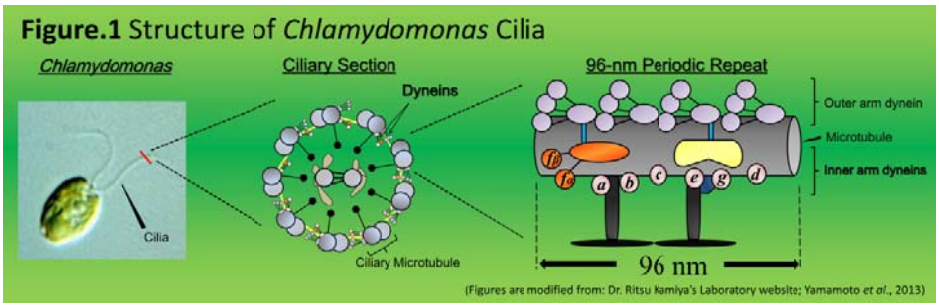
Japanese-Swiss Science and Technology Programme

Young Researchers Exchange Programme between Japan and Switzerland
Scientific & Financial Report

Project No.	EG10-2014
Project title	Searching for the Factor(s) that Determine the Periodic 960nm Repeat of Ciliary/Flagellar Inner Arm Dyneins
Fellowship period	14.10.2014 – 13.01.2015
Swiss Principal Investigator / Host	Dr. Takashi Ishikawa (Paul Scherrer Institute)
Japanese Fellow	Dr. Ryosuke Yamamoto (Univ. Tsukuba)

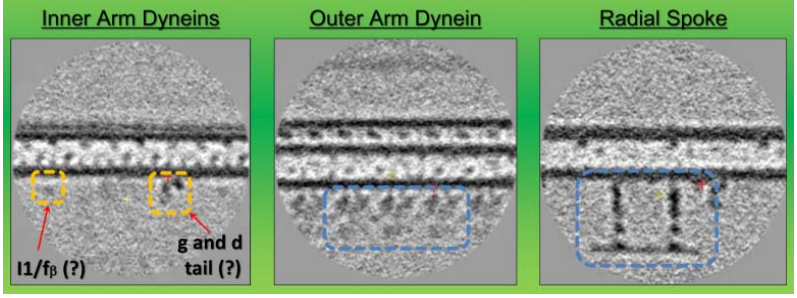
1. Summary of scientific achievements (max ½ page; incl. pictures, suitable for publication)

Dr. Ryosuke Yamamoto carried out three-dimensional structural analysis by cryo-electron tomography of flagella (Figure 1) using a mutant strain of green algae *Chlamydomonas*, pf23, which he has been biochemically and physiologically characterizing in his laboratory in Japan. Dr. Yamamoto brought the cells from Japan, culture and prepare samples for electron tomography studies in PSI. The members of the Ishikawa group made a cryo-preparation and data acquisition of electron tomography. Dr.



Yamamoto learned 3D image analysis in the Ishikawa group to reconstruct 3D structure of pf23 mutant. He succeeded 3D reconstruction (Fig.2) and analyzed internal conformation of component molecules of flagella. He successfully visualized the whole regulatory complex, radial spokes and outer arm dyneins, known as an accelerator of flagellar motility. Some parts of inner arm dyneins, which determines bending form of flagellar motion, are visualized, but still to be further analyzed. The collaboration will be continued to finalize the analysis of inner dynein arms.

Figure.2 Averaged Images of *pf23* Cilia



2. Scientific project achievements (max 2 pages)

Dr. Yamamoto stayed in our laboratory at Paul Scherrer Institute (PSI) from October 14th, 2014 to January 13th, 2015. His visit to our laboratory was timely and of importance to us since we are interested in the arrangement of inner-arm dyneins in cilia, and would like to determine the precise and detailed location of various ciliary proteins including ciliary dyneins in high resolution. Dr. Yamamoto aimed to solve the ciliary structure of the inner-arm-dynein-deficient mutant called *pf23*, to visualize which inner-arm dynein subspecies are missing in *pf23*, and also to reveal the docking mechanism(s) in which inner-arm dyneins are attached to the ciliary microtubule.

To this end, first Dr. Yamamoto and we tried to isolate cilia from the *pf23* mutant. The *pf23* mutant either did not grow cilia or at best grew extremely short cilia. To solve this problem, we changed the growing media from rich TAP (Tris-Acetate-Phosphate) to low-nutrient SG (Sager and Granick) media. This change resulted in ciliary growth and greatly improved the final amount of isolated *pf23* cilia required to prepare samples for the cryo-electron microscopic observation.

Second, using our well established procedures and cryo-electron microscopy (JEOL JEM-2200FS) we acquired about 60 images per sample of *pf23* cilia ranging from -60° to +60°, 2° difference between adjacent slices. It was difficult to obtain good images of *pf23* cilia compared to wild-type cilia since the *pf23* cilia are easily compressed during the procedure used. However, during the period of Dr. Yamamoto's visit, we were successful in acquiring slice images from 9 cilia.

Third, Dr. Yamamoto learned to use the Linux-based program (called "ETomo") for tomography reconstruction, and our custom-designed Linux-based program to perform sub-tomogram averaging calculations. The calculations required to reconstruct the 3-dimentional tomography were performed by the PSI supercomputer. Most of the calculation programs are not based on Windows or Macintosh. Therefore, we also trained Dr. Yamamoto in various Linux commands to run and complete jobs in the PSI supercomputer. This was Dr. Yamamoto's first time to use and apply the Linux system to his biological experiments: we believe this training will greatly benefit Dr. Yamamoto for his future structural biological studies.

Finally, as an initial effort, Dr. Yamamoto and we completed sub-tomogram averaging calculation using 2 tomogram data sets from *pf23* cilia. The work went quite well, and we found, unexpectedly, that most of the

densities of inner-arm dyneins are missing in *pf23* cilia. Remaining structures appear to include inner-arm dynein f/I1β located at the proximal end of the ciliary 96-nm repeat, and the base of inner-arm dynein *g* and *d* located close to the ciliary N-DRC (Nexin-Dynein Regulatory Complex). These results suggest that many inner-arm-dynein subspecies are either missing or very flexible and lost during averaging. The inner-arm-dynein defects in *pf23* appear more profound than previously expected. Notably, in contrast to inner-arm dyneins, the densities of radial spokes and outer-arm dyneins appear normal in *pf23* cilia.

We will complete this project by collecting more *pf23* slice images in PSI. Dr. Yamamoto will continue the analysis using PSI supercomputer in Japan. One problem we continue to face is that *pf23* cilia are very easy to be compressed during preparation. Therefore, we must eliminate some tomographies from sub-tomogram averaging calculation since the preservation quality of cilia is not sufficient. We anticipate that Dr. Yamamoto and we will complete sub-tomogram averaging and 3-dimentional reconstructions using 5 to 6 high-quality *pf23* data sets. These calculations will be completed once Dr. Yamamoto sets the Linux environment in Japan which is able to access PSI supercomputer. We anticipate that we will solve the *pf23* ciliary structure in high resolution with a high signal/noise ratio.

3. Partnership

- Is the exchange based on an existing partnership between the Japanese and Swiss research groups?

  This exchange triggers a new partnership between the Ishikawa group in Switzerland and Dr. Yamamoto in Japan.

- Did the cooperation between fellow and host go well?
  Yes, it went very well.

- Will there be a continued collaboration after the return home of the Japanese fellow?
  Yes, we will continue collaboration.

4. Please describe how the Swiss host and Japanese fellow have benefited from this exchange

Dr. Yamamoto learned and got access to state-of-art cryo-EM infrastructure in PSI and our know-how of cryo-electron tomography. This allows him to analyze 3D structure of his mutant, which has been his research target for biochemical analysis, but has never been analyzed at the ultrastructure level. The Ishikawa group obtained valuable technique of endogenous gene expression in Chlamydomonas, one of the most popular model organisms for flagella/cilia studies.

5. Outlook

As stated above, Dr. Yamamoto and we will continue doing sub-tomogram averaging calculation to obtain the high-resolution structure of *pf23* cilia. With high resolution and high signal/noise ratio, we should be able to make the density map of ciliary 96-nm repeat and definitively determine which inner-arm dyneins are missing/flexible in the *pf23* cilia. These results will permit iso-surface rendering images of *pf23* ciliary structure with the publication quality. We also anticipate that we should define new details of the tail domains of each inner-arm-dynein subspecies. Such

data will provide important clues as to how various inner-arm dyneins are precisely located in the ciliary 96-nm repeat. In addition to these structural analyses, in collaboration with other labs in the US, Dr. Yamamoto will complete analysis of the *pf23* mutant using biochemistry and genetics, and he will write a paper that combines these new structural studies with the biochemical and genetic studies of the *Chlamydomonas pf23* mutant and function of the PF23 protein.

6. List of Publications

Yamamoto, R., Ishikawa, T. “Structural and biochemical characterization of motility mutant of Chlamydomonas p23” manuscript in preparation

7. Miscellaneous

- Do you expect any patents coming out of this project? No
- Do the results of this project have commercial potential? Do you think there could be an industrial partner involved in this project in the next phase? No

8. Suggestions for the next phase of the exchange programme

It would be nice if the money is already transferred before the trip. This time Dr. Yamamoto had to pay his flight, make a money exchange from his Japanese account. If the fellowship was already deposited in the account at his arrival, it would be convenient. (the problem might be located in the system to create account in PSI, though)

9. Financial report

- Please include a copy of the account report from your financial department concerning this project. Original receipts need not accompany this financial report. However, please keep the original receipts for 5 years after the project has finished.

Income:

7369.75CHF (Transferred to PSI)

Outcome:

1500CHF (paid to PSI Guesthouse for 91 nights stay on 19.01.2015 and 18.02.2015)

2140CHF (Transferred to Dr. Yamamoto for his daily expenses and domestic trip by bank transfer on 04.03.2015)

3729.75CHF (paid to Dr. Yamamoto to cover his flight and daily expense on 15.12.2014)

Sum: 7369.75CHF

Copy of the account is attached.

10. Appendix (please attach any additional documentation such as pictures or links to media coverage)