

Bankhead-Coley Cancer Research Program

Bloom, Linda

*Biochemistry and Molecular Biology
University of Florida*

*2008 Program
Bridge (1-year project)*

Project Title: Dynamic Eukaryotic Replication Machines

Project Summary: The genome is replicated prior to every cell division. DNA damage encountered during replication can cause point mutations and genetic rearrangements both of which can lead to cell transformation and cancer. Cells have a surveillance mechanism to monitor levels of DNA damage during replication and halt the process when damage is severe. One component of this checkpoint system is a complex of five proteins, four of which are also found in the clamp loader, a component of the replisome required for synthesizing DNA. The mechanism of action of the replication clamp loader is reasonably well established, but that of the checkpoint complex is less understood. Studies show this single subunit difference alters the substrate specificity of the checkpoint complex. The checkpoint complex interacts with a checkpoint clamp, the "9-1-1 complex," and lacks the specificity for DNA with double-stranded/single-stranded junctions containing 3' recessed ends that the replication clamp loader has. The major goal of this grant is to compare the biochemical activities of the replication and checkpoint complexes to determine how substitution of a single subunit can alter the function. Specifically, our aims are to compare 1) the DNA-binding activities and 2) the clamp-binding activities of the replication and checkpoint clamp loaders. Our approach is to use fluorescence-based assays with purified proteins to measure these dynamic interactions directly in solution and in real time. One hypothesis is that the checkpoint complex functions as a clamp unloader to help stop replication. Many types of cancer and syndromes predisposing individuals to cancer result directly from defects in cell cycle checkpoint responses. The goal of this research is to increase our understanding of the biochemical basis for a checkpoint response that functions during DNA replication and contribute to the knowledge base required to develop effective therapeutic strategies against cancer.