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Displacement of Formins from Growing Barbed Ends by Bud14 Is Critical for Actin Cable Architecture and Function

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SUMMARY

Normal cellular development and function require tight spatiotemporal control of actin assembly. Formins are potent actin assembly factors that protect the growing ends of actin filaments from capping proteins. However, it is unresolved how the duration of formin-mediated actin assembly events is controlled, whether formins are actively displaced from growing ends, and how filament length is regulated in vivo. Here, we identify Bud14 as a high-affinity inhibitor of the yeast formin Bnr1 that rapidly displaces the Bnr1 FH2 domain from growing barbed ends. Consistent with these activities, *bud14Δ* cells display fewer actin cables, which are aberrantly long, bent, and latrunculinA resistant, leading to defects in secretory vesicle movement. Moreover, *bud14Δ* suppressed mutations that cause abnormally numerous and shortened cables, restoring wild-type actin architecture. From these results, we propose that formin displacement factors regulate filament length and are required in vivo to maintain proper actin network architecture and function.

INTRODUCTION

Formins are a conserved family of actin assembly-promoting factors that employ a novel mechanism for building actin networks that drive cell movement, morphogenesis, and division (Faix and Grosse, 2006; Goode and Eck, 2007; Kovar, 2006). The conserved FH2 domain of formins catalyzes de novo actin polymerization, and processively associates with growing barbed ends of nascent filaments, protecting them from capping proteins. The adjacent FH1 domain accelerates filament growth by recruiting profilin-actin. These activities of the FH1 and FH2 domains are employed in vivo to assemble diverse actin-based structures, including polarized cables, stress fibers, filopodia, cytokinetic actin rings, and lammelipodia.

How formin activities are regulated spatially and temporally in vivo remains only partially understood. A large class of formins are autoinhibited via intramolecular interactions between their N-terminal DID and C-terminal DAD domains and are released/activated by Rho GTPase binding. However, it has been unclear whether additional, non-Rho factors contribute to regulating formin transitions between their active and inactive states. It is also unclear how the length of actin filaments produced by formins is controlled. This is particularly important given that many actin networks assembled by formins are comprised of relatively short filaments 0.3–13 μm in length (e.g., cytokinetic rings, stress fibers, and cables) (Cramer et al., 1997; Kamasaki et al., 2005). Factoring in actin monomer concentrations and actin growth rates in vivo, the length of these filaments suggests that the duration of a formin activity cycle must be very rapid (<5 s). However, purified formins persist on barbed ends much longer than could account for the short filament lengths produced in vivo, even when challenged with capping proteins in vitro (Kovar and Pollard, 2004; L. Blanchoin, personal communication). Thus, additional cellular factors may be required to catalyze formin displacement from barbed ends to restrict filament length.

In this study, we have investigated formin temporal regulation in *S. cerevisiae*. Yeast contain two prominent F-actin structures visible throughout the cell cycle, cortical patches assembled by the Arp2/3 complex, which mediate endocytosis, and cables assembled by formins, which direct polarized secretion and cell morphogenesis (Moseley and Goode, 2006). *S. cerevisiae* expresses two formins, Bni1 and Bnr1, which have distinct localization patterns and functions. Bni1 particles are transiently recruited from the cytoplasm to the bud cortex (Buttery et al., 2007), where they assemble short actin filaments organized into cables (Pruyne et al., 2004), then are released and/or incorporated into the cables. In contrast, Bnr1 remains stably anchored to the neck region and assembles filaments incorporated into cables that fill the mother cell. The actin cables assembled by these two formins serve as polarized tracks for myosin V-dependent transport of secretory vesicles and other cargoes required for growth of the daughter cell. Genetic evidence has suggested that Bni1 and Bnr1 are autoinhibited, then activated by Rho GTPases (Evangelista et al., 2003). Furthermore, Bud6 has been shown to function as a cofactor for Bni1-mediated actin assembly (Moseley et al., 2004). Little else is known about how Bni1 and Bnr1 activities are regulated in vivo, but the stable localization of Bnr1 suggests that it must be regulated through rapid and tightly controlled cycles of activation/inactivation.

Here, we identify the *S. cerevisiae* cell polarity factor Bud14 as a Bnr1 regulator. Previous studies showed that Bud14 interacts with Glc7 (protein phosphatase type 1) to control

dynein-dependent microtubule sliding at the bud cortex and facilitate nuclear migration (Knaus et al., 2005). In addition, deletion of *BUD14* caused a poorly understood hyperelongated bud phenotype (Cullen and Sprague, 2002). The observation that Bud14 overexpression was conditionally lethal and exacerbated by *bni1Δ* hinted that it may have additional roles in actin-based cell morphogenesis events (Cullen and Sprague, 2002). We show that Bud14 directly controls formin activity to regulate actin cable architecture and function.

RESULTS

Deletion of *BUD14* Causes Altered Actin Cable Architecture

To identify novel regulators of Bnr1-mediated actin cable assembly, we visually screened yeast strains carrying deletions of nonessential genes that encode bud neck resident proteins for actin cable abnormalities in the mother cell compartment. Of the 28 strains examined, one (*bud14Δ*) showed clear defects in cable organization. A large percentage of *bud14Δ* cells contained at least one cable that was exaggerated in length and kinked or buckled where it reached the cell cortex (Figure 1A, arrows in lower panels; quantified in Figure 1B). Such hyperextended cables were rarely observed in wild-type cells, and cable "turns" at the cortex in wild-type cells were not as sharp as in *bud14Δ* cells (Figure 1A, arrows in upper panels). In addition, *bud14Δ* cells showed a significant decrease (~50%) in the number of visible cables compared to wild-type cells (Figure 1C; see Figure S1 available online).

One model to explain these observations is that *bud14Δ* cells contain fewer cables comprised of abnormally long individual filaments. We tested this possibility by using two independent methods. First, we compared wild-type and *bud14Δ* cells for retention of visible cables after treatment with the actin monomer-sequestering drug latrunculin A (LatA) (Figure 1D). A single cable is comprised of many short, bundled actin filaments and is highly dynamic. Cables are continuously synthesized and rapidly turned over. As a result, visible cable staining in wild-type cells is completely lost after less than 1 min of exposure to LatA (Okada et al., 2006; Yang et al., 2001). Therefore, a cable that is comprised of abnormally short filaments should be hypersensitive to LatA treatment, whereas a cable that is comprised of abnormally long filaments should be resistant to LatA. Wild-type and *bud14Δ* cells were treated for different times with a low concentration of LatA (20 μM) and scored for the percentage of cells with visible actin cables (Figure 1E). Furthermore, from a plot of these data we derived the time required for half of the cells to lose visible cables (see Experimental Procedures): wild-type, $T_{1/2}$ = 27 s; *bud14Δ*, $T_{1/2}$ = 53 s. These data indicate that *bud14*Δ cells contain LatA-resistant cables. Furthermore, these cables appeared to be the same hyperextended and/or kinked cables described above, suggesting a correlation between abnormal architecture and increased LatA resistance. These observations support the model that the altered cables in *bud14*Δ cells are comprised of abnormally long filaments.

An alternative model is that *bud14*Δ changes the decoration of cables by stabilizers and/or destabilizers to reduce cable turnover. However, this model is inconsistent with our observations that Bud14 does not directly bind F-actin, does not affect F-actin dynamics in vitro, and does not decorate F-actin in vivo (see below).

As a second test of the cable architecture model, we performed genetic crosses to examine *bud14Δ* interactions with two other mutations that affect cable architecture, *tpm1Δ* and *aip1Δ*. Loss of the filament stabilizer Tpm1 results in greatly diminished actin cable staining and loss of polarity, resulting in enlarged cell morphology and temperature-sensitive growth (Liu and Bretscher, 1992). In contrast, loss of the filament destabilizer Aip1 results in thickened, hyperstabilized cables without obvious defects in cell morphology (Okada et al., 2006; Rodal et al., 1999). Notably, *bud14Δ* strongly suppressed the temperature-sensitive cell growth defects and partially rescued the cell morphology and actin defects of *tpm1Δ* (Figures 1F and 1H; Figure S2). This result supports our model, since *bud14Δ* cells have exaggerated cables and *tpm1Δ* cells have diminished, shortened cables. To further assess rescue, we transformed each strain with a low-copy plasmid expressing GFP-Sec4, a marker for secretory vesicles (Schott et al., 2002). Delivery of secretory vesicles to the bud tip requires type V myosin (Myo2) transport on actin cables, which makes polarized accumulation of GFP-Sec4 a sensitive in vivo assay for cable function. Each strain was scored for the percentage of cells with a bright spot of GFP-Sec4 at the bud tip (Figure 1G): wild-type, 97%; *bud14Δ*, 93%; *tpm1Δ*, 3%; and *bud14Δtpm1Δ*, 49%.

In contrast to the rescue of *tpm1Δ* by *bud14Δ*, *bud14Δaip1Δ* double mutants showed compounded defects in cell morphology, with ~34% of double mutant cells being grossly elongated and misshapen (Figure 1H). Thus, *bud14Δ* has opposite genetic interactions with *tpm1Δ* and *aip1Δ*. Taken together with the observation that *bud14Δ* cells contain LatA-resistant cables, these data support the model that loss of Bud14 results in elongated and bent cables comprised of abnormally long actin filaments.

Bud14 Functions Upstream of Bnr1 to Control Actin Cable Assembly and Polarized Cell Growth

We next tested the genetic relationship between Bud14 and the formins Bni1 and Bnr1. Since *BNI1* and *BNR1* function in genetically redundant pathways for cable assembly, mutation of a gene that regulates Bnr1 is predicted to have synthetic interactions with *bni1Δ*. Indeed, *bni1Δbud14Δ* double mutants showed synthetic defects in cell growth at 37°C, whereas *bnr1Δbud14Δ* double mutants displayed no synthetic defects in cell growth (Figure 2A). Synthetic defects in the *bni1Δbud14Δ* strain were supported further by an examination of GFP-Sec4 localization (Figure S3). Together, these data reveal that, in cells lacking Bni1, the further loss of Bud14 impairs polarized cell growth, and they suggest that *BUD14* functions in the same pathway as *BNR1*, in parallel to *BNI1*.

We also compared the cell morphologies of the single and double mutants (Figure 2B). *bud14Δ* cells showed a high-penetrance elongated bud phenotype, as previously reported (Cullen and Sprague, 2002) (Figure 2C). The elongated bud phenotype can arise from a delay in the switch from anisotropic to isotropic bud growth at G2/M, a transition controlled by the *SWE1* checkpoint (Lew and Reed, 1993). Accordingly, we found that deletion of *SWE1* suppressed the elongated bud phenotype of *bud14Δ* (Figure 2C). Interestingly, *bnr1Δ* also suppressed the elongated