Science and Technology Group
Annual Report FY2023

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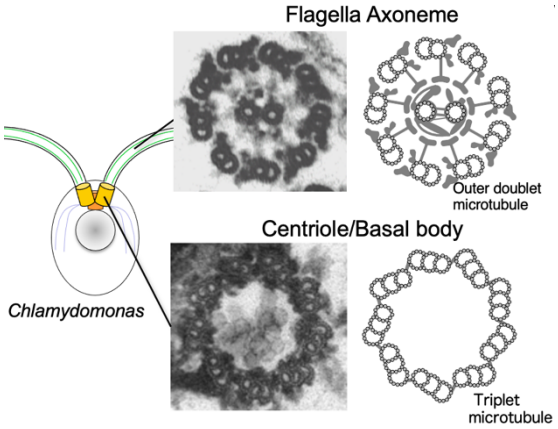
1 Introduction

Centrioles are highly conserved organelles that have important functions in controlling cell division and eukaryotic cilia/flagella assembly. Their structures consist of nine *triplet* microtubules arranged in rotational symmetry. When they function as templates for cilia/flagella assembly, The inner two of the triplets are continuous to become the outer doublets of cilia/flagella. Thus, the ciliary/flagellar characteristic "9+2" pattern is determined by the base structure, centrioles.



Recently, many proteomic studies have identified flagellar and centriole proteins, and super-resolution imaging studies have revealed flagellar and centriole structures precisely. However, it is still unclear how their characteristic structures assemble and how their components function.

To understand them, I have been using mutants of a green alga *Chlamydomonas reinhardtii*, a model organism useful for these studies.

2 Activities and Findings

・ Lab set-ups

In FY2023, lab move to Lab5 and setup in Lab5 was completed.

・ About a novel mutant *bld13*

From the results obtained so far, my collaborators and I have shown that the mutated gene product Bld13p is involved in triplet microtubule assembly and stabilization at the proximal end of the centriole. We have prepared a manuscript and figures about these mutants in FY2023 (Nakazawa et al., in preparation).

・ About a transition zone protein

In FY2023, I analyzed one protein (S6L) localized in the transition zone, the area between the flagellum and centriole. Previously, my collaborators and I have shown that this protein forms a dimer and localizes to the lumenal side of the transition zone (Nakazawa et al., unpublished). To investigate its function in the transition zone, I induced a mutation in the predicted dimerization site and tried to transform the plasmid containing the gene with the mutation into wild-type cells. Eight strains expressing HA-tagged protein with the mutation were obtained by completing the experimental setup, including gene introduction into E. coli, plasmid extraction, sequencing, gene introduction into Chlamydomonas cells, and expression check. I just started phenotypic analysis of the strains and plan to observe their localization precisely.

・ About other mutants

I have several novel mutants with mutations in centriolar microtubule-related proteins. Most of these proteins are predicted to be embedded deep within the centriole structure. In that case, a post-embedding immuno-EM is required to determine their localization. In FY2023, I confirmed the equipment and suitable protocols and prepared reagents for the post-IEM with support from the OIST IMG-section and the RIKEN CSRS Microscopy Facility. Regarding antibodies that I plan to use for it, unfortunately, most centriole antibodies available from companies do not cross-react with Chlamydomonas homologs. Therefore, I outsourced the production of antibodies

specific to the four target Chlamydomonas centriolar proteins. I am currently confirming whether the antisera react with the proteins.

3 Collaborations

- Dr. Masafumi Hirono, Dr. Hiroko Kawai-Toyooka (Hosei University)
- Dr. Ken-ichi Wakabayashi (Tokyo Institute of Technology, Kyoto Sangyo University)
- Dr. Mikito Owa, Dr. Akira Noga (University of Tokyo)
- Dr. Manuel Hilbert, Dr. Michel O. Steinmetz (Paul Scherrer Institute)

4 Publications and other output

Presentation

○KUBOTA N, NOGA A, JI J, NAKAZAWA Y, KAWAI-TOYOOKA H, HIRONO M
"Analyses of a novel *Chlamydomonas* mutant that has a defect in determining the number of centriole microtubules"
The 94th Annual Meeting of the Zoological Society of Japan, Sep. 2023.

Grant

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