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Postprint: Identification and Functional Study of Vesicle Protein YlSec15 in Yarrowia lipolytica

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Abstract

During budding reproduction, Yarrowia lipolytica does not randomly select the budding site that determines the future division plane; instead, it buds at the position opposite the previous cell division site, a process known as bipolar budding. The molecular regulatory mechanism underlying bipolar budding in Yarrowia lipolytica is currently unclear. In this study, we investigated the function of the vesicle protein YlSec15 in Yarrowia lipolytica by examining its protein localization and overexpression. The results show that YlSec15 exhibits pronounced polarized localization in cells, enriching in small buds and at the necks of medium and large buds. Overexpression of YlSec15 inhibited hyphal formation and caused the budding site selection pattern in some cells to transition from bipolar budding to random budding, possibly because excess YlSec15 could not achieve proper polarized localization in cells. Additionally, YlSec15 may regulate hyphal formation and bipolar budding through the YlRas2-mediated signaling pathway. These findings enhance our understanding of the molecular regulatory mechanisms of bipolar budding in Yarrowia lipolytica and demonstrate the mutual influence between polarized growth and vesicle trafficking.

Full Text

Identification and Characterization of the Vesicle Protein YlSec15 in *Yarrowia lipolytica*

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Abstract

Yarrowia lipolytica reproduces through budding, and the budding site that determines the future division plane is not selected randomly. Instead, daughter cells bud at the site opposite the previous division location, following a bipolar budding pattern. However, the molecular mechanisms regulating bipolar budding in *Y. lipolytica* remain poorly understood. This study investigated the function of the vesicle protein YlSec15 through protein localization analysis and overexpression experiments. The results demonstrate that YlSec15 exhibits distinct polarized localization in cells, accumulating in small buds and at the necks of medium and large buds. Overexpression of YlSec15 inhibited hyphal formation and caused a shift in budding pattern from bipolar to random in a subset of cells, likely due to mislocalization of excess YlSec15. Furthermore, YlSec15 may regulate hyphal formation and bipolar budding through the YlRas2-mediated signaling pathway. These findings enrich our understanding of the molecular mechanisms underlying bipolar budding in *Y. lipolytica* and confirm the interplay between polarized growth and vesicle transport.

Keywords: *Yarrowia lipolytica*; bipolar budding; hyphae; vesicle protein; YlSec15

Introduction

Yeast reproduce through budding, a process that involves polarized distribution of the cytoskeleton, vesicle transport, and membrane fusion and remodeling once the budding site is determined. Both budding site selection and bud growth are highly polarized processes, with cell polarity establishment and maintenance subject to strict temporal and spatial regulation. Current understanding of the molecular mechanisms governing budding site selection primarily comes from studies of the model organism *Saccharomyces cerevisiae*, where a preliminary complete signaling pathway has been elucidated. However, research on budding site selection mechanisms in other non-conventional yeasts remains limited. This raises an important question: are the molecular mechanisms identified in *S. cerevisiae* universally applicable?

Yarrowia lipolytica is a non-conventional yeast that has attracted increasing research interest due to its unique physiological, biochemical, and metabolic characteristics. Evolutionarily distant from *S. cerevisiae*, *Y. lipolytica* exhibits bipolar budding, meaning daughter cells assemble new buds at sites opposite the previous budding location, while mother cells bud either opposite or adjacent to the previous site, resulting in bud scars distributed at both cell poles. This bipolar budding pattern is remarkably stable in *Y. lipolytica*, unlike in *S. cerevisiae* or *Candida albicans*, as it does not change with cell type or environmental conditions. This stability makes the underlying molecular regulatory pathway particularly intriguing.

Previous studies suggest that two independent signaling pathways may regulate bipolar budding in *Y. lipolytica*: the YlRsr1-mediated pathway and the

YlRas2-mediated pathway. The YlRsr1 pathway appears to function analogously to the bipolar budding regulation pathway in *S. cerevisiae*, operating through YlRsr1→YlCdc24→YlCdc42. The YlRas2 pathway, however, may be more closely associated with polarized cell growth, though its additional components remain unidentified. Vesicle transport is a highly polarized process during cell growth, involving vesicle formation, transport, clustering, and membrane fusion, with the exocyst complex playing a crucial role. This evolutionarily conserved eight-protein complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84) exhibits high dynamics during vesicle transport. This raises the question: is there a connection between these two highly polarized processes—bipolar budding and vesicle transport? Do vesicle proteins participate in regulating bipolar budding?

Research by Guo et al. in *C. albicans* revealed that Sec15 participates not only in vesicle transport but also in regulating polarized growth and budding site selection. *SEC15* deletion prevents hyphal formation and shifts budding patterns from axial to random. They further demonstrated that proper Sec15 localization depends on Rsr1 and Bem1, both known regulators of budding site selection. This suggests that in *C. albicans*, vesicle proteins are indeed involved in hyphal formation and budding site selection. The question remains whether a similar mechanism exists in *Y. lipolytica*.

In *Y. lipolytica*, both YlRsr1 and YlBem1 regulate budding site selection, with their deletion or mutation causing a shift from bipolar to random budding. Therefore, we investigated whether a Sec15 homolog exists in *Y. lipolytica* and whether it participates in regulating bipolar budding. This study identified YlSec15 and demonstrated its polarized localization in small buds and at bud necks. Functional analysis revealed that YlSec15 regulates hyphal formation and bipolar budding, potentially interacting with YlRas2. These findings advance our understanding of the relationship between budding site selection and vesicle transport and further elucidate the regulatory pathway for bipolar budding in *Y. lipolytica*.

Materials and Methods

Strains and Plasmids The strains used in this study are listed in Table 1 . The main primers are listed in Table 2 .

Media Preparation **YPD medium (rich medium):** 20 g peptone, 10 g yeast extract, and 20 g glucose dissolved in 1000 ml deionized water.

YNBD synthetic medium: 6.7 g/L yeast nitrogen base and 20 g/L glucose, supplemented with 0.08 g/L leucine or 0.02 g/L uracil as needed.

YNDC7 hyphal induction medium: 6.7 g/L yeast nitrogen base and 20 g/L glucose, with pH adjusted to 7.0 using citric acid and sodium citrate, supplemented with 0.08 g/L leucine or 0.02 g/L uracil as needed.

LB medium for *E. coli*: 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl (pH 7.2-7.4). LA medium was prepared by adding ampicillin to 100 g/ml. For solid media, agar was added to a final concentration of 2%.

Yeast Culture and Morphology Observation YPD solid medium: Strains were streaked onto YPD plates and incubated at 30°C for 3 days to observe colony morphology, followed by examination under low-power microscopy (10$×$10).

YNBD solid medium: Fresh strains were streaked onto YNBD plates and incubated at 30°C for 15 hours. Fluorescence microscopy was then used to observe fusion protein localization in cells.

YPD liquid medium: Fresh strains were inoculated into 3 ml YPD liquid medium and cultured with shaking at 30°C for 12 hours. Cell morphology was observed under high-power microscopy (10$×$100).

YNDC7 medium: Fresh strains were inoculated into 3 ml YNDC7 liquid medium and cultured with shaking at 30°C for 20 hours. Cell morphology was observed under high-power microscopy (10$×$100).

Microscopic observations were performed using an Olympus BX51 microscope with a DP80 camera and QCapture system.

Bud Scar Staining and Budding Pattern Analysis Fresh yeast colonies were inoculated into 3 ml YPD liquid medium and cultured with shaking at 30°C for 12 hours. Cells were harvested and washed twice with 1 ml ddH_2O, then resuspended in ddH_2O. Five microliters of 0.01% Calcofluor white stain were added to the suspension. Two microliters of stained suspension were placed on a microscope slide, covered with a coverslip, and pressed firmly to flatten cells uniformly without breaking the coverslip. Bud scar distribution was observed under UV fluorescence.

The cell surface was divided into three regions: both poles and the middle section. Cells with bud scars exclusively at both poles were classified as bipolar budding, while those with randomly distributed scars were classified as random budding. At least 200 cells were counted per experiment to calculate the percentage of each budding pattern.

Plasmid Construction For YlSec15-EGFP localization: Using wild-type PO1a genomic DNA as template and primers YlSec15-1F/YlSec15-1R, a 1103 bp fragment containing the YlSec15 promoter and complete ORF was amplified. After digestion with BglII and BamHI, the fragment was ligated into similarly digested pYL14 vector, creating pYL14-YlSEC15 with GFP fused to the C-terminus of YlSec15. This plasmid was transformed into wild-type *Y. lipolytica* for localization studies.

For EGFP-YlSec15 overexpression: Using wild-type PO1a genomic DNA as template and primers YlSec15-3F/YlSec15-TR, a fragment containing the YlSec15 ORF and 1022 bp terminator sequence was amplified. After BamHI and ClaI digestion, the fragment was ligated into pYL15 (BamHI/ClaI sites), creating pYL15-YlSEC15 with GFP fused to the N-terminus under the strong YlTEF1 promoter. This plasmid was transformed into wild-type cells to observe overexpressed EGFP-YlSec15 localization.

For YlSec15 overexpression: Using wild-type PO1a genomic DNA as template and primers YlSec15-3F/YlSec15-3R, a fragment containing the YlSec15 ORF and 550 bp terminator sequence was amplified. After BamHI and EcoRI digestion, the fragment was ligated into pYL4 (BamHI/EcoRI sites), creating pYL4-YlSEC15 with YlSec15 expression driven by the strong YlTEF1 promoter. The constructed plasmid was linearized with EcoRI and transformed into wild-type yeast. At least three independent transformants were examined to assess the effects of YlSec15 overexpression on cell morphology.

Yeast Transformation Rapid transformation of *Y. lipolytica* was performed as described previously [14].

Results

2.1 Identification and Structural Analysis of YlSec15 In *C. albicans*, Sec15 regulates vesicle transport, polarized growth, and budding site selection. *SEC15* deletion prevents hyphal formation and shifts budding from axial to random patterns [12]. We therefore investigated whether *Y. lipolytica* possesses a Sec15 homolog and whether it regulates hyphal formation and bipolar budding. Using *C. albicans* and *S. cerevisiae* Sec15 amino acid sequences, we searched the *Y. lipolytica* database and identified protein YALI0F12969p, which shares 35% and 39% homology with the two Sec15 proteins, respectively. This 829-amino-acid protein contains the conserved Sec15 superfamily domain (Figure 1 [Figure 1: see original paper]a), a signature domain that participates in vesicle-plasma membrane fusion [10]. The Sec15 superfamily domain of YALI0F12969p shows 44% and 47% homology with those of *C. albicans* and *S. cerevisiae* Sec15, respectively (Figure 1b). Based on these analyses, we designated this protein YlSec15 and proceeded to characterize its function.

2.2 Localization of YlSec15-EGFP Fusion Protein Protein localization patterns are directly related to function. We constructed a YlSec15-EGFP fusion protein with EGFP fused to the C-terminus of YlSec15 to examine its cellular distribution. YlSec15-EGFP exhibited clear polarized localization, accumulating in small buds and at the necks of medium and large buds. When small buds first emerged, YlSec15 was enriched in these nascent structures with no detectable signal in mother cells. As buds enlarged, this specific localization disappeared, and the fusion protein became diffusely distributed throughout the cytoplasm. Later in the cell cycle, YlSec15-EGFP reappeared at the bud neck

until cell separation (Figure 2 [Figure 2: see original paper]). This localization pattern mirrors that of Sec15 in *S. cerevisiae* and *C. albicans* [13,15], suggesting functional conservation. The protein' s localization to bud necks particularly suggests a potential role in budding site selection. To test this hypothesis, we further investigated YlSec15 function in *Y. lipolytica.*

2.3 Overexpression of YlSec15 Affects Hyphal Formation In *S. cerevisiae*, Sec15 deletion is lethal, and evidence suggests Sec15 interacts with Bem1 to direct polarized growth [16]. In *C. albicans*, Sec15 deletion is viable but causes slow growth and defective polarized growth, preventing hyphal formation [12]. We attempted to delete YlSec15 via homologous recombination but failed to obtain correct knockout mutants after multiple attempts, suggesting YlSec15 may be essential for viability in *Y. lipolytica*—a conclusion requiring further experimental verification.

To study YlSec15 function, we overexpressed the protein using the strong YlTEF1 promoter and examined its effects on growth and morphology. On YPD solid medium, YlSec15 overexpression did not affect growth rate, with colonies reaching sizes comparable to wild-type strains. However, colony morphology differed significantly. Wild-type PO1a formed colonies with raised edges, central depressions, and fluffy margins after 3 days, whereas YlSec15-overexpressing colonies were smooth, centrally raised, and had neat edges (Figure 3 [Figure 3: see original paper]a). Low-power microscopy revealed pseudohyphae extending from wild-type colony edges, while overexpressing strains showed neat edges without pseudohyphal extensions (Figure 3b), indicating potential morphological differences.

Examination of cells in liquid culture confirmed these differences. In YPD medium, wild-type PO1a cells were oval-shaped, whereas YlSec15-overexpressing cells became rounder (45%, n=200) and slightly larger, displaying mildly compromised polarity. In hyphal-inducing YNDC7 medium, wild-type cells elongated to form germ tubes and hyphae, while YlSec15-overexpressing cells failed to elongate or form hyphae, maintaining a slightly oval shape (Figure 3c). These results indicate that excess YlSec15 impairs polarized growth and hyphal formation.

2.4 Overexpression of YlSec15 Disrupts Bipolar Budding In *C. albicans*, Sec15 deletion shifts budding from axial to random patterns [13]. Since YlSec15 overexpression affected polarized growth in *Y. lipolytica*, we examined its impact on bipolar budding. Overexpression caused a subset of cells (59%, n=200) to switch from bipolar to random budding (Figure 4 [Figure 4: see original paper]), with only 41% maintaining bipolar patterns. In contrast, 93% of wild-type cells (n=200) exhibited bipolar budding. These data demonstrate that YlSec15 participates in regulating bipolar budding in *Y. lipolytica*, with excess protein causing mislocalization and defective bipolar budding.

2.5 Excess YlSec15 Loses Polarized Localization Given that YlSec15 overexpression affects hyphal formation and bipolar budding, we investigated whether excess protein exhibits altered localization. We fused EGFP to the N-terminus of YlSec15 and expressed it under the strong YlTEF1 promoter. While expression levels were high, as evidenced by bright green fluorescence, the fusion protein lost polarized localization and appeared as granular, disordered cytoplasmic aggregates. In pre-budding cells, EGFP-YlSec15 formed one or multiple granules in the cytoplasm. Upon bud emergence, the fusion protein accumulated at the mother-bud junction but failed to enter small buds. As buds enlarged, EGFP-YlSec15 appeared as granules within buds, and in larger buds, the protein was distributed in both mother and daughter cells without specific enrichment, with bud neck localization absent (Figure 5 [Figure 5: see original paper]). Thus, excess YlSec15 cannot achieve proper polarized localization, and this mislocalization impairs its normal function, affecting hyphal formation and bipolar budding.

2.6 YlSec15 May Regulate Hyphal Formation and Bipolar Budding Through Interaction with YlRas2 Our results show YlSec15 regulates hyphal formation and bipolar budding, but what is the underlying pathway? Previous studies indicate two independent signaling pathways control bipolar budding in *Y. lipolytica*: the Rho GTPase YlRsr1 pathway and the Ras-related protein YlRas2 pathway [8]. YlRas2 also regulates hyphal formation [9]. We therefore investigated whether YlSec15 functions through these pathways by examining YlSec15-EGFP localization in Ylrsr1Δ and Ylras2Δ deletion mutants.

In Ylrsr1Δ cells, EGFP-YlSec15 localized normally to small bud tips and bud necks (Figure 6 [Figure 6: see original paper]), identical to its pattern in wild-type cells. However, in Ylras2Δ cells, EGFP-YlSec15 failed to enrich in small buds, and only weak localization was occasionally observed at bud necks (Figure 6). These results suggest YlSec15' s polarized localization depends on YlRas2, indicating YlSec15 may regulate hyphal formation and bipolar budding through the YlRas2-mediated pathway. This differs from *S. cerevisiae* and *C. albicans*, where Sec15 interacts with Rsr1 to regulate bipolar budding and polarized growth. In *C. albicans*, Sec15 mislocalizes in rsr1Δ mutants, whereas YlSec15 maintains normal localization in Ylrsr1Δ cells. This suggests YlSec15 functions primarily through the YlRas2 pathway rather than the YlRsr1 pathway in *Y. lipolytica*, implying that YlRas2-mediated signaling may be the dominant pathway for bipolar budding regulation in this yeast, unlike in the model organism *S. cerevisiae*.

Discussion

Through overexpression and localization studies, we characterized the vesicle protein YlSec15 in *Y. lipolytica*. Our findings reveal: (1) *Y. lipolytica* contains a Sec15 homolog, YlSec15, whose localization pattern—enriching in small buds and at bud necks—is conserved with Sec15 in *S. cerevisiae* and *C. albicans*; (2)

Overexpressed YlSec15 loses polarized localization and becomes disordered in the cytoplasm, leading to defective hyphal formation and a shift from bipolar to random budding in some cells, highlighting the importance of proper localization for YlSec15 function; (3) YlSec15 likely regulates hyphal formation and budding site selection through the YlRas2-mediated pathway rather than the YlRsr1-mediated pathway.

These discoveries further elucidate the molecular mechanisms of bipolar budding in *Y. lipolytica* and establish a direct relationship between polarized growth and vesicle transport. However, this study has limitations, as it relied solely on overexpression and localization analysis. More comprehensive investigations are needed to deepen our understanding of YlSec15 function.

Acknowledgments

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References

[1] Chiou J G, Balasubramanian M K, and Lew D. Cell polarity in yeast. *Annual Review of Cell and Developmental Biology*, 2017, 33: 77-101.
[2] Howell A S, and Lew D J. Morphogenesis and the cell cycle. *Genetics*, 2012, 190(1): 51-77.
[3] Park H O, and Bi E. Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiology and Molecular Biology Review*, 2007, 71(1): 48-96.
[4] Nicaud J M. *Yarrowia lipolytica*. *Yeast*, 2012, 29(10): 409-418.
[5] Herrero A B, López M C, Fernández L L, et al. *Candida albicans* and *Yarrowia lipolytica* as alternative models for analysing budding patterns and germ tube formation in dimorphic fungi. *Microbiology*, 1999, 145(10): 2727-2737.
[6] Veses V N, and Gow N A. Pseudohypha budding patterns of *Candida albicans*. *Medical Mycology*, 2008, 47(3): 268-275.
[7] Cullen P J, and Sprague G F. The roles of bud-site-selection proteins during haploid invasive growth in yeast. *Molecular Biology of the Cell*, 2002, 13(9): 2990-3004.
[8] Li Y Q, Li M, Zhao X F, et al. A role for the Rap GTPase YlRsr1 in cellular morphogenesis and the involvement of YlRsr1 and the Ras GTPase YlRas2 in bud site selection in the dimorphic yeast *Yarrowia lipolytica*. *Eukaryotic Cell*, 2014, 13(5): 580-590.
[9] Li M, Li Y Q, Zhao X F, et al. Roles of the three Ras proteins in the regulation of dimorphic transition in the yeast *Yarrowia lipolytica*. *FEMS Yeast Research*, 2014, 14(3): 451-463.
[10] TerBush D R, Maurice T, Roth D, et al. The Exocyst is a multiprotein com-

plex required for exocytosis in *Saccharomyces cerevisiae*. *The EMBO Journal*, 1996, 15(23): 6483-6494.
[11] Guo P P, Yong J Y, Wang Y M, et al. Sec15 links bud site selection to polarised cell growth and exocytosis in *Candida albicans*. *Scientific Report*, 2016, 6: 26464.
[12] Hurtado C A, and Rachubinski R A. Isolation and characterization of YlBEM1, a gene required for cell polarization and differentiation in the dimorphic yeast *Yarrowia lipolytica*. *Eukaryotic Cell*, 2002, 1(4): 526-537.
[13] Richard M, Quijano R R, Bezzate S, et al. Tagging morphogenetic genes by insertional mutagenesis in the yeast *Yarrowia lipolytica*. *Journal of Bacteriology*, 2001, 183(10): 3098-3107.
[14] Zhao X F, Li M, Li Y Q, et al. The TEA/ATTS transcription factor YlTec1p represses yeast-to-hypha transition in the dimorphic yeast *Yarrowia lipolytica*. *FEMS Yeast Research*, 2013, 13(1): 50-61.
[15] TerBush D R, and Novick P. Sec6, Sec8, and Sec15 are components of a multi-subunit complex which localizes to small bud tips in *Saccharomyces cerevisiae*. *The Journal of Cell Biology*, 1995, 130(2): 299-312.
[16] France Y E, Boyd C, Coleman J, et al. The polarity-establishment component Bem1p interacts with the exocyst complex through the Sec15p subunit. *Journal of Cell Science*, 2005, 119(5): 876-888.

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