Single-Cell Transcriptome Profiling Reveals Mechanisms of Host-Control and Nutrient Exchange in Acantharea-Phaeocystis Photosymbioses

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Abstract

Microbial eukaryotes (protists) are important contributors to marine biogeochemistry and play essential roles as both producers and consumers in marine ecosystems. Among protists, mixotrophs—those that use both heterotrophy and autotrophy to satisfy their energy requirements—are especially important to primary production in oligotrophic regions where nutrient availability is otherwise limiting. For instance, acantharians accomplish mixotrophy by hosting Phaeocystis spp. as endosymbionts. Despite their ecological importance, Acantharea-Phaeocystis symbioses are understudied due to host fragility and inability to survive in culture. We investigated the evolution and ecological functioning of these symbioses by sequencing single-cell transcriptomes from sixteen acantharians. Since hosts harbor multiple Phaeocystis species, we prepared transcriptomes for the two most common symbiont species available in culture—P. cordata and P. jahnii—and evaluated differential gene expression between symbiotic and free-living cells. Results indicate photosynthesis genes are upregulated in symbiosis for both symbiont species, suggesting symbionts are photosynthesizing at elevated rates within hosts. However, biosynthesis and metabolism of storage carbohydrates and lipids are downregulated in symbiosis, indicating that extra energy captured through elevated photosynthesis is not retained. Symbiont gene expression suggests symbionts relinquish fixed carbon as small organonitrogen compounds, such as amides and amino acids, while receiving host-supplied nitrogen as urea and ammonium. Importantly, genes associated with protein kinase signaling pathways that promote cell proliferation are deactivated in symbionts. Manipulation of these pathways may prevent symbionts from overgrowing hosts and therefore represents a key component of maintaining the symbiosis. This study illuminates mechanisms of host control and nutrient transfer in an important microbial symbiosis in oligotrophic waters.

Symbiont maintenance and host control in Acantharea-*Phaeocystis* photosymbioses revealed through single-holobiont transcriptomics

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Background

Photosymbioses are important to primary production in low-nutrient regions[1,2] and are opportunities to study the early stages of plastid acquisition[3]. Although traditionally assumed mutualistic—with hosts benefiting from organic carbon fixed by symbionts and symbionts benefiting from nutrients supplied by hosts—it is now doubted whether many photosymbioses, including among acantharians, are truly mutualisms[4].

Acantharians are the most abundant photosymbiotic Rhizaria in oligotrophic surface waters, where they create localized productivity hot spots[2]. Acantharians maintain *Phaeocystis* (Haptophyta) symbionts without systematically digesting them[5], but symbionts undergo a dramatic phenotypic transformation, including increased cell size and chloroplast proliferation[6]. However, it is unclear how acantharians manage symbiont populations or whether symbionts benefit from the relationship.

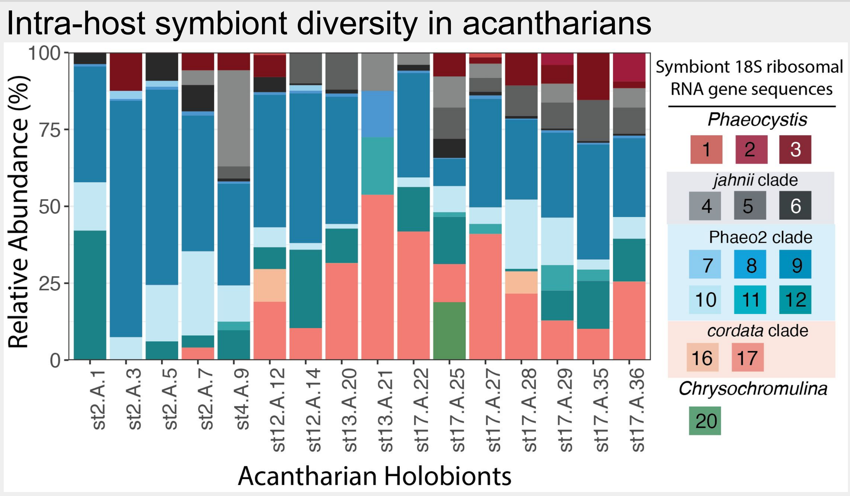
Aims

Determine molecular mechanisms involved in nutrient transfer and symbiont population control in Acantharea-*Phaeocystis* symbioses by comparing gene expression in symbiotic and free-living cells of two *Phaeocystis* species.

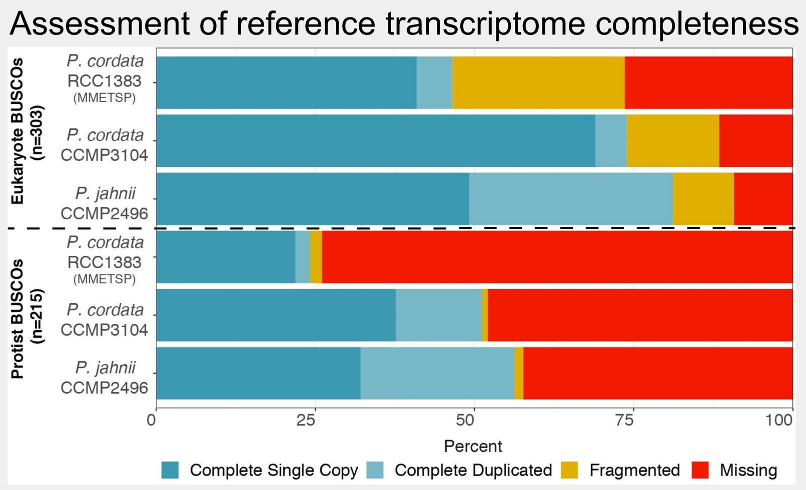
Methods

Sequenced single holobiont transcriptomes for 16 individual acantharians with diverse symbiont communities.

Intra-host symbiont diversity in acantharians



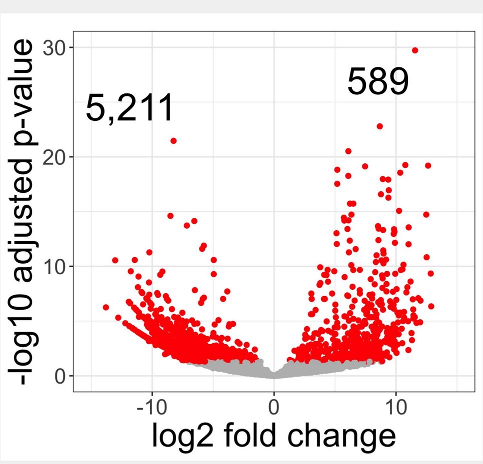
Assessment of reference transcriptome completeness



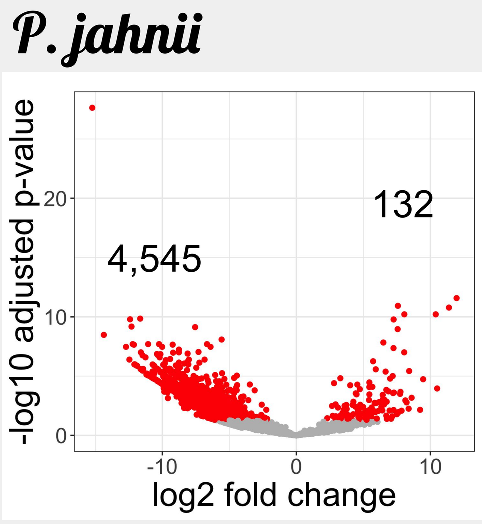
Sequenced transcriptomes from three biological replicates each for two symbiont species, ***P. cordata*** and ***P. jahnii***.

Performed differential expression testing (DESeq2) for free-living replicates compared to holobiont replicates.

P. cordata



P. jahnii



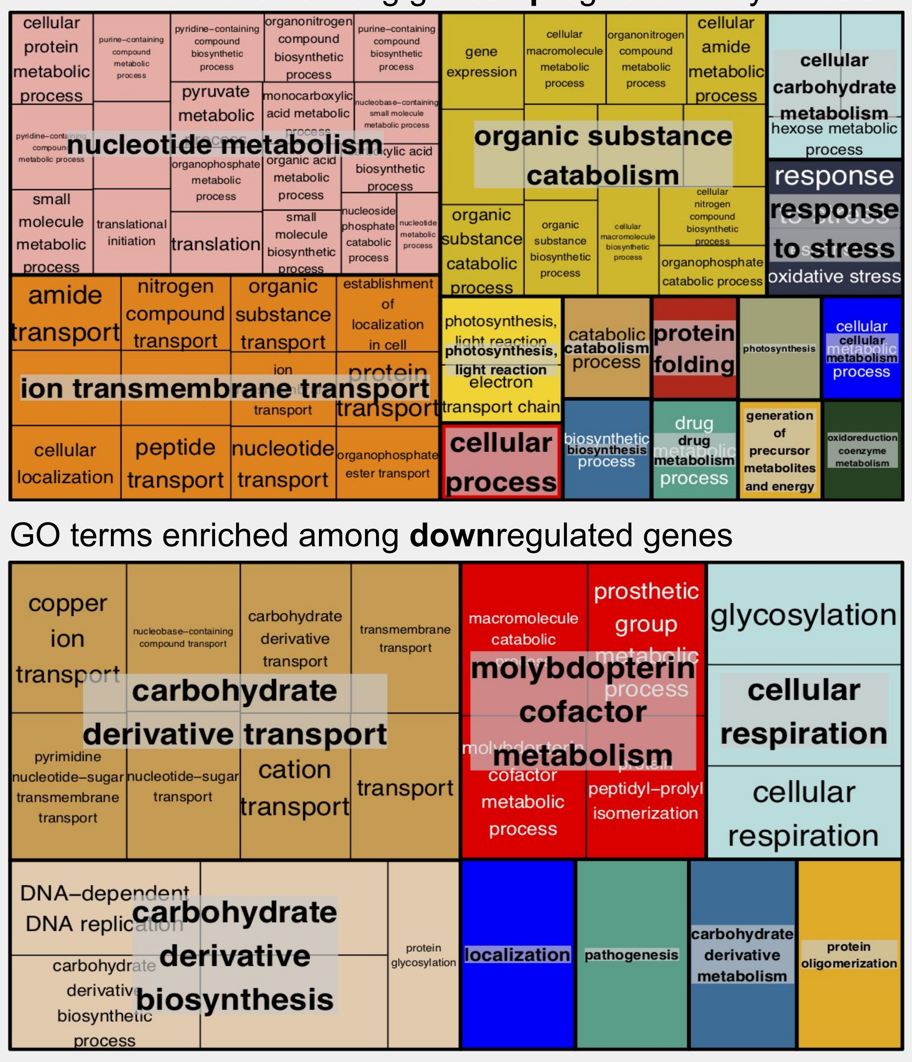
Summary of significantly (red) up- and downregulated genes in symbiotic *Phaeocystis* cells within acantharian hosts

Performed GO term and KEGG pathway enrichment testing with differentially expressed gene sets.

Results

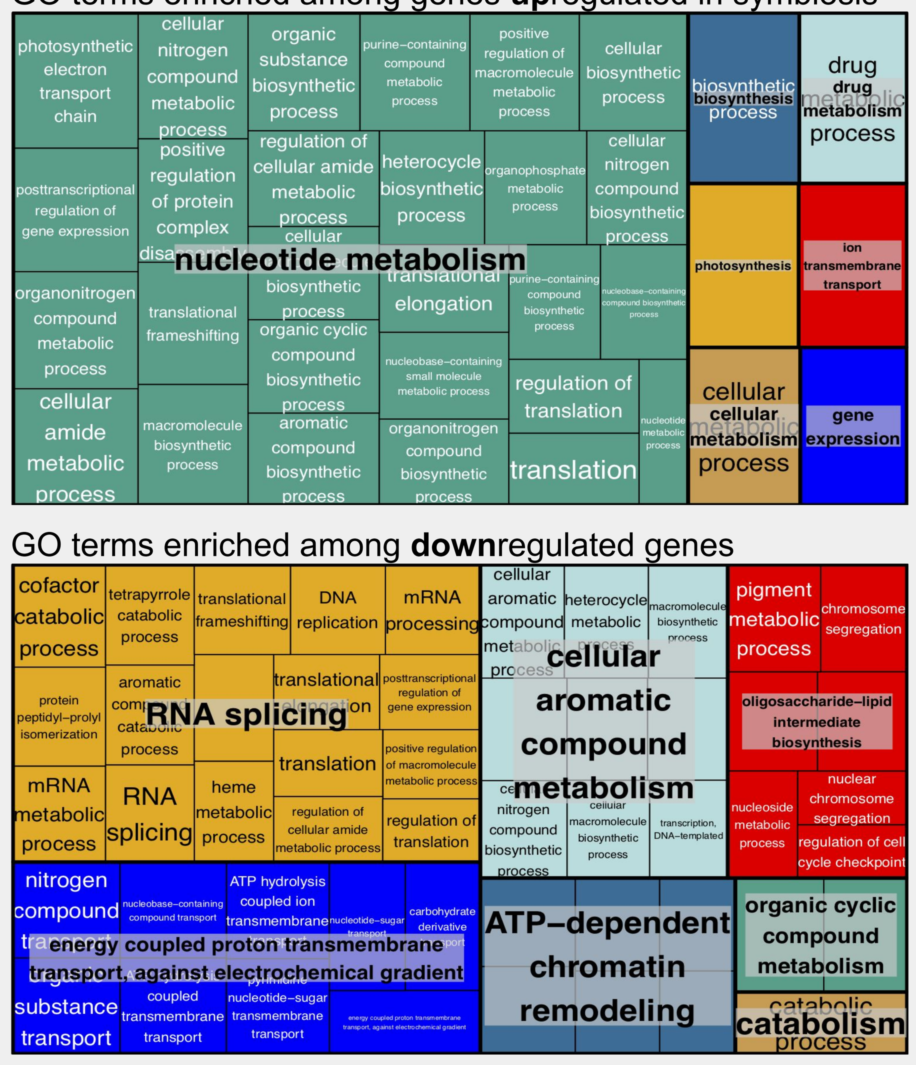
P. cordata

GO terms enriched among genes **up**regulated in symbiosis
GO terms enriched among genes **down**regulated genes



P. jahnii

GO terms enriched among genes **up**regulated in symbiosis
GO terms enriched among genes **down**regulated genes

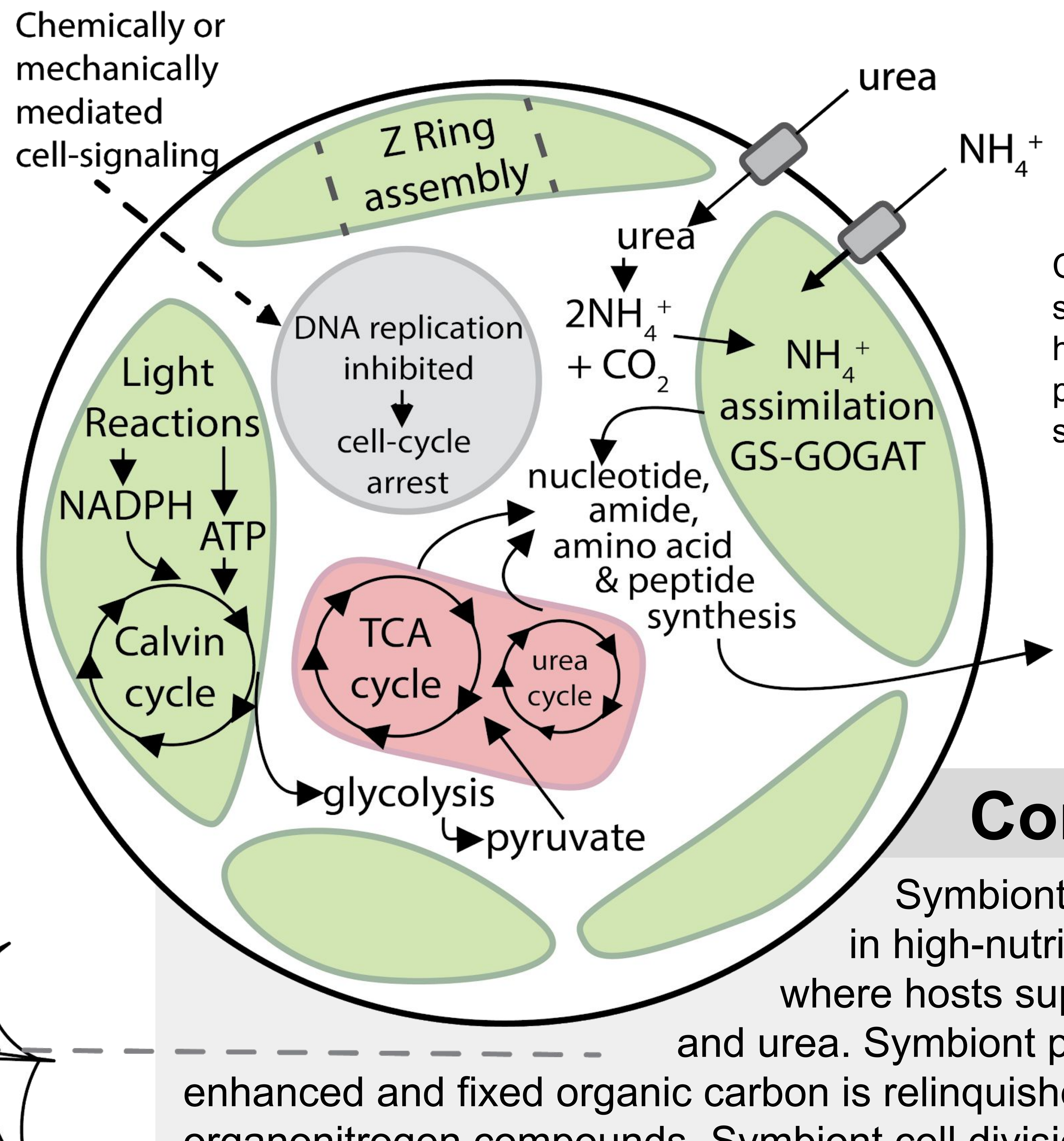


Photosynthesis GO terms are enriched among genes upregulated in symbiosis for both species. Biosynthesis of storage carbohydrates and lipids is downregulated.

DNA replication GO terms are enriched among downregulated genes in both species, as are DNA replication and cell-cycle KEGG pathways. Genes in the Mitogen Activated Protein Kinase (MAPK) pathway that influence cell proliferation are downregulated in symbiosis.

Nuclear encoded chloroplast division genes are expressed at similar levels in symbiotic and free-living cells in both species.

Neither species expressed genes associated with P or N limitation in symbiosis. Urea and ammonium transporter genes and ammonium assimilation genes are expressed in symbiosis.



Conceptual model summarizing results and highlighting pathways and processes important in symbiotic *Phaeocystis*

Conclusions

Symbionts are maintained in high-nutrient conditions, where hosts supply ammonium and urea. Symbiont photosynthesis is enhanced and fixed organic carbon is relinquished to hosts as small organonitrogen compounds. Symbiont cell division is inhibited by hosts, but chloroplasts continue dividing. Instead of controlling symbiont populations by limiting nutrients, hosts manipulate cell-signaling pathways to prevent symbiont proliferation. This gives hosts finer control and ensures symbionts maintain high photosynthetic output.

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