

Kwansei Gakuin University

Report of Research Outcome

2022/03/16

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	<input type="checkbox"/> Sabbatical leave with grant <input type="checkbox"/> Sabbatical leave with no grant <input type="checkbox"/> KGU Joint Research <input type="checkbox"/> Individual Special Research <input checked="" type="checkbox"/> Postdoctoral fellow ※Please report by designated form as for "International Research Collaboration".
Research Theme	Characterized pyrenoid and CCM factor in diatom
Research Site/Venue	Matsuda Laboratory, Graduate School of Science and Technology
Research period	2021/04/01 ~ 2022/03/31 (12 month)

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

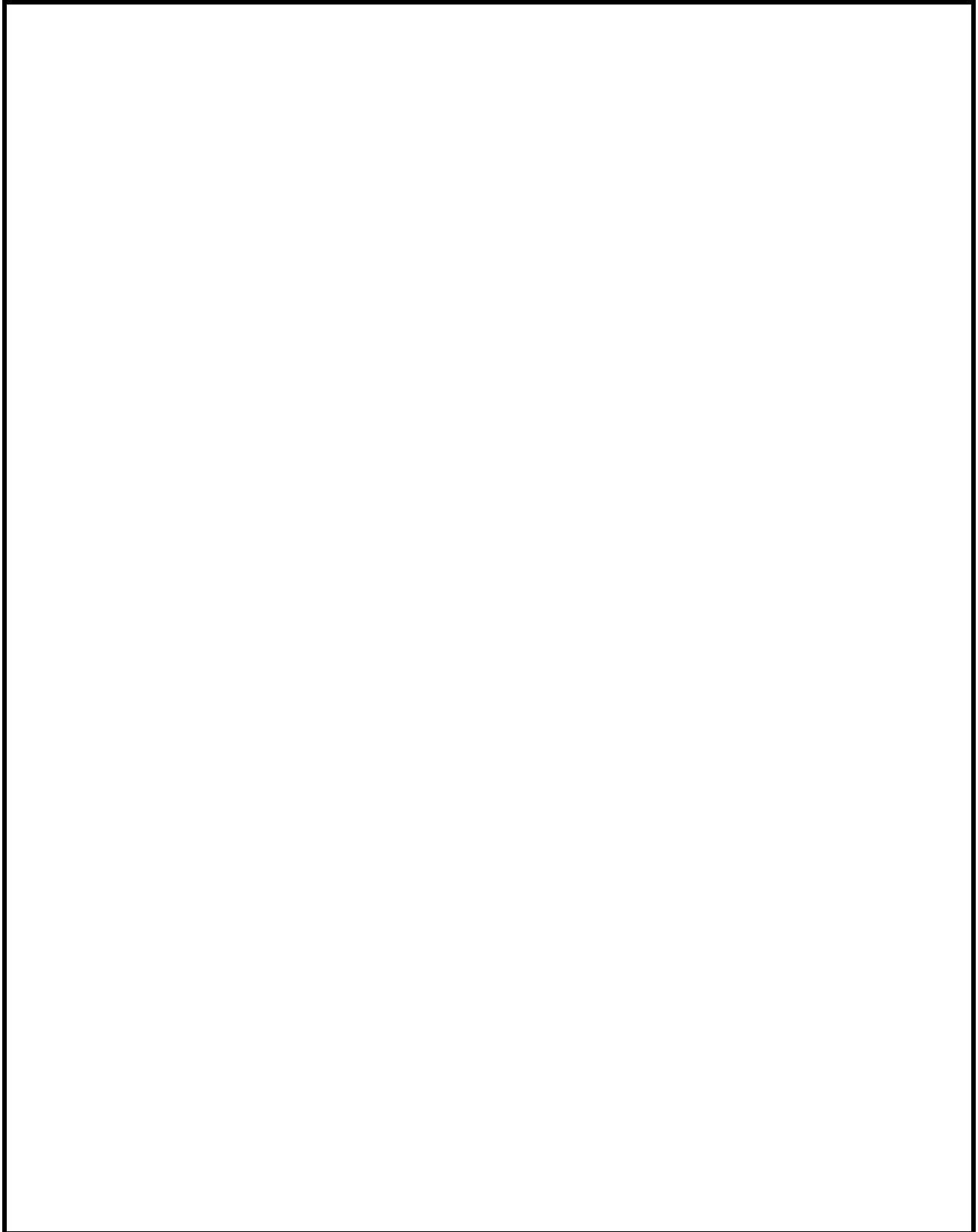
Please write down the outcomes in detail regarding the research theme above.

In seawater, the concentration of CO₂ is very limited in the range of 10 and 25 μM. In such constrained conditions, diatoms could successfully adapt and become one of the ocean's primary producers, contributing to 20% of global primary production. The mechanism to take up inorganic carbon, CO₂, and HCO₃⁻ from the environment and accumulate in the chloroplast, well known as CO₂ concentrating mechanism (CCM), is an evolutionary adaptation from aquatic photosynthesis organisms, including diatoms.

In the diatom CCM model, carbonic anhydrases (CAs) located in various cellular compartments were hypothesized to have a crucial role. CA is an enzyme facilitating equilibrium between CO₂ and HCO₃⁻. θ-type CA is the newest type CA, which was recently identified in the marine diatom,

Phaeodactylum tricornutum. In *Thalassiosira pseudonana*, its orthologous were identified, TpθCA1, TpθCA2, TpθCA3, and TpθCA4. Their localization was confirmed except for TpθCA4. In this time, I focused on TpθCA2, which localized in the lumen of thylakoid-penetrating pyrenoid. Using the knock-down approach by Cas13d, I could obtain four candidates mutants, which showed lower cell density in low CO₂ conditions relative to high CO₂. Further characterization for these four mutants is still required. In addition, localization of TpθCA4 was confirmed in the cytoplasm.

Pyrenoid is a large protein body that widely occurs in the chloroplast of eukaryotic algae. Most ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is sequestered in the pyrenoid of diatom, strongly suggesting the function of this body as a hub for the diatom biophysical CO₂-concentrating mechanism (CCM) and photosynthesis. A proper arrangement of pyrenoidal components is believed to be critical to achieving high [CO₂] at the proximity of RubisCO. However, the molecular bases of the pyrenoid structure are not yet clear. Previous work in our lab identified a new pyrenoid factor. This pyrenoid factor previously identified in *P. tricornutum*. Its orthologous was identified in *T. pseudonana*, and two out of six candidates showed high expression levels in high and low CO₂ conditions. In the current work, we used the knock-out (KO) approach by Cas9 (D10A) to double KO the two target genes. Mutants KO was acquired, mutant 1 (M1) and mutant 2 (M2). M1 showed reducing of specific growth rate compared to wild type (WT) in low and high CO₂. This indicated that pyrenoid factor protein is an important factor for *T. pseudonana*. Further characterization for these mutants is still required.



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.