Distinct roles of the polarity factors Boi1 and Boi2 in the control of exocytosis and abscission in budding yeast

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ABSTRACT Boi1 and Boi2 (Boi1/2) are budding yeast plasma membrane proteins that function in polarized growth, and in cytokinesis inhibition in response to chromosome bridges via the NoCut abscission checkpoint. How Boi1/2 act in these two distinct processes is not understood. We demonstrate that Boi1/2 are required for a late step in the fusion of secretory vesicles with the plasma membrane of the growing bud. Cells lacking Boi1/2 accumulate secretory vesicles and are defective in bud growth. In contrast, Boi2 is specifically required for abscission inhibition in cells with chromatin bridges. The SH3 domain of Boi2, which is dispensable for bud growth and targets Boi2 to the site of abscission, is necessary and sufficient for abscission inhibition. Gain of function of the exocyst, a conserved protein complex involved in tethering of exocytic vesicles to the plasma membrane, rescued secretion and bud growth defects in boi mutant cells, and abrogated NoCut checkpoint function. Thus Boi2 functions redundantly with Boi1 to promote the fusion of secretory vesicles with the plasma membrane at sites of polarized growth, and acts as an abscission inhibitor during cytokinesis in response to chromatin bridges.

INTRODUCTION

Exocytosis, the delivery of secretory vesicles containing new membranes and membrane-remodeling factors to the plasma membrane (PM), is essential for cell growth and division. The molecular principles of exocytosis have been well characterized in the budding yeast \textit{Saccharomyces cerevisiae}. In this organism, secretory vesicles are transported toward growth sites in the bud by actin-based trans-

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Abbreviations used: EM, electron microscopy; ESCRT, endosomal sorting complexes required for transport; GFP, green fluorescent protein; NAA, 1-naphthaleneacetic acid; PH, pleckstrin homology domain; PM, plasma membrane; PS, primary septum; SH3, SRC homology 3; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex formation. Prior to SNARE-mediated fusion with the target membrane, secretion requires a rate-limiting step known as “tethering,” mediated by an evolutionarily conserved octamer complex, the exocyst (Wu et al., 2007; He and Guo, 2009). Exocyst function is essential for cell growth and its inactivation leads to accumulation of exocytic vesicles in the cytoplasm (Novick et al., 1980; Guo et al., 1999; He et al., 2007; Wu et al., 2010). The Sec3 and Exo70 subunits associate directly with the PM in a manner that is largely independent of the actin cytoskeleton, whereas the other six subunits (Sec5, 6, 10, 15, and 84) are transported to growth sites on membrane vesicles. This has led to the hypothesis that assembly of all subunits at the PM mediates vesicle tethering before fusion (Boyd et al., 2004).

Exocytosis is also important for completion of cytokinesis. During this process, contraction of a membrane-associated actomyosin ring guides progression of the PM at the site of cell division. Membrane resolution then splits the cell into two distinct topological units, in a process known as abscission (Green et al., 2012;...
Schi and Prekeris, 2013). In HeLa cells, inactivation of the exocyst during cytokinesis leads to abscission defects (Gromley et al., 2005). In budding yeast, actomyosin ring contraction at the site of division, called the bud neck, is coupled to synthesis of a chitin-based primary septum (PS). Yeast exocyst mutants show aberrant ring contraction dynamics, mislocalization of the PS chitin synthase Chs2, and cytokinesis defects suggesting that exocytosis is also required for late cell division steps in this organism (Dobbelzaer and Barral, 2004; VerPlank and Li, 2005). The timing of abscission is monitored by a mechanism known as NoCut in budding yeast, and the abscission checkpoint in animal cells, which inhibits abscission in cells with chromatin bridges caught in the path of the cell division machinery (Steigemann et al., 2009; Amaral et al., 2016, 2017; Nähse et al., 2016). Whether NoCut impinges on the exocytic machinery is not known.

The functionally redundant yeast cortical proteins Boi1 and Boi2 (Boi1/2) were previously implicated in polarized growth (Bender et al., 1996; Matsui et al., 1996) and in the NoCut abscission checkpoint (Norden et al., 2006; Mendoza et al., 2009). Boi1/2 associate with the bud cortex during bud growth and with the bud neck during cytokinesis, but how they act in these two distinct processes remains unclear. Here, we demonstrate that Boi1/2 promote vesicle exocytosis during bud growth. In contrast, the SH3 domain of Boi2, which targets the protein to the bud neck, is dispensable for bud growth but is specifically required to inhibit abscission in cells with chromatin bridges. Our results raise the possibility that Boi2 acts as both an activator of exocytosis and an inhibitor of abscission, depending on the cell cycle stage and the cellular response to chromosomal segregation defects during cytokinesis.

RESULTS

Boi1 and Boi2 are essential for bud growth

Single boi1Δ and boi2Δ cells show normal growth and morphology but double deletion mutants display severe morphogenesis and growth defects (Supplemental Figure S1A; Bender et al., 1996; Matsui et al., 1996). To investigate the role of Boi1/2 during cell growth, we generated a conditional boi1 boi2 null strain using an auxin-inducible degron (AID) to rapidly target Boi2 for polyubiquitination and proteasome-dependent degradation in the presence of 1-naphthaleneacetic acid (NAA) and the plant E2 ligase Tir1 (Figure 1A; Nishimura et al., 2009). A boi2-aid strain expressing Tir1 grew well in complete media but failed to form colonies in the presence of NAA specifically in a boi1Δ background, consistent with an essential role of Boi1/2 in cell viability (Figure 1B).

To determine the consequences of Boi1/2 depletion on cell growth, wild-type and boi1Δ boi2-aid cells were examined by differential interference contrast (DIC) time-lapse microscopy 2 h after addition of 0.25 mM NAA, when Boi2-aid protein levels were reduced to nearly undetectable levels (Figure 1C). Wild-type and boi1Δ boi2-aid cells had similar morphology; however, Boi-depleted cells were severely impaired in surface growth. NAA-treated boi1Δ boi2-aid cells with small or medium buds grew at a slower rate or turned dark and stopped growing altogether (Figure 1D). Moreover, large round boi1Δ boi2-aid cells were observed 24 h after NAA addition (Figure 1E). Thus Boi1/2 function in cell growth, and depolarized growth previously reported in boi1Δ boi2Δ mutants (Bender et al., 1996; Matsui et al., 1996) might represent secondary defects associated with long-term Boi1/2 depletion.

Boi1/2 are dispensable for organization of actin and cell polarity factors

To assess whether Boi1/2 are required for maintenance of a polarized actin cytoskeleton, F-actin was visualized with fluorescently labeled phalloidin in wild-type and boi1Δ boi2-aid cells treated with NAA for 2 h. Actin patches and cables appeared similarly organized in wild-type and Boi-depleted cells (Figure 2A). In addition, we determined the localization of various cell polarity proteins fused to green fluorescent protein (GFP) in wild-type and Boi-depleted cells. Lack of Boi1/2 did not severely affect the localization of the Cdc42 guanine nucleotide exchange factor Cdc24, whereas it did moderately reduce that of the Boi-interacting protein Bem1 (McCusker et al., 2007) and of the exocyst component Exo70 to the bud cortex. Furthermore, Boi-depleted cells showed slightly increased frequency of bud cortex localization of the Rab-like protein residing on post-Golgi vesicles, Sec4 (Figure 2B; Goud et al., 1988). Thus Boi1/2 are not required for the maintenance of a polarized actin cytoskeleton and do not play a major role in the localization of polarity factors and exocytic vesicles to sites of polarized growth.

Identification of putative boi1 boi2 suppressors by genome sequencing

To gain insight into the molecular functions of Boi1 and Boi2, we took advantage of a previously described boi1Δ boi2Δ strain, which was viable. These cells show no obvious morphological defects but are defective in the NoCut abscission checkpoint, which inhibits completion of cytokinesis in the presence of chromosome segregation defects (Norden et al., 2006; Mendoza et al., 2009). We hypothesized that this strain might contain one or more suppressor mutations (SUP) ensuring cell viability in the absence of Boi1/2. To identify putative suppressors, whole genome sequencing of this viable boi1Δ boi2Δ strain was performed and compared with its wild-type parent. Sequence analysis showed that gene order and copy number were identical between the two strains, ruling out aneuploidy and gross genome rearrangements in boi1Δ boi2Δ SUP (see Materials and Methods and Supplemental Figure S2). However, assessment of variation at the single nucleotide level identified 19 single nucleotide polymorphisms (SNPs) between boi1Δ boi2Δ SUP and its parental strain. Of these, seven were predicted to introduce amino acid changes in the encoded proteins (see Supplemental Table S1).

To determine linkage of SNPs to survival of boi1Δ boi2Δ SUP cells, genetic crosses were performed between this mutant and a BOI1 BOI2 strain. As expected, a fraction of boi1Δ boi2Δ spores from this cross gave rise to viable colonies (Supplemental Figure S1B). One boi1 boi2 SUP clone was selected and backcrossed four more times; a BOI1/boi1Δ BOI2/boi2Δ SUP/+ zygote that produced four viable spores was identified after the fifth backcross and its meiotic products were characterized by whole genome sequencing. Candidate suppressors should be present in both viable boi1Δ boi2Δ spores and absent in the two BOI1 BOI2 clones; SNPs fulfilling these criteria are shown in Supplemental Table S2. Several SNPs clustered around specific genomic regions as expected by a selection sweep effect, presumably due to genetic background differences between boi1Δ boi2Δ SUP and the BOI1 BOI2 strain (see Materials and Methods). However, the only SNP common to both the original and the backcrossed boi1Δ boi2Δ SUP segregants was located in the gene encoding the exocyst subunit Exo70, where it introduces one amino acid substitution (EXO70-G388R). Notably, the same mutation was independently isolated as a suppressor of cdc42 and rho3 mutants. The mutant protein, hereafter termed Exo70*, is expressed at a similar level to the wild-type protein and is incorporated into endogenous exocyst complexes, where it acts as a dominant, gain-of-function allele that suppresses the lethality and exocytosis defects of cdc42 and rho3 mutants (Wu et al., 2010).
FIGURE 1: Boi1 and Boi2 are essential for bud growth. (A) Schematic representation of the auxin-induced degron system. SCF: Skp1, Cullin, and F-box complex. E2: E2 ubiquitin ligase. Aid: AID. (B) Serial dilutions of the indicated strains spotted onto the indicated plates and incubated for 3 d at 30°C. (C) GAL-AtTIR1 boi1Δ boi2-aid-HA cultures were grown in YPR to log phase, transferred to YPG for 2 h, and Boi2-aid-HA was detected by immunoblotting at the indicated time points after addition of dimethyl sulfoxide (DMSO) or the indicated concentrations of NAA. G6PDH was used as a loading control. (D) DIC time-lapse imaging of wild type and boi1Δ boi2-aid mutants expressing GAL-AtTIR1. Cells were grown in YPR to log phase, transferred to YPG for 2 h, and imaged in the presence of 250 µM NAA for the following 6 h. Cell images (4-µm-thick stacks spaced 0.8 µm) were acquired every 5 min; selected frames are shown. Time is represented in minutes. Scale bars, 2 µm. Cells were classified in three groups as indicated; the relative frequencies of these groups are indicated in the graph. N > 23 cells pooled from two independent experiments. (E) DIC images of wild-type and boi1Δ boi2-aid cells 24 h after addition of NAA. Scale bar, 10 µm.
Boi1/2 roles in exocytosis and abscission

Boi1/2 are required for vesicle exocytosis

To test whether EXO70* is a dominant suppressor of the boi1 boi2 mutant, centromeric plasmids encoding either the wild type or the dominant version of EXO70 under the control of the regulatable GAL1,10 promoter were introduced in boi1boi2-aid strains. Galactose-driven expression of Exo70* was sufficient to restore the growth of Boi-deficient cells in the presence of NAA, whereas overexpression of wild-type Exo70 did not (Figure 3A). EXO70* expressed from its natural promoter at the endogenous locus also supported the growth of both boi1boi2-aid cells in NAA-containing media (Figure 3B) and of boi null strains, although boi1boi2 EXO70* strains grew at slower rates than wild type (Supplemental Figure S1C). Quantification of bud growth by DIC time-lapse microscopy showed that boi1boi2-aid EXO70* cells grew in a polarized manner and at slower rates than wild-type cells (Figure 3C). Together these results indicate that Boi1/2 promote exocyst-dependent vesicle fusion with the PM during bud growth.
FIGURE 3: Boi1/2 are required for vesicle exocytosis. (A) Serial dilutions of wild-type or boi1Δ boi2-aid strains bearing EXO70, EXO70*, or empty centromeric (CEN) plasmids were grown on galactose-containing media with and without NAA for 4 d. The plasmid-borne EXO70 gene was under the control of the GAL1 promoter. (B) Serial dilutions of cells of the indicated strains grown on YPG with and without NAA for 3 d. EXO70* is expressed from the EXO70 promoter at the endogenous locus. (C) DIC time-lapse images of boi1Δ boi2-aid cells transformed with EXO70 or EXO70* CEN plasmids. Cells were grown in SC Gal-Leu to log phase, and imaged 2 h after the addition of 250 µM NAA at 25°C. Numbers indicate time in minutes; scale bar, 5 µm. The graph represents the relative increase in volume of individual buds over time. N = 10 cells per condition. Error bars indicate SEM. (D) EM images of small budded cells in the indicated mutants. Cells were treated as in Figure 1C and fixed 2 h after NAA addition; scale bar, 500 nm. The number of vesicles/cell/section is represented in the graph (N > 15). Lines represent the mean; bars are SEM. (E) Serial dilutions of the indicated strains grown for 4 d at 30°C. (F) DIC time-lapse images of cells treated as in C, but imaged at 27°C. Time is indicated in minutes; scale bar, 2 µm. (G) Relative increase in bud volume over time for cells of the indicated strains as in C. N = 19 (WT), 13 (bem2-84), 14 (boi1Δ boi2-aid), 10 (bem2-84 boi1Δ boi2-aid).
To directly assess this, electron microscopy (EM) analysis was performed in wild-type and Boi-depleted cells. In wild-type cells few secretory vesicles are detected by EM due to high basal secretion rates. In contrast, a higher number of 80–100-nm-diameter vesicles were visualized in NAA-treated boi1Δ boi2-aid cells (Figure 3D). Secretory vesicles were particularly abundant near the surface of small and medium-sized buds, in a manner reminiscent of exocyst loss-of-function mutants. Conversely, vesicle accumulation was largely alleviated in boi1Δ boi2-aid EXO70* cells (Figure 3D). Thus Boi1 and Boi2 may promote the tethering function of the exocyst to allow secretory vesicles to fuse with the PM.

Cdc42 and Rh3 GTPases are thought to directly activate the exocyst through their interaction with Exo70 (Wu et al., 2010). As viability of boi1 boi2 cells is rescued by overexpression of Rh3 or its functionally related GTPase Rho4 (Bender et al., 1996; Matsui et al., 1996) and by expression of EXO70* (this study), Boi proteins might act together with Rh GTPases in exocyst activation. Deletion of the GTPase-activating proteins (GAP) RGA1 or RGA2 (targeting Cdc42) or RGD1 (acting on Rh3 and Rh4) (Stevenson et al., 1995; Roumanie et al., 2000; Smith et al., 2002) did not restore the growth of boi1Δ boi2-aid strains in NAA (Supplemental Figure S3). In contrast, the GAP-deficient bem2-84 mutation, which leads to Cdc42 hyperactivation (Atkins et al., 2013) allowed Boi-depleted cells to survive and restored their normal growth, suggesting a specific role for Bem2 in the control of exocytosis through hyperactivation of Rh-GTPase signaling (Figure 3, E–G). Thus Boi1/2 might promote activation of the exocyst together with multiple Rho-like GTPases regulated by Bem2, such as Cdc42 and potentially Rh4 (Gong et al., 2013) during bud growth.

**Boi1/2 are required for secretion of specific exocytic vesicles**

We then tested whether Boi1 and Boi2 are required for the secretion of specific protein cargoes. Budding yeast cells have two major types of exocytic vesicles, which can be distinguished by their density and protein content: light vesicles containing cell wall–related protein such as the endoglucanase Bgl2, and dense vesicles (e.g., containing invertase (Harsay and Bretscher, 1995). The activity of secreted periplasmic invertase in wild-type and Boi-depleted cells was determined using a standard assay (Goldstein et al., 1995; Lampen, 1975). Exocyst mutant cells (sec6Δ) known to be impaired in secretion of invertase showed a strong reduction in invertase activity after incubation with NAA to deplete Boi2, sug-

This opens the possibility that Boi proteins are recruited to exocytic sites by the exocyst complex, either directly or indirectly, to regulate sorting of specific vesicles for exocyst-dependent secretion.

**Boi2 inhibits cytokinesis in cells with catenated chromatin bridges**

We next addressed the role of Boi1/2 in cytokinesis. Anaphase chromatin bridges lead to inhibition of abscission; this inhibition was bypassed in boi1Δ boi2Δ cells treated with NAA for 2 h, and of sec6-4 cells shifted to 37°C for 2 h. Invertase secretion is the ratio of external invertase activity relative to total (internal + external) activity. Invertase data are the mean ± SEM of three independent experiments performed in triplicate. (B) Internal Bgl2 levels of cells of the indicated strains after incubation with DMSO or NAA for 2 h (boi1Δ boi2-aid), or after shift to 37°C for 2 h (sec14-1). Bgl2 levels were determined by Western blot and represent the ratio between internal and total (internal + external). G6PDH was used as a loading control. Bgl2 data are the mean and SD of three independent experiments. *: p < 0.05, Student’s t test.

FIGURE 4: Boi1/2 are essential for secretion of Bgl2 but not invertase. (A) Activity of secreted invertase in wild-type and boi1Δ boi2-aid cells treated with NAA for 2 h, and of sec6-4 cells shifted to 37°C for 2 h. Invertase secretion is the ratio of external invertase activity relative to total (internal + external) activity. Invertase data are the mean and SEM of five independent experiments performed in triplicate. (B) Internal Bgl2 levels of cells of the indicated strains after incubation with DMSO or NAA for 2 h (boi1Δ boi2-aid), or after shift to 37°C for 2 h (sec14-1). Bgl2 levels were determined by Western blot and represent the ratio between internal and total (internal + external). G6PDH was used as a loading control. Bgl2 data are the mean and SD of three independent experiments. *: p < 0.05, Student’s t test.

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Live fluorescence microscopy showed that wild-type cells underwent ingression of the PM at the bud neck after entry of the SPB in the bud, and this was followed by membrane resolution into two distinct layers as previously reported (abscission) (Figure 6, A and B). In boi1Δ and boi2Δ cells, ingressed bud neck membranes resolved with dynamics similar to those of wild-type cells (Figure 6, A and B). In contrast, top2-4 and top2-4 boi1Δ mutants showed ingression of the PM at the bud neck but were impaired or severely delayed in its resolution, consistent with a defect in abscission in top2 mutants (Figure 6, A and C) as previously observed by fluorescence microscopy and EM tomography (Amaral et al., 2016). However, analysis of GFP-CAAX showed that unlike top2-4 and top2-4 boi1Δ mutants, most top2-4 boi2Δ cells were able to complete abscission (Figure 6, A and C). Therefore Boi2 is specifically required for inhibition of abscission in cells with catenated chromatin bridges, whereas Boi1 is dispensable for this inhibition.

The SH3 domain of Boi2 is necessary and sufficient for inhibition of cytokinesis

Boi1 and Boi2 share a similar domain organization, featuring a Pleckstrin-homology (PH) domain at their C-terminus and a Src-homology 3 (SH3) domain at their N-terminus. Boi1/2 PH domains can interact with PM lipids and associate with the bud cortex; moreover, the PH domain of Boi1 is required for viability of boi1 boi2 mutant cells (Hallett et al., 2002; Yu et al., 2004). Therefore PH domains mediate the essential function of Boi1/2 in bud growth. In contrast, the SH3 domain of Boi1 is not required for viability of boi null mutants and targets Boi1 to the bud neck (Hallett et al., 2002). We therefore asked whether the SH3 domain of Boi2 might be required for its bud neck localization and its function in abscission inhibition. A version of Boi2 expressed under the control of the weak Gal5 promoter, and lacking the 102 N-terminal amino acids spanning the SH3 domain, did not perturb Boi2 targeting to the bud cortex, but abrogated its localization to the bud neck during cytokinesis (Figure 7A). This Boi2 mutant version (Boi2ΔSH3) also supported normal bud growth and abscission in boi1Δ cells (Figure 7B). However, boi1Δ Gal5-boi2ΔSH3 cells did not inhibit abscission in the presence of chromatin bridges caused by topoisomerase-II inactivation (Figure 7B). Furthermore, a C-terminal truncation of Boi2 at position 103, which removed most of the protein but retained only the SH3 domain (SH3Boi2) was recruited to the bud cortex and bud neck (Figure 7C) and supported abscission inhibition in cells with chromatin bridges (Figure 7D). Thus the SH3 domain of Boi2 is not essential for bud growth, but is specifically required for Boi2 targeting to the bud neck and is sufficient for its function in the NoCut abscission checkpoint.

Exocyst gain of function restores abscission in cells with chromatin bridges

As inhibition of the exocyst perturbs the completion of yeast cytokinesis (Dobbelaere and Barral, 2004; VerPlank and Li, 2005), we asked whether gain of function of the exocyst can prevent its inhibition. To this end, the GFP-CAAX reporter was used to monitor abscission in EXO70Δ cells with normal and defective chromosome segregation. EXO70Δ cells completed abscission with similar kinetics to those of wild type, and importantly, failed to inhibit abscission in the presence of catenated DNA bridges (Figure 8A). These results raise the possibility that abscission inhibition in cells with chromatin bridges requires modulation of exocyst-dependent, membrane-remodeling events at the abscission site. To gain insight into cytokinesis membrane trafficking events in cells with chromatin bridges, we followed the kinetics of the vesicle marker Sec4, which accumulates in the bud neck at the end of mitosis and controls membrane trafficking during cytokinesis (Figure 8B; Lepore et al., 2016). The levels of Sec4-GFP accumulating in the bud neck relative to total Sec4 cellular levels were significantly
signaling (in EXO70* and bem2-ts mutants, respectively). Because the exocyst subunit Exo70 is an effector of the GTPases Cdc42 and Rho3 (Wu et al., 2010), our results raise the possibility that Boi1/2 act by GTPase-mediated up-regulation of the exocyst. Boi1/2 could achieve this by facilitating the proper assembly of exocyst-activating complexes. Supporting this model, Boi1 and Boi2 interact with multiple proteins involved in polarized growth including the Cdc42 exchange factor Cdc24 and Bem1 (McCusker et al., 2007), which in turn associates with exocyst complex (Liu and Novick, 2014). Moreover, Boi1/2 interact with exocyst components in vitro and by two-hybrid assays (Tonikian et al., 2009) and we find that recruitment of Boi2 to sites of membrane remodeling depends on exocyst function. Boi1/2-dependent up-regulation of the exocyst could then promote vesicle

FIGURE 6: Boi2 is required for inhibition of abscission in topoisomerase-II deficient cells. (A) Membrane ingestion and abscission in representative cells of the indicated strains. Z-stacks spaced 0.3 µm apart and spanning the whole cell were acquired at 2-min intervals, but only central Z-planes are shown. Whole-cell images are shown at the top and enlargements of the bud neck region at the bottom. The spindle pole marker Spc42–GFP (visible only in some Z-sections; arrows) allowed the simultaneous visualization of spindle elongation. Numbers indicate time in minutes; time 0 marks the frame before membrane ingestion. To analyze the status of the bud neck membrane, the GFP fluorescence intensity was measured across the cleavage plane in the central Z-plane (yellow lines) shown in the right. A reduction in local intensity marked membrane resolution and was scored as abscission (top and bottom graphs); a single peak denoted the preabsission stage (middle). (B, C) Graphs show the fraction of cells completing abscission relative to the time of membrane ingestion. Cells were synchronized in G1 with α-factor in YPD at 25°C and shifted to 37°C 15 min before release from the G1 block. Data are from cells pooled from two to four independent experiments.

Delaying higher in top2-4 cells than in wild-type cells. Moreover, increased Sec4 levels at the bud neck in the presence of chromatin bridges were restored in EXO70* cells but not in boi2Δ cells (Figure 8, B and C, and Supplemental Figure S5). Thus cytokinetic membrane remodeling is altered in response to DNA decatenation defects, in a process that can be bypassed by gain of function of the exocyst.

DISCUSSION
Role of Boi1/2 in vesicle exocytosis during polarized growth
In this study, we demonstrate that Boi1/2 are essential for exocytosis of specific vesicle types, and for bud growth. We find that inhibition of exocytosis caused by Boi1/2 depletion is rescued by gain of function of the exocyst and by hyperactivation of GTPase

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Our results suggest that Boi1/2 function in secretion of Bgl2-containing vesicles, but not of invertase vesicles. Most exocyst mutants (including exo70 alleles) are blocked in secretion of both vesicle types (Novick et al., 1980; Harsay and Bretscher, 1995; Guo et al., 1999; Wu et al., 2010). However, the hypomorphic exo70-35 and

**FIGURE 7:** The SH3 domain of Boi2 is necessary and sufficient for abscission inhibition. (A) Cells of the indicated strains were released from a G1 block and imaged at time intervals in galactose-containing media. The localization of Boi2 to either the cortex or the bud neck (left) was determined by wide-field microscopy (≥30 cells/time point). (B) Abscission analysis as in Figure 6, except that the cultures were grown to mid log phase in galactose-containing media. Data are from cells pooled from two to three independent experiments. (C) Localization of Boi2-GFP and SH3_{Boi2}-GFP as in A, except that cells were released from a G1 block and imaged in glucose-containing media (N ≥ 70 cells/time point). (D) Abscission analysis as in B except that cells were grown in glucose-containing media. Data are from cells pooled from three independent experiments.

fusion with the PM by SNARE complexes. Accordingly, a recent study reported that both the exocyst and the SNARE-associated protein Sec1 interact with Boi1/2, and that overexpression of Sec1 restores exocytosis in Boi-depleted cells (Kustermann et al., 2017).
FIGURE 8: EXO70* is a suppressor of abscission defects in topoisomerase-II-deficient cells. (A) Abscission analysis as in Figure 5, A and B. Data are from cells pooled from two to three independent experiments. (B) Localization of Sec4-GFP and histone 2B (Htb2) fused to mCherry in late mitotic cells. Arrows mark chromatin bridges in top2-4 mutants. Time 0 marks the appearance of Sec4 at the bud neck. Levels of Sec4-GFP at the bud neck, expressed as percent of the total GFP fluorescence in the cell, were measured in sum projections of confocal Z-stacks spanning the entire cell. Mean and SEM are shown. Data are from cells pooled from two to four experiments. (C) Maximal levels (mean and SEM) of Sec4-GFP at the bud neck of the indicated strains. Asterisks denote statistically significant differences from the wild type (p < 0.05, Student’s t test).
exo70-38 mutants, like Boi1/2-depleted cells, are specifically impaired in secretion of Bgl2 but not invertase vesicles (He et al., 2007). Therefore Boi1/2 might specifically target Bgl2 (and perhaps other) vesicles for tethering by exocyst complexes during bud growth.

**Role of Boi2 in inhibition of abscission**

In both yeast and human cells, chromatin bridges can cause inhibition of abscission through the NoCut abscission checkpoint, but the molecular mechanisms involved remain poorly understood. In human cells, this process involves regulation of the endosomal sorting complex required for transport (ESCRT) III complex, which associates with the PM at the site of cytokinesis and regulates abscission timing (Carlton et al., 2012). Our data indicates that in yeast, abscission inhibition relies on association of the SH3 domain of Boi2 with the abscission site. Although both Boi-dependent functions, in polarized growth and abscission, may involve PM-remodeling processes, the relevant molecular mechanisms could be distinct. Indeed, the essential role of Boi1 and Boi2 for polarized growth and exocytosis depends on their PH domains, which are required for association with the bud cortex and are essential for cell viability (Hallett et al., 2002; Kustermann et al., 2017). In contrast, we find that the SH3 domain of Boi2 is dispensable for viability, but is both necessary and sufficient for bud neck targeting and inhibition of abscission. Interestingly, the SH3 domains of Boi1 and Boi2 can interact with exocyst components (Tonkian et al., 2009), and the exocyst is required for Boi2 recruitment to the bud neck. Whether direct Boi2-exocyst interactions are relevant for the control of abscission in cells with chromosome segregation defects remains an important question for future studies.

It has been suggested that Rho-like GTPases mediate exocyst activation through the induction of conformational changes in Exo70 (Wu et al., 2008). This allosteric regulation model was further supported by the identification of point mutations in EXO70, including EXO70A, that support growth of cdc42 and rho3 mutants defective in exocytosis (Wu et al., 2010). Our finding that EXO70A mutants are defective in the NoCut checkpoint suggests that allosteric regulation of the exocyst may play a key role in the regulation of abscission timing in yeast, and perhaps also in human cells. Exo70A complexes might be refractory to modulation by Boi1/2, thereby enabling multiple exocytic pathways independently of Boi1/2 or topoisomerase-II defects, and explaining proper growth of boi1 boi2 EXO70A and normal cytokinesis in top2-ts EXO70* mutants. Accumulation of Sec4-GFP at the abscission site in top2-ts cells may thus represent inhibition of exocytosis during cytokinesis. Consistent with this idea, inhibition of the exocyst prevents completion of abscission in budding yeast (Dobbelaere and Barral, 2004). EM imaging failed to detect vesicles in the vicinity of the bud neck during cytokinesis in top2 mutants (Amaral et al., 2016, and unpublished data), but this could be due to the short time during which Sec4 accumulates at the bud neck during cytokinesis. Alternatively, accumulation of Sec4 in the bud neck of top2 mutant cells may reflect alterations in a nonexocytic membrane-remodeling process such as endocytosis, which can also be regulated by the exocyst (Jose et al., 2015). A detailed characterization of specific membrane and protein trafficking events during abscission, and the potential role of exocyst complexes in regulating these processes, should provide valuable insight into the mechanisms of abscission regulation in response to chromatin bridges.

**MATERIALS AND METHODS**

**Strains and media**

*S. cerevisiae* strains are derivatives of S288c, except boi1Δ boi2Δ and its parental wild-type strain (kind gifts from E. Bailly, INSERM) which have the BFA264-15D background. Yeast cells were grown in YPD/YPG/YPR (1% bacto-yeast extract; 2% bacto-peptone; 2% dextrose, galactose or raffinose; and 0.004% adenine). Gene tagging and deletions were generated by standard PCR-based methods. The EXO70A (G488R) plasmid was generated by site-directed mutagenesis (Quick-change; Stratagene) of a GAL1-EXO70 centromeric vector (Open Biosystems). To integrate EXO70A* in its native locus in S288c strains, EXO70A* was tagged with the HA-natNT2 cassette (Janke et al., 2004) in boi1Δ boi2A SUP, and a PCR-derivated fragment of EXO70A*-HA:natNT2 was used in a one-step allele replacement; correct strains were identified by sequencing. PB3065 (bem2-84 LEU2/Bgl2) (Atkins et al., 2013) was used for one-step gene replacement at the BEM2 locus, and the mutation was confirmed by sequencing.

**Time-lapse and fluorescence microscopy**

Time-lapse microscopy was performed on cells in log phase, or after synchronization with α-factor as indicated, plated in minimal synthetic medium in concanavalin A-coated Lab-Tek chambers (Nunc) and placed in a preequilibrated temperature-controlled chamber. Imaging was performed using a Cell Observer HS microscope with a 100×, 1.4 NA objective and an AxioCam MRx camera (Zeiss) (Figures 1 and 3), an AF6000 wide-field microscope (Leica) with a 100×, 1.4 NA objective and an iXon 885 DU EM-CCD camera (Andor Technology; Figures 2 and 5C), or a Revolution XD spinning disk confocal microscope with a 100×, 1.45 NA objective and an iXon 897E Dual Mode EM-CCD camera (Andor Technology; Figures 5 and 6 and Supplemental Figure S4). Bud volumes were calculated from DIC stacks using ImageJ and the BudJ plug-in (Ferrezuelo et al., 2012) customized for analysis of Z-stacks. To visualize F-actin, cells were fixed with 3.7% formaldehyde for 1 h, washed in phosphate-buffered saline (PBS), incubated with 0.2 U/ml Alexa 488-phalloidin for 1 h at 25°C, washed in PBS and visualized immediately in 80% glycerol/20% PBS. Abscission assays were performed as in Amaral et al. (2016). Briefly, the PM was visualized via GAL1-10 promoter driven GFP-CAAX integrated in the HIS3 locus. Expression of GFP-CAAX in glucose media was driven by the chimeric ADH1-pr-Gal4-ER-VP16 transcription factor (Louvion et al., 1993) (URA3; gift from Francesc Posas, Universitat Pompeu Fabra) and addition of 90 nM β-estradiol (Sigma) 2 h before imaging. Only cells starting cytokinesis (membrane ingestion) at least 40 min before the end of image acquisition were considered for the quantifications of abscission. Fluorescence intensities were measured from single sections of each cell to score membrane separation (absission).

**Electron microscopy**

Cells were cryoimmobilized using an EM HPM 100 high-pressure freezer (Leica), freeze-substituted in anhydrous acetone containing 2% glutaraldehyde and 0.1% uranyl acetate (Figure 3) or 2% OsO4 and 0.1% uranyl acetate (Figure 5) and warmed to room temperature (EM AFS-2; Leica). After several acetone rinses, cells were incubated with 1% tannic acid, washed, incubated with OsO4 at 1% acetone, washed again, infiltrated with Epon resin and polymerized at 60°C. Ultrathin sections were obtained using an Ultracut UC6 ultramicrotome (Leica) and observed in a Tecnai Spirit electron microscope (FEI, The Netherlands) equipped with a MegaView III CCD camera.

**Sequencing and bioinformatics analysis**

Strains were sequenced using Illumina Genome Analyzer II or HiSeq paired-end technology. Reads were trimmed before the genome assembly at the base with quality lower than 20 using FASTX-Toolkit (Cold Spring Harbor Laboratory; http://hannonlab.cshl.edu/fastx_toolkit). Subsequently reads shorter than 31 bases (and their
pairs) were discarded. Assemblies for each strain were created de novo using Velvet v. 1.1.02 (Zerbino and Birney, 2008). The insert size of the paired-end reads was estimated by aligning reads onto the assembly created from single reads. The optimal k-mer length (k) for each strain was chosen using VelvetOptmiser.pl to maximize the total number of bases in contigs longer than 1 kb (Lbp). In addition, we used autoestimation of coverage cutoff and removed contigs shorter than 1 kb. Suplemental Table S3 lists detailed information about each resulting assembly. Genomic reads were aligned onto the Saccharomyces Genome Database (SGD) reference strain using Bowtie2 v. 2.0.0-beta7 (Langmead and Salzberg, 2012). SNPs were detected using bam2snpe ref.py v1.0. In brief, SNPs were called at sites with different genotypes in a given strain than in the wild-type strain. Only SNPs with high coverage (>10x) and low ambiguity (>90% of aligned reads call the same nucleotide at that position) at both the mapped reads of the given mutant strain and the reference were accepted. This filtering procedure was able to discard likely false-positive SNPs.

Depth-of-coverage variation was analyzed with IGV v 2.3.32 (Thorvaldsdóttir et al., 2013) to identify potential duplications or deletions. In addition, we analyzed read pairs with incongruent distance or orientation to identify possible rearrangements, that is, inversions, deletions, duplications, or translocations using bam2sv.py v1.0b. Finally, we used de novo contigs aligned against wild-type reference assembly as an independent line of evidence for potential rearrangements detection. Alignments were created by MUMer v3.07 with default parameters (Kurtz et al., 2004). Only best reciprocal matches were further accepted, but partial overlaps between matches were allowed. A minimal alignment length of 65 base pairs was set as a threshold. In addition, cutoff of 200 base pairs was set for deletion detection.

Genomic libraries were deposited to Short Read Archive (PRJEB7711). bam2sv.py and bam2snp.ref.py are available at https://bitbucket.org/lpryszcz/bin.

**Secretion assays**

Invertase secretion was assessed essentially as described previously (Goldstein and Lampen, 1975). Briefly, cells were grown to logarithmic phase and transferred to 0.1% glucose YPDA to induce invertase expression, either in the presence of 90 nM Boi1∆ or incubated with 0.5 mM NAA (boi1∆ boi2-aid). At 37°C (for sec6-4), after 2 h, cells were centrifuged and washed with cold 10 mM Na3PO4, and equal cell numbers were transferred to tubes with 10 mM Na3PO4/0.2% Triton X-100, which were then vortexed and freeze-thawed (total invertase expression). Reactions were stopped after 30 min with 0.2 M β-mercaptoethanol; 10 mM Na3PO4; 50 mM KH2PO4/KOH, pH 7.4; 1.4 M sorbitol; 10 mM NaN3. Then cells were resuspended in spheroplasting buffer containing 167 μg/ml zymolyase 100T (Seikagaku Biobusiness) and incubated with gentle mixing. Spheroplasts were harvested and resuspended in sample buffer before separation by SDS–PAGE and Western blotting to detect Bgl2 (specific antibody gift from Randy Schekman, University of California, Berkeley) and glucose-6-phosphate dehydrogenase (G6PDH) as a loading control (Sigma).

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