Antarctic Bursary Award Project Report (2020 Round). Sarah Andrew.

Can Antarctic diatoms use Proteorhodopsin to generate energy instead of photosynthesis?

General overview

Proteorhodopsins are light-absorbing proteins that function as proton pumps, harnessing solar energy using the chromophore retinal; to drive proton transport across cellular membranes. Initially discovered in bacteria, proteorhodopsins have since been found in a variety of marine organisms, including phytoplankton such as diatoms. Measured retinal values normalized to phytoplankton Chl a in the West Antarctic Peninsula, suggests that proteorhodopsins are more abundant in phytoplankton communities in these iron-limited, offshore regions. These findings suggest that PPRs may be particularly important in supporting phytoplankton energy production and survival in environments where iron is scarce and light conditions are highly variable. Proteorhodopsins are especially relevant due to their potential role in supplementing energy production, particularly under conditions where traditional photosynthesis (PS) is limited.

Project summary

This project sought to understand the role of the light-driven proton pump, proteorhodopsin, in cellular energy synthesis for survival strategies in the iron-limited polar Southern Ocean. The major goals of the project were to 1) determine if all Antarctic diatoms in the UNC culture collection contained proteorhodopsin, 2) determine if Antarctic diatoms that possess proteorhodopsin are able to grow in the absence of photosynthetic activity. To address these aims, I cultured two Antarctic diatoms: under broad spectrum light, that stimulates photosynthesis versus spectral tuned light that discourages light absorption by chlorophyll and stimulates diatom-PR (511nm, Andrew et al., 2023) in Fe-replete and Fe limited media (Figure 1). Samples were collected for gross primary productivity, ATP, gene expression (high-throughput sequencing of RNA) chlorophyll a (pigment used by photosynthesis), and retinal (pigment used by diatom-PR).

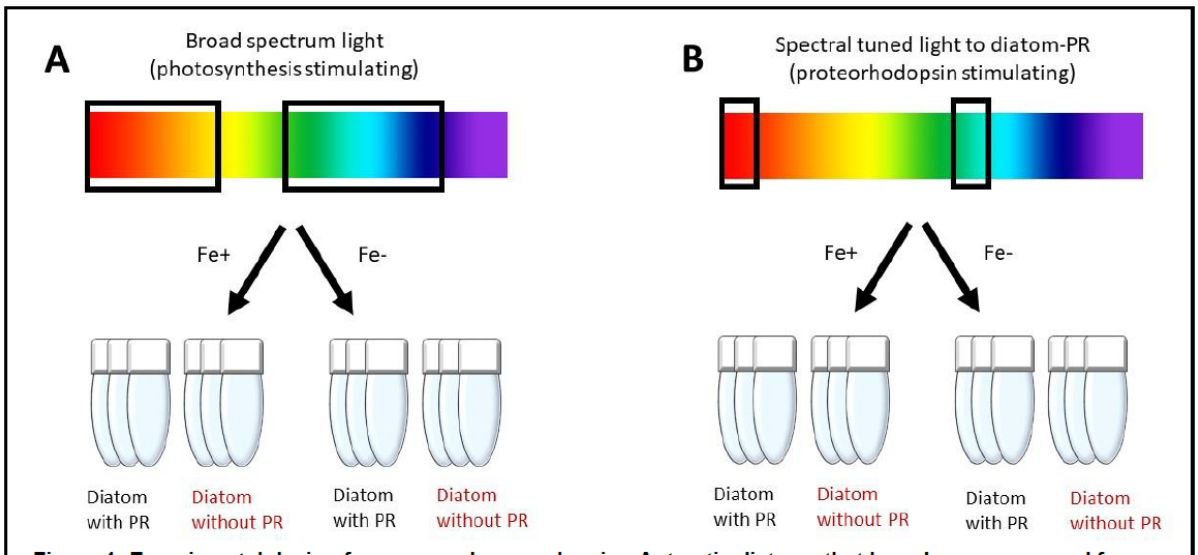

Figure 1: Experimental design for proposed research using Antarctic diatoms that have been sequenced for presence or absence of diatom-PR. Light experiments will be set up with a unique light spectrum to (A) stimulate photosynthesis or (B) stimulate proteorhodopsin over photosynthesis. Both diatoms will be grown in Fe-replete or Fe-limited media in both light experiments.

Results

From previous research (Andrew et al., 2023) a monoclonal antibody was created for diatom proteorhodopsin against the *Pseudo-nitzschia subcurvata* diatom. This antibody, in combination with Sanger sequencing was used to create a primary screen for diatoms that may, or may not possess the diatom proteorhodopsin gene. From this screen, two diatoms were chosen for further study and cultured under the different light spectrums. Upon completion of the experiment, high-throughput sequencing of RNA from both species showed that they possessed different sequences for diatom proteorhodopsin (Andrew et al., 2023).

Preliminary findings showed that growth of both diatoms was negatively impacted by iron supply under green light suggesting that diatoms were still able to access part of the light spectrum to drive photosynthesis. In addition, the comparison of growth rate between green and white light for the iron replete cultures showed no significant difference in growth rate. As of April 2022, culturing was complete and all samples were collected. Samples include: differential gene expression (transcriptomics), pigment analysis (HPLC), cellular C and N, chlorophyll a, and estimates of proteorhodopsin (retinal).

A small part of this data has been published in 2023 in the journal Proceedings of the National Academy of Sciences, and the sequences for the diatom proteorhodopsin genes are available in NCBI (accession number OQ799912 and OQ856872).

Another manuscript sharing the remainder of this research is being prepared. Upon publication, all data associated with this project will be made available in a public data repository.

Publications associated with this project

Andrew, S. M., Moreno, C. M., Plumb, K., Hassanzadeh, B., Gomez-Consarnau, L., Smith, S. N., Schofield, O., Yoshizawa, S., Fujiwara, T., Sunda, W. G., Hopkinson, B. M., Septer, A. N., & Marchetti, A. (2023). Widespread use of proton-pumping rhodopsin in Antarctic phytoplankton. In Proceedings of the National Academy of Sciences (Vol. 120, Issue 39). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.2307638120