atar Formation, Atar Group, Mauritania, Late Mesoproterozoic (S3, S4), sample provided by A. Maloof]. (A) Mosaic photomicrograph of the central zone (thin section). The white rectangle surrounds the area shown in B, and the arrows point to fossil bubbles. (Scale bar, 1 cm.) (B) A typical nearly-circular submillimeter feature surrounded by dark laminae in the middle of the stromatolite (white arrow). This fossil bubble is found on top of a larger, irregularly shaped feature surrounded by contorted laminae (outlined by a dashed line) that may be a former mat-trapped bubble. (Scale bar, 1 mm.) (C) Fossil bubbles are absent from laminae on the sides of the same conical stromatolite. (Scale bar, 1 mm.) (D) Histogram showing the diameters (in millimeters) of fossil bubbles in 18 well-preserved Proterozoic conical stromatolites. Bubbles were identified as submillimeter- and millimeter-diameter features with nearly-circular cross-sections enclosed by laminae.

**Fig. 2.** Oxygen and bubble production in modern conical aggregates dominated by cyanobacteria. (A) Bubbles often form at the tips of cones and are partly or completely covered by biofilm. Note that these bubbles did not lift the mat up into a conical structure. (Scale bar, 5 mm.) (B) Profiles of oxygen (filled circles) and gross photosynthetic rate (white bars) and oxygen uptake rate (gray bars) within a cone. Depth 0 mm denotes the tip of the cone and ~6 mm is the bottom of the cone. (C) Transmitted-light micrograph of a 30-μm-thick thin section of a cone showing disrupted fabrics, large voids (former blisters), and bubbles in the center. Multiple pores surrounded by cyanobacterial filaments were present in the series of successive thick sections, confirming that the porosity was not an artifact. The sample had an elliptical cross-section, and it was sectioned along the minor axis. (Scale bar, 1 mm.)
the former lack continuous laminae, their porosity is not spatially restricted to topographic highs, and they contain carbonate minerals exhibiting characteristically depleted carbon isotopes (17) that are not observed in well-preserved stromatolites (18). The presence of methanogens in communities with anoxygenic photosynthetic microbes could potentially account for the large-scale conical shape of stromatolites, and, possibly, the localization of bubbles at the tips. This model is unlikely because anoxygenic phototrophs and methanogens actually compete for substrates in some modern environments (19), suggesting that intense production of methane should not be expected in the zones of highest photosynthetic activity. Moreover, processes such as microbial iron and sulfate reduction and fermentation zones of highest photosynthetic activity. Moreover, processes such as microbial iron and sulfate reduction and fermentation would be energetically better poised to degrade of organic matter close to the photosynthetic layer in the thin bubble-enclosing laminae, diminishing the likelihood of abundant methanane production. Although further search for cone-building and gas-producing anaerobic photosynthetic communities is warranted, such communities are not known currently. We also note that hydrogen-based anoxygenic photosynthesis would take up H₂ and CO₂, thereby reducing the local concentration of gases needed to form bubbles. Overall, stromatolite growth in the presence of strongly phototactic cyanobacteria best accounts for the presence of inferred fossil bubbles at the tips of nondivergent conical stromatolites.

Gas-bubble entrapment has been suggested as the origin of axial porosity in the central zone of some Proterozoic stromatolites (13). Here, we explicitly link the photosynthetic production of oxygen to the deformed laminae commonly observed in the central zones of Proterozoic conical stromatolites (12, 13, 20, 21). Cyanobacteria were thus involved in the formation of some well-preserved Paleoproterozoic and Mesoproterozoic cones as well as some much younger Neoproterozoic conical stromatolites (22). Intriguingly, features consistent with fossil bubbles and contorted laminae in the crestal zone may be present in rare conical stromatolites as early as 2.7 Ga (23–25) and in more widespread cones on Late Archean carbonate platforms (26, 27) (Table 1 and Fig. 3). The apparent absence of fossil bubbles in the few Archean cones older than 2.7 Ga (Table 1 and Fig. 3) may be attributed both to poor preservation and to a very small number of described conical stromatolites (28), but further experiments are needed to determine whether these cones could have grown in the presence of anoxygenic photosynthetic microbes. The origin of gas-related features in Archean conical stromatolites thus deserves focused investigation because these features may be the first detectable traces of oxygen exhaled under an anoxic atmosphere that forever changed the surface environment on our planet.

**Table 1. Presence of contorted laminae, a distinct axial zone, and bubble-like structures in Archean conical stromatolites**

<table>
<thead>
<tr>
<th>Age, Ga*</th>
<th>Contorted laminae and/or distinct axial zone</th>
<th>Cement-filled contorted laminae (possible fossil bubbles)</th>
<th>Reference†</th>
<th>Continent</th>
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<tr>
<td>3.4</td>
<td>Absent</td>
<td>Absent</td>
<td>Allwood et al., 2007 (37); Hofmann et al., 1999 (38); Lowe, 1980 (39)</td>
<td>Australia</td>
</tr>
<tr>
<td>3.1–2.9</td>
<td>Absent</td>
<td>Absent</td>
<td>Beukes and Lowe, 1989 (40)</td>
<td>Africa</td>
</tr>
<tr>
<td>3.0–2.8</td>
<td>Unclear‡</td>
<td>Unclear‡</td>
<td>Hofmann et al., 1985 (41)</td>
<td>North America</td>
</tr>
<tr>
<td>2.9–2.7</td>
<td>Unclear‡</td>
<td>Unclear‡</td>
<td>Wilks and Nisbet, 1985 (42)</td>
<td>North America</td>
</tr>
<tr>
<td>2.8 ± 7</td>
<td>Probably absent‡</td>
<td>Probably absent‡</td>
<td>Donaldson and deKemp, 1998 (43)</td>
<td>North America</td>
</tr>
<tr>
<td>2.7</td>
<td>Present</td>
<td>Present</td>
<td>Hofmann and Masson, 1994 (23)</td>
<td>North America</td>
</tr>
<tr>
<td>2.7–2.6</td>
<td>Poorly defined and narrow</td>
<td>Unclear‡</td>
<td>Sakurai et al., 2005 (44); Van Kranendonk et al., 2006 (25)</td>
<td>Australia</td>
</tr>
<tr>
<td>2.7</td>
<td>Present</td>
<td>Present</td>
<td>Grey, 1981 (45)</td>
<td>Australia</td>
</tr>
<tr>
<td>2.6–2.9</td>
<td>Present</td>
<td>Unclear‡</td>
<td>Hofmann et al., 1991 (24)</td>
<td>North America</td>
</tr>
<tr>
<td>2.7–2.6</td>
<td>Present</td>
<td>Unclear‡</td>
<td>Srinivasan et al., 1990 (46)</td>
<td>Asia</td>
</tr>
<tr>
<td>2.7–2.6</td>
<td>Present</td>
<td>Unclear‡</td>
<td>Walter, 1983 (47); Abell et al., 1985 (48)</td>
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<tr>
<td>&lt;2.63</td>
<td>Unclear‡</td>
<td>Unclear‡</td>
<td>Lambert, 1998 (49)</td>
<td>North America</td>
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<tr>
<td>2.63–2.52</td>
<td>Present</td>
<td>Present</td>
<td>Murphy and Sumner, 2008 (50)</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>Beukes, 1987 (26); Buck, 1980 (51); Altermann, 2008 (27)</td>
<td>Africa</td>
</tr>
</tbody>
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* Ages based on the references listed in Table 1 in Schopf (52) and Hofmann (28).
† References only given for the sources that contain detailed descriptions and/or images of conical stromatolites.
‡ Biogenicity and stromatolitic nature questionable (41).
§ Stromatolites are poorly preserved.
¶ Insufficient detail given in the published pictures or preserved.
† References only given for the sources that contain detailed descriptions and/or images of conical stromatolites.
* Ages based on the references listed in Table 1 in Schopf (52) and Hofmann (28).
** Contorted laminae consistent with the former presence of a bubble can be seen in the middle of a well-developed central zone can be seen in Van Kranendonk et al. (25), although we are not aware of any published photographs showing a thin section.
Materials and Methods

Culturing Media and Growth Conditions. Cone-forming cyanobacteria from Yellowstone National Park were obtained under the permit number YELL-2008-SCI-5758 from National Park Services. Two cyanobacterial clones dominate the 16s rDNA clone libraries from our enrichment cultures. One of these sequences (GenBank accession no. FJ933259) is 99% similar to a sequence found in cone-forming mats in Yellowstone National Park (29), whereas the sequences (GenBank accession no. FJ933259) is 99% similar to a sequence nate the 16s rDNA clone libraries from our enrichment cultures. One of these

2008-SCI-5758 from National Park Services. Two cyanobacterial clones domi-

Yellowstone National Park were obtained under the permit number YELL-

10942

iments. Two cyanobacterial clones dominate the 16s rDNA clone libraries from our enrichment cultures. One of these sequences (GenBank accession no. FJ933259) is 99% similar to a sequence found in cone-forming mats in Yellowstone National Park (29), whereas the other one is 97% similar to a sequence reported from mats growing at 50–55 °C in Yellowstone (30). The cultures were grown on solid substrate (agar, silica sand, or aragonite sand) at 45 °C in modified Castenholz D medium (31) containing low concentrations of nitrate and phosphate (2.3 mM NO3 and 0.8 mM PO4), respectively) at pH 8. The medium was initially in equili-

rium with an atmosphere of 5% CO2, 5% H2, and 90% N2, although cone-

forming cultures grow well even in the medium equilibrated with air. The cultures were grown at various distances from a fluorescent cold light source with a 12-h day–12-h night cycle, and the medium was exchanged periodically (every week). Lightification in the medium was promoted by lowering phos-

phate concentration to 80 μM, and 0.23 mM NO3, and increasing calcium and magnesium concentration to 3.5 mM and 4 mM, respectively, by the addition of CaCl2 and MgCl2. The initial pH of the lithifying medium was 7.6, and sterile

medium prepared in this manner did not contain visible precipitates.

Microscopy. Samples were removed from actively growing cultures by a sterile surgical blade, fixed with 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate (pH 7.4) for 2 h. After the fixative was washed away, the samples were embedded in Sakura TissueTek O.C.T. compound (VWR) and frozen for at least 2 h, and sectioned to a thickness of 30–50 μm at −20 °C by using a cryostat (Leica). O.C.T. was washed away by 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h. After the fixative was washed away, the samples were embedded in embedding medium prepared as above. The initial pH of the lithifying medium was 7.6, and sterile medium prepared in this manner did not contain visible precipitates.

Measurement of Oxygen Profiles and Photosynthetic Rates. Culture flasks were labeled with 1.5 μCi of Na14C2O3. The flasks were illuminated using a 12-h day–12-h night cycle, and the medium was exchanged periodically (every week). In the steady state, the photosynthetic oxygen production is equal to the loss of oxygen due to respiration and to diffusion. After the termination of illumination, the photosynthesis stops instantaneously, whereas diffusion and respiration are initially unchanged. This assumption should be valid as long as the decrease in oxygen concentration is linear with time (38). We measured the decrease in oxygen concentration within 5 s after the darkening and calculated the oxygen uptake (respiration) rates from the difference between the net and gross photosynthetic rates.

Supporting Information. For more information, see SI Text.

ACKNOWLEDGMENTS. A. Maloof (Princeton University) and S. Ono (Massachusetts Institute of Technology) provided the samples of Proterozoic and Archaean conical stromatolites; A. Thieraut and J. Dougherty enabled the analysis of the Geochemical Survey of Canada samples; G. Geese and S. Gunther assisted with field work in Yellowstone National Park; S.-H.D. Shim and G. Brent helped with Raman spectroscopy; D. Rothman, H. Hofmann, S. Golubic, B. Weiss, J. Kirschvink, V. Sergeev, 3 anonymous reviewers, and members of the Massachusetts Institute of Technology Laboratory for Geomicrobiology and Microbial Sedimentology provided useful comments; T. Grove (Massachusetts Institute of Technology) and S. Bowring (Massachusetts Institute of Technology) provided the rock saw and the petrographic microscopes; and J. Barr generously assisted with the cutting.


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