Reports

How Old Are the Eukaryotes?

Abstract. *Evidence from Precambrian sediments appears to indicate that nucleated (eukaryotic) organisms had become well established and relatively diverse about 850 ± 100 million years ago and that eukaryotes were probably extant, and may have first appeared, as early as 1400 ± 100 million years ago.*

Among major events in biologic evolution, the origin of the eukaryotic cell type stands out as having had special impact—an event that both changed the course and apparently altered the rate of early evolutionary advance. Although paleobiologic data can be expected to provide only limited insight into the mode of origin of eukaryotes, evidence indicative of the timing of this event should be preserved in the fossil record. Assessment of such evidence, however, is likely to be difficult. The earliest eukaryotes seem certain to have been simple, microscopic unicells. Such eukaryotes, similar in gross morphology to their prokaryotic precursors, would be expected to be difficult to distinguish from prokaryotes in the early fossil record. Indeed, resemblances between degraded, modern blue-green algae (prokaryotes) and Precambrian unicells generally regarded as eukaryotes have recently led Knoll and Barghoorn (*1*) to suggest that the Precambrian fossil record has been misinterpreted; stating that "there is simply no compelling evidence" for the existence of eukaryotes significantly earlier than the oldest known (Ediacaran) metazoan fossils (about 650 ± 50 million years old), they speculate that "eukaryotic cells may not have existed until very near the end of the Precambrian." In contrast with this view, we regard available evidence as indicating that nucleated microorganisms were extant prior to about 850 ± 100 million years ago [the time of deposition of the microfossiliferous Bitter Springs cherts of central Australia (*2–6*)] and that such organisms may have been established as early as 1400 ± 100 million years ago.

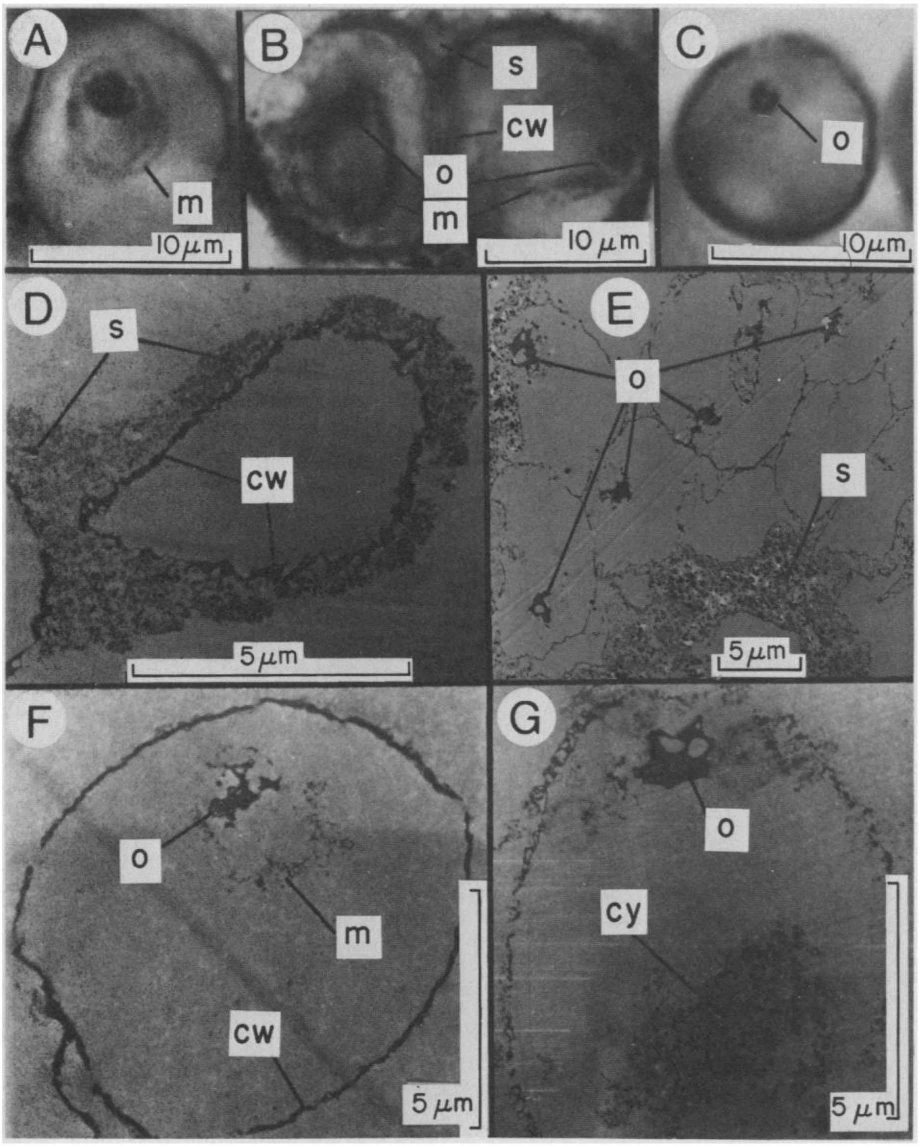
In moribund cultures of modern prokaryotic algae, Knoll and Barghoorn (*1*) have observed degraded protoplasts which they regard as identical to organelle-like bodies occurring in some unicells of the late Precambrian Bitter Springs microflora (Fig. 1, A to C, E to G). On the basis of such comparison, they interpret the Bitter Springs unicells as prokaryotes—the organelle-like bodies are regarded as condensed whole cells (cytoplasm plus cell wall) preserved within spheroidal remnants of an originally mucoidal sheath (*1*). This interpretation is not implausible; partially degraded protoplasts are known to occur within some Bitter Springs fossils (*2, 3, 7, 8*). Nevertheless, as is discussed below, recent studies of these unicells have shown that there are evident differences between such protoplasmic remnants and the organelle-like bodies, and that the three-dimensional morphology of these microfossils is generally preserved by the cell wall and not solely by extracellular sheath material. Current data suggest that certain of the Bitter Springs microfossils are of eukaryotic affinities.

In populations of degraded cells of modern and fossil algae (*2, 3, 7–9*), cellular remnants characteristically vary in size from large, seemingly unaltered protoplasts to condensed structures about one-fourth the cell diameter; such degraded protoplasts are composed of loosely particulate organic matter (Fig. 1G) generally concentrated in centrally located globular masses that conform in outline to the shape of the enclosing cell (*8*). In contrast, the organelle-like bodies measured in about a thousand specimens of the Bitter Springs unicell *Glenobotrydion aenigmatis* (*4, 8*) are of small and relatively constant size (about one-tenth the cell diameter) and are composed of dense, finely granular organic matter occurring in eccentrically located bodies that are independent of the cell shape in outline (Fig. 1, C and E to G) (*8*). Such organelle-like bodies do not occur in Bitter Springs cells that are known on other grounds to be prokaryotic; for example, although many of the filamentous blue-green algae of the assemblage contain degraded protoplasts, organelle-like bodies have not been detected in any of the more than 1850 cells measured in these fossil trichomes (*2, 3*). These observations, coupled with the fact that cytoplasmic remnants and organelle-like bodies occur together within the same cells (Fig. 1G), lead us to conclude that these two types of intracellular structures are of differing origin.

In diameter, the Bitter Springs organelle-like bodies [generally 0.7 to 2.3 μm in size (*4*)] are comparable to pyrenoids [0.8 to 2.1 μm in size (*8*)] and nuclei [1.5 to 2.2 μm in size (*4*)] which occur in many modern eukaryotic algae that are similar in dimensions to the Bitter Springs unicells. Like virtually all organelles known from the Phanerozoic fossil record (*4, 10, 11*), organelle-like bodies in the Bitter Springs algae have been preserved by permineralization, the cells having been embedded in a fine-grained, chemically precipitated siliceous matrix. Also like younger fossil organelles, the Bitter Springs bodies occur in three-dimensionally preserved cells that are delimited by a well-defined, relatively electron opaque, organic layer that we interpret as the cell wall; such layers are optically and ultrastructurally similar to cell walls of Phanerozoic fossil plants (*11*) and of artificially permineralized modern algae (*12*), and they are readily distinguished from encompassing, loosely particulate, fossilized sheath material (Fig. 1, B, D, and E).

These data, together with other evidence discussed and tabulated by Oehler (*8*), seem to indicate that the Bitter Springs organelle-like bodies are not remnants of degraded, condensed whole cells. Rather, the bodies seem more reasonably interpreted as remnants of original biologic structures occurring within spheroidal unicells that are delimited by their organically preserved cell walls. The bodies have no obvious morphological counterparts among prokaryotes but are comparable in many respects to nuclei and pyrenoids of modern eukaryotic algae; it thus seems warranted to interpret Bitter Springs unicells containing such bodies as eukaryotic (*13*).

Among other evidence from the Bitter Springs cherts that relates to the possible existence of eukaryotes is the occurrence of a tetrahedral tetrad of sporelike unicells, interpreted as being of eu-



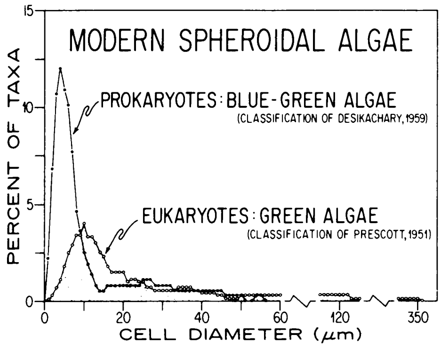


Fig. 1 (left). Optical photomicrographs (A to C) and transmission electron micrographs (D to G) of organically preserved algal microfossils from the late Precambrian Bitter Springs cherts (*2–4, 7, 8*). Abbreviations: *cw*, cell wall; *cy*, cytoplasmic remnant; *m*, membranous structure; *o*, organelle-like body; *s*, sheath. Fig. 2 (above). Size distributions of modern spheroidal unicellular algae: prokaryotic (*15*) and eukaryotic (*16*). Fig. 3 (below). Size ranges, mean cell sizes, and modal size groupings ("mode") of simple, spheroidal, algal microfossils measured in petrographic thin sections of algal laminated (stromatolithic) sediments from the 20 Precambrian formations indicated.

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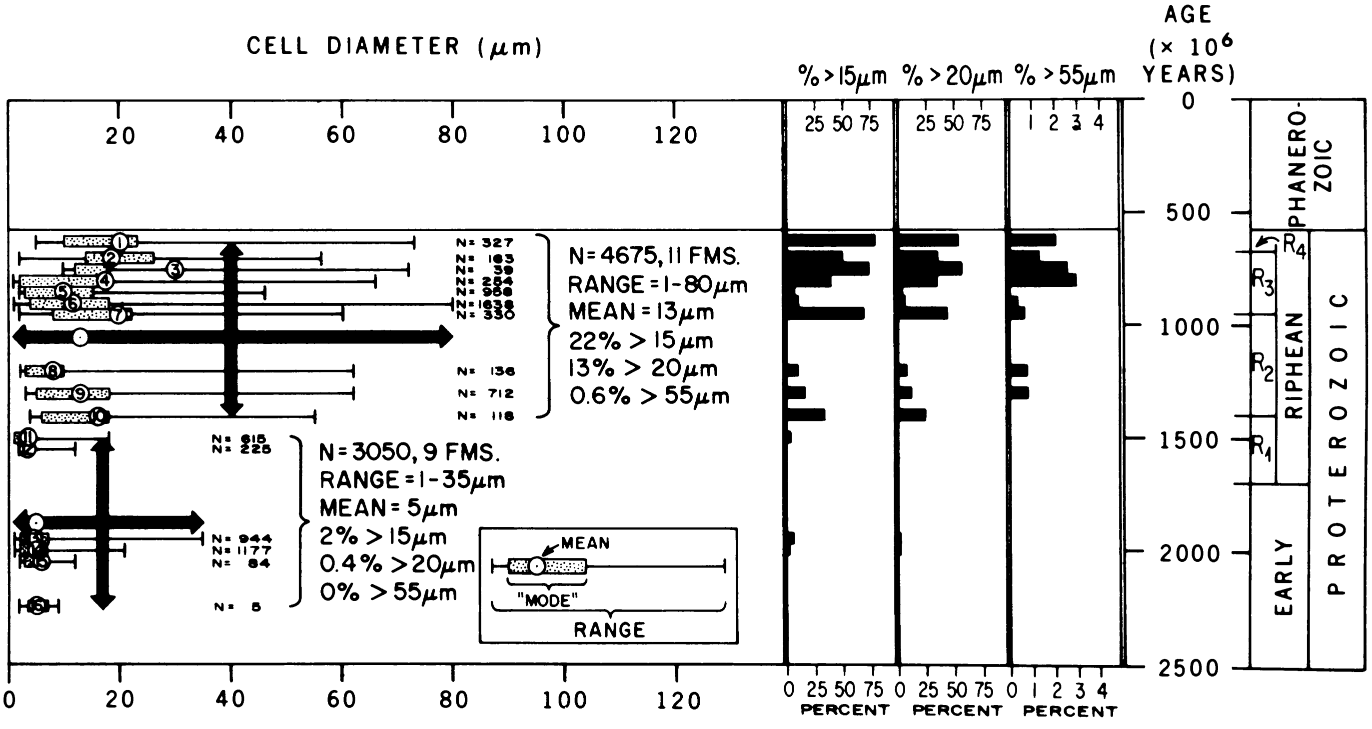

karyotic (green or red algal), meiotic origin (*3*). Margulis (*14*) has suggested that, if it is eukaryotic, the tetrad might be more closely compared with products of mitotic (green algal or fungal) division. Thus, both meiotic and mitotic analogs exist among modern eukaryotic thallophytes for this configuration, a configuration that is of rare occurrence among cyanophytes. Such tetrads, although they possibly represent rearranged products of prokaryotic fission (*5*), are "far more characteristic of eukaryotes than prokaryotes" (*14*, p. 18); as such, their occurrence represents strong supportive evidence for the existence of eukaryotic organization during Bitter Springs time.

As is shown in Fig. 2, prokaryotic and eukaryotic algal unicells differ in their patterns of size distribution. Modern taxa of spheroidal cyanophytes (prokaryotes) range in diameter from less than 1 to about 55 μm, but are commonly about 4 μm in diameter and rarely have a maximum cell size greater than 15 μm [about 10 percent are greater than 15 μm; about 6 percent are greater than 20 μm (*15*)]. In contrast, spheroidal chlorophytes (eukaryotes) range in diameter from 1 to about 350 μm, exhibit a modal size class at about 10 μm, and include many taxa with a maximum cell size greater than 20 μm [about 50 percent are greater than 15 μm; about 35 percent are greater than 20 μm (*16*)]. In Fig. 3 are data for more than 7700 simple spheroidal microfossils measured in petrographic thin sections of algal laminated sediments from 20 Precambrian formations (*17*). As is shown, unicells reported from sediments more than about 1400 million years old are of small dimensions (1 to 35 μm in diameter; 2 percent are greater than 15 μm, 0.4 percent are greater than 20 μm) with an average diameter of about 5 μm; in cell size, these forms are quite comparable to modern prokaryotes. In contrast, all of the Precambrian assemblages younger than about 1400 million years old contain relatively large unicells (up to 80 μm in diameter; 22 percent are greater than 15 μm, 13 percent are greater than 20 μm) with an average diameter of about 13 μm; all contain cells larger than 45 μm in diameter and most contain cells in the 60- to 80-μm size range [larger than described prokaryotes (*15*)]. Timofeev (*18*) has observed a similar increase in cell size (coupled with a marked increase in taxonomic diversity) beginning at about 1400 ± 100 million years ago in microfossils studied in acid-resistant residues of shales. Moreover, he has detected unusually large unicells ("Megasphaeromorphida"), from 100 to more than 250 μm in diameter, in macerations of shales thought to be about 850 ± 50 million years old (*18*). We interpret these data as indicating that unicellular eukaryotes were probably extant—and in fact may have first appeared—as early as 1400 ± 100 million years ago and that the lineage had become well established and relatively diverse by Bitter Springs time.

Organic-walled, branching filaments of large diameter (15 to 60 μm) occur in the approximately 1300-million-year-old Beck Spring Dolomite of California (*19*) and in the younger Skillogalee Dolomite of South Australia (*5, 20*). The discovery of rare but well-defined cross walls in these filaments (*19, 21*) indicates that they are probably not remnants of blue-green algal sheaths or tubes formed by boring cyanophytes, as previously suggested (*5, 20*). They seem most reasonably interpreted as being siphonaceous green or golden-green (Vaucheriacean?) algae (*19, 21*). Other recent discoveries suggest that the fossil record of both microscopic (*22*) and megascopic (*23*) eukaryotes may extend appreciably further into the Precambrian than has generally been supposed.

Thus, we cannot agree with those who have suggested that "there is no good evidence" for the presence of eukaryotes earlier than the Ediacaran faunas (*1*). It seems to us that the totality of evidence indicates the contrary—that the eukaryotic lineage predates both the megafossils of Ediacara and the microfossils of Bitter Springs.

Note added in proof: The early occurrence of eukaryotes seems further evidenced by the recent discovery of spheroidal unicells more than 500 μm in diameter (and more than ten times larger than known prokaryotes) in shales of the Soviet Union thought to be 1000 ± 50 million years in age (*24*).

J. WILLIAM SCHOPF
Department of Geology, University of California, Los Angeles 90024
DOROTHY ZELLER OEHLER
Baas Becking Geobiological Laboratory, Post Office Box 378, Canberra City, A.C.T., Australia 2601

References and Notes

1. A. H. Knoll and E. S. Barghoorn, *Science* **190**, 52 (1975).
J. W. Schopf, *J. Paleontol.* **42**, 651 (1968).
——— and J. M. Blacic, *ibid.* **45**, 925 (1971).
J. W. Schopf, *Origins Life* **5**, 119 (1974).
5. —— ., *Annu. Rev. Earth Planet. Sci.* **3**, 213 (1975).
6. M. R. Walter, *Spec. Pap. Palaeontol. No. 11* (1972).
7. D. Z. Oehler, *Abstr. Geol. Soc. Am.* **6** (No. 7), 895 (19 *J. Paleontol.* **50**, 90 (1976).
8. ———, *J. Paleontol.*, in press.
9. W. N. Croft and E. A. George, *Bull. Br. Mus. (Nat. Hist.) Geol.* **3**, 339 (1959).
10. A. Eisenack, *Nat. Mus.* **95**, 473 (19 J. M. Schopf, *Rev. Palaeobot. Palynol.* **20**, 27 (1975).
M. A. Millay and D. A. Eggert, *Am. J. Bot.* **61**, 1067 (1974); D. Z. Oehler and J. H. Oehler, in preparation.
12. J. H. Oehler, *Geol. Soc. Am. Bull.* **87**, 117 (1976).
13. It should be stressed, however, that the occurrence of intracellular organic structures in fossil unicells should not, by itself, be considered conclusive evidence of eukaryotic affinity. In light of the results summarized here, it seems apparent that use of transmission electron microscopy, especially in studies of numerous examples of individual taxa and, if present, of single cells containing coexisting organelle-like bodies and cytoplasmic remnants (Fig. 1G), may be required to elucidate the nature and probable origin of organic structures known to occur in other Precambrian unicells (*5*).
14. L. Margulis, *BioSystems* **6**, 16 (1974).
15. Comparison of the taxonomy of T. V. Desikachary [*Cyanophyta* (Indian Council of Agricultural Research, New Delhi, 1959)], who recognizes 97 spheroidal species and forms, with the taxonomy of F. Drouet and W. A. Daily [*Butler Univ. Bot. Stud.* **12**, entire volume (1956)], who recognize only 15 such taxa, indicates that this pattern of size distribution is not an artifact of classification.
16. Comparison of the taxonomy of G. W. Prescott [*Cranbrook Inst. Sci. Bull.* **31**, entire volume (1951)] with that of G. Lindau and H. Melchior [*Die Algen, Kryptogamflora für Anfänger* (Springer-Verlag, Berlin, 1930), vol. 4, part 2] indicates that this pattern of size distribution is not an artifact of classification.
17. J. W. Schopf, in *Correlation of the Precambrian, International Geological Correlation Programme* (Academy of Science and Ministry of Geology of the USSR, Moscow, 1975), p. 39 (abstract). Data summarized in Fig. 3 are from (*2–4, 19, 21*) and from M. Akiyama and N. Imoto, *Earth Sci.* (*J. Assoc. Geol. Collaboration Jpn.*) **29**, 280 (1975); E. S. Barghoorn and S. A. Tyler, *Science* **147**, 563 (1965); B. Bloeser, J. W. Schopf, R. J. Horodyski, W. J. Breed, in preparation; D. G. Darby, *Geol. Soc. Am. Bull.* **85**, 1595 (1974); W. L. Diver, *Nature (London)* **247**, 361 (1974); J. A. Donaldson and G. Delaney, *Can. J. Earth Sci.* **12**, 371 (1975); H. J. Hofmann, *Nature (London)* **249**, 87 (1974); *J. Paleontol.*, in press; ——— and G. D. Jackson, *Can. J. Earth Sci.* **6**, 1137 (1969); R. J. Horodyski, J. W. Schopf, J. A. Donaldson, in preparation; G. R. Licari and P. Cloud, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2500 (1972); —— ., W. D. Smith, *ibid.* **62**, 56 (1969); M. D. Muir, *Origins Life* **5**, 105 (1974); L. A. Nagy, *Science* **183**, 514 (1974); *J. Paleontol.*, in press; J. W. Schopf and T. R. Fairchild, *Nature (London)* **242**, 537 (1973); in preparation; J. W. Schopf and Yu. K. Sovietov, *Science*, in press; J. W. Schopf and K. N. Prasad, in preparation; J. W. Schopf, T. D. Ford, W. J. Breed, *Science* **179**, 1319 (1973); M. R. Walter, in preparation; ———, A. D. T. Goode, W. D. M. Hall, *Nature (London)*, in press.
18. B. V. Timofeev, in *Microfossils of the Oldest Deposits, Proceedings of the Third International Palynological Conference* (Nauka, Moscow, 1973), vol. 7.
19. G. R. Licari, in preparation.
20. T. R. Fairchild and J. W. Schopf, *Am. J. Bot.* **61** (Suppl. No. 5), 15 (1974).
21. T. R. Fairchild, in preparation.
22. H. Tappan, *Geol. Soc. Am. Bull.* **87**, 633 (1976); J. Kazmierczak, *Lethaia* **9**, 39 (1976).
23. W. Sabrodin [*Ideen des exakten Wissens* **12**, 835 (1971)] has discussed and figured millimeter-sized structures, 900 to 1000 million years old, that he interprets as worm burrows and possible fecal pellets; and M. R. Walter, J. H. Oehler, and D. Z. Oehler (*J. Paleontol.*, in press) have interpreted ribbonlike organic structures in approximately 1300-million-year-old sediments of the Belt Supergroup as remnants of megascopic, and presumably eukaryotic, algae.
24. B. V. Timofeev, T. N. Hermann, N. S. Mikhaylova, *Plant Microfossils of the Precambrian, Cambrian and Ordovician* (Nauka, Leningrad, 1976), pp. 85, 88–90.

25. We thank B. Bloeser, T. R. Fairchild, C. A. Hall, R. J. Horodyski, Y. Leih, J. H. Oehler, G. Oertel, E. C. Olson, D. F. Satterthwait, J. M. Schopf, and M. R. Walter for helpful discussion, and Y. Leih for the micrograph shown in Fig. 1E.

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