

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

**Effects of checkpoint mutation on DNA replication and re-replication within fission yeast**

By

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Though much is known about the process of replication, little is known about origin selection in eukaryotic cells. Previous studies characterizing origin selection have been limited to techniques which allow characterization of one origin per experiment. Here we report the use of new microarray technology available for the simple eukaryotic model organism, *Schizosaccharomyces pombe*, to characterize origin selection on a genome-wide level.

First we studied the use of origins in wild-type cells and checkpoint-mutant (*cds1Δ* and *rad3Δ*) cells in the presence of hydroxyurea (HU). HU induces depletion of deoxyribonucleoside triphosphates (dNTPs), causing a block in replication fork progression. Previous studies indicated that the replication checkpoint in budding yeast slows progress through S phase by inhibiting replication origin firing while the replication checkpoint in mammalian cells slows progress through S phase by inhibiting both origin firing and replication fork movement. In our study, fission yeast replication origins were not significantly inhibited by the replication checkpoint suggesting that checkpoint-dependent slowing of S phase in fission yeast is probably accomplished primarily by the slowing of replication forks.

We also studied origin selection under DNA re-replicating conditions in *S. pombe*. Similar to recent budding yeast results, we found that the *S. pombe* genome was re-replicated unevenly, with several broad regions re-replicated more than others. The regions of excess re-replication remained present even when Cds1 or Rad3 were deleted, indicating that Cds1 and Rad3 do not affect the choice of origins used for re-replication. Amplified regions in *rad3Δ* cells tended to be smaller and have sharper boundaries than those of wild-type or *cds1Δ* cells, probably as a consequence of the unique failure of *rad3Δ* cells to prevent cell division during re-replication. This failure suggests that cell-cycle arrest is maintained, at least in part, by a Rad3-dependent, Cds1-independent DNA-damage checkpoint response during DNA re-replication.

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**Dissertation Advisor:** Dr. Janet Leatherwood