Science and Technology Group
Annual Report FY2022

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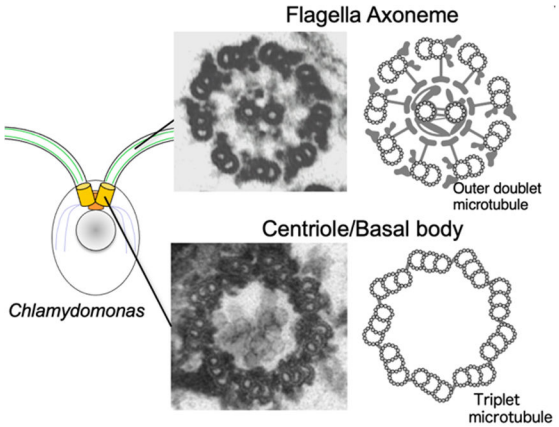
1 Introduction



Eukaryotic cilia/flagella are highly conserved organelles that have various functions, including motility and cell signaling. They have an internal cytoskeletal structure called an axoneme, which contains nine doublet microtubules surrounding two central microtubules and their associated structures. This characteristic “9+2” pattern is determined by the base structure, centriole/basal body.
Centrioles/basal bodies are also highly conserved organelles that have two main functions: first as templates for cilia/flagella assembly, and second as a core of the centrosome. Their structures consist of with nine triplet microtubules arranged in rotational symmetry. The inner two of the triplets are continuous to become the axonemal outer doublets.

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.

For understanding 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

Since arriving at OIST in 2020, lab-setup has been almost completed through FY2022. And I have also begun preparations for lab move to Lab5 in 2023.

・ About a novel mutant *bld13*

I have increased the observation number to get fine cross-sectional images of the centrioles in *bld13-1* and *bld13-2* cells and found that 1) the defects were concentrated at specific areas in the A- and C-tubules of the triplet microtubules, and 2) the defects were more severe in the proximal part than in the distal part. Including these data, I am preparing a paper about the function of the mutated gene product (Bld13p) on triplet microtubule assembly/stabilization.

・ About a suppressor mutant of an allele of *bld10*

In FY2022, I carefully observed ultra-thin sections of the suppressed mutant cells by TEM and found that this double mutant, named *supbld10-bld10-2*, has defects in centriole orientation and position. To easily understand these defects, I started my first trial of 3D FIB-SEM imaging using whole cell samples.

・ About other mutants

To count the triplet microtubule number from mother-daughter centriole pairs in the mutant *bld12*, which has defects in the 9-fold symmetry of the centriole, I tried 3D FIB-SEM imaging using isolated cytoskeletal structures. I have been analyzing the FIB-SEM images to re-construct the three-dimensional structure of the control (wild type) centrioles. So far, the current methodology still requires improvement in the z-dimensional resolution.

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To investigate the relationship between each centriole protein involved in triplet microtubule assembly, I produced some triple/double mutants of them by genetic crossing.

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.
“Phenotypic analyses of a novel *Chlamydomonas* mutant that have a defect in the 9-fold symmetry of the centriole.” The 55th Annual meeting of Japan Society of Protistology, Sep. 2022.

Grant

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