Functional and evolutionary distinctiveness of fatty acid and polyketide synthesis in microbial eukaryotes

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Introduction

Fatty acid synthesis (FAS) is an essential metabolic process. Despite its significance, little is known about this process in protists, which comprise ~90% of eukaryotic lineages. Elaborate polyketide compounds are produced by some protists, in particular, the toxins released during harmful algal blooms (HABs). While FAS is a primary metabolic process, other polyketide compounds are secondary metabolites involved in processes such as predation defense. Surprisingly, some protist studies suggest that the two processes are catalyzed by the same enzymes.

Aims

1. Identify the genetic basis of FAS and polyketide synthesis (PKS) in the major protist lineages
2. Infer the constraints and processes in the evolutionary history of protist FAS and PKS genes

Methods

Transcriptomic libraries (213 strains/152 genera) from the Marine Microbial Eukaryotic Sequencing Project (MMETSP) were screened for FAS and PKS genes using the following key steps:

RNA Extraction & Illumina RNASeq sequencing → Assembly via CLCBio or ABySS → PKS & FAS gene hunting via BLAST and Hmmer → Phylogenetic Analysis RAxML V8.0 (GAMMA, 1000 bootstraps)

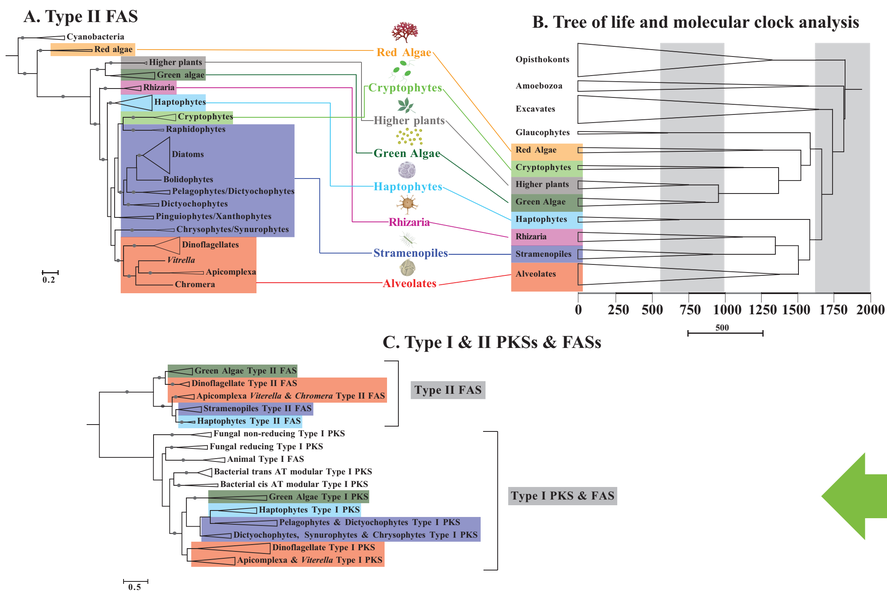

Figure 2: **Comparative evolution of fatty acid and polyketide synthase.** A. Concatenated phylogeny of type II FAS genes. B. Dated molecular clock phylogeny of the eukaryotes, showing absolute time scale (million years)[1]. C. Phylogeny of 3-ketoacyl ACP synthase II (FAS) and type I ketosynthase (PKS) domains. Solid circles indicate bootstrap values above ≥ 90.

Type II FAS genes recently transferred from the plastid to the nucleus of its tertiary hosts (in some cases, e.g. haptophytes & diatoms, then to their dinoflagellate hosts)

Evolution of type II FAS genes broadly follows the evolution pattern of the organism

Clear distinction between FAS and PKS genes in protists

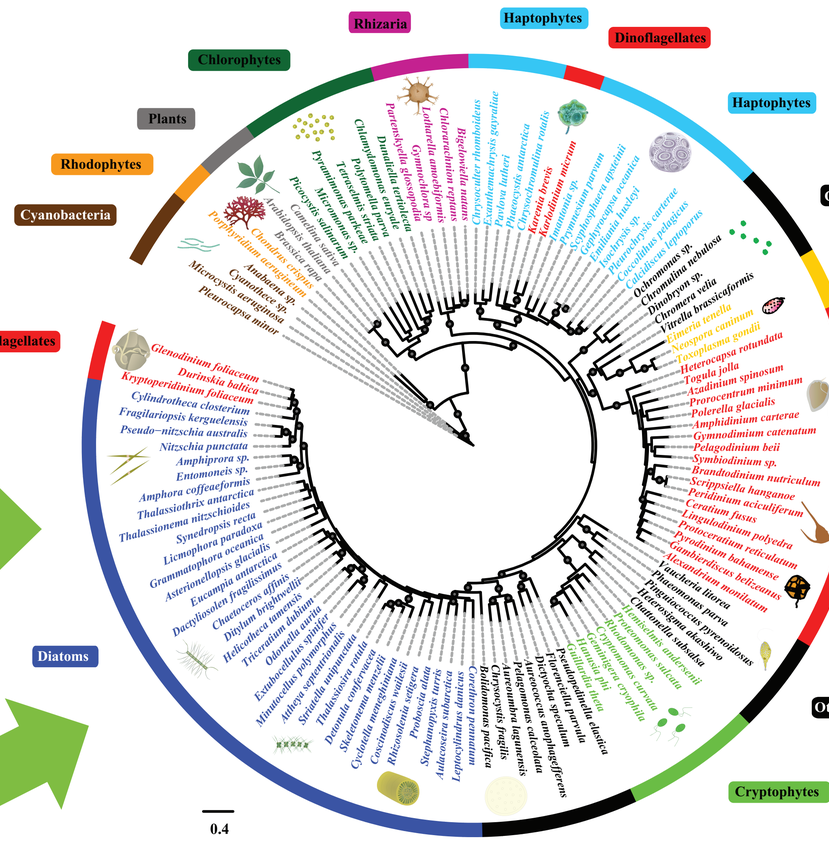

Figure 1: **Type II Fatty acid synthase genes are present in all phototrophic lineages**

Results

- Six key type II FAS enzyme are present in all phototrophic lineages (Figure 1).
- Evidence supports type II FAS genes being nuclear encoded, and initial fatty acid synthesis steps occur in the chloroplast (transit peptides; polyA tail, dinoflagellate spliced leader; genes encoded in *E. huxleyi* genome).
- Enormous diversity of PKS genes are found in selected lineages of protists (Figs 3 & 4).

PKS gene family is highly expanded in dinoflagellates and haptophytes

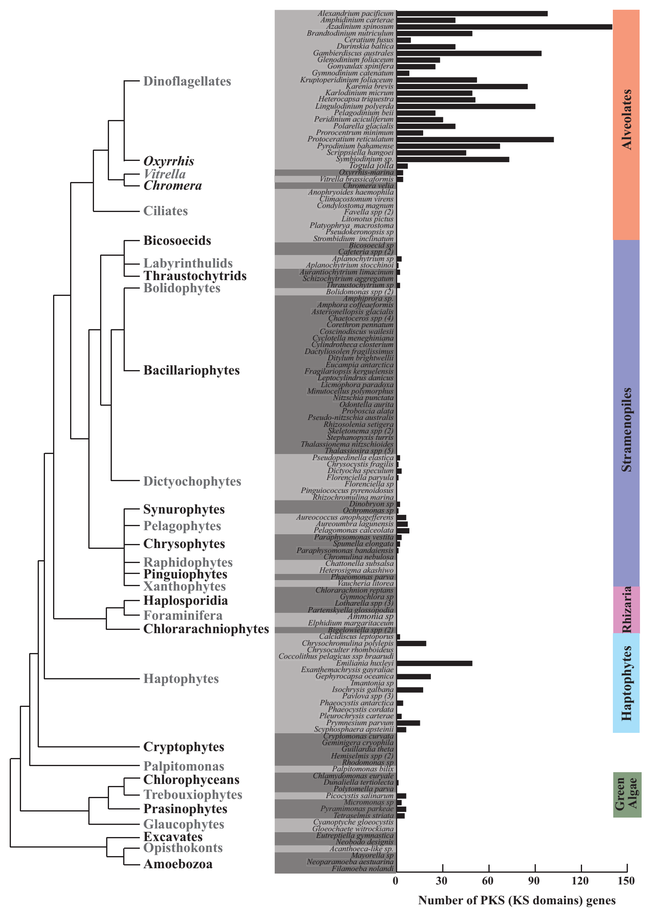

Figure 3: Abundance of expressed type I PKS ketosynthase domains among protist lineages

Dinoflagellates type I PKS ketosynthase domains form three distinct clades, but the pattern of distribution is not related to the species phylogeny and/or the chemical structures these organisms produce

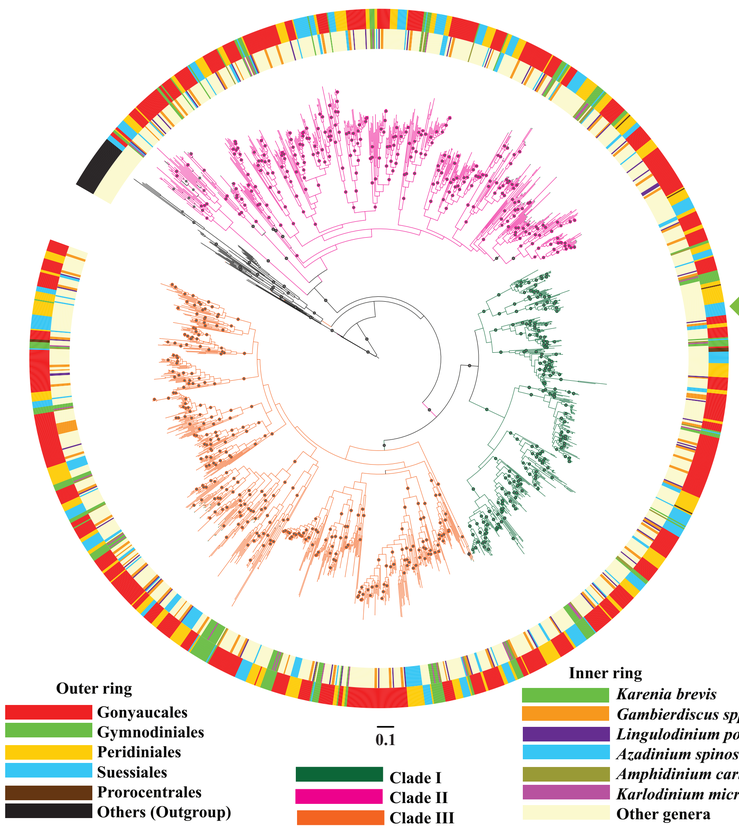

Figure 4: Polyketide synthase gene phylogeny in dinoflagellates: Phylogenetic analysis of 1633 type I PKS ketosynthase domains.

Conclusions:

- FAS is essential for survival. Thus, protists likely retained genes in the nucleus due to strong selective pressure;
- Large PKS gene diversity in dinoflagellates, suggests multiple gene duplication events, domain shuffling and losses;
- Relaxed selection pressure may have acted on the evolution of PKS genes acquired or lost based on functionality;
- Differentiation of PKS and FAS will facilitate investigation of harmful algal toxin biosynthesis pathways in protists.

References: [1]Parfrey LW, Lahr DJG, Knoll AH, Katz LA. (2011). *Proc Natl Acad Sci* **108:** 13624-13629

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