# The Role of Cdc42p Adaptors in Regulating the Filamentous Growth MAP Kinase Pathway

Sukanya Basu [a], Beatriz Gonzalez [a], Boyang Li [a], Garrett Kimble [a], Keith Kozminski [b], and Paul J. Cullen [a]
[a] Department of Biological Sciences, University at Buffalo, Buffalo, NY 14260
[b] Departments of Biology and Cell Biology University of Virginia School of Medicine, Charlottesville, Virginia 22908

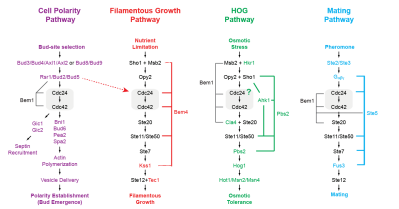
University at Buffalo
The State University of New York

University of Virginia
School of Medicine

## Abstract

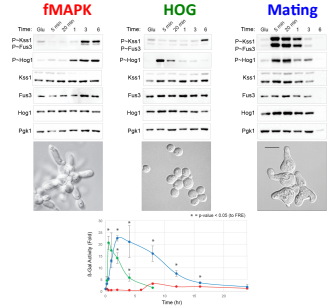
Rho GTPases regulate cell polarity and signal transduction pathways to control morphogenetic responses in different settings [1]. In yeast, the Rho GTPase Cdc42p regulates cell polarity and mitogen-activated protein kinase (MAPK) pathways [mating, filamentous growth or fMAPK, and HOG [2]]. Although much is known about how Cdc42p regulates cell polarity and mating, how Cdc42p is activated in the fMAPK pathway is not clear. This question is relevant because filamentous growth is a developmental fungal response to nutrient limitation that occurs in mnay fungal species, including pathogens [3-5]. Moreover, although the signaling glycoprotein (Msb2) that regulates the fMAPK pathway has been characterized [6-7], how Msb2p connects to Cdc42p is not clear. To begin to address this question, Cdc42p-dependent MAPK pathways were compared in the filamentous (Σ1278b) strain background. Each MAPK pathway showed a unique activation profile, with the fMAPK pathway exhibiting slow activation kinetics compared to the mating and HOG pathways. A previously characterized version of Cdc42p, Cdc42p$^{E100A}$ [8], that is specifically defective for fMAPK pathway signaling, was also defective for interaction with Bem4p, the pathway-specific adaptor for the fMAPK pathway [9]. Corresponding residues in Bem4p were identified that were required for interaction with Cdc42p and fMAPK pathway signaling. The polarity adaptor Bem1p also regulated the fMAPK pathway. In the fMAPK pathway, Bem1p recruited the p21-activated kinase (PAK) Ste20p to the plasma membrane, cycled between an open and closed conformation, and interacted with the GEF Cdc24p. Bem1p also regulated effector pathways in different ways, behaving as a multi-functional adaptor in some pathways and like a passive scaffold in others. Genetic suppression tests showed that Bem4p and Bem1p, together with Rsr1p – the bud site GTPase that also regulates the fMAPK pathway [10] - regulated the fMAPK pathway in an ordered sequence. Collectively, the study demonstrates the unique and sequential roles that Rho GTPase adaptors have in regulating MAPK pathways.

## MAPK Pathways Share Components



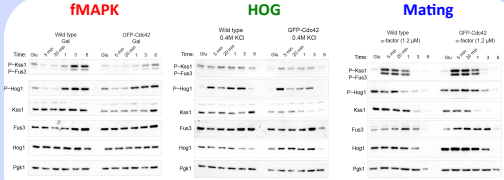

The fMAPK pathway (red) shares components with two other MAPK pathways (HOG or osmotic stress pathway, green; and mating, blue). Cdc42p also regulates cell polarity, purple.

## MAPK Pathways Are Insulated



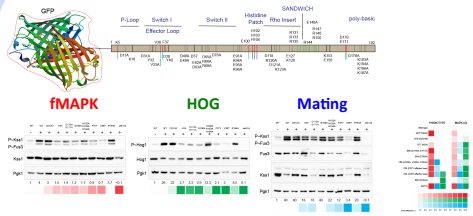

To compare MAPK pathway activity, a filamentous (Σ1278b) strain lacking the redundant branch of the HOG pathway (*ssk1Δ*) was examined by immunoblots for P~MAPK and transcriptional reporters by β-galactosidase assays. Cell morphology was also examined.

## Cdc42p is required for fMAPK signaling



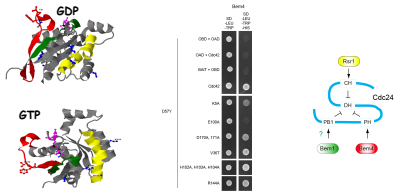

P~MAPK levels in wild-type (WT) cells or cells carrying GFP-Cdc42p. Cells were induced by galactose, salt, or alpha factor.

## Cdc42p Exhibits MAPK Pathway-Specific Phenotypes



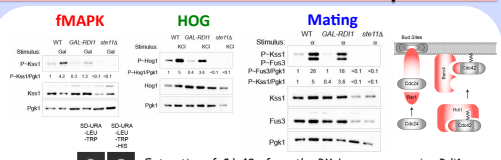

A collection of Cdc42 alleles was examined for MAPK activity. Several alleles showed pathway-specific phenotypes.

## Bem4p Associates with Cdc42p



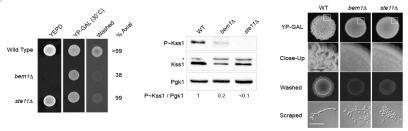

Rsr1p and Bem4p interact with the inactive or (GDP) conformation of Cdc42p but not with a fMAPK-defective version, Cdc42$^{E100A}$.

## PM Association of Cdc42p



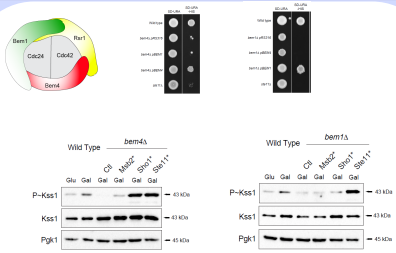

Extraction of Cdc42p from the PM by overexpressing Rdi1p selectively dampens the fMAPK pathway. Overexpressing Rdi1 interferes with the interaction between Cdc42p and Bem4p.

## Bem1 Regulates fMAPK pathway



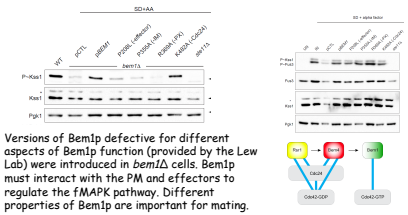

Bem1p regulates Cdc42p function in cell polarity, mating and osmotic stress. Bem1p also regulated the fMAPK pathway.

## Bem1 Acts After Bem4




Genetic tests indicate that Rsr1p (Basu 2016) and Bem4p act at an early step in fMAPK signaling, whereas Bem1p acts at a later step. This is consistent with the fact that Rsr1p and Bem4p associate with GDP-Cdc42p, and Bem1p associates preferentially with GTP-Cdc42p.

## Bem1p regulates MAPK pathways

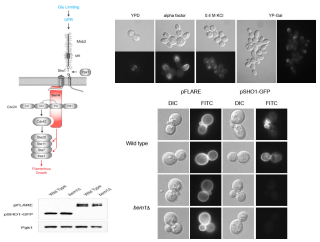



Versions of Bem1p defective for different aspects of Bem1p function (provided by the Lew Lab) were introduced in *bem1Δ* cells. Bem1p must interact with the PM and effectors to regulate the fMAPK pathway. Different properties of Bem1p are important for mating.

## References

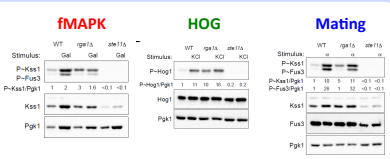
1. Park, H. O.; Bi, E., Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiology and molecular biology reviews : MMBR* **2007,** *71* (1), 48-96.
2. Saito, H., Regulation of cross-talk in yeast MAPK signaling pathways. *Current opinion in microbiology* **2010,** *13* (6), 677-83.
3. Gimeno, C. J.; Ljungdahl, P. O.; Styles, C. A.; Fink, G. R., Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. *Cell* **1992,** *68* (6), 1077-90.
4. Cullen, P. J.; Sprague, G. F., Jr., Glucose depletion causes haploid invasive growth in yeast. *Proceedings of the National Academy of Sciences of the United States of America* **2000,** *97* (25), 13619-24.
5. Finkel, J. S.; Mitchell, A. P., Genetic control of Candida albicans biofilm development. *Nature reviews. Microbiology* **2011,** *9* (2), 109-18.
6. Cullen, P. J.; Sabbagh, W., Jr.; Graham, E.; Irick, M. M.; van Olden, E. K.; Neal, C.; Delrow, J.; Bardwell, L.; Sprague, G. F., Jr., A signaling mucin at the head of the Cdc42- and MAPK-dependent filamentous growth pathway in yeast. *Genes & development* **2004,** *18* (14), 1695-708.
7. Vadaie, N.; Dionne, H.; Akajagbor, D. S.; Nickerson, S. R.; Krysan, D. J.; Cullen, P. J., Cleavage of the signaling mucin Msb2 by the aspartyl protease Yps1 is required for MAPK activation in yeast. *The Journal of cell biology* **2008,** *181* (7), 1073-81.
8. Mosch, H. U.; Kohler, T.; Braus, G. H., Different domains of the essential GTPase Cdc42p required for growth and development of Saccharomyces cerevisiae. *Molecular and cellular biology* **2001,** *21* (1), 235-48.
9. Pitoniak, A.; Chavel, C. A.; Chow, J.; Smith, J.; Camara, D.; Karunanithi, S.; Li, B.; Wolfe, K. H.; Cullen, P. J., Cdc42p-interacting protein bem4p regulates the filamentous-growth mitogen-activated protein kinase pathway. *Molecular and cellular biology* **2015,** *35* (2), 417-36.
10. Basu, S.; Vadaie, N.; Prabhakar, A.; Li, B.; Adhikari, H.; Pitoniak, A.; Chow, J.; Chavel, C. A.; Cullen, P. J., Spatial landmarks regulate a Cdc42-dependent MAPK pathway to control differentiation and the response to positional compromise. *Proceedings of the National Academy of Sciences of the United States of America* **2016,** *113* (14), E2019-28.

## Bem1 Localizes Sho1



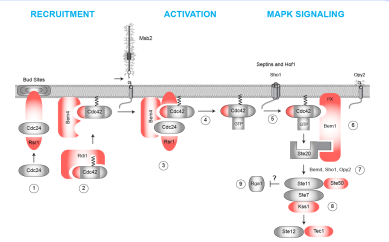

The adaptor Sho1p, which is activated by Msb2p in the fMAPK pathway, was mis-localized in the *bem1Δ* mutant. Total Sho1 protein levels were not altered. pFLARE (provided by the Emr lab), which marks the plasma membrane, served as a control. Active Cdc42p, as measured by pGic2-Cherry (Bi lab) was distributed differentially in response to different stimuli.

## Rga1 Is Excluded from Active MAPKs




How is activated Cdc42 in MAPK pathways turned off? The main Cdc42p GAP Rga1p which can reduce basal MAPK signaling was not effective at attenuating active MAPK pathways. Cdc42p PAK may be protected from Rga1p.

## Model




**RECRUITMENT**
1. Rsr1 recruits Cdc24 to bud sites in $G_1$.
2. Bem4 competes with Rdi1 to promote PM recruitment of Cdc42.

**ACTIVATION**
3. Bem4 and Rsr1 stabilize Cdc24 with Cdc42-GDP.
4. Cdc24 activates Cdc42.
5. Sho1 is activated by Msb2 and recruits Ste20 and Ste11/Ste50 to PM.

**MAPK SIGNALING**
6. Bem1 brings Ste20 to GTP-Cdc42 at the PM.
7. Bem4, Sho1, and Opy2 bring Ste11 to the PM for activation by Ste20.
8. MAPK cascade is activated.
9. Rga1 is prevented from inhibiting
10. MAPK signaling in an unknown way.

NIGMS