Reprint Series
10 July 1992, Volume 257, pp. 232–235

SCIENCE

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Hundreds of specimens of spirally coiled, megascopic, carbonaceous fossils resembling *Grypania spiralis* (Walcott), have been found in the 2.1-billion-year-old Negaunee Iron-Formation at the Empire Mining Partnership in Michigan. This occurrence of *Grypania* is 700 million to 1000 million years older than fossils from previously known sites in Montana, China, and India. As *Grypania* appears to have been a photosynthetic alga, this discovery places the origin of organelle-bearing eukaryotic cells prior to 2.1 billion years ago.

Megascopic fossils resembling *Grypania spiralis* (Walcott) from the late Proterozoic of Montana (*1*, *2*), China (*2*, *3*), and India (*4*) occur within the lower part of the Negaunee Iron-Formation (IF), northern Michigan (Figs. 1 and 2). The Negaunee IF (Marquette Range) and correlative Biwabik IF (Mesabi Range) have given an isotopic date (Sm-Nd isochron) of 2110 ± 52 million years (*5*) and are early Proterozoic in age. This new horizon predates previously known occurrences of *Grypania* by 700 to 1000 million years; the fossils are the oldest known remains of megascopic organisms.

Grypania spiralis was a corkscrew-shaped, spaghetti-like organism that grew to maximum size of about half a meter in length and 2 mm in diameter (Fig. 3E). It is normally preserved as unbranched, ribbon-like films or impressions on bedding planes, but a unique specimen from India illustrated by Beer (*6*) is uncompacted, showing

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that *Grypania* was originally circular in cross section. Rarely seen terminations are rounded; well-preserved specimens from India and China have transverse markings (*2*, *4*), which are best interpreted as the trace of spiral elements within the wall of the organism (*7*). Thus the corkscrew shape of *Grypania* appears to have been due to supercoiling that was maintained in life and death by helical filaments within the body wall. As a result, *Grypania* is almost always preserved as a compressed coil, sinuous ribbon, or cuspate ribbon. Walter *et al.* (*2*) regarded the cuspate ribbons as specimens of *Grypania* that had been stretched by currents; it was therefore thought that *Grypania* might have been tethered during life.

The Negaunee fossils are from thinly bedded magnetite-carbonate-silicate-chert IF (silicate horizon) in the Empire Mine (*8*). The fossiliferous zone is exposed on the east side of the pit; it is about 335 m above sea level and about 180 to 210 m stratigraphically above the base of the Negaunee IF (Fig. 2). The fossils occur on top of and at the base of 1- to 10-mm-thick magnetite-rich layers. They are abundant, carbonaceous in composition, and are of two main types: coiled, thin filaments resembling *Grypania spiralis* from Montana (Fig. 3, A to D); and coiled, thicker forms (Fig. 1) that may represent a new genus or species. The thinner forms are coiled or curved, smooth, uniformly wide filaments, 0.7 to 1.1 mm in width, and up to 90 mm in length. Many are preserved as tightly wound coils of three or fewer turns but on some surfaces the filaments lie in irregular, sinuous or cusp-shaped curves (some bedding surfaces are covered with fragments of many different sizes). The coils are typically oval in shape due to post mortem deformation, from 5 to 30 mm in average diameter, and of various sizes on the same surface. Terminations are smoothly rounded except where obviously torn. The thicker forms are wider (about 1.5 mm), more tightly coiled (5 to 9 mm in diameter) and shorter (up to 30 mm), but in other respects are not obviously different from the thinner forms. Neither type shows evidence of transverse markings.

Grypania has no certain living relatives but is regarded as a probable eukaryotic alga because of its complexity, structural rigidity, and large size. Spiral-shaped fossil cyanobacteria known as *Obruchevella* and *Spirellus* are widespread in late Proterozoic and early Cambrian strata (*9*) but even the exceptionally large Cambrian forms (*Spirellus*) are orders of magnitude smaller than *Grypania* (Fig. 4). Similarly, it is unlikely that *Grypania* was the abandoned giant sheath of a sulfide-oxidizing bacterium (Beggiatoaceae) as has been suggested for late Proterozoic carbonaceous megafossils known as vendotaenids (*10*); the largest known bacterial sheaths (0.5 mm in diameter) are narrower than large specimens of *Grypania* and they lack rounded ends, a coiled morphology, and transverse structures (*11*). A third possibility, that *Grypania* represents an aggregate of bacterial or cyanobacterial filaments, may also be discarded because even man-made bacterial fibers are less well organized and narrower than *Grypania* (*12*). Natural aggregates of filaments (for example, *Nostoc* colonies) may be cylindrical in form and up to 0.5 mm in width, but they lack structures that could maintain a coiled morphology.

The best modern analog for *Grypania* may be the giant unicellular dasycladacean alga *Acetabularia* (*13*). Before the formation of an umbrella-shaped reproductive cap, *Acetabularia* grows as a narrow cylinder about 0.4 mm in diameter and up to 180 mm in length. The single nucleus remains within a holdfast; there is a large, central, sap-filled vacuole that occupies most of the stalk of the alga so that the cytoplasm, which contains numerous chloroplasts and mitochondria, is restricted to the periphery. Although *Acetabularia* is unicellular, its close relatives have a coenocytic organization, as might have *Grypania*. In summary, *Grypania* is interpreted as a sessile, eukaryotic alga that may have been unicellular but is more likely to have been either multinucleate or multicellular. Few uninucleate organisms have achieved the cytoplasmic volume (~1.5 ml) of Indian specimens of *Grypania* (Fig. 3E). As *Grypania* has no obvious modern counterpart, it may belong to an extinct algal group.

Phylogenetic trees constructed from the sequences of genes that were duplicated before the existence of the latest common ancestor of all living organisms have re-

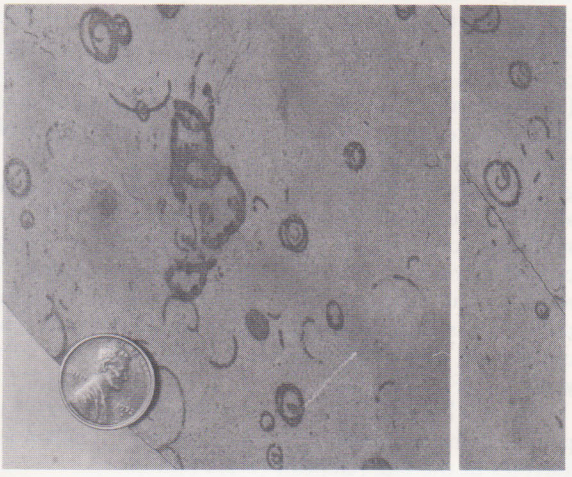

Fig. 1. Bed surface of Negaunee Iron-Formation with numerous fragments of *Grypania* and some thicker filaments. Line represents 2-cm-wide strip of unfossiliferous rock; coin is 18.5 mm in diameter.

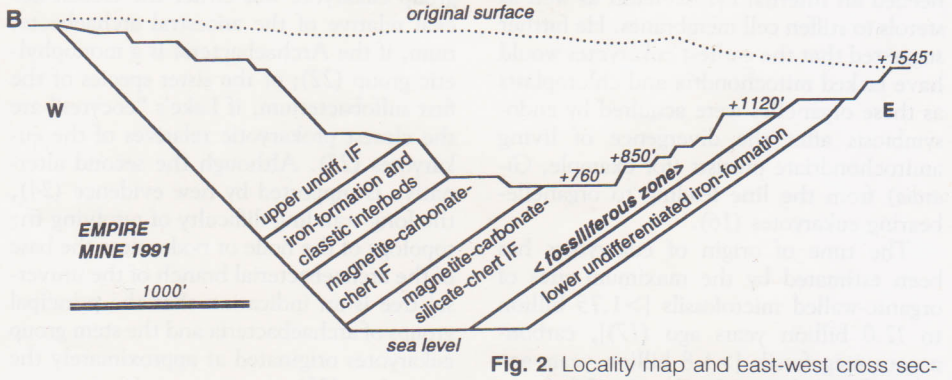

Fig. 2. Locality map and east-west cross section of the Empire Mine showing the 1991 and projected positions of the pit surface.

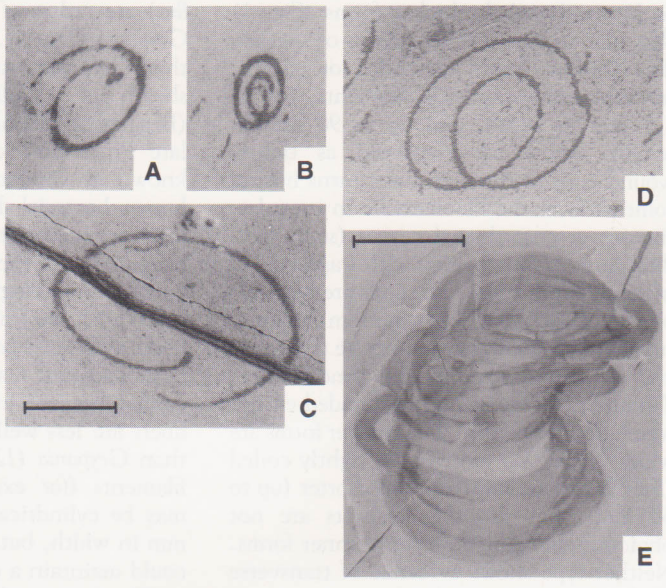

Fig. 3. (**A** to D) Specimens of *Grypania* from the Negaunee Iron-Formation, Empire Mine. (**E**) Large specimen of *Grypania spiralis*, about 1100 million years old, Rohtas Formation, Semri Group, Vindhyan Supergroup, central India. Scale bar in (C) (applies to A to D), 1 cm; scale bar in (E), 1 cm.

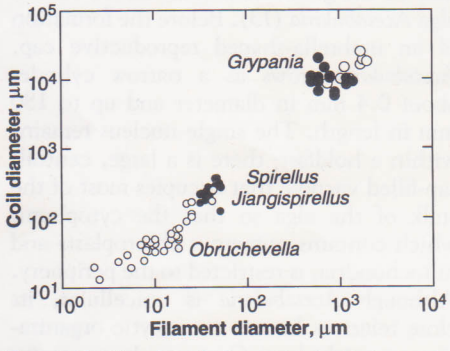

Fig. 4. Scatterplot of sizes of late Proterozoic and Cambrian spirally coiled cyanobacteria (*Obruchevella*, open circles; *Spirellus* and *Jiangispirellus*, solid circles) for comparison with specimens of *Grypania spiralis* (open circles) and *Grypania* from the Negaunee Iron-Formation (solid circles).

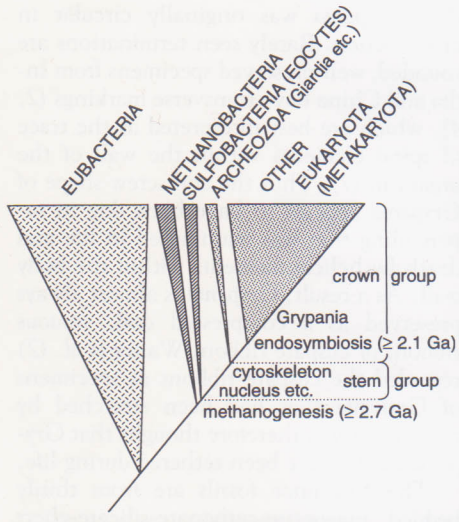

Fig. 5. Cartoon showing the major branches of the universal tree of life and the presumed position of *Grypania* in the tree.

vealed that eukaryotes are more closely related to archaebacteria than they are to eubacteria (*14*). Cavalier-Smith (*15*) has described one plausible scenario for the origin of the eukaryotic cell from eubacteria that had lost the ability to manufacture murine-based cell walls and consequently needed an internal cytoskeleton as well as sterols to stiffen cell membranes. He further suggested that the earliest eukaryotes would have lacked mitochondria and chloroplasts as these organelles were acquired by endosymbiosis after the divergence of living amitochondriate protists (for example, *Giardia*) from the line leading to organelle-bearing eukaryotes (*16*).

The time of origin of eukaryotes has been estimated by the maximum sizes of organic-walled microfossils [>1.75 billion to ?2.0 billion years ago (*17*)], carbonaceous megafossils [>1.8 billion years ago (*18*)], biomarker molecules (modified sterols) extracted from Proterozoic rocks [>1.7 billion years ago (*19*)], and the molecular clock [1.8 billion ± 0.4 billion years ago (*20*)]. If the Negaunee fossils are correctly interpreted as the remains of eukaryotic algae, the origin of organelle-bearing eukaryotes must have occurred prior to 2.1 billion years ago. Although this date is 300 million years older than most previous estimates, it may also underestimate significantly the times of origin of (i) the first stem group eukaryote (latest common ancestor of all living and extinct eukaryotes); (ii) eukaryotic organization (membrane sterols, cytoskeleton, nucleus, Golgi apparatus, mitosis, and so on); (iii) the first crown group eukaryote (latest common ancestor of all living eukaryotes); and (iv) the endosymbiotic conversion of purple bacteria and cyanobacteria into mitochondria and chloroplasts in an early eukaryote (*21*). Each of these events must have taken place in the order given above before about 2.1 billion years ago (Fig. 4).

There is limited geological evidence that points to the existence of stem group eukaryotes in the late Archean (>2.5 billion years ago). By definition, the first stem group eukaryote was either the closest coeval relative of the ancestral archaebacterium, if the Archaebacteria is a monophyletic group (*22*); or the sister species of the first sulfobacterium, if Lake's "eocytes" are the closest prokaryotic relatives of the eukaryotes (*23*). Although the second alternative is supported by new evidence (*24*), the long-standing difficulty of resolving the topology of the node or nodes near the base of the archaebacterial branch of the universal tree is an indication that the principal groups of archaebacteria and the stem group eukaryotes originated at approximately the same time (*25*).

Exceptionally light carbon isotope ratios ($\delta^{13}C_{PDB} \leq -40$ per mil) obtained from organic matter found in late Archean and early Proterozoic rocks (*26*) have been interpreted as evidence for the existence, at those times, of eubacterial methylotrophs that were using methane produced by archaebacterial methanogens (*27*). Thus the original stem group eukaryote may have lived before 2.5 billion to 2.7 billion years ago (Fig. 5). It follows that the divergence between eukaryotes and eubacteria may be much deeper than the about 1.8 billion year date obtained by extrapolating rates of protein evolution (*20*).

The earliest crown group eukaryotes lacked mitochondria and were therefore incapable of aerobic respiration (*15–16*). However, *Grypania* probably respired and consequently needed at least 1% of the present atmospheric level (PAL) of oxygen to survive (*13*). This suggests that oxygen was a significant component of the earth's atmosphere ($\geq 10^{-2}$ PAL O_2) during the main period of banded iron formation deposition, some 2.0 billion to 2.5 billion years ago.

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28. We thank J. W. Villar and G. L. LaBerge for their enthusiastic support of this work and the Management of Cleveland-Cliffs, Inc. for permission to publish this paper.

31 January 1992; accepted 18 May 1992