FEBS Letters 589 (2015) 513–520

FEBS Letters

journal homepage: www.FEBSLetters.org

# Ssn6 has dual roles in *Candida albicans* filament development through the interaction with Rpd31

Ji-Eun Lee [a,1], Jang-Hyun Oh [a,1,2], MyungHee Ku [a], Jueun Kim [b], Jung-Shin Lee [b,*], Sa-Ouk Kang [a,*]

[a] Laboratory of Biophysics, School of Biological Sciences, and Institute of Microbiology, Seoul National University, Seoul 151-742, Republic of Korea
[b] Department of Molecular Biosciences, College of Biomedical Sciences, Kangwon National University, 1 Kangwondaehak-gil, Chuncheon-si, Gangwon-do 200-701, Republic of Korea

ARTICLE INFO

*Article history:*
Received 11 November 2014
Revised 9 January 2015
Accepted 9 January 2015
Available online 17 January 2015

Edited by Dietmar J. Manstein

*Keywords:*
Transcriptional repressor
Ssn6
Rpd31
Filamentous growth
*Candida albicans*

ABSTRACT

**Ssn6 is a crucial regulator of morphological transition and virulence in the fungal pathogen *Candida albicans*. Ssn6 has previously been reported to act in complex with the transcriptional repressor Tup1. Here, we report that Ssn6 also interacts with the histone deacetylase Rpd31, independently of Tup1. The *ssn6*/*rpd31* double mutant strain formed elongated filaments, but failed to form filament extension, and this coincided with the down-regulation of the filament extension gene *UME6*. Occupancy patterns of Ssn6 and Rpd31 differed at the promoters of *UME6* and the metabolic gene *INO1*. These findings indicate that, in *C. albicans*, Ssn6 has dual roles in filament development, depending on the interaction with Rpd31.**
**© 2015 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.**

## 1. Introduction

*Candida albicans* is one of the most common opportunistic fungal pathogens in humans [1,2]. Its pathogenicity is directly linked to its ability to undergo a morphological transition from the yeast-form to filamentous hyphae [3–9]. This morphological change is induced in response to a variety of environmental signals such as serum, proline, N-acetylglucosamine, and different carbon sources. From signal induction to morphological transition, there are complex networks of signal transduction pathways [10,11].

Ssn6 is known as a general transcriptional repressor, which exists as a complex with the transcriptional repressor Tup1 through its tetratricopeptide repeat (TPR) motifs [12–16] and regulates the genes of many cellular processes including nutrient utilization, osmotic stress, meiosis, mating, and sporulation in *Saccharomyces cerevisiae* [17,18]. In *C. albicans*, Ssn6 also functions as a complex with Tup1 [19], which is a distinct repressor for morphogenesis [20–24], but our previous data revealed that Ssn6 is a critical factor for filamentous growth and virulence independently of Tup1 [25]. Despite many studies focusing on the Ssn6-Tup1 complex in *S. cerevisiae* [26–28] and Tup1 in *C. albicans* [19–21,24,29–31], how Ssn6 can regulate the morphological transition and virulence of *C. albicans* in Tup1-independent manner has not been well understood.

Therefore, we first screened the interaction partner of Ssn6 through tandem affinity purification (TAP) and identified histone deacetylase Rpd31 interacting with Ssn6 in Tup1-independent manner. Because Ssn6 is a transcriptional regulator and histone deacetylases (HDACs) also regulate genes transcriptionally through direct binding to the chromatin, we speculated that Ssn6 and Rpd31 might share the regulatory mechanism. We found that Rpd31 is one of the critical factors regulating for morphogenesis and virulence as Ssn6 in *C. albicans*. We also found that deletion of *SSN6* and *RPD31* induced the development of elongated filaments, but caused to be defective in filament extension. Surprisingly, chromatin immunoprecipitation (ChIP) assays of Ssn6 and Rpd31 showed different patterns of enrichment at the promoters of metabolic gene *INO1* and hyphal extension gene *UME6*. Our results suggest that Ssn6 has dual functions as a repressor or an activator, depending on its target genes and participation of Rpd31 for regulation, which is the critical point determining the roles of Ssn6.

*Abbreviations:* TAP, tandem affinity purification; ORF, open reading frame; TPR, tetratricopeptide repeat; MALDI, matrix-assisted laser desorption/ionization; PAGE, polyacrylamide gel electrophoresis

* Corresponding authors. Fax: +82 2 888 4911 (S.-O. Kang), +82 33 259 5641 (J.-S. Lee).
*E-mail addresses:* jungshinlee@kangwon.ac.kr (J.-S. Lee), kangsaou@snu.ac.kr (S.-O. Kang).
[1] These authors contributed equally to this work.
[2] Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA.

http://dx.doi.org/10.1016/j.febslet.2015.01.011
0014-5793/© 2015 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

## 2. Materials and methods

### 2.1. C. albicans strains and culture conditions

All of the *C. albicans* strains used in this study are listed in Table 1. The clinical isolates SC5314 and BWP17 were used as parental wild-type strains. For yeast growth, cells were grown in YPD (1% yeast extract, 2% peptone and 2% dextrose) at 28 °C. For hyphal-growth, pre-cultured cells in YPD were inoculated into YPD supplemented with 10% Fetal bovine serum (FBS) or Spider (1% mannitol, 1% nutrient broth and 0.2% $K_2HPO_4$) and grown at 37 °C. Cells for gene disruption were cultured in modified synthetic defined (SD) with supplements.

### 2.2. Tandem affinity purification and MALDI-TOF mass spectroscopic analysis

A calmodulin-binding peptide (CBP) TAP tag was fused to the C-terminus of Ssn6 following the strategy described by Puig et al. [32]. The resulting plasmid, pJO-1, was used as a template for amplifying a CBP-TAP cassette containing at each end regions homologous to the target region of *SSN6*. To express Rpd31-HPM-TAP in *C. albicans*, the pHPM53U was used as described in Table 2. The *RPD31* open reading frame (ORF) was placed under the *ADH1* promoter. Transformation of *C. albicans* cells was performed according to a previously described protocol [33,34]. The CBP-TAP-tagged Ssn6 complex was purified according to a previously described protocol [32] with minor modifications. The peptide eluents treated with trypsin were concentrated with a vacuum evaporator, and analyzed by MALDI-TOF mass spectroscopy.

### 2.3. Gene disruption of RPD31 in wild type and ssn6Δ in C. albicans

Disruption of *RPD31* in *C. albicans* was carried out as described by Gola et al. [35]. Briefly, each allele of *RPD31* was disrupted by *HIS1* and *ARG4* auxotrophic markers flanked at the 5′-end by the promoter region and at the 3′-end by the terminator region of *RPD31*. The cassettes were generated by PCR. For reintegration of *RPD31*, the flanking region of *RPD31* ranging from 540 bp upstream of the start codon to 450 bp downstream of the stop codon was cloned with a *URA3* marker yielding pRPDREV2. It was cleaved at the *Bgl*II site for integration. Disruption of *SSN6* in the *rpd31Δ* strain was carried out as described by Fonzi and Irwin [36]. All transformants were confirmed by diagnostic PCR and Southern blot.

### 2.4. Northern blot analysis

The coding regions of indicated genes were amplified by PCR and labeled with [$\alpha$-$^{32}$P]-dATP. The primers used for production of probes are listed in Table 3. The resultant blots were visualized using the BAS-2500 system (Fuji Film).

### 2.5. Western blot analysis

The sample was separated by 10% SDS–PAGE and transferred onto a membrane according to the method described by Towbin et al. [37]. The membrane was incubated with primary antibody overnight. After washing with TBST, the membrane was incubated for 1 h with alkaline phosphate-conjugated secondary antibody. The TBST-washed membrane was developed with BCIP and NBT at 25 °C.

**Table 1**
*Candida albicans* strains used in this study.

| Strain | Genotype | Source |
|---|---|---|
| SC5314 | Wild type | Clinical isolate |
| CAI4 | *ura3Δ::imm434/ura3Δ::imm434* | Fonzi and Irwin [36] |
| BWP17 | *ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG/arg4 Δ::hisG his1Δ::hisG/his1Δ::hisG* | Wilson et al. [55] |
| Bca2-9 | *ura3Δ::imm434/ura3Δ::imm434 tup1Δ::hisG/tup1Δ::hisG* | Braun and Johnson [20] |
| CH403 | *ura3Δ::imm434/ura3Δ::imm434 ssn6Δ::hisG/ssn6Δ::hisG-URA3-hisG* | Hwang et al. [25] |
| CH404 | *ura3Δ::imm434/ura3Δ::imm434 ssn6Δ::hisG/ssn6Δ::hisG* | Hwang et al. [25] |
| JO103 | *ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG/arg4Δ::hisG his1Δ::hisG/his1Δ::hisG rpd31Δ::HIS1/rpd31Δ::ARG4* | This study |
| JO1031 | *RPS10::RPS10-URA3*-pGEM-T Easy in JO103 | This study |
| JO103R | *rpd31Δ::RPD31-URA3*-pGEM-T Easy in JO103 | This study |
| JO203 | *ssn6Δ::hph/ssn6Δ::hph-URA3-hph* in JO103 | This study |
| JO500 | *ura3Δ::imm434/ura3Δ::imm434 SSN6::CBPTAP-URA3* | This study |
| JO700 | *ura3Δ::imm434/ura3Δ::imm434 RPD31::CBPTAP-URA3* | This study |
| JO710 | *ura3Δ::imm434/ura3Δ::imm434 ssn6Δ::hisG/ssn6Δ::hisG RPD31::CBPTAP-URA3* | This study |
| JO720 | *ura3Δ::imm434/ura3Δ::imm434 arg4Δ::hisG/arg4Δ::hisG his1Δ::hisG/his1Δ::hisG rpd31Δ::HIS1/rpd31Δ::ARG4 SSN6::CBPTAP-URA3* | This study |
| JO730 | *SSN6::CBPTAP-ARG4 RPD31::HPM TAP-HIS1* in BWP17 | This study |
| JO740 | *tup1Δ::hph/tup1Δ::hph SSN6::CBPTAP-ARG4 RPD31::HPM TAP-HIS1* in BWP17 | This study |

**Table 2**
Plasmids used in this study.

| Plasmid | Description | Sources |
|---|---|---|
| pBS1479 | Shuttle vector containing CBPTAP tag | Puig et al. [32] |
| pJO-1 | pBS1479 containing *CaURA3* | This study |
| pJS-HPM53H | Shuttle vector containing HPMTAP tag | Graumann et al. [54] |
| pHPM53U | pJS-HPM53H containing *CaURA3* | This study |
| PADH | pGEM T-Easy vector containing *ADH* promoter | This study |
| pRPDREV2 | pGEM T-Easy vector containing *RPD31* ORF region with *URA3* marker for reintegration | This study |
| pSSN-1441 | pGEM T-Easy vector containing *SSN6* ORF | This study |
| pQF181 | Disruption vector containing *hph-URA3-hph* cassettes (*URA3*:5′ → 3′ direction) | Hwang et al. [25] |
| pQF182 | Disruption vector containing *hph-URA3-hph* cassettes(*URA3*:3′ ← 5′ direction) | Hwang et al. [25] |
| pSSN-181 | *hph-URA3-hph* cassettes containing homologous recombination region of *SSN6* in pQF181 | This study |
| pSSN-182 | *hph-URA3-hph* cassettes containing homologous recombination region of *SSN6* in pQF182 | This study |
| pRP10-URA3 | pGEM T-Easy vector containing *RPS10-CaURA3* | This study |

Download English Version:

https://daneshyari.com/en/article/10870005

Download Persian Version:

https://daneshyari.com/article/10870005

Daneshyari.com