

# MECHANISMS OF NEGATIVE REGULATION OF THE CDT1 DNA REPLICATION FACTOR

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# Background and Goals

- In my research I am studying the regulation of a protein called Cdt1. This protein is part of a pre-replicative (pre-RC) complex that is essential for DNA replication. After replication, Cdt1 is promptly inhibited. If Cdt1 is not inhibited it will result in an over-replication of DNA, destroying the integrity of the genome. Recent studies in cultured cell lines have shown that there are two regulatory mechanisms that inhibit Cdt1; Cullin dependent degradation and Geminin binding.
- In my research leading up to the summer, I determined that Cullin degradation played an important role in regulating Cdt1 in developing fruit flies, not just in cells growing in a culture dish.
- This summer I hoped to determine how deregulating Cdt1 would affect DNA replication, and in so doing also determine how important each of these two regulation pathways were to the inhibition of Cdt1.

# Results

- My results over the summer showed that without Cullin-mediated degradation, Cdt1 is over-expressed, leading to a moderate degree of cell death in follicle cells during oogenesis. This suggested to me that there were at least some problems with DNA replication
- Further experimentation, however, showed that eliminating Cullin-mediated degradation of Cdt1 in follicle cells did not disrupt oogenesis and the ability of flies to produce viable offspring.
- These results suggests that Geminin binding, the other mechanism involved in negatively regulating Cdt1, is either more important than, or redundant with, Cullin-mediated degradation for maintaining functional levels of Cdt1 in the cell.



A photomicrograph of fruit fly ovarian follicle cells expressing a mutant version of Cdt1 (green) that cannot be regulated by Cullin dependent degradation. DNA of nuclei is stained in blue, and dying cells in red.