Title of the project: Cell cycle of unicellular N_2 fixing cyanobacteria *Crocosphaera watsonii* and *Cyanothece* sp. ATCC51142

Supervisor: Takako Masuda

For how many student/s:1-2

Description of the project:

Nitrogen fixation is the biochemical conversion of the inert, atmospherically abundant nitrogen gas (N_2) into biologically available ammonium (NH_4^+) by a few microbial species (known as diazotrophs) including some cyanobacteria. This process is globally important, because it provides essential nitrogen (N) to ecosystems, where bio-productivity is primarily controlled by N availability[1]. The appearance of photosynthesis and consequent high levels of ambient O_2, resulted in a paradoxical situation for anaerobic N_2 fixation (for which O_2 is highly toxic), where one critical biological process to sustain growth was inhibited by another. Therefore continuing N_2 fixation-while also relying on oxygenic photosynthesis to provide energy and organic carbon (C) to sustain metabolism and growth- became the ultimate challenge. Cyanobacteria managed to circumvent the O_2 problem, through a series of biochemical and/or structural adaptations. Some species of unicellular diazotrophs like *Crocosphaea watsonii* and *Cyanothece* spp. confine N_2 fixation to night-time, when photosynthesis is "turned off"[2].

Recently our group found out the inter-cellular variability in N_2 fixation activity in both two model-unicellular diazotrophs *Crocosphaera watsonii* and *Cyanothece* sp. ATCC51142 – some cells actively fix N_2, some cells moderately fix N_2, some cells do not fix N_2 at all. One of the proposed hypotheses of the cause of variability in such metabolisms is "combination of circadian rhythm in cellular metabolisms and cell cycle" [3,4]. Therefore, we would like to understand the detail cell cycle in both *Crocosphaera watsonii* and *Cyanothece* sp. ATCC51142 under different growth condition, and evaluate the relationship between variability in metabolisms and cell cycle.

We aim to analyse following parameters

(1) Visualization of DNA content and topology by fluorescence probe and flow cytometry
(2) Visualization of nitrogenase enzyme using antibody
(3) Cell density and cell size using coulter counter
(4) Shape of the cell and frequency of cell division by microscope

Requirements:

- Good practice with teamwork
- Enjoy co-learning processes
- Communication in English

[1]Karl et al., 2002. Biogeochemistry 57: 47-98. The nitrogen cycle at regional to global scales.

[2]Zehr et al., 2001. Nature 412: 635-638. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean.

[3]Levine et al., 2013. Science 342: 1193-1200. Functional roles of pulsing in genetic circuits.

[4] Caudron et al. 2013. Cell 155: 1244–1257. A super assembly of Whi3 encodes memory of deceptive encounters by single cells during yeast courtship.