"The roles of Dbf4-dependent kinase and Chromatin Assembly Factor I in epigenetic gene silencing in *Saccharomyces cerevisiae*

Dbf4-dependent kinase (DDK) is a conserved kinase with an essential role in the initiation of DNA replication. DDK also phosphorylates non-essential substrates, but the significance of these functions remains enigmatic. *In vitro*, DDK targets Chromatin Assembly Factor I (CAF1), a histone chaperone that reassembles nucleosomes behind replication forks. This process is critical for the maintenance and transmission of chromatin structure, and thereby chromatin-mediated gene expression, across generations. Phosphorylation of CAF1 by Cyclin-Dependent Kinase (CDK), but not DDK, is required for loading CAF1 to chromatin, suggesting a role of DDK in post-initiation events. The mutation of a putative DDK phosphorylation site, in conjunction with a CDK target site, leads to loss of heterochromatin-mediated gene silencing at several loci. This implies that DDK-directed CAF1 phosphorylation is crucial to CAF1 activity. Separate lines of evidence show that DDK and CAF1 are present at paused replication forks. Thus, I hypothesize that DDK targets CAF1 and modulates its activity at paused replication forks. My goal is to demonstrate that DDK phosphorylates CAF1 *in vivo* and establish a connection between the activities of DDK and CAF1 in the model organism *Saccharomyces cerevisiae*. To accomplish this, I will mutate DDK target sites on CAF1 and analyze the properties of these mutants in strains with wildtype or defective DDK, paying specific attention to loss of gene silencing at two typically silenced loci. The proposed study will provide mechanistic insight into the roles of CAF1 and DDK in replication-mediated chromatin assembly and explicate details surrounding this highly conserved process.