Plasma Membrane Proteolipid 3 Protein Modulates Amphotericin B Resistance through Sphingolipid Biosynthetic Pathway

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Invasive opportunistic fungal infections of humans are common among those suffering from impaired immunity, and are difficult to treat resulting in high mortality. Amphotericin B (AmB) is one of the few antifungals available to treat such infections. The AmB resistance mechanisms reported so far mainly involve decrease in ergosterol content or alterations in cell wall. In contrast, depletion of sphingolipids sensitizes cells to AmB. Recently, overexpression of PMP3 gene, encoding plasma membrane proteolipid 3 protein, was shown to increase and its deletion to decrease, AmB resistance. Here we have explored the mechanistic basis of PMP3 effect on AmB resistance. It was found that ergosterol content and cell wall integrity are not related to modulation of AmB resistance by PMP3. A few prominent phenotypes of PMP3 delete strain, namely, defective actin polarity, impaired salt tolerance, and reduced rate of endocytosis are also not related to its AmB-sensitivity. However, PMP3 overexpression mediated increase in AmB resistance requires a functional sphingolipid pathway. Moreover, AmB sensitivity of strains deleted in PMP3 can be suppressed by the addition of phytosphingosine, a sphingolipid pathway intermediate, confirming the importance of this pathway in modulation of AmB resistance by PMP3.

Fungi cause superficial and invasive infections. Opportunistic invasive infections, though less prevalent, are of much greater concern because of high mortality (often over 50%) associated with them1. Many fungal species are responsible for these invasive infections, killing over one and half a million people every year, which is higher than that due to tuberculosis or malaria1. The treatment options for invasive infections are quite limited2. Amphotericin B (AmB) is a commonly used antifungal for over five decades. In spite of its toxicity, it is preferred for its broad-spectrum and fungicidal mode of action, particularly for treating invasive infections. Though echinocandins are also used for treating such infections, their use is limited in resource poor settings due to high cost. Moreover, Cryptococcus species do not respond to echinocandins and thus AmB alone (or in combination with flucytosine) is the mainstay to treat invasive infections caused by these species2,3.

AmB is currently considered to kill fungi by forming large, extramembranous fungicidal sterol sponge that depletes ergosterol from lipid bilayers4. Leakage of intracellular ions due to pore formation is thought to be a secondary effect of AmB5. Though AmB resistance is rare, it is seen in a significant percentage of pathogenic Candida species and filamentous fungi6–7. The AmB resistance mechanisms reported so far mainly involve reduction in ergosterol content or alterations in cell wall8–11. We have recently shown that sphingolipids also modulate AmB resistance12. A better understanding of AmB resistance/sensitivity mechanisms would facilitate developing therapeutic strategies to minimize evolution of AmB resistance, or to sensitize fungi to AmB such that lower AmB dose can be used to reduce toxicity.

While investigating apparent elevated AmB resistance of yeast cells in presence of farnesol (unpublished), we identified Saccharomyces cerevisiae PMP3 gene as conferring increased AmB resistance when present in a multicopy plasmid. Deletion of this gene rendered the cells hypersensitive to AmB. During the course of our studies, PMP3 gene’s role in AmB resistance was also reported by Huang et al13, but the mechanism underlying this phenotype was not clear. PMP3 was first reported as a non-essential gene whose deletion results in plasma membrane hyperpolarization and salt sensitivity14. It encodes a 55 amino acid hydrophobic protein of plasma membrane. A homologous plant protein could complement salt sensitivity of a yeast strain deleted in PMP315. Here we have explored the mechanistic basis of PMP3 effect on AmB resistance. We show that certain prominent phenotypes of PMP3 delete.
strain, namely defects in salt tolerance, actin polarity and endocytosis, are not responsible for AmB-sensitivity of this strain. Instead, we demonstrate that modulation of AmB resistance by PMP3 is mediated through sphingolipid biosynthetic pathway.

**Results and Discussion**

**PMP3 modulates AmB resistance.** The *S. cerevisiae* PMP3 gene was isolated from a multicopy overexpression library (in plasmid pFL44L) as conferring higher resistance to AmB. A PMP3 clone with 165 bp ORF along with 1196 bp upstream and 275 bp downstream regions was used in further studies. To confirm the phenotype, PMP3 deletion and overexpression strains were compared with their parent strain for AmB resistance (Fig. 1a). While the delete strain was 8-fold more sensitive to AmB than the parent strain, the overexpression strain was about 4-fold more tolerant compared to the parent strain. During the course of this study, Huang et al.\(^6\) showed that modulation of AmB resistance by *PMP3* is not dependent on ergosterol or Hsp90 or cell wall integrity. As far as the mechanistic basis of PMP3 effect on AmB resistance is concerned, Huang et al.\(^6\) showed that it is not related to its role in ion homeostasis. Absence or severe reduction in the amount of ergosterol in the fungal membranes and its replacement with certain other sterols results in AmB resistance in fungi\(^7,10,11\). To address this possibility total cellular content of ergosterol was estimated, as described\(^6\). The ergosterol content, as % wet weight of cells, of parent, delete and overexpression strains, was 0.021 ± 0.001, 0.023 ± 0.002 and 0.023 ± 0.001, respectively. Though these values are comparable, it is possible that the intracellular

![Figure 1](https://www.nature.com/scientificreports/)

**Figure 1** | *S. cerevisiae* PMP3 and its homologs from *C. albicans* and *C. glabrata* modulate AmB resistance. (a) Multicopy overexpression of *S. cerevisiae* PMP3 (ScPMP3) and its homologs from *C. glabrata* (CgPMP3) and *C. albicans* (CaPMP3) in *S. cerevisiae* enhance AmB resistance by about 4-fold with respect to wild-type strain (BY4741) and about 32-fold with respect to *pmp3Δ* strain. The relative growth of the strains on 0.1 μg/ml AmB (not shown) was comparable to that of respective strains on 0.2 μg/ml AmB. (b) AmB sensitivity of *C. glabrata* strain deleted in PMP3 ortholog (Cgmpmp3Δ) and *C. albicans* strains deleted in both alleles of PMP3 ortholog (Capmp3*-OΔ/Δ* and PMP3* best hit (Capmp3*-BΔ/Δ*), with respect to their respective parent strains CG462 and SN95. Five μl of 10-fold serial dilutions of cells were spotted starting from about 10^6 cells/spot, as described in Methods.
distribution of ergosterol might be affected. To check this, cells were stained with filipin, which is specific for sterols\textsuperscript{19}, and observed (Fig. S3a). While wild-type and PMP3 overexpression strains showed intense fluorescent spots within cells, pmp3\textsuperscript{Δ} strain lacked such spots. Thus, it is possible that more ergosterol is distributed in the plasma membrane of the delete strain, rendering it more accessible for AmB binding and killing. If this is true, then the delete strain should be more sensitive to other polynenes which also act by binding to ergosterol. However, the sensitivity pmp3\textsuperscript{Δ} strain to the polynenes nystatin, natamycin and filipin was found to be comparable to that of erg6\textsuperscript{Δ} strain. The sensitivity of this strain to radicicol and oxidative stress was checked along with erg6\textsuperscript{Δ} strain as positive control (Table 1). The AmB resistance of erg6\textsuperscript{Δ} strain and PMP3 overexpression strain was comparable. However, while erg6\textsuperscript{Δ} strain was 8-fold and 4-fold, respectively, more sensitive to radicicol and oxidative stress, the sensitivity of PMP3 overexpression strain was comparable to wild-type, implying that Pmp3p is not dependent on Hsp90 for conferring AmB resistance. Cell wall alterations also can affect AmB resistance\textsuperscript{2}. Compared to parent strain, PMP3 delete strain showed normal chitin deposition (Fig. S4a), as well as similar resistance to cell wall disrupting agents calcofluor white, sodium dodecyl sulphate and congo red (Fig. S4b), implying that AmB sensitivity of delete strain is not related to cell wall integrity.

Actin polarity and endocytosis, though impaired in pmp3\textsuperscript{Δ} strain, are not responsible for its AmB sensitivity. To gain further insight into PMP3 mechanism of action, we tried to predict its possible functions on the basis of biological roles of genes that interact with PMP3. The list of interacting genes was analyzed using DAVID Bioinformatics Resources\textsuperscript{16} for enrichment of gene ontology terms for biological processes. The top-two annotation clusters corresponded to endocytosis and actin cytoskeleton (Table 2). To

### Table 1 | AmB resistance mediated by PMP3 is not dependant on HSP90

| Strain | MIC (AmB, µg/ml) | Radicicol, µM | TBH, mM |
|--------|-----------------|--------------|--------|
| BY4741/vector | 0.4 | 16 | 2 |
| pmp3Δ/vector | 0.05 | 8 | 2 |
| pmp3Δ/SMP3 | 1.6 | 16 | 2 |
| erg6Δ/vector | 1.6 | 2 | 0.5 |

MIC for AmB and radicicol was determined in SC-ura broth at 30 °C. Sensitivity to oxidative stress was determined by dilution spotting on SC-ura agar medium with tert-butyl hydroperoxide (TBH) at 37 °C. MIC is the concentration at which no growth was observed.

### Table 2 | Functional Annotation Clustering of PMP3 interacting genes

#### Cluster 1 - Enrichment Score: 2.82

| Term | Count | P-Value |
|------|-------|---------|
| GO:0006970 ~ endocytosis | 12 | 2.07E-04 |
| GO:00130324 ~ membrane invagination | 13 | 1.32E-03 |
| GO:0016192 ~ vesicle-mediated transport | 23 | 4.18E-03 |
| GO:0016044 ~ membrane organization | 18 | 4.57E-03 |
| ACT1, AGE1, CMD1, CSR2, CYC2, ERG3, FEN1, GTS1, INP52, MGM1, MVB12, MYO5, PHO86, RUD3, SHR3, SLA1, SSO2, SUR7, VAM10, VPS24, VPS27, VPS30, VPS8, VRP1, WHI2. |

#### Cluster 2 - Enrichment Score: 2.40

| Term | Count | P-Value |
|------|-------|---------|
| GO:0006970 ~ response to osmotic stress | 14 | 2.38E-05 |
| GO:0048308 ~ organelle inheritance | 10 | 2.89E-04 |
| GO:0030036 ~ actin cytoskeleton organization | 11 | 1.88E-03 |
| GO:0008064 ~ regulation of actin polymerization or depolymerization | 4 | 2.39E-03 |
| GO:0032271 ~ regulation of protein polymerization | 4 | 2.39E-03 |
| GO:0030833 ~ regulation of actin filament polymerization | 4 | 2.39E-03 |
| GO:0030832 ~ regulation of actin filament length | 4 | 2.39E-03 |
| GO:0033043 ~ organelle organization | 10 | 2.63E-03 |
| GO:0043254 ~ regulation of protein complex assembly | 5 | 2.76E-03 |
| GO:0030029 ~ actin filament-based process | 11 | 2.85E-03 |
| GO:0032970 ~ regulation of actin filament-based process | 4 | 5.84E-03 |
| GO:0032956 ~ regulation of actin cytoskeleton organization | 4 | 5.84E-03 |
| GO:0013333 ~ negative regulation of protein complex assembly | 3 | 9.69E-03 |
| GO:0007010 ~ cytoskeleton organization | 15 | 1.13E-02 |
| GO:0044087 ~ regulation of cellular component biogenesis | 5 | 1.16E-02 |
| GO:0015493 ~ regulation of cellular cytoskeleton organization | 5 | 3.28E-02 |
| GO:0032535 ~ regulation of cellular component size | 9 | 3.42E-02 |
| GO:007015 ~ actin filament organization | 5 | 1.50E-01 |
| ABP1, ACT1, ARC15, ARC18, BHM1, CAP1, CAP2, CDC13, CMD1, DFG16, EMP70, ENA1, GCL7, GTS1, HSC82, HSP82, INP52, IST2, KTI12, MGM1, MVB12, MYO5, NIP100, NPT1, PET122, SHE4, SKY1, SLA1, VMA9, VRP1, WHI2. |

List of PMP3 interacting genes were downloaded from SGD\textsuperscript{16} and analyzed with DAVID Functional Annotation Clustering tool (http://david.abcc.ncifcrf.gov/home.jsp) of DAVID Bioinformatics Resources v6.7\textsuperscript{21} for enrichment of gene ontology terms using default parameters, but restricted to biological processes (GO.Term_BP_Fat category). Only the top-two annotation clusters with greater than 2-fold enrichment are listed, along with the genes grouped under each cluster.
check if impaired endocytosis would result in AmB sensitivity, we screened mutants of several genes having role in endocytosis for their AmB sensitivity. Deletants of RVS161 and RVS167 were about 4-fold more sensitive to AmB compared to the parent strain (Fig. S5). These strains, besides defects in endocytosis have several other phenotypes including salt sensitivity and altered actin cytoskeleton\(^\text{22-25}\). SUR7, encoding an eisosome protein involved in endocytosis, partially suppresses several of these phenotypes upon multicopy overexpression\(^\text{26-28}\). Thus, we exploited overexpression of SUR7 to understand if AmB sensitivity of \textit{pmp3Δ} strain is a consequence of defects in actin cytoskeleton or endocytosis, or it is an independent phenotype.

A large scale survey using GFP-Snc1-Suc2 reporter has indicated that endocytosis is decreased in \textit{pmp3Δ} strain\(^\text{29}\). We monitored rate of endocytosis with a different reporter, namely methionine permease (Mup1) tagged with eclipsic pHluorin, which is a pH-sensitive green fluorescent protein variant that does not fluoresce after internalization to an acidic compartment like vacuole\(^\text{30,31}\). Mup1-pHluorin is

**Figure 2** | Slow rate of endocytosis of \textit{pmp3Δ} strain is restored to normal level by overexpression of \textit{ScSUR7}. (a) Wild type strain 3818 (SEY6210-Mup1pHluorin) and \textit{pmp3Δ} strain (3818 \textit{pmp3Δ::HIS3}) transformed with either vector or \textit{ScSUR7}, were grown without methionine to promote accumulation of Mup1-pHluorin in the plasma membrane. After addition of 20 µg/ml methionine, random fields of cells were imaged at different time intervals. All images were obtained at identical exposure conditions. (b) After addition of methionine, Mup1-pHluorin fluorescence was measured at indicated time intervals in a flow cytometer, as described in Methods. The values shown are average of two replicates from one representative experiment. Experiments were repeated thrice with comparable results.
internalized rapidly upon exposure to methionine. Wild-type cells showed substantial decrease in Mup1-pHluorin intensity within 20 min after adding methionine (Fig. 2a). However, in pmp3 strain 40 min was needed for a similar decrease, confirming that the rate of endocytosis is slowed down in this strain.

SUR7 expressed from a multicopy plasmid restored the rate of endocytosis of pmp3 strain to normal level (Fig. 2a). Mup1-pHluorin fluorescence was also monitored by flow cytometry (Fig. 2b). Though background fluorescence was high for all the strains, the rate of decrease in fluorescence is indicative of rate of endocytosis. While it was slow in the pmp3 strain, it was restored to wild-type level upon SUR7 overexpression.

Actin cytoskeleton plays a central role in endocytosis and rvs161A and rvs167Δ strains impaired in endocytosis also have actin polarization defects. Moreover, as PMP3 interacts with genes having role in actin cytoskeleton (Table 2), we visualized actin in PMP3 strains. The pmp3Δ strain showed pronounced defect in actin polarity, which is suppressed by overexpression of SUR7 (Fig. 3 and Fig. S6). SUR7 also suppressed the sensitivity of pmp3Δ, rvs161A and rvs167A strains to NaCl (Fig. 4a). However, it could not reverse the sensitivity of these strains to AmB (Fig. 4b), demonstrating that AmB sensitivity of these mutants is not mediated by defects in actin polarity, endocytosis or NaCl tolerance.

Sphingolipid biosynthetic pathway is essential for PMP3 mediated increase in AmB resistance. We had recently shown that sphingolipid biosynthetic pathway genes FEN1 (ELO2) and SUR4 (ELO3) modulate AmB resistance. While inhibition of sphingolipid biosynthesis with myriocin sensitized cells to AmB, addition of phytosphingosine, a sphingolipid pathway intermediate, reversed this phenotype. To check the importance of this pathway for PMP3 mediated increase in AmB resistance, multicopy ScPMP3 was transformed into a few sphingolipid pathway mutants and the resistance was checked (Fig. 5a). In the wild-type parent strain (BY4741) ScPMP3 could increase AmB resistance at least by 4-fold. However, it increased AmB resistance by 2-fold or less in mutants of sphingolipid biosynthetic genes FEN1 and SUR4, and regulatory genes YPK1 and SAC1. If PMP3 overexpression effect is independent of sphingolipid pathway, then fold-increase in AmB resistance by PMP3 in these mutants should have been comparable to that of the parent strain. Only 2-fold or less increase in resistance shows that PMP3 is dependent on this pathway for enhancing AmB resistance. Even this increase appears to be due to genetic redundancy. FEN1 and SUR4 are involved in fatty acid elongation and can partly compensate for each other’s loss, since double deletion is lethal. YPK1 and YPK2 are synthetic lethal and arose from the whole genome duplication. Sac1p is a phosphatidylinositol phosphate phosphatase, and its catalytic domain (Sac1-like domain) is seen among several phosphatases with partially overlapping function. Sac1p is known to modulate sphingolipid metabolism. Physical interaction of

Figure 3 | Actin polarization defect of pmp3Δ strain is suppressed by multicopy SUR7 overexpression. Cells were grown to log phase and actin was visualized by rhodamine phalloidin staining. About 200 cells with small buds were scored according to their polarization state. Cells with actin patches concentrated in the small bud, with fewer than four patches in the mother cell, were classified as polarized cells. Other cells with more actin patches in the mother cell than in the small bud were classified as depolarized cells. Representative images are shown in Figure S6. Mean values of two independent experiments are given. The error bars indicate the range.

Figure 4 | SUR7 overexpression can suppress salt sensitivity (a), but not AmB sensitivity (b) of strains deleted in PMP3, RVS161 or RVS167. Wild-type (BY4741) and PMP3 overexpression strains are included as controls.
Pmp3p and Sac1p has also been reported in a large-scale study. Thus it appears likely that Pmp3p modulates sphingolipid biosynthesis and AmB resistance by interacting with Sac1p. Dependence of Pmp3p on Sac1p provides possible link between Pmp3p and sphingolipid pathway.

Myriocin inhibits the first committed step of sphingolipid biosynthesis catalyzed by serine palmitoyltransferase. Sphingolipid pathway regulatory genes YPK1 and SAC1 modulate myriocin resistance. To test if PMP3 also regulates sphingolipid pathway, we checked myriocin resistance of deletion and overexpression strains. While deletion of PMP3 decreased myriocin resistance by 2-fold, its overexpression increased myriocin resistance by 4-fold, both with respect to parent strain (Fig. 5b), indicating that PMP3 is possibly involved in regulation of this pathway in S. cerevisiae. We also checked the myriocin sensitivity of C. glabrata and C. albicans strains deleted in PMP3 orthologs (Figs. 6b and 6c). These results further establish that PMP3 modulates AmB resistance through sphingolipid pathway in S. cerevisiae as well as in pathogenic Candida species.

Sphingolipid bases and complex sphingolipids have multiple roles in cells, both as structural components and as signalling molecules. Mutants of sphingolipid pathway show pleiotropic phenotypes, of which those affected in actin cytoskeleton, endocytosis and AmB resistance are pertinent here. Since actin is critical for endocytosis, defective endocytosis could be a consequence of impaired actin polarity. Thus, impaired actin cytoskeleton and slow rate of endocytosis of pmp3 strain are consistent with the regulatory role played by PMP3 in sphingolipid pathway.

In conclusion, we have shown that a few striking phenotypes of PMP3 mutant, such as impaired actin polarity, endocytosis and salt tolerance are not related to its AmB-sensitivity. Rather, we show that modulation of AmB resistance by PMP3 is dependent on sphingolipid biosynthetic pathway, since AmB sensitivity of PMP3 deletants is suppressed by phytosphingosine, a sphingolipid pathway intermediate. Moreover, enhanced AmB resistance conferred by overexpression of PMP3 is dependent on functional sphingolipid biosynthetic and regulatory genes. Efforts are underway to elucidate the precise mechanism underlying PMP3 effect or dependence on sphingolipid pathway for modulating AmB resistance.

Methods

Fine chemicals and yeast synthetic drop-out medium supplements without uracil were procured from Sigma. All other media components were obtained from BD (Difco). Oligonucleotides were custom synthesised from Sigma-Genosys, India. Restriction enzymes, DNA polymerases and other DNA modifying enzymes were obtained from New England Biolabs, and DNA purification kits were obtained from Qiagen.

Strains, media and growth conditions. S. cerevisiae and Candida strains and plasmids used in this study are listed in Table S1 and S2. The Escherichia coli strain DH5α was used as a cloning host. YPD and Synthetic complete (SC) media were prepared and used as described. Uracil supplement is omitted in SC medium to provide SC-ura medium. Yeast transformations were carried out using the modified
lithium acetate method\(^4\). Stock solutions of AmB (2 mg/ml), myriocin (5 mM), phytosphingosine (15 mM) and radicicol (5 mM) were prepared in DMSO. Stock solutions of nourseothricin (200 mg/ml) and tert-butyl hydroperoxide (500 mM) were made in water.

**Growth assays by dilution spotting.** For dilution spotting assays, the strains/transformants were grown overnight in SC or SC-ura medium, reinoculated in fresh medium to an \(A_{600}\) of 0.1 and grown for 6 h. The exponential phase cells were harvested, washed and resuspended in sterile water to an \(A_{600}\) of 1.0 (\(2 \times 10^7\) cells/ml). Ten-fold serial dilutions were made in water and 5 ml of each dilution was spotted on SC or SC-ura plates with desired concentration of compounds, as mentioned in Figures. DMSO alone was included in control plates, corresponding to its concentration in experimental plates, where appropriate. Plates were incubated for 2 days at 30°C before taking photographs. These experiments were repeated at least three times with comparable results.

**Cloning methods.** The ORFs of putative homologs of ScPMP3 in C. albicans (CaPMP3-ortholog (orf19.1655.3), CaPMP3-Best hit (orf19.2959.1)) and C. glabrata (CAGL0M08552g) were PCR amplified from the genomic DNA of C. albicans and C. glabrata with specific primers sets (Table S3). The PCR products were then used to replace the ScPMP3 ORF in a ScPMP3 clone in multicopy vector pFL44L, using Circular Polymerase Extension Cloning (CPEC) method\(^4\), thereby retaining the ScPMP3 promoter and terminator regions for all PMP3 orthologs as well. For cloning ScSUR7 gene, the SUR7 ORF of S. cerevisiae along with its promoter and terminator (568 to 326 bp) was amplified from strain BY4741 with forward primer ScSUR7-OCS1 and reverse primer ScSUR7-OCA1 (Table S3) and cloned in pFL44L by CPEC method\(^4\).

**Construction of C. albicans strains deleted in CaPMP3-ortholog and CaPMP3-Best hit.** Both alleles of CaPMP3-ortholog (Capmp3-O\(\Delta\)/\(\Delta\)) or CaPMP3-Best hit (Capmp3-B\(\Delta\)/\(\Delta\)) were deleted in C. albicans, using HAH2 cassette and gene-specific primers, as described\(^12\), and confirmed by diagnostic PCR with appropriate primers (Table S3).

**Construction of C. glabrata strain deleted in CgPMP3.** PMP3 ortholog in C. glabrata (CAGL0M08552g) was deleted using a selection cassette conferring nourseothricin resistance containing CaNAT1 gene with codon usage adapted for Candida species\(^5\). A 508 bp region upstream of, and 472 bp region downstream of CgPMP3 ORF were PCR amplified from wild type genomic DNA using primers for upstream (CgPMP3-US1 and CgPMP3-UA1) and downstream regions (CgPMP3-DS1 and CgPMP3-DA1). The upstream flanking region was fused with the 5’ region of CaNAT1 cassette using amplified upstream region and plasmid (pCR2.1-NAT\(^5\)) with CaNAT1 as templates and primers CgPMP3-US1 and CaNAT1-US-R1 to generate upstream split marker. Similarly, the downstream flanking region was fused.
13. Huang, Z. were 488 nm and 530 nm, respectively. For each sample 10^4 cells were analysed.

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with filipin as described with slight modification. Exponentially growing cells (0.5 OD cells/ml) were fixed with 3.7% paraformaldehyde for 10 min at 30°C, washed with phosphate-buffered saline (PBS) and incubated with 5 μg/ml of filipin (Sigma F9765) in the dark at 30°C for 5 min. The stained cells were directly observed under a confocal laser scanning microscope (Nikon A1R) using 405 nm laser and images were analysed using NIS element software.

Flow cytometry.

Log-phase cells were grown in SC medium without uracil and methionine for 6 hours, and then methionine was added to 20 μg/ml

attached in yeast cell wall material.

The endocytic vacuolar protein sorting (VPS) pathway is essential for the degradation of many endocytosed proteins. The VPS5 gene, which is required for the formation of the endosomal sorting complex required for transport (ESCRT) machinery, is a key player in the endocytic pathway. Several studies have reported that the endocytosis of Candida albicans is impaired in the vps5 mutant, indicating that the VPS5 pathway is involved in the internalization of this fungus.

In this study, we investigated the role of the VPS5 pathway in the endocytosis of Candida albicans. We used a fluorescence microscopy approach to visualize the internalization of a pH-sensitive green fluorescent protein (pHluorin) in the yeast cells. We observed a significant reduction in the internalization of pHluorin in the vps5 mutant compared to the wild-type strain, suggesting that the VPS5 pathway is necessary for efficient endocytosis of Candida albicans.

Our findings provide new insights into the molecular mechanisms underlying the endocytosis of Candida albicans and the role of the VPS5 pathway in this process. Further studies are needed to understand the specific steps of the endocytic pathway that are affected by the vps5 mutation and to identify potential targets for antifungal therapy.

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Acknowledgments

We thank Beverly Wendland and Suzanne Noble for *S. cerevisiae* and *C. albicans* strains, and Rupinder Kaur for *C. glabrata* strain and pCR2.1-NAT plasmid. Vinay K. Bari, Sushma Sharma and Md. Alfatah acknowledge the Council of Scientific and Industrial Research, New Delhi, for fellowships. This work was supported by a CSIR project “Understanding the molecular mechanism of diseases of national priority: Developing novel approaches for effective management” (SIP10), and a Supra Institutional Project on Infectious Diseases (BSC0210).

Author contributions

K.G. designed the project and provided overall guidance. V.K.B. and S.S. carried out the experiments and collected data. V.K.B. and K.G. drafted and finalized the manuscript. A.K.M. provided technical inputs and guidance for confocal microscopy. S.S., M.A. and A.K.M. provided critical input during group meetings and on the manuscript. All authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Bari, V.K., Sharma, S., Alfatah, M., Mondal, A.K. & Ganesan, K. Plasma Membrane Proteolipid 3 Protein Modulates Amphotericin B Resistance through Sphingolipid Biosynthetic Pathway. *Sci. Rep.* 5, 9685; DOI:10.1038/srep09685 (2015).

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