Kwansei Gakuin University
Report of Research Outcome

2025/03/21

To President

Department : Science and Technology
Position : Postdoctoral fellow
Name : Matthew Brown

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	JST CREST -
Research Site/Venue	Kwansei Gakuin University, Department of Biosciences
Research period	2024/04/01 ～ 2025/03/31 （ 12 month）

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

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

The diatom CO2-concentrating mechanism (CCM) is a system of pumps and enzymes that serve to transport inorganic carbon towards the site of photosynthesis, artificially raising the concentration of CO2 around the pyrenoid (a specialized organelle formed from the carbon-fixing enzyme Rubisco) to increase photosynthetic efficiency. In the pennate diatom *Phaeodactylum tricornutum*, one of the major components of the CCM is the bicarbonate transporting protein Bestrophin. Multiple bestrophin proteins exist in the cell, differing in location. BEST1 is found within the pyrenoid itself and plays an important role in maintaining the CCM.

Recently, a novel protein (J49151) was discovered that appeared to be an interacting partner with BEST1. Bioinformatics analysis showed that it contains a protein domain involved in NADPH binding and thus may play a role in facilitating redox reactions. Analysis of expression of J49151 showed that it is highly expressed under low CO2 conditions, at a similar level to CCM components like BEST1 and carbonic anhydrase. Based on this, we decided to determine if J49151 plays a role in regulating or facilitating CO2 transport.

To do so, knockout strains of J49151 were created using a custom CRISPR/Cas9 system for *P. tricornutum*. Plasmids containing the CRISPR/Cas9 protein were transformed into WT cells using bacterial conjugation. Subsequently, cells were plated onto ½ f/2 containing selection anti-biotic (zeocin). Strains were isolated using multiple rounds of colony PCR using primers for the target gene. Once a prospective monoclonal strain was isolated, genomic DNA was extracted and DNA sequence was examined via Sanger sequencing.

Two strains containing out-of-frame deletions were successfully generated (7.1.2 and 9.40.7.1). To ensure that the detected deletions resulted in the abolishment of gene expression, I performed Western Blotting using an antibody specific to J49151. Cells of WT and mutant strains of *P. tricornutum* were cultured under low and high CO2. Cells were harvested in exponential growth phase, concentrated, and flash frozen via liquid N2 before being stored at -80 C. Proteins were then extracted using temperature-controlled sonication. Protein extract was then separated via gel electrophoresis, blotted onto blotting paper, and the antibody applied. Analysis of the results showed the presence of the protein in WT cells under low CO2 but not under high CO2, with mutant strains showing no expression in either condition.

To determine whether removal of J49151 affected cellular growth rate, WT and mutant strains were cultured under atmospheric CO2 and very low (<0.04%) CO2. No significant differences in growth rate were observed. In response to high light, phytoplankton cells can engage protective mechanisms called NPQ, which can convert excess light energy into heat. The process is measured using a technique called chlorophyll fluorescence. Examination of NPQ in WT and mutant strains showed no significant differences.

My next step will be to determine whether elimination of J49151 has any effect on photosynthesis under low and saturating CO2 conditions. To determine this, I will culture cells under low and very low CO2 conditions, then measure O2 production across a range of inorganic carbon concentrations. In addition, as J49151 is likely involved in redox reactions, I will induce redox stress by growing the cells under high light and in the presence of H2O2. Subsequently, I will examine growth, photosynthesis, and chlorophyll fluorescence under these conditions.

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.