Evidence for life on Earth before 3,800 million years ago

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It is unknown when life first appeared on Earth. The earliest known microfossils (~3,500 Myr before present) are structurally complex, and it is assumed that the associated organisms required a long time to develop this degree of complexity, then the existence of life much earlier than this can be argued. But the known examples of crustal rocks older than ~3,500 Myr have experienced intense metamorphism, which would have obliterated any fragile microfossils contained therein. It is therefore necessary to search for geochemical evidence of past biotic activity that has been preserved within minerals that are resistant to metamorphism. Here we report ion-microprobe measurements of the carbon-isotope composition of carbonate inclusions within grains of apatite (basic calcium phosphate) from the oldest known sediment sequences—a ~3,800-Myr-old banded iron formation from the Isua supracrustal belt, West Greenland; and a similar formation from the nearby Ailikia island that is possibly older than 3,850 Myr (ref. 3). The carbon in

for velocity measurements. For instance, attempts to record arrivals after multiple consecutive reflections have not achieved the anticipated improvement in accuracy. This can be understood in terms of interference between increasing numbers of waves.

Figure 3 shows a section of a thick cylindrical pipe containing a weld, probed by leaky waves (Fig. 3a, b). Different frequencies produce differing amounts of penetration and permit control over the depth of material that is sampled. The top surface of the sample was ground. The intensity map obtained at 15 MHz (Fig. 3c) shows no appreciable variation, consistent with the absence of stress near the surface. Homogeneity between weld and parent material is also demonstrated. The corresponding map at 2 MHz (Fig. 3b) shows the outline of the weld, because it samples down to a depth where residual stress is present. Stress throughout the bulk of the weld is confirmed in Fig. 3c, which was obtained by transmission and reflection through the thickness of the specimen, of the longitudinal wave generated in out-of-focus mode.

Many other examples are available; further information is available on the World Wide Web (http://www.ctcms.nist.gov/~kfrankii/stresses.html). Detailed theoretical analysis which we believe will enhance understanding and lead to improved technique for practical deployment is in progress.

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the carbonaceous inclusions is isotopically light, indicative of biological activity; no known abiotic process can explain the data. Unless some unknown abiotic process exists which is able both to create such isotopically light carbon and then selectively incorporate it intoapatite grains, our results provide evidence for the emergence of life on Earth by at least 3,800 Myr before present.

Because of the unique chemical properties of phosphate and its fundamental role in a large variety of biochemical processes, the participation of phosphorus in biogeochemical cycles must be a primitive phenomenon. Authigenic phosphate minerals are a significant sedimentary component of sediments. The principal constituent of modern authigenic phosphate minerals in marine sediments is carbonate (hydroxy)fluorapatite (CHFA), Ca₅(OPO₄)₃(PO₄)(OH)₂. In the marine environment, organisms participate in concentrating organic phosphate from solution and by recycling organic phosphate species from decaying P-rich bioorganic matter. Microorganisms are well known to segregate calcium from magnesium, and actively nuclease CHFA by means of specific oligopeptides. Due to this common formation of authigenic CHFA, microcrystalline aggregates ofapatite in marine sediments are intergrown with organic matter. During diagenesis the aggregates recrystallize, eventually forming single apatite crystals with inclusions of carbonaceous material, which after extensive metamorphism crystallizes to graphite.

Organisms are capable of depositing apatite outside thermodynamic equilibrium in sea water with pH < 8.5 and [Mg] : [Ca] > 0.1 (refs 10–12), which indicates the potential value of phosphate microminerals and their associated carbonaceous inclusions as indicators of biological activity in ancient sedimentary chemical precipitates, such as chert and banded iron formations (BIFs). The potential biogenic significance of the apatite alone can only be realized if the range of Mg/Ca ratios and the pH in the source solution can be estimated independently. Even in exceptional cases, BIFs have at most minor fractions of clastic apatite derived from the weathering of igneous rocks. However, such igneous apatite is free of carbon inclusions and is resistant to dissolution in natural waters; it therefore has minimal interaction with marine biogeochemical cycles and, moreover, is a relatively minor mineral constituent in most igneous rocks and their weathering detritus. But metasedimentary apatite from early Archaean BIFs, if found to contain isotopically light carbonaceous inclusions diagnostic of a biogenic origin, might be one of the few distinguishable traces of early life in the Earth’s sediments.

Carbon isotopic measurements of carbonaceous matter in sedimentary rocks have provided insights into biogeochemical pathways and the evolution of early life with or without the presence of identifiable microfossils. However, conventional methods of mass spectrometry lack the sensitivity to analyse carbonate isotopes in individual apatite inclusions which are typically ~10 μm² and contain ~20 pg carbon. The ion microprobe permits the study of isotopic variations at the scale of 10–20 μm spots. To enhance the accuracy of measurements, sputtering of each inclusion was generally continued until a large fraction of the target had been consumed. The required high sensitivity must be maintained at the relatively high mass load of 254 ions/μm² using a needle with a 4,000 nm aperture to separate interfering ¹⁴CH⁺ ions from ¹³C⁺. We have tested the hypothesis that carbonaceous inclusions contained in apatite from early Archaean sediments are biogenic by using an ion microprobe to perform in situ carbon isotope measurements of such mineral microdomains in cherts and BIFs from Western Australia (≥3,250 Myr) and from West Greenland (≥3,700 Myr).

The Pilbara craton of northwestern Australia contains well preserved volcano-sedimentary sequences with ages between ~3,000 and 3,500 Myr (refs 20, 21). Within the Warrawoona Group, cherts from the Apex Basalt (3,450 ± 16 Myr) contain the oldest microfossils yet identified, of which resemble extant chemosynthetic and photosynthetic prokaryotic morphotypes. Whole-rock carbon isotope ratios of kerogen in the Warrawoona sediments have been interpreted to infer that photosynthesizing, or even cyanobacterium-like, organisms were already active by 3,500 Myr (refs 1, 2, 16–19). The apatite intergrowths with organic matter we report here are from lower greenstreak facies chert of the (>3,250 Myr) Nickol Well unit of the Roeboorne belt, west Pilbara Archean succession. (R. Buick, personal communication).

The Isa supracrustal belt in West Greenland contains large volumes of early Archaean BIF and meta-chem. As BIFs are of sedimentary origin, these rocks are at least as old as their metamorphic age of 3,700 Myr. Isa rocks used in this study have been metamorphosed to amphibolite facies; details of the mineralogy and petrographic relationships of the Isa rocks have been given elsewhere. An early Archaean BIF encompassed within a layered amphi-
FIG. 2 Isotope compositions of carbonaceous inclusions in individualapatite grains from early Archaean sediments measured by ion microprobe. a, West Pilbara sediments, Roebourne Belt, Western Australia (3,250 Myr), samples courtesy of K. Sugitani; the data indicated by ‘2’ are previous whole-rock measurements of Warrawoona Group sediments for comparison; b, Isua supracrustal belt BIF (>3,700 Myr; field sample no. 3381, Isua) and West Greenland; courtesy of E.J. Robbins and P.W.U. Appel; ‘2’ and ‘3’ indicate respectively previously whole-rock measurements reported by Schilder et al. and Hayes and BIF from Akilia island (>3,850 Myr) in southern West Greenland. c, Carbon isotope variations found in nature. Standard deviations for the ion microprobe data are indicated by the vertical lines (1σ). Dotted lines above and below the weighted means of the data correspond to the 2σ confidence interval.

METHODS. Cleaned rock chips, taken several centimetres away from weathering surfaces and free from cracks, were cored to yield 25-mm-diameter rock disks of 5-mm thickness. These were purified in distilled water and drilled with an ultrasonic microcorder to produce a 3-mm-diameter hole at their centres, then sonically cleaned in successive ethanol and ultrapure water baths before being dried in air. Plugs 3 mm in diameter of pelletized USGS 24 graphite standard (δ¹³C carbo = −16.0%) were inserted into the central hole and the section was then Au-coated. Carbon isotope inclusion in the apatite were sputtered with a focused ion beam in the CAMECA ims1270 ion microprobe at UCLA, and charge compensation during negative ion extraction was maintained using a normal-incidence electron gun. Carbon isotope measurements were performed by standard ion microprobe techniques utilizing magnetic peak switching at high mass-resolving power and ion counting with an electron multiplier. The carbon isotope ratios, corrected for deadtime and instrumental mass fractionation, are reported relative to the VPDB standard using the conventional delta notation. The mass-fractionation correction procedure assumes that the degree of bias for the lighter isotope inherent in the sputtering process is the same for the carbonaceous inclusions in the apatite as for the graphite standard. Measurements on a suite of kerogen samples differing by a factor of ~7 in δC/H ratio show that the effect of such structural and compositional variations between the standard and the sample is small (<2σ) in agreement with earlier, less precise findings. The inorganic carbon field is the region of carbon isotopic compositions defined as characteristic of inorganic carbonaceous material.

bolite and ultramafic complex on Akilia island, southern West Greenland, is cut by a deformed quartz-diorite sheet dated at 3,860 ± 10 Myr (ref. 3), providing a possible minimum age for the transected sedimentary unit. The sample used in this study (G91-26) comes from a well-preserved layer of BIF consisting of quartz (50%), clinopyroxene (25%), orthopyroxene (20%), amphibole (15%), magnetite (5%), sulphides (1%) and other minerals (<1%) including apatite, but no observable carbonate. The unit meets the criteria of James (ref. 26) for a silicate facies to low-grade oxide facies BIF. Anhedral oblate to lozenge-shaped apatite grains, occurring either individually or in groups, and resembling those found in the younger Pilbara cherts and Isua supracrustal belt samples cited above, are typically 10–15 μm in diameter and 30–40 μm in length (Fig. 1), and frequently contain inclusions and envelopes of graphitized carbon. Apatite grains occur in the pyroxenes, quartz, amphibole and (rarely) in magnetite, and when found in groups, are present as trains parallel to banding. In contrast, ~3,860–3,870 Myr orthogneisses from the same locality, and transsecting and encompassing the supracrustals, contain igneous apatites that are devoid of graphite inclusions, are compositionally distinct, and are associated with common igneous phases such as feldspar not found in chemically precipitated sediments like BIF.

The stable isotopes of carbon are partitioned as a result of both equilibrium exchange reactions and kinetic effects, which are due to metabolic mechanisms as well as inorganic processes such as evaporation, diffusion and condensation. Kinetic isotopic fractionation between organic and inorganic carbon results in marked enrichment of the light isotope in the biogenic component by several per cent (refs 16, 17). Hence, biogenic materials, including carbonaceous fossils, are typically characterized by δ¹³C values of −20 to −35‰ in the case of most photoautotrophic bacteria, and can be as light as −50 or −60‰ for products of microbial communities apparently involved in the recycling of methane. In contrast, inorganic carbon is usually heavier than about −10‰, with a typical range between −5 to −5‰ (refs 16, 17).

In situ ion-microprobe measurements of occluded carbon in apatite micrograins from the Akilia island BIF yield a range of δ¹³C values from −21(±2)% to −49(±7)% with a weighted mean of −37(±3)% (Fig. 2). Because of the micrometre size of the irregular samples embedded in apatite, the precision and accuracy of individual measurements are typically ±5% (1σ) and encompass counting statistics plus an extra component for fluctuation of count-rates during analysis. Isotope results for carbonaceous inclusions in the Pilbara sediments and Isua BIF yield weighted means of −26(±3)% and −30(±3)% respectively. The results for the Pilbara sediments agree with previous whole-rock values obtained by conventional mass-spectrometric techniques for Warrawoona Group sediments. All measured values from our early Archaean apatite inclusions are well resolved from what are generally considered to be inorganic carbon values.

To evaluate the presence of life in the previously oldest known sedimentary rocks, carbon isotope ratios were measured in acid insoluble carbonaceous residues (kerogens) of bulk samples from the BIF. These measurements yielded mean δ¹³C values of −11 to −15 (±5‰) that have been interpreted as indicating photoautotrophic carbon fixation. However, these previous values are close to the range of inorganic carbon δ¹³C ratios, possibly owing to isotopic exchange with
carbonate carbon present in the Isua rocks during metamorphism, so they have been regarded as ambiguous. Some carbon in igneous rocks is observed to have intermediate isotope ratios in the range of −10 to −20‰, but these could reflect biogenic contamination from assimilated sediments. Miller–Urey spark-discharge laboratory experiments, carried out to simulate hypothetical hydrogen-rich primitive Earth atmospheres, yield organic bulk reaction products which are isotopically heavier than −10‰ and that could not have contributed to the carbon in the apatites. We can rule out reduced carbon from carbonaceous meteorites (the richest contain −3 mass% reduced C), as carbon isotope ratios for these generally cluster at −11 to −18‰ and there is no reason to expect meteoritic carbon to be selectively associated with apatite in BIF. Regardless of the modes of origin for the carbon components in the various materials mentioned above, the δ13C values for the carbon inclusions in apatite are 10–15‰ lighter than the δ13C values seen in such biogenic samples, and are characteristic of the range of carbon isotope compositions for biogenic matter (Fig. 2).

For strongly negative carbon isotope values in metamorphosed sediments to be convincingly interpreted as unaltered products of biogenic fractionation, it is necessary to analyse the magnitude and sign of such effects that could have perturbed an original distribution. Empirical studies have shown that the loss process of CO2 from the oxidation of organic matter is kinetically controlled, and the evolved CO2 is isotopically lighter than the source organic carbon. Hence, loss of volatiles from thermally degrading organic matter leads to the residual organic matter being isotopically enriched in 13C. However, to investigate theoretical scenarios where progressive thermal metamorphism in principle could lead to enrichment of residual carbon in 13C, we evaluated possible changes in the δ13C value of organic matter included in apatite under both thermodynamically open-system (Rayleigh) and closed-system (single-step) behaviours. Depending on the oxygen fugacity of the system, metamorphism can result in the release from carbonaceous matter of different proportions of CO2 and CH4 fluids, but a loss of methane which partitions the light isotope can never produce isotopic compositions lighter than the starting materials. On the other hand, the escape (during diagenesis and metamorphism) of isotopically heavy CO2 evolved from the organic matter trapped within the apatites could, in such a theoretical model, drive the residue to lighter isotopic values. The most extreme isotopic shifts would result from a Rayleigh distillation process, which assumes a continuously reactive (graphitic) residue and immediate removal of CO2 from the system. The progressive change in 13C/12C of the graphite residue by such a Rayleigh-type process is shown in Fig. 3 as a function of the fraction of carbonaceous matter consumed in degassing to either CO2 or CH4 in the apatite. This figure shows that, for any degree of degassing and without consuming all of the carbon inclusion in the process, it is not possible to generate a final value (C) of δ13C = −35‰ from an inorganic material (C) with δ13C > −10‰. In fact the thermodynamically determined Rayleigh evolution lines do not intersect the inorganic carbon field except toward the limit of F = 1/∞, where F is the mole fraction of remaining carbon. We assert that neither by kinetic nor by thermodynamic arguments, can loss of volatiles by thermal degradation of organic matter modify isotopically heavy abiotic reduced carbon to make is resemble biogenic organic carbon.

In the CHFA mineral structure, carbonate primarily substitutes for [PO4]3− and less frequently for [OH]−. Thermally induced decarbonation of CHFA occurs between 400 and 800 °C via the decomposition reaction of structural carbonate: 2[CO3]2− + [CO2]− + [F−] → 3[CO2]− + [F−]. With the formation of stable fluorapatite. However, there is no known mechanism that can reduce structural CO2− in CHFA, or its decarbonation product CO2, at low partial pressure of hydrogen, to produce carbonaceous inclusions in apatite. Any such hypothetical carbon formed by reduction of carbonate or the evolved CO2 would remain within the inorganic field of carbon isotope ratios anyway. Moreover, a simple mass-balance calculation shows that even 100% efficiency of such an assumed carbonate-reduction process could not supply the observed volume of carbonaceous inclusion contained in each of the apatites. The association of apatite with carbon is observed in the Pilbara sediments as well as in other younger, unaltered modern sediments, and in laboratory cultures of microorganisms; these features cannot be explained by metamorphism. We therefore conclude that metamorphic effect
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are not responsible for the association of isotopically light carbonic anhydride inclusions in metasedimentaryapatite.

Together with the intergrowth of carbonatic material withapatite in BIF from Akilia island, we conclude that the isotopic results reported here give strong evidence for life on Earth by 3,850 Myr. Although this finding pushes back the horizon for the emergence of life by 300–400 million years, it is not entirely unexpected, given also the apparently evolved nature of lifeforms at ~3,500 Myr. However, the ‘late heavy bombardment’ (>3,800 Myr), documented in the lunar record, has been speculated to place an upper limit on the age of a continuous terrestrial biosphere. The evidence for life presented here overlaps this critical time period and shows that if the accretion models are realistic, such a bombardment did not lead either to the extinction of life or the perturbation of the finely laminated >3,850-Myr BIF preserved on Akilia island.

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Activation of floral meristem identity genes in Arabidopsis

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The Arabidopsis floral meristem-identity genes APETALA1 (AP1) and LEAFY (LFY) confer floral identity on developing floral primordia1–4, whereas TERMINAL FLOWER (TFL) is required to repress their expression within shoot and inflorescence meristems5–7. LFY and AP1 are expressed in floral primordia in response to environmental conditions, such as day length, which regulate the onset of flowering and simultaneously also in response to the action of genes that influence flowering time. However, the relationship between these flowering-time genes and the floral meristem-identity genes has been difficult to assess because flowering time is determined by several interacting genetic pathways5–7. Here we describe a method to regulate expression of the flowering-time gene CONSTANS (CO) and demonstrate that CO expression is sufficient to trigger flowering, irrespective of day length. In response to CO expression, transcription of LFY and TFL is initiated rapidly, whereas transcription of AP1 occurs much later. We propose that CO acts within a genetic pathway that is sufficient to activate LFY and TFL transcription, but that rapid activation of AP1 requires an additional pathway.

Flowering of Arabidopsis occurs rapidly under long days (LDs) containing 16 hours light, and is delayed under short days (SDs) of 10 hours light (Fig. 1). Over 20 mutations that delay flowering under LDs have been described. The genes affected in these

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mutants have been placed in at least three pathways based upon double-mutant phenotypes and the response of the mutants to environmental conditions5–7. One of the pathways is required to promote flowering in response to daylength, and includes the CO gene. Mutations in CO delay flowering under LDs, but have no effect under SDs8–10, suggesting that CO promotes flowering in response to LDs. Flowering time is likely to be mediated, at least in part, by transcriptional regulation of CO, because CO messenger RNA is more abundant under LDs than SDs10. Furthermore, the CO mRNA is rare, and reducing CO gene dosage in heterozygous plants or increasing CO dosage in transgenic plants delays or promotes flowering respectively.

To examine the effect of CO expression on floral meristem-identity gene transcription and on the regulation of flowering by day length, we constructed a transgene designed to express the CO mRNA at high levels independently of day length, and to permit the activation of CO function at different stages of development. A transcriptional fusion between the CO complementary DNA and a strong viral promoter (cauliflower mosaic virus (CaMV) 35S) was used to increase expression of the mRNA. Furthermore, in order to regulate activity of the CO protein, the translational stop codon of CO was removed and replaced with a 287-amino-acid segment of the rat glucocorticoid receptor. This segment can inactivate plant transcription factors in the absence of the steroid ligand, but activity of the protein is restored in the presence of the ligand dexamethasone11–12. Transgenic co-2 mutant plants containing the gene fusion between CO and the glucocorticoid receptor (CO-GR) were constructed.

To assess the effect of CO-GR on flowering time, the response to applications of dexamethasone at different stages of development was measured in detail for plants grown under both LDs and SDs (Figs 1 and 2). Dexamethasone was supplied through the soil to the roots of the plants at various times from day 0 until 26 days after sowing (see Methods). This treatment had no effect on the flowering time or morphology of wild-type plants, nor of co-2