and the molars are larger, increasing slightly in size from M1 to M3 (see Table S1) (*32*).
24. B. Wood, *Nature* **355**, 783 (1992).
25. Several authors have argued that *H. habilis* (*sensu stricto*) and/or *H. rudolfensis* should be removed from *Homo* and placed instead with *Australopithecus*. J. T. Robinson (*33*) suggested this, and A. Walker (*34*) pointed out that the KNM-ER 1470 cranium exhibits a number of resemblances to *Australopithecus*. Recently, this view has been advanced by M. H. Wolpoff (*35*) and B. Wood and M. Collard (*36*).
26. P. V. Tobias, *Olduvai Gorge*, vol. 4, *The Skulls, Endocasts and Teeth of* Homo habilis (Cambridge Univ. Press, Cambridge, UK, 1991), pp. 1–921.
27. B. Wood, *Koobi Fora Research Project*, vol. 4, *Hominid Cranial Remains* (Clarendon, Oxford, UK, 1991).
28. W. H. Kimbel *et al.*, *J. Hum. Evol.* **31**, 549 (1996).
29. W. H. Zagwijn, *Mededeling. Nederl. Ins. Toegepast. Geowetensch. TNO* **60**, 19 (1998).
30. A. S. Tesakov, *Mededeling. Nederl. Ins. Toegepast. Geowetensch. TNO* **60**, 71 (1998).
31. B. Asfaw *et al.*, *Nature* **416**, 317 (2002).
32. L. L. Gabunia, M. A. De Lumley, A. Vekua, D. Lordkipanidze, *C. R. Acad. Sci.*, in preparation.
33. J. T. Robinson, *Nature* **205**, 121 (1965).
34. A. Walker, in *Earliest Man and Environments in the Lake Rudolf Basin*, Y. Coppens, F. C. Howell, G. Ll. Isaac, R. F. Leakey, Eds. (Univ. of Chicago Press, Chicago, IL, 1976), pp. 484–489.
35. M. H. Wolpoff, *Paleoanthropology* (McGraw-Hill, New York, ed. 2, 1999).
36. B. Wood, M. Collard, *Science* **284**, 65 (1999)
37. Research at Dmanisi is funded by the Georgian Academy of Sciences (grant N1318), National Geographic Society, and The Leakey Foundation (grants awarded to D.L.). Aspects of our interdisciplinary studies have been supported by Fulbright Foundation, Projects DGICYT-PB97-0157 (Spanish Ministry of Science) and ACE-38 (Generalitat de Catalunya), University of Zurich, the Eckler Fund of Binghamton University and the American School of Prehistoric Research, and the Peabody Museum of Harvard University. We thank all members of the 2001 Dmanisi research expedition, particularly J. Kopaliani, G. Kiladze, M. Mayer, G. Nioradze, S. Ediberidze, T. Shelia, D. Taktakishvili, and D. Zhvania, We are grateful to O. Bar-Yosef, F. C. Howell, H. de Lumley, M. A. de Lumley, and A. Walker for their help and assistance. Our work benefited from discussions with E. Delson, D. Lieberman, A. Justus, D. Pilbeam, O. Soffer, I. Tattersall, M. Wolpoff, and B. Wood. CT scans were produced at the Medical-Diagnostic Center of Tbilisi University. Photographs and illustrations were made by G. Davtiani, S. Holland, and G. Tsibakhashvili.

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Table S1
Figs. S1 and S2

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Rooting the Eukaryote Tree by Using a Derived Gene Fusion

Alexandra Stechmann and Thomas Cavalier-Smith

Single-gene trees have failed to locate the root of the eukaryote tree because of systematic biases in sequence evolution. Structural genetic data should yield more reliable insights into deep phylogenetic relationships. We searched major protist groups for the presence or absence of a gene fusion in order to locate the root of the eukaryote tree. In striking contrast to previous molecular studies, we show that all eukaryote groups ancestrally with two cilia (bikonts) are evolutionarily derived. The root lies between bikonts and opisthokonts (animals, Fungi, Choanozoa). Amoebozoa either diverged even earlier or are sister of bikonts or (less likely) opisthokonts.

One of the most challenging evolutionary problems is locating the root of the eukaryote tree. The widespread view that early eukaryotes were amitochondrial has recently been dramatically overturned (*1*). Multigene trees, though more reliable than single-gene trees, leave many possibilities open (*2*). We use a derived gene fusion between dihydrofolate reductase (DHFR) and thymidylate synthase (TS), previously known from a few eukaryotes (*3*), to greatly narrow down the position of the root. In eubacteria, both genes are separately translated, often in one operon, TS preceding DHFR (Fig. 1). Animals and fungi also have separately translated DHFR and TS genes (not in an operon), presumably the original eukaryotic condition (*3*). Plants, alveolates, and Euglenozoa instead have a bifunctional fusion gene with both enzyme activities in one protein (*3*). As this fusion is clearly derived compared with separate genes, it suggests that the eukaryote tree's root must be below the common ancestor of plants, alveolates and Euglenozoa (*3*). The root cannot lie among groups all having the fusion gene, because they share this derived character that arose in their common ancestor. As those with separate genes have the primitive condition, the root must lie adjacent to or within one of them.

This reasoning is valid only if the genes fused just once and were never secondarily split or laterally transferred within eukaryotes. Although evolutionary gene splitting is known for a few bacterial genes, it is a priori many orders of magnitude less likely for eukaryotic protein-coding genes, requiring simultaneous evolution at four separate, correctly ordered positions, not just two as in bacteria: we know no examples. Secondary splitting might also theoretically occur by gene duplication and differential deletions within each copy; even this would involve three independent mutations, two positionally precise, so is very improbable.

We amplified and sequenced DHFR-TS fusion genes from four previously unstudied groups: the heterokont chromist '*Cafeteria*' *marsupialis* and three protozoan phyla (centrohelid Heliozoa, Apusozoa, Cercozoa); plus, as positive controls, additional Euglenozoa and Ciliophora (*4*). Multiple alignment shows that all are authentic DHFR-TS fusion genes with one open reading frame. A further control was the choanozoan *Corallochytrium limacisporum*; as expected, because Choanozoa are probably sisters to animals (*5*), we found no fusion gene. Only in one other protist phylum (Amoebozoa, represented by *Phreatamoeba*, *Phalansterium solitarium*) could we similarly detect no fusion gene. In *Phreatamoeba* and *Corallochytrium*, we successfully amplified TS genes alone (*4*).

The presently known phylogenetic distribution of DHFR-TS fusion genes is shown in Fig. 1; strikingly, their origin coincides with that of the biciliate condition. All organisms above the apparent point of origin of the fusion protein in Fig. 1 are ancestrally biciliate and collectively called bikonts (*5*). Bikont monophyly is also shown by trees for 123 genes with ~25,000 amino acid positions (*6*), if rooted as in Fig. 1. In plants, chromalveolates, and excavates, biciliate cells, differentiate their cilia and roots over two successive cell cycles; this developmental complexity strongly indicates that bikont ciliary transformation is derived (*5*). The distribution of the DHFR-TS fusion supports this interpretation. We cannot exclude the possibility that the fusion occurred not at the very origin of bikonts, but after some small and obscure unstudied bikont lineage diverged from the rest. Our conclusion strongly contradicts recent assumptions that the root is among the excavate bikonts [e.g., beside Parabasalia (*7*) or jakobid Loukozoa (*8*)]; the two single amino-acid enolase deletions suggesting early divergence of Parabasalia (*7*) are much more easily reversible than the DHFR-TS fusion.

Archezoa (Parabasalia and metamonads) were formerly considered possible primitive eukaryotes because of absence of mitochondria and deep branching in sequence trees (*7*, *9*), but several lines of evidence now indicate that they are a relatively advanced group within excavates. Neither DHFR nor TS enzymatic activity is detectable in *Giardia intestinalis* (Metamonada), *Trichomonas vaginalis* and *Tritrichomonas foetus* (Para-

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basalia), suggesting dependence on exogenous thymidine (*10-12*), and the gene is absent from the virtually complete *Giardia* genome. Possibly these parasites have lost the fusion gene. rRNA trees not using long-branching archaebacteria as an outgroup place Parabasalia and Metamonada together with high bootstrap support (*5*, *13*, *14*) and often place Archezoa as sisters to Percolozoa. The complex tetrakont ciliary apparatus of Archezoa and Percolozoa was long an obstacle to considering them the most primitive eukaryotes; more likely, they evolved from simpler biciliate eukaryotes (*5*, *9*, *14*). As parabasalids and diplomonads share two derived laterally transferred genes of cyanobacterial origin [glucokinase (GK) and glucose-phosphate isomerase (GPI); Fig. 1] (*15*, *16*), Archezoa are almost certainly holophyletic, so the root cannot lie within them, whether beside metamonads, as rRNA trees rooted on archaebacteria suggested, or Parabasalia, as suggested by two single amino-acid insertions in enolase that are shared with prokaryotes (*7*). The enolase insertions are far weaker evidence than the DHFR-TS fusion, as they could easily have been secondarily acquired by replication slippage mutations or by a single gene conversion using as template a bacterial enolase gene taken into the phagotrophic ancestor of Parabasalia after it diverged from Metamonada and other excavates. Sequence evidence that retortamonads arose from diplomonads (*17*) eliminates them as candidate "early amitochondrial eukaryotes" by placing them firmly within the ancestrally tetrakont metamonads.

Jakobid Loukozoa have also been considered possible primitive eukaryotes because their mitochondria retain more prokaryotic features than others (*8*); retention of the proteobacterial RNA polymerase by *Reclinomonas* does not mean that it is the most ancient eukaryote—if the viral-type polymerase that replaced it was present in the ancestral eukaryote, the bacterial enzymes could have been lost independently in several lineages.

Although most published distance trees do not show excavate monophyly, probably because of long-branch problems, one maximum likelihood rRNA tree not rooted on archaebacteria shows the monophyly of excavates including *Reclinomonas* (*5*), with reasonably strong bootstrap support (87%) in the corresponding distance tree. In view of this and of the ultrastructural unity of excavates and their complex ciliary roots (*5*), we predict that jakobid Loukozoa also have the gene fusion. Whether Loukozoa are monophyletic or polyphyletic is uncertain, as is the precise position of excavates. But given the strong evidence for bikont monophyly, no uncertainty in their internal branching order would be relevant to our conclusion unless an unstudied lineage consistently branches closer to Amoebozoa than any other bikonts and also has separate DHFR and TS genes.

Like shared laterally transferred genes, symbiogenetic acquisitions of chloroplasts are derived characters that help narrow down the position of the eukaryotic root, for it cannot lie within any group created by a single such event. Thus the root cannot be within Plantae or chromalveolates (*5*, *18*). If euglenoids and chlorarachneans got their chloroplasts in one event in a common ancestor, for which good evolutionary arguments exist (*18*), then it cannot lie in any of its descendants (i.e., not within excavates or the Cercozoa/Retaria clade; Fig. 1). Even if euglenoids acquired chloroplasts independently (*5*), an early timing of that event would exclude the root from some groups: the *gnd* gene of plastid affinity in Percolozoa (*19*) implies a photosynthetic common ancestor of Percolozoa and Euglenozoa, ruling out both discicristates and Archezoa, if the latter really are sisters of Percolozoa (Fig. 1) [even the highly divergent *gnd* of Archezoa (*19*) might have the same origin].

Although the DHFR-TS gene fusion is the strongest available evidence for the position of the eukaryote root—among uniciliate protozoa (*5*), independent corroboration is desirable to rule out the theoretical possibility of its reversal in opisthokonts (and possibly Amoebozoa). Such reversal might in principle occur by horizontal acquisition from bacteria of separate DHFR and/or TS genes and partially or totally deleting the fusion gene. Our multiple alignments argue clearly against this; derived signature sequences indicate that the separate opisthokont DHFR and TS genes are distinctly more similar to the fusion genes than to the separate bacterial genes. Unfortunately, both genes are too short to make robust trees.

A unique insertion in EF-1α of animals

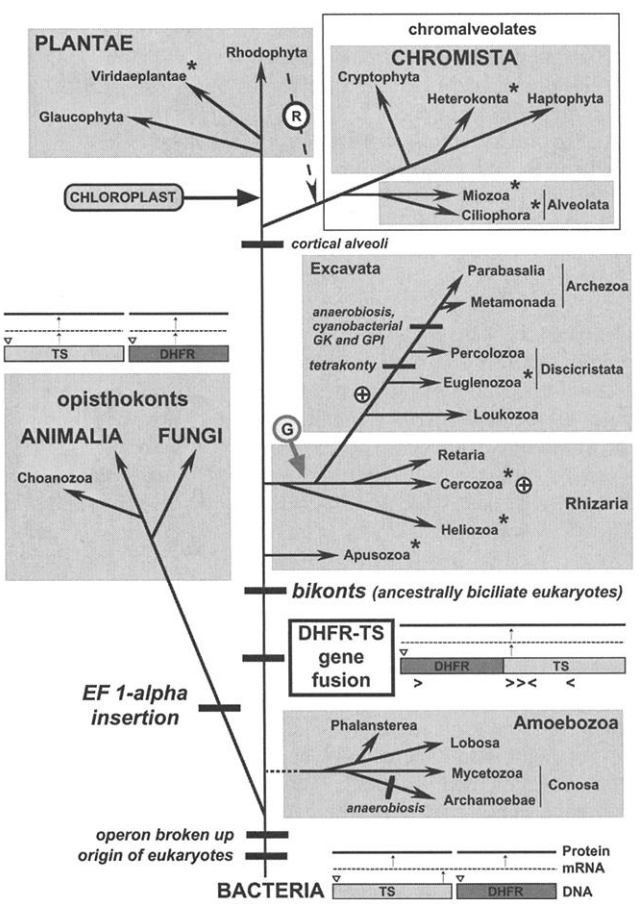

Fig. 1. Phylogenetic relationships of the major eukaryote groups [modified from (*5*)]. Opisthokonts have separate DHFR and TS genes. Asterisks mark all eight groups positive for the bifunctional DHFR-TS fusion gene. The origin of the fusion gene at the same time as the bikont flagellates is indicated [< and > mark primer sites used here; for details and a list of the nine taxa studied see (*4*)]. Triangles on the gene organization diagrams indicate the position of translation initiation codons. Although the branching order among the bikont groups is uncertain in places (notably the precise position of Heliozoa and excavates), these uncertainties are irrelevant to the conclusion that the root lies below the common ancestor of all these bikont groups. The monophyly and internal branchings of excavates are also uncertain, involving both sequence and ultrastructural evidence (*5*); the topology (not the rooting) of this tree is congruent with several (not all) recent sequence trees (e.g., *2*, *5*, *6*, *9*). The uncertain position of Amoebozoa relative to the root is emphasized by a dashed branch. Symbiogenetic events that created eukaryotic algae are shown: the primary origin of chloroplasts from cyanobacteria to form the plant kingdom (*23*); the secondary symbiogenetic implantation of a red algal cell (circled R) into a heterotrophic host to form chromalveolates (*5*) and lateral transfer of green algal chloroplasts to create euglenoid and chlorarachnean algae—whether both got plastids in a single ancestral event [circled G (*18*)] or separately [circled plus sign (*5*)] is uncertain. Miozoa comprise dinoflagellates, Sporozoa and protalveolates; Loukozoa include jakobids and anaeromonads; Retaria comprise Foraminifera and Radiolaria (*5*).

and fungi (*20*) is a derived character indicating that the root cannot be within opisthokonts. If it is outside bikonts and opisthokonts, it must be at or near the bifurcation between them. Our inability to amplify fusion genes in Amoebozoa (*Phreatamoeba*, *Phalansterium*) does not prove their absence. We also searched the genomic/EST databases of other Amoebozoa (*Dictyostelium*, *Entamoeba histolytica*) for the fusion and individual genes, without success. This is not surprising, for *E. histolytica* and *invadens* lack DHFR or TS enzymatic activity (*21*) and presumably also the genes, whereas *Dictyostelium* probably replaced TS by a nonhomologous enzyme (*22*). If other Amoebozoa have the fusion gene, contrary to present indications, they must be sisters to bikonts and the tree is rooted precisely as in Fig. 1, i.e., between opisthokonts and Amoebozoa/bikonts. If they genuinely lack it, their position will remain ambiguous; they could be sisters of bikonts or opisthokonts or branch below either.

Three arguments suggest, albeit indecisively, that the root may be between opisthokonts and Amoebozoa/bikonts (*5*). First, opisthokonts typically have flat mitochondrial cristae, whereas Amoebozoa/bikonts would ancestrally have had tubular cristae; this difference could reflect divergent specialization immediately following the symbiogenetic origin of mitochondria (*23*). Second, the single cilium is posterior in opisthokonts, but anterior in Amoebozoa; the latter character is shared with bikonts, ancestrally with one anterior and one posterior cilium (*5*). Third, bootstrap support for the bipartition between opisthokonts and bikonts/Amoebozoa is typically much stronger on single-gene trees than that between Amoebozoa and other eukaryotes (*2*, *5*, *7*, *20*); a significantly earlier divergence between opisthokonts and Amoebozoa/bikonts would simply explain this (*5*). Only if Amoebozoa turn out to branch below the opisthokont/bikont bifurcation would they be early diverging eukaryotes—the only ones. If Amoebozoa are sisters of bikonts or opisthokonts, there would be no extant eukaryote lineages that diverged before the common ancestor of animals and plants; the recent extensive searches for early diverging eukaryotes would have been wild goose chases. Further study of genetic diversity within Amoebozoa should clarify their position and thereby precisely pinpoint the root.

References and Notes

1. A. J. Roger, *Am. Nat.* **154**, S146 (1999).
2. S. Baldauf, A. J. Roger, I. Wenk-Siefert, W. F. Doolittle, *Science* **290**, 972 (2000).
3. H. Philippe *et al.*, *Proc. R. Soc. London Ser. B* **267**, 1213 (2000).
4. Supplementary information is available on *Science* Online.
5. T. Cavalier-Smith, *Int. J. Syst. Evol. Microbiol.* **52**, 297 (2002).
6. E. Bapteste *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 1414 (2002).
7. P. J. Keeling, J. D. Palmer, *Nature* **405**, 635 (2000).
8. B. F. Lang *et al.*, *Nature* **387**, 493 (1997).
9. T. M. Embley, R. P. Hirt, *Curr. Opin. Genet. Dev.* **8**, 624 (1998).
10. S. M. Aldritt, P. Tien, C. C. Wang, *J. Exp. Med.* **161**, 437 (1985).
11. C. C. Wang, H. W. Cheng, *Mol. Biochem. Parasitol.* **10**, 171 (1984).
12. C. C. Wang, R. Verham, S. F. Tzeng, S. Aldritt, H. W. Cheng, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2564 (1983).
13. T. Cavalier-Smith, in *Evolutionary Relationships Among Protozoa*, G. H. Coombs, K. Vickerman, M. A. Sleigh, A. Warren, Eds. (Kluwer, London, 1998), pp. 375–407.
14. T. Cavalier-Smith, in *The Flagellates*, B. S. C. Leadbeater, J. C. Green, Eds. (Taylor & Francis, London, 2000), pp. 361–390.
15. G. Wu, K. Henze, M. Müller, *Gene* **264**, 265 (2001).
16. K. Henze *et al.*, *Gene* **281**, 123 (2001).
17. J. D. Silberman *et al.*, *Mol Biol. Evol.* **19**, 777 (2002).
18. T. Cavalier-Smith, *J. Eukaryot. Microbiol.* **46**, 347 (1999).
19. J. O. Anderson, A. J. Roger, *Curr. Biol.* **12**, 115 (2002).
20. S. Baldauf, *Am. Nat.* **154**, S178 (1999).
21. C. Garrett *et al.*, *Mol. Biochem. Parasitol.* **11**, 257 (1984).
22. J. L. Dynes, R. A. Firtel, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7966 (1989).
23. T. Cavalier-Smith, *Biol. J. Linn. Soc.* **17**, 289, (1982).

24. We thank E. E-Y. Chao for all the DNA samples, H. Philippe for gene alignments, and NERC for a research grant. TC-S thanks NERC and the Canadian Institute for Advanced Research Evolutionary Biology Programme for Fellowship support. DHFR-TS and TS sequences obtained in this study are deposited under GenBank accession numbers AF450268-AF450274, AF485373.

Supporting Online Material

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Materials and Methods

Table S1

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Rapid Regulation of Light Harvesting and Plant Fitness in the Field

Carsten Külheim,[1] Jon Ågren,[2] Stefan Jansson[1]*

We used *Arabidopsis thaliana* mutants to examine how a photosynthetic regulatory process, the qE-type or ΔpH-dependent nonphotochemical quenching, hereafter named feedback de-excitation, influences plant fitness in different light environments. We show that the feedback de-excitation is important for plant fitness in the field and in fluctuating light in a controlled environment but that it does not affect plant performance under constant light conditions. Our findings demonstrate that the feedback de-excitation confers a strong fitness advantage under field conditions and suggest that this advantage is due to the increase in plant tolerance to variation in light intensity rather than tolerance to high-intensity light itself.

The ability to adjust metabolic processes to a variable environment should be crucial for the Darwinian fitness of plants and other sessile organisms, which cannot move away from unfavorable conditions. In recent years, the molecular basis of various short-term regulatory processes has been identified in plants, but the adaptive importance of these processes has never been explored under field conditions. One metabolic pathway that must be strictly controlled is the photosynthetic light reaction because it has potentially dangerous side effects. If the incident light increases or the photosynthetic dark reactions are retarded (for example, due to a drop in temperature or closure of stomata), then there is the risk that the production of adenoside triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide (NADPH) by the photosynthetic light reactions becomes greater than the capacity to catabolize these compounds, which causes photo-oxidative stress. Plants have evolved several protective mechanisms that have been suggested to represent adaptations against photo-oxidative stress (*1*). They operate at different time scales, and one, the qE-type of nonphotochemical quenching (NPQ) or feedback de-excitation, is a very rapid process that is induced seconds after a plant has been exposed to extreme light ("high light"). Feedback de-excitation accounts for about 80% of NPQ (*2*) and works by switching the photosynthetic antenna into a state of thermal dissipation instead of efficient solar energy utilization (*3*). Two proteins have been shown to be essential for feedback de-excitation. One is the enzyme violaxanthin de-epoxidase (VDE), which converts one carotenoid species (violaxanthin) to another (zeaxanthin) in the so-called xanthophyll cycle (*4*). The other is the PsbS protein that undergoes a conformational change when the "excitation pressure" rises, resulting in a nonradiative energy dis-

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