Science and Technology Group
Annual Report FY2025

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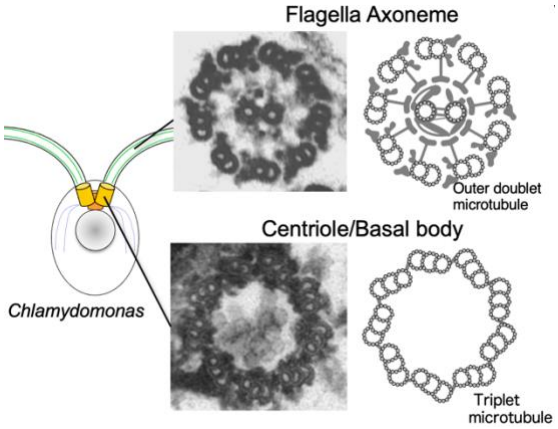
1 Introduction

Centrioles are highly conserved organelles that have essential functions in controlling cell division and eukaryotic cilia/flagella assembly. Their structures consist of nine *triplet* microtubules (MTs) arranged with rotational symmetry. When they function as templates for cilia/flagella assembly, the inner two MTs of each triplet extend to form 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 well-established model organism for studying centriole and cilia biology.

2 Activities and Findings

・ About a novel mutant *bld13*

By 2023, I identified a previously uncharacterized functional role for the mutated gene product Bld13p in maintaining the structural stability of centriolar triplet MTs at their proximal ends. This insight was uniquely enabled by genetic analysis of a viable *Chlamydomonas bld13* mutants with subtle phenotypes. The manuscript was submitted in FY2024 and underwent external peer review. In FY2025, I incorporated additional experimental analyses and refined the presentation of the data, enabling a more detailed mechanistic interpretation of the study.

・ About a Bld13p related proteins

Building on these findings, in FY2025 I initiated a new line of investigation to examine whether Bld13p functions as part of a larger protein complex involved in triplet MT stabilization. To address this question, I obtained one previously characterized mutant (div49) and two additional candidate mutants affecting putative components of the complex from the *Chlamydomonas* Resource Center. I confirmed the insertion of DNA fragments within the coding regions of the target genes and completed initial phenotypic characterization, establishing a foundation for further mechanistic analysis.

・ About other mutants

In parallel with the studies described above, I continued exploratory investigations of other centriole-associated proteins. During this period, I generated and validated multiple new mutant strains, including higher-order combinations produced through genetic crosses. These resources provide a broader genetic framework for future analyses aimed at dissecting additional mechanisms underlying centriole assembly and triplet MT organization.

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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)
・Dr. Kazumasa Z. Tanaka (OIST)

4 Publications and other output

Presentation

*Kubota N, Midorikawa R, Ji J, Noga A, **Nakazawa Y,** Kawai-Toyooka Hiroko, Hirono M.
“Function of centriolar protein Rtn1 in centriole assembly”
The 58th Annual Meeting of the Japan Society of Protistology (Sep. 2025)