Defining the Cellular Systems in Marine Diatoms That Provide for Their Predominant Acquisition of Dissolved Nitrate in World Oceans

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The ecological prominence of diatoms in the contemporary ocean environment largely results from their superior competitive ability for dissolved nitrate (NO_3^-). To investigate the cellular and genetic basis of diatom NO_3^- assimilation, we generated a knockout in the nitrate reductase gene (*NR*-KO) of the model pennate diatom *Phaeodactylum tricornutum*. In *NR*-KO cells, N assimilation was abolished although NO_3^-transport remained intact. Unassimilated NO_3^- accumulated in *NR*-KO cells resulting in swelling and associated changes in biochemical composition and physiology. Elevated expression of genes encoding putative vacuolar NO_3^- chloride channel (ClC) transporters, combined with electron micrographs (EM)indicating enlarged vacuoles, suggested vacuolar storage of NO_3^-. Triacylglycerol concentrations in the *NR*-KO cells increased immediately after the addition of NO_3^- and were concurrent with elevated gene expression of key TAG biosynthesis components. Simultaneously, notable induction of transcripts encoding proteins involved in thylakoid membrane lipid recycling suggested more abrupt repartitioning of carbon resources in *NR*-KO cells compared to WT. Conversely, ribosomal structure and photosystems genes were immediately deactivated in *NR*-KO cells after NO_3^- addition, followed within hours by those encoding for chlorophyll biosynthesis, and carbon fixation and metabolism. N-assimilation pathway genes respond uniquely, apparently induced simultaneously by both NO_3^- replete and deplete conditions. To confirm and advance our characterizations of these cellular systems, we have recently constructed gene knockouts of two putative vacuolar transporters, and two chloroplast-targeted nitrite reductases, and are screening for phenotypic changes. With plans to characterize N-stress signaling components in diatoms, we are initially using GC-MS analysis to monitor changes in metabolite concentrations between WT and *NR*-KO and *GSII*-KO mutants as cells respond N-replete and N-stressed conditions.

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