Horizontal gene transfer maintains chloroplasts stolen from prey in the algae *Dinophysis acuminata*

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Background

Chloroplasts evolved through multiple endosymbioses

Endosymbiosis has been a fundamental process in evolution, giving rise to cell organelles including chloroplasts (the center for photosynthesis in plants and algae).

Primary chloroplasts in green algae and land plants resulted from an ancient endosymbiotic association with photosynthetic bacteria. Subsequent secondary endosymbioses spread chloroplasts of green and red algae across the tree of life (Fig. 1) [1].

Some algae have undergone even more recent, tertiary endosymbioses, while others contain klepto-chloroplasts (temporary chloroplasts acquired from prey) [2].

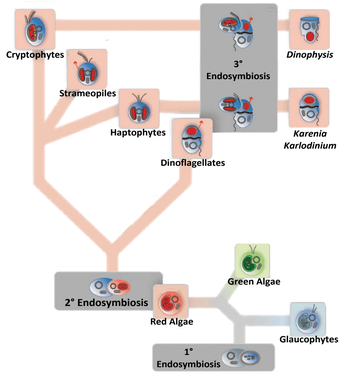

Figure 1. Origin and spread of chloroplasts through endosymbiosis. Modified from Archibald, 2005, IUBMB Life

A few kleptoplastidic organisms can maintain their temporary chloroplasts for months to years without having to feed [3].

Klepto-chloroplasts may represent an early stage of endosymbiosis before the organelle is under the complete control of the host and may provide insights into the first steps of this important evolutionary process.

The System

The algae *Dinophysis* has klepto-chloroplasts stolen from photosynthetic prey

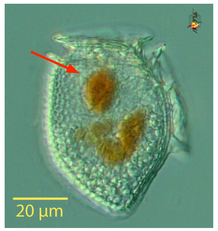

Figure 2. Image of the dinoflagellate *Dinophysis*. Colored compartments indicated by the red arrow are klepto-chloroplasts acquired through feeding. Image courtesy of Micro*scope.

Dinophysis is an exceptional dinoflagellate algae that possesses klepto-chloroplasts acquired from the crypytophyte *Geminigera cryophila* (Fig. 2).

Although *Dinophysis* can be maintained in pure culture for several months, the genus is ultimately mixotrophic and needs to feed to reacquire chloroplasts. The extended length of time between feedings is surprising considering that *Dinophysis* presumably lacks thousands of genes required for chloroplast function.

Possible mechanism for klepto-chloroplast longevity: *Dinophysis* has acquired its own suite of chloroplast-related genes through horizontal gene transfer.

I sequenced cDNA from *Dinophysis acuminata* and identified chloroplast-targeted proteins encoded in the nuclear genome of *D. acuminata* that function in photosystem stabilization, carbon fixation, and metabolite transport.

Results

Chloroplast-targeted genes in *D. acuminata* function in photosystem stabilization, carbon fixation, and metabolite transport

Genes found in *D. acuminata* include auxiliary light harvesting proteins, photosystem subunits, and genes involved in electron transport and metabolite exchange between the chloroplast and the cytosol of *D.acuminata*. However, some important genes are missing from *D. acuminata*, including PsbO (an important photosystem II subunit) and petC (the major iron-sulfur protein of the the cytochrome b6f complex) (Fig. 3).

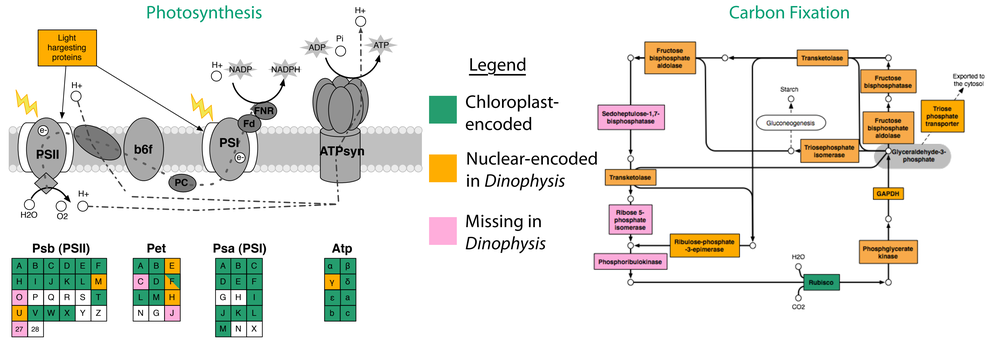

Figure 3. Genes involved in photosynthesis and carbon fixation in the *D. acuminata*. Missing genes are shown in pink.

Chloroplast-targeted genes in *D. acuminata* have been horizontally acquired

Transcriptome analysis of *D. acuminata* identified several nuclear-encoded chloroplast genes derived from multiple algal lineages. Genes group phylogenetically with either other dinoflagellates, the klepto-chloroplast donor *G. cryophila*, or other algae such as haptophytes (Fig. 4).

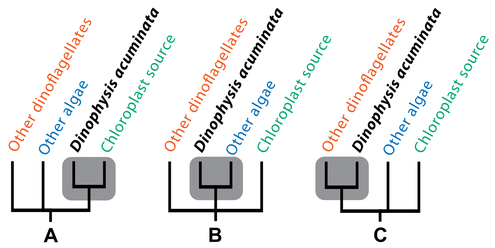

Figure 4. Schematic representations of phylogenies of plastid-targeted, nuclear-encoded genes in *D. acuminata*. A) Genes have been horizontally acquired from the algal source of the plastid, *G. cryophila*. B) Some genes have been horizontally acquired from other algae. C) Lastly, other genes group with other dinoflagellates and were likely retained from a photosynthetic ancestor.

D. acuminata is lacking genes to maintain the stolen chloroplast permanently

Analysis of gene ontology (GO) suggests that the percentage of genes in *D. acuminat*a that function in the chloroplast are about a fourth of what is typically found in other fully photosynthetic algae (Fig. 5).

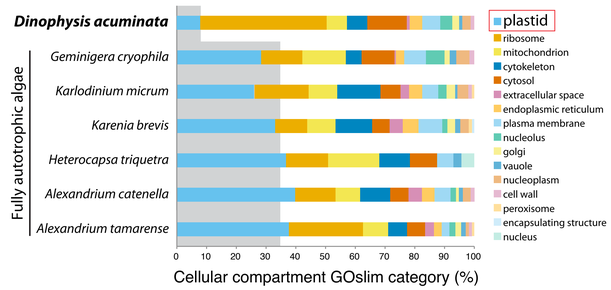

Figure 5. Percent cellular compartment GO terms in *D. acuminata* compared to six other algae. The amount of GO terms is expressed as a percentage of the total number of unigenes annotated.

Methods

454 sequencing: *D. acuminata* cDNA was sequenced with a 454 FLX pyrosequencing machine at the Arizona Genomics Institute. Reads assembled using an in house cleaning, clustering, and assembling pipeline developed for 454 transcriptome sequences (Figure 6).

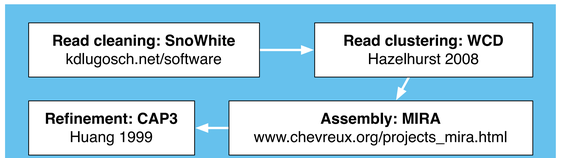

Figure 6. De novo transcriptome assembly pipeline for 454 sequencing.

Annotation: Blast2GO and KEGG were used to annotate genes.

Phylogenetics: Amino acid sequences were aligned using ProbCons. Distance analyses were performed in PAUP*. The best-fit evolutionary model was identified by ProtTest and applied to maximum likelihood (ML) and Bayesian analyses. ML trees were inferred using RAxML. Bayesian analyses were performed in BEAST.

Conclusions

D. acuminata has some functional control of its klepto-chloroplast, and may be able to extend the useful life of this stolen organelle by replacing damaged transporters and protecting components of the photosystem from stress. Phylogenetic analyses show that the genes are derived from multiple algal sources indicating a complex evolutionary history involving horizontal gene transfer.

However, the overall dearth of chloroplast-related genes compared to other fully phototrophic algae suggests that *D. acuminata* does not have the nuclear repertoire necessary to maintain the chloroplast permanently.

These findings suggest that horizontal gene transfer occurs early in, and may even facilitate the development of, chloroplast endosymbioses.

References

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2. Hackett JD, Anderson DM, Erdner DL, Bhattacharya D (2004) Am J Bot 91:1523-1534.
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