

Nitrate sensing with a model pennate diatom, *Phaeodactylum tricornutum*

Maxine H. Tan¹, Zheng Wang², Hong Zheng³, Jill Meisenhelder², James McCarthy³, Ahmed Moustafa⁴, John McCrow³, Tony Hunter², **Andrew Allen (aallen@jcv.org)**^{1,3*}

¹Scripps Institution of Oceanography, UCSD, La Jolla, CA

²Salk Institute of Biological Studies, La Jolla, CA

³J. Craig Venter Institute, La Jolla, CA

⁴The American University of Cairo, Egypt

Nitrate sensing mechanisms have been studied for more than 30 years in organisms such as heterotrophic bacteria, cyanobacteria, green algae, yeast, fungi and plants. However, mechanisms by which diatoms sense and respond to nitrate availability are currently not known. We have identified a putative nitrate sensing protein, undescribed or characterized to date, in the genome of the model pennate diatom *Phaeodactylum tricornutum*. NitK contains a nitrate sensing Pfam (NIT) fused to a kinase domain and we hypothesize that it assists in sensing intracellular nitrate status. A search of available data indicated that this domain combination is prevalent in marine diatoms, dinoflagellates, haptophytes, and is also found in various green lineage prasinophyte species. In *P. tricornutum*, NitK transcript abundance appears to be regulated by addition of nitrate and nitrogen stress. Functional assays on NitK show autophosphorylation and that it is a serine/threonine kinase. Secondary structure predictions and analysis of membrane isolation fractions, complemented by preliminary localization of NitK, proposes that NitK is membrane bound. Mass spectrometry and yeast two-hybrid results suggest that NitK complexes with other nitrogen metabolism proteins, assisting in maintaining cellular nitrate homeostasis. Using *P. tricornutum* as a model for functional analysis, we strive to obtain an enhanced understanding of nitrogen metabolism and sensing mechanisms in major groups of marine primary producers which will improve our ability to forecast the impact of environmental change on marine ecosystems.

Funding Statement

Research supported by United States Department of Energy Genomics Science program grants DE-SC0008593 and DE-SC0018344.