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Characterization of Salt Response Mechanisms in the Marine Diatom *Phaeodactylum tricornutum*

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Summary

Diatoms are the most dominant phytoplankton in the ocean and contribute to a quarter of the global primary production. In this study, responses of critical cell physiology and biochemical processes to salt concentrations were investigated in the marine diatom, *Phaeodactylum tricornutum*. Salt responsive genes were also screened and isolated by using the amplified fragments lengths polymorphism (cDNA-AFLP) technique. Cell of *P. tricornutum* could grow in a wide range of NaCl concentrations from 0.1 to 1.0 M. However cells exhibited a benthic oval form when grown in 0.1 M NaCl whereas in 0.5 and 1.0 M NaCl a floating fuciform was observed. In the presence of low (0.2 M) Cl^-, Na^+ showed mitigation effects against Li^+ and K^+ stresses in a concentration dependent manner. Na^+ also stimulated PSII activity whereas Cl^- was inhibitory to the PSII. The active thylakoid membrane was prepared by freezing in liquid nitrogen and thawing cells grown in 0.5 M NaCl-ASW. *p*BQ-dependent-O_2-evolution rates were measured as the PSII activity. The PSII activity was stimulated by the addition of NaCl, NaF, or Na_2SO_4 whereas there was no stimulation by the addition of NaBr, NaI, Cholin-Cl, or Cholin-SO_4. These results indicate that the PSII in *P. tricornutum* is directly stimulated by Na^+. *P. tricornutum* cells were grown in artificial seawater containing 0.5 M NaCl (0.5 M NaCl-ASW). Cells at mid-logarithmic phase were transferred to 0.1 M NaCl-ASW. A growth lag for 2 days was observed after starting low-salt treatment. Cells were harvested before the low-salt treatment, during 2 days of the early acclimation period, and the late acclimation period to low-salt after 4 days of the treatment. cDNA libraries were constructed from cells of each stage and cDNA-AFLP analysis was carried out using these cDNA libraries. As a result of cDNA-AFLP analysis, 74 low-salt responsive genes were screened and cloned. Of these, 40, 14, and 20 genes were identified as inducible at the early acclimation stage, the late acclimation stage to low salt, and repressive under low-salt condition, respectively. 40% of these 74 genes were found to be diatom-specific genes. Within the low-salt inducible genes, there were 11 transporter genes including Na^+/solute symporter and Na^+/H^+ antiporter, suggesting that marine diatom cells actively maintain Na^+, pH, and ionic homeostasis in response to low salt. Also, there were significant numbers of stress responsible genes. These results suggest that low salt environment is recognized as a stress by marine diatom cells. Finally, localization of intracellular Na^+ and Cl^- by using specific fluorescent indicators, Sodium green and MEQ, showed that these ions are likely to accumulate in the four-layered-membrane system surrounding the chloroplast when cells were grown in the ordinary and low-salt seawater.