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Bem3

Filling the GAP between cell polarity and secretion

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A highly conserved member of the Rho family of small GTPases, Cdc42 functions as the "master regulator of cell polarity." It has been reported that for proper establishment and maintenance of cell polarity, Cdc42 regulates and requires vesicle trafficking. Importantly, we recently discovered that in budding yeast, vesicle trafficking also controls the localization and function of Bem3, a GTPase activating protein for Cdc42. Specifically, we observed that Bem3 partitioned between the plasma membrane and an internal membrane-bound compartment. This Bem3-containing compartment was present during extended periods of apical growth, required actin tracks for trafficking to polarized sites and functioned as a recycling station that was positioned at the junction of endocytic and secretory pathways. Strikingly, many of these features are reminiscent of the Spitzenkörper, a dynamic structure involved in polarized growth during hyphal development in several filamentous fungi. Furthermore, Bem3 was not merely a passive cargo but actively recruited the secretory Rab GTPase Sec4 to this Spitzenkörper-like compartment. Importantly, this function of Bem3 was independent of its GAP activity. Our work demonstrates the existence of a complementary regulation between Bem3, a regulator of Cdc42 signaling and Sec4, a key component of the secretory machinery.

Keywords: vesicle trafficking, secretory pathway, recycling pathway, cell polarity, Spitzenkörper, Bem3

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Cell polarity is fundamental for the execution of a diverse set of cellular functions, such as cell differentiation, proliferation, and migration.[1-3] A plethora of polarity regulators were identified using the budding yeast *Saccharomyces cerevisiae*, including Cdc42 (Cell Division Cycle 42, also called the "master regulator of cell polarity").[1,2,4] A highly conserved member of the Rho family of small GTPases, Cdc42 acts as a molecular switch that alternates between a signaling-active GTP-bound form and a signaling-inactive GDP-bound form. This cycling between the 2 Cdc42 activation states is regulated by GTPase Activating Proteins (GAPs) and Guanine nucleotide Exchange Factors (GEFs).[5-7] Thus, while the GAPs accelerate the GTPase mediated hydrolysis of GTP to GDP, leading to Cdc42 inactivation, the GEFs support the reversion of inactive Cdc42 to the active GTP bound form, thereby continuing the cycle.[6,8]

For proper establishment and maintenance of cell polarity, Cdc42 activation needs to be precisely regulated spatio-temporally.[9] Indeed, the coordinated action of the GAPs and GEFs is essential for regulating Cdc42 activity and distribution. Cdc24, the only known Cdc42 GEF in budding yeast, is recruited to specific sites at the beginning of the cell cycle following spatial cues (such as the previous budding site) where it triggers the local activation of Cdc42.[10] Once activated, a positive feedback mechanism plays a crucial role in maintaining this concentrated and polarized distribution of Cdc42, often designated as the polar cap.[11] The feedback loop is generated by Cdc42-dependent organization of actin cables, which in turn reinforce the polarized trafficking of this membrane-anchored signaling molecule by targeting the delivery of Cdc42-containing secretory vesicles to the polar cap (**Fig. 1**).[12,13] Furthermore, to counter

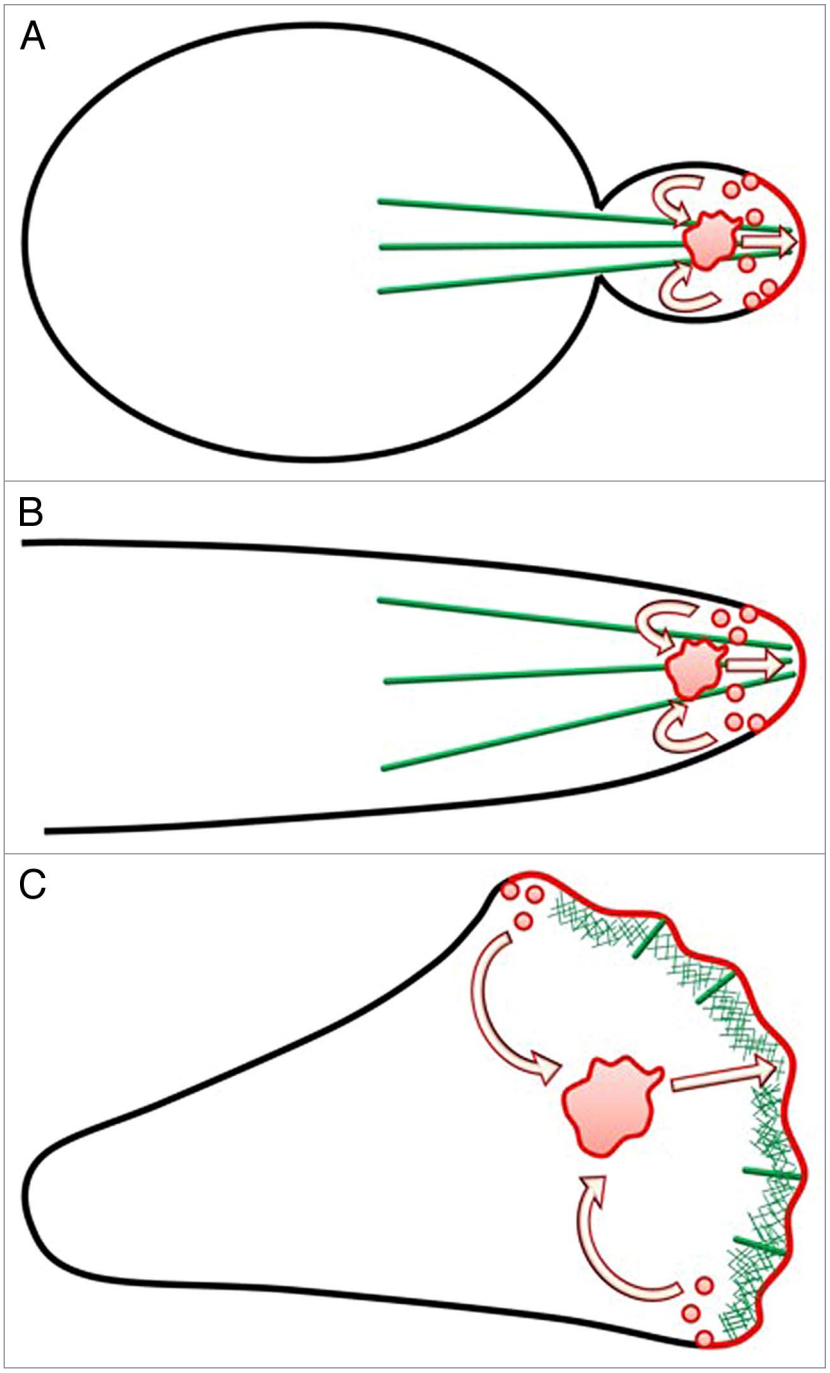

Figure 1. The Polarized distribution of RhoGTPases, such as Cdc42, is maintained by vesicle trafficking. The processes of endocytosis and exocytosis are spatially segregated at the polarized site to counteract diffusion of Cdc42. (**A**) budding yeast at an early stage of the cell cycle; (**B**) filamentous fungi undergoing hyphal growth; (**C**) migratory mammalian cell.

the effect of Cdc42 diffusion away from the concentrated patch, endocytosis and exocytosis are spatially separated to complement each other, where these processes have been hypothesized to gather the diffused Cdc42 and redirect it back to the polar cap (**Fig. 1**).[14-16] Thus, activated Cdc42 not only regulates vesicle trafficking, but also requires it for the maintenance of its own polarized localization and of the cell as a whole.[14,16-20] Further strengthening the link between polarity and vesicle trafficking, previous studies from our lab have demonstrated that the epsin endocytic adaptors in yeast and mammals can directly interact with and inhibit the GAPs for Rho GTPases.[21,22] This Epsin-GAP complex then performs an essential function that leads to the accumulation of activated Cdc42 and promotes its signaling downstream.

Further, we recently discovered that in budding yeast, vesicle trafficking not only controls Cdc42 localization and function as previously demonstrated, but at least one of the 3 Cdc42 GAPs, Bem3.[23] This is striking because no vesicle trafficking-mediated regulation has been previously reported for peripherally associated membrane proteins such as Bem3. In the study by Mukherjee and co-authors, we showed that polarization of Bem3 is mediated by vesicle trafficking and depends on the integrity of both the endocytic and secretory pathways. We observed a bipartite localization of Bem3 where it was present cortically at the plasma membrane as reported before, as well as in a novel internal membrane-bound compartment. Immunoelectron microscopy revealed that these Bem3-containing compartments were present as a cluster of vesicles at the vicinity of the polar caps. Time-lapse imaging unveiled their dynamic behavior where the compartments appeared to closely interact with cortically localized Bem3. In addition, Fluorescence Recovery after Photobleaching (FRAP) analysis revealed that Bem3 itself displayed a dynamic behavior, associating and dissociating with this compartment (DM, AS, and RCA unpublished data).

In this compartment, Bem3 coexisted with proteins such as Snc1, a v-SNARE that recycles from endosomes, and underwent recycling mediated by Rcy1, an effector of Ypt31/32 (the yeast homolog of Rab11). Indeed, this Bem3-containing compartment functioned as a recycling station that was present at the junction of endocytic and secretory pathways and required actin tracks for trafficking to polarized sites. Interestingly, many of these features were reminiscent of the Spitzenkörper, a dynamic structure involved in polarized growth during hyphal development in several filamentous and pathogenic fungi.[24-27] The Spitzenkörper maintains the necessary supply of secretory vesicles to support hyphal development, and disappears

upon cessation of apical growth.[28-30] As mentioned before, and similar to the Spitzenkörper, the Bem3-containing compartment in budding yeast was temporally regulated during the cell cycle where it predominantly occurred during periods of robust apical growth—that is, in unbudded and small-budded cells. Interestingly, under conditions that simulate pseudohyphal growth in budding yeast, this Bem3-containing Spitzenkörper-like endomembrane-compartment underwent significant enlargement. Taken together, our data suggested that this Bem3-containing compartment played a crucial role specifically during apical growth.

Furthermore, an additional remarkable discovery was that Bem3 was not merely a passive secretory cargo delivered to polarized sites on secretory vesicles. Instead, Bem3 actively regulated the assembly of the Spitzenkörper-like compartment. Our results suggested that Bem3 actively influenced the delivery of secretory vesicles to polarized sites since deletion of Bem3 led to a significant reduction in the localization of the yeast Rab8 homolog and secretory vesicle marker, Sec4, to the bud tips specifically during early stages of cell cycle. Further, Bem3 dosage strongly correlated with the size of the Spitzenkörper-like compartment.

Most importantly, Bem3 overexpression enhanced the recruitment of the activated GTP-bound form of Sec4 to the Spitzenkörper-like compartment in a dose-dependent manner. Interestingly, a Bem3 mutant for its Pleckstrin Homology (PH) domain unable to efficiently bind PI(4,5)P_2, or cellular membranes in general, could no longer lead to the recruitment of GTP-bound Sec4 to endomembranes when overexpressed. However, mutation of the GAP domain of Bem3 had no effect on its ability to localize Sec4 on the Spitzenkorper-like compartment. Indeed, this is the first report of a GAP-independent activity of Bem3.

To summarize, this study demonstrated for the first time that the coordinated interplay of both endocytosis and secretion was essential for the polarized distribution of a Cdc42 regulator. Furthermore, reminiscent of the reciprocal regulation between Cdc42 and vesicle trafficking, we determined that the Cdc42 GAP Bem3 was not just a passive secretory vesicle cargo, but actively influenced the trafficking of secretory vesicles to the polar cap during bud expansion. Indeed, Bem3 was crucial for recruiting the secretory Rab GTPase Sec4, to Bem3-containing Spitzenkörper-like compartments. Thus, for the first time, we showed the existence of a complementary regulation between Bem3, a regulator of Cdc42 signaling and Sec4, a key component of the secretory machinery. Importantly, this function of Bem3 was independent of its GAP activity and is the first discovery of its kind. Most relevantly, the parallels between the Bem3-containg compartment in budding yeast and the Spitzenkörper in filamentous fungi were striking. Indeed, the Bem3-homolog from *Candida albicans* could also sustain the recruitment of Sec4 when overexpressed in budding yeast. Taken together, our data indicate the presence of a conserved function of Bem3 which is critical for Cdc42—and Sec4-dependent polarity establishment. Since the formation of the Spitzenkörper is essential for pathogenicity in filamentous fungi, we believe that the tractable genetics of the budding yeast system will play a crucial role in better understanding the basis of hyphal development in filamentous fungi.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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