

Mechanisms that Control the Protein Phosphatase Cdc14, an Essential Cell Cycle Regulator

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Activities 2007. Maintenance of genetic integrity from one generation to the next requires the accurate replication of chromosomes during S phase and their faithful segregation during mitosis. Accomplishing these critical tasks requires the coordination of several events. Errors during cell division, in particular chromosome mis-segregation, lead to genetic instability, a molecular hallmark of cancer. In all eukaryotes, cell cycle progression is triggered and coordinated by a set of interacting proteins including cyclins, cyclins dependent kinases (CDKs) and their inhibitors (CKI). My interests lie in understanding how a dividing cell exits from mitosis. For cells to exit the mitotic cycle and enter the next G₁ phase, mitotic CDKs need to be inactivated. In budding yeast, the phosphatase Cdc14 plays a critical role in promoting this event. Given Cdc14's key role in promoting exit from mitosis, it is essential to understand how this protein is itself regulated. Cdc14 activity is controlled by an inhibitory subunit Cfi1 (also known as Net1). For most part of the cell cycle, from G₁ up to metaphase, Cdc14 is sequestered and kept inactive in the nucleolus by binding to Cfi1. As soon as cells start to segregate their genetic material, Cdc14 is released from Cfi1 and spreads into the nucleus and cytoplasm, allowing it to reach its targets and trigger mitotic exit. Two regulatory networks have been identified that control the association of Cdc14 with Cfi1: the FEAR (Cdc Fourteen Early Anaphase Release) network and the MEN (Mitotic Exit Network). At present, we have some understanding of the players involved in releasing Cdc14 from the nucleolus. However it remains unclear (1) which mechanisms are responsible for the dissociation between Cdc14 and Cfi1, and (2) how Cdc14 is recruited back into the nucleolus.

Question 1: We intend to dissect the mechanisms that control the Cdc14/Cfi1 association by:

- Examining the contribution of the two networks in promoting the release of Cdc14.
- Testing the hypothesis that phosphorylation regulates the dissociation of Cdc14 from Cfi1 (as suggested by my own preliminary data)



- Identifying the protein kinases that mediate the phosphorylation of these sites (once determined that phosphorylation of certain residues in Cdc14 and/or Cfi1 is important for regulating their association.
- Dissecting the role of protein kinases Cdc5 and Dbf2, already identified as critical for this release to occur.

Question 2: Based on the knowledge that inactivation of the ubiquitin ligase APC/CCdh1 delays the return of Cdc14 into the nucleolus, we will:

- Test the hypothesis that degradation of one or more MEN and FEAR network components is important for this event to take place.
- Consider other APC/C substrates that are degraded at the end of mitosis.
- Look for proteins involved in this process that have not been identified yet.

By integrating both lines of research, I strongly believe that we will shed some light on to the three fundamental processes of activation, maintenance, and termination of Cdc14 release, responsible for mediating progression through and exit from mitosis.

Because Cdc14, Cfi1, and the FEAR and MEN networks are highly conserved among eukaryotes, this research will provide key insights into the regulation of mitosis and maintenance of genomic integrity in humans.