Internship proposal

Host laboratory: Structure Biology Research Center (SBRC) – Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK)

Supervisor: Professor Toshiya Senda (toshiya.senda@kek.jp)

Internship project: Structural biology of infection and carcinogenesis by *Helicobacter Pylori*.

Research Term: Sep. to Dec., 2014

Helicobacter pylori is a bacterium that is found in the stomach of more than half of the world's population. *H. pylori* of *cagA*-positive strains are associated with atrophic gastritis and peptic ulcers, and those chronically infected by these strains are considered to be at high risk of gastric cancer. Oncoprotein CagA is encoded in *cag* pathogenicity islands in the bacterial genome. Upon infection of gastric epithelial cells, CagA is injected into the cell *via* the type four-secretion system, encoded in the same genetic island. CagA, then, localizes to the inner surface of the plasma membrane and promiscuously interacts with human signalling protein molecules, thereby disrupting signal transduction. As a consequence, a morphological change in the infected cells, known as "hummingbird phenotype", is induced.

Structurally CagA can be divided into two regions: an N-terminal structured region, and a C-terminal intrinsically disordered region. The C-terminus contains sites that interact with human signalling molecules. Recently, we determined the crystal structure of the N-terminal region and elucidated the interaction between CagA and the inner plasma membrane. In addition, biochemical analyses of the C-terminal region enabled us to propose a model, where C-terminal helices in the N-terminal region (NBS; N-terminal binding site) intramolecularly interact with residues in the C-terminal region (CBS; C-terminal binding site). This interaction may stabilize the disordered C-terminal portion and induce secondary structure formation, perhaps facilitating the interaction with target proteins.

The objectives of this project are to identify the interactions between CagA and human proteins, and also to unveil the molecular mechanisms of CagA-related stomach cancer. This internship will provide students with the opportunity to work on cutting-edge research alongside experienced scientists. We have state-of-the-art facilities and regular access to Photon Factory (PF), an X-ray source from synchrotron radiation. Experimental protocols include gene cloning, protein expression and purification, biochemical analyses, and crystal screening *via* our high-throughput screening system. If protein crystals are obtained, diffraction experiments will be performed in our synchrotron facility (PF and PF-AR).

Reference: Hayashi, T. *et al.,* (2012). *Cell Host Microbe.* **12**, 20-33.