Kwansei Gakuin University
Report of Research Outcome

2023/03/13

To President

Department : Science and Technology
Position :Postdoctoral fellow
Name : Hermanus Nawaly

I report the outcome of the research as follows.

Name of the Fund/Program	□Sabbatical leave with grant □Sabbatical leave with no grant □KGU Joint Research □Individual Special Research ☒Postdoctoral fellow ※Please report by designated form as for “International Research Collaboration”.
Research Theme	Molecular research on environmental responses in marine diatoms
Research Site/Venue	Matsuda Laboratory, Graduate School of Science and Technology
Research period	2022/04/01 ～ 2023/03/31 （ 12 month）

◆ **Summary of the research outcome** （approx. 2,500 words）

Please write down the outcomes in detail regarding the research theme above.

In seawater, the concentration of CO2 is very limited. It is between 10 and 25 μM. Under such limited conditions, diatoms have been able to successfully adapt and become one of the primary producers of the ocean, contributing up to 20% of the global primary production. An evolutionary adaptation of aquatic photosynthetic organisms, including diatoms, is the mechanism to take up inorganic carbon, CO_2 and HCO_3^-, from the environment and accumulate it in the chloroplast, known as the CO_2 concentrating mechanism (CCM).

In the marine diatom *Phaeodactylum tricornutum*, solute carrier (SLC) 4-2, a plasma membrane HCO_3^- transporter, was previously identified. Two paralogs, PtSLC4-1 and PtSLC4-4, both localized to the plasma membrane, were discovered and we try to understand their roles in photosynthesis. Their overexpression stimulated the uptake of HCO3, and this uptake was inhibited by the anion channel blocker 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Similar to SLC4-2, PtSLC4-1 specifically required a Na^+ concentration of ~100 mM for its maximal HCO_3^- transport activity. In contrast to PtSLC4-1 and PtSLC4-2, the HCO_3^- transport of PtSLC4-4 was equally dependent on Na^+, K^+ or Li^+, suggesting a broad selectivity for cations. Transcript analysis revealed that PtSLC4-1 was the most abundant HCO_3^- transporter under atmospheric CO_2, whereas PtSLC4-4 showed little transcript induction under atmospheric CO_2, but transient induction to levels comparable to PtSLC4-1 during initial acclimation from high CO_2 (1%) to very low CO_2 (<0.002%). Our results strongly suggest an important role for PtSLC4-1 in HCO_3^- transport, with a relatively minor role for PtSLC4-2, and that PtSLC4-4 functions in a non-selective manner under severe CO_2 limitation when the other SLC4s do not function to support HCO_3^- uptake.

Publication: Nawaly, H., Matsui, H., Tsuji, Y., Iwayama, K., Ohashi, H., Nakajima, K. and Matsuda, Y., 2023. Multiple plasma membrane SLC4s contribute to external HCO_3^- acquisition during CO_2 starvation in the marine diatom *Phaeodactylum tricornutum*. *Journal of Experimental Botany*, *74*(1), pp.296-307.

Carbonic anhydrase (CA) is essential for the operation of CO2-concentrating mechanisms (CCMs) in the vast majority of aquatic photoautotrophs that sustain global primary production. In the genome of Thalassiosira pseudonana, a centric marine diatom, there are four putative gene sequences that encode θ-type CA, a recently identified type of CA in marine diatoms and green algae. T. pseudonana was used to express GFP-fused proteins of four CAs, TpθCA1, TpθCA2, TpθCA3, and TpθCA4, in order to determine their subcellular localization. Consequently, the C-terminal GFP fusion proteins of TpθCA1, TpθCA2, and TpθCA3 were all localized in the chloroplast; TpθCA2 was in the central chloroplast region, and the other two TpθCAs were dispersed throughout the chloroplast. Anti-GFP monoclonal antibody immunogold-labeling transmission electron microscopy was performed on transformants expressing TpθCA1:GFP and TpθCA2:GFP. TpθCA1:GFP was localized in the free stroma, including the pyrenoid periphery. TpθCA2:GFP was clearly located as a lined distribution at the central portion of the pyrenoid structure, which was likely the thylakoid that penetrated the pyrenoid. Given that the TpθCA2 gene encodes the N-terminal thylakoid-targeting domain, this localization was likely the lumen of the pyrenoid-penetrating thylakoid. TpθCA4:GFP, on the other hand, was localized in the cytoplasm. TpθCA2 and TpθCA3 were upregulated in atmospheric CO2 (0.04% CO2, LC), whereas TpθCA1 and TpθCA4 were highly induced under 1% CO2 (HC) conditions, as revealed by transcript analysis. The genome-editing knockout (KO) of TpθCA1 by CRISPR/Cas9 nickase resulted in a silent phenotype in T. pseudonana under LC–HC conditions, which was in striking agreement with the previously reported case of TpθCA3 KO. In contrast, TpθCA2 KO has thus far been unsuccessful, suggesting that TpθCA2 serves a maintenance function. The silent phenotype of KO strains of stromal CAs suggests that TpαCA1, TpθCA1, and TpθCA3 may have functional redundancy, but their distinct transcript regulation in response to CO2 suggests that they may play independent roles.

Publication: Nawaly, H., Tanaka, A., Toyoshima, Y., Tsuji, Y. and Matsuda, Y., 2023. Localization and characterization θ carbonic anhydrases in Thalassiosira pseudonana. *Photosynthesis Research*, pp.1-13.

Deadline : Within two months after finishing the research period.

Sabbatical leave with grant: Submit this report to President with confirmation by the dean of school you belong to.

※Postdoctoral fellow is required to submit this report with confirmation by the dean of graduate school before the end of employment period.

Where to submit : Organization for Research and Development and Outreach (NUC)

◆We put this report on the web of KGU. If there is any problem about it because of difficulties on your research, please let us know.