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

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November 24, 2022

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 acquisition³. 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 mutualisms⁴.

Results

copper

ion

ranspo

DNA-dep

DNA rep

carboh

P. cordata

GO terms enriched among genes upregulated in symbiosis



P. jahnii

GO terms enriched among genes upregulated in symbiosis

photosynthetic electron transport chain	cellular nitrogen compound metabolic	organic substance biosynthetic process	purine-containing compound metabolic process	positive regulation of macromolecule metabolic process	cellular biosynthetic process	biosynthetic biosynthesis process	drug drug metabolism	
	positive regulation	regulation o cellular amid	f le heterocycl	e organophosphate	cellular e nitrogen		process	
posttranscriptional regulation of gene expression	of protein complex	metabolic process cellular	biosynthet process	IC metabolic process	compound biosynthetic process			
	^{disa} nucle	eotide n		photosynthesis	ion transmembrane			
organonitrogen	translational	biosynthetic process	elongati	ON purine-contair compound biosynthetic	ning nucleobase-containing compound biosynthetic c process		transport	

Acantharians are the most abundant photosymbiotic Rhizaria in oligotrophic surface waters, where they create localized productivity hot spots². Acantharians maintain *Phaeocystis* (Haptophyta) symbionts without systematically digesting them⁵, but symbionts undergo a dramatic phenotypic transformation, including increased cell size and chloroplast proliferation⁶. 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.

ion transmembrane transport cellular proce

GO terms enriched among **down**regulated genes

1	metabolic	frameshifting	organic cyclic					
1	process		compouna biosynthetic	nucleobase-containing small molecule	rogulation	of		
	cellular		process	metabolic process	translatic	on	cellular	dene
÷.	amide	macromolecule	aromatic	organonitrogen		nucleotide metabolic	metabolism	expression
i	metabolic	process	biosynthetic	compound biosvnthetic	translati	on	process	
1	process		process	process				

GO terms enriched among downregulated genes

											<u>J</u>			J	
nucleobase-containing compound transport	carbohydrate derivative transport	transmembrane transport	macromolecule catabolic	prosthetic group metabolic	glycosylation		cofactor catabolic process	tetrapyrrole catabolic process	translational frameshifting	DNA replication	mRNA processing	cellular aromatic compound metabolic	heterocycle metabolic	macromolecule biosynthetic process	pigment metabolic process
carboh rivative	ydrate transpo	ort	cofa	opterin process ctor olism	cellular respiration	-	protein peptidyl–prolyl isomerization	aromatic company catabolic process	A spli	anslational cing on ranslation	posttranscriptional regulation of gene expression positive regulation of macromolecule	ar COI	omati mpou	ic nd	oligosacchario intermedi biosynthe
nucleotide-sugar transport	transport	transport	cofactor metabolic process	peptidyl–proly isomerization	cellular respiration		mRNA metabolic process	RNA splicing	heme metabolic process _m	regulation of cellular amide etabolic process	metabolic process regulation of translation	ce me nitrogen compound biosynthetic	ceilular macromolecule biosynthetic process	transcription, DNA-templated	nucleoside metabolic process cycle
carboh carboh ate deriv	ydrate ative	protein glycosylation	localization	c pathogenesis	arbohydrate derivative netabolism		nitrogen compoun traenerg transpo	d ^{nucleobase–cor} compound trai y couple rt,₄agạin	ATP hydrogram coupled transmen d protom stielectrogram	rolysis d ion nbranenucleotide- transmem chemical	sugar derivative brane ^{sport} gradient	ATP-	depei roma	ndent tin	organic o compo metabo
biosyn	thesis						substanc transpor	e transmemb t transpo	d nucleotide orane transmen ort transp	e-sugar hbrane energy cou transport, ag:	pled proton transmembrane ainst electrochemical gradient	ren	nodel	ing	catabo catabo proce

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.

Methods

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



Assessment of reference transcriptome completeness



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

P. cordata

P. jahnii

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*

organonitrogen

Conclusions

Symbionts are maintained



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.

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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