Cellular Impact of Inactivation of the Nitrate Reductase Gene in the Marine Diatom, *Phaeodactylum tricornutum*

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A knockout of *Phaeodactylum tricornutum's* nitrate reductase (NR) gene, resulting in the absence of the NR protein, was produced by TALEN-enabled homologous recombination. In place of NR, a stable, selectable marker was introduced into the *P. tricornutum* genome. Investigations of the NR knockouts by confocal microscopy and deep-etch freeze fracture EMs show a dramatic reorganization of normal pennate diatom physiology. KO cells are swollen and deformed with chloroplasts reduced and pushed to the sides or ends of the cells. FTIR results indicate a dramatic rearrangement of protein and carbohydrate fractions in the scans of WT and NR-KO samples. UV-Spectroscopy showed that NR-KO cells had, within 3 days, accumulated greater than 1 mM NO_3^-, whereas in WT cells, by assimilating the NO_3^-, there was almost no NO_3^- in the cell extracts. An increase was also observed in the NR-KO lipid fraction as compared to the wild type. FAME analysis of cell lipids showed an immediate, substantial redistribution of TAG fatty acids in the knockout line. Whole transcriptome analysis, comparing NR-KO lines vs WT expression profiles and differential expression over a 10-day time series, confirmed and helped characterize the physiological and biochemical transformation of *P. tricornutum* caused by the loss of function of nitrate reductase.