The Plastid Terminal Oxidase does not play a photoprotective role in the marine diatom *Phaeodactylum tricornutum*.

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Project Goals: Overall goal - Reprogram metabolic networks using *in vivo* synthetic modules to increase the flux of energy and carbon into biofuel precursors. Goal 1) Profiling the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. Goal 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and regulation of lipid accumulation. Goal 3) Forward genetic library generation, screening and genotyping. These approaches complement our development of *Phaeodactylum* genome reconstruction /modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing productivity.

Algae, cyanobacteria and plants require a dynamic photoprotective system to maintain high photosynthetic efficiencies in dynamic light environments. These systems need to be rapidly induced in excess light to reduce the incidence of photooxidative stress. Conversely, they need to be turned off to maintain high rates of photochemistry in lower light. The Plastid Terminal Oxidase (PTOX) has been shown to play an important role in photoprotection in the green alga Chlamydomonas. PTOX accepts electrons from the photosynthetic electron transport chain and donates them to reduce oxygen to water. This alleviates excitation pressure and increases fitness during growth in excess light. We investigated the role of PTOX in the marine diatom Phaeodactylum tricornutum by creating a double knockout of 2 PTOX isoforms using a CRISPR-Cas9/HDR approach. The mutants displayed a distinct rise in chlorophyll a fluorescence following intense illumination, suggesting it plays a role in dark chlororespiration. We found no changes in fitness between the WT and KO strains in a variety of stress conditions including excess constant light, nitrogen limitation, carbon limitation, or variable light. We also found no changes in light-dependent oxygen consumption (LDOC) between the WT and KOs in any of these conditions. The rates of LDOC remained low in all culture conditions, which is contrast to previous observations of cyanobacteria and green algae. Other processes, such as non-photochemical quenching or perhaps mitochondria-plastid coupling, appear to be the predominant forms of photoprotection in this diatom. This work suggests that increasing energy fluxes to carbon fixation through engineering PTOX levels is not viable in this species.

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