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Tracing back EFL gene evolution in the cryptomonads–haptophytes assemblage: Separate origins of EFL genes in haptophytes, photosynthetic cryptomonads, and goniomonads

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ABSTRACT

A recently identified GTPase, elongation factor-like (EFL) protein is proposed to bear the principal functions of translation elongation factor 1α (EF-1α). Pioneering studies of EF-1α/EFL evolution have revealed the phylogenetically scattered distribution of EFL amongst eukaryotes, suggesting frequent eukaryote-to-eukaryote EFL gene transfer events and subsequent replacements of EF-1α functions by EFL. We here determined/identified seven new EFL sequences of the photosynthetic cryptomonad *Cryptomonas ovata*, the non-photosynthetic cryptomonad (goniomonad) *Goniomonas amphinema*, the foraminifer *Planoglabratella opecularis*, the haptophyte *Chrysochromulina* sp., the centroheliozoan *Raphidiophrys contractilis*, and two red algae *Chondrus crispus* and *Gracilaria changii*. The analyses of these EFL sequences successfully brought new insights into lateral EFL gene transfer amongst eukaryotes. Of most interest is a complex EFL evolution in a monophyletic assemblage comprised of cryptomonads and haptophytes. Since our analyses rejected any phylogenetic affinity amongst the EFL sequences from *Goniomonas*, photosynthetic cryptomonads, and haptophytes, the EFL genes of the three lineages most likely originated from different phylogenetic sources.

1. Introduction

Elongation factor 1 (EF-1α) in eukaryotes and archaebacteria (homologous to EF-Tu in eubacteria) is one of the principal protein factors involved in nascent peptide synthesis (Andersen et al., 2003). The GTP-bound EF-1α can bind and deliver aminoacyl-tRNA (aa-tRNA) to the A site of the ribosome. Subsequently, EF-1α hydrolyzes GTP coupled with the release of aa-tRNA. GDP-bound EF-1α then interacts with EF-1β (or EF-Ts in eubacteria) to recharge GTP before participating in the second round of peptide elongation process. In addition to both "core" functions described above, eukaryotic EF-1α bears arbitrary functions (e.g., interaction to cytoskeleton and proteosomes, Negrutskii and El'skaya, 1998). Due to the constraints from the core and arbitrary functions in cells, the primary, secondary, and tertiary structures of EF-1α/EF-Tu are highly conserved.

It has been generally believed that the evolution of EF-1α/EF-Tu is relatively simple: EF-1α/EF-Tu genes have been vertically inherited from the last universal common ancestor, and the gene products are ubiquitous in all extant cells. However, large-scale sequence data from phylogenetically diverged organisms have exposed both duplication and lateral transfer of EF-1α/EF-Tu genes. Firstly, there is literature describing lateral EF-1α/EF-Tu gene transfers (Ke et al., 2000; Inagaki et al., 2002; Inagaki et al., 2006). In addition, EF-1α paralogues, which were produced by gene duplication followed by functional divergence, are regularly found in both prokaryotic and eukaryotic genomes (Inagaki and Doolittle, 2000; Inagaki et al., 2003). Most importantly, recent complete genome data questioned the absolute ubiquity of EF-1α. No EF-1α gene has been identified in the complete nuclear genome of the chlorophycean green alga *Chlamydomonas reinhardtii* (genome.jgi-psf.org/Chlre3/Chlre3.home.html), or in those of prasinophycean green algae *Ostreococcus lucimarinus* (genome.jgi-psf.org/Ost9901_3/Ost9901_3.home.html) and *O. tauri* (genome.jgi-psf.org/Ostta4/Ostta4.home.html). Instead, these genomes possess genes encoding "elongation factor-like (EFL)" proteins that bear sequence similarity to, but are clearly divergent from canonical EF-1α (Keeling and Inagaki, 2004). An *in silico* analysis comparing the functions of EFL and those of EF-1α suggested that EFL holds at least the primary EF-1α functions (Keeling and Inagaki, 2004). Although EFL and EF-1α are considered as functionally equivalent molecules, the evolutionary mode proposed for EFL genes is drastically different from that for EF-1α genes in light of EF-1α/EFL gene distribution in global eukaryotic phylogeny.

A current hypothetical eukaryotic tree and the EF-1α/EFL distribution including the results in this study are shown in Fig. 1. The

Abbreviations: AU, approximately unbiased; BP, bootstrap probability; EF-1α, elongation factor 1α; EFL, elongation factor-like; ML, maximum likelihood; PCR, polymerase chain reaction.

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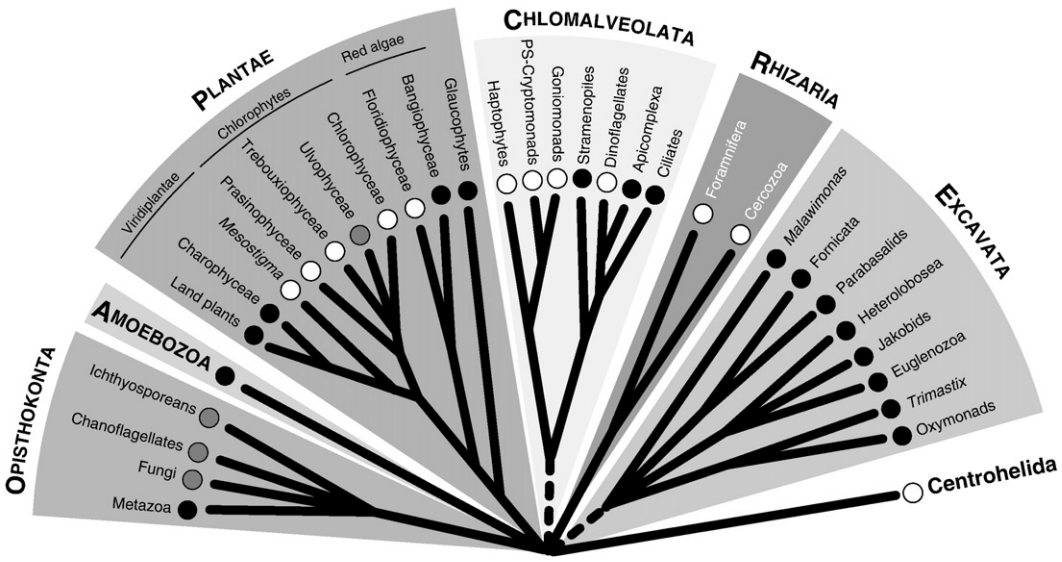

Fig. 1. The distribution of EF-1α/EFL in the global eukaryotic tree. Closed and open circles indicate that EF-1α-containing and EFL-containing groups, respectively. Groups including both EF-1α-containing and EFL-containing lineages are indicated by grey circles. Since neither monophyly of Chromalveolata nor that of Excavata has been phylogenetically recovered, the branch leading to these supergroups are shown in dotted lines.

monophylies of the supergroups Opisthokonta, Amoebozoa, Plantae, and Rhizaria have been demonstrated by multigene analyses (Bapteste et al., 2002; Burki and Pawlowski, 2006; Rodrìguez-Ezpeleta et al., 2005; Seenkamp et al., 2006). On the other hand, the monophyly of the lineages included in the supergroup Excavata has been proposed mainly based on morphological and ultrastructural data (Simpson and Roger, 2004). The supergroup Chromalveolata contains four major protist groups — alveolates (dinoflagellates, ciliates, and apicomplexans), stramenopiles, haptophytes, and cryptomonads. The chromalveolate hypothesis (Cavalier-Smith, 1999) assumes that a common ancestor of "chromalveolates" acquired plastids through the endosymbiosis of a red alga, and non-photosynthetic relatives (e.g., ciliates and goniomonads) were secondarily lost the red alga-derived plastids. To our knowledge, no nucleus-encoded gene phylogeny has recovered the host monophyly of the four "chromalveolata" groups. Nevertheless, the robust sisterhood between haptophytes and cryptomonads was constantly recovered in multigene analyses (e.g., Patron et al., 2007). In addition, some eukaryotic lineages show no clear phylogenetic affinity to any of the six supergroups described above. Centrohelida is typically considered *incertae sedis* (Sakaguchi et al., 2005; Sakaguchi et al., 2007).

EFL genes have so far been identified in (i) the members of Chlorophyta (except the ulvophycean green alga *Acetabularia*), (ii) the mesostigmatophycean green alga *Mesostigma viride*, (iii) dinoflagellates and the perkinsid *Perkinsus marinus*, (iv) photosynthetic cryptomonads, (v) haptophytes, (vi) chytrid and zygomycete fungi, (vii) choanoflagellates, (viii) ichthyosporeans, and (ix) chlorarachniophytes (Keeling and Inagaki, 2004; Gile et al., 2006; Ruiz-Trillo et al., 2006; Noble et al., 2007). Keeling and Inagaki (2004) originally pointed out that "EFL-containing" organisms phylogenetically associate with "EF-1α-containing" organisms as shown in Fig. 1. In fungi, EFL genes were identified only in limited species, while the majority of fungal species utilizes EF-1α (Keeling and Inagaki, 2004). Similarly, Viridiplantae is comprised of both EF-1α-containing and EFL-containing organisms — land plants, charophycean green algae, and the ulvophycean green alga *Acetabularia acetabulum* utilize EF-1α, while other green algal species possess EFL (Noble et al., 2007). The large protist assemblage Alveolata is comprised of two EF-1α-containing sub-groups (apicomplexans and ciliates) and an EFL-containing sub-group (dinoflagellates plus perkinsids) (Keeling and Inagaki, 2004). Such mosaic EF-1α/EFL distribution was most likely achieved by EFL gene transfer between distantly related eukaryotes. Nevertheless, an alternative hypothesis in which invokes parallel loss events of EFL genes cannot completely be excluded. If one hesitates to invoke an extremely high rate of lateral EFL gene transfer in eukaryotic evolution (e.g., LGT amongst very closely related lineages), the "gene-loss" scenario can be an alternative explanation for complex EF-1α/EFL distribution in choanoflagellates (Keeling and Inagaki, 2004), ichthyosporeans (Ragan et al., 2003; Ruiz-Trillo et al., 2006), and ulvophycean green algae (Noble et al., 2007), which are highlighted by grey circles in Fig. 1.

In this study, we newly determined/identified seven EFL sequences, and updated the EFL distribution in global eukaryotic phylogeny. Of particular interest is the EFL evolution in a monophyletic assemblage comprised of the cryptomonads, haptophytes, and their closely related lineages (henceforth designated as the cryptomonads–haptophytes assemblage). Our EFL phylogenetic analyses failed to recover the monophyly of the sequences from the member of the cryptomonad–haptophyte assemblage, suggesting that the EFL origins of haptophytes, goniomonads, and photosynthetic cryptomonads are separate from one another.

2. Materials and methods

2.1. Cell culture and cDNA preparation

Raphidiophrys contractilis cells were cultured as described by Sakaguchi and Suzaki (1999). *Goniomonas amphinema* was kindly donated by N. Yubuki (University of Tsukuba). *Cryptomonas ovata* (NIES-274) was given by I. Inouye (University of Tsukuba). The haptophyte *Chrysochromulina* sp. (NIES-1333) was purchased from the Microbial Culture Collection at the National Institute for Environmental Studies (NIES; 16-2, Onogawa, Tsukuba, Ibaraki 305-8506, Japan). Poly(A)+ RNA samples were prepared from the cultured cells by using a QuickPrep Micro mRNA Purification Kit (Amersham Biosciences). cDNA were subsequently synthesized from the poly(A)+ RNA by Superscript III RNase H-Reverse Transcriptase (Invitrogen). *Planoglabratella opecularis* cDNA prepared in Takishita et al. (2005) was used in this study.

2.2. EFL gene amplification and sequencing

Short DNA fragments (~300 bp), which corresponds to the guanidine nucleotide binding region of *Raphidiophrys*, *Cryptomonas*, and *Goniomonas* EFL, were amplified from cDNA by polymerase chain