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Ph.D. THESIS DEFENSE

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"Structural mechanisms of the sliding clamp and sliding clamp loader: insights into disease and function"

Chromosomal replication is an essential process in all life. This dissertation highlights regulatory roles for two critical protein complexes at the heart of the replication fork: 1) the sliding clamp, the major polymerase processivity factor, and 2) the sliding clamp loader, a spiral-shaped AAA+ ATPase, which loads the clamp onto DNA.

The clamp is a promiscuous binding protein that interacts with at least 100 binding partners to orchestrate many processes on DNA, but spatiotemporal regulation of these binding interactions is unknown. Remarkably, a recent disease-causing mutant of the sliding clamp showed specific defects in DNA repair pathways. We aimed to use this mutant as a tool to understand the binding specificity of clamp interactions, and investigate the disease further. We solved three structures of the mutant, and biochemically showed perturbation of partner-binding for some, but not all, ligands. Using a fission yeast model, we showed that mutant cells are sensitive to select DNA damaging agents. These data revealed significant flexibility within the binding site, which likely regulates partner binding.

Before the clamp can act on DNA, the sliding clamp loader places the clamp onto DNA at primer-template (p/t) junctions. The clamp loader reaction couples p/t binding and subsequent ATP hydrolysis to clamp closure. Here we show that composition (RNA vs. DNA) of the primer strand affects clamp loader binding, and that the order of ATP hydrolysis around the spiral is likely sequential. These studies highlight additional details into the clamp loader mechanism, which further elucidate general mechanisms of AAA+ machinery.

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