Poster Presentation

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Structural and Functional Analysis of the interaction between PCNA and ATAD5

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Proliferating cell nuclear antigen (PCNA) is a DNA sliding clamp that coordinates DNA replication and repair. PCNA is loaded on and unloaded off of DNA during replication. It is loaded onto DNA by the replication factor C (RFC) complex. The mechanism of PCNA unloading is not fully understood however. ATPase family AAA domain containing protein 5 (ATAD5) interacts with subunits of RFC to form a RFC-like complex that functions in unloading PCNA. Reduced expression of ATAD5 extends the life of replication factories by retaining PCNA and other repliosome proteins on chromatin. ATAD5 also plays a role in maintaining genomic stability, as mutations to ATAD5 cause genomic instability in mice and human cells. The region of ATAD5 that interacts with PCNA had yet to be determined. Proteins interacting with PCNA usually have a PCNA-interaction protein (PIP) motif termed the PIP-box. Through sequence analysis, we identified a putative PIP-box in ATAD5. Using GST pull-down experiments, we found that the putative PIP-box bound to PCNA. Mutation of the key residues in the PIP-box abolished the binding. The interaction was also confirmed by isothermal titration calorimetry (ITC) analysis of PCNA and a synthetic peptide containing the PIP-box. The binding constant (Kd) of the PCNA-peptide interaction is 6.2µM, determined by the ITC assays. To further characterize the interaction, we co-crystallized PCNA and the ATAD5 peptide. The complex crystals diffracted to a resolution of 3.5Å. The crystals belong to the space group H3, with unit-cell parameters a = 83.6Å and c = 211.4Å. Our molecular replacement solution indicates that the peptide binds on PCNA at the conserved PIP-box binding pocket. Taken together, our work demonstrates that ATAD5 does contain a PIP motif that directly interacts with PCNA. The structure-function studies on the interaction will provide insights into the molecular mechanism of PCNA unloading and the role of ATAD5 in DNA replication and repair.

Keywords: PCNA, DNA replication and repair