Structural View of the Clamp-loading Mechanism onto DNA

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DNA replication is a highly coordinated process, which involves numerous proteins in nuclei. To promise the integrated system, proteins involved in this event constitute several kinds of molecular machinery. Archaeal systems generally exhibit attractive properties to study DNA metabolism; Their DNA binding proteins are very similar to those from eukarya both functionally and structurally, irrespective of their morphological difference from eukaryotic ones, and hence they are good model systems for understanding eukaryotic DNA processing. We have been working on several DNA processing proteins from a hyperthermophilic archaeon, *Pyrococcus furiosus*.

Replicative DNA polymerase requires two essential protein factors, a sliding clamp and a clamp loader, for rapid and accurate DNA duplication. In eukarya and archaea, a homo-trimeric proliferating cell nuclear antigen (PCNA) and a hetero-pentameric replication factor C (RFC) function as the clamp and the clamp loader, respectively. The ATP-dependent clamp-loading mechanism is particularly intriguing, because it requires opening and resealing of the PCNA ring. We have determined the three-dimensional structure of an archaeal RFC–PCNA–DNA clamp-loading complex by electron microscopy-single particle reconstruction. Importantly, the structure of the complex presents the first direct view of a washer-like open conformation of the PCNA ring in contact with RFC. In combination with the two X-ray structural data reported previously, our EM model implies an intriguing clamp loading mechanism.

Keywords: DNA replication, clamp-loading complex, single-particle reconstruction