Saccharomyces Cerevisiae Cdc7 Homology in Drosophila Melanogaster

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Saccharomyces cerevisiae Dbf4(Dumbbell former 4) and Cdc7(Cell Division Cycle 7) form a complex that phosphorylates Mcm2 (Minichromosome maintenance 2) to initiate DNA replication. Cdc7 is a target for cancer research because there is a Cdc7 ortholog in humans that is necessary for DNA replication and cell survival. Our goal is to characterise a putative Cdc7 homolog in Drosophila melanogaster (dCdc7). We have previously shown that expression of the known Drosophila Dbf4 ortholog, Chiffon, and dCdc7 can rescue yeast cells deficient in active Cdc7. Our hypothesis is that the dCdc7 is activated by Chiffon to phosphorylate MCM2. To test this hypothesis, we will determine if Chiffon interacts with dCdc7 and if Chiffon triggers kinase activity of dCdc7. To do this we purified dCdc7, a putative kinase dead substitution mutant of dCdc7, Chiffon, and Chiffon truncations that are predicted to contain the interacting domains. To produce these proteins we performed a combination of subcloning, SLIC(sequence and ligation independent cloning), and MultiBac Baculovirus techniques. SLIC is a recently developed method of cloning that uses single strand sequence complements instead of ligation to combine vector and insert. This allows us to clone any insert without regard to enzyme restriction sites within the insert's DNA sequence. MultiBac is a system that allows us to infect insect cells with a Baculovirus to coexpress multiple proteins. We purified our proteins through affinity chromatography using various resins that bind to tags on the proteins. These proteins will then be used for protein binding pulldowns and kinase activity assays to determine if Chiffon and dCdc7 have a physical interaction, and if dCdc7 is a kinase. The results from the protein binding pulldown and kinase assay are pending. The results from these experiments will show us whether or not this protein is the Cdc7 ortholog in Drosophila.