

Reduction of UDP-glucose diphosphorylase (UGP2) gene expression does not reduce accumulation of the diatom storage sugar chrysolaminarin in *Phaeodactylum tricornutum*

Yun (Leo) Zhang, Michael Caballero, Graham Peers, Department of Biology, Colorado State University

Introduction

Diatoms are ecologically significant microalgae and responsible for 40% of the ocean's primary productivity. Diatoms distribute fixed carbon into metabolic pools such as carbohydrate, lipid, and protein. We are interested in exploring the unusual storage carbohydrate of diatoms, chrysolaminarin, which has the same composition and function as starch from plants, but a different structure (β -1,3 and α -1,4 linked glucans, respectively). Decreasing carbon partitioning into chrysolaminarin may increase diatom lipid productivity for biofuels. The synthesis and degradation pathways for chrysolaminarin are unknown. Biochemical evidence suggests that making UDP glucose is the first step of chrysolaminarin synthesis.

The sequenced genome of the model diatom *Phaeodactylum tricornutum* encodes two predicted gene products that may make UDP glucose (UGP1, UGP2)¹. UGP1 has already begun to be investigated, and appears to significantly modify carbon partitioning². Interestingly, UGP2 mRNA accumulation varies over day:night cycles³, as does the storage carbohydrate chrysolaminarin. Therefore, we wanted to investigate the role of UGP2 in chrysolaminarin biology by generating RNAi knockdown lines and studying the physiology of these mutants.

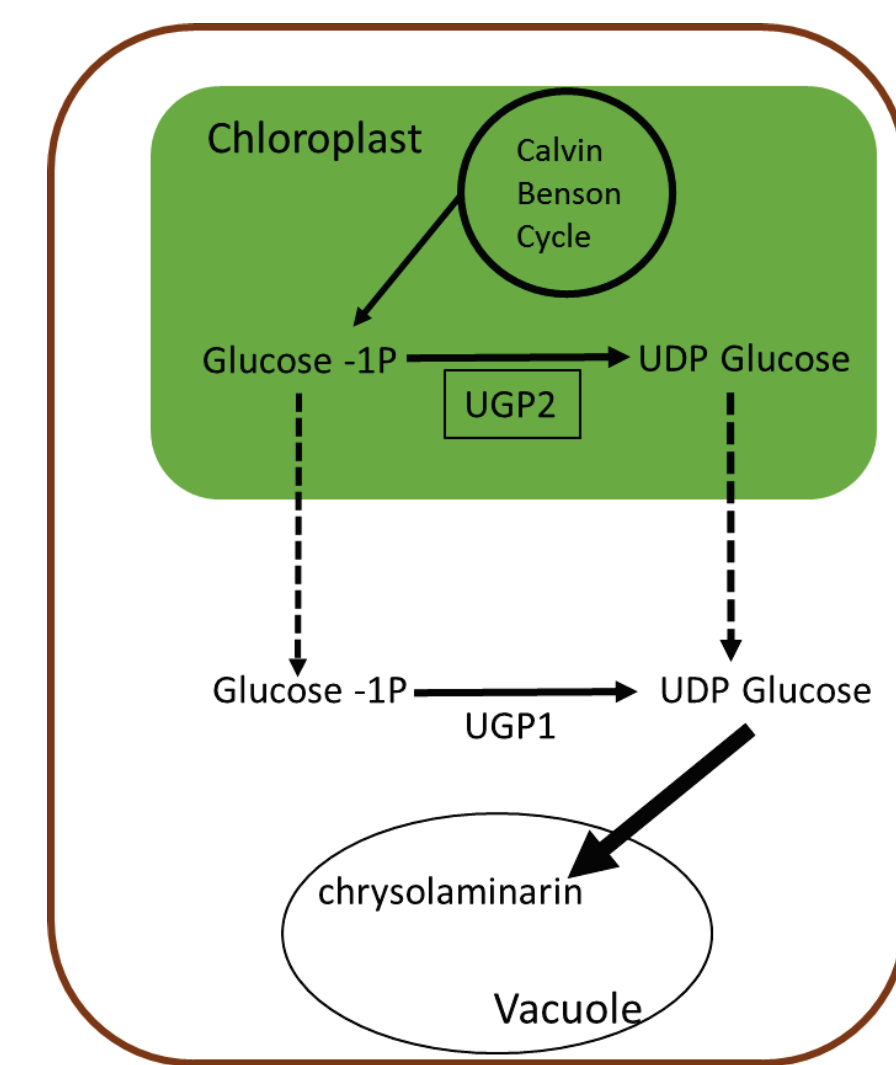


Figure 1. The two annotated UDP-glucose diphosphorylases (UGP1, UGP2) from *P. tricornutum* and their predicted localizations. These enzymes may be important for chrysolaminarin synthesis.

Experiment workflow

A. Cloning knockdown vectors

Two independent knockdown vectors were generated by cloning an antisense fragment specific to UGP2 into the pMAC3 plasmid⁴. These two vectors (A & B lines, results) were linearized prior to electroporation.

B. Transformation by electroporation

Both UGP2 knockdown vectors were transformed into *Phaeodactylum* by electroporation⁵. After electroporation, *Phaeodactylum* was plated on selective Zeocin plates.

C. Screening with qRT-PCR

mRNA was extracted from 16 randomly selected RNAi lines, harvested 2h after dawn (12:12 light:dark, 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Relative abundance of UGP2 mRNA was determined by qRT-PCR using TATA binding protein mRNA as the reference transcript⁶. This process was repeated, yielding 3 biological replicates for selected RNAi lines.

D. Phenotyping with physiology

I. Growth Rate. 1 mL of culture was harvested at the same time each day and cell density was determined by flow cytometry. Cells were maintained in exponential growth.

II. Carbohydrates. A modified reducing sugar assay (MBTH assay) was used⁷ which permitted quantification of chrysolaminarin; soluble, reducing carbohydrates; and insoluble carbohydrates in parallel.

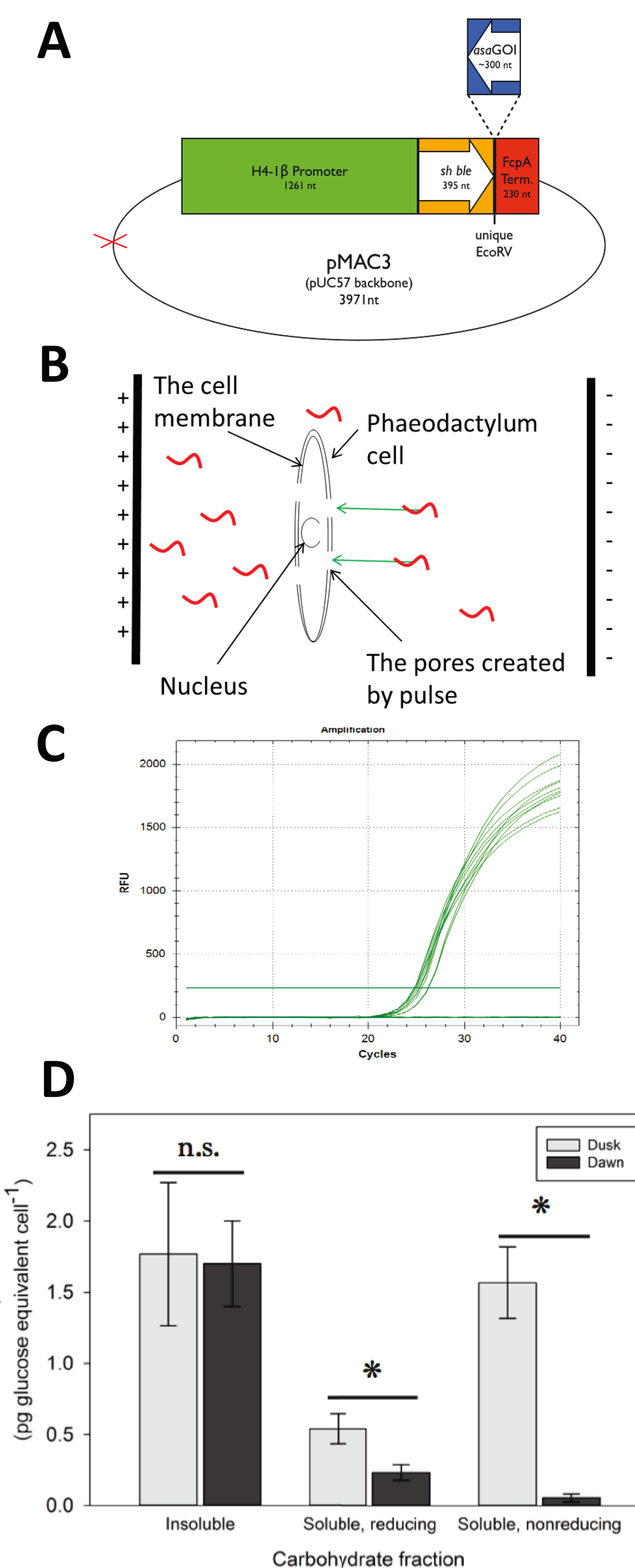


Figure 2. An experimental workflow overview: (A) the pMAC3 vector map, (B) a summary of the electroporation mechanism, (C) amplification of positive and negative qRT-PCR controls, and (D) The insoluble, soluble reducing, soluble nonreducing carbohydrate change during day-night cycle in *P. tricornutum*. (* = $p < 0.05$)

Results

UGP2 RNAi lines accumulate about 50% UGP2 mRNA relative to WT

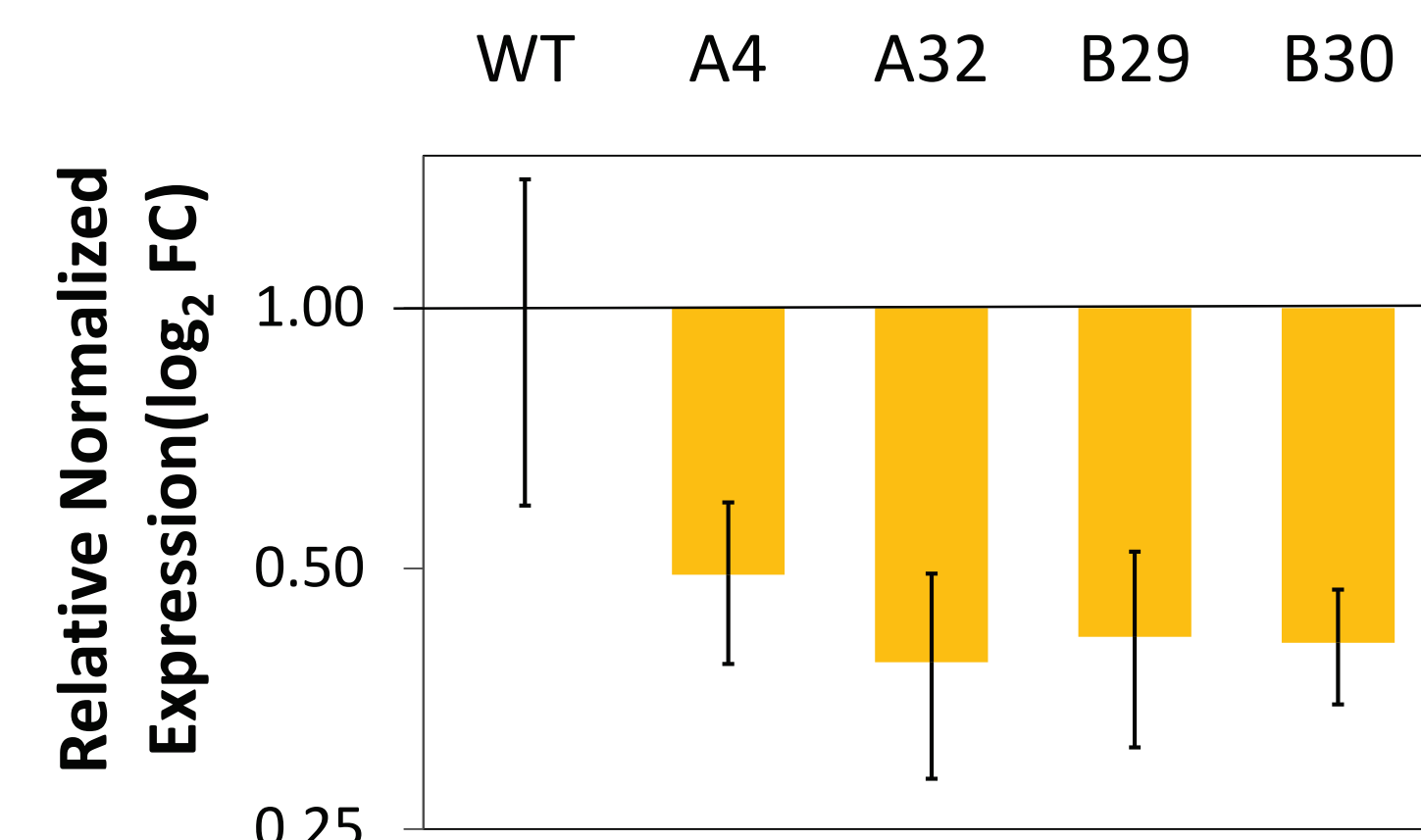


Figure 3. Identification of UGP2 RNAi lines. Cells were harvested 2 h after dawn, when UGP2 mRNA abundance is at its peak. Two "A" lines and two "B" lines were identified from the qRT-PCR screen. (n = 3, error = SD)

- Four UGP2 knockdown lines were identified from the qRT-PCR screen with at least a 50% decrease of UGP2 mRNA abundance.

No change in growth rate for UGP2 RNAi lines relative to WT

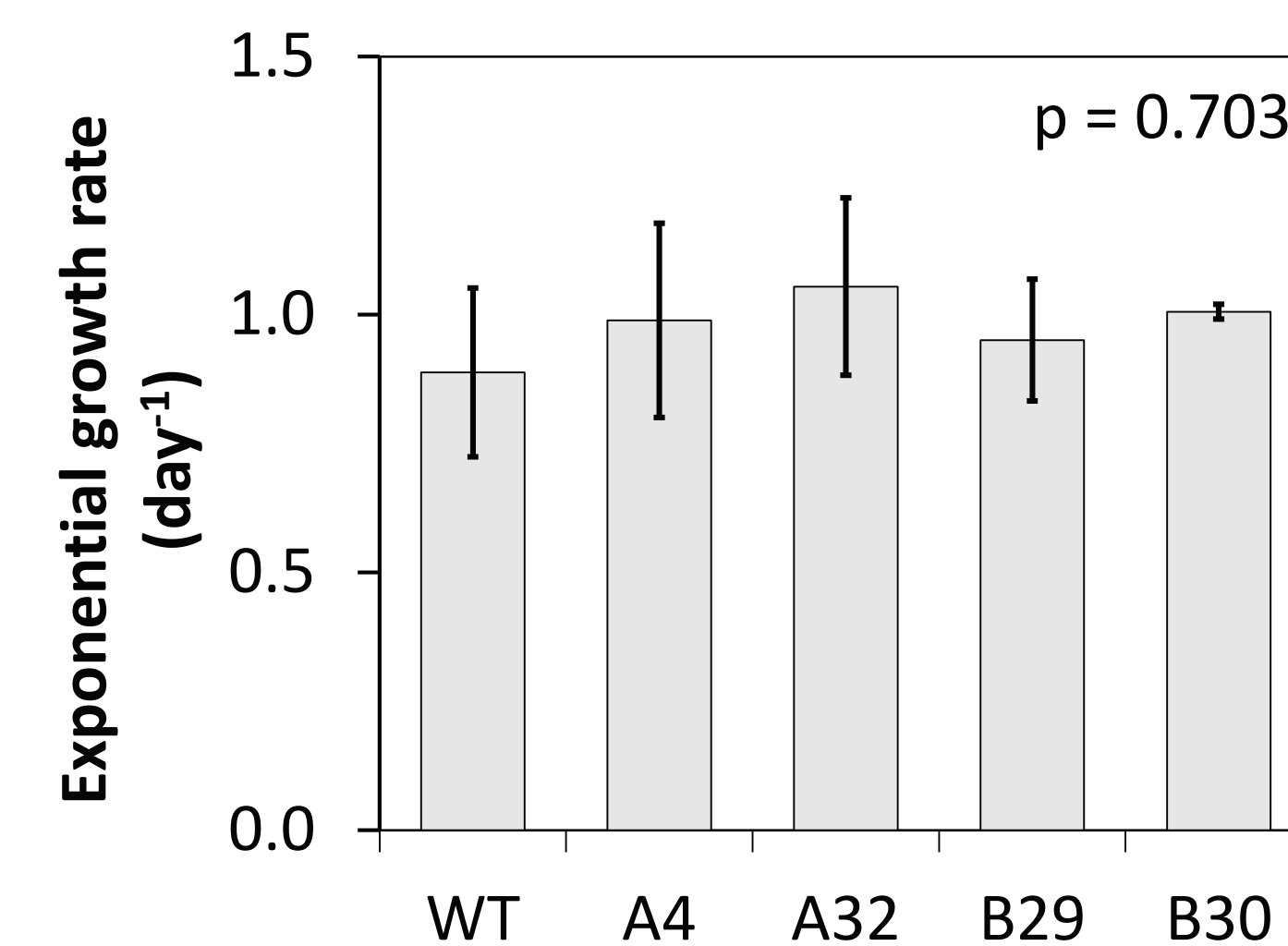


Figure 4. A comparison of exponential growth rates between UGP2 RNAi lines and WT. Cell densities were determined by flow cytometry at the same time over at least a three day period (n = 3, error = SD, One way ANOVA)

- There is no statistically significant difference for growth rate between UGP2 RNAi lines and WT.

Major carbohydrate pools remain unchanged in UGP2 RNAi lines relative to WT

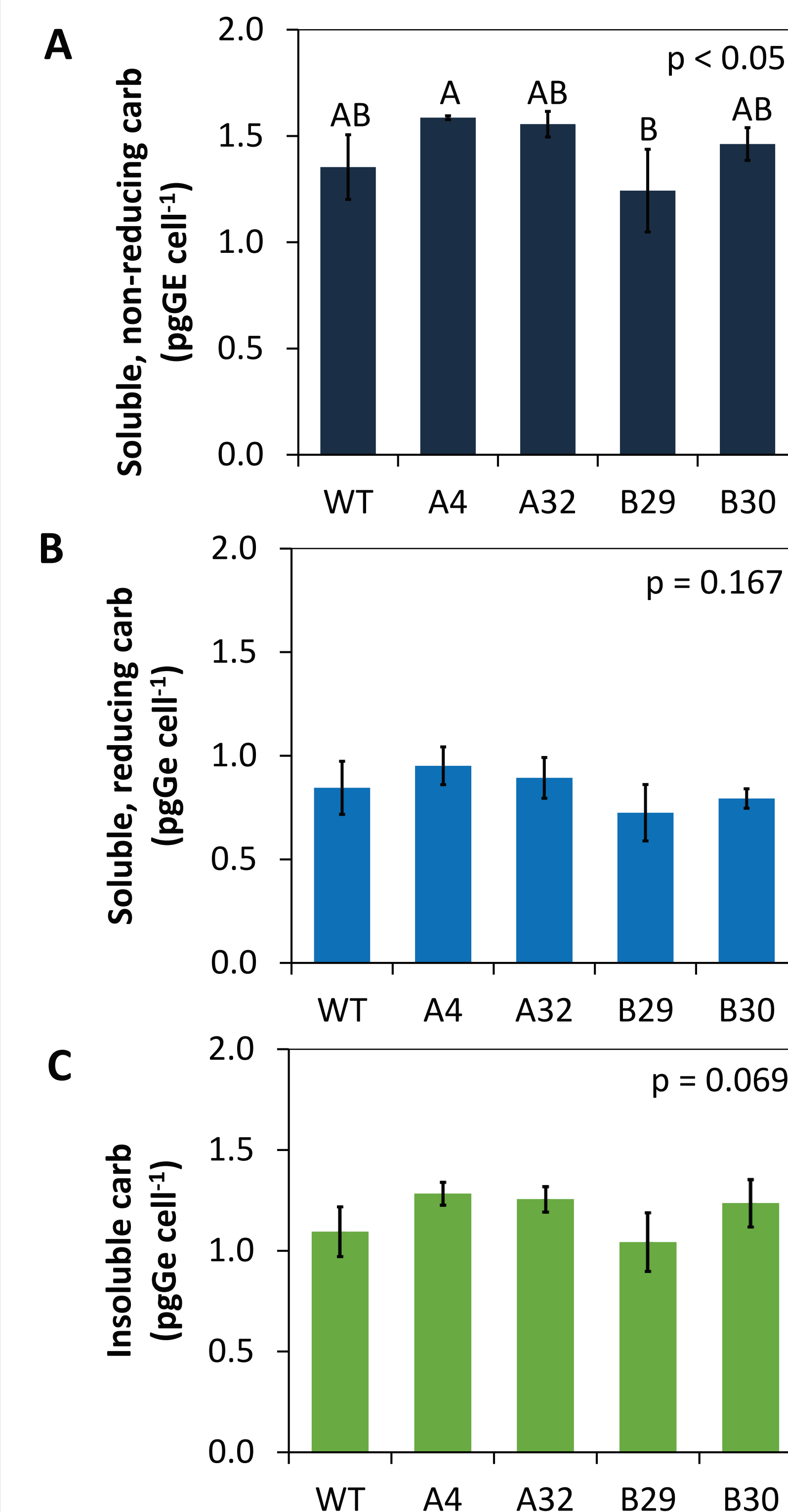


Figure 5. Quantification of major carbohydrate pools extracted from UGP2 RNAi lines and WT. (A) Soluble, non-reducing carbohydrate, (B) Soluble, reducing carbohydrate, (C) Insoluble carbohydrate were determined in parallel from the same harvested cell pellet. Cells were harvested 2 h prior to dusk, which enabled quantification of maximum carbohydrate accumulation per cell. (n = 3, error = SD, One way ANOVA with a Tukey HSD test as appropriate, letters represent statistically significant groups)

- A novel quantification approach⁷ enables quantification of three major carbohydrate pools in parallel:

- Soluble, non-reducing carbohydrate is composed of polysaccharides. Other studies in our lab suggest that this fraction is mostly chrysolaminarin.
- Soluble, reducing carbohydrate is made up of monosaccharides present in central metabolism.
- Insoluble carbohydrate is mostly structural sugars and are likely associated with the cell wall.
- There is no statistically significant difference between WT and any UGP2 RNAi line for any of the three major carbohydrate pools.

Discussion

Re-evaluating the role of UGP2 in chrysolaminarin biology

We did not observe any significant physiological changes in terms of growth rate or carbohydrate accumulation between WT and any of our UGP2 RNAi lines. We developed three hypotheses to explain these results.

- A 50% decrease of UGP2 mRNA may not be adequate to alter carbohydrate accumulation.
- UGP1 may be upregulated, compensating for the reduced accumulation of UGP2 mRNA.
- UGP2 may not be essential for chrysolaminarin synthesis; instead, UGP2 may be involved in some other aspect of cellular metabolism.

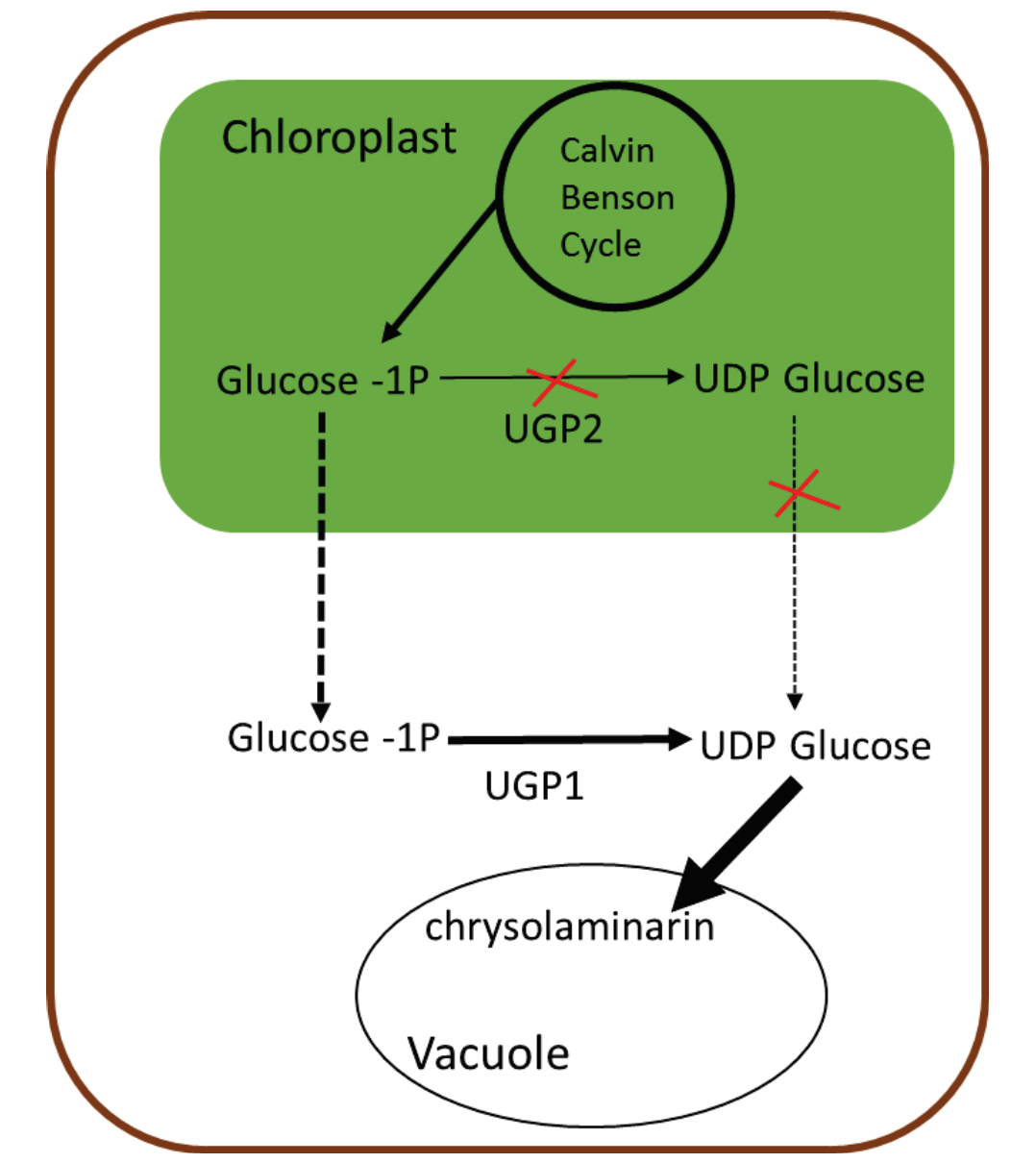


Figure 6. Experimental result that UGP2 knockdown does not reduce accumulation of chrysolaminarin in *P. tricornutum*.

Alternative potential roles of UDP glucose in diatom biology

- We assumed that UDP-glucose produced by UGP2 is important for chrysolaminarin synthesis. However, UDP-glucose is a common sugar intermediate⁸, and the UDP glucose made by UGP2 may not be used for carbohydrate synthesis.
- Glycolipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are located in the chloroplast and require UDP-glucose⁹. Decreasing the UDP-glucose supply could decrease MGDG and DGDG abundance, potentially influencing photosynthetic efficiency and fitness.
- Glycoproteins also require UDP-glucose for their synthesis⁸.
- If UGP2 is participate in glycolipids or glycoprotein synthesis, insoluble carbohydrate should decrease in the MBTH assay result for our UGP2 knockdown. However, we did not see any significant change in the result.

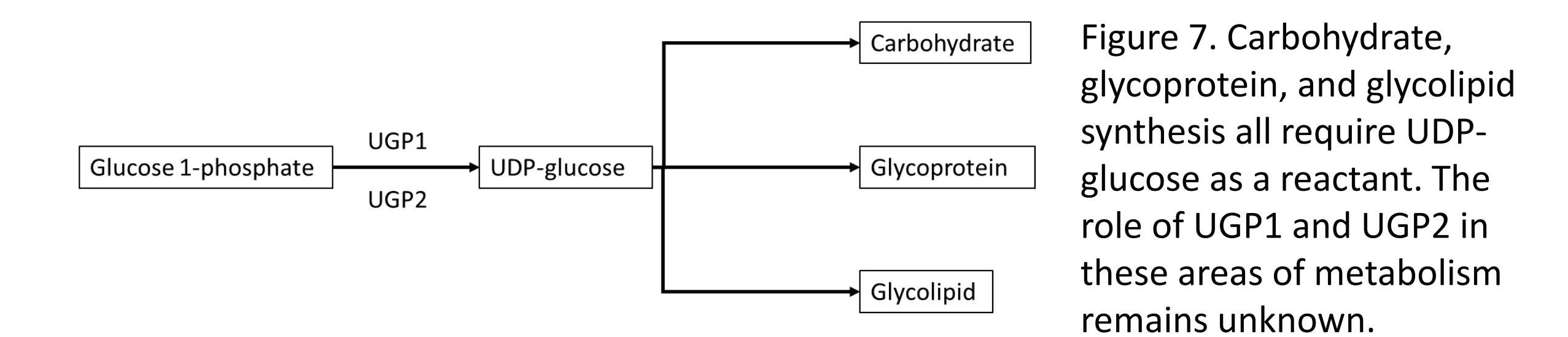


Figure 7. Carbohydrate, glycoprotein, and glycolipid synthesis all require UDP-glucose as a reactant. The role of UGP1 and UGP2 in these areas of metabolism remains unknown.

Next steps

- We are generating both UGP1 and UGP2 knockout lines with a CRISPR/Cas9 approach. Investigating these knockout lines will allow us to resolve the potential role of each UGPase in chrysolaminarin biology.
- We are also attempting a functional screen for UGPase activity using a *Phaeodactylum* cDNA library because The UGP glucose diphosphorylase activity for UGP 2 has not been verified, only bioinformatically predicted.

References

- Kroth, P. G., et al. (2008). A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. *PLoS ONE*, 3: e1426.
- Daboussi, F., et al. (2014). Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat. Commun.* 5:3831 doi: 10.1038/ncomms4831.
- Chaumont, M. S., Winge, P., Brembu, T., Vadstein, O., & Bones, A. M. (2013). Gene Regulation of Carbon Fixation, Storage, and Utilization in the Diatom *Phaeodactylum tricornutum* Acclimated to Light/Dark Cycles. *Plant Physiology*, 161(2), 1034-1048. doi:10.1104/pp.112.206177
- De Riso, V., et al. (2009). Gene silencing in the marine diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res.* 37: e96.
- Zhang, C., and Hu, H. (2014). High-efficiency nuclear transformation of the diatom *Phaeodactylum tricornutum* by electroporation. *Marine genomics* 16, 63-66. doi:10.1104/pp.111.174706
- Boulié, L., et al. (2014). Glycolipids in photosynthesis: Composition, synthesis and trafficking. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, Volume 1837, Issue 4, April 2014, Pages 470-480, ISSN 0005-2728
- Caballero et al. in prep.
- Kieczkowski, L. A., Decker, D., & Wilczynska, M. (2011). UDP-Sugar Pyrophosphorylase: A New Old Mechanism for Sugar Activation. *Plant Physiology*, 156(1), 3-10. doi:10.1104/pp.111.174706
- Boulié, L., et al. (2014). Glycolipids in photosynthesis: Composition, synthesis and trafficking. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, Volume 1837, Issue 4, April 2014, Pages 470-480, ISSN 0005-2728

Acknowledgments

Project funded by DOE-BER (DE-SC0008595).
MAC supported by NSF GRFP (DGE-1321845) & IGERT MASB.
Special thanks to the Sloan Lab (Biology) for training and equipment use.

