*THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE*

# Yeast Two-Hybrid Library Screen Reveals Novel Binding Partners of Vps1 and Links Vps1 to a Novel Role in Budding

**Bryan T. Banh**
Student, Department of Biology, Missouri State University, 901 S National, Springfield, Missouri, United States of America (USA)
**Hyoeun McDermott**
Student, Department of Biology, Missouri State University, 901 S National, Springfield, Missouri, United States of America (USA)
**Michelle Williams**
Ph. D. Student, Department of Biology, Missouri State University, 901 S National, Springfield, Missouri, United States of America (USA)
**Shiva Kumar Goud Gadila**
Student, Division of Biological Sciences, University of Missouri, 105 Tucker Hall, Columbia, USA
**Kyoungtae Kim**
Professor, Department of Biology, Missouri State University, 901 S National, Springfield, Missouri, United States of America (USA)

***Abstract:***
*Vacuolar protein sorting 1 (Vps1) is a dynamin-like GTPase involved in membrane remodeling and protein sorting in multiple cellular trafficking pathways. It appears that Vps1 functions through interaction with a selective group of proteins that reside at eachorganelle. Though Vps1's role in membrane remodeling has been well characterized, a majority of its biochemical interactions at each traffic location still elude us. A yeast two-hybrid library screen was performed in Saccharomyces cerevisiae in search of novel Vps1 binding partners. These results reveal seventeen as-yet-unidentified Vps1 binding proteins implicated in various pathways. Notably, our study present evidence forVps1'sinteraction with Ste24, an ER membrane protein and proteins implicated in chitin distribution, suggesting Vps1's functional association with chitin regulation required for yeast budding.*

***Keywords:*** *Vps1, Dynamin, Yeast two-hybrid Screen*

## 1. Introduction

Dynamin is a membrane remodeling GTPase, best characterized by its role inendocytic fission and vesicle fusion (Anantharam et al., 2011; Peters et al., 2004; Sundborger and Hinshaw, 2014). Mammalian systems possess three isoforms of dynamin proteins; dynamin1, 2, and 3, sharing 80% homology between one another. Dynamin-1 and dynamin-3are primarily found in nerve cells, while dynamin-2 is expressed ubiquitously(Cao et al., 1998). Mutations in dynamin have been associated with certain neurodegenerative diseases such as Alzheimer's and Charcot Marie-Tooth disease (Ferguson et al., 2007; Liu et al., 2011).

Classical dynamins are composed of five major domains, with an N-terminal GTPase (G-domain), a middle domain (stalk), a pleckstrin homology domain (PH), a GTPase effect or domain (GED), and a C-terminal proline-rich domain (PRD) (Chappie and Dyda, 2013).In addition, a small bundle signal element (BSE) region exists on both sides of the G-domain as well as the C-terminal end of the GED, and BSE is required for bringing the G-domain and stalk/GED together in dynamin's tertiary structure(Chappie and Dyda, 2013). During endocytosis, dynamin self-assembles through their stalk-to-stalk interactions in a criss-cross manner(Faelber et al., 2012). As a result, two GTPase heads are orientated on the same side, while the lipid binding PH domains are situated on the opposite side of the catalytic GTPase heads (Chappie and Dyda, 2013). A tetrameric dynamin forms in the cytoplasm as two dimers joined by a short stretch located at the stalk-GED region (Chappie and Dyda, 2013). Dynamin tetramers are then recruited to the membrane via their PH and PRD domains, followed by a high-order assembly around the neck of the invaginated pit (Faelber et al., 2012; Mehrotra et al., 2014). A conformational change in the BSE region driven by the association and hydrolysis of GTP allows the homo-oligomeric structure to constrict and elongate, which results in the fission of the nascent vesicle (Chappie et al., 2011; Faelber et al., 2012).

The budding yeast, *Saccharomycescerevisiae,* expresses three dynamin-like proteins (DLPs)homologous to mammalian dynamins. Dynamin-related GTPase (Dnm1) and mitochondrial genome maintenance (Mgm1) primarily function in fission and fusion at the mitochondrial membrane (Abutbul-Ionita et al., 2012; Mears et al., 2011), whereas vacuolar sorting protein (Vps1) localizes at a wider range of cellular structures, including the Golgi, endosome, vacuole, peroxisome and plasma membrane (Kuravi et al., 2006; Nothwehr et al., 1995; Peters et al., 2004; Smaczynska-de Rooij et al., 2010; Williams and Kim, 2014).Among its diverse roles, Vps1 was initially characterized in cargo sorting at the late Golgi due to the fact that *vps*1 mutant cells exhibited an impaired carboxypeptidase Y (CPY) trafficking bound for the vacuole, namely in anterograde traffic (Nothwehr et al., 1995; Robinson et al., 1988).Severe defects in the retrograde traffic from the endosome to the late Golgi have also been observed in Vps1-deficient cells(Arlt et al., 2015; Chi et al., 2014; Lukehart et al., 2013). Additionally, endocytosis and degradation traffic from the endosome to the vacuole was disrupted in *vps1* mutant strains (Hayden et al., 2013; Smaczynska-de et al., 2015). Together, this evidence suggests Vps1'simplication in a broad spectrum of membrane traffic pathways.

In addition, evidence has supported Vps1's role in membrane remodeling required for membrane fusion and fission. Upon oligomerization, Vps1 can interact with and tabulate liposomal membranes *in vitro*(Smaczynska-de Rooij et al., 2010). Vps1-mediated scission of the nascent vesicle during endocytosis appears to depend on the negatively-charged glutamate residue at position 461 and phosphorylation of the 599 residue(Palmer et al., 2015; Smaczynska-de et al., 2015). Surprisingly, Vps1 also plays a role in vacuolar homotypic fusion (Alpadi et al., 2013; Peters et al., 2004), which requires Vps1's self-assembly ability(Kulkarni et al., 2014).

Significant progress has been made in the past two decades for understanding the molecular features of dynamin-related protein functioning in a variety of cellular processes. However, many details regarding the precise action mode of Vps1in these traffic pathways and its binding partners at different sub-cellular locations still elude us. An investigation of its interactions with different partners may provide deeper insights into potential or uncharacterized roles of Vps1. To establish a Vps1 interaction network, we performed a genome wide yeast two-hybrid screen for novel binding partners. We present here seventeen novel partners that bind to Vps1 in yeast. We tested the validity of the genome screen via an alternative protein-protein interaction assay using two selected proteins.

## 2. Experimental Procedures

### *2.1. Strains/Cell Maintenance*

*Saccharomyces cerevisiae* Y187, Y2HGOLD and *Escherichia coli* HST08, acquired through Clontech, and *S. cerevisiae* BY4741 from Invitrogen were stored at -80˚C until use. Plasmid vectors pGADT7-Rec (prey) and pGBKT7 (bait) were used as an over expression vector for the protein of interest tagged with the Activator Domain (in prey) or Binding Domain (in bait), respectively. Stellar competent *E. coli* cells carrying these vectors were kept at -80˚C and purified vectors were kept at -20˚C until use. During cell culturing, yeast cells were grown in 30˚C and bacterial cells at 37˚C.

### *2.2. Construction of Bait Vector and Yeast cDNA library*

Purified genomic DNA from *S. cerevisiae* (BY4741, Invitrogen) was used as a template for amplification of *VPS1* DNA sequence using Phusion Green High-Fidelity DNA Polymerase (F-534L, Thermo Scientific) with forward primer 5'CATGGAGGCCGAATTCATGGATGAGCATTTATTTTCTAC 3' and reverse primer 5'GCAGGTCGACGGATCCAACAGAGGAGACGATTTGACTAG 3'. The amplified sequence was ligated by using In-Fusion® HD Cloning Kit (Clontech) into the linearized BD (DNA Binding Domain, pGBKT7) vector using EcoRI and BamHI. The recombinant vector was then transformed into Stellar Competent *E. coli*, using the corresponding protocol PT5055-2 from Clontech. The cells were plated on LB agar plates, containing 25 µg/ml kanamycin. Positive colonies were confirmed by bacterial colony PCR and restriction enzyme digestion with EcoRI and BamHI. The bait vector, pGBKT7-Vps1, was then purified using PureYieldTM Plasmid Miniprep System (A1223, Promega), prior to performing a one-step transformation into competent Y2HGOLD yeast cells as previously described(Chen et al., 1992).

A library of potential Vps1 binding partners was constructed in AD (Activation Domain, pGADT7-rec) vector using Clontech's Make Your Own "Mate & Plate™" Library System (PT4085-1). Total mRNA was first extracted from wild type *S. cerevisiae* (KKY002) using Masterpure Yeast RNA Purification Kit (MPY03100, Epicentre). The purified mRNA product was then converted into cDNA using SMART MMLV Reverse Transcriptase with CDS III primers poly-A specific primers containing EcoRI and BamHI restriction sites. The cDNA product was amplified using Long Distance PCR and purified with CHROMA SPIN+TE-400 Columns. Following Matchmaker Yeast Transformation System 2 Usual Manual (Clontech), 5 µg of the purified cDNA and 3 µg pGADT7-Rec (Clontech) were co-transformed into Y187 strain, allowing *in vivo* recombination in yeast. Transformants were plated on selective media, lacking leucine (SD/-Leu), and were incubated at 30°C for 3-4 days.

### *2.3. Yeast two-hybrid Screening*

The yeast two-hybrid screen was performed using the Matchmaker Gold Yeast Two-Hybrid System (Clontech) with minor adjustments. The harvested prey library pool (pGADT7-Rec cDNA) was mated with the bait strain (pGBKT7-Vps1) according to Clontech's Mate and Plate System. The culture was then plated on Triple Drop Out (-leu, -trp, -his), Quadruple Drop Out (-leu, -trp, -his, -ade), and Double Drop Out with X-alpha-gal and Aureobasidin (DDO/X/A) plates for 3-5 days for screening. Colonies that survived on the QDO and turned blue on DDO/X/A were selected for DNA sequencing. The vectors were then isolated from the positive colonies using Zymoprep™ Yeast Plasmid Miniprep I (D2001, Epigenetics Company). These vectors were amplified in

Stellar competent *E. coli* cells as previously described and grown in LB medium containing ampicillin. The vectors were then isolated from *E. coli* using QIAGEN Miniprep Kit and then quantified using an Implen Nanophotometer.

*2.4. Genomic Analysis*

Vectors containing the gene of interest were isolated from positive colonies and delivered to a biotechnology company (Eurofins) for sequencing. Proteins were identified by comparing genetic data using Basic Local Alignment Search Tool (BLAST) search from the National Center for Biotechnology Information (NCBI) and Saccharomyces Genomic Database (SGD) websites.

*2.5. One-on-One Yeast Two-Hybrid Assay*

Matchmaker Gold Yeast Two-Hybrid system (Clontech) was used to further test the interaction of Spa2 and Ste24 with Vps1. The C-terminal 642 amino acids of Spa2 (2476-4401 bp) and Ste24 cytosolic (1195-1362 bp) sequences were amplified by Phusion HiFi PCR (Thermo Sci) and ligated with In-Fusion HD Cloning (Clontech) into pGADT7 vector using XmaI and BamHI restriction sites. These vectors were introduced into Y187 yeast cells using a transformation protocol, previously described (Chen et al., 1992). pGBKT7-Vps1 bait vector-carrying cells were mated with yeast cells containing prey vector. The resulting diploid cells were screened on DDO, TDO, and QDO media using a spotting assay in which the diploid cells were subjected to threefold serial dilutions before plating on corresponding medium yeast growth.

**3. Results and Discussion**

Vps1 is a cytoplasmic protein involved in several trafficking pathways (Vater et al., 1992; Williams and Kim, 2014).Our current understanding suggests that Vps1's membrane remodeling roles at multiple organelles may be attributed to its ability to interact with a variety of proteins (Figure 1). In our search for novel Vps1 binding partners, we performed a yeast two-hybrid library screen using Clontech's Make Your Own "Mate & Plate" Library System (Cat. No. 630490). For this, full length *VPS1*sequence was cloned into BD (DNA Binding Domain, pGBKT7) vector to screen a yeast cDNA library fused to gal-4 AD (Activation Domain, pGADT7-rec) vector. Our initial screen yielded sixty-nine positive colonies on Triple Drop Out (TDO) medium, which were picked and patched onto new TDO medium (Fig. 2A). Upon replica plating ontoa more stringent Quadruple Drop Out (QDO) medium, sixty-four colonies grew (Fig. 2B).Fifty-eight colonies grew and produced blue color when replica plated onto Double Drop Out medium with X-alpha-galand aureobasidin (DDO/X/A) (Fig. 2C). The isolated prey vectors from the positive colonies grown on these stringent tests were analyzed by comparing their cDNA inserts with the BLAST (Basic Local Alignment Search Tool).

After excluding both the cloned DNA sequences originated from rRNA species and low-purity sequencing errors, the sequencing analysis revealed seventeen novel binding partners for Vps1 (Table 1).These partners appear to be involved in a wide range of cellular processes, including stress responses, signaling transduction pathways, ribosomal activities, and mating processes. For example, Fes1 and Pre10 are implicated in targeting misfolded proteins for ubiquitinationin different stress responses (Gowda et al., 2013; Janse et al., 2004), while Pbs2 is involved in signal transduction(Zarrinpar et al., 2004). Eight proteins (Drs1, Pxr1, YLR154C, Rps1B, RPL18A, RPL26B, RNH203, and YDR341C)are involved in ribosome-associated activity.Spa2, Ste24 and SCW4 localizes to the plasma membrane and ER membranes, and are all implicated in the yeast mating process (Cappellaro et al., 1998; Meissner et al., 2010; Noma et al., 2005).

In order to validate the results, we selected two prey proteins and tested their ability to interact with Vps1: Spa2, a cytoplasmic protein, and Ste24,an integral protein. Though previous studies have not linked Vps1 and Spa2, Ste24 has been shown to genetically interact with Vps1 (Hoppins et al., 2011; Jonikas et al., 2009; Surma et al., 2013). Sequencing analysis from our library screen showed that the C-terminal ends of Spa2 and Ste24 were inserted into the library vector. For one-on-one interaction study, pGADT7 vector was engineered to contain the C-terminal 642 aa of Spa2 (825-1467aa) or the C-terminal 56 aa of Ste24 (398-453aa),and then the yeast strains expressing the recombinant protein fragments were mated with the strain carrying Vps1-BD bait vector. The mated cells were spotted on TDO medium in serial dilutions (Figure 2D). Strains expressing both prey and bait vectors grew on TDO medium, but not on QDO medium (Data not shown), suggesting Spa2 and Ste24 appear to interact with Vps1, but at lower efficiency than previously tested in the library screen (Fig. 2A-C). One possible explanation for a weak binding between Vps1 and these binding partners would be that the cloned C-terminal fragments of Spa2 and Ste24 used in this one-on-one two-hybrid assay are larger than those cloned in the library vectors, and thus the increase in peptide size may have altered the conformation of these proteins.

The obtained result of Vps1 interaction with Spa2 and Ste24creates new insights into possibleroles for Vps1. For example, given that Spa2, a polarisome subunit (Snyder, 1989), plays a role in act in organization like Vps1(Virag and Harris, 2006; Yu and Cai, 2004), it is possible that Vps1 works together with Spa2 synergistically or redundantly to help reorganize the act in cytoskeleton for cellular polarization. Importantly, recent studies demonstrated that Vps1 interacts genetically with Ste24,an ER transmembrane protein with the C-terminal exposed to the cytosol (Hoppins et al., 2011; Jonikas et al., 2009; Pryor et al., 2013; Surma et al., 2013).In light of the finding of the physical interaction between Vps1 and the C-terminal fragment of Ste24, we speculate that Vps1 can be targeted to the periphery ER, but this speculative idea awaits further testing.

Previously, *vps1*Δ cells have been shown to exhibit abnormal budding and altered chitin deposition(Yu and Cai, 2004). However, the molecular details behind this defect had not been documented. According to our Vps1 interaction results, Vps1 physically interacts with proteins involved in the recruitment (Spa2), maintenance (Scw4), and targeting (Ste24) of chitin synthases (Figure 3), suggesting a model that links Vps1 to multiple factors involved in chitin distribution for cellular budding. In this model, we propose that when induced by mating factors, the Spa2-containing polarisome localizes to the nascent bud to aid in act in reorganization and cellular signaling, which promotes the recruitment of new cell materials for polarized growth(Sheu et al., 1998; Snyder, 1989). In response to

mating signaling, chitin synthase III (Chs3), a major player in chitin production, is delivered to the plasma membrane from the ER by passing the *trans*-Golgi network (Starr et al., 2012). We speculate that Ste24 and Vps1 work together at the ER,as explained above, to sort and aid in fission of Chs3-containing vesicles. Upon arriving at the plasma membrane, Scw4, a soluble cell wall glucanase, is then involved in maintaining the integrity of the cell wall (Sestak et al., 2004). Taken together, it is likely that Vps1 is involved in several steps of the Chs3 trafficking pathway. However, additional studies are required to further validate these ideas.

While the yeast two-hybrid is a commonly used tool in protein-protein interaction, certain limitation should be kept under consideration. In particular, all the binding partners of Vps1 in our library screen present C-terminal domains of their corresponding proteins; unnaturally exposing certain domains or affecting overall conformation. Also, intrinsic factors within the *in vivo* model may be involved or interfere with these interactions. Ultimately, this study has revealed novel binding partners with a wide array of functional capabilities; suggesting possibilities for alternative roles in Vps1. Due to Vps1's dynamic nature, its significance with each partner remains plausible. In one example, we have proposed that Vps1 serves as a new link between three membrane peripheral proteins in yeast budding. While this idea is still speculative, future research will be focused towards pursuing the authenticity of these implications.

**4. Acknowledgement**

This work was supported by a Faculty Research Grant (to K. Kim) and by thesis funding from Missouri State University (to B. Banh). B. Banh contributed to the preparation of the figures and substantial contributions to the writing of this review. K. Kim provided the intellectual input and participated in the manuscript correction, proofreading, and revision of the manuscript.

| Gene Name | Accession Number | # of Clones Recovered | Function from Saccaromyces Genome Database |
|---|---|---|---|
| SPA2 | X53731.1 | 1 | A subunit of the polarisome; functions in act in cytoskeletal organization during polarized growth; acts as a scaffold for Mkk1p and Mpk1p cell wall integrity signaling components |
| STE24 | U77137.1 | 1 | Highly conserved zinc metalloprotease; functions in two steps of a-factor maturation, C-terminal CAAX proteolysis and the first step of N-terminal proteolytic processing |
| SCW4 | Z73064.1 | 1 | Cell wall protein with similarity to glucanases; |
| FES1 | NM_001178449.3 | 2 | Hsp70 nucleotide exchange factor |
| PRE10 | Z75270.1 | 1 | Alpha 7 sub-unit of the 20S proteasome |
| PBS2 | Z49403.1 | 1 | MAP kinase kinase of the HOG signaling pathway; mitophagy-specific regulator; plays a role in regulating Ty1 transposition |
| YNL054W-B | NM_001281536.1 | 1 | Type B Gag-Pol protein; proteolytically processed to make the Gag, RT, PR, and IN proteins that are required for retrotrans position |
| DRS1 | NM_001181828.1 | 2 | Nucleolar DEAD-box protein required for ribosome assembly and function |
| PXR1 | NM_001181409.1 | 1 | Essential protein involved in rRNA and snoRNA maturation; a possible role in the negative regulation of telomerase |
| YLR154c/RNH203 | Z73326.1 | 1 | Ribonuclease H2 subunit; required for RNase H2 activity; role in the ribonucleotide excision repair |
| RPS1B | NM_001182422.1 | 1 | Ribosomal protein 10 (rp10) of the small (40S) subunit |
| RPL18A | Z74862.1 | 1 | Ribosomal 60S subunit protein L18A |
| RPL26B | Z72819.1 | 1 | Ribosomal 60S subunit protein L26B |
| RNH203 | Z73326.1 | 1 | Ribonuclease H2 subunit; role in ribonucleotide excision repair; related to human AGS3 that causes Aicardi-Goutieres syndrome |
| YDR341C | NM_001180649.3 | 1 | Arginyl-tRNAsynthetase |
| YLR157C-C | Z73326.1 | 5 | A protein of unknown function; Paralog of ASP3-2, implicated in asparagine catabolism |
| YLR179C | NM_001182066.1 | 1 | A protein of unknown function with similarity to Tfs1p; transcription is activated by paralogous proteins Yrm1p and Yrr1p along with proteins involved in multidrug resistance |

*Table 1: Yeast two hybrid screen of Vps1 binding partners*

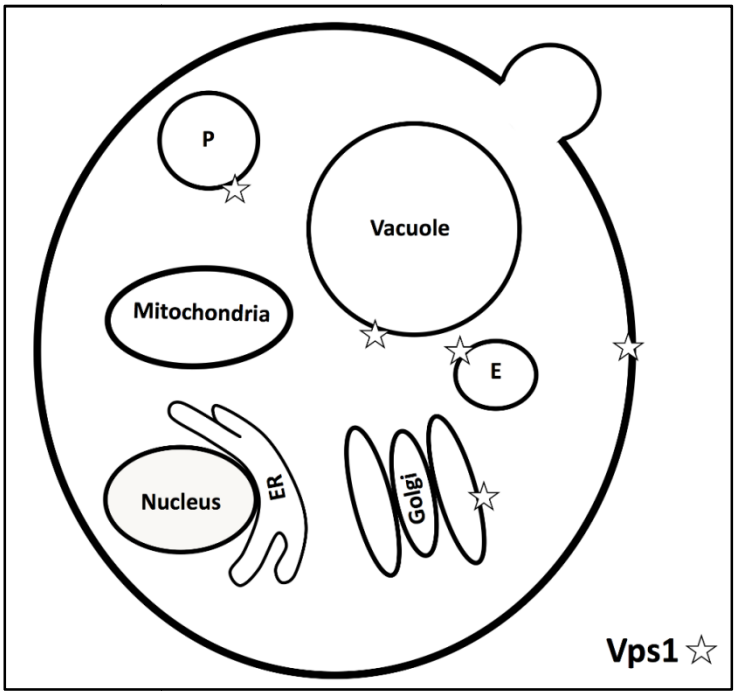


*Figure 1: Localization of Vps1 in S. cerevisiae. Dynamin-like protein, Vps1 is a cytoplasmic protein that is targeted to the periphery of various membrane-bound organelles, including the peroxisome (P), endosome (E), Golgi, vacuole, and the plasma membrane. Vps1 is implicated in membrane remodeling activity, such as membrane tubulation and scission of vesicles emerging from these organelles. Vps1 locations are indicated by stars*

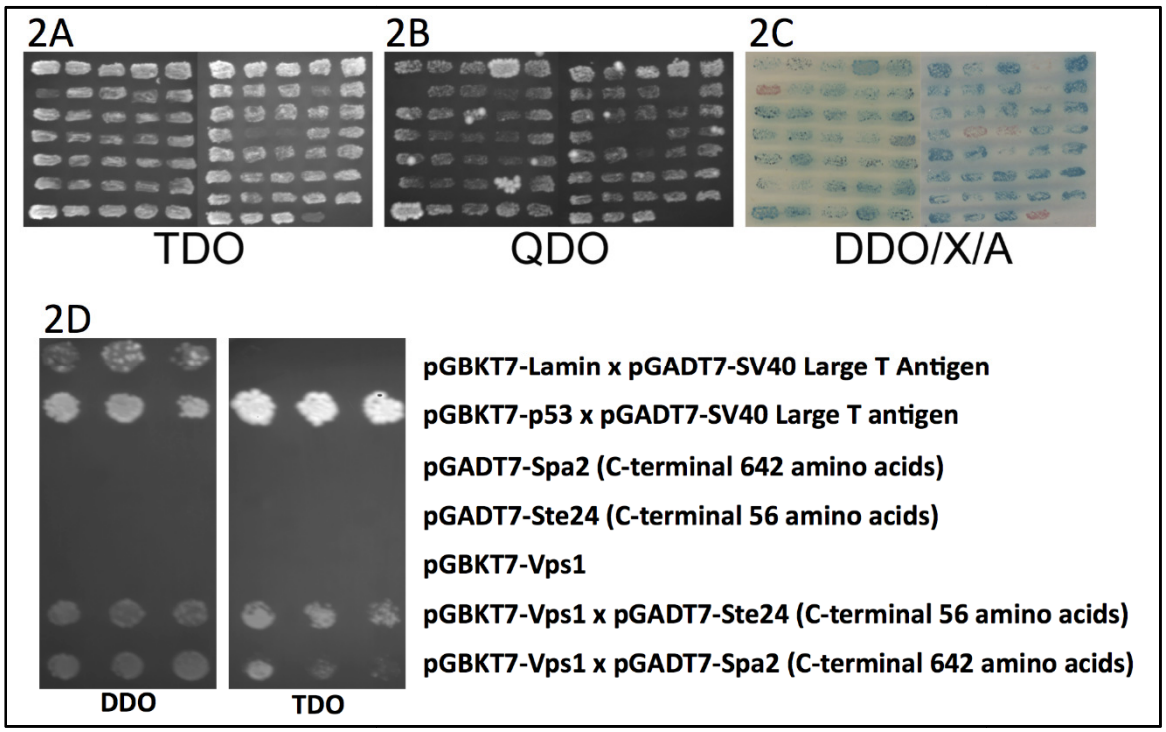


*Figure 2: Yeast two hybrid genome wide assay for searching Vps1 binding partners in S. cerevisiae. 2A-C. The harvested prey library pool (pGADT7-Rec cDNA) was mated with the bait strain (pGBKT7-Vps1), and the mated diploid cells were plated onto TDO (2A), QDO (2B), and DDO/X/A (2C). 2D. Verification of our library assay by one-on-one yeast two-hybrid assay. For a positive control, cells expressing BD-p53 and AD-SV40 large T antigen were utilized; p53 was inserted into the bait vector (pGBKT7) and SV40 large T antigen into the prey vector (pGADT7). Cells expressing BD-Lamin and AD-SV40 large T antigen, which do not bind to each other, served as the negative control. Following the same method, the full length of VPS1 was inserted into the bait vector, and the indicated C-terminal regions of Spa2 and Ste24 were cloned into the prey vector. Diploid cells harboring both the bait and prey vectors were plated on DDO (Double Drop Out) and TDO (SD/-Leu/-Trp/-His). Only positive control strain and the strains containing both the bait and prey grew on TDO medium, indicating that Vps1 interacts with Ste24 and Spa2.*

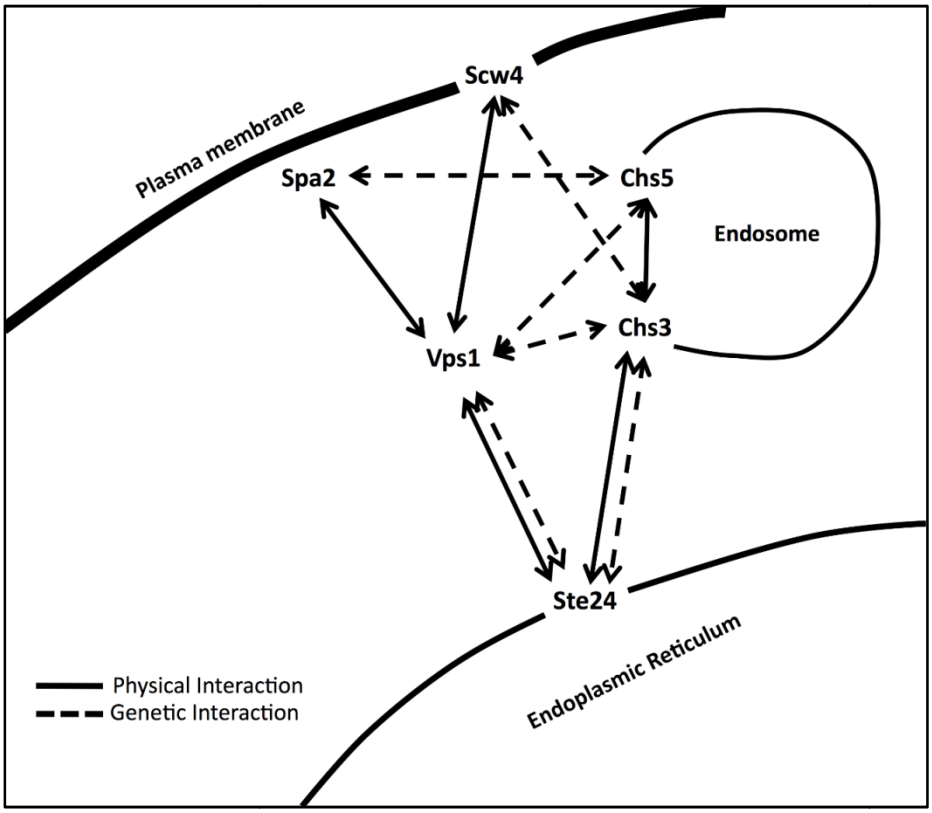


*Figure 3: Interaction mapping of Vps1 in chitin deposition. Our results show that Vps1 physically interact with factors involved in mating induced budding; Spa2, Ste24, and Scw4 (see the main text). These proteins have all been implicated in targeting, recruiting, or maintaining chitin synthases (Chs3 and Chs5).*

**5. References**


i. Abutbul-Ionita, I., Rujiviphat, J., Nir, I., McQuibban, G.A., and Danino, D. (2012). Membrane tethering and nucleotide-dependent conformational changes drive mitochondrial genome maintenance (Mgm1) protein-mediated membrane fusion. J Biol Chem *287*, 36634-36638.

ii. Alpadi, K., Kulkarni, A., Namjoshi, S., Srinivasan, S., Sippel, K.H., Ayscough, K., Zieger, M., Schmidt, A., Mayer, A., Evangelista, M., *et al.* (2013). Dynamin-SNARE interactions control trans-SNARE formation in intracellular membrane fusion. Nat Commun *4*, 1704.

iii. Anantharam, A., Bittner, M.A., Aikman, R.L., Stuenkel, E.L., Schmid, S.L., Axelrod, D., and Holz, R.W. (2011). A new role for the dynamin GTPase in the regulation of fusion pore expansion. Mol Biol Cell *22*, 1907-1918.

iv. Arlt, H., Reggiori, F., and Ungermann, C. (2015). Retromer and the dynamin Vps1 cooperate in the retrieval of transmembrane proteins from vacuoles. J Cell Sci *128*, 645-655.

v. Cao, H., Garcia, F., and McNiven, M.A. (1998). Differential distribution of dynamin isoforms in mammalian cells. Mol Biol Cell *9*, 2595-2609.

vi. Cappellaro, C., Mrsa, V., and Tanner, W. (1998). New potential cell wall glucanases of Saccharomyces cerevisiae and their involvement in mating. J Bacteriol *180*, 5030-5037.

vii. Chappie, J.S., and Dyda, F. (2013). Building a fission machine--structural insights into dynamin assembly and activation. J Cell Sci *126*, 2773-2784.

viii. Chappie, J.S., Mears, J.A., Fang, S., Leonard, M., Schmid, S.L., Milligan, R.A., Hinshaw, J.E., and Dyda, F. (2011). A pseudoatomic model of the dynamin polymer identifies a hydrolysis-dependent powerstroke. Cell *147*, 209-222.

ix. Chen, D.C., Yang, B.C., and Kuo, T.T. (1992). One-step transformation of yeast in stationary phase. Curr Genet *21*, 83-84.

x. Chi, R.J., Liu, J., West, M., Wang, J., Odorizzi, G., and Burd, C.G. (2014). Fission of SNX-BAR-coated endosomal retrograde transport carriers is promoted by the dynamin-related protein Vps1. The Journal of cell biology *204*, 793-806.

xi. Faelber, K., Held, M., Gao, S., Posor, Y., Haucke, V., Noe, F., and Daumke, O. (2012). Structural insights into dynamin-mediated membrane fission. Structure *20*, 1621-1628.

xii. Ferguson, S.M., Brasnjo, G., Hayashi, M., Wolfel, M., Collesi, C., Giovedi, S., Raimondi, A., Gong, L.W., Ariel, P., Paradise, S., *et al.* (2007). A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. Science *316*, 570-574.

xiii. Gowda, N.K., Kandasamy, G., Froehlich, M.S., Dohmen, R.J., and Andreasson, C. (2013). Hsp70 nucleotide exchange factor Fes1 is essential for ubiquitin-dependent degradation of misfolded cytosolic proteins. Proc Natl Acad Sci U S A *110*, 5975-5980.

xiv. Hayden, J., Williams, M., Granich, A., Ahn, H., Tenay, B., Lukehart, J., Highfill, C., Dobard, S., and Kim, K. (2013). Vps1 in the late endosome-to-vacuole traffic. J Biosci *38*, 73-83.

xv. Hoppins, S., Collins, S.R., Cassidy-Stone, A., Hummel, E., Devay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., and Nunnari, J. (2011). A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. The Journal of cell biology *195*, 323-340.

xvi. Janse, D.M., Crosas, B., Finley, D., and Church, G.M. (2004). Localization to the proteasome is sufficient for degradation. J Biol Chem *279*, 21415-21420.

xvii. Jonikas, M.C., Collins, S.R., Denic, V., Oh, E., Quan, E.M., Schmid, V., Weibezahn, J., Schwappach, B., Walter, P., Weissman, J.S.*, et al.* (2009). Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. Science (New York, NY *323*, 1693-1697.

xviii. Kulkarni, A., Alpadi, K., Sirupangi, T., and Peters, C. (2014). A dynamin homolog promotes the transition from hemifusion to content mixing in intracellular membrane fusion. Traffic (Copenhagen, Denmark) *15*, 558-571.

xix. Kuravi, K., Nagotu, S., Krikken, A.M., Sjollema, K., Deckers, M., Erdmann, R., Veenhuis, M., and van der Klei, I.J. (2006). Dynamin-related proteins Vps1p and Dnm1p control peroxisome abundance in Saccharomyces cerevisiae. Journal of cell science *119*, 3994-4001.

xx. Liu, Y.W., Lukiyanchuk, V., and Schmid, S.L. (2011). Common membrane trafficking defects of disease-associated dynamin 2 mutations. Traffic *12*, 1620-1633.

xxi. Lukehart, J., Highfill, C., and Kim, K. (2013). Vps1, a recycling factor for the traffic from early endosome to the late Golgi. Biochem Cell Biol *91*, 455-465.

xxii. Mears, J.A., Lackner, L.L., Fang, S., Ingerman, E., Nunnari, J., and Hinshaw, J.E. (2011). Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission. Nat Struct Mol Biol *18*, 20-26.

xxiii. Mehrotra, N., Nichols, J., and Ramachandran, R. (2014). Alternate pleckstrin homology domain orientations regulate dynamin-catalyzed membrane fission. Mol Biol Cell *25*, 879-890.

xxiv. Meissner, D., Odman-Naresh, J., Vogelpohl, I., and Merzendorfer, H. (2010). A novel role of the yeast CaaX protease Ste24 in chitin synthesis. Mol Biol Cell *21*, 2425-2433.

xxv. Noma, S., Iida, K., and Iida, H. (2005). Polarized morphogenesis regulator Spa2 is required for the function of putative stretch-activated Ca2+-permeable channel component Mid1 in Saccharomyces cerevisiae. Eukaryot Cell *4*, 1353-1363.

xxvi. Nothwehr, S.F., Conibear, E., and Stevens, T.H. (1995). Golgi and vacuolar membrane proteins reach the vacuole in vps1 mutant yeast cells via the plasma membrane. The Journal of cell biology *129*, 35-46.

xxvii. Palmer, S.E., Smaczynska-de, R., II, Marklew, C.J., Allwood, E.G., Mishra, R., Goldberg, M.W., and Ayscough, K.R. (2015). A Charge Swap mutation E461K in the yeast dynamin Vps1 reduces endocytic invagination. Commun Integr Biol *8*, e1051274.

xxviii. Peters, C., Baars, T.L., Buhler, S., and Mayer, A. (2004). Mutual control of membrane fission and fusion proteins. Cell *119*, 667-678.

xxix. Pryor, E.E., Jr., Horanyi, P.S., Clark, K.M., Fedoriw, N., Connelly, S.M., Koszelak-Rosenblum, M., Zhu, G., Malkowski, M.G., Wiener, M.C., and Dumont, M.E. (2013). Structure of the integral membrane protein CAAX protease Ste24p. Science *339*, 1600-1604.

xxx. Robinson, J.S., Klionsky, D.J., Banta, L.M., and Emr, S.D. (1988). Protein sorting in Saccharomyces cerevisiae: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. Mol Cell Biol *8*, 4936-4948.

xxxi. Sestak, S., Hagen, I., Tanner, W., and Strahl, S. (2004). Scw10p, a cell-wall glucanase/transglucosidase important for cell-wall stability in Saccharomyces cerevisiae. Microbiology *150*, 3197-3208.

xxxii. Sheu, Y.J., Santos, B., Fortin, N., Costigan, C., and Snyder, M. (1998). Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. Mol Cell Biol *18*, 4053-4069.

xxxiii. Smaczynska-de, R., II, Marklew, C.J., Allwood, E.G., Palmer, S.E., Booth, W.I., Mishra, R., Goldberg, M.W., and Ayscough, K.R. (2015). Phosphorylation Regulates the Endocytic Function of the Yeast Dynamin-Related Protein Vps1. Molecular and cellular biology *36*, 742-755.

xxxiv. Smaczynska-de Rooij, I.I., Allwood, E.G., Aghamohammadzadeh, S., Hettema, E.H., Goldberg, M.W., and Ayscough, K.R. (2010). A role for the dynamin-like protein Vps1 during endocytosis in yeast. J Cell Sci *123*, 3496-3506.

xxxv. Snyder, M. (1989). The SPA2 protein of yeast localizes to sites of cell growth. J Cell Biol *108*, 1419-1429.

xxxvi. Starr, T.L., Pagant, S., Wang, C.W., and Schekman, R. (2012). Sorting signals that mediate traffic of chitin synthase III between the TGN/endosomes and to the plasma membrane in yeast. PLoS One *7*, e46386.

xxxvii. Sundborger, A.C., and Hinshaw, J.E. (2014). Regulating dynamin dynamics during endocytosis. F1000Prime Rep *6*, 85.

xxxviii. Surma, M.A., Klose, C., Peng, D., Shales, M., Mrejen, C., Stefanko, A., Braberg, H., Gordon, D.E., Vorkel, D., Ejsing, C.S.*, et al.* (2013). A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. Mol Cell *51*, 519-530.

xxxix. Vater, C.A., Raymond, C.K., Ekena, K., Howald-Stevenson, I., and Stevens, T.H. (1992). The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in Saccharomyces cerevisiae, is a GTPase with two functionally separable domains. The Journal of cell biology *119*, 773-786.

xl. Virag, A., and Harris, S.D. (2006). Functional characterization of Aspergillus nidulans homologues of Saccharomyces cerevisiae Spa2 and Bud6. Eukaryot Cell *5*, 881-895.

xli. Williams, M., and Kim, K. (2014). From membranes to organelles: emerging roles for dynamin-like proteins in diverse cellular processes. Eur J Cell Biol *93*, 267-277.

xlii. Yu, X., and Cai, M. (2004). The yeast dynamin-related GTPase Vps1p functions in the organization of the actin cytoskeleton via interaction with Sla1p. Journal of cell science *117*, 3839-3853.

xliii. Zarrinpar, A., Bhattacharyya, R.P., Nittler, M.P., and Lim, W.A. (2004). Sho1 and Pbs2 act as coscaffolds linking components in the yeast high osmolarity MAP kinase pathway. Mol Cell *14*, 825-832.