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Position paper

Eukaryosis: Phagocytosis and hydrogenases

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Abstract

Members of the International Symbiosis Society are interested in many aspects of eukaryosis and symbiogenesis, especially those aspects discussed in the recent special issue of *Symbiosis* (Volume 44) resulting from the International Symbiosis Congress in Vienna (Margulis et al., 2007; Chapman and Alliegro, 2007; Gontier, 2007). Since eukaryosis and symbiogenesis are central foci of our society, we draw attention in this position paper to the relevant protistological, biochemical and genomic literature included in the recent de Duve-Meyer reviews (de Duve, 2007; Meyer, 2007). We fully agree with de Duve's analysis and his conclusion that evolution of the nucleocytoskeleton most likely preceded acquisition of chloroplasts and mitochondria (Margulis et al., 2006). We present additional information to elucidate his analysis.

Keywords: Cytoskeleton, hydrogenosome, intracellular motility, karyomastigont, nucleus, seme, symbiogenesis

1. Commentary

Christian de Duve (2007) in a *Perspectives* Essay entitled "*The origin of eukaryotes: A reappraisal*" (see below) contrasts two hypotheses with respect to "eukaryosis": the problem of how nucleated cells originated. Both accept the symbiotic origin of mitochondria from oxygen-respiring alpha proteobacteria and the later acquisition in some lineages of plastids from oxygenic chlorophyll-*a* bearing cyanobacteria. Thus evolved the two classes of membrane-bounded hereditary organelles: mitochondria and chloroplasts. However, de Duve then asks "what types of cells, precursors to the nucleocytoplasm, 'adopted' the eubacterial ancestors?" With regard to this problem he outlines the great differences between all prokaryotes and the "ground cytoplasmic" features of any eukaryote. De Duve outlines the astonishing, unique and universal characteristics of eukaryotic cells, summarized below, to which we have added further detail:

1. Cell movement, visible with light microscopy, in living organisms and known as the phenomenon of "cell motility":

A. The ability to perform phagocytosis, the actin (and other protein-based) ingestion process, required for the "adoption" of any prokaryote that, with time and integration, potentially evolves to become an organelle;

B. Exocytosis, cyclosis (=cytoplasmic streaming), endocytosis, plasmodial circulation, pseudopod formation and retraction and other directed cell movement, mainly internal, but (in the formation of scales, spines, and cell wall plates such as coccoliths) sometimes external, i.e., extending beyond the plasma membrane;

C. Mitotic cell division, cytoskeleton and its associated motor proteins with all the biochemistry of locomotion and protracted microtubule-microfilament-NTPase activities. Meiosis, the corollary of mitosis, in animal, plant, fungal and protoctist cells that undergo sexual fusion involves the reduction of chromosome number by the use of the spindle, also used in mitosis, where the centromere-kinetochore motors attach to DNA. The microtubular spindle and its associated MAPS (*m*icrotubule-*a*ssociated *p*roteins) are also a form of intracellular motility.

2. Pore-studded nuclear membrane that encloses chromatin-chromosomes (histone and other alkaline-rich proteins that form nucleosomes around their DNA).

3. Oxygen-related organelles such as mitochondria, plastids, and peroxisomes.

4. "Eukaryotic signature proteins" (revealed by molecular investigation, these "ESPs" are apparently absent so far in genome sequences of any prokaryote).

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5. Intracellular digestion via lysosome vesicle formation followed by protein release and absorptive nutrition through the lysosome membranes.

The two hypotheses that de Duve evaluates are:

1. The proposal that the main features of the "ground-cytoplasm" of eukaryotes were already present in the ancestors of those cells that "adopted" (acquired the prokaryotic genomes that became) the mitochondria and plastids. The major feature of interest is the "ability to capture food by endocytosis and digest it intracellularly;" it must have preceded organelle acquisition and "later had a key role in the adoption of endosymbionts."

2. The idea that the eukaryosis transformation "was triggered by an interaction between two typical prokaryotic cells, one of which became the host and the other the endosymbiont."

After a masterful discussion of biochemical, molecular and cytological details de Duve concludes "re-examination of this question in the light of cell biological and phylogenetic data leads to the conclusion that the first model [#1 here] is more likely to be the correct one". We strongly encourage the readers of *Symbiosis* to read his review.

We were inspired both by de Duve's review and by the profound body of biochemical evolutionary data that was described and critically reviewed by Meyer (2007). We are persuaded to support de Duve's hypothesis #1. Both quantity and quality of genomic-proteomic details amassed and evaluated in an evolutionary context by Meyer are astounding. Meyer and colleagues over the years thoroughly have traced genomic-proteomic sequences that are inexplicable except by symbiotic acquisition of "coherent collections of enzymes" (de Duve's phrase). We identify these as a set of genes and their enzymes inherited together that determine an evolutionary trait of identifiable selective advantage, i.e., "a seme" (Margulis et al., 2007). Examples include hydrogenases in hydrogenosomes that evolved from hydrogen-gas producing prokaryotes, e.g., clostridia.

The de Duve and Meyer reviews, taken together, underscore the relevance of this research to the history of cell symbiosis of such "coherent collections of [hydrogen-handling] enzymes." Through amino acid sequences in the Fe-Fe hydrogenases (including some present in green algal chloroplasts, but not in their cyanobacterial ancestors) both the symbiotic and direct filiation modes of origin of these polyphyletic hydrogen-productive organelles were deduced (Meyer, 2007).

Following acceptance of hypothesis #1, the next question is how did the ancestors (of cells that "adopted" oxygen-respiring alpha proteobacteria and lineages of plastids from oxygenic chlorophyll-*a* bearing cyanobacteria) evolve? Neither de Duve (2007) nor Poole and Penny (2007) satisfactorily resolve this dilemma. In the attached paper, we discuss the relevant research data, primarily from the protistological literature. This was written as a response to de Duve (2007) and published in abstract form in the November issue of *Nature Reviews – Genetics*. The full version, including the two figures, is only available online. The present commentary together with our online paper, re-published with permission of the journal, comprises this position paper.

In our *Nature Reviews – Genetics* response, we reply to those who ask whether the membrane-bounded nucleus evolved in the same series of evolutionary innovations in which mitochondrial ancestors were symbiotically acquired. We think not. Did peroxisomes evolve from bacterial symbioses? We think the data are consistent with de Duve's idea that they did. Did all mitosomes, hydrogenosomes and/or "anaerobic mitochondria" evolve from oxygen-respiring ATP-coupled standard mitochondria? No doubt some did, for example, those in the anaerobic chytrids, plagyopylid ciliates and mutant yeast. However, we propose, on the basis of comparative protist cell biology, that many others did not. Is the protoctist phylum ARCHAEAPROTISTA (=direct descendants of amitochondriates, e.g., metamonads, retortamonads, parabasalids including trichomonads and hypermastigotes, etc) a valid classification, as we claim? We insist, in fact, that there is no missing link in the story of the symbiogenetic origin of nucleated cells in the sulfur-rich waters of the Proterozoic-eon; but we also claim, as does Meyer (2007), that the most relevant organisms to this evolutionary scenario are the least studied: smaller members of the kingdom Protoctista (i.e., protists) in anoxic environments. We encourage study of protoctist "imperfections and oddities" (in Darwin's phrase, see Margulis et al., 2005) to elucidate their origins. Indeed, "eukaryosis" can be documented because modern descendants of each step still can be found, studied and photographed live in their natural anoxic habitats (Margulis and MacAllister, 2004).

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Semes for analysis of evolution: de Duve's peroxisomes and Meyer's hydrogenases in the sulphurous Proterozoic eon

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Although de Duve's review, "The origin of eukaryotes: A reappraisal" (de Duve, 2007), is masterful and relevant, certain lesser-known but important work was overlooked. Support for his idea abounds: phagocytotic intracellular motility preceded the 'adoption' of mitochondria and plastids (Fig. 1). What de Duve calls "coherent collections of enzymes" are 'semes', the units of evolutionary analysis (Margulis et al., 2006).

The archaeabacterial-eubacterial merger (Gupta's chimaera) (Gupta, 2005) resulted in membrane fusion; archaeabacterial lipids and proteins formed the endoplasmic reticulum whereas the Golgi components evolved from eubacterial membrane biosynthesis (Helenius and Aebi, 2001). The archaeabacterial-eubacterial symbiotic merger of a thermoacidophilic sulphidogenic heterotroph (Searcy, 2003; Searcy and Lee, 1998) with a motile sulphide-to-sulphur oxidizing heterotroph occurred under the threat of oxygen toxicity. From this syntrophy, hundreds of protists evolved. Their descendants thrive in anoxic habitats (for example, pelomyxids, mastigamoebae, devescovinids, oxymonads, trichomonads and other parabasalids). The presence of phagocytosis, mitosis and endocytosis in these amitochondriates testifies to the evolution of cytoskeletal motility (Fig. 2) before mitochondria (Margulis et al., 2006). The contributor of motility to the chimaera was the ancestral '*Perfil'ieva*', a free-living, aerotolerant, sulphurous mud-scum-mat *Spirochaeta*-like eubacterium of geochemical significance (Dubinina et al., 1993a,b) (now banked in the German culture collection in Braunschweig, with strain accession numbers Str. P=DSMZ 19205 and Str. SR=DSMZ19230). By use of Hall's new algorithm, more than 50 genes for the synthesis of eukaryotic enzymes and lipids were acquired by the chimeric eukaryotes in the transition from a *Spirochaeta*-like eubacterium to the [9(2)+2] motility organelle (J.L. Hall and L.M., unpublished observations). At lower stringency, even more eukaryotic sequences in *Perfil'ieva* should be detected.

Permanent bacterial conjugation-nucleoid membrane formation (Fuerst and Webb, 1991) generated the nucleus tethered to [9(2)+2] motility organelles (such as undulipodia including cilia) and its attachment apparatus, which became the centriole-centrosome system (*Mixotricha* is analogous) (König et al., 2007). The intron-less eubacterial DNA (now in the nucleolinus of the nucleolus) became the centrosome-centriole DNA (Alliegro and Alliegro, in press). Specific centrosomal RNAs and proteins for assembly and maintenance of the centriole-centrosome system (Alliegro et al., 2006) are discussed in Chapman and Alliegro (2007). Redundancy reduction followed fusion. Genetic and metabolic systems acquired from intracellular motile symbionts were integrated and redeployed (comparable with what occurred in the evolution of *Staurojoenina* [Wier et al., 2007], *Peridinium balticum*, *Mesodinium rubrum* and *Hatena*) (Oklamoto and Inouye, 2005; Margulis, 1993). Natural selection in microoxic habitats maintained heterotrophic chimaeras.

Information-molecule loss from centriole-kinetosomes (such as gene loss in plastids and mitochondria) occurred in the sulphurous Proterozoic eon (2500–541 million years ago) (Knoll, 2003) during which time peroxisomes were acquired. Besides peroxisomes, organelles that are probably of bacterial origin that continued the trend of loss of genes to the nucleus until completion include some hydrogenosomes, γ-particles of *Blastocladiella*, and mitosomes.

Genome analysis of hydrogenase distribution is explicable only if hydrogen gas production entered anaerobic protists via at least two distinct events (Meyer, 2007): acquisition of a cytosolic enzyme complex or of a symbiotic bacterium (or both). The hydrogenase seme

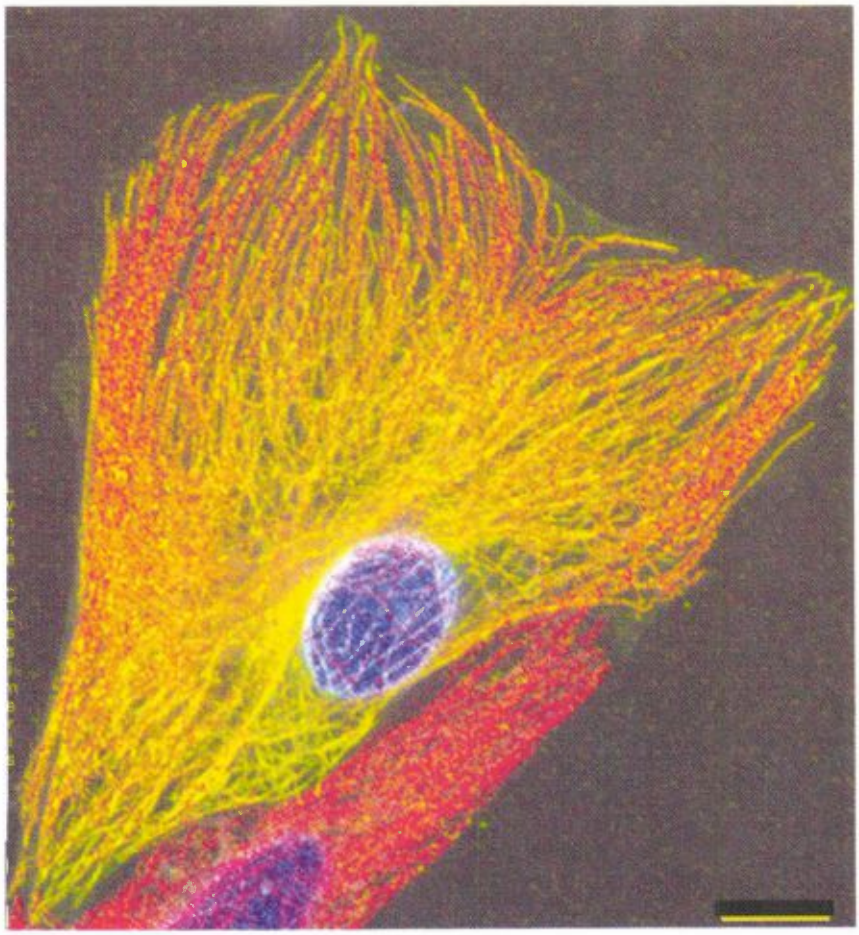

Figure 1. Did this cell originate by direct filiation from an archaeabacterium? The image shows a porcine epithelial cell (cell line LLCPK) fixed in –20°C methanol. The microtubules, stained in red, were labeled with an Alexa-568 fluorescent antibody to alpha-tubulin. The microtubule tip-binding protein EB1 was stained green with an Alexa-488 labeled antibody (see Piehl and Cassimeris, 2003). The DAPI-stained blue nucleus can be clearly distinguished. EB1 binds to the tips of growing microtubules, highlighting the dynamic nature of the eukaryotic cytoskeleton. Growth and shortening of microtubules allows them to probe throughout the cytoplasmic volume and to connect distant regions of the cell via transport by molecular motors. Confocal fluorescent light micrographic unpublished image courtesy of Lynne Cassimeris, Department of Biological Sciences, Lehigh University, Bethlehem, PA, USA. Scale bar = 10 micrometers. *See cover illustration.*

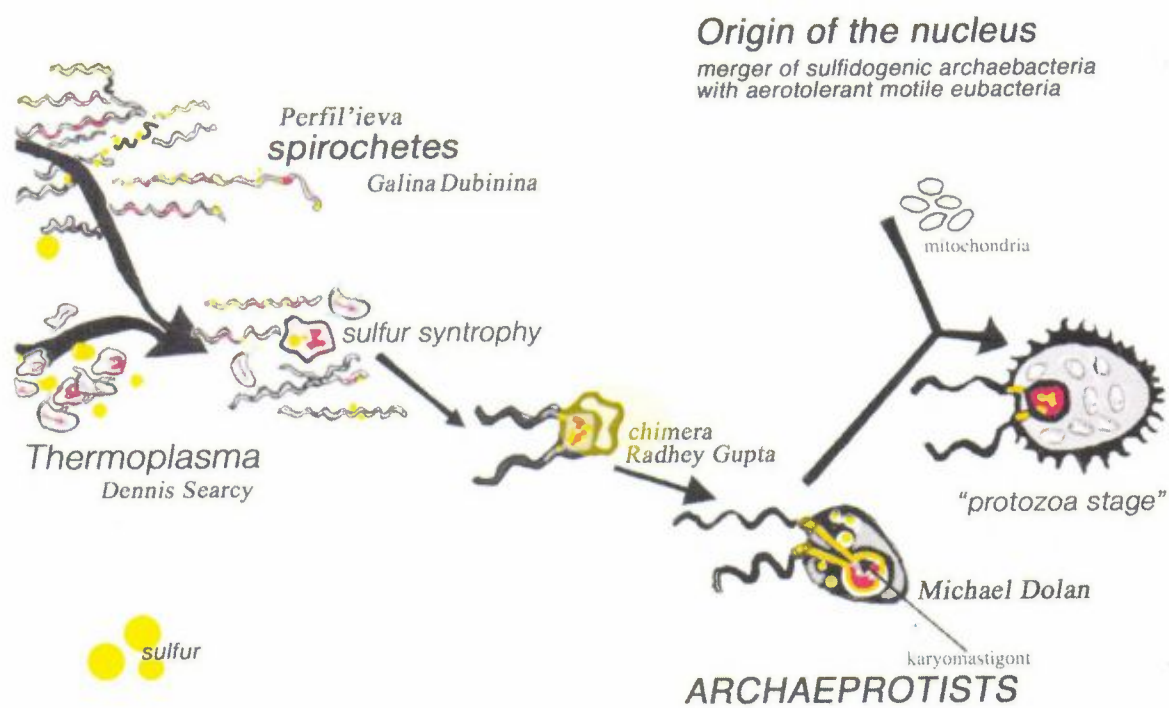

Figure 2. Karyomastigont model of the origin of nucleocytoplasm (earliest protists) in the Proterozoic eon (summarized). See Margulis et al. (2006) for a detailed explanation.

derives from the chimaera's eubacterial partner (aerotolerant sulphide-oxidizing *Perfil'ieva*) or some other 'adopted' eubacterium. The hydrogenosomal Fe–Fe hydrogenases – for example, those of *Trichomonas vaginalis* and most parabasalids (many of which are multinucleate, but none of which are mitochondriate) – evolved from *Clostridium*-like bacteria. But the hydrogenases of *Spironucleus*, *Giardia* and *Entamoeba histolytica* are cytosolic; presumably they retained enzymes from the eubacterial ancestor (for example, the sulphur syntrophic *Perfil'ieva*). Anaerobic chytrids, even in the same genus, differ markedly (*Neocallimastix ovalis* versus *Neocallimastix frontalis*), confirming hydrogenase–hydrogenosome polyphyly (Hackstein and Yarlett, 2005). The origin of Fe-Fe hydrogenases that are incapable of generating hydrogen gas in 'crown taxa eukaryotes' (animals, plants and fungi) noted by Meyer (Meyer, 2007) is implied by the data that de Duve discussed. The eubacterial cytosolic or periplasmic hydrogenase complex that was acquired from eubacterial ancestors changed during eukaryosis as intracellular motility evolved in amitochondriates. The hydrogenase system hypertrophied, mutated or was lost in response to the rising oxygen threat. Dispensable hydrogen gas production was not selected for, but rather hydrogenases and/or hydrogenosomes and their components were retained for myriad other semes.

Semes must be identified. Amino-acid or nucleotide homologies without seme identification lead to systematic inaccuracy in evolutionary reconstruction. Molecular sequencing techniques may resolve origins, but not in absence of the knowledge of whole organisms in their paleoenvironments.

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