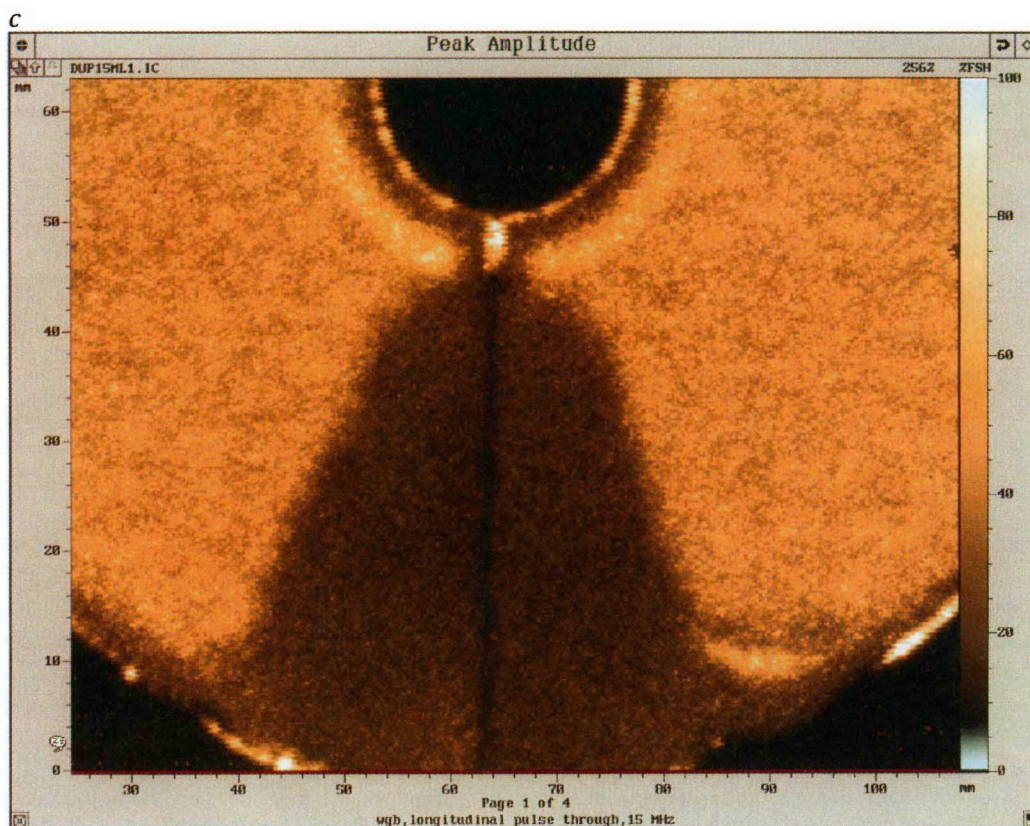




for velocity measurements. For instance, attempts to record arrivals after multiple consecutive reflections have not achieved the anticipated improvement in accuracy⁴. This can be understood in terms of interference between increasing numbers of waves.

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. 3a) 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/~kfrankli/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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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 if 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^{1,2}. 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 carbonaceous 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 Greenland³⁵, and a similar formation from the nearby Akilia island that is possibly older than 3,850 Myr (ref. 3). The carbon in

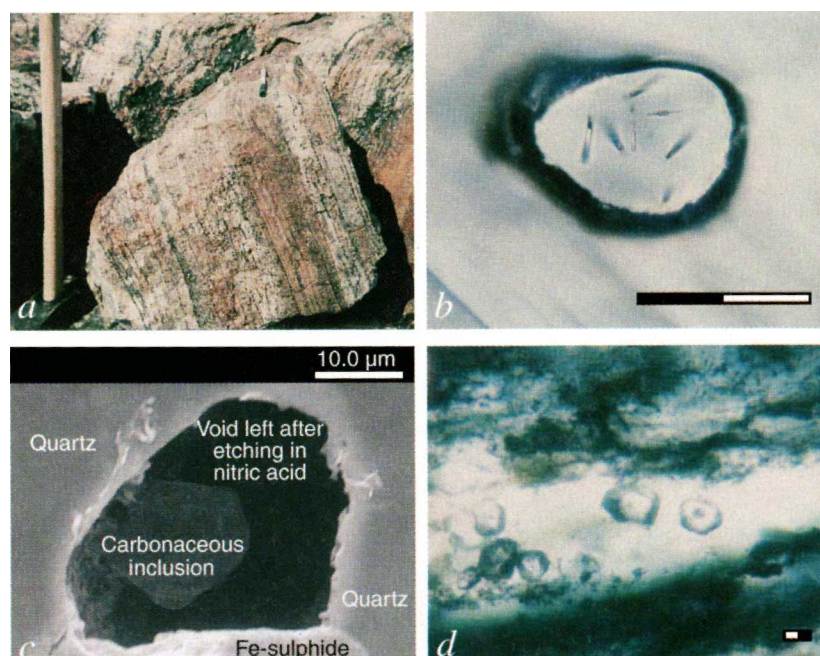


FIG. 1 a, Field exposure of the $\geq 3,850$ -yr-old Akilia island BIF³ (collected 150 km south of Isua, 63° 55' 40" N, 51° 41' 30" W; photograph by A. Nutman) in southern West Greenland. Finely alternating bands of magnetite (dark) and silicates (light) are evident on the broken-off boulder which is part of a ~ 5 -m-thick section of BIF (background) on the island; the rock hammer is ~ 40 cm tall. b–d, micrographs of anhedral, oblate apatite grains and associated carbon in early Archaean BIF. b, View of an apatite crystal in amphibole (grunerite), from the Akilia island BIF. The apatite was etched in 2% HNO₃ (120 s at room temperature) to uncover an envelope of opaque carbonaceous matter at the grain boundary; linear features in the crystal are fission tracks from the decay of intrinsic radionuclides revealed by the etching process (b and d are optical micrographs in transmitted light, oil immersion lens, plane polarized). c, Scanning electron micrograph of void left after treating apatite in 2% HNO₃ (1,800 s at room temperature), revealing an acid-resistant carbonaceous inclusion (centre) typical of those analysed by ion microprobe. d, Quartzitic microband from the Pilbara craton sediments, Western Australia, containing groupings of apatites with cores of organic matter along thin laminae of organic rich chert. Scale bars in b and d, 20 μ m; scale bar in c, 10 μ m.

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 into apatite 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 processes⁴, the participation of phosphorus in biogeochemical cycles must be a primitive phenomenon. Authigenic phosphate minerals are a significant sedimentary component of sediments^{5–8}. The principal constituent of modern authigenic phosphate minerals in marine sediments is carbonate (hydroxy)fluorapatite (CHFA), $\text{Ca}_{10}(\text{PO}_4)_6-x(\text{CO}_3)_x(\text{F}, \text{OH})_{2+x}$. In the marine environment, organisms participate in concentrating organic phosphate from solution and by recycling organic phosphate species from decaying P-rich bioorganic matter^{7–11}. Microorganisms are well known to segregate calcium from magnesium, and actively nucleate CHFA by means of specific oligopeptides^{8–10}. Due to this common formation of authigenic CHFA, microcrystalline aggregates of apatite in modern as well as ancient sediments characteristically are intergrown with organic matter^{6–11}. 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 $\text{pH} < 8.5$ and $[\text{Mg}]:[\text{Ca}] > 0.1$ (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)¹¹. 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^{13,14}. 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 bioorganic origin, might be one of the few distinguishable traces of early life in the Earth's sediments.

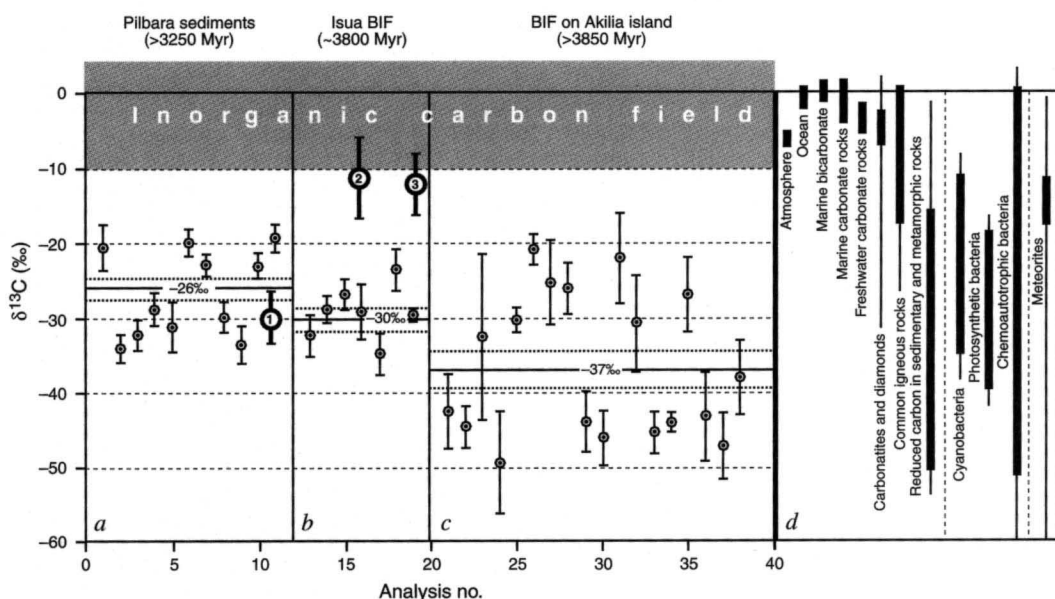
Carbon isotopic measurements of carbonaceous matter in sedimentary rocks have provided insights into bioorganic pathways and the evolution of early life with or without the presence of identifiable microfossils^{1,2,15–18}. However, conventional methods of mass spectrometry lack the sensitivity to analyse carbon isotopes in individual apatite inclusions which are typically $\sim 10 \mu\text{m}^3$ and contain ~ 20 pg carbon. The ion microprobe permits the study of isotopic variations at the scale of 10–20 μm spots²⁰. 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 resolving power ($M/\Delta M \approx 4,000$) necessary to separate interfering $^{12}\text{CH}^-$ ions from $^{13}\text{C}^-$. 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 ($\geq 3,250$ Myr) and from West Greenland ($\geq 3,700$ Myr).

The Pilbara craton of northwestern Western Australia contains well preserved volcano-sedimentary sequences with ages between $\sim 3,000$ and 3,500 Myr (refs 20, 21). Within the Warrawoona Group, cherts from the Apex Basalt ($3,450 \pm 16$ Myr)²² contain the oldest microfossils yet identified, some of which resemble extant chemoautotrophic and photoautotrophic prokaryotic morphotypes^{1,2}. 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 greenschist facies chert of the ($> 3,250$ Myr) Nickol Well unit of the Roebourne belt, west Pilbara Archaean succession²² (R. Buick, personal communication).

The Isua supracrustal belt in West Greenland contains large volumes of early Archaean BIF and meta-chert^{14,23–25,35}. As BIFs are of sedimentary origin, these rocks are at least as old as their metamorphic age of 3,700 Myr^{3,23–26,35}. Isua rocks used in this study have been metamorphosed to amphibolite facies; details of the mineralogy and petrographic relationships of the Isua rocks have been given elsewhere^{23,24,35}.

An early Archaean BIF encompassed within a layered amphi-

FIG. 2 Isotope compositions of carbonaceous inclusions in individual apatite 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 '1' are previous whole-rock measurements^{15–17} of Warrawoona Group sediments for comparison; **b**, Isua supracrustal belt BIF ($>3,700$ Myr; field sample no. 3381, Isukasia, West Greenland; courtesy of E.I. Robbins and P.W.U. Appel); '2' and '3' indicate respectively previously whole-rock measurements reported by Schidlowski *et al.*¹⁸ and Hayes¹⁹. **c**, BIF from Akilia island ($\geq 3,850$ Myr) in southern West Greenland³. **d**, 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 polished with alumina powder in distilled water and drilled with an ultrasonic microcorer 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 ($\delta^{13}\text{C}_{\text{PDB}} = -16.0\text{‰}$) were inserted into the central hole and the section was then Au-coated. Carbonaceous inclusions in the apatite were sputtered by a focused Cs^+ beam in the CAMECA ims1270 ion microprobe at UCLA, and charge compensation during negative ion extraction was maintained by using a normal-incidence electron gun. Carbon isotopic measurements were per-

formed by standard ion microprobe techniques^{19,32} 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 H/C ratio show that the effect of such structural and compositional variations between the standard and the sample is small ($<2\text{‰}$) in agreement with earlier, less precise findings³³. The inorganic carbon field is the region of carbon isotopic compositions defined as characteristic of inorganic carbonate carbon and non-bioorganic reduced carbon.

bolite and ultramafic complex on Akilia island, southern West Greenland³⁴, is cut by a deformed quartz-dioritic sheet dated at $3,860 \pm 10$ 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 (30%), clinopyroxene (25%), orthopyroxene (20%), amphibole (15%), magnetite (5%), iron sulphides ($\sim 1\%$) 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 $10\text{--}15\text{ }\mu\text{m}$ in diameter and $30\text{--}40\text{ }\mu\text{m}$ 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, $\sim 3,860\text{--}3,870$ Myr orthogneisses from the same locality³, and transecting 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 bioorganic component by several per cent (refs 16, 17). Hence, bioorganic materials,

including carbonaceous fossils, are typically characterized^{15–18} by $\delta^{13}\text{C}$ values of -20 to -35‰ in the case of most photoautotrophic bacteria, and can be^{16–18} 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 $\delta^{13}\text{C}$ values from $-21(\pm 2)\text{‰}$ to $-49(\pm 7)\text{‰}$, with a weighted mean of $-37(\pm 3)\text{‰}$ (Fig. 2). Because of the micrometre size of the irregular samples embedded in apatite, the precision and accuracy of individual measurements are typically $\pm 5\text{‰}$ (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 $-26(\pm 3)\text{‰}$ and $-30(\pm 3)\text{‰}$, respectively. The results for the Pilbara sediments agree with previous whole-rock values obtained by conventional mass-spectrometric techniques for Warrawoona Group sediments^{16–18}. 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 $\geq 3,700$ -Myr Isua supracrustal belt BIF¹⁸. These measurements yielded mean $\delta^{13}\text{C}$ values of -11 to $-15(\pm 5)\text{‰}$ that have been interpreted as indicating photoautotrophic carbon fixation. However, these previous values are close to the range of inorganic carbon $\delta^{13}\text{C}$ ratios, possibly owing to isotopic exchange with

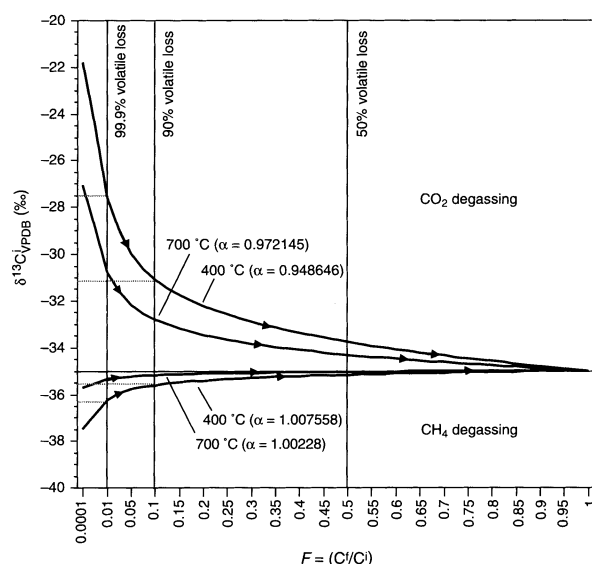


FIG. 3 Theoretical changes in the $\delta^{13}\text{C}$ values of organic carbon by degassing of CO_2 and CH_4 using a Rayleigh distillation process (open-system behaviour)³⁴ $\delta^{13}\text{C}^f = [\delta^{13}\text{C}^i / F^{(\alpha-1)}]$ which assumes a continuously reactive (graphitic) residue and immediate loss of gas from the system with lack of continuous isotopic equilibrium between the evolved gas and the residue. The F -values are the mole ratios of the final residuum (C^f ; carbon inclusions) to the initial carbon contents of the apatites (C^i), where $\delta^{13}\text{C}^f$ is

the final value of the organic carbon inclusions as measured *in situ* in the apatites. The α -values ($\alpha = K_B/K_A$, which is the ratio of the equilibrium constants of isotopic exchange reactions at a given temperature) for the equilibrium isotopic fractionation factors between the evolved species CH_4 (graphite-methane; $\text{C}-\text{CH}_4$), CO_2 ($\text{C}-\text{CO}_2$) versus graphite (C) at temperatures between 400 and 700 °C, the thermal metamorphic conditions experienced by the Akilia island BIF³⁴, are based on thermodynamical calculations^{33,36,37}. Depending on the oxygen fugacity (f_{O_2}) of the system, metamorphism can result in the release of different proportions of CO_2 and CH_4 . In both the CO_2 and CH_4 degassing, the isotopic ratio of the inclusion ('residue') would tend to increase or decrease depending on whether the expelled fluid preferentially partitions the light isotope (CH_4) or the heavy isotope (CO_2). The $[\text{CO}_2/\text{CH}_4]$ ratio of evolved fluids from graphite tends to increase with increasing temperature and/or decreasing pressure because of the reaction $2\text{C} + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{CH}_4$. Under high f_{O_2} conditions and at high temperatures, graphite reacting to form CO_2 could yield in the thermodynamical model an isotopically lighter residue during progressive metamorphism; the graphite content in the apatite would tend in all of these cases to continually decrease. Assigning $\delta^{13}\text{C}^i = -35\text{‰}$, the figure demonstrates that even under these hypothetical and extreme conditions resulting in the loss of CO_2 of 99.9% ($F = 0.001$) of the original carbon contained in the apatites, derivation of $\delta^{13}\text{C}^f$ from the inorganic isotopic field ($+5\text{‰} \geq \delta^{13}\text{C}^f \geq -10\text{‰}$) cannot be achieved, rather the range obtained is $-27.5\text{‰} \geq \delta^{13}\text{C}^f \geq -36.5\text{‰}$. A theoretical F -value of 2.54×10^{-11} is required to go from $\delta^{13}\text{C}^i = -10\text{‰}$ (the lowermost bounds of the inorganic field in Fig. 2) to $\delta^{13}\text{C}^f = -35\text{‰}$. These calculations provide extremes in both starting compositions and open-system behaviour of the carbonaceous inclusions during metamorphism. A kinetic model would give results that only increase the $^{13}\text{C}/^{12}\text{C}$ ratio, and lead to $\delta^{13}\text{C}^f$ values $\geq \delta^{13}\text{C}^i$. The x -axis reflects a scale change in F from linear ($F = 1.0-0.1$) to nonlinear ($F = 0.1-0.0001$) as a means of highlighting changes in $\delta^{13}\text{C}^f$ under extreme conditions of conversion of graphite to CO_2 or CH_4 .

carbonate carbon present in the Isua rocks during metamorphism, so they have been regarded as ambiguous¹. Some carbon in igneous rocks is observed to have intermediate isotope ratios in the range of -10 to -20‰ , but these could reflect bioorganic contamination from assimilated sediments²⁷. Miller-Urey spark-discharge laboratory experiments, carried out to simulate hypothetical hydrogen-rich primitive Earth atmospheres, yield organic bulk reaction products which are²⁸ 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 at²⁹ -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 $\delta^{13}\text{C}$ values for the carbon inclusions in apatite are 10 – 15‰ lighter still than the $\delta^{13}\text{C}$ values seen in such abiogenic samples, and are characteristic of the range of carbon isotopic compositions for bioorganic matter (Fig. 2).

For strongly negative carbon isotope values in metamorphosed sediments to be convincingly interpreted as unaltered products of bioorganic 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 CO_2 from the oxidation of organic matter is kinetically controlled, and the evolved CO_2 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 ^{13}C . However, to investigate theoretical scenarios where progressive thermal metamorphism in principle could lead to enrichment of residual carbon in ^{12}C , we evaluated possible changes in the $\delta^{13}\text{C}$ 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 CO_2 and CH_4 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 dia-

genesis and metamorphism) of isotopically heavy CO_2 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 CO_2 from the system. The progressive change in $^{13}\text{C}/^{12}\text{C}$ 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 CO_2 or CH_4 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^f) of $\delta^{13}\text{C}^f = -35\text{‰}$ from an inorganic initial material (C^i) with $\delta^{13}\text{C}^i > -10\text{‰}$. In fact the thermodynamically determined Rayleigh evolution lines do not intersect the inorganic carbon field except toward the limit of $F = 1/\infty$, 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 abiogenic reduced carbon to make it resemble biogenic organic carbon.

In the CHFA mineral structure, carbonate primarily substitutes for $[\text{PO}_4^{3-}]$, and less frequently for $[\text{OH}^-]$. Thermally induced decarbonation of CHFA occurs between 400 and 800 °C via the decarboxylation of structural CO_3^{2-} , and possibly of $[\text{CO}_3^{2-}, \text{OH}^-]$ and $[\text{CO}_3^{2-}, \text{F}^-]$ ion pairs³⁰ leading to the formation of stable fluorapatite. However, there is no known mechanism that can reduce structural CO_3^{2-} in CHFA, or its decarbonation product CO_2 , at low partial pressure of hydrogen, to produce carbonaceous inclusions in apatite. Any such hypothetical carbon formed by reduction of carbonate or the evolved CO_2 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

are not responsible for the association of isotopically light carbonaceous inclusions in metasedimentary apatite.

Together with the intergrowth of carbonaceous matter with apatite 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 unexpected¹, 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 biosphere³¹. 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* (*API*) and *LEAFY* (*LFY*) confer floral identity on developing floral primordia^{1,4}, whereas *TERMINAL FLOWER* (*TFL*) is required to repress their expression within shoot and inflorescence meristems^{1,5}. *LFY* and *API* are expressed in floral primordia in response to environmental conditions, such as day length, which regulate the onset of flowering, and presumably 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 pathways^{6,7}. 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 *API* 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 *API* 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

mutants have been placed in at least three pathways based upon double-mutant phenotypes and the response of the mutants to environmental conditions^{6,7}. 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 SDs^{8–10}, 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 plants⁹ or increasing *CO* dosage in transgenic plants¹⁰ 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 dexamethasone^{11,12}. 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*

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