Science and Technology Group
Annual Report FY2024

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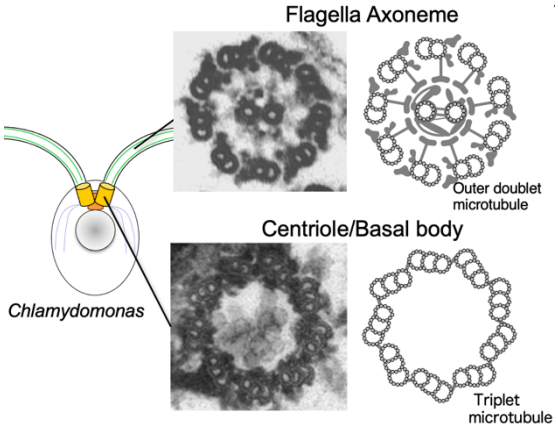
1 Introduction

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

・ About a novel mutant *bld13*

By 2023, I found that the mutated gene product, Bld13p, plays a crucial role in stabilizing centriolar triplet microtubules at their proximal ends. In FY2024, I further refined our manuscript and figures, incorporating additional experiments to enhance our findings on these mutants (Nakazawa et al., 2025 preprint; presentation #1).

・ About a transition zone protein

In FY2024, to investigate the function of the transition zone protein S6L (Nakazawa et al., unpublished), I produced wild-type strains expressing HA-tagged wild-type S6L and mutated S6L predicted to be incapable of self-assembly and function within the cell. Initial phenotypic analysis of these strains revealed no discernible differences. Consequently, I obtained available mutant candidates from *Chlamydomonas* Resource Center. Further analysis identified a DNA fragment insertion in the 5' UTR of one strain, and premature stop codons near the N-terminus part in two other strains due to the insertion. I plan to proceed to characterize the phenotypes of these mutant strains.

・ About other mutants

In FY 2024, I tried to generate specific antibodies against several centriole proteins. Unfortunately, most of these were found to be non-specific to the target antigens in cells. Additionally, we obtained several new mutant candidates for proteins implicated in the assembly and/or maintenance of centriolar microtubules. After confirming the mutations in these candidates, I successfully created several multiple mutant strains through crosses between existing and newly generated mutants. I also plan to proceed to characterize the phenotypes of these mutant strains.

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3 Collaborations

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

4 Publications and other output

Paper

1. **Nakazawa Y,** Horii M, Noga A, Yoshikazu Koike, Hiroko Kawai-Toyooka, Hideo Dohra, Katsushi Yamaguchi, Shuji Shigenobu, Wakabayashi K, and Hirono M. (2025)
"*Chlamydomonas* γ-tubulin mutations reveal a critical role of γ-TuRC in maintaining the stability of centriolar microtubules."
bioRxiv, preprint, DOI: 10.1101/2025.03.18.643570

Presentation

1. ○**Nakazawa Y,** Horii M, Noga A, Wakabayashi K, and Hirono M.
"Dominant negative mutations in γ-tubulin cause partial loss of protofilaments in centriole triplet microtubules."
International Union for Pure and Applied Biophysics (IUPAB) (June, 2024)

2. ○Kubota N, Midorikawa R, JI J, Noga A, **Nakazawa Y**, Kawai-Toyooka H, and Hirono M.
"Retention mechanism of the cartwheel for establishing the nine-fold symmetry of the centriole"
The 95th Annual meeting of the Zoological Society of Japan （Sep, 2024）