Contributions of Dna2 and the Tim/Tipin Complex to Genomic Stability

Thesis by

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To My Family

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Abstract

This thesis describes the essential roles of Dna2 and the Tim/Tipin complex in the maintenance of genomic stability. Dna2 participates in DNA replication and double-strand break repair by homologous recombination. Meanwhile, the Tim/Tipin complex is required for efficient checkpoint activation upon replication stress, which can be caused by stalled DNA replication forks.

While yeast genetics and experiments with purified proteins have revealed much about yeast Dna2, we chose to pursue characterization of metazoan Dna2 using *Xenopus* cell-free extracts. We show that binding of Dna2 to origins of replication is dependent upon formation of pre-replication complexes but independent of CDK2 activity. Upon initiation of DNA replication, Dna2 travels with replication forks. Physical interactions with Mcm10 and And-1, proteins involved in lagging strand DNA replication, are indicative of a role in replication of the lagging strand; this result is consistent with genetic results in yeast and *in vitro* biochemical experiments.

Dna2 also participates in the response to double-strand breaks and accumulates on chromatin containing double-strand breaks. We show that Dna2 binds to free DNA ends after the Mre11-Rad50-Nbs1 complex and ATM, but before RPA. Dna2-depleted extracts exhibit delayed processing of DNA ends, indicating that other nucleases do not easily compensate for the lack of Dna2. Consistent with genetic results in yeast, we find that the Mre11-Rad50-Nbs1 protein complex is essential for the processing of free DNA ends, but inhibition of Mre11 nuclease activity only slows processing. This observation indicates that other nucleases, possibly Dna2, can compensate for loss of Mre11 nuclease

activity. Despite the role of Dna2 in double-strand break processing, Dna2 is not required for checkpoint activation.

Timeless (Tim) and Tipin participate in the checkpoint response to stalled replication forks. We demonstrate here that Tim and Tipin form a complex, associate with chromatin in S phase, and physically interact with many proteins at the replication fork. Human cells lacking the Tim/Tipin complex do not exhibit robust checkpoint activation in response to stalled replication forks. Finally, we show that Tipin is also a target of both the ATR and Cdc7 kinases, which respond to stalled replication forks.

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