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Role of the mRNA export factor Sus1 in oxidative stress tolerance in *Candida albicans*

Chenpeng Xiao, Qilin Yu, Bing Zhang, Jianrong Li, Dan Zhang, Mingchun Li*

Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China

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ABSTRACT

In eukaryotes, the nuclear export of mRNAs is essential for gene expression. However, little is known about the role of mRNA nuclear export in the important fungal pathogen, *Candida albicans*. In this study, we identified *C. albicans* Sus1, a nucleus-localized protein that is required for mRNA export. Interestingly, the *sus1*Δ/Δ displayed hyper-sensitivity to extracellular oxidative stress, enhanced ROS accumulation and severe oxidative stress-related cell death. More strikingly, although the mutant exhibited normal activation of the expression of oxidative stress response (OSR) genes, it had attenuated activity of ROS scavenging system, which may be attributed to the defect in OSR mRNA export in this mutant. In addition, the virulence of the *sus1*Δ/Δ was seriously attenuated. Taken together, our findings provide evidence that the mRNA export factor Sus1 plays an important role in oxidative stress tolerance and pathogenesis.

1. Introduction

In eukaryotes, the nucleus and the cytoplasm were separated by the nuclear envelop, which makes that mRNA biogenesis in the nucleus and proteins translation in the cytoplasm [1]. The eukaryotic gene expression is consisted of complicated processes, including gene transcription, mRNA biogenesis and mature mRNA transport [2]. In *Saccharomyces cerevisiae*, several multiprotein complexes are coordinated to ensure this process. The SAGA (Spt-Ada-Gcn5 acetyltransferase) complex participates in gene transcription and mRNA nuclear export. This complex regulates genes transcribed by polymerase II, possesses histone acetyltransferase and deubiquitination, and facilitates the actively transcribing genes such as *GAL1* tethering to the nuclear periphery [3–5]. The TREX-2 complex, which locates at the nuclear pore complexes, functions in mRNA export and transcription elongation [6–8].

In *Saccharomyces cerevisiae*, Sus1 is a component of both SAGA complex and TREX-2 complex [9]. In SAGA, Sus1 is a member of the deubiquitinating module (DUBm), which comprises four proteins: Sus1, Sgf11, Sgf73 and the ubiquitin-specific protease Ubp8. DUBm mediates the histone H2B deubiquitination, and Sus1, along with Sgf73, also participate the mRNA export [10,11]. In TREX-2 complex, Sus1 and Cdc31 directly interact with the CID motif of Sac3. Sus1 is essential for nuclear pore complex (NPC) targeting of TREX-2, and deletion of *SUS1* strongly impairs mRNA nuclear export [12].

Candida albicans is a commensal pathogenic fungus, causing both superficial and systemic infections in immune-compromised patients, such as those with AIDS or cancer related treatments [13,14]. When this pathogen attacks host immune system, it is frequently confronted with oxidative stress caused by reactive oxygen species (ROS) [15]. Oxidative stress response (OSR) is essential for this pathogen surviving in host tissues. Hence, exploring the factors functioning in OSR is important for development of novel antifungal targets.

In *C. albicans*, the proteins that function in mRNA export remains to be investigated. Therefore, in our work, we identified the *C. albicans* mRNA export factor Sus1, and investigated its physiological function in *C. albicans*. Our results reveal that Sus1 plays an important role in mRNA export, oxidative stress response and virulence.

Abbreviations: DCFH-DA, 2′, 7′-dichlorodihydro-fluorescein diacetate; ROS, reactive oxygen species; DAPI, 4′,6-diamidino-2-phenylindole; PI, propidium iodide; MDA, Malondialdehyde.

* Corresponding author. Department of Microbiology, College of Life Science, Nankai University, Tianjin 300071, China.
E-mail address: nklimingchun@163.com (M. Li).

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2. Materials and methods

2.1. Growth conditions

The strains were grown in the YPD medium (1% yeast extract, 2% peptone, 2% glucose) supplemented with 80 μg/mL uridine, or in synthetic drop-out medium (0.67% yeast nitrogen base (YNB) without amino acids, 2% glucose, 0.2% complete mixture lacking specific amino acids) for selection of specific transformants.

2.2. Strains and plasmids

Plasmids and primers in this study are listed in Tables 1 and 2. For constructing the *SUS1* reconstituted plasmid pDDB78-SUS1, the *SUS1* fragment containing the 360 bp of ORF plus 352 bp of the promoter region and 356 bp of the terminator sequence was amplified from the DAY1 genome by PCR using the primers SUS1-5com and SUS1-3com, digested by *Kpn*I/*EcoR*I and cloned into the plasmid pDDB78. For generating the Sus1-location plasmids, the *SUS1* ORF was amplified from the BWP17 genome using the primers SUS1-5GFP and SUS1-3GFP, digested by *Sma*I/*BamH*I, and inserted into pAU34M-GFP to obtain pAU34M-GFP-SUS1.

The strains used in this study were listed in Table 1. All *C. albicans* strains were generated in the wild-type strain DAY1 background. Construction of the homozygous *sus1*Δ/Δ was described previously [16]. To construct the *SUS1* reconstituted strain, the *sus1*Δ/Δ strain was transformed with *Nru*I-digested pDDB78-SUS1. For Sus1 localization, the plasmid pAU34M-GFP-SUS1 was digested by *Bgl*II, and transformed into the wild-type strain DAY1.

2.3. Fluorescence in situ hybridization (FISH) of mRNA

RNA FISH was performed as previously described [17]. Overnight cultures were diluted to OD_{600} of 0.1 in YPD and grown for indicated time. Then cells were harvested and fixed with 4% formaldehyde at room temperature for 30 min. The cells were then washed twice with PBS buffer and resuspended in the same buffer. 500 μL of the suspension were incubated for 1 h at 30 °C with 0.5 mg/mL of Zymolyase 100T to digest the cell wall. The obtained protoplasts were spotted onto poly-$_L$-lysine-coated slides. Attached protoplasts were rehydrated with 2 × SSC and then incubated in prehybridization buffer overnight at 37 °C with the Cy3-end-labeled oligo$(dT)_{18}$ primer or primers of *GLR1*, *TRR1* and *CAT1* in a humid chamber. The slides were then washed five times with 1 × SSC, and incubated in 1 × SSC buffer with 5 μg/mL DAPI at room temperature for 30 min. Slides were air-dried, mounted using VECTASHIELD Mounting Medium, and observed with a fluorescence microscope (BX41, Olympus, Japan).

2.4. ROS assays

The ROS levels were measured using 2′,7′-dichlorodihydrofluorescein deacetate (DCFH-DA, Molecular Probes, USA). Overnight cultures were diluted in fresh YPD, adjusted to OD_{600} of 0.1 and cultivated for 4 h with shaking at 30 °C. H_2O_2 was then added to the final concentration of 5 mM. The cells were further treated with H_2O_2 for indicated time. Then cells were harvested, washed twice with PBS buffer, resuspended with PBS containing 20 μg/mL DCFH-DA and incubated at 30 °C for 30 min. The stained cells were washed again with PBS buffer, and the fluorescence intensity (FLU, excitation wave 488 nm, emission wave 520 nm) was detected by a fluorescent microplate reader (Perkin Elmer, USA). The results were normalized to cell concentration through measuring the optical density (OD_{600}).

2.5. Malondialdehyde (MDA) assays

MDA contents were determined using the thiobarbituric acid (TBA) method. The cells were cultured as described above, and then treated with 5 mM H_2O_2 for indicated time, washed twice with PBS, and suspended in 50 mM Tris-HCl (pH 7.5) containing 1 mM PMSF. Cells were then blocked with glass beads and the lysates were centrifuged. MDA contents were determined using a MDA assay kit (Nanjing Jiancheng Bioengineering Institute, China), and total protein contents were analyzed by the Coomassie protein assay reagents.

2.6. Assays of CAT and SOD activity

To determine the activity of CAT and SOD, the cells were cultured as described above, and then treated with 5 mM H_2O_2 for indicated time. Cells were harvested and washed with distilled water. Protein extracts were prepared using the cell breakage buffer (50 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride) with glass beads. Activity of CAT and SOD was determined by cellular CAT and

Table 1
Strains and plasmids used in this study.

Strains/Plasmids	Genotype	Source
Stains		
WT (DAY1)	*ura3Δ::λimm434/ura3Δ::λimm43 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	Dana Davis
sus1/SUS1	*ura3Δ::λimm434/ura3Δ::λimm43 his1::hisG/his1::hisG arg4::hisG/arg4::hisG sus1::ARG4/SUS1*	This study
*sus1*Δ/Δ	*ura3Δ::λimm434/ura3Δ::λimm43 his1::hisG/his1::hisG arg4::hisG/arg4::hisG sus1::ARG4/sus1::URA3*	This study
*sus1*Δ/Δ + *SUS1*	*ura3Δ::λimm434/ura3Δ::λimm43 his1::hisG/his1::hisG arg4::hisG/arg4::hisG sus1::ARG4/sus1::URA3, pDDB78-SUS1*	This study
WT-GFP-Sus1	*ura3Δ::λimm434/ura3Δ::λimm43 his1::hisG/his1::hisG arg4::hisG/arg4::hisG PACT1-GFP-SUS1*	This study
Plasmids		
pRS-ArgΔ*Spe*I	Amp^{R+}, *ARG4*	Dana Davis
pDDB57	Amp^{R+}, *URA3*	Dana Davis
pDDB78	Amp^{R+}, *HIS1*	Dana Davis
pDDB78-SUS1	Amp^{R+}, *SUS1*, *HIS1*	This study
pAU34 M	Amp^{R+}, P_{ACT1}-MCS, *URA3*	Qilin Yu [25]
pAU34M-GFP-SUS1	Amp^{R+}, P_{ACT1}-GFP-*SUS1*, *URA3*	This study

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