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A Memory System of Negative Polarity Cues Prevents Replicative Aging

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SUMMARY

Cdc42 is a highly conserved master regulator of cell polarity. Here, we investigated the mechanism by which yeast cells never re-establish polarity at cortical sites (cytokinesis remnants [CRMs]) that have previously supported Cdc42-mediated growth as a paradigm to mechanistically understand how Cdc42-inhibitory polarity cues are established. We revealed a two-step mechanism of loading the Cdc42 antagonist Nba1 into CRMs to mark these compartments as refractory for a second round of Cdc42 activation. Our data indicate that Nba1 together with a cortically tethered adaptor protein confers memory of previous polarization events to translate this spatial legacy into a biochemical signal that ensures the local singularity of Cdc42 activation. "Memory loss" mutants that repeatedly use the same polarity site over multiple generations display nuclear segregation defects and a shorter lifespan. Our work thus established CRMs as negative polarity cues that prevent Cdc42 reactivation to sustain the fitness of replicating cells.

INTRODUCTION

The establishment of cell polarity sites is fundamental for a plethora of cellular functions related to morphogenesis, differentiation, and/or proliferation of uni- and multicellular organisms (Bloch and Yalovsky, 2013; Dworkin, 2009; Heasman and Ridley, 2008; Howell and Lew, 2012; Iden and Collard, 2008; Li and Bowerman, 2010; Martin-Belmonte and Perez-Moreno, 2011; McCaffrey and Macara, 2009; Nelson, 2009; Noatynska et al., 2013). The Rho-GTPase Cdc42, initially described in yeast (Johnson and Pringle, 1990), is a master regulator of cell polarization and highly conserved among eukaryotes (Boureux et al., 2007; Etienne-Manneville, 2004). How Cdc42 polarity sites are regulated in space and time has been extensively studied over the past years (Casamayor and Snyder, 2002; Johnson et al., 2011; Park and Bi, 2007). However, much less is known about the molecular mechanisms involved in the establishment of polarity cue refractory to Cdc42 activation.

During G1 phase, yeast cells establish a polarity site from which the daughter cell will emerge. Polarity establishment depends on Rho GTPase Cdc42 (Park and Bi, 2007). The site of bud emergence (bud neck) will later be used in cytokinesis to separate the daughter from mother cell (Figure 1A) (Park and Bi, 2007; Wloka and Bi, 2012). The remnants of the cytokinetic machinery (bud scar or cytokinesis remnant [CRM]) are retained in the mother cell and can be visualized by transmission electron microscopy (TEM) or by specific dyes (Figure 1B) (Meitinger et al., 2013). CRMs mainly consist of extracellular matrix, which is encircled by a chitin-rich ring (Cabib et al., 1993). In addition, transmembrane proteins, including Rax1 and Rax2, protrude into the extra- and intracellular spaces (Kang et al., 2004). This indicates that CRMs may modulate intracellular processes. However, relatively little is known about the composition and function of CRMs. During the replicative lifetime, the aging mother cell accumulates increasing numbers of these CRMs over its cell surface (Casamayor and Snyder, 2002). Importantly, a new polarity site is never established within CRMs, indicating that Cdc42 activation cannot occur twice at the same site, even though the initiation event could be many generations after the cytokinesis that generated the remnant.

The molecular basis for the prevention of Cdc42 activation at CRMs remains to be established. Different GTPase-activating proteins (GAPs) contribute to the inhibition of Cdc42 at the cell-division site (Atkins et al., 2013; Tong et al., 2007). In addition, the scaffold protein Gps1 inhibits Cdc42 at the site of cytokinesis as part of a pathway working in parallel to the Cdc42 GAP Rga1 (Meitinger et al., 2013). However, neither Cdc42 GAPs nor Gps1 accumulate at old CRMs. Using a combination of proteomics, cell biology, and biochemical approaches, we identified a protein complex that is recruited to the cell-division site by Gps1 and inherited to CRMs in a Gps1 and Rax1-Rax2-dependent manner. We show that Nba1, a bud-neck-associated protein of unknown

function (Calvert et al., 2008), is the core component of this complex that is responsible for the prevention of Cdc42 activation in the remnant. Mutant cells that are unable to inhibit Cdc42 activation at CRMs display nuclear segregation defects and have markedly shorter lifespans. This study therefore identifies the molecular mechanism for a long-recognized phenomenon that keeps CRMs inactive for Cdc42-reactivation, thereby ensuring the longevity of asymmetrically dividing yeast cells.

RESULTS

Nap1, Nba1, and Nis1 Function with Gps1 in Cdc42 Inhibition

Nap1, Nba1, and Nis1 were previously identified as putative Gps1-interacting proteins (Meitinger et al., 2013). Nap1 is a conserved histone chaperone involved in chromatin assembly and morphogenesis (Ishimi and Kikuchi, 1991; Mortensen et al., 2002), whereas Nba1 and Nis1 are proteins of unknown function associated with the cell-division site (Calvert et al., 2008; Iwase and Toh-e, 2001). Using coimmunoprecipitation experiments, we established that Nap1, Nba1, and Nis1 interact with Gps1 (Figure 1C). Fluorescence microscopy analysis revealed that GFP-tagged Gps1, Nap1, Nba1, and Nis1 localize to the cell-division site (Figure 1D). This localization was cell-cycle-dependent for Nap1, Nba1, and Nis1 (Figures S1A and S1B available online). In contrast, only Nba1 and Nis1 localized to CRMs (Figure 1E).

Gps1 localized to the cell-division site independently of *NAP1*, *NBA1*, or *NIS1* (Figures 1F and S1C). Similarly, Nap1 localization to the cell-division site was not affected in *gps1*Δ, *nba1*Δ, or *nis1*Δ cells (Figures 1F and S1C). Nba1 failed to localize to the cell-division site in the absence of *GPS1* (Figures 1F and S1C), although its protein levels were unaffected by deletion of *GPS1* (Figure S1D). Nis1 localization to the cell-division site also required *GPS1*. Moreover, *NAP1* and *NBA1* were necessary for Nis1 localization to the cell-division site (Figures 1F and S1C). Consistent with this observation, coimmunoprecipitation of Nis1 with Gps1 was impaired in the absence of *NAP1* or *NBA1* (Figure 1C).

Interestingly, although GFP-tagged Gps1 and Nap1 did not localize to CRMs, both were necessary for Nba1 and Nis1 localization to CRMs (Figures 1G and S1C). Impaired localization of Nis1 to CRMs in *nap1*Δ cells was at least partially explained by reduced levels of Nis1-GFP in this mutant (Figure S1D). Furthermore, localization of Nba1 and Nis1 to CRMs was mutually dependent (Figure 1G). Together, we conclude that Gps1 and Nap1 are involved in recruiting Nba1 and Nis1 to the cell-division site, and in loading Nba1 and Nis1 to CRMs (Figures 1H).

Gps1 coordinates the activity of Cdc42 and Rho1, two members of the Rho family of small GTPases (Meitinger et al., 2013). In the absence of *GPS1,* abnormal activation of the Cdc42 GTPase causes rebudding inside the old cell-division site, resulting in the appearance of one or more concentric collars of cell wall material at the cell-division site in 30%–40% of the cells (Figure 1I) (Meitinger et al., 2013). In addition, deletion of *GPS1* also compromises the Rho1 cytokinetic pathway, leading to a thinning of the secondary septum (Meitinger et al., 2013). Transmission electron microscopy of *nap1*Δ, *nba1*Δ, and *nis1*Δ cells revealed multiple collars at the bud neck without any thinning of the secondary septum in all three mutants (Table 1A), suggesting that Nap1, Nba1, and Nis1 are involved in regulating Cdc42 but not Rho1. The multiple collar phenotype was more frequent in the *nba1Δ* strain (Table 1A), indicating that Nba1 plays a major role in Cdc42 inhibition. Together, these results established Nap1, Nba1, and Nis1 as Gps1-interacting proteins that are specifically involved in inhibiting the Cdc42 pathway. Furthermore, our findings suggest that inhibition of Cdc42 might not be restricted to the cell-division site but could also occur at CRMs, where Nba1 and Nis1 localize.

Nba1 Is Necessary and Sufficient to Inhibit Cdc42

We sought to understand how Gps1 interacts with Cdc42, Nap1, Nba1, and Nis1. Yeast two-hybrid experiments with Gps1 truncations suggested that Gps1 has specific binding domains for each of its interaction partners (Figures 2A and S2A). In vitro, recombinant Gps1$^{293–422}$ (a fragment of Gps1 containing the amino acid residues 293–422), Gps1$^{514–600}$ and Gps1$^{443–530}$ interacted directly and specifically with recombinant Nap1, Nba1, and Nis1, respectively (Figure 2B). Based on these results we generated a Gps1$^{\Delta513–598}$ mutant in which the Nba1 interaction domain was deleted. Nba1 failed to interact with Gps1$^{\Delta513–598}$ in yeast two hybrid (Figure 2C) and showed impaired localization to the cell-division site and CRMs in *gps1*$^{\Delta513–598}$ cells (Figures 2D and 2E). Notably, *gps1*$^{\Delta513–598}$ cells showed no defects in Rho1 regulation, as judged by the thickness of the secondary septum, yet exhibited multiple collar phenotype with frequency similar to that of *gps1Δ* and *nba1Δ* mutants (Table 1B). This observation suggests that Nba1 functions downstream of Gps1 in the Cdc42 inhibition pathway, and that the main role of Gps1 is to recruit Nba1 to the cell division site and CRMs.

If this model is correct, then artificial targeting of Nba1 to the cell-division site should eliminate the need for Gps1 in Cdc42 inhibition. We tested this possibility using the septin Shs1 tagged with the GFP-binding protein (GBP) (Rothbauer et al., 2008) to recruit GFP fusions to the cell-division site (Meitinger et al., 2013). Expression of *SHS1-GBP* in *NBA1-GFP gps1*Δ cells resulted in permanent association of Nba1-GFP with the cell-division site, partially restored the localization of Nba1-GFP to CRMs and partially rescued the growth defect of the *gps1Δ* mutant (Figures 2F–2H). Notably, artificial tethering of Nba1 to Shs1 rescued the multiple collar phenotype but not the secondary septum defect of *gps1Δ* cells (Table 1C), indicating that *NBA1-GFP gps1*Δ *SHS1-GBP* cells bypassed the requirement of Gps1 in the regulation of Cdc42 but not of Rho1. Similar results were obtained with *NIS1-GFP gps1Δ SHS1-GBP* cells (Figures S2B–S2D; Table 1D). However, Shs1-tethered Nis1 required *NBA1* for function, whereas Shs1-tethered Nba1 suppressed the *gps1*Δ growth defect even in the absence of *NIS1* (Figures 2H and S2D). Collectively, our data indicate that Gps1 recruits Nba1 and Nis1 to the cell-division site and CRMs, where Nba1 is necessary and sufficient to inhibit Cdc42 activation.

Nba1 Inhibits Binding of the GEF Cdc24 to GTP-Bound Rsr1

Next, we concentrated on the mechanism of Cdc42 inhibition by Nba1. Two parallel mechanisms contribute to the site-directed