Institut Européen de Chimie et Biologie

I E C B

Soutenance de thèse

Régulation par la phosphorylation d'un module Rho GTPase dans la levure saccharomyces cerevisiae.

Romain MITTEAU
IECB/IBGC, Equipe D. McCusker

The eukaryotic cell cycle is characterized by abrupt and dynamic changes in cellular polarity as chromosomes are duplicated and segregated. Those dramatic cellular events require coordination between the cell cycle machinery and polarity regulators. The mechanisms underlying this coordination are not well understood. In the yeast *S. cerevisiae,* as in other eukaryotes, the GTPase Cdc42 plays an important role in the regulation of cell polarity. Cdc42 regulators constitute a GTPase module that undergoes dynamic phosphorylation during the cell cycle by conserved kinases including Cyclin Dependent Kinase 1 (Cdk1) and p21Cactivated kinase (PAK). These kinases and substrates may link cell polarity to the cell cycle progression. Using *in vitro* approaches, we have reconstituted the phosphoregulation of the Cdc42 Guanine Nucleotide Exchange Factor (GEF), Cdc24. We have identified a possible mechanism of Cdc24 regulation involving a scaffold dependent increase in Cdc24 phosphorylation by Pak and Cdk1. This phosphorylation moderately increases the affinity of Cdc24 for another GTPase module component, the scaffold Bem1. Moreover, by testing the effect of other GTPase module components on the phosphorylation of Cdc24, and thus on its interaction with the scaffold, we identified an antagonistic function for the GTPase Activating Protein (GAP) Rga2. Our *in vivo* data of *rga2* mutants suggest that Rga2 phosphorylation by Cdk1 inhibits its GAP activity. We propose a tentative model to explain the inhibition of Cla4 by Rga2 and its presence in a complex containing Cdc24 and Bem1. The presence of the GAP protein in the complex may be a mechanism that reduces Cdc24 phosphorylation in case of a mis-targeting of the complex in order to downregulate the GEF/Scaffold dimer. Since the PAK component of the GTPase module is itself activated by Cdc42 activity, our results are consistent with a model in which inputs from the cell cycle lead to autoamplification of the Cdc42 GTPase module. In *S. pombe*, polarized growth requires a gradient of activation of Cdc42 due to GEF and GAP segregation. Here we show that all Cdc42 GAPs localize to the polarized site during the cell cycle. Those localizations are consistent with a requirement of Cdc42 cycling to maintain a polarity cap. Our results may suggest that Cdc42 GAPs localizations in *S. cerevisiae* are different from current knowledge in *S. pombe.*

Institut Européen de Chimie et Biologie
2, rue Robert Escarpit - 33607 Pessac, France
Tél. : +33(0)5 40 00 30 38 - Fax. : +33(0)5 40 00 22 15
www.iecb.u-bordeaux.fr