Dynamics of Cell Growth & Division

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The most basic unit of life is the cell and the most fundamental function of cells is to grow and divide, thus propagating life. Cells grow and divide via a controlled series of events termed the cell cycle. Given the fundamental nature of the cell cycle, the core regulatory mechanisms responsible for cell cycle progression have proven remarkably conserved among eukaryotes. This conservation has enabled principles gleaned from simple unicellular eukaryotes to be applied to more complex multicellular systems, including humans. The impetus to understand the logic of the cell cycle in humans is driven by the fact that cancer, a major cause of mortality, is defined by uncontrolled growth and division. During the cell cycle, the pattern and amount of cell growth, combined with the timing of division contributes to the final size and shape of cells. The aim of this project was to understand the mechanisms that coordinate cell growth with progression through the cell cycle using the unicellular eukaryote *Saccharomyces cerevisiae*, budding yeast, as a model system. Specifically, the project addresses the following questions:

1) The role of Cdk1 in regulating membrane dynamics during the cell cycle.

In budding yeast, cyclin-dependent kinase 1 (Cdk1) plays an essential role in the establishment of a polarity axis and the initiation of polarized growth early in the cell cycle. However, the mechanisms underlying the initiation of polarized growth were unclear. Using state-of-the-art imaging and a "chemical-genetics" approach, we found that inhibition of Cdk1 activity resulted in a cell surface growth defect that was comparable to that seen after depolymerization of actin. This was a surprising result, since Cdk1 inactivation was not thought to play such a prominent role in the maintenance of polarized cell surface growth. Cdk1's function in polarized growth may reflect its role in regulating the actin cytoskeleton via the Rho-family GTPase Cdc42. However, we found that, unlike actin depolymerization, Cdk1 inhibition did not result in the accumulation of post-Golgi vesicles in the cytoplasm. Instead, high-speed evenescent-field imaging *in vivo* revealed that inhibition of Cdk1 activity resulted in the anterograde streaming of post-Golgi vesicles from the plasma membrane into the mother cell, where these vesicles may be consumed in the vacuole. In summary, these observations suggest that Cdk1 plays a role in regulating membrane trafficking to coordinate membrane growth with cell cycle progression. Direct control of membrane trafficking by Cdk1 would offer an attractive model for linking membrane growth to cell cycle progression. This work was published in Molecular Biology of the Cell.

While studying how a polarity axis is established in a Cdk1-dependent fashion, we uncovered a novel endocytosis-based cortical corralling mechanism. We found that during polarity establishment, Cdk1 activates Cdc42, resulting in the reorganization of endo- and exocytic membrane trafficking domains from a non-polarized to polarized state (see Figure 1). Evanescent-field imaging was used to demonstrate that endocytic vesicles surround a central zone of exocytosis, corralling the exocytic zone into a vertex that establishes a polarity axis during the ensuing cell cycle. Moreover, we found that the rate of individual endocytic events changed during polarity establishment, increasing in frequency at the site of polarization. Consistent with endocytic corralling providing a positive contribution to polarity establishment, endocytic mutants displayed weakened polarity axes that often became unfocussed. These results were reproduced using a novel mathematical model that we developed. In the model, polarity was established and maintained via membrane

trafficking-mediated delivery and removal of Cdc42. This work was published in the Journal of Cell Biology.

We next set out to understand what proteins are required for the bull's eye-like organization of endo- and exocytic trafficking domains. We performed a quantitative imaging-based screen of 400 candidate mutants that identified critical components required for the apposition of endo- and exocytic compartments during cell polarization. Moreover, we developed photo-conversion methods to track and quantitate the dynamics of exocytic vesicles in wild type and mutant cells. Using these methods, we are now able to follow more than a thousand vesicles per cell, providing powerful quantitative insight into vesicle trafficking dynamics. A manuscript describing this work is currently submitted.

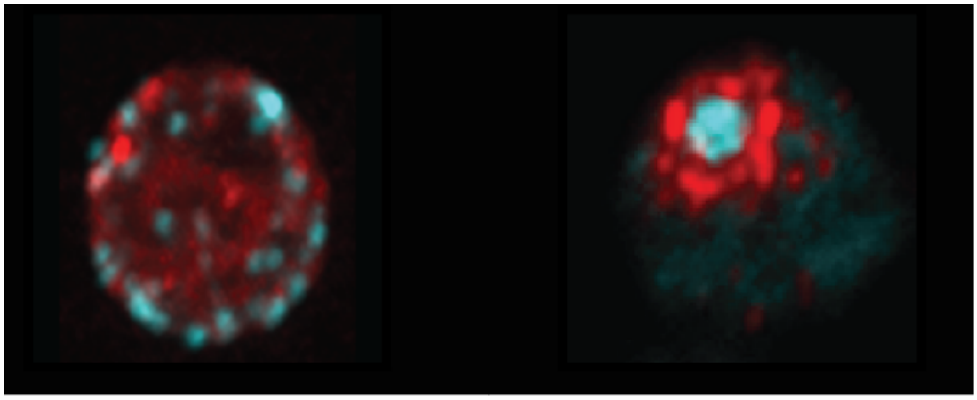

Figure 1. Cell cycle-dependent establishment of cell polarity in the budding yeast *Saccharomyces cerevisiae*. Early in the cell cycle, membrane trafficking compartments reorganize to establish a polarity axis for the ensuing cell cycle. In the cell on the left, which is non-polarized and in early G1 of the cell cycle, endocytosis-associated actin patches (red) and exocytic vesicles marked by GFP-Sec4 (cyan) are organized randomly. During polarity axis establishment, endocytic vesicles form a ring that surrounds a central zone of exocytic vesicles (cell on the right). The bull's-eye organization of these compartments depends on Cyclin-dependent kinase 1 (Cdk1) activity and Cdc42.

2) The molecular mechanisms by which Cdk1 activates Cdc42 to initiate and maintain polarized growth.

Having purified full-length Cdc42 GTPase module components from bacteria, we verified that the recombinant GEF, GAP and scaffold proteins were folded. We then purified active G1-cyclin Cdk1 and the p21 Activated Kinase (Pak) Cla4 and used the GTPase components for *in vitro* kinase assays. We have been able to quantitatively phosphorylate the GEF and then test how phosphorylation affects its affinity for the scaffold. These experiments identified a mechanism whereby the scaffold in the GTPase module greatly increases the rate at which the GEF is phosphorylated by both Cdk1 and PAK. Preliminary results indicate that this phosphorylation alters the affinity of the GEF-scaffold interaction. Consistent with this data, we have obtained evidence by single protein tracking that both the scaffold and PAK are required for the normal dynamics of Cdc42 *in vivo*. When completed, this work will be submitted for publication.

In conclusion, the project identified a new role for Cdk1 in the control of membrane trafficking. In addition, we discovered a novel corralling mechanism by which endocytic activity focuses exocytosis, establishing a polarity axis during the cell cycle. Our identification of mutants that disrupt this cell cycle-dependent membrane trafficking organization will shed further light on the links between the cell cycle, polarity and cell growth.