ing huge volcanic hydrogen outgassing rates or assuming a reduced mantle. The efficient production of organics in a hydrogen-rich early Earth's atmosphere would have led to an organic soup in the oceans and ponds on the early Earth. The world ocean could have been the birthplace of life (14).

References and Notes

9. Both CH4 and NH3 in the atmosphere of early Earth would have been subject to rapid loss driven by solar UV radiation. It is unlikely that the volcanic outgassing rate of CH4 or NH3 could have been adequate to maintain high concentrations of these gases.
10. The hydrogen concentration is determined by the balance between volcanic and hydroxyl radical outgassing rates, hydrogen escape of hydrogen to space. The modern volcanic hydrogen outgassing rate is 1.8 x 10^26 hydrogen molecules cm^-2 s^-1 (29). Because of the higher heat flow in the past, the overall outgassing rate of gases, and hydrogen in particular, might have been ~5 times greater on ancient Earth (30). In the present Earth's atmosphere, oxygen is dominant at the exobase level (defined as the boundary beyond which rapid-ion moving molecules may escape with collision). The current exobase temperature is high (1000 to 2500 K) because of the efficient absorption of solar UV radiation by oxygen. If the exospheric temperature on the early Earth were as high or as low as that of today, the overall hydrogen escape rate would have been efficient, and the diffusion of hydrogen through the background gases to the homopause level would have been the limiting process. The rate of diffusion-limited escape can be expressed as f(H2) = 2.5 x 10^11 fmax molecules cm^-2 s^-1, with fmax defined as the total mixing ratio of hydrogen (in all chemical forms) at the homopause (31). By balancing the diffusion-limited escape rate of hydrogen with the hydrogen outgassing rate, the hydrogen mixing ratio up to the homopause in early Earth's atmosphere should be of the order of 10^-6 or below to (21, 31), unless the oxidation state of Earth's mantle was more reduced than its current oxidation state. But Earth's mantle has been suggested to be in a similar oxidation state to that of today for the past 3.96 billion years (32). The common consensus among planetary scientists for the past 30 years has been that early Earth's atmosphere had a low hydrogen concentration.
11. However, experiments to date generate only methane or formate in realistic hydrothermal-like systems (33). The exogenous flux of organic materials at about 4 billion years ago (Ga), primarily interplanetary dust particles (IDPs), may be less than 150 times the present value (34), although the interpretations of the Alkilla rocks are debatable (35).
14. Materials and methods are available as supporting material on Science Online.
18. Nonthermal escape from a hydrogen-rich early Earth has not been studied in detail. Although a similar upper limit of nonthermal hydrogen escape rate should apply to early Earth, it is important to note that nonthermal hydrogen escape processes may also be rather different for Earth than for Venus because of the presence of a strong magnetic field on Earth. Future work should include these processes in escape models to produce more accurate estimates.
20. Assuming that the linear relation between escape flux and UV still holds for even higher solar EUV input, the hydrogen escape flux would be about 7.5 x 10^26 cm^-2 s^-1 for a solar EUV level 100 times that of today if the homopause hydrogen density is kept at ~5 x 10^10 cm^-3. This escape rate is still slower than the diffusion-limited escape rate (~1 x 10^22 cm^-2 s^-1) for the same homopause hydrogen density. Hence, the diffusive flux does not become limiting except for extreme EUV input.
22. H2 is not chemically reactive gas. So in the steady state, hydrogen has an equilibrium mixing ratio all the way from the surface to the homopause, as does CO2 (21). Therefore, the homopause mixing ratio of hydrogen is representative of the whole homosphere.
23. The amino acid production rate is found to be ~0.4 nmol cm^-2 year^-1 (8) in electric discharge experiments when H2/CO2 = 4, equivalent to 2 x 10^21 kg/year assuming a mean molecular weight of 100. This estimate is based on an annual electric discharge rate ~2 x 10^21 kg/year, which is ~20 times the contemporary electric discharge rate. ~1 x 10^18 kg/year (36). If the electric discharge rate on early Earth is the same as that of today, the rate of amino acid production by electric discharge would be 1 x 10^21 kg/year when H2/CO2 = 4, extrapolating the contemporary data back to early Earth faces large uncertainty. So here the conservative estimate (1 x 10^21 kg/year) of the amino acid production rate by electric discharge is taken.
24. Assuming the ocean volume is 1.4 x 10^20 liters and that there is no loss of organics within the ocean, the amino acid concentration in the ocean can reach 7 x 10^-10 kg/liter (equivalent to 7 x 10^-7 mole/liter, assuming an amino acid molecular weight of 100) in 10 million years, which is the time scale for the entire ocean to circulate through submarine vents at 300°C, potentially destroying the organisms (25).
28. It is difficult to estimate accurately how much organic material was delivered to early Earth by comets because of the large uncertainty in the impact record (37). The delivery of organic compounds by IDPs is more definitive, although still debatable. For the present Earth, the mass flux of all IDPs with particle mass lower than 10^-6 g is 10^-9 kg/year (38). It is suggested that the IDP flux at 4 Ga could be up to ~150 times that of today (34), although the interpretation of the geological record leading to this suggestion is debatable. Bearing that in mind, a reasonable estimate of the organic delivery rate by IDP at 4 Ga is in the order of ~10^-8 kg/year, assuming 10% of the mass is organic (7). The formation rate of prebiotic organic compounds in hydrothermal vents is also in the order of 10^-8 kg/year (39). Therefore, the production of prebiotic organic compounds by UV in a hydrogen-rich atmosphere is ~2 orders of magnitude greater than the delivery of organic compound from outer space or the synthesis of organic compounds in hydrothermal systems at ~3.8 Ga.
40. We thank two anonymous reviewers and J. F. Kasting for valuable comments. This paper was supported by the NASA Astrobiology Institute.

Lichen-Like Symbiosis 600 Million Years Ago
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The fossil record of fungi and lichens is scarce. Here we report the discovery of lichen-like fossils, involving filamentous hyphae closely associated with coccolid cyanobacteria or algae, preserved in marine phosphorite of the Doushantuo Formation (between 551 and 635 million years old) at Weng'an, South China. These fossils indicate that fungi developed symbiotic partnerships with photautotrophs before the evolution of vascular plants.

Fungi are a major eukaryote kingdom and perform critical ecological roles in nutrient recycling. Many living fungi maintain facultative or obligate interactions with marine and terrestrial photautotrophs (1, 2). However, the fossil record of fungi is poor and includes Ordovician [460 million years ago (Ma)] glomales (3) and microfossils interpreted as probable fungi dating to ~720 Ma (4). Fossil evidence for fungal interactions (such as cyanolichenization, mycoparasitism, and vesicular arbuscular mycorrhizal symbiosis) with other organisms comes from the ~400-million-year-old Rhynie chert in Scotland, which also preserves a diverse
fungal assemblage, including chytridiomycetes and ascomycetes (5). In addition, some Ediacara fossils (575 to 542 Ma) have been interpreted, on the basis of taphonomic observations, as fungi (6) and lichens (7).

Here we describe three specimens of lichen-like fossils occurring in thin sections of two phosphorite samples from the upper Doushantu Formation at Weng’ an, South China (8) (Fig. S1). The samples were collected from a 0.5- to 5-m-thick unit of black bituminous phosphorite immediately above a karstification surface in the middle Doushantu Formation (9). This unit was probably deposited in a shallow subtidal environment and contains abundant algal fossils (10, 11). The Doushantu Formation in the Yangtze Gorges area is bracketed by U-Pb ages between 635 ± 1 and 551 ± 1 Ma (12), and direct Pb-Pb dating of upper Doushantu phosphorite at Weng’an indicates that the fossils described here are probably 599 ± 4 million years old (13); however, Condon and colleagues argue that the fossiliferous upper Doushantu Formation may be between 580 and 551 million years old (8, 12).

The lichen-like fossils are completely phosphatized. They consist of two closely associated components: coccoidal cells and thin filaments (Figs. 1 and 2). The coccoid cells are 6 to 15 µm in diameter (average = 9 µm, SD = 2 µm, n = 25 cells) and are usually clustered (Figs. 1A, 2A, and 2C). They typically consist of an opaque central body surrounded by a hyaline envelope 1 to 2 µm thick (Fig. 2E). In some, the remains of organic sheaths are visible in the hyaline envelope. These coccoidal cells are interpreted as sheathed cyanobacteria (similar to modern Gloecapsa, Entophysalis, and Chroococcus) or possibly green algae (similar to modern colonial chlorococcales).

The filaments are about 0.5 to 0.9 µm wide (average = 0.6 µm, SD = 0.1 µm, n = 20 filaments). They are up to 50 µm long, although they may be longer, because the 30-µm-thick thin section captures only a segment of the filaments. It is unclear whether they are septate, because they are opaque. Some filaments branch dichotomously (Fig. 2, E and G). Many bear opaque, pyriform terminal structures (Fig. 2, B and D to F) that are smaller than the coccoidal cells described above, about 3 to 6 µm in maximum dimension (average = 5 µm, SD = 1 µm, n = 6 terminal structures) and 2 to 4 µm in minimum dimension (average = 3 µm, SD = 1 µm, n = 6 terminal structures). Some terminal structures show evidence of possible transverse splits (Fig. 2, D to E). A number of filaments appear to envelop coccoidal cells or are arranged in loops (Fig. 2C). In some cases, a single filament connects two pyriform structures, or a single pyriform structure is connected to multiple filamentous appendages. The filaments lack hyaline sheath-like envelopes that characterize filamentous cyanobacteria, and can be distinguished from pseudoparenchymatous multicellular algae preserved in the same deposit (10, 11). In one specimen (Fig. 1A), which was probably fragmented during post-phosphatization reworking, the filaments can be found throughout the entire specimen. In another (Fig. 1B), the filaments occur on only one side of the specimen. However, because the specimens were found in thin sections, it remains impossible to reconstruct the three-dimensional structure of the coccoidal/filament association.

We interpret these filaments as fungal hyphae and the pyriform terminal structures as resting spores, reproductive structures, or some type of fungal vesicle. Alternative interpretations (such as filamentous cyanobacteria) are inconsistent with the combination of morphological features (thin filaments, dichotomous branching, pyriform terminal structures, and absence of sheaths). The diameter of the hyphae may have been reduced during phosphatization (14), but modern marine fungal hyphae can be <1 µm in diameter (1). The pyriform terminal structures are similar to, although smaller than, modern and fossil glomalean spores or vesicles (2, 3, 15). Furthermore, glomalean (such as Entrophospora) hyphae can bear terminal sporiferous sacules and lateral spores (2), which are similar to those illustrated in Fig. 2E (white arrowheads).

It is unlikely that the fungal hyphae were saprophytic or were accidentally preserved with the coccoidal cells. In all three specimens, the hyphae are associated only with coccoidal thalli; they do not occur in pseudoparenchymatous red algae in the same deposit (Fig. 3A) (10, 11), which would be expected if they were saprophytic. Furthermore, the coccoidal cells would be expected to show a greater degree of decomposition if the fungal hyphae were saprophytic; instead, the preservation of coccoidal cells is not inferior to that of the fungal hyphae. Third, the hyphae appear to be structurally (and not accidentally) associated with the cyanobacterial coccoids; the coccoid clusters are distinctly compartmentalized and surrounded by abundant hyphae (Figs. 1A, 2A, and 2C) similar to the hyphal nets described in the Devonian cyanolichen (16, 17). This structural association make the coccoidal clusters appear different from structures described as “cell islands” in Doushantu multicellular algae (10); cell islands (Fig. 3B) are surrounded by ellipsoidal cells rather than hyphae. In addition, some hyphae are in close contact with coccoidal cells (Fig. 2, C and G), suggesting that there was direct physiological interaction between them.

The association between coccoidal cells and fungal hyphae is interpreted to be symbiotic, not parasitic. The coccoidal thalli show no evidence of host reaction to mycoparasitism. Neither do the coccoid cells in contact with hyphae show morphological abnormality. On the other hand, there are numerous similar coccoidal thalli in the same deposit that are not associated with fungal hyphae (Fig. 3B). Thus, the coccoidal thalli may have functioned as facultative photobionts that could form loose lichen-like or lichenoidal (1) association with filamentous mycobionts.

Terrestrial lichens, involving ascomycetes or basidiomycetes as mycobionts and cyanobacteria or chlorophytes as photobionts, have affected global weathering since the Devonian (5). Modern marine fungi (mostly ascomycetes) also form a wide range of interactions with cyanobacteria, chlorophytes, phaeophytes, and rhodophytes. These interactions can be loose lichenoid association with microscopic photobionts, mycophycobiosis with macroscopic algae, mycoparasitism, or obligate lichen association (1). Lichenized fungi are phylogenetically widespread within the Dikaryomycota (Ascomyota + Basidimyota), which suggests that fungal lichenization may have evolved multiple times (18-20). However, the broadly defined symbiotic life-style (including arbuscular mycorrhizal symbiosis) has a broader phylogenetic distribution and characterizes the Symbiomycota (Glomeromycota + Dikaryomycota) (21, 22). Although most glomeromycetes are arbuscular mycorrhizal fungi with vascular plants,
**References and Notes**

8. Stratigraphic information is available as supporting material on Science Online.
The Structure of a pH-Sensing Mycobacterial Adenylyl Cyclase Holoenzyme

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Class III adenylyl cyclases contain catalytic and regulatory domains, yet structural insight into their interactions is missing. We show that the mycobacterial adenylyl cyclase Rv1264 is rendered a pH sensor by its N-terminal domain. In the structure of the inhibited state, catalytic and regulatory domains share a large interface involving catalytic residues. In the structure of the active state, the two catalytic domains rotate by 55° to form two catalytic sites at their interface. Two α helices serve as molecular switches. Mutagenesis is consistent with a regulatory role of the structural transition, and we suggest that the transition is regulated by pH.

Adenylyl cyclases (ACs) synthesize the universal second messenger 3',5'-cyclic adenosine monophosphate (cAMP) (1). Most ACs belong to class III, such as all mammalian and many bacterial enzymes (2), and are multidomain proteins (2, 3). In the genome of the bacterium Mycobacterium tuberculosis (4), 15 putative class III ACs (5) with eight different domain compositions have been identified. For comparison, the similarly sized genome of Escherichia coli contains a single AC gene, and even in the human genome only 10 AC genes have been identified (6, 7). This suggests that mycobacteria can respond to changing extra- and intracellular conditions by cAMP formation.

The mycobacterial AC Rv1264 is autoinhibited by its N-terminal domain (8). A knockout of the single Streptomyces AC, which has an identical domain composition to Rv1264, abolishes the bacterial response to an acidic milieu that affects differentiation processes (9). Because M. tuberculosis must counteract acidification of phagolysosomes during host invasion for intracellular survival (10, 11), we examined the pH sensitivity of Rv1264 (Fig. 1A) (12). At pH 8, AC activity was 3 nmol of cAMP·mg⁻¹·min⁻¹ at 0.5 mM adenosine triphosphate (ATP) with a maximal velocity (V_max) of 34 nmol of cAMP·mg⁻¹·min⁻¹ and a substrate affinity (SC₅₀) of 1.5 mM ATP. At pH 6, AC activity increased almost 40-fold to 115 nmol and V_max increased 12-fold to 420 nmol of cAMP·mg⁻¹·min⁻¹. The substrate affinity increased slightly to 0.8 mM ATP. The Hill coefficient of 1.9 was unaffected. In contrast, the isolated catalytic domain (Rv1264_211-397) displayed uniformly high AC activity between pH 5.5 and 8 (Fig. 1A). Thus, in Rv1264, pH sensitivity is mediated by a distinct regulatory domain, and the activation by far exceeds the usual pH dependence of an enzyme. Biochemically, Rv1264 qualifies as a pH-sensing AC and is a likely candidate for mycobacterial pH sensing. To understand the molecular mechanism of pH sensing and AC regulation, two crystal forms of Rv1264 were analyzed (12). Anisotropic crystals in a hexagonal space group with a diffraction limit of 3.3 Å were grown from Li₂SO₄, and the resulting model was designated the active form of Rv1264 (Fig. 2A); the 2.3 Å resolution structure obtained from monoclinic crystals grown from polyethylene glycol was designated the inhibited form.

**Fig. 1.** The pH dependence of the AC activity of the Rv1264 wild type and mutants. AC activities of purified recombinant enzymes were measured from pH 4.8 to 8.0, with 0.5 mM ATP as a substrate. Standard deviation (SD) is given by error bars, if they exceed the size of the symbols. The symbol size itself corresponds to an SD of 10%. (A) Rv1264 catalytic domain (Rv1264_211-397) (●) and holoenzyme (○). To facilitate comparisons, these curves are included as dotted lines in the other panels. (B) Rv1264 M193P/M194P (●). (C) Rv1264 R309A (○) and E195A (●). (D) Rv1264 H192A (●) and H192E (○).
**Supporting Online Text**

**Lichen-like Symbiosis 600 Million Years Ago**

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**Stratigraphic Information**

The Doushantuo Formation at Weng’an is about 40 m thick and consists five units (Fig. S1), in ascending stratigraphic order, 1) 8 m-thick cap carbonate overlying glacial deposit of the Ghaub-age Nantuo Formation (S1, S2); 2) 8 m-thick, thin-bedded, peloidal phosphorite; 3) 2–4 m-thick massive dolostone with a karstification surface at its top; 4) 10 m-thick intraclastic phosphorite and dolostone; and 5) 10 m-thick phosphatic dolostone. The fourth unit begins with 0.5–5 m-thick black bituminous phosphorite (unit 4A) but gradually shifts to gray dolomitic phosphorite (unit 4B). The lichen-like fossils reported in this paper were collected from the basal part of unit 4A, which also yields abundant celluarly preserved algal fossils (S3-5).
Unit 4A also contains spherical structures interpreted as sponges (S6) and eumetazoans (S7-9); these interpretations have been questioned (S10-12). Gray dolomitic phosphorite in the lower part of unit 4B contains abundant phosphatized and secondarily concentrated animal embryos and algal fossils (S5, S13, S14).

The depositional age of Doushantuo Formation is constrained by two ash beds, in the basal and topmost Doushantuo Formation in the Yangtze Gorges area, which give two U-Pb ages of, respectively, 635±1 Ma and 551±1 Ma (S15). Doushantuo phosphorite (unit 4) at Weng’an has been dated using the Pb-Pb method and gives an age of 599±4 Ma (S16). A similar but less precise Pb-Pb age (598±26 Ma) has been obtained from the same unit at Weng’an (S17). However, Condon and colleagues (S15) argue that the diagenesis of Doushantuo phosphorite may compromise the reliability of these Pb-Pb dates, and they interpret the mid-Doushantuo karstification surface as a glacio-eustatic response to the 580 Ma Gaskiers glaciation (S18). This interpretation, which needs to be substantiated by more evidence, would suggest that the lichen-like fossils are about ~580 Ma.
Fig. S1. Geographic location and stratigraphic of the Doushantuo Formation at Weng’an. The five lithostratigraphic units of the Doushantuo Formation are labeled. Arrow points to fossil horizon. Radiometric ages—U-Pb ages from the Yangtze Gorges area (S15) and Pb-Pb ages from the Weng’an area (S16)—are marked on the
stratigraphic column based on lithostratigraphic and biostratigraphic correlation (S19, S20).

References


