Rvs161p and Sphingolipids Are Required for Actin Repolarization following Salt Stress

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Received 15 July 2002/Accepted 29 September 2002

In *Saccharomyces cerevisiae*, the actin cytoskeleton is depolarized by NaCl stress. In this study, the response was maximal after 30 min, and then actin patches repolarized. Rvs161p was required for actin repolarization because the rvs161Δ mutant did not repolarize actin patches after growth in a salt medium. Mutations suppressing the rvs161Δ-related salt sensitivity all occurred in genes required for sphingolipid biosynthesis: *FEN1, SUR4, SUR2, SUR1*, and *IPT1*. These suppressors also suppressed *act1-1*-related salt sensitivity and the defect in actin repolarization of the rvs161Δ mutant, providing a link between sphingolipids and actin polarization. Indeed, deletion of the suppressor genes suppressed the rvs161Δ defect in actin repolarization in two ways: either actin was not depolarized at the wild-type level in a set of suppressor mutants, or actin was repolarized in the absence of Rvs161p in the other suppressor mutants. Rvs161p was localized as cortical patches that concentrated at polarization sites, i.e., bud emergence and septa, and was found to be associated with lipid rafts. An important link between sphingolipids and actin polarization is that Rvs161p was required for actin repolarization and was found to be located in lipid rafts.

Membrane lipid microdomains called rafts are formed by the lateral association of sphingolipids and cholesterol (ergosterol in yeasts). In mammalian cells, rafts are involved in endocytosis and exocytosis and more generally in lipid-mediated membrane trafficking (reviewed in reference 22), in the immunological response (23), and in establishing platforms for vesicle-linked actin polymerization, visualized as comets (36).

Rafts recruit specific sets of membrane proteins and exclude others. They harbor signaling molecules (phosphatidylinositol bisphosphate), kinases (Src) involved in signal transduction (29, 40), SNAREs (10, 26), and glycolipid-protein-anchored proteins (1, 22). This specific lipid-protein association forms insoluble complexes in cold, nonionic detergents such as Triton X-100 (9) that can be separated by floatation in density gradients in the form of detergent-resistant membranes (DRMs).

In the yeast *Saccharomyces cerevisiae*, rafts have been defined biochemically as DRMs and proven to be important for protein sorting through the endoplasmic reticulum and Golgi apparatus (1, 2). Importantly, sphingolipid biosynthetic intermediates are also signaling molecules (Fig. 1). In particular, the sphingoid base phytosphingosine is a signaling molecule required for endocytosis (21, 45) and ubiquitin-dependent proteolysis following heat stress (13).

The yeast *RVS161/END6* gene is involved in cell polarity (20), actin cytoskeleton polarization (41), endocytosis (30), and secretory vesicle trafficking (6). *rvs161* mutant cells die during stationary phase and are sensitive to NaCl (3, 16). *rvs161Δ*, together with a specific set of actin alleles, leads to synthetic lethality (5). Furthermore, the *end6-1* allele of *RVS161* displays nonallelic noncomplementation with the *act1-1* allele, while *rvs161Δ* complements *act1-1* (5, 30). Therefore, the primary defect of the *rvs161Δ* mutant lies in actin cytoskeleton organization.

*rvs161*-related salt sensitivity and cell death during starvation are suppressed by mutations in the *SUR1, SUR2, SUR4*, and *FEN1* genes (18, 34). Moreover, mutations in these suppressor genes lead to abnormal lipid composition (18), and they were recently shown to encode enzymes required for sphingolipid biosynthesis (19, 37). Therefore, we designed experiments to unravel the relationships between actin cytoskeleton organization, Rvs161p, and sphingolipids.

In this paper, we show that both Rvs161p and sphingolipids have major roles in depolarization and repolarization of actin following salt stress. We also establish that Rvs161p is associated with lipid rafts, suggesting for the first time a link between rafts and actin in *S. cerevisiae*. Finally, we show that Wsc1p is a sensor for actin depolarization after salt stress.

MATERIALS AND METHODS

Strains, media, and growth conditions. The *S. cerevisiae* strains used in this study are listed in Table 1. Complete medium was YPD (10 g of yeast extract, 20 g of Bactopeptone, and 20 g of glucose per liter) solidified with 20 g of agar per liter when required.

Minimal medium was SD (6.7 g of yeast nitrogen base [Difco] per liter) containing 20 g of glucose as the carbon source. SD-casa was SD supplemented with Casamino Acids (0.2%). To check for sodium chloride sensitivity, this medium contained 7% NaCl. To assay for the ability to grow under different conditions, droplets of cells resuspended in water containing 5 × 10⁷ cells/ml and serial dilutions were laid on different media, and growth was checked after 48 h.

For microscopic observations of the green fluorescent protein (GFP) fusion proteins, yeast cells were grown exponentially (<10⁷ cells/ml) in SD-casa supplemented with tryptophan when required. For NaCl-stress-induced experiments, cells were grown in liquid YPD to 3 × 10⁸ to 5 × 10⁹ cells/ml. Cells were collected by centrifugation and resuspended for different times in 10 ml of YPD or YPD containing 3% NaCl, an NaCl concentration that is sublethal for the *rvs* mutants. Matings, sporulations, and dissections were carried out according to standard

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FIG. 1. Sphingolipid biosynthetic pathway in _S. cerevisiae_, adapted from Dickson and Lester (19) and Schneiter (37). Genes are shown in capital italic letters. _LCB1_ encodes a subunit of the serine palmitoyl transferase, _FEN1_ and _SUR4_ encode the elongases and are partially redundant, _SUR2_ encodes a hydroxylase, _SUR1_ encodes a mannosyl transferase, and _IPT1_ encodes an inositol phosphotransferase. Abbreviations: CoA, coenzyme A; VLCFA, very long chain fatty acid; PI, phosphatidylinositol; DHS and DHS, sphingoid bases dihydrophosphogin and phytosphingosine, respectively. IPC, inositolphosphorylcereamide; MIPC, mannosyl-inositolphosphorylcereamide; and _M(IP)_2C, mannosyl-diinositolphosphorylcereamide.

DNA manipulations. Restriction enzymes and other DNA modification enzymes were purchased from Promega and used according to the specifications of the manufacturer. Amplifications of DNA fragments were done by PCR on a DNA Thermal Cycler (Perkin Elmer Cetus). Custom oligonucleotides were provided by Genset. Transformation of *Escherichia coli* was carried out by the CaCl2 method (31). Yeast cells were transformed by the one-step method (11).

**Plasmid construction.** The _RVS161-GFP_ gene was obtained in two steps. First, _RVS161_ was amplified by PCR with primers R161Bam (5'-GGGATCCGTAAGCAAGGTACGGTTA-3') and R161Kpn (5'-GGGATCCGGTAAGCAAGGTACGGTTA-3'). The PCR product was cloned with _BamHI_ and _KpnI_ into the _pFL44_ plasmid (4) after digestion of the GFP gene was directly cloned in the _pFL44_ plasmid (4) after digestion of the upstream region of _TCI_. The _Flp_ recombinase was used to delete the _FLP_ gene, which is the selectable marker, from the construct. The final construct was confirmed by sequencing.

**Visualization of polymerized actin.** Alexa-phalloidin staining of actin was carried out by a modification of the method of Kaiser et al. (25). Cells grown in standard concentration of 3.7% were centrifuged at 800 × g for 2 min and resuspended in ice-cold PBS. The actin cytoskeleton organization was assessed by immunofluorescence microscopy using anti-phalloidin antibody (Sigma). The nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). Images were acquired using an Axioskop 2 microscope (Carl Zeiss). The fluorescence intensity was quantified using ImageJ software.

**Centrifugation of the membrane fraction.** The membrane fraction was isolated from yeast cells by ultracentrifugation. The fraction was washed twice with PBS and resuspended in 20 mM Tris-HCl, pH 7.4, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Phosphorimager analysis.
FIG. 2. Rvs161p is required for actin repolarization following salt stress. Wild-type (WT, A to E) and rvs161Δ mutant (F to J) strains were grown in YPD at 30°C, and at time zero, NaCl was added to a final concentration of 3.4%. Aliquots were removed at different times as indicated. Samples were fixed, stained with Alexa-phalloidin, and observed by fluorescence and Nomarski microscopy to visualize the actin cytoskeleton and whole cells, respectively.
RESULTS

Rvs161p is required for actin repolarization following salt stress. Actin patch polarization is slightly impaired in the rvs161 mutant, and this defect is dramatically enhanced by salt stress (41). Moreover, it has previously been shown that actin is transiently depolarized in wild-type cells after salt stress (12, 27). Further characterization of actin depolarization and repolarization by kinetic analysis following salt stress in the wild type revealed that after 35 min in NaCl medium, there were few cables, and when present, they appeared diffuse and the actin patches were depolarized. However, after 90 min, cables were present along the axis in the mother cell and the patches were fully repolarized (Fig. 2). In rvs161 mutant cells, actin patches were depolarized after 35 min in salt-containing medium, as in the wild-type strain. However, further incubation of the rvs161 mutant in salt-containing medium (90, 150, or 240 min) resulted in the appearance of some faint unoriented cables, while the actin patches never repolarized (Fig. 2). It should be noted that the actin patches were slightly depolarized in the rvs161 mutant cells grown in regular medium (32% of cells partially depolarized versus 0% in the wild type; see Fig. 5). This could be due to small stresses occurring during normal growth with which the rvs161 mutant was unable to cope.

Therefore, while the wild-type cells depolarized actin following salt stress and repolarized after a period of adaptation, the rvs161 mutant was able to depolarize actin in response to salt stress but was unable to repolarize afterwards. Therefore, the Rvs161p protein is required for actin repolarization following salt stress.

Search for a signal to salt stress. Wsc1p is required for the actin patch depolarization response to heat stress (17). The timing of this heat stress response was similar to what we found above during salt stress (see also Fig. 5).

Wsc1p and Mid2p are thought to function as sensors following different stresses, such as heat and cell wall stress induced by Calcofluor or α-factor (32). Wsc1p proteins encompass a family of four homologous proteins with an N-terminal extracellular domain that is rich in cysteine, serine, and threonine, followed by a transmembrane domain and a C-terminal cytoplasmic tail (43). Mid2p is not homologous to Wscp but is like it structurally.

Therefore, we studied actin cytoskeleton polarization following salt stress in wsc1, wsc2, wsc3, wsc4, and mid2 mutant strains. While the wsc2, wsc3, wsc4, and mid2 mutants were able to depolarize actin patches comparably to the wild type (data not shown), the wsc1 mutant was severely impaired in depolarizing actin (Fig. 3). Indeed, 30 min after salt stress, the wsc1 mutant still presented 52% fully polarized cells (Fig. 3), compared to 8% in the wild type (Fig. 5), and only 25% fully depolarized cells, compared to 43% in the wild type. No more cells depolarized subsequently. Rather, repolarization occurred as in the wild type. Therefore, Wsc1p, a heat shock sensor for actin depolarization (17), also behaved as a sensor responding to salt stress.

Genetic relationships between mutations in sphingolipid metabolism genes and rvs161A or act1-1. The salt sensitivity phenotype of the rvs161A mutant was suppressed by mutations in the FEN1, SUR4, SUR2, and SURL genes (Fig. 4A and 4B) (18, 34). Since all these suppressor genes encode enzymes required for sphingolipid biosynthesis (Fig. 1), we assayed whether a mutation in the IPT1 gene, also involved in this pathway (Fig. 1), behaved as a suppressor of rvs161A. Indeed, the ipt1 rvs161 double mutant was no longer sensitive to salt, strengthening the hypothesis of a connection between Rvs161p function and sphingolipid synthesis (Fig. 4A and B).

According to growth on SD-casa at 30°C, the sphingolipid mutants fell into three classes: the fen1 rvs161 and sur4 rvs161A mutants grew at the same slow rate as the fen1 and sur4 mutants (class I); the sur2 rvs161A mutant grew at the rate of the sur2 mutant, close to the wild-type rate (class II); the sur1 and ipt1 mutants grew at the wild-type rate, but the double sur1 rvs161A and ipt1 rvs161A mutants grew much more slowly than the single mutants, showing a strong negative synergistic effect on growth between sur1 or ipt1 and the rvs161A mutation (class III) (Fig. 4A and B).

Since end6-1, an RVS161 allele, and act1-1 display nonallelic noncomplementation (30), Rvs161p and actin most probably belong to the same functional complex. This prompted us to test whether the fen1, sur4, sur1, sur2, and ipt1 mutations were also able to suppress the act1-1-related salt sensitivity phenotype. Indeed, all the sphingolipid biosynthesis mutations also partially suppressed the salt sensitivity of the act1-1 mutant (Fig. 4A and C). The resistance of the sur2 act1-1 mutant to salt was more obvious on YPD medium (not shown).

Therefore, these data further substantiate the involvement of Rvs161p and actin in the same function and strongly suggest that the latter involves sphingolipids.

Role of sphingolipid metabolism genes in salt stress and actin polarization. The fact that rvs161 suppressors were also able to alleviate the act1-1-related growth defects prompted us to study actin depolarization-repolarization kinetics following salt stress in fen1, sur4, sur2, sur1, and ipt1 mutants and in the double mutants (Fig. 5). All the single fen1, sur4, sur2, sur1, and ipt1 mutants were able to lose actin cables, to depolarize actin patches, and subsequently to repolarize, although the timing and extent of depolarization varied among the different mutants. Indeed, the fen1 and sur4 mutants depolarized faster than the wild type (maximal depolarization occurred at 20 min, while it was 30 min for the wild type [Fig. 5]) and repolarized like the wild type. The sur2, sur1, and ipt1 mutants were not able to depolarize actin to the same degree as the wild type.

Indeed, 30 min after salt stress, twice as many polarized cells were present in the mutants (16%, versus 8% in the wild type) and fewer fully depolarized cells were present (12%, versus 43% in the wild type), while a higher percentage of cells in an intermediate stage of depolarization (72%, versus 49% in the wild type) was observed. The sur2 and sur1 mutants repolarized to the same degree as the wild type, while ipt1 repolarization was lower and incomplete after 60 min (50%, versus 84% in the wild type).

Therefore, fen1 and sur4 mutants, carrying mutations in the genes encoding the first two enzymatic steps of the sphingolipid pathway, clearly depolarized faster than the wild-type cells, while the sur2, sur1, and ipt1 mutants, carrying mutations in the genes involved in subsequent biosynthetic steps, never fully depolarized. Roughly the same results were obtained when the double mutant strains between the rvs161A mutant and all these suppressor mutant genes were analyzed (Fig. 5).
Thus, as expected, all the suppressive mutations were epistatic on the rvs161 disruption. The suppressors allowed the rvs161Δ mutant to grow on salt-containing medium as a consequence of suppression of the defect in actin repolarization. Therefore, sphingolipids and biosynthetic intermediates might be involved in actin depolarization and repolarization.

**Rvs161p is linked to raft microdomains.** Complex sphingolipids are major components of lipid rafts. Since mutations in genes required for sphingolipid biosynthesis are suppressors of the rvs161Δ mutation, we could hypothesize that Rvs161p and other proteins required for actin depolarization and repolarization are localized in raft microdomains. In G1, Rvs161p-GFP was visualized as small cortical patches (Fig. 6) localized close to the plasma membrane, as revealed by the equatorial and top views. Later, while the patches remained cortical, Rvs161p-GFP was more concentrated at bud emergence (Fig. 6B). Finally, during cytokinesis, Rvs161p-GFP concentrated at the mother bud neck, where it could be visualized as a disk or a bar in most of the images (Fig. 6C). These results are in good agreement with previous data (7) showing that Rvs161p is found at polarization sites, in particular at the tip of the shmoo.

We then studied the localization of Rvs161p-GFP in the sphingolipid single fen1, sur4, sur2, sur1, ipt1, and lcb1-100 mutants; the last is a temperature-sensitive mutant impaired in the first step of sphingolipid biosynthesis (Fig. 1) that has been shown to be impaired in establishing DRMs (1). In sur2, sur1, and ipt1 deletants, the localization of Rvs161p was similar to what was seen in the wild-type strain (Fig. 7B), while Rvs161p-GFP was clearly mislocalized in the fen1 and sur4 deletants and in the lcb1-100 mutant. In the fen1 and sur4 mutants, cortical patches were still present but in a lower amount. No fluorescence could be detected in the septa, while undefined structures visible with Nomarski optics were stained (Fig. 7A). In the lcb1-100 mutant at the permissive temperature (26°C), cortical patches were present, but the septa were not stained, although the fluorescence increased in the mother bud neck area on the mother side. Importantly, when the lcb1-100 mu-

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**FIG. 3.** *wsc1* mutant impaired for actin cytoskeleton depolarization following salt stress. (A) The *wsc1* strain was grown and treated as described for Fig. 2. (B) Cells (~100) were classified and quantified according to their polarization state. Only cells with small buds were scored. Cells with actin patches concentrated in the small bud, with fewer than four patches in the mother cell, were classified as polarized cells (black bars). Cells with actin patches concentrated in the small bud but with more than four patches in the mother cell were classified as partially depolarized cells (grey bars). Cells with more actin patches in the mother cell than in the small bud were classified as totally depolarized cells (white bars).
amount of Rvs161p can be recovered from membranes, further substantiating the cortical localization of Rvs161-GFP. In addition, at least some of these membranes were resistant to detergent treatment, as are the sphingolipids and ergosterol contained in raft microdomains. Consistently, in the lcb1-100 mutant, which is impaired in DRM assembly, the Rvs161p-DRM fraction dropped from 28% to 17% at the permissive temperature and from 20% to 12% at the restrictive temperature (Fig. 9B).

In the sur4 and fen1 mutants, the Rvs161 protein was still found in DRMs at the same level as in the wild type (not shown), although Rvs161p was mislocalized in these mutants (Fig. 7A). Therefore, we hypothesize that in these mutants, Pma1p or Rvs161p accumulates in DRMs in which the sphingolipids are different from those in the wild type, such that they are improper for the protein to be routed any further. Taken together, these data show that Rvs161p is associated with lipid rafts.

**DISCUSSION**

We now confirm and extend previous observations on actin depolarization under salt stress (12, 27). Following the shift to NaCl-containing medium, actin patch depolarization was maximal after 30 min, and full repolarization of actin patches occurred between 60 and 90 min. These rearrangements occurred with the same time course as those following heat stress (17). Therefore, both salt and heat stress lead to the same physiological response, actin patch depolarization.

Following salt stress, the rvs161 mutant was able to depolarize actin patches even faster than the wild type, but the actin patches did not repolarize later. The rvs161 mutant responded to salt stress but was unable to recover from it. Therefore, Rvs161p is required for actin repolarization following salt stress.

From these data, we propose that rvs161Δ cells die when they are grown in NaCl-containing medium because the Rvs161 protein is required for repolarization and a polarized actin cytoskeleton is required for cell survival. Also, this could explain why the rvs161Δ mutant dies following starvation, since the actin cytoskeleton is depolarized during stationary phase (3) and repolarizes when cells enter a new cycle. Indeed, Rvs161p could be required to repolarize actin whatever the primary signal for actin depolarization. The fact that exponentially growing rvs161Δ mutant cells display a partially depolarized actin cytoskeleton further substantiates this model. Likely, actin patches are transiently depolarized during normal growth following small stresses occurring during the culture. This phenomenon is only visible in a strain that is unable to repolarize actin, such as the rvs161Δ mutant.

Also, it is interesting that the rvs161 mpk1/slt2 double mutation is lethal (6). Since Mpk1p is required for actin repolarization following heat stress (17), both Rvs161p and Mpk1p could be required for actin repolarization following a wide range of stresses.

Mutations in the suppressor genes have an effect on actin depolarization and actin repolarization: mutations in class I genes (fen1 and sur4) cause faster depolarization, while those in class II (sur2) and III genes (sur1 and ipt1) cause lesser extents of depolarization. The same suppressors of the rvs161Δ
FIG. 5. Sphingolipid mutants are impaired in actin depolarization and suppress the repolarization defect of the rvs161Δ mutant. The wild-type (WT) and the indicated mutant strains were grown as described for Fig. 2. Cells (~100) were classified and quantified according to their polarization state as in Fig. 3.
mutation suppressed the inability of the act1-1 mutant to sustain salt stress and were all able to suppress the defect of the rvs161Δ mutant in actin repolarization. This reinforces the notion that the primary function of Rvs161p lies in actin cytoskeleton repolarization. Because the Fen1p, Sur4p, Sur2p, Sur1p, and Ipt1p proteins are not likely to act by themselves in actin polarization or depolarization, sphingolipids or biosynthetic intermediates are involved in actin polarization. Indeed, sphingolipids or biosynthetic intermediates are missing or are present in greater amounts in the different suppressor mutants (14, 45) (Fig. 1). Either biosynthetic intermediates could act as signaling molecules, or complex sphingolipids could act as structural components of lipid rafts. These two mechanisms might not be exclusive.

The intermediate sphingoid base phytosphingosine has been described as a signaling molecule for resistance to heat stress (13) and protein phosphorylation required for endocytosis (21). The fen1 mutant is known to accumulate phytosphingosine (14), and phytosphingosine signals actin repolarization via the Pkh1/Pkh2-Pkc1-Mpk1 pathway (17, 21). In the class I suppressor mutants (fen1 or sur4), the excess of phytosphingosine could exacerbate the pathway in such a way that Rvs161p is no longer required for actin repolarization.

Complex sphingolipids together with cholesterol (ergosterol in yeasts) are the main components of the raft membrane microdomains. In S. cerevisiae, the three main complex sphingolipids, inositolphosphoceramide, mannosyl-inositolphosphorylceramide, and mannosyl-diinositolphosphorylceramide, together with variations in the chain lengths of the sphingoid moiety and the very long chain fatty acid and different levels in hydroxylation, make up a variety of more than 30 different molecular species (37) that potentially lead to a variety of different rafts (35). The proteins associated with specific rafts could be present in lower or higher amounts in the suppressor mutants. In the sur2, sur1, and ipt1 mutants, the proteins signaling salt stress or proteins essential for actin depolarization could be missing in their proper environment. We found that the sensor Wsc1p responds to salt stress for actin depolarization. Moreover, Wsc1p is an integral membrane protein that is likely associated with lipid rafts because it is resistant to detergent extraction (28). Wsc1p could be mislocalized in sur2, sur1, and ipt1 mutants, explaining why these mutants do not
FIG. 7. Rvs161p-GFP localization is impaired in fen1, sur4, and lcb1-100 mutants. Different mutant cells grown in SD-casa medium were observed by Nomarski and fluorescence microscopy to visualize whole cells and Rvs161p-GFP localization, respectively. (A) In the fen1 and sur4 mutants, the arrow on the Nomarski images indicates the structures that contain Rvs161p-GFP. (B) In the sur2, sur1, and ipt1 mutants, the arrow on the Nomarski images indicates the septum between the mother cell and the bud. (C) Rvs161p-GFP localization in the lcb1-100 mutant and the corresponding wild-type (RH1800) cells grown at 26°C and shifted to 33°C for 90 min. The arrow on the Nomarski images indicates the septum between the mother cell and the bud.
completely signal salt stress to actin patch depolarization. Here the suppression can be explained by the fact that depolarization only reaches a level at which Rvs161p is no longer required for repolarization.

Rvs161p is required for actin repolarization, and several lines of evidence show that this protein is associated with lipid rafts. Although the RVS161-GFP fusion did not complement the rvs161Δ mutant, Rvs161p-GFP was localized to very specific areas of the cell, i.e., in cortical patches that polarized to bud emergence and septum. In accordance, Rvs161p was recovered from a membranous fraction and part of it was found in DRMs. The fraction that was associated with DRMs was relatively low (40% of membrane-associated protein). Nevertheless, considering that Rvs161p has no glycosphingolipid or glycosphingolipid anchor or transmembrane domain, it cannot be directly integrated in rafts. Most probably, Rvs161p is associated with a protein(s) integrated in rafts. Moreover, Rvs161p is delocalized in the fen1 and sur4 mutants, and Fen1p and Sur4p could be involved in the establishment of rafts. Indeed, Pma1p, a raft protein, is mislocalized in the fen1 and sur4 mutants. This is consistent with previous results demonstrating a role for lipid rafts in Pma1p sorting (2). Also consistent with this is that, the rvs161Δ mutant depolarized actin patches faster than the wild type, just like the fen1 and sur4 mutants. Therefore this behavior could result from the mislocalization of Rvs161p in the fen1 and sur4 mutants.

In the raft hypothesis, Wsc1p could be required for actin depolarization and Rvs161p for actin repolarization. Therefore, in S. cerevisiae, lipid rafts could function as platforms for actin depolarization and actin repolarization.

Rafts are difficult to visualize by microscopy unless there is clustering (8, 24, 33). Therefore, the cortical patches where Rvs161p localizes could be viewed as clustered rafts. After Sur7p, this is the second report of a protein localizing as small cortical patches in S. cerevisiae (44). Interestingly, SUR7 was uncovered as a multicopy suppressor of both the rvs167 and rvs161Δ mutations. Actin patches are not delocalized in the rvs161Δ mutant overexpressing SUR7 following salt stress (42). Raft clustering could be a physical means to bring together partners for actin repolarization in S. cerevisiae.
ACKNOWLEDGMENTS

We are greatly indebted to Bertrand Daignan-Fornier and Kai Simons for discussions and reviewing the manuscript. Special thanks to Mafalda Escober-Henriques and Isabelle Sagot for supportive discussions. We also thank Howard Riezman for the gift of strains and Ray Cooke for editing the manuscript.

This work was supported by the Association Française pour les Myopathies (AFM grant 7314), the CNRS, and the University of Bordeaux 2.

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