Electron Microscopic Single Particle Analysis of the Clamp Loading Complex from *Pyrococcus furiosus*

Tomoko Miyata[a], Hirofumi Suzuki[a], Takuji Oyama[a], Kouta Mayanagi[a], Yoshizumi Ishino[b], Kosuke Morikawa[a], [a]*Biomolecular Engineering Reserch Institue.* [b]*Osaka, Japan. Kyushu Univ. Kyushu, Japan.* E-mail: miyata@beri.or.jp

Ring-shaped sliding clamps and clamp loader ATPases are essential factors for rapid and accurate DNA replication. The clamp ring is once opened and resealed at the primer-template junctions by ATP-fueled clamp loader function. Processivity of DNA polymerase is conferred by attachment to the clamp loaded onto DNA. In eukarya and archaea, the hetero-pentemeric replication factor C (RFC) and the proliferating cell nuclear antigen (PCNA) trimer play crucial roles as the clamp loader and the sliding clamp, respectively [1]. Here we report an EM structure of an archaeal RFC–PCNA–DNA complex at 12 Å resolution. This complex exhibits excellent fitting of each atomic structure of RFC, PCNA, and a primed DNA with the convincing positions of 3' and 5' termini into the map. The PCNA ring is opened by extensive interactions with RFC, with the distorted structural view of a washer-like conformation. The RFC–PCNA contact mode is distinct from that in the yeast RFC–PCNA crystal structure [2]. Thus, the complex appears to represent a scene, where the PCNA ring is kept open before ATP hydrolysis by RFC.

[1] Waga S., Stillman B., *Annu. Rev. Biochem.*, 1998, **67**, 721. [2] Bowman G.D., O'Donnell M., Kuriyan J., *Nature*, 2004, **429**, 724-730.

Keywords: DNA replication, ATPase, electron microscopy