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Abstract

Back to Hit List

Grant Number:	1Z01DK057803-03
PI Name:	COHEN FIX, ORNA
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PI Title:	
Project Title:	The Molecular Mechanism of Cell Cycle Regulation in Budding Yeast

Abstract: The accurate transmission of genetic material during cell division depends on the ability of the cell to coordinate the various processes involved in chromosome segregation. This is accomplished by the existence of regulatory mechanisms whose function is to ensure that late cell cycle steps are executed only after earlier ones have been successfully completed. We study the regulation of cell cycle progression through mitosis, using the budding yeast Saccharomyces cerevisiae as a model system. We have previously shown that an anaphase inhibitor, the Pds1 protein (Pds1p), acts as a key regulator of mitotic events; it controls the timing of anaphase initiation and it prevents the premature exit from mitosis. This function of Pds1p is especially important under conditions of intracellular damage (e.g. DNA damage or spindle damage), where progression through mitosis must be halted until the damage is repaired. To understand how Pds1p carries out its role as a mitotic inhibitor and to identify additional components of this regulatory pathway we have been engaged in the following experimental approaches: (1) A genetic screen aimed at identifying mutants that abrogate the ability of Pds1 to induce a mitotic arrest (conducted by Dr. Katie Farr). This screen is expected to reveal proteins that interact directly with Pds1 as well as downstream components of this regulatory pathway. In the past year this screen has yielded 4 classes of mutants: (a) mutants that fail to localize Pds1 to the nucleus; (b) mutants that are defective in spindle orientation and thus have defects in proper nuclear segregation; (c) mutants that fail to inhibit the exit of mitosis and go on to divide without chromosome segregation; and (d) mutants that initiate a new cell cycle without completing the previous one. We are currently cloning the mutant genes responsible for these phenotypes. (2) A biochemical approach aimed at identifying proteins that interact with Pds1 under normal cell cycle conditions and under conditions of DNA damage (conducted by Dr. Ritu Agarwal). We have previously shown that in the presence of DNA damage Pds1 is phosphorylated via the DNA damage checkpoint pathway, but the function of this phosphorylated form is unknown. So far we have been able to detect Pds1 as part of at least two high molecular weight complexes, one of which is observed only in the presence of DNA damage. We intend to identify the components of these complexes using mass spectrometry. In addition we are trying to reconstitute the DNA damage induced phosphorylation of Pds1 in a cell free system and we expect that this approach will lead us to the molecular mechanism that

underlies the DNA damage checkpoint response. (3) A yeast two-hybrid screen for identifying Pds1-interacting proteins (conducted by Dr. Zoe Hilioti). In this screen we have so far identified one Pds1-interacting protein, the Cdc20 protein. Cdc20 is thought to be involved in the ubiquitination of Pds1, leading to Pds1?s degradation at onset of anaphase. Cdc20 is also a target of the spindle assembly checkpoint. Our observation allows us now to examine how the spindle assembly checkpoint affects Pds1 ubiquitination, whether the DNA damage checkpoint pathway also affects Pds1 degradation through Cdc20, and how Cdc20 acts to promote Pds1 ubiquitination. (4) A structure and functional analysis of Pds1 (conducted by Dr. Ritu Agarwal and Dr. Orna Cohen-Fix). We are currently identifying the functional domains of Pds1p by mutational analysis and we are attempting to crystallize the purified protein. We are also beginning to study the interaction between Pds1 and one of its known targets, the anaphase activator Esp1. Earlier work in budding yeast suggested that Pds1 is both a positive and negative regulator of Esp1, and Dr. Karen Ross will be studying this question.

Thesaurus Terms:
Saccharomyces cerevisiae, cell cycle, cell cycle protein, cell growth regulation, microorganism reproduction
DNA damage, fungal genetics

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