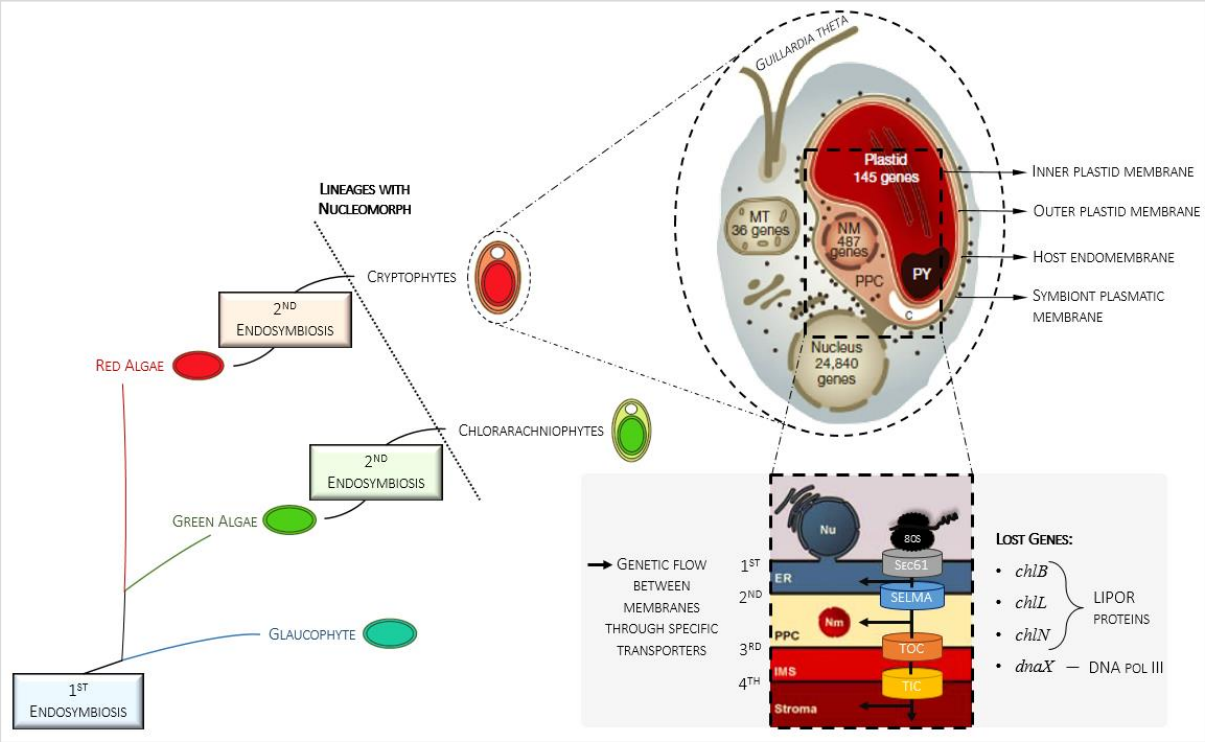
The Cryptophyte *Guillardia theta*: "– Where's your genome, Nucleomorph?"

Ricardo Cerqueira, 54492. Biology of Unicellular Eukaryotes, Msc Molecular and Microbial Biology, 2018/2019. Faculty of Science and Technology (FCT), Universidade do Algarve (UAlg), Campus de Gambelas, Faro, Portugal.

Graphical Abstract



Abstract

During evolution, the second endosymbiosis, with the uptake of a red algae and green algae, gave rise to two distinct lineages with nucleomorph, cryptophytes and chlorarachniophytes, respectively. The nucleomorph, a remnant nucleus was retained in the periplastidial space as a consequence of this event. The study of nucleomorph was based on information of the cryptophyte, *Guillardia theta*, with the objective to find how is it possible the interchange of genetic information between the plastidial nucleus and host nucleus, through specific membrane transporters. TIC, TOC, SELMA and Sec61 were found to play an important role in the genetic flow between membranes. The genes *chlB, chlL, chlN* (LIPOR proteins) and *dnaX* (DNA polymerase III) were targets of genetic loss due to massive gene transfer.

Keywords: Nucleomorph, Gene Loss, *Guillardia theta*

1. Origin and Evolution of Cryptophytes: A Taxonomic Approach

Coating Keeling (2009) plastids and mitochondria arose by a primary endosymbiotic uptake of a cyanobacterium and proteobacterium, respectively. Three major algal lineages (glaucophytes, green algae and red algae) were derived (Cavalier-Smith, 2004; Gilson & McFadden, 2002). By secondary endosymbiosis, the uptake of a red algae gave rise to a few organisms, including cryptophytes (Gilson & McFadden, 2002) which phylogeny still unclear as it will be explained next. In the hacrobian hypothesis, the phylogenomic analysis based on nuclear and plastid tree supported the monophyly of cryptophytes and haptophytes. The same analysis was based on the presence of a bacterial *rpl36* gene from a horizontal gene transfer (HGT) event (ANNEX 1 **Fig. 1**) (Hackett *et al*., 2007; Khan *et al*., 2007; Patron *et al.*, 2007; Yoon *et al.*, 2002). Still, the monophyly of hacrobia group was challenged by nuclear gene data (Keeling, 2013). The same occurs to chromalveolate hypothesis, which is explained that all red algae were forthcoming from a single secondary event of endosymbiosis, but in fact evidences has shown that sometimes haptophytes belongs to SAR but cryptophytes do not (ANNEX 1 **Fig. 2**) (Keeling, 2013). There is data based on different analysis of the 18S rRNA and small protein-coding genes (actin, a-tubulin, b-tubulin, EF2, HSP70, HSP90) challenging the position of cryptophytes (Burki *et al*., 2016; Keeling, 2013) and antagonizing hacrobia hypotheses. Evidences suggested that red plastid was preceded by a green plastid (Keeling, 2013). In recent studies, it was shown that cryptophytes have affinity to heterotrophic eukaryotes (Kim, *et al*., 2018). The main storage products of haptophytes are paramylon or chrysolaminaran, while in cryptophytes are starch and lipid (Borowitzka, 2018; Deschamps *et al*., 2006). Analysis based on glycogen and starch supports cryptophytes as a sister group with archaeplastida and shows haptophytes as a sister group with SAR (ANNEX 1 **Fig. 2**) (Burki *et al*., 2016; Ball *et al*., 2011).

2. The Singleness of Cryptophytes

In marine and freshwaters, cryptophytes represents a group constituted by photosynthetic and nonphotosynthetic organisms (Moore *et al.*, 2012). Chlorophyll *a*, chlorophyll c_2, as well as phycobilins are the main photosynthetic pigments (Borowitzka, 2018; Kim *et al*., 2017). Cryptophytes are formed by four plastid membranes which are (from inside outward): two membranes of the primary plastid, the plasma membrane of the engulfed red algae and the host endomembrane (Cavalier-Smith, 2018; Burki *et al.*, 2016; Keeling, 2010; Gilson & McFadden, 2002). They have four genomes: host-derived nuclear and mitochondrial, and endosymbiotic-derived plastid and nucleomorph (Kim *et al*., 2018). It was believed that nucleomorph was a

relic nucleus of the engulfed red algae (Gilson & McFadden, 2002). Nowadays, nucleomorph is considered a highly reduced genome which persisted until the present (Wong *et al*., 2018; Keeling, 2010; Douglas *et al*., 2001; Gillott & Gibbs, 1980). Relatively to genome composition, the tiny nucleomorph in the periplastid compartement, presents only three chromosomes (Gilson & McFadden, 2002; Douglas *et al*., 2001). It was thought nucleomorphs had an extensive collection of genes for plastid-targeted proteins (Keeling, 2010), but cryptophytes were found to have only 30 identified proteins (Douglas *et al*., 2001; Zauner *et al*., 2000). The majority of genes responsible for the maintenance and expression of the nucleomorph genome are housekeeping genes (Keeling, 2010; Douglas *et al*., 2001) sufficient for all necessary functions (Keeling, 2010; Douglas *et al*., 2001). Chlorarachniophytes (retained green plastid) and cryptophytes are two lineages with a relic nucleus nested in the periplastidic space (Cavalier-Smith, 2018; Keeling, 2013).

3. Transport between Membranes: The Origins of Gene Transfer?

Cryptophytes have four plastid membranes as explained above. Those membranes were obtained through the evolution time by the primary and secondary endosymbiosis (Keeling, 2013). Over time, many exchanges of gene information between symbiont and host cell were important to evolve protein transport complexes such as protein-targeting systems (Keeling, 2013). Transit peptides and TIC/TOC (Translocon Inner-membrane Chloroplast/Translocon Outer-membrane Chloroplast) system were developed in the primary endosymbiosis (ANNEX 1 **Fig. 3**) (Keeling, 2013; Patron & Waller, 2007; Gould *et al*., 2006). Chaperones (Hsp70) were also described to have a biding-relation to transit peptides in the periplastidal compartment (Hempel *et al*., 2007; Patron & Waller, 2007; Rensing *et al.*, 1994). In secondary endosymbiosis, other systems were identified, such as, BTS (Bipartite N-terminal Topogenic Signal) which marks the leader proteins targeted to secondary plastids (Keeling, 2013; Gould *et al*., 2006), consisting by a signal peptide followed by a transit peptide (Keeling, 2013). Quoting Hempel *et al.* (2007), the BTS transit peptides are important not only for crossing the plastid envelope, but also for passing the second outermost membrane. For example, the starch which is deposited in the periplastid compartment needs to be synthesised by UDP glucose-starch glycosyl transferase, which in the other hand, needs to across the membrane with N-terminal BTS sequence (Hempel *et al*., 2007; Patron & Waller, 2007; Deschamps *et al*., 2006). Involved in the second outermost plastid membrane were found the ERAD (Endoplasmic Reticulum-Associated Degradation) complex partially encoded by the nucleomorph *(Felsner et al*., 2010). There are evidences demonstrating that cryptophytes possesses a specific system

associated to the host ERAD system designated by SELMA (Symbiont-specific ERAD-Like Machinery) system (ANNEX 1 **Fig. 3**) (Felsner *et al*., 2010). SELMA is a multi-protein encoded in the nucleomorph that is associated to the second outermost plastid membrane (Kim *et al*., 2017). Sec61 system is present in the fourth membrane associated to the endoplasmic reticulum (ANNEX 1 **Fig. 3**) (Felsner *et al*., 2010).

4. *Guillardia theta*: "-Did I lost my nucleomorph genes?"

Endosymbiotic Gene Transfer (EGT) is a type of gene transfer between symbiont-host where evolution depends on (Nosenko & Bhattacharya, 2007). It is crucial the establishment of new transport routes for proteins encoded in host-symbiont as a consequence of EGT, as well as the synchronization of the prokaryotic division with the eukaryotic host cell cycle (Vries & Gould, 2018). In the secondary host is present a regulatory machinery evolved in the primary host, necessary to control nuclear and plastid division (Vries & Gould, 2018). Investigations showed that the division of cryptophyte nucleomorph precedes the division of the cell nucleus regulated by host factors (Onuma *et al.*, 2017; Gould *et al*., 2006). To explain how gene loss occurs, it is necessary to explain EGT, which is, quoting Vries & Gould (2018), the process of gene loss from the organelle to the nuclear genome with the consequence of gain control by the host, which promotes the loss of autonomy of the endosymbiont. Vries & Gould (2018) proposed a monoplastidic bottleneck as an evolutionary promoter. The success of EGT events could be explained by massive transfer of gene information between host-endosymbiont (Tanifuji & Onodera, 2017), possibly by continuous events of attempts and errors. Nucleomorph analysis of *Guillardia theta* showed 548 genes present in three chromosomes, of which 485 to 487 are tightly packed protein-coding genes, 217 are housekeeping genes and only 31 plastid-associated genes (Gentil *et al.*, 2017). In *G. theta*, the nuclear genome composition revealed higher levels of G+C (53%), compared to the 25% to 35% of G+C to nucleomorph genome. It was annotated 17 introns showing high specific splice patterns (Wong *et al*., 2018) and it was shown a trend for intron insertion and large retention in mature transcripts of nucleomorph (Wong *et al*., 2018; Kim *et al*., 2017), exhibiting unique properties of gene regulation and transcription control observed by elevated antisense transcripts and gene expression compared to nucleus (Wong *et al.*, 2018). Recent studies showed complete gene loss to *chlB*, *chlL* and *chlN* genes, which encoded LIPOR (Light-Independent Protochlorophyllide OxidoReductase) proteins and *dnaX* gene, which encodes the gamma component of DNA polymerase III (Kim *et al*., 2017).

5. Conclusion

Several authors tried to propose a clear and solid classification of the cryptophytes. First, hacrobia group was supported by *rpl36* gene analysis. Further, the SAR megagroup were proposed as a new classification, which comprised chromalveolates (stramenopiles and alveolata) and rhizaria. Haptophytes were included in this group and cryptophytes not, challenging the chromalveolate hypothesis. Herein, analysis with 18S rRNA, glycogen and starch revealed similarities between archaeplastida and cryptophytes. However, doing a taxonomic approach and establishing a position for cryptophytes, we must consider all the variables, since the morphologic/structure to genetic analysis, comparing results, and it still an *art* to be defined.

The cryptophyte and chlorarachniophytes both share common characteristics, such as the presence of the nucleomorph. Relative to *Guillardia theta*, it was observed the presence of three chromosome with only 31 plastid-associated genes and highly conserved 217 housekeeping-genes in a total of 485-487 protein-coding genes. Given this information, why and how nucleomorph remained until nowadays? The *G. theta* nucleomorph is a remarkable case of EGT study. It was shown the existence of specific membrane transporters (TIC, TOC, SELMA and Sec61) for the interchange of gene information between Red plastid - Nucleomorph - Host nucleus. As an example, the information in *chlB, chlL, chlN* and *dnaX* genes were lost during evolution in *G. theta*. Gene loss was described as a consequence of massive gene transfer by EGT and only the remaining functional genes were considered the necessary for nucleomorph maintenance. It will be instinct in the future? To further studies, it would be interesting to find what controls nucleomorph division or why nucleomorph is capable of controlling genetic information to precede the division of cell nucleus and to find different intron patterns obtained by splicing and their role in the host-symbiont system.

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ANNEX 1 - Supplement analysis

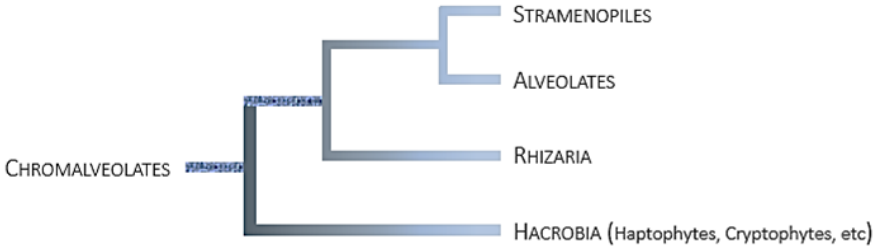


Figure 1 - Phylogenetic tree representing the hacobria group formed by haptophytes and cryptophytes. Textured bars represents undefined taxonomic classification. Developed and adapted graphic based on Tree of Life Web. http://tolweb.org/Eukaryotes/3 (Accessed: 31.10.2018 18:57).

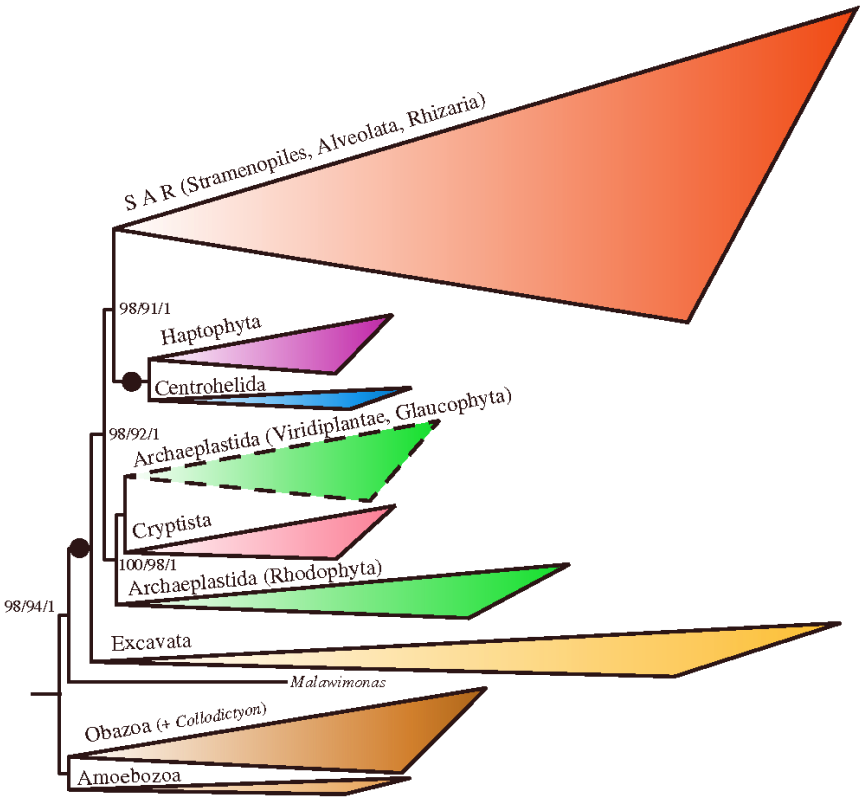


Figure 2 - Eukaryotic tree emphasizing for the establishment of SAR group, sister with Haptophyta, and Archaeplastida sister group with Cryptista. Phylogenetic tree source: Burki *et al.*, 2016.

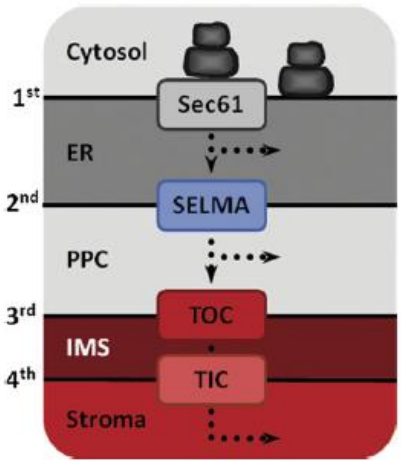


Figure 3 - Schematic representation of specific membrane transporters (TIC, TOC, SELMA and Sec61). Scheme source: Felsner *et al.*, 2010