Foraminifera and Cercozoa Are Related in Actin Phylogeny: Two Orphans Find a Home?

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In recent years, the increased sampling of protein-coding genes from diverse eukaryotes has revealed that many aspects of each gene tree are at odds with other phylogenies. This has led to the belief that each gene tree has unique strengths and weaknesses, suggesting that an accurate picture of eukaryotic relationships will be achieved only through comparative phylogeny using several different genes. To this end, actin genes were characterized from two genera of chlorarachniophytes, *Chlorarachnion* and *Lotharella*, and three species of the cercomonad flagellate *Cercomonas*. Phylogenetic trees including these new actin genes confirm the recently proposed relationship between chlorarachniophytes and cercomonads (Cercozoa) and, more importantly, also show a close relationship between Cercozoa and Foraminifera. Both of these are major eukaryotic groups encompassing extremely diverse organisms, yet there is no strong evidence for the evolutionary position of either from morphological or molecular data. The union of Cercozoa and Foraminifera suggested by actin phylogeny represents a novel step in the long process of determining the broad relationships between all major eukaryotic groups.

Introduction

The chlorarachniophytes are green amoeboflagellate algae that are primarily distinguished by the presence of a plastid of secondary endosymbiotic origin. Primary plastids (those of plants, green algae, red algae, and glaucocystophytes) arose through the endosymbiotic uptake of a cyanobacterium by a eukaryote, but the ancestor of chlorarachniophytes acquired its plastid by swallowing a photosynthetic eukaryote and, rather than simply digesting it as a food source, retaining the alga to perform photosynthesis. Now the algal endosymbiont is severely reduced and is completely integrated with its amoeboflagellate host such that the two are regarded as a single organism (McFadden and Gilson 1995).

The origins of both the host and the endosymbiont components of chlorarachniophytes have proved to be quite puzzling, since both are unusual and extremely highly adapted to their endosymbiotic association. Before secondary endosymbiotic plastid origin was understood, it was thought that *Chlorarachnion* was likely a relative of heterokont algae (Geitler 1930); however, plastid pigmentation eventually suggested that the endosymbiont was some kind of green alga (Hibberd and Norris 1984). This has recently been confirmed by molecular phylogeny (Van de Peer et al. 1996; Ishida et al. 1997), but still no strong evidence from either pigmentation or molecular data has been able to demonstrate conclusively what kind of green alga it was. Indeed, many molecular studies have provided conflicting results on this issue (see, e.g., McFadden, Gilson, and Waller 1995; Van de Peer et al. 1996; Ishida et al. 1997). The evolution of the host component of chlorarachniophytes has been no less enigmatic. When *Chlorarachnion* was first discovered, the presence of a plastid naturally tempted investigators to suggest that the whole cell was related to other algal groups (Geitler 1930). However, even since the secondary, green algal origin of chlorarachniophyte plastids was recognized, the evolutionary position of the host has remained unclear, since the host amoeboflagellate bears no particular resemblance to any other eukaryote (Hibberd and Norris 1984). The host has been proposed to be related to a variety of protist groups based on morphology (Geitler 1930; Grell 1990; Cavalier-Smith, Allsopp, and Chao 1994), and the first molecular data from the host genome did not help this situation, as chlorarachniophyte sequences also showed no particular similarity to any other eukaryote. Accordingly, the evolutionary nature of the host in this unusual association remained completely unclear until very recently.

The first clues as to the origin of the chlorarachniophyte host came from molecular data from other organisms. Small-subunit rRNA (SSU rRNA) genes of euglyphids, nonflagellated amoebae with filose pseudopods and hard tests (Bovee 1985), were the first to show any strong association with the SSU rRNA of the *Chlorarachnion* host (Bhattacharya, Helmchen, and Melkonian 1995). The subsequent characterization of SSU rRNA from a tremendous variety of other eukaryotes, including amoeboflagellate cercomonads, thaumatomonads, and (more weakly) the plasmodiophorid plant pathogens (Cavalier-Smith and Chao 1996), has further expanded the group, which Cavalier-Smith (1998) has collectively called the Cercozoa. Because of this diversity, the various members of the Cercozoa were previously not considered to be closely related to one another: cercomonads were thought to be related to bodonids or opalinids, thaumatomonads to heterokonts, and plasmodiophorids to fungi or slime molds (Hollande 1952; Olive 1975; Vickerman 1976). Yet, the rRNA trees uniting these taxa are quite robust (with the possible exception of *Plasmodiophora*), and the relationship between the *Chlorarachnion* host and cercomonads has now been confirmed by both alpha-tubulin and beta-tubulin phylogenies (Keeling, Deane, and McFadden 1998; Keeling

Key words: Cercozoa, Foraminifera, chlorarachniophytes, actin, phylogeny.

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Mol. Biol. Evol. 18(8):1551–1557. 2001

et al. 1999). Altogether, the Cercozoa are a formidable group of eukaryotes, encompassing a wide variety of organisms and including some of the most abundant nonphotosynthetic amoebae, flagellates, and amoeboflagellates known.

The morphological, ecological, and genetic diversity of the Cercozoa is enormous, so the characterization of these relationships was a major advance in the reconstruction of eukaryotic evolution. However, because of this very diversity, Cercozoa are presently only defined by molecular data, so it is very important that this relationship is confirmed by additional data, both molecular and otherwise. Moreover, with the difficulty in even defining the Cercozoa, it is not surprising that it is also extremely difficult to say exactly how they fit into the larger picture of eukaryotic evolution, since they retain little or no obvious similarity to any other eukaryotic group. Here, we characterized actin genes from several chlorarachniophytes and cercomonad flagellates to address their relationship to one another and to other eukaryotes. The phylogeny of these genes provides additional support for the union of the Cercozoa and, more importantly, also provides the first strong evidence for the exact relationship of Cercozoa to other eukaryotes. Actin phylogeny shows a consistent and well-supported relationship between Cercozoa and Foraminifera. Foraminifera, or forams, are abundant marine and freshwater amoebae with granulose, reticulating pseudopodia. They are typically surrounded by organic or mineralized tests, which can be very large and intricate structures (Bock, Hay, and Lee 1985; Lee 1990). Like that of Cercozoa, the evolution of foraminifers has been something of an enigma. Molecular data from large- and small-subunit rRNAs have suggested several conflicting solutions but have often indicated that Foraminifera were an ancient lineage (Pawlowski et al. 1994, 1996, 1999*a*, 1999*b*; Wade et al. 1996). This is almost certainly because foraminifer rRNA, and indeed most foraminiferan genes studied to date (a notable exception being actin), are highly divergent, and the phylogeny of these genes is not easily interpreted (Pawlowski et al. 1996). The sister relationship between Cercozoa and Foraminifera supported by actin phylogeny is the first concrete evidence for the position of either of these two diverse and enigmatic groups within the tree of eukaryotes. This represents another step toward resolving the branching order of the major eukaryotic lineages.

Materials and Methods

Strains and Amplification Conditions

Chlorarachnion sp. strain CCMP 621 was cultured as described (McFadden, Gilson, and Sims 1997), and DNA was prepared by repeated extraction with phenol-chloroform. DNA and cultures of *Lotharella amoeboformis* (recently described strain Ryukyu; Ishida, Ishida, and Hara 2000) were kindly provided by K. Ishida, and DNA from *Cercomonas* sp. RS/21 (1B001 ATCC 50317), *Cercomonas* sp. RS/22 (ATCC 50318), and *Cercomonas* sp. RS/23 (ATCC 50319) were kindly provided by E. E. Chao and T. Cavalier-Smith.

Genomic DNA was used to amplify actin gene sequences using primers GAGAAGATGACNCARATHAT GTTYGA and GGCCTGGAARCAYTTNCGRTGNAC in all cases except for Chlorarachnion, for which products were also amplified using CGGCTTCGCNGGNGAYGA YGCNCC and GGCCTGGAARCAYTTNCGRTGNAC. This primer combination was used with other Cercozoa but did not result in amplification. All amplifications were carried in 5–30-μl capillary tubes using an Idaho Rapidcycler with the following protocol: a 2-min 92°C denaturing step, followed by 35 cycles consisting of 15 denaturing at 92°C, 15 s annealing at 40°C, and 1 min extension at 72°C, followed by 2 min at 72°C. Products corresponding to at least the expected size were gel-isolated and cloned using TOPO-TA cloning vector pCR 2.1 (Invitrogen). Three to six clones from each organism were sequenced with ABI big-dye terminator chemistry, generally resulting in multiple closely related copies of actin from each organism. For Cercomonas strains RS/21 and RS/22, different-sized products were characterized and found to contain introns or protein insertions. New sequences were deposited in GenBank as accessions AF363528–AF363539.

Phylogenetic Analyses

Inferred translations of new actins were aligned with homologs from existing databases using PIMA (Smith and Smith 1992). The alignment was inspected manually, but the actin protein contains very few insertions, deletions, or ambiguously aligned regions, so few changes were made. The alignment is available on request. Distances were calculated using PUZZLE 4.0.1 (Strimmer and von Haeseler 1996) with the JTT substitution frequency matrix and correction for site-to-site rate variation modeled on a gamma distribution with eight rate categories plus invariable sites (the estimated alpha parameters for the 95- and 77-OTU trees were 0.59 and 0.51, respectively, and in neither case were any invariant sites estimated). Distance trees were constructed using BIONJ, WEIGHBOR, and Fitch-Margoliash (Felsenstein 1993; Gascuel 1997; Bruno, Socci, and Halpern 2000). No significant difference was noted between trees generated by these methods. One hundred distance bootstraps were calculated using the same method (employing puzzleboot; M. Holder and A. Roger), with the shape parameter estimated from the original tree imposed on each replicate. Individual bootstrap trees were examined manually to identify any trends in the placement of either cercozoan or foraminiferan actins. Maximum-likelihood trees were inferred by quartet puzzling using PUZZLE, the settings described above, and 10,000 puzzling steps. The quartet puzzling tree was not well resolved, but those relationships that were resolved did not differ from those of distance analyses, and they are not shown. Alternate topologies were compared using Kishino-Hasegawa tests (Kishino and Hasegawa 1989) with PUZZLE and the settings described above. The gamma shape parameter was estimated from the original distance calculation. In no case was any significant discrepancy found between these two sets of

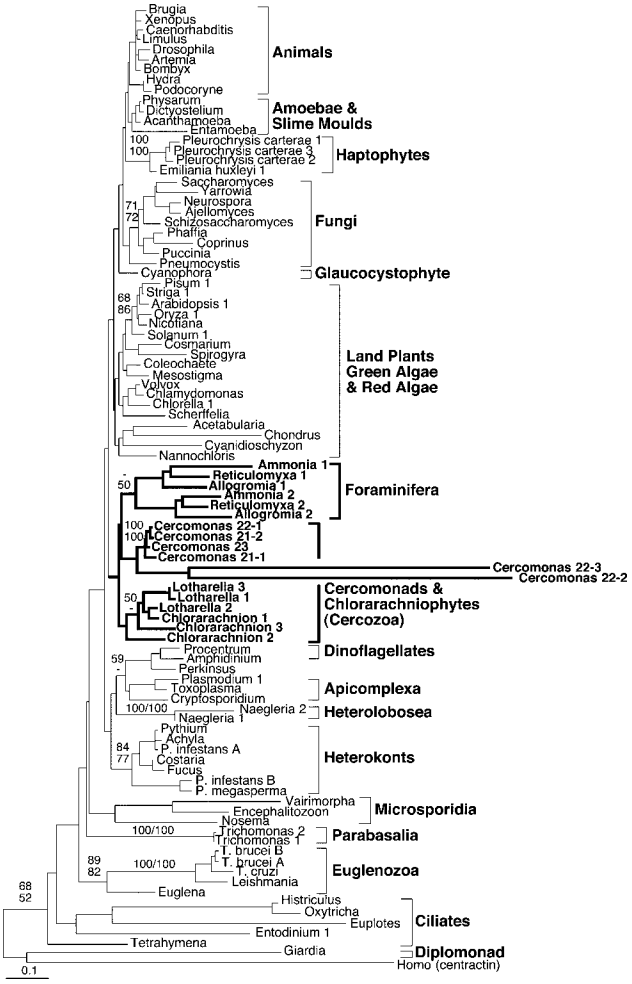

FIG. 1.—Global actin phylogeny showing the overall topology of the actin tree; BIONJ tree of gamma-corrected maximum-likelihood distances using human centractin as an outgroup. Bootstrap proportions are based on gamma-corrected distances using BIONJ (top) and Fitch-Margoliash (bottom). Groups are bracketed and named to the right. The scale bar indicates 0.1 substitutions (corrected) per site.

tests. Test trees were constructed by constraining groups that were well supported by either actin phylogeny or other data, leaving the topology within these groups unresolved and leaving the relations between these groups unresolved. These groups were as follows: (1) animals, amoebae and slime molds, and fungi (constrained as three separate groups); (2) plants, green algae, and red algae (all unresolved); (3) *Cyanophora*; (4) foraminifers; (5) cercomonads and chlorarachniophytes (as two separate groups); (6) euglenozoa and heterolobosea (as two separate groups); (7) heterokonts; and (8) alveolates (as two separate groups of ciliates and apicomplexa with *Perkinsus*). The tests were conducted in two ways. First, either foraminifers, cercomonads, and chlorarachniophytes were placed together (in each of three possible topologies) or Foraminifera or Cercozoa were placed individually with each of the other six groups. (In other tests, Cercozoa were divided into cercomonads and chlorarachniophytes, but all such alternatives were rejected.) In the second test, two sets of trees were constructed, one in which foraminifers were placed with each other group, including Cercozoa, and one in which Cercozoa were placed with each other group, including foraminifers. It has been shown that the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) is more appropriate than the Kishino-Hasegawa test for comparing multiple trees, but the results of a Kishino-Hasegawa test may still be considered valid if the *P* value is halved (i.e., if it is treated as a one-tailed test; Goldman, Anderson, and Rodrigo 2000). Accordingly, we determined the confidence intervals of each test as both one- and two-tailed. Every alternative that failed at a 5% level of confidence also failed a one-tailed test (some alternatives failed at 1% in the two-tailed test, but only at 5% in the one-tailed test).

Results and Discussion

Cloning and Sequencing Cercozoan Actin Genes

Actin gene sequences were amplified from two chlorarachniophytes (*Chlorarachnion* sp. CCMP 621 and *Lotharella amoeboformis*) and from three cercomonad flagellates (*Cercomonas* species strains RS/21, RS/22, and RS/23). Primer combinations to amplify over 90% of the actin gene (346 codons) were used in all cases but generated only two copies of actin from *Chlorarachnion*. All other actins from Chlorarachnion and other Cercozoa were amplified using primers to generate approximately two thirds of the gene (244 codons).

Products of the size expected for intron-free actin genes were observed in all amplifications, and the sequence of these products confirmed this to be the case. In addition, several clones were sequenced for each organism, and in all but *Cercomonas* strain RS/23, several copies of the actin gene were characterized. In amplifications from *Cercomonas* strains RS/21 and RS/22, larger-than-expected products were also observed, suggesting that additional copies of the gene might contain introns. These were also sequenced, revealing that one *Cercomonas* strain RS/21 actin contained two small introns, while two genes from RS/22 (numbered 2 and 3) were found to be highly divergent and to contain several large protein insertions, and one of these products (number 2) was truncated at the 3′ end within an intron positioned two codons before the expected end of the PCR product. In total, six protein insertions were found in these two genes, they vary from 1 to 21 amino acids in length, occur at positions where no other insertions have been characterized in actin (which is typically very highly conserved in size), and none of the insertions are common to both genes. Nevertheless, both sequences are uninterrupted open reading frames, suggesting that both are likely functional actin-like proteins even though their high degree of divergence, along with the presence of several unique insertions, suggests that these genes encode proteins with some unusual function in the cell.

Actin Phylogeny

A phylogeny of 95 actin amino acid sequences from a very broad diversity of eukaryotes rooted with the closest actin paralog, centractin, is shown in figure 1. Actin phylogeny, like many other eukaryotic trees, is

characterized by a large number of highly divergent sequences at the base of the tree, followed by a radiation of more conserved sequences at the so-called ''crown.'' Like other eukaryotic molecular phylogenies, the validity of these basal taxa is extremely dubious, especially since the ciliates are consistently basal in actin trees, but it is abundantly clear from molecular and ultrastructural evidence that ciliates are actually very closely related to dinoflagellates and apicomplexa. Such problems are seen in the deep branches of most or all eukaryotic phylogenies (Embley and Hirt 1998; Keeling 1998; Philippe and Adoutte 1998; Roger et al. 1999). Nevertheless, as with other molecular trees, most of the major groups in the upper portion of the actin tree are in close agreement with other data (for recent analysis, see Baldauf et al. 2000): land plants form a clade with green algae and red algae; animals, fungi, slime molds, and some amoebae are closely related (a grouping that also unexpectedly includes haptophytes); heterokonts form a group; Foraminifera branch with *Reticulomyxa* (recently shown to be a naked foraminifer; Pawlowski 1999*a*, 1999*b*); and the apicomplexa and dinoflagellates also group together (but without the highly divergent ciliates, as indicated above). While some other characteristics of this tree are unusual and likely artifactual (e.g., the position of the haptophytes, certain green algae, and *Cyanophora*), overall, the more conserved branches of the actin tree are consistent with much of the current understanding of eukaryotic evolution.

Within this framework, the positions of the cercomonads and chlorarachniophytes are extremely interesting. Cercomonads and chlorarachniophytes have been proposed to share a close ancestry based entirely on molecular data from rRNA (Bhattacharya, Helmchen, and Melkonian 1995; Cavalier-Smith and Chao 1996; Cavalier-Smith 1998), alpha-tubulin (Keeling, Deane, and McFadden 1998), and beta-tubulin (Keeling et al. 1999). Actin supports this relationship but also includes Foraminifera in this grouping with all methods used. Indeed, in all analyses, the foraminifers actually branch within the Cercozoa, either with chlorarachniophytes or cercomonads, but never as a sister to the group as a whole. The bootstrap support for the Cercozoa-Foraminifera clade was consistently low (<50%) in global analyses, but this is perhaps not surprising given the divergent nature of the two insertion-containing *Cercomonas* strain RS/22 genes and that of many of the ''deep-branching'' sequences.

To address the relationship between cercomonads, chlorarachniophytes, and foraminifers without the potential problems caused by the presence of sequences with accelerated rates of substitution, the phylogeny was reconstructed after excluding the most divergent sequences. Here (fig. 2), the relationship between cercomonads, chlorarachniophytes, and foraminifers is recovered once again, and in these analyses this clade is supported by much higher bootstrap values (70%–72%). Although the support seen here is not overwhelming, examination of the bootstrap trees reveals that it is likely substantially underestimated. Of the 30 trees in which a relationship between Cercozoa and Foraminifera is not

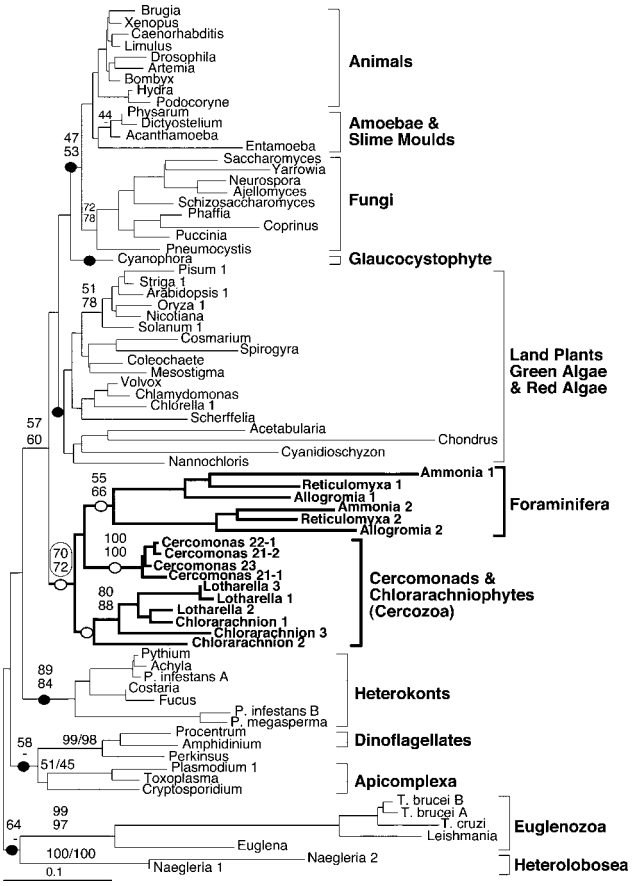

FIG. 2.—Actin phylogeny restricted to the more conserved actin sequences; BIONJ tree of gamma-corrected maximum-likelihood distances with bootstrap proportions from BIONJ (top) and Fitch-Margoliash (bottom). Circles at nodes indicate positions at which Cercozoa or Foraminifera were positioned in partially unresolved trees and tested by Kishino-Hasegawa tests. Filled circles indicate positions rejected at the 5% level for both groups (many were rejected at the 1% level—see text), while open circles indicate positions not rejected. All topologies separating Cercozoa and Foraminifera were rejected. Groups are bracketed and named to the right. The scale bar indicates 0.1 substitutions (corrected) per site.

recovered in neighbor-joining analysis, in only 3 are the two groups monophyletic and unrelated. In half of these 30 trees, the Cercozoa-Foraminifera clade is intact, but some other taxon branches within it (commonly the relatively long branched Chondrus). Conversely, in four trees, the foraminifers are polyphyletic (typically the two foraminiferan actin paralogs are unrelated), and in nine trees, the Cercozoa are polyphyletic (importantly, in five of these cases, the polyphyly was not simply the splitting of chlorarachniophytes and cercomonads, but instead specifically involved *Chlorarachnion* actin 2). Altogether, these data do not support any viable alternative relationship for either Cercozoa or Foraminifera, nor do they provide any reason to doubt their close relationship. Instead, they appear to reflect the relatively low information content in actin and the unstable position of a handful of individual sequences. Indeed, the union of Cercozoa and Foraminifera is one of the best supported relationships in actin phylogeny, being substantially more robust than the union of many widely

believed groups, such as animals, animals and fungi, or land plants and green algae.

The relationship between Cercozoa and Foraminifera was also tested directly against numerous alternative topologies using Kishino-Hasegawa tests. The major groups in figure 2 were constrained and left unresolved as described in *Materials and Methods.* Fifteen topologies were then constructed where the Cercozoa or Foraminifers were joined to one another (with the relative relationships between cercomonads, chlorarachniophytes, and foraminifers in any of three possible combinations) or to each of six other major lineages individually. Of these topologies, those which placed the Cercozoa and Foraminifera together were favored (although their relative orders were indistinguishable), and each of the alternatives where either group was placed with any other eukaryotic lineage was rejected at the 5% confidence level for both one- and two-tailed tests. These tests were also repeated where the relative positions of Cercozoa or Foraminifera were tested independently in two sets of alternative trees, and once again the tree that placed them together was favored in both sets of tests while all other alternatives were rejected at at least the 5% level for both one- and two-tailed tests (7 out of 10 alternative position for Cercozoa and 2 out of 10 for Foraminifera were also rejected at the 1% level when the confidence level was estimated for a one-tailed test).

Cercozoa and Foraminifera: Two Pieces in the Puzzle of Eukaryotic Phylogeny

A believable and comprehensive phylogeny of eukaryotes has been an elusive goal. The introduction of molecular phylogeny initially appeared to provide a solution to this problem by offering the first large set of universally comparable characters with which to reconstruct evolutionary events. The first such trees, based mostly on SSU rRNA, appeared to support the notion that molecules would quickly provide unambiguous answers, and the apparently well supported characteristics of the SSU rRNA tree soon became the gold standard for eukaryotic evolution (Sogin 1989). In the past decade, however, the increased sampling of diverse genes has revealed a significant lack of consistency between various gene trees, leading to the widely held belief that no single gene tree adequately represents all relationships perfectly (Embley and Hirt 1998; Keeling 1998; Philippe and Adoutte 1998; Roger et al. 1999). This period of deconstruction has seen a fully resolved universal tree degenerate into a poorly resolved bush in which many major eukaryotic groups are recovered but the relationships between them are unclear. Despite this retreat from a fully resolved single gene tree, there have been a number of extremely important advances from global eukaryotic phylogenies on two fronts: in working out the relationships between several of the major eukaryotic groups, and in defining some of the groups themselves.

In the first instance, several eukaryotic ''supergroups,'' composed of a handful of major eukaryotic lineages, have been defined or confirmed using many molecular data sets and appear to be very robust. These include the alveolates, the heterokonts, and the relationship between them; the red algae, the green algae and the glaucocystophyte algae; and the animal/fungal clade (for recent analysis, see Baldauf et al. 2000). Using these data can be thought of as rebuilding the eukaryotic tree from the ground up, based not on a single gene, but on a composite of several genes and other molecular, biochemical, and morphological information. In the second instance, a few previously unanticipated relationships between eukaryotic lineages have been identified based on molecular data, and sometimes only after this has happened have morphological similarities been identified. A few examples of this include the recognition that apicomplexa are related to dinoflagellates and ciliates (Wolters 1991), that Pneumocystis and microsporidia are fungi (Stringer et al. 1989; Keeling and Doolittle 1996), that the parasite *Blastocystis* is a heterokont (Silberman et al. 1996), and that slime molds are closely related to animals and fungi (Baldauf and Doolittle 1999), and perhaps the best example is the Cercozoa. The Cercozoa comprise a group of phototrophic and heterotrophic flagellates, amoeboflagellates, and amoebae that are phenotypically so diverse that no unifying feature other than molecular phylogeny can be found to define them (Cavalier-Smith 1998). Nevertheless, four molecular data sets have been used to test the Cercozoa (SSU rRNA, alpha-tubulin, beta-tubulin, and now actin), and all strongly support the union of at least cercomonad flagellates and chlorarachniophytes.

The grouping together of Cercozoa represents a significant slice of eukaryotic diversity and therefore a major advance in our understanding of the universal tree of eukaryotes. Now, the grouping of Cercozoa with Foraminifera further consolidates and simplifies our understanding of eukaryotic relationships by linking two groups that were previously ''adrift'' in the eukaryotic tree. Until now, the position of Cercozoa had been analyzed with SSU rRNA, alpha-tubulin, and beta-tubulin, and none of these analyses has led to a clear conclusion as to the relationship of Cercozoa to other eukaryotes (Bhattacharya, Helmchen, and Melkonian 1995; Cavalier-Smith and Chao 1996; Keeling, Deane, and McFadden 1998). Similarly, foraminiferan SSU and LSU rRNAs have been extensively analyzed, while beta-tubulin and actin have also been used somewhat less, and none of these has revealed a robust grouping of Foraminifera with any other group of eukaryotes (Pawlowski et al. 1994, 1996, 1999*a*, 1999*b*; Wade et al. 1996; beta-tubulin trees were inferred here, and no robust position was found for either Cercozoa or Foraminifera [data not shown]).

Similarly, no morphological characteristics of either group have suggested any obvious relationship to other eukaryotes, although with hindsight we can identify a handful of similarities between Cercozoa and Foraminifera. These characteristics are generally weak, as they either are found in other eukaryotes or are not universal among Cercozoa. For instance, both groups have tubular mitochondrial cristae, but this is a common trait among protists (Taylor 1999). Similarly, reticulate pseu-

dopodia are found in both groups, but in Cercozoa this trait is restricted to chlorarachniophytes (Hibberd 1990). While these characters are not strong evidence by themselves, they certainly are consistent with a relationship between the two groups. Moreover, it should be noted that with the vast morphological diversity of Cercozoa, such differences would not be unexpected; indeed, the Foraminifera are no more dissimilar to Cercozoa in general than many Cercozoa are to one another. In light of this, it is interesting that actin phylogeny typically places foraminifers within the Cercozoa, specifically with the cercomonads at the exclusion of chlorarachniophytes (see figs. 1 and 2). This is not well supported, and no topology is supported by Kishino-Hasegawa tests, and so it should be considered very cautiously. Nevertheless, the fact that the cercomonads and chlorarachniophytes fail to form a strong group at the exclusion of foraminifers could indicate that foraminifers are part of the Cercozoa rather than being sisters to them.

Finally, the relationship between Cercozoa and Foraminifera, like any other, will need to be corroborated by additional evidence. As already stressed, the majority of our present problems in discerning the tree of eukaryotes likely arise from variations in the rates of evolution among different lineages. Fortunately, it appears that different genes are prone to accelerated rates in different lineages, so an accurate picture of eukaryotic evolution will likely emerge from collection of data from many different genes, since each gene phylogeny will have different strengths and weaknesses. However, until such a battery of molecular and morphological evidence unites to support or refute a relationship, it must remain hypothetical. Nevertheless, until now there has been no good hypothesis for the position of either Cercozoa or Foraminifera, but actin provides a strong starting point. Moreover, with the current paucity of molecular data from both of these groups, the finding of a strong relationship between them provides good reason to be optimistic that increased molecular sampling from these groups will quickly yield a strong conclusion.

Acknowledgments

We would like to thank E. E. Chao, T. Cavalier-Smith, and K. Ishida for providing DNA to make this work possible. We would also like to thank J. Archambault for technical assistance, and N. M. Fast and K. Ishida for comments on the manuscript. This work was supported by a grant from the Natural Science and Engineering Research Council of Canada (227301-00). P.J.K. is a Scholar of the Canadian Institute for Advanced Research.

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MANOLO GOUY, reviewing editor

Accepted April 19, 2001