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Defining the Destruction Box: Understanding How the APC Recognizes Its Substrates

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ABSTRACT

Cell division is the process by which mother cells give rise to two genetically identical daughter cells. All cells have protein networks to ensure that cell division is completed correctly because mistakes during the cell division may cause diseases. The Anaphase-promoting complex (APC) is an important regulatory enzyme that ensures successful completion of mitosis. It acts by removing inhibitors of chromosomal segregation and cytokinesis, as well as other important cell division regulators. Existing chemotherapies, like taxol, act by indirectly inhibiting APC function. This makes APC a potential target for new cancer chemotherapies. However, designing APC inhibitors is challenging because how APC interacts with its substrates is not fully understood. What is currently known is that APC recognizes a short linear sequence containing R-x-x-L, called the destruction box (D-box). A D-box is needed for efficient proteolysis of most APC substrates, but what makes a functional D-box is still unclear. The goal of my project is to define the minimal functional D-box using an artificial reporter substrate containing the known D-box motif from the budding yeast APC substrate Fin1. To accomplish this goal, reporter expression plasmids are mutated and the stability of the mutant proteins are measured and compared using a cycloheximide chase assay. Any mutation in the D-box will cause slower decay in the immunoblotting signal for the reporter substrate after protein synthesis is terminated. By defining the minimal functional D-box, we can understand how APC interacts with its substrates, helping the development of chemotherapy drugs to kill cancer cells.

KEYWORDS

Anaphase-promoting Complex (APC), Ubiquitin, Substrate