Thioautotrophic symbiosis: towards a new step in eukaryote evolution?

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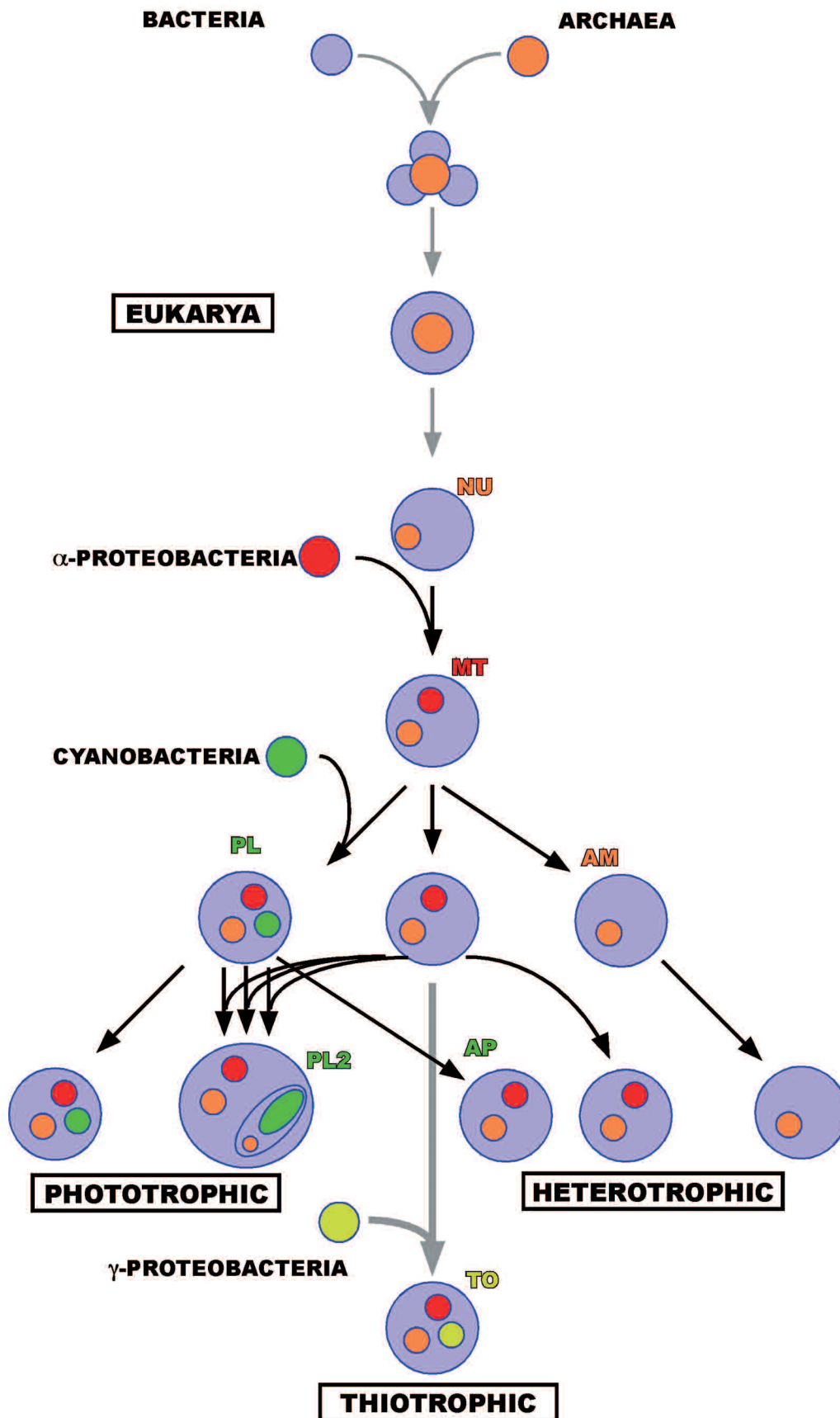
Abstract: Endosymbiosis is a key feature of the origin and history of eukaryotes which possibly involved a trophic symbiosis between bacteria and archaea at the very beginning, and whose mitochondria and plasts certainly stemmed out of endosymbiosis events. However the detailed history of these pioneers may well be buried too deep in the past to be ever completely elucidated. Endosymbiosis examples abound in the contemporary marine environment either between bacteria and eukarya or even between several eukarya. Some of the most prominent examples of such trophic symbiosis are the chemoautotrophic symbiosis between bivalves or tubeworms and sulfo-oxydant bacteria which dominate deep-sea hydrothermal vents and cold seeps. These different endosymbiosis experiences may provide biological models for the various steps these associations must go through to evolve from a simple trophic assemblage between two or more organisms into a true organelle status resulting in a single organism. In the case of sulfo-oxidant symbionts one of these examples could be the stem for a new eukaryotic lineage, the thiotrophic eukaryotes. In this short review, some thiotrophic assemblages from chemosynthetic ecosystems serve as examples to highlight the major features exhibited by these endosymbiosis and illustrate how genomic approaches provide us with tools that can help resolve the intricate relationships between host and symbionts.

Keywords: Endosymbiosis ● Eukaryote evolution ● Hydrogen sulfide ● Chemoautotrophy ● *Riftia* ● *Calyptogena* ● *Bathymodiolus*

The importance of endosymbiosis in the evolution of life on Earth

A century ago, Mereschkowsky (1905) proposed that chloroplasts were derived from bacterial ancestors that merged with "regular" eukaryotes. The symbiogenesis theory was born but awaited 70 years to grow and spread following the work of Lynn Margulis (1970 & 1992). Endosymbiosis is now firmly recognized as a key feature of the origin of all eukaryotic life on Earth (Fig. 1, middle). This is widely supported by numerous molecular data for mitochondria (MT) and chloroplasts (PL), allowing for the existence of heterotrophic and phototrophic eukaryote lineages. In the latter case the picture is further complicated

BACTERIA

ARCHAEA

EUKARYA

NU

α-**PROTEOBACTERIA**

MT

CYANOBACTERIA

PL

AM

PL2

AP

PHOTOTROPHIC

HETEROTROPHIC

γ-**PROTEOBACTERIA**

TO

THIOTROPHIC

by several and independant secondary endosymbiosis (PL2) events (Falkowski et al., 2004), involving the merging of two eukaryotic cells from each of the two lineages, i.e. a phototrophic and an heterotrophic eukaryotes. Loss of these organelles may also occur, as evidenced by amitochondriate (AM) eukaryotes and some heterotrophic lineages of primarily phototrophic eukaryotes (AP) such as heterotrophic Heterokonta or Apicomplexa (Nozaki, 2005; Rodriguez Ezpeleta et al., 2005). These "simple" eukaryotes were first considered as primitive but recent phylogenies tend to consider them as secondarily "simplified" (Adl et al., 2005; Keeling et al., 2005).

The more ancient events that have brought up the first eukaryotic cells may also have involved trophic symbiosis between Bacteria and Archaea (Fig. 1, top). Nuclear genes of Eukaryotes have undoubtedly links with both archaeal and bacterial genes (Horiike et al., 2001; Horiike et al., 2002), with some genes that appear to be truly eukaryotic. Archaeal genes tend to run processes involving replication, transcription and translation, the so-called "informational" functions, whereas bacterial genes would be more responsible for metabolic and housekeeping chores, the "operational" functions. From the jumble of genes, some evolutionary biologists have proposed that this division of labor arose from the ancient symbiotic partnership eventually between bacteria and archaea, a partnership that gave rise to eukaryotes. The nucleus (NU) ancestor could thus have been the first endosymbiont (Lake & Rivera, 1994) but the exact mechanisms and partners involved are still debated (Martin & Muller, 1998; Moreira & Lopez-Garcia, 1998). The general scheme is centered around a methanogen Archaea forming a consortium with either an α- or δ-proteobacteria, the latter producing hydrogen, acetate and carbon dioxide from organic compounds that are further metabolized by the Archaea to form methane. It should be stressed that alternative hypotheses for the origin of Eukaryotes are otherwise proposed (e.g. Pennisi, 2004), with either a filiation with planctomycetes Bacteria (Fuerst, 1995), sisterhood with Archaea and common descent from neomuran bacteria (Cavalier-Smith, 2006) or the existence of primitive Eukarya directly issued from the RNA world and later transformed by DNA viruses (Forterre, 2005). However, the detailed history of these pioneers may well be buried too deep in the past to be ever completely elucidated (Baldauf, 2003; Rivera & Lake, 2004), the picture being even more complicated since the recent recognition that horizontal gene transfer may be much more frequent in Eukarya than previously thought (Andersson, 2005; Bapteste et al., 2005; Keeling et al., 2005). Hence the idea that studying contemporary endosymbiosis may shed light on the mechanisms that prevailed at the origins.

Endosymbiosis examples are abound in the marine environment either between bacteria and eukarya or between several eukarya. Some of the most prominent examples of such trophic symbioses are the various associations between algae and cnidarians/molluscans found among tropical coral reefs. Because they involve two Eukarya species, one being diverse (the cnidarian or molluscan host) while the other is constrained to the *Symbiodinium* genus (the symbiont), these symbioses are reminiscent of a secondary endosymbiosis in progress, just like the events that brought up Chromista algae (Falkowski et al., 2004; Yoon et al., 2004). Dinoflagellates may also represent good phototrophic study models with their remarkable ability to remodel their genomes through endosymbiosis (Yoon et al., 2005). Alternatively, the chemoautotrophic symbiosis between molluscs or tubeworms and sulfo-oxydant bacteria which dominate deep-sea hydrothermal vents and cold seeps (Childress & Fischer, 1992) may in turn provide appropriate examples of a primary endosymbiosis in progress. The trophic nature of the association of a heterotrophic invertebrate with a chemoautotrophic bacterium, resulting in a symbiosis with the ability to use chemical energy for carbon fixation, could thus give rise to a third metabolic type of Eukarya, the thiotrophic Eukarya (Fig. 1, bottom), an idea already put forward 15 years ago by Vetter (1991).

Figure 1. Hypothetical schematic evolution of Eukarya and endosymbiosis. Top: the origin of Eukarya may have involved the merging of an Archaea and a Bacteria, the former giving birth to the nucleus (NU). Middle: primary endosymbiosis events gave birth to the mitochondria (MT) from γ-Proteobacteria, and to chloroplasts (PL) from Cyanobacteria. Later events include secondary endosymbiosis (PL2) and loss of primary symbionts (AM, AP) to form the various phototrophic and heterotrophic lineages of Eukarya. Bottom: tentative addition of new primary endosymbioses with γ-Proteobacteria (TS) to give rise to thiotrophic Eukarya (see text for further details).

Figure 1. Schéma hypothétique de l'évolution des Eucaryotes et de l'endosymbiose. En haut : l'origine des Eucaryotes peut avoir impliqué la fusion d'une Archée et d'une Bactérie, la première étant à l'origine du noyau (NU). Au milieu : les endosymbioses primaires sont à l'origine des mitochondries (MT) issues d'une γ-Protéobactérie et des chloroplastes (PL) issus d'une Cyanobactérie. Les événements suivants comprennent les endosymbioses secondaires (PL2) et les pertes des symbiontes primaires (AM, AP) pour former les différentes lignées phototrophes et hétérotrophes des Eucaryotes. En bas : tentative d'ajout de nouvelles endosymbioses primaires issues de γ-Protéobacteries (TS) à l'origine d'Eucaryotes thiotrophes (voir le texte pour plus de détails).

What are the successive steps a symbiotic association must go through for the symbiont to reach an organelle status?

Obviously the first step is for the symbiont to get inside the host cells, i. e. an anatomical integration; then interdependency of metabolic needs and products should take place to reach a physiological integration of the two species. At last, the life cycle of the symbiont may completely take place inside the host, resulting in a genetic integration. At this stage, symbiont genome reduction and gene transfer to the nuclear genome of the host may take place, as observed for mitochondria and plastids. The different endosymbiosis examples that can be found in the deep-sea hydrothermal/cold seeps environments may provide appropriate biological models to study the various steps these associations must go through to evolve from a simple trophic assemblage between two organisms into a single organism with a new cellular organelle, the thioplast. Below, we will briefly review the characteristics of three of the most studied examples in the taxa Bathymodiolinae, Vesicomyidae and Siboglinidae, all of which harboring sulfo-oxydant chemoautotroph γ-Proteobacteria from three distinct phylogenetic lineages (Nelson & Fisher, 1995; Cavanaugh et al., 2005).

Mussels from the genus *Bathymodiolus* and closely related genus within the sub-family Bathymodiolinae harbor their symbionts in their gill cells. Sulfo-oxidizing bacteria are mostly intracellular with some bacteriocyte apical vesicles opened to the environment (Gustafson et al., 1998; McKiness et al., 2005), and they are acquired by juveniles from the environment (Won et al., 2003). Some species also harbor intracellular methanotrophic symbionts, with dual-symbiosis apparently restricted to Atlantic species (McKiness & Cavanaugh, 2005). Both symbiosis types may be facultative since the mussels retain the ability to feed by themselves through filtration. Furthermore, recent reports indicate that in dual-symbiont species relative proportions of each type is related to the CH_4/H_2S ratio in the environment (Salerno et al., 2005) and that when deprived of CH_4 and/or H_2S, the respective symbionts are progressively lost (Kadar et al., 2005). This could provide aposymbiotic individuals to test reinfestation and the concommitent symbiosis-related expression of genes. *Bathymodiolus* could thus serve as a model with an environmental transmission and high plasticity for symbiont acquisition. Comparative studies with littoral Lucinid species such as *Codakia* (Gros et al., 1996) could be beneficial since they share the same general characteristics.

Siboglinid polychaetes, the most studied of which being *Riftia pachyptila*, all harbor sulfo-oxidizing γ-proteobacteria in an internal organ, the trophosome. The symbiosis is obligatory for adult worms with a profound physiological and biochemical integration of the host and symbiont metabolisms (Goffredi et al., 1998; Minic & Herve, 2004). However, symbionts are newly acquired by recruits from the environment at each generation, as evidenced by cytological (Nussbaumer et al., 2006), molecular (Cary et al., 1993) and phylogenetic (Feldman et al., 1997; Laue & Nelson, 1997; Nelson & Fisher, 2000) studies. Siboglinids, and in particular *Riftia pachyptila*, could thus represent a model with high host-symbiont physiological integration but retaining an environmental transmission of symbionts even after 100 million years of evolution (Little & Vrijenhoek, 2003).

In the Vesicomyidae, sulfo-oxidizing γ-proteobacteria are located intracellularly in the gill cells. Physiological integration has been less studied than in Siboglinids (Kennish & Lutz, 1992) but we know that the symbiosis is obligatory even though the host has retained a rudimentary gut. But, in contrast to the two previous examples, the symbionts are provided to the next generation through the eggs (Endow & Ohta, 1990; Cary & Giovannoni, 1993): it is a vertical transmission, with a maternal heritage just as for mitochondria, as confirmed recently by co-evolution studies (Hurtado et al., 2003). *Calytpogena* spp and other vesicomyid clams could thus serve as the most achieved models for genetic integration studies of thiotrophic symbioses.

How genomic approaches may help us resolve the intricate relationships between host and symbionts?

The thiotrophic symbioses discussed above are not easy to work with: they are deep-sea dwellers that can be kept alive only if maintained under pressure. Furthermore, the symbiotic bacteria can be isolated and are able to live for a few hours but all attempts to culture them outside their respective hosts have failed so far. Transcriptomic and proteomic studies thus constitute valid alternative approaches to disclose physiological and biochemical adaptations to the symbiotic way of life. When symbiotic and aposymbiotic individuals are available (Lucinidae and possibly Bathymodiolinae), the interindividual differential expression of genes and proteins involved in the symbiotic relationship can be studied along the course of re-infestation. Even when the symbiosis is obligatory, the restriction of symbiotic bacteria to one specific tissue allows for an intra-individual transcriptomic or proteomic approach. First attempts of Subtractive Suppressive Hybridation (SSH) banks in *Riftia* have already revealed interesting proteins expressed specifically in the gills or

trophosome (Sanchez et al., 2005) and work is in progress to characterize the *Riftia* endosymbionts physiology on the proteomic level (Markert et al., 2005).

However the lack of extensive sequences for these species in the databases force to use homology approaches to infer functions from sequences, a process that is both time consuming and a potential source of mislead. We need to promote large EST and/or full genome sequencing projects for the symbionts and hosts of some selected models on which a large community of people would agree. Symbiont genomes sequencing projects, given their small size, are already underway: *Riftia* symbiont(s) genome(s) should be made available in some time (Felbeck, pers. comm.), projects for *Calyptogena* exist in the US (Cavanaugh, http://www.jgi.doe.gov/sequencing/DOE microbes2005.html) and Japan. Genome size reduction often occur in vertically transmitted endosymbionts and the first evidence of such a genome size reduction has been presented in *Calyptogena* during the meeting (Kuwahara et al., 2005). To examine potential gene transfer and losses we will need host genomes. In the cases of environmental transmission, free-living bacteria transcriptomes will need to be compared with that of symbionts to reveal the ways hosts regulate the symbionts population.

The advent of comparative genomics has dramatically changed our appreciation of the mosaic nature of the eukaryotic nuclear genome, and the ongoing role endosymbiosis plays in shaping its content (Archibald, 2005). Comparative genomic analyses of thiotrophic eukaryotes will undoubtedly contribute to the comprehension of the broad biological meaning of endosymbiosis, both in physiological and evolutionnary perspectives.

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