Interaction among Bt1p, Bt2p, and Ist2p Reveals Potential Interplay among the Vacuole, Amino Acid Levels, and Ion Homeostasis in the Yeast *Saccharomyces cerevisiae*

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Bt2p, a novel cytosolic coiled-coil protein in *Saccharomyces cerevisiae*, was previously shown to interact with and to be necessary for the correct localization of Rhb1p, a regulator of arginine uptake, and Yif1p, a Golgi protein. We now report the biochemical and physical interactions of Bt2p with Ist2p, a plasma membrane protein that is thought to have a function in salt tolerance. A deletion in Bt2p (*btn2Δ* strains) results in a failure to correctly localize Ist2p, and strains lacking Bt2p and Ist2p (*btn2Δ ist2Δ* strains) are unable to grow in the presence of 0.5 or 1.0 M NaCl. Bt2p was originally identified as being up-regulated in a *btn1Δ* strain, which lacks the vacuolar-lysosomal membrane protein, Bt1p, and serves as a model for Batten disease. This up-regulation of Bt2p was shown to contribute to the maintenance of a stable vacuolar pH in the *btn1Δ* strain. Bt1p was subsequently shown to be required for the optimal transport of arginine into the vacuole. Interestingly, *btn1Δ ist2Δ* strains are also unable to grow in the presence of 0.5 or 1.0 M NaCl, and *ist2Δ* suppresses the vacuolar arginine transport defect in *btn1Δ* strains. Although further investigation is required, we speculate that altered vacuolar arginine transport in *btn1Δ* strains represents a mechanism for maintaining or balancing cellular ion homeostasis. Bt2p interacts with at least three proteins that are seemingly involved in different biological functions in different subcellular locations. Due to these multiple interactions, we conclude that Bt2p may play a regulatory role across the cell in response to alterations in the intracellular environment that may be caused by changes in amino acid levels or pH, a disruption in protein trafficking, or imbalances in ion homeostasis resulting from either genetic or environmental manipulation.

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BTN2 in the yeast *Saccharomyces cerevisiae* encodes a non-essential novel coiled-coil protein, Bt2p, which was originally identified by gene expression profiling as one of only two proteins up-regulated in yeast strains lacking Bt1p (*btn1Δ* strains), the yeast model for Batten disease (24). This up-regulation of Bt2p in *btn1Δ* strains was originally presumed to occur in response to altered vacuolar pH because deletion of either *BTN1* or *BTN2* results in an alteration of the ability of yeast cells to maintain balanced pH homeostasis in the vacuole (1, 24). It was subsequently determined that Bt2p interacts with Rhb1p (previously designated Rsg1p), which regulates the activity of plasma membrane Can1p arginine and lysine permease (2, 27). Furthermore, deletion of *BTN2* results in altered arginine uptake, a phenotype exhibited by rhb1Δ strains. This altered arginine uptake in *btn2Δ* strains was shown to result from a failure to localize Rhb1p to a distinct peripheral structure, thereby causing a loss of the regulation of arginine uptake by the Can1p permease (2). Therefore, Bt2p was implicated in regulating intracellular levels of arginine, an assertion supported by the fact that Bt1p has been shown to have a role in the transport of arginine across the vacuolar membrane (12). Thus, the original observation that Bt2p was up-regulated in *btn1Δ* strains was interpreted to be a compensatory mechanism for balancing intracellular arginine levels, although the functions of both Bt1p and Bt2p still require further study.

Bt2p has also been shown to interact with Yif1p (3), an essential protein that is a component of a complex at the Golgi apparatus that interacts with transport GTPases, such as Ypt1p, Ypt31p, and Sec4p, and that functions in transport from the endoplasmic reticulum to the Golgi apparatus (16). In the absence of Bt2p (*btn2Δ* strains), Yif1p becomes mis-localized to or accumulates in the vacuole. Therefore, disruption of Bt2p function alters the trafficking of both Rhb1p and Yif1p, which have different functions in different parts of the cell. These results implicate Bt2p as having a function in intracellular protein trafficking and in maintaining intracellular metabolite homeostasis and suggest that disturbances in intracellular homeostasis and protein trafficking may be linked.

We report a third protein interaction for Bt2p. We demonstrate that Ist2p, a plasma membrane protein that has been reported to show homology to sodium and calcium channel proteins (15) and to have a function in salt tolerance (5), interacts physically and biochemically with Bt2p. Similar to the situation for other Bt2p-interacting proteins, the disruption of Bt2p function results in a failure to correctly localize Ist2p. Moreover, the disruption of both Bt2p and Ist2p (*btn2Δ ist2Δ* strains) reveals a potential functional interaction between these two proteins through sensitivity of growth in the presence of NaCl. Therefore, *btn2Δ* results in the altered subcellular...
localization of the interacting proteins Rhb1p, Yif1p, and Ist2p, which have been implicated in a variety of cellular processes, such that btn2Δ results in pleiotropic phenotypes. Therefore, the up-regulation of Btn2p in btn1Δ strains may represent a compensatory mechanism for balancing disturbances in each of these biological processes, namely, the regulation of amino acid levels, protein trafficking, and ion homeostasis. In addition, we demonstrate that btn1Δ ist2Δ strains also exhibit NaCl sensitivity and that ist1Δ can suppress the vacuolar arginine transport defect in btn1Δ strains. As human Cln3 has been shown to complement Bn1p function in btn1Δ strains (9, 12, 23), the implications of these results are also discussed in the context of Batten disease.

MATERIALS AND METHODS

Yeast strains and plasmids. The S. cerevisiae strains used in this work are listed in Table 1. Yeast strain BY1178 was acquired from the American Type Culture Collection, and strain YPH499 was obtained from Stratagene. Strains B-13048 (btn1Δ), B-14248 (btn1Δ btn2Δ), B-14252 (btn1Δ btn2Δ), and B-14399 (btn2Δ btn1Δ) were previously described (2).

Strains constructed for this study were generated by using theloxP-KanMX-loxP disruption cassette (7). The btn2Δ::loxP-KanMX-loxP disruption cassette was amplified by using forward primer 5′-CAAGAAATTAAAGACATCACTAGCAGACATAATGATACCC-3′ and reverse primer 5′-CGGCCCATTGCGTACGGATCTGTGAGACCTTAAATGAGATTTGATAAAAAGATGGG-3′. The parental strain BY1178 competent cells were transformed with Btn1p::KanMX-Btn2p::KanMX disruption cassette. The transformants were selected on 5-fluoroorotic acid plate medium containing 5-FOA (0.67% yeast nitrogen base without amino acids, 2% dextrose, 1% Bacto yeast extract, 2% Bacto peptone) supplemented with 200 μg of G418 (G-418 sulfate; Invitrogen)/ml.

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### TABLE 1. Yeast strains used in this study

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All integration events were verified by PCR analyses. The primers used in the PCR to validate replacement of the genes were kanFW (5′-CCT CGA CAT CAT CTG CCC-3′) and kanRE (5′-GGA TGT AGC TGC TAA ATG-3′). These primers anneal to regions inside the two loxP sequences. Also, primers annealing to regions 350 to 500 bp upstream of the ATG translational start site and 350 to 500 bp downstream of the stop codon of BTN1, BTN2, or IST2 were used in combination with primers kanFW and kanRE to confirm homologous integration at the desired locus.

Double-deletion strains were constructed by using the Cre-loxP recombination technique. Plasmid pCP133 contains the Cre recombination gene under the control of the GAL1 promoter. Expression of the Cre recombinase results in precise excision of the KanMX marker, allowing for the selection marker to be reused for the construction of double-deletion strains. The primers used to validate homologous integration (see above) were used to confirm excision of the KanMX marker at the BTN1 or IST2 locus. Plasmid pCP133 was removed from each of the strains lacking the KanMX marker by streaking 108 cells onto a solid medium (2% glucose and 0.67% yeast nitrogen base with amino acids) containing 5-fluoorotic acid and selecting for uracil auxotrophs.

For complementation studies, IST2-c·myc and enhanced green fluorescent protein (EGFP·IST2) were independently subcloned into centromeric single-copy expression vector pAB625 (1). NaCl sensitivity. To assay NaCl sensitivity, growth on agar-solidified media was assessed by spotting 5 μl of cell suspension containing approximately 106, 105, 104, or 103 cells onto YPD medium, YPD medium-0.5 μM NaCl, or YPD medium-1.0 μM NaCl. The plates were incubated for 2 to 3 days at 30°C.

Two-hybrid studies and coimmunoprecipitation of Btn2p and Ist2p. The S. cerevisiae yeast strain YPH499 (Table 1). Protein-protein interactions were confirmed by immunoprecipitation followed by Western analysis as previously described (2). The blot was probed with anti-c·myc mouse monoclonal antibody (1:1,000; New England Markers) or anti-FLAG mouse monoclonal antibody (Sigma) and with horse-radish peroxidase-tagged anti-mouse secondary antibody (1:3,000; Amersham). The blot was stained by using an ECLplus kit (Amersham) and developed on ECL film.

Localization of EGFp·Bnt2p and EGFp·Ist2p. EGFp·Bnt2p was previously described (2). Plasmid pDAP124 contains yeast EGFp at the N terminus of the IST2 ORF, which is downstream of the MET promoter. To construct plasmid pDAP124, a 2,885-bp BamHI/Sall fragment containing the IST2 ORF was isolated from plasmid pDAP106 and ligated into the corresponding sites of pUG34. Cells were grown to stationary phase in synthetic dextrose minimal medium (0.67% yeast nitrogen base without amino acids, 2% dextrose, 1.3 g of amino acid

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dropout powder/600 ml) lacking histidine or both methionine and histidine on a rotary shaker at 30°C. Cells were diluted 1:100 in synthetic dextrose minimal medium lacking histidine or both methionine and histidine and grown to an optical density at 600 nm of 0.2. Vacuoles were stained with FM4-64 (Molecular Probes) and prepared for confocal microscopy as previously described (2). Confocal microscopy was performed with a Leica TCS SP microscope equipped with argon, krypton-argon, and UV lasers and 100 × 1.3NA lenses. Images were processed by using Photoshop 7.0 (Adobe).

**Isolation of yeast vacuoles.** Yeast cells grown to an optical density at 600 nm of 0.6 to 0.7 in 1 liter of medium were washed once with sterile distilled water and then once with 1.0 M sorbitol. Cells were converted to spheroplasts by suspension of the cell pellet in 100 ml of 1.0 M sorbitol containing 400 U of Zymolase 10100T (ICN Pharmaceuticals). The culture was gently shaken for 90 min at 30°C. Spheroplasts were collected by centrifugation at 800 × g for 5 min and then washed twice with 1.0 M sorbitol. All subsequent manipulations were carried out at 4°C. The pellet was suspended in 25 ml of buffer A (10 mM morpholineethanesulfonic acid [MES]-Tris [pH 6.9], 0.1 mM MgCl₂, 12% Ficoll 400) and homogenized by six or seven strokes in a Dounce homogenizer. The lysate was cleared by centrifugation at 26,600 × g for 35 min. The top wafer layer was collected and placed into a Dounce homogenizer containing 6 ml of buffer A, and clumps were broken up by five or six strokes. The homogenate was transferred to an ultracentrifuge tube and layered with 6 ml of buffer B (10 mM MES-Tris [pH 6.9], 0.1 mM MgCl₂, 12% Ficoll 400) and homogenized by six or seven strokes in a Dounce homogenizer. The lysate was cleared by centrifugation at 26,600 × g for 35 min. The top wafer layer was collected and placed into a tube containing 6 ml of buffer B (10 mM MES-Tris [pH 6.9], 0.1 mM MgCl₂, 8% Ficoll 400). The mixture was centrifuged at 26,600 × g for 30 min. The top wafer layer was collected and placed into a tube containing 6 ml of buffer C (10 mM MES-Tris [pH 6.9], 5 mM MgCl₂, 25 mM KCl). Vacuoles were converted to vesicles by the addition of 2 volumes of buffer C, and a pellet was obtained by centrifugation at 26,600 × g for 20 min. The purity of the vacuoles obtained by this isolation procedure was verified by confocal microscopy, and vesicle enrichment was confirmed by Western analysis with vesicular markers (1, 12).

**Assays of arginine transport.** Arginine uptake assays were performed as previously described (13, 17, 18, 12). The accumulation of 1⁴C-arginine by vacuolar vesicles at each time point, at time zero, and at 30-s increments was assayed with argon, krypton-argon, and UV lasers and 100 × 1.3NA lenses. Images were processed by using Photoshop 7.0 (Adobe).

**RESULTS**

**Btn2p interacts with Ist2p.** We used the Cytotraps two-hybrid system to screen for Btn2p interactions by cotransformation of yeast strain CDC25H with BTN2-pSOS and a yeast cDNA library in pMyr as previously described (2, 3). We had previously characterized Rbh1p and Yif1p as the most common candidates for interactions with Btn2p (2, 3). In addition, we isolated a single clone of IST2 that codes for a putative Na⁺/K⁺ ATPase (Myt1p) (2). We therefore tested whether functional Btn2p-EGFP localized to the cytosol (2). Because the disruption of Btn2p function alters the localization of its interacting partners, Rbh1p and Yif1p, we tested whether the absence of Btn2p affected the localization of Ist2p. Ist2p has been reported to be localized at the plasma membrane (26). We confirmed that EGFP-Ist2p (N-terminal fusion of EGFP to Ist2p) in ist2Δ strains localizes to the plasma membrane (Fig. 2A). Furthermore, we demonstrated that in btn2Δ ist2Δ strains, EGFP-Ist2p is no longer localized to the plasma membrane but appears predominantly as a punctate entity in the cytosol (Fig. 2B). Interestingly, a small amount of EGFP-Ist2p appears to be localized in the vacuolar membrane; however, subcellular fractionation studies will be required to confirm this finding. These data suggest that the absence of Btn2p alters the localization of Ist2p and that Btn2p may therefore be involved in the trafficking or localization of Ist2p to the plasma membrane. Each image shown is typical of what was seen for the entire cell population for each strain. EGFP-Ist2p is functional, as determined by functional complementation of a phenotype that is described later (see Fig. 3). Note that Btn2p localization to the cytosol is not altered in ist2Δ strains (data not shown).

**The growth of btn2Δ ist2Δ strains is NaCl sensitive.** Ist2p has been reported to show similarity to higher eukaryotic sodium and calcium channel proteins (15). We therefore tested the sensitivity of the growth of ist2Δ strains to elevated concentrations of sodium or calcium in the media. Deletion of IST2 was previously reported to result in salt tolerance (15); however, we found that ist2Δ strains did not show an obvious growth defect in the presence of elevated levels of sodium or calcium. However, btn2Δ ist2Δ strains failed to grow in media.

**FIG. 1.** Btn2p interacts with Ist2p in vivo. Immunoprecipitation was performed with anti-FLAG antibody and cell extracts derived from strain YPH499 expressing Btn2p-FLAG or both Btn2p-FLAG and Ist2p–c-myc. (Upper panel) Western analysis performed with anti-c-myc monoclonal antibody. (Lower panel) Western analysis performed with anti-FLAG monoclonal antibody. Lanes: 1, cell extract of YPH499 expressing Btn2p-FLAG; 2, cell extract of YPH499 expressing Btn2p-FLAG and immunoprecipitated with anti-FLAG antibody; 3, cell extract of YPH499 expressingBtn2p-FLAG and Ist2p-c-myc; 4, cell extract of YPH499 expressing Btn2p-FLAG and Ist2p-c-myc and immunoprecipitated with anti-FLAG antibody. Size markers are indicated on the right. The arrow indicates the Ist2p–c-myc band.
containing 0.5 or 1.0 M NaCl (Fig. 3). There was no effect on growth for the same strains in the presence of elevated calcium levels (data not shown). We also found that deletion of Rhb1p, which also interacts with Btn2p, does not result in an NaCl sensitivity phenotype. Furthermore, deletion of Ist2p does not result in the canavanine resistance phenotype previously reported for strains lacking Rhb1p or Btn2p (2). The NaCl sensitivity phenotype exhibited by btn2Δ/ist2Δ strains could be complemented by the expression of plasmid-borne BTN2 or IST2 (data not shown) and by c-myc- or EGFP-tagged IST2, as shown in the coimmunoprecipitation and localization studies described above, respectively (Fig. 3).

The growth of btn1Δ/ist2Δ strains is also NaCl sensitive. Btn2p was originally identified as being up-regulated in a btn1Δ strain. We therefore tested whether btn1Δ results in sensitivity of growth in media containing NaCl. Although the growth of btn1Δ strains did not show NaCl sensitivity, btn1Δ btn2Δ and btn1Δ ist2Δ strains were both unable to grow in media containing 0.5 or 1.0 M NaCl (Fig. 3). There was no effect on growth for the same strains in the presence of elevated calcium levels (data not shown).}

**FIG. 2.** Localization of Ist2p to the plasma membrane and alteration of the localization of Ist2p in btn2Δ strains. (A) EGFP-Ist2p in ist2Δ strain B-14915. (B) EGFP-Ist2p in btn2Δ/ist2Δ strain B-14983. (a) EGFP fluorescence. (b) Differential interference contrast images. (c) FM4-64 staining showing the vacuolar membrane. (d) Merged images. EGFP-Ist2p in the ist2Δ strain localizes to the plasma membrane. EGFP-Ist2p in the btn2Δ/ist2Δ strain appears to be mislocalized to the cytoplasm and the vacuolar membrane (arrows 1 and 2, respectively). Each image is presented at a magnification of ×100 and is typical of that seen for the entire cell population.

**FIG. 3.** btn2Δ/ist2Δ strains are sensitive to NaCl. Wild-type (B-11718), ist2Δ (B-14884), btn2Δ (B-14847), and btn2Δ ist2Δ (B-15013) strains were serially diluted. Wild-type, ist2Δ, and btn2Δ cells grew normally, whereas btn2Δ ist2Δ cells exhibited sensitivity of growth. Plates were incubated at 30°C for 2 days. For complementation studies, IST2–c-myc and EGFP-IST2 were independently subcloned into centromeric single-copy expression vector pAB625 and similarly plated (1).
media containing 0.5 or 1.0 M NaCl (Fig. 4). Because Btn2p has been shown to interact with Rhb1p, we demonstrated that the growth of btn1Δ/Rhb1Δ and btn2Δ/Rhb1Δ strains did not show NaCl sensitivity. Therefore, the NaCl sensitivity phenotype is not a result of interactions with Btn2p but is a result of the loss of distinct interactions between Btn2p and Ist2p or Btn1p and Ist2p. Strains lacking Yif1p, which also interacts with Btn2p, could not be tested, as this is an essential protein.

We also performed FM4-64 staining to test the vacuolar integrity and morphology of cells exposed to NaCl. We found that strains containing btn1Δ appear to have more fragmented vacuoles. However, as the dynamics of this technique have not been fully characterized in the presence of high salt concentrations, we report this finding as a qualitative assessment while we work to establish a reliable means of isolating vacuoles from salt-exposed strains to quantify the possible effects that NaCl may have on vacuoles of btn1Δ strains as opposed to other strains.

ist2Δ suppresses the defect in vacuolar arginine transport in btn1Δ strains. We previously reported that vacuoles isolated from btn1Δ strains have a decreased ability to transport arginine into the lumen of this organelle (12). To test whether...
Ist2p exerts an effect on the transport of arginine into the vacuole, we examined the effect of deleting IST2 (ist2Δ) on vacuolar arginine transport. Compared to the wild type, ist2Δ did not significantly alter arginine transport into the vacuole (Fig. 5). However, btn1Δ ist2Δ strains showed nearly normal levels of arginine transport into the vacuole, indicating that ist2Δ suppressed this defect in btn1Δ strains and revealing a distinct interaction between Btn1p and Ist2p.

**DISCUSSION**

**BTN2** encodes a 410-amino-acid novel coiled-coil protein that has a role in localizing proteins to different subcellular compartments. Btn2p was originally identified as being up-regulated in a btn1Δ strain. The functional implications of Btn1p and Btn2p interactions as a result of btn1Δ are summarized in Fig. 6. Btn1p is a vacuolar protein, and btn1Δ results in altered vacuolar pH and decreased sequestration of the basic amino acids arginine and lysine in the vacuole (Fig. 6A) (4, 12, 24). A previous study revealed that btn2Δ results in elevated activity of the vacuolar H⁺-ATPase, suggesting that the up-regulation of BTN2 expression in btn1Δ strains may contribute either directly or indirectly to normalization of the vacuolar pH in btn1Δ strains (24). However, btn2Δ does not result in altered vacuolar pH, and btn1Δ does not result in altered vacuolar H⁺-ATPase activity, suggesting that there is no direct correlation between vacuolar pH and vacuolar H⁺-ATPase activity (1). Interestingly, the only other gene up-regulated in btn1Δ strains in addition to BTN2 is HSP30 (24). The up-regulation of HSP30 was shown to down-regulate the activity of the plasma membrane H⁺-ATPase, which was presumed to result in elevated use of ATP as well as elevated proton pumping out of the cell (Fig. 6A). However, as we discuss below, we now believe that btn1Δ results in an alteration in ion homeostasis, perhaps due to altered amino acid levels, and this alteration in the regulation of plasma membrane H⁺-ATPase activity through Hsp30p could be interpreted as a means of balancing the ionic content of cells.

Btn2p is a cytosolic protein that has now been implicated in localizing Rhb1p to the cell periphery, Yif1p to the Golgi apparatus, and now Ist2p to the plasma membrane (Fig. 6B). There is little to link Rhb1p, Yif1p, and Ist2p at the functional level other than the fact that each interacts with Btn2p and that each is mislocalized in the absence of Btn2p. We have therefore discovered a novel link among Rhb1p, Yif1p, and Ist2p that implies that Btn2p has the potential to affect several cellular processes. Disruption of Btn2p function can result in a variety of different phenotypes that are associated with the mislocalization of Bnt2p-interacting proteins. In this study, we have shown that Btn2p physically interacts with Ist2p and that Btn2p is necessary for the correct localization of Ist2p. In addition, we have shown that btn2Δ or btn1Δ in combination with ist2Δ results in a similar phenotype, sensitivity to NaCl. The role of Btn2p and its multiple interactions are summarized in Fig. 6B. Btn2p may have a role in regulating the trafficking around the cell of a very specific set of proteins that have a functional link essential to maintaining a biological balance. In this sense, Btn2p may be a sensor of an imbalance or an effector that acts upon an imbalance. What Btn2p senses or responds to requires further study. However, clues that further identify the role of Bnt2p come from studies of btn1Δ strains that up-regulate Bnt2p, which are further discussed below and which suggest that Btn2p may have a role in sensing or responding to changes in cation levels in cells.

We demonstrate that btn1Δ strains lacking either Ist2p or Btn2p show decreased growth in the presence of high salt concentrations. Several Na⁺/H⁺ antiporters present in the vacuolar membrane have been implicated as functioning in vacuolar transport, vacuolar acidification, and ion homeostasis (10). Furthermore, several studies have concluded that H⁺ antiporters represent the principal mechanism of transporting both inorganic and organic cations across the vacuolar membrane (19). In addition, a family of seven proteins has been identified as mediating bidirectional amino acid transport at the vacuolar membrane (25). Btn1p has been shown to be involved in the transport of arginine into the vacuole (12), and arginine transport across the vacuolar membrane requires the generation of a proton motive force and an intact vacuolar ATPase (12, 17, 25). Disruption of Btn1p function disrupts the transport of arginine into the vacuole, resulting in a 10-fold decrease in the levels of sequestered arginine and lysine in the vacuole (12). Therefore, one could predict that an environmental insult, such as high salt concentrations, would alter the activity of vacuolar Na⁺/H⁺ antiporters and precipitate further disruptions in transport processes at the vacuolar membrane. Thus, disruption of both Btn1p at the vacuole and Ist2p at the plasma membrane (btn1Δ ist2Δ strains) results in cells being unable to maintain cellular cationic balance upon exposure to high salt concentrations. Similarly, the lack of Btn2p and its multiple roles in cells precipitate a similar situation in btn1Δ btn2Δ and btn1Δ ist2Δ strains.

It is fascinating that ist2Δ suppresses the vacuolar arginine transport defect exhibited by btn1Δ strains. Although further studies are required, it is apparent that disruptions that result in altered cation levels, whether they are organic, such as arginine, or inorganic, such as salt, have revealed a link that indicates that yeast cells may work to maintain an overall ionic balance of all or some of these ions. Although there appears to be no apparent correlation between vacuolar pH and vacuolar

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**FIG. 6.** Schematic representation of the cellular roles of Btn1p and Btn2p. (A) Step 1 shows the deletion of btn1Δ. Step 2 shows that btn1Δ results in decreased arginine uptake into the vacuole (12). Step 3 shows that btn1Δ results in the up-regulation of Btn2p (23). Step 4 shows that btn1Δ results in the up-regulation of Hsp30p (11, 23). Step 5 shows that the up-regulation of Hsp30p down-regulates overactive Pma1p (23). Step 6 shows that the up-regulation of Hsp30p decreases excess proton pumping across the plasma membrane (23). (B) Step 1 shows that Bnt2p interacts with Yif1p and is involved in correctly localizing this protein to the Golgi apparatus (3). Step 2 shows that Bnt2p interacts with and is involved in correctly localizing Rhb1p (2). Step 3 shows that Rhb1p negatively regulates the Can1p permease, which transports arginine and lysine into the cell (27). Step 4 shows decreased uptake of arginine into the cell (2). Step 5 shows that Bnt2p interacts with Ist2p, which shows homology to ion channel proteins and may be involved in the transport of an as-yet-unidentified cation across the plasma membrane.
H\(^+\)-ATPase activity, future experiments will focus on determining whether cation levels influence both vacuolar pH and vacuolar H\(^+\)-ATPase activity. We note that studies of other organisms have revealed that plants have been shown to mediate salt tolerance through ion uptake into the tonoplast (28). A complete understanding of this phenomenon, in particular, in \textit{bni1Δ} strains, will require a complete understanding of which proteins facilitate the transport of all molecules into and out of cells and also into and out of each subcellular compartment.

It was previously demonstrated that the protein associated with Batten disease, Cln3, and Btn1p have conserved functions. Btn1p is 39% identical and 59% similar to human Cln3 protein, mutations in which result in the lysosomal storage disorder Batten disease (11, 22). Batten disease is characterized by accumulation of lipopigments in the lysosome (6, 8, 14, 20, 21). In summary, \textit{bni1Δ} cells are known to have an altered regulation of vacuolar pH and a decrease in the ability to transport basic amino acids into the vacuole, features which presumably account for the decreased levels of basic amino acids in the vacuole. Our new findings suggest that these alterations in vacuolar content might result in these cells having an altered ability to maintain cellular and vacuolar ionic homeostasis. A limited ability to utilize the vacuolar compartment as a means to maintain ionic homeostasis might precipitate further vacuolar-lysosomal dysfunction. It is obviously difficult to compare ion homeostasis mechanisms between single-celled organisms, such as yeasts, and humans. However, a change in lysosomal function mediated by altered ionic content could conceivably result in the accumulation or aggregation of proteins that are usually targeted to the lysosome for degradation and might contribute to the characteristic accumulation of storage materials in the lysosome in Batten disease.

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