“The role of POL30 (PCNA) in coordinating the association of Chromatin Assembly Factor-1 (CAF-1) and Rrm3p with the replication forks”

Epigenetics studies the heritable transmission of the state of chromatin and the associated epigenetic marks. This process is orchestrated by the Proliferating Cell Nuclear Antigen (PCNA) and several histone chaperones (CAF-1, ASF1, FACT, Rtt106) at the advancing replication fork. Previous studies in our lab has indicated that paused forks could be major sites of epigenetic conversions. It has been shown that certain mutations in PCNA preclude its binding to CAF-1 and reduce gene silencing. It is also known that the DNA helicase Rrm3p is recruited at paused replication forks via PCNA, but it is not known if the PCNA mutations affect the Rrm3p-PCNA interaction. I propose to investigate the interaction between Rrm3p and the PCNA mutants defective in gene silencing by yeast two-hybrid assay and co-immunoprecipitation. Failure of these PCNA mutants to bind Rrm3p will indicate that PCNA-mediated loss of gene silencing cannot be attributed to CAF-1 alone. I will also analyze epistatic interactions of Rrm3p with these PCNA mutants. If the association of Rrm3p with PCNA is independent of the mutations, I would expect additional effects of the destruction of RRM3 on gene silencing. Finally, I will develop a Chromatin immunoprecipitation (ChIP) assay to investigate the composition of paused replication forks. Based on earlier genetic evidence, I expect that both Rrm3p and CAF-1 are retained at pause sites. The study will shed light on the composition of paused forks and provide mechanistic insights into the protein-protein interactions and epigenetic conversions.