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Protists – A textbook example for a paraphyletic taxon ☆

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Abstract

Protists constitute a paraphyletic taxon since the latter is based on the plesiomorphic character of unicellularity and does not contain all descendants of the stem species. Multicellularity evolved several times independently in metazoans, higher fungi, heterokonts, red and green algae. Various hypotheses have been developed on the evolution and nature of the eukaryotic cell, considering the accumulating data on the chimeric nature of the eukaryote genome. Subsequent evolution of the protists was further complicated by primary, secondary, and even tertiary intertaxonic recombinations. However, multi-gene sequence comparisons and structural data point to a managable number of such events. Several putative monophyletic lineages and a gross picture of eukaryote phylogeny are emerging on the basis of those data. The Chromalveolata comprise Chromista and Alveolata (Dinoflagellata, Apicomplexa, Ciliophora, Perkinsozoa, and Haplospora). Major lineages of the former 'amoebae' group within the Heterolobosa, Cercozoa, and Amoebozoa. Cercozoa, including filose testate amoebae, chlorarachnids, and plasmodiophoreans seem to be affiliated with foraminiferans. Amoebozoa consistently form the sister group of the Opisthokonta (including fungi, and with choanoflagellates as sister group of metazoans). A clade of 'plants' comprises glaucocystophytes, red algae, green algae, and land vascular plants. The controversial debate on the root of the eukaryote tree has been accelerated by the interpretation of gene fusions as apomorphic characters. In the more traditional view, based on sequence comparisons using archaebacteria as outgroup representatives, parabasaleans and diplomonads branch off first, rendering the biflagellate eukaryotes paraphyletic. In sharp contrast, the root is placed between Bikonta and Opisthokonta plus Amoebozoa on the argument of a single enzyme gene fusion which is postulated to have occurred in the stem species of the Bikonta, and of a double enzyme gene fusion weighed as a synapomorphy of the Opisthokonta and Amoebozoa. We conclude that the paraphyletic taxon 'protists' may be maintained for practical reasons. However, introduction of new, clearly recognizable paraphyletic taxa should be avoided.
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Introduction

Protists are paraphyletic

The contemporary protists form a paraphyletic group, as the representative taxa do not contain all

Table 1. The chimeric nature of eukaryotic genomes and evolutionary hypotheses of the eukaryotic cell

Host	Endosymbiont	Result	Name/authorship
Archaeon	Bacterium	Pre-eukaryote without nucleus	Simple chimeric fusion
Archaeon	Bacterium	Mitochondrion in eukaryote	Endosymbiosis (Altmann 1890, 1994; Margulis 1970, 1981)
Archaeon	Bacterium	Nucleus in eukaryote	Mereschkowsky (1910)
H_2-dependent archaeon	H_2-producing bacterium	Mitochondrion in pre-eukaryote	Hydrogen hypothesis (Martin and Müller 1998)
Bacterium	Archaeon	Nucleus in eukaryote	Endokaryon hypothesis (Gupta et al. 1994; Horiike et al. 2001)
'Neomuran'	Bacterium?	Mitochondrion in eukaryote	Phagotrophy hypothesis (Cavalier-Smith 2002)
Bacterium	Archaeon	Nucleus in chronocyte	Chronocyte hypothesis (Hartman and Fedorov, 2002)
Community of primitive cells		Intensive lateral gene transfer	"You are what you eat" hypothesis (Doolittle 1998, 1999); "Pre Darwinian evolution" hypothesis (Woese 1998, 2002)

descendants of their stem species, i.e. the multicellular taxa, which evolved several times independently. In other words: they stand for those eukaryotes that are founded on nothing but the primitive feature of being unicellular organisms. The multicellular Metazoa as part of the Opisthokonta have a unicellular sister group, the choanoflagellates. Multicellularity also arose within the higher fungi, the heterokonts (with brown algae and oomycetes as main representatives), red algae, and distinct lineages of green algae: Likewise, all these higher taxa contain several unicellular representatives besides the multicellular ones.

Thus, the question arises whether a monophyletic taxon Eukaryota including the multicellular groups including the stem species and all its descendants can be argued for.

Discussion

Evolution of the eukaryotic cell

Both the evolutionary emergence of the eukaryotic cell and particularly the nature of the stem species are still far from being reasonably understood, and therefore are a matter of controversial debate. Accumulating information from gene sequence comparisons between prokaryotes and eukaryotes has yielded a confusing picture. Many genes, such as elongation factor 1α, vacuolar-type ATPase, large- and small-subunit ribosomal RNA, suggest closer relations to the archaebacteria or Archaea, whereas basic housekeeping genes such as those coding for aldolase type II and Fe-containing super oxide dismutase support sisterhood between Eukaryota and Eubacteria (Smith et al. 1992; Lake and Rivera 1994; Doolittle 1998). The eukaryotic genome appears to have a mosaic structure (for review see Horiike et al. 2001).

Several different hypotheses on the evolution of the eukaryotic cell have been developed (see Table 1); these are reviewed briefly here.

(1) The endokaryon hypothesis postulates the origin of eukaryotes as a fusion event between a gram-negative bacterium (the host) and an archaebacterium (the symbiont) eventually evolving into the nucleus. The argument is a shared 23 amino acid insertion in the hsp 70 gene of gram-negative bacteria and eukaryotes (Gupta et al. 1994). Likewise, the chimeric nature of the eukaryotic genome was explained by such a symbiosis (Horiike et al. 2001).

(2) In a comparable way but with different results, the hydrogen hypothesis assumes a symbiotic association between a hydrogen-dependent, autotrophic archaean methanogen (the host) and a heterotrophic aerobic eubacterium (the symbiont) which evolved into the mitochondrion (Martin and Müller 1998).

(3) Another hypothesis, postulating a community of primitive cells in early evolution that underwent intensive horizontal gene transfer, was promoted predominantly by W.F. Doolittle. This transfer is supposed to have been driven towards eukaryotes after the development of phagocytosis ("you are what you eat" hypothesis). It was also claimed that lateral gene transfer was significantly reduced only after the evolution of multicellularity and separation of germ line and soma (Doolittle 1998, 1999). Similarly, C.R. Woese proposed a pivotal role of horizontal gene transfer driving early cellular evolution. In stark contrast to Darwinian concepts of evolution, Woese postulates that early cell designs were simple and loosely organized, and that all cellular components could be altered or
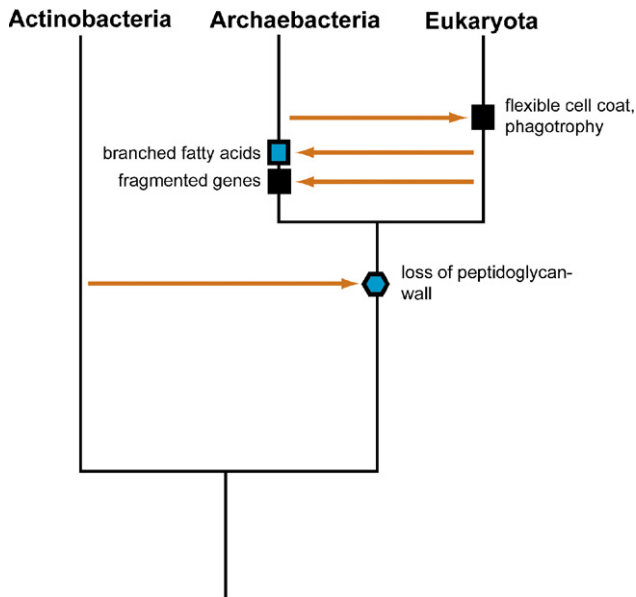
Fig. 1. Phagotrophy hypothesis of the evolution of eukaryotes. Archaebacteria and Eukaryota share the loss of the peptidodoglycan wall and development of N-linked glycoproteins as a synapomorphy ('Neomura'). Replacement of acyl ester lipids by isoprenoid ether lipids, and fragmentation of RNA polymerase into two separate genes and of glutamate I into three separate genes are autapomorphies of Archaebacteria. Acquisition of a flexible cell coat and of phagotrophy are autapomorphies of Eukaryota.

Fig. 2. Chronocyte hypothesis of the evolution of eukaryotes. The chronocyte was a temporarily existing cell type with RNA-based information storage. The nucleus is derived from a DNA-based endobiotic eubacterium.

displaced by horizontal gene transfer without a stable organismic genealogical trace. Later, as cell design became more complex and interconnected, a critical point is supposed to have been achieved, with a more integrated cellular organization and increasing importance of vertical inheritance. This "Darwinian threshold" is postulated to mark the origin of species (Woese 1998, 2002).

(4) A radically different scenario is proposed by the phagotrophy hypothesis (Cavalier-Smith 2002). According to this theory, eukaryotes are the sister taxon of Archaebacteria, with the loss of the peptidoglycan wall and development of N-linked glycoproteins as a synapomorphic character (Fig. 1; for colour version see the online edition). Cavalier-Smith coined the name Neomura ('new-wall' organisms) for these two sister taxa. The new glycoproteins served as an 'exoskeleton' in Archaebacteria. Both the replacement of acyl ester lipids by isoprenoid (isopranyl) ether lipids – as an adaptation to hyperthermophily (Cavalier-Smith 1987, 1998) – and the fragmentation of RNA polymerase into two separate genes and of glutamate I into three separate genes are interpreted as autapomorphic characters of Archaebacteria. However, the eukaryotes evolved a flexible surface coat and the capability for phagocytosis that led to fast development of cytoskeleton, endomembrane system, nuclear envelope, mitosis,
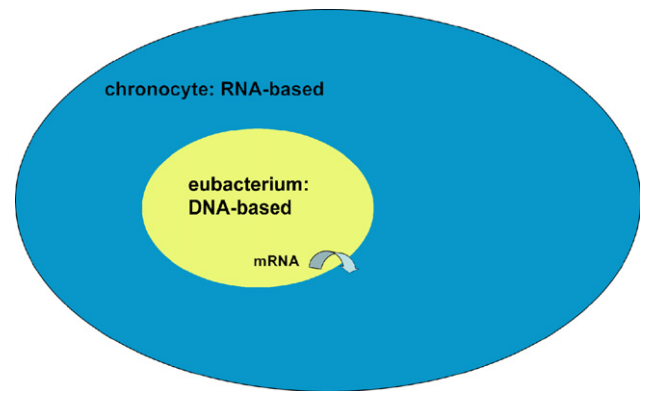
meiosis, and syngamy. An argument favouring this hypothesis is the low probability of a re-unification of the fragmented genes in the eukaryotes, which one has to assume when advocating the endokaryon hypothesis. Eubacterial genes in eukaryotes are interpretable as plesiomorphic characters that were retained from the neomuran ancestor. However, it is difficult to imagine how phagocytosis may have evolved prior to the presence of a cytoskeleton being a prerequisite for invagination processes and the formation of food vacuoles.

(5) The chronocyte hypothesis was developed by H. Hartmann and A. Federov (2002). They identified 347 eukaryote signature proteins (ESPs) by genome comparisons between members of all three domains, including *Giardia lamblia* as a 'basal' eukaryote. ESPs are affiliated with the cytoplasm and membrane system (cytoskeleton and calcium ion control system in signal transduction), the endoplasmic reticulum, and the cyclin cycle (as a master for the cell cycle). The authors postulate the temporary existence of a further cell type, the chronocyte, which possessed ESPs, a nucleus derived from a eubacterium and additional genetic information from an archaebacterium, and which became the precursor of the eukaryotes. The chronocyte is considered as a remnant from the RNA world, with RNA-based information storage, whereas the eubacterium-derived nucleus was DNA-based. This would be a reasonable explanation for the separation of nuclear transcription and cytoplasmic translation (Fig. 2; for colour version see the online edition).

Primary, secondary and tertiary intertaxonic recombination

Regardless of how the mitochondrion evolved, whether by a fusion event or by endobiosis (shortly) after the evolution of the eukaryotic cell (Gray 1989,
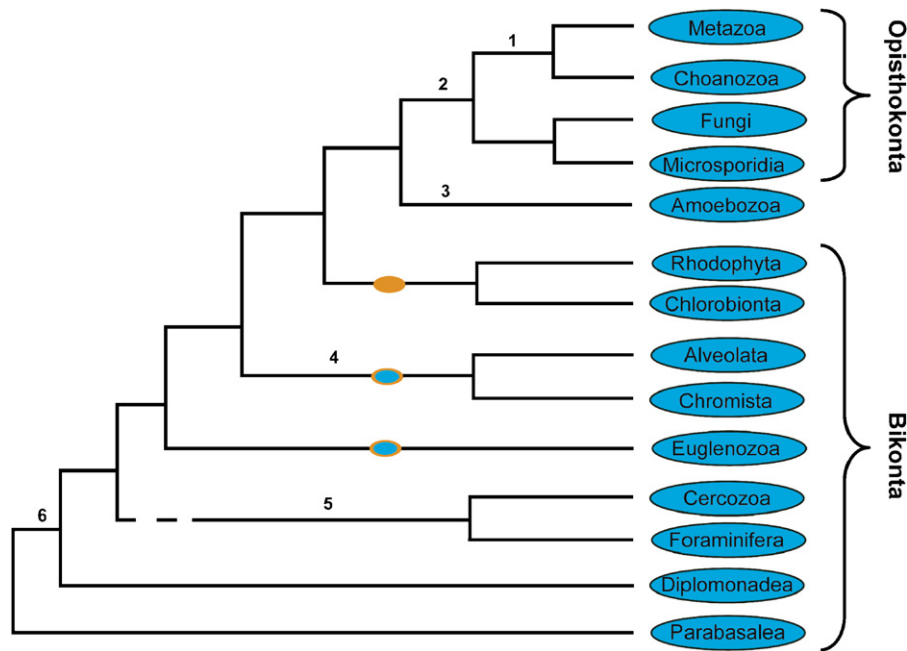
Fig. 3. Phylogenetic tree of eukaryotes based on multi-gene sequence comparisons (Baldauf et al. 2000; Bapteste et al. 2002; redrawn from Schlegel 2003). Numbers indicate evolutionary events that can be seen as autapomorphies for the respective clade: (1) fusion of the epidermal growth factor gene with the tyrosine kinase to a receptor tyrosine kinase; (2) 12–17 amino acid insertion in the elongation factor 1α gene; (3) fusion of the COX I and COX II genes; (4) duplication and plastid targeting of nuclear glyceraldehyde-3-phosphate dehydrogenase genes; (5) serine and/or alanine insertion in the functionally important monomer–monomer junction which is involved in correct polyubiquitin processing; (6) two amino acid deletion in the enolase gene. Solid ellipse indicates unique primary plastid acquisition; open ellipses indicate secondary plastid acquisitions.

1999), subsequent intensive gene transfer renders the monophyly of eukaryotes problematic. This chimerical nature is even more characteristic for the autotrophic organisms, although it is widely agreed now that plastids were acquired also only once and that the host cell then separated into the glaucocystophytes, red and green algae (Delwiche 1999; Keeling 2004). Besides these primary intertaxonic recombinations, secondary acquisition also occurred several times by the uptake of unicellular green algae (in euglenids, dinoflagellates, chlorarachnids) or of red algae (in cryptomonads, heterokonts, haptomonads, dinoflagellates, apicomplexans). Even tertiary recombination occurred through engulfment of a haptomonad (or diatom) by several dinoflagellates (Delwiche 1999). However, multi-gene sequence comparisons and structural data point to a limited number of such events. For example, T. Cavalier-Smith's concept of the Chromalveolata is supported, which reduces the number of secondary endosymbiotic events (Fig. 3; for colour version see the online edition) (Baldauf et al. 2000). This taxon encompasses the Chromista (Cavalier-Smith 1986, 2004) and the Alveolata (dinoflagellates, apicomplexans, ciliates, and two smaller taxa: the Perkinsozoa and Haplosporidia). It is obvious that the apicomplexans, although completely parasitic, carry the remnant of an algal plastid; whether of red- or green-algal ancestry

remains to be determined (Palmer 2003). Interestingly, the Chromalveolata concept demands the former possession of plastids in ancestral ciliates, too. On the other hand, the relative rareness of examples for evolutionary events from an endosymbiotic algal cell to a plastidal organelle contrasts with the common presence of endosymbiotic algae in food vacuoles of ciliates with non-photosynthetic lifestyle, such as *Paramecium bursaria*.

Monophyletic lineages within protists

Several other monophyletic lineages can be postulated based on sequence comparisons and structural data (Fig. 3) (Baldauf et al. 2000; Baldauf 2003; Schlegel 2003; Steenkamp and Baldauf 2004). One of the promising results is the emergence of a clearer picture of the phylogeny of the former 'amoebae', which form several independent lineages (for example the Heterolobosa, Cercozoa and Amoebozoa). Amoebozoa contains the largest portion of amoeboid taxa, including the lobose amoebae, the amitochondriate, 'primitive' mastigamoebae, the cellular and acellular slime moulds, and the naked filopodial amoebae. These amoebozoans consistently form the sister group of the Opisthokonta, including the fungi and choanoflagellates with
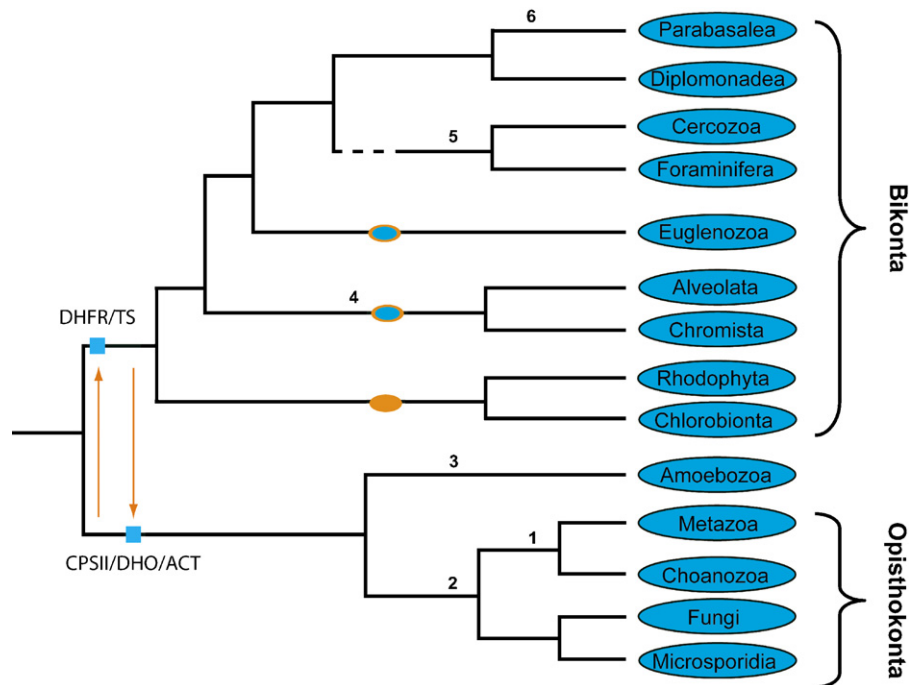
Fig. 4. Alternative rooting of the eukaryote tree. Arrows indicate the putative dihydrofolate reductase (DHFR) and thymidylate synthase (TS) gene fusion, which is interpreted as an autapomorphy for the Bikonta, and the three enzyme genes fusion of carbamoyl-phosphate synthase II (CPSII), dihydroorotase (DHO) and aspartate carbamoyl transferase (ACT), seen as autapomorphic for the Opisthokonta and Amoebozoa.

metazoans (Baldauf et al. 2000; Bapteste et al. 2002). The Cercozoa represent another recently defined taxon, including filose testate amoebae, chlorarachnids, and the plant-parasitic plasmodiophoreans (Bhattacharya et al. 1995; Cavalier-Smith 1998; Wylezich et al. 2002). Most recently, an affiliation of cercozoans with foraminiferans has been hypothesized on the evidence of actin gene sequence comparisons (Keeling 2001) and a peculiar polyubiquitin structure. Usually, ubiquitin genes are organized in head-to-tail polymers whose products are post-translationally processed to ubiquitin monomers. In cercozoans and foraminiferans, they deviate from the canonical polyubiquitin structure in having an amino acid insertion at the monomer–monomer junction, suggesting that polyubiquitin processing in these organisms is unique among eukaryotes (Archibald et al. 2002). In addition, phylogenetic analyses of RNA polymerase II phylogenies point in the same direction (Longet et al. 2003).

In addition, it is worth mentioning a clade ('plants') comprising glaucocystophytes, red algae, green algae, and land vascular plants (Baldauf et al. 2000).

Where is the root of the tree?

With regard to the basal branching order and thus the position of the eukaryotic root of these lineages, two radically differing hypotheses are competing. In analyses of gene sequences or derived amino acid sequences, and rooting the tree with archaebacteria as outgroup representatives, parabasaleans and diplomonads branch off first, which would render the biflagellate eukaryotes paraphyletic (Fig. 3) (Keeling and Palmer 2000). However, it has been argued that this early branching may be an artifact due to high substitution rates in these sequences, which would place them artificially in a deep phylogenetic position (so called 'long branch attraction') (Bapteste et al. 2002). Stechmann and Cavalier-Smith (2002, 2003) proposed an alternative branching order based on an apomorphic dihydroxyfolate oxidase-thymidilate synthase gene fusion in bikonts. Conversely, and in contrast to the traditional view, the opisthokont flagellation is regarded as plesiomorphic, whereas bikont flagellation is apomorphic, and bikonts form a monophyletic group (Fig. 4; for colour version see the online edition). However, since all opisthokonts show an 11 amino acid insertion within the elongation factor 1α, there remains evidence for their monophyly. Moreover, a multi-enzyme gene fusion of carbamoyl-phosphate synthase, dihydroorotase, and aspartate carbamoyl transferase (enzyme genes involved in pyrimidine synthesis) points to a sister-group relationship between opisthokonts and amoebozoans (Stechmann and Cavalier-Smith 2002, 2003). However, other schemes (Cavalier-Smith 1998; Hausmann et al. 2003) consider

the fact that bikonty (biflagellation, biciliation) or – in a more general form – the possession of paired kinetosomes (= centrioles) is a primitive character of all eukaryotes, including the opisthokonts.

New clades detected by rRNA analyses of environmental samples are changing the view of protistan biodiversity

Ribosomal RNA analyses are also informative at the species level and have even led to the discovery of many new protistan taxa, often in extreme habitats, for example in the Rio Tinto in Spain with a pH of 2. These results challenge traditional ideas about the phylogenetic range of organisms capable of living in extreme habitats (Amaral-Zettler et al. 2002). Likewise, with freshwater Foraminifera detected using gene probes (Holzmann et al. 2003), the group is by no means exclusively marine. So-called 'picoeukaryotes' were also discovered in the Antarctic marine polar front. Ribosomal RNA sequence comparisons have revealed, among others, two lineages within the alveolates, related to the dinoflagellates (Lopez-Garcia et al. 2001). Their sequence diversity is equivalent to that displayed by all dinoflagellates. However, there has been a drawback to this approach until now. We do not know more of these organisms than the ribosomal RNA sequences; important morphological, physiological and genetic data are missing. In addition, deep-phylogenetic relationships reconstructed with ribosomal RNA have to be interpreted with caution (Philippe et al. 2000). An elegant approach to overcome this problem has been made recently by Massana et al. (2002) and further developed by Stoeck (Stoeck et al. 2003). The latter author uses gene-specific rRNA oligonucleotides for fluorescence in situ hybridization to detect the cells in water samples where the rRNA gene had been isolated before. Then, he cuts out the region containing the target cell and prepares it for scanning electron microscopy. Thus, at least the gap to morphology has been bridged now.

Conclusions

Should we abolish using protists as a taxon in textbooks? Formal arguments to do so come from the consequent phylogenetic approach, since a natural classification of protists is not achievable. Moreover, there are doubts concerning a genetically homogeneous stem species, and phylogeny was by no means always dichotomous, but several times reticulate.

However, the basis for scientific discussion of phylogeny is the actual tree hypothesis; the written classification is subordinate and may be handled more conservatively (Sudhaus and Rehfeld 1992). In addition,

general conclusions can be drawn from paraphyletic groups, too, based on plesiomorphic characters of diagnostic value. Consequently, paraphyletic taxa may be maintained for reasons of easier communication and for teaching purposes, as long as they are clearly labelled in a classification. Thus, for the time being, we make a plea for maintaining the 'protists', both in science and teaching, as many contemporary colleagues may also live with 'algae', a group that is even polyphyletic, when understanding the term as an organization level rather than a natural group. On the other hand, introduction of new taxa clearly recognizable as paraphyletic should be avoided.

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