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Stress-associated endoplasmic reticulum protein 1 (SERP1) and Atg8 synergistically regulate unfolded protein response (UPR) that is independent on autophagy in *Candida albicans*

Jianrong Li, Qilin Yu, Bing Zhang, Chenpeng Xiao, Tianyu Ma, Xiao Yi, Chao Liang, Mingchun Li*

Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, College of Life Science, Nankai University, Tianjin, PR China

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ABSTRACT

Cellular stresses could activate several response processes, such as the unfolded protein response (UPR), autophagy and oxidative stress response to restore cellular homeostasis or render cell death. Herein, we identified the *Candida albicans* stress-associated endoplasmic reticulum protein 1 (SERP1), also known as Ysy6, which was involved in endoplasmic reticulum (ER) stress response. We found that deletion of both *SERP1/YSY6* and *ATG8* led to hypersensitivity to tunicamycin (TN), and resulted in severe mitochondrial dysfunction under this stress. UPR reporting systems illustrated that the double mutation attenuated splicing of *HAC1* mRNA, followed by decreased level of UPR activation. In addition, the *atg8*Δ/Δ *ysy6*Δ/Δ double mutant had normal autophagic degradation of the ER component Sec63 under ER stress, suggesting that SERP1/Ysy6 and Atg8 synergistically regulated UPR that is independent on autophagy. We also found that deletion of both *SERP1/YSY6* and *ATG8* caused the loss of virulence. This study reveals the important role of SERP1/Ysy6 and Atg8 in ER stress response and virulence in *C. albicans*.

1. Introduction

The endoplasmic reticulum (ER) is the site of synthesis and folding of secretory proteins, which is essential for most cellular activities and survival. Disruption of ER homeostasis may cause ER stress such as accumulation of unfolded or misfolded proteins (Oyadomari and Mori, 2004; Szegezdi et al., 2006). Eukaryotic cells have evolved different strategies to respond to these stresses. For example, this stress causes translational decay, reducing synthesis of new proteins and preventing further accumulation of unfolded proteins (Harding et al., 2002). Moreover, the ER-associated degradation (ERAD) pathway and the unfolded protein response (UPR) also function in alleviation of ER stress. On the one hand, the ERAD pathway is the major degradation mechanism in response to accumulation of misfolded proteins (Meusser et al., 2005). On the other hand, the UPR pathway is activated to stimulate expression of proteins that can relieve the stress (Patil and Walter, 2001; Travers et al., 2000). In the yeast *Saccharomyces cerevisiae*, Ire1 is a major UPR sensor to regulate unconventional splicing of the *HAC1* mRNA (Back et al., 2005; Ron and Walter, 2007). Spliced form of *HAC1* mRNA produces a potent transcription factor that induces expression of UPR genes needed for re-establishment of ER functions (Tam et al., 2014). In addition, autophagy, an evolutionarily conserved process, is also activated by ER stress. Previous studies have found that activation of the UPR pathway in yeast also induces a new branch of macroautophagy that selectively targets the ER (Bernales et al., 2007).

Stress-associated endoplasmic reticulum protein 1 (SERP1), also known as Ysy6 or ribosome-associated membrane protein 4 (RAMP4), is a Sec61-associated polypeptide that is induced by ER stress(Hori et al., 2006)[11]. This protein may suppress the secretion defect of a *secY* mutant in *Escherichia coli* (Hori et al., 2006). Other studies reveal that the *SERP1-/-* mice showed growth retardation, increased mortality and impaired glucose tolerance. SERP1 may form a stable complex with Sec61β and functions in stabilization and glygosylation of membrane proteins after induction of ER stress (Yamaguchi et al., 1999). Moreover, its expression is strongly induced by the UPR transcription factor Hac1, suggesting its association with UPR (Pool, 2009).

In this study, we identified SERP1 (encoded by *YSY6*) from *Candida albicans*, which is involved in ER stress response. We here demonstrated that mutation of both *SERP1/YSY6* and *ATG8* severely attenuated activation of the UPR pathways, followed by hypersensitivity to ER stress, but did not impair the ER phagy-related degredation. Moreover, SERP1/Ysy6 and Atg8 synergistically maintain mitochondrial function under ER stress and is involved in virulence of this pathogen. This study reveals an autophagy-independent mechanism of UPR regulation

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

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Table 1
C. albicans strains and plasmids in this study.

Strains or Plasmids	Genotype	Source
Strains		
WT (BWP17)	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG*	Dana Davis
ysy6/YSY6	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG ysy6:ARG4/YSY6*	This study
ysy6Δ/Δ	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG ysy6:ARG4/ysy6:URA3*	This study
ysy6Δ/Δ-URA3	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG ysy6:ARG4/ysy6:URA3-dpl200*	This study
YSY6c	*ura3:imm434/ura3:imm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG ysy6:ARG4/ysy6:URA3-dpl200 YSY6*	This study
atg8Δ/Δ-URA3	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8: dpl200*	Qilin Yu
atg8Δ/Δysy6/YSY6	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8:dpl200 ysy6:URA3-dpl200/YSY6*	This study
atg8Δ/Δysy6/YSY6-URA3	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8:dpl200 ysy6:dpl200/YSY6*	This study
atg8Δ/Δysy6Δ/Δ	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8:dpl200 ysy6:dpl200/ysy6:URA3-dpl200*	This study
atg8Δ/Δysy6Δ/Δ-URA3	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8:dpl200 ysy6:dpl200/ysy6:dpl200*	This study
WT + *YSY6*-GFP	*ura3:imm434/ura3:imm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG YSY6-GFP*	This study
WT + *SEC63*-GFP	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG SEC63-GFP*	This study
ysy6Δ/Δ + *SEC63*-GFP	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG ysy6:ARG4/ysy6:URA3 SEC63-GFP*	This study
atg8Δ/Δ + *SEC63*-GFP	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8: dpl200 SEC63-GFP*	This study
atg8Δ/Δysy6Δ/Δ + *SEC63*-GFP	*ura3Δ:λimm434/ura3Δ:λimm434 his1:hisG/his1:hisG arg4:hisG/arg4:hisG atg8:ARG4/atg8:dpl200 SEC63-GFP ysy6:dpl200/ ysy6:dpl200*	This study
Plasmids		
pRS-ArgΔ*Spe*I	Ap^R*ARG4*	Dana Davis
pDDB57	Ap^R *URA3*	Dana Davis
pDDB78	Ap^R *TRP1 HIS1*	Dana Davis
pGFP-URA3	Ap^R *GFP-URA3*	Dana Davis
P_{PMT4}-GFP	Ap^R P_{PMT4}*-GFP URA3*	Qilin Yu
P_{PRB1}-GFP	Ap^R P_{PRB1}*-GFP URA3*	Qilin Yu
pLUBP	Ap^R *URA3*	Gerald Fink

governed by the synergy of SERP1/Ysy6 and Atg8.

2. Materials and methods

2.1. Construction of plasmids and C. albicans strains, growth conditions

The strains and plasmids used in this study are listed in Table 1, and the primers used are listed in Table 2. All *C. albicans* strains were generated in the BWP17 (WT) background. For deletion of one copy of *YSY6*, the wild-type strain BWP17 was transformed with the PCR product amplified from the pRS-ArgΔ*Spe*Iplasmid with the deletion primers YSY6-5DR and YSY6-3DR using the *ARG4* marker, and the heterozygous mutant *ysy6/YSY6* was confirmed by PCR method with the detection primers YSY6-5det and YSY6-3det. The strain *ysy6/YSY6* was then transformed with the *URA3* cassette amplified from the plasmid pDDB57, obtaining the homozygous mutant *ysy6Δ/Δ*. To facilitate the following genetic manipulation, the homozygous mutant was plated on SC agar (2% glucose, 0.67% yeast nitrogen base, 0.2% amino acid mixture, 2% agar) containing 0.1% 5-FOA (BBI, USA), generating the *URA3*-depleted strain *ysy6Δ/Δ-URA3*. To construct the *YSY6* reconstituted strain, *ysy6Δ/Δ-URA3* was transformed with the *Nru*I-digested pDDB78-YSY6 obtaining *YSY6c*. Based on the previous research, we got the *atg8Δ/Δ-URA3* from our laboratory. The *atg8Δ/Δysy6Δ/Δ* double mutant, in which both *ATG8* and *YSY6* were disrupted, was constructed in the *atg8Δ/Δ-URA3* background. Firstly, the *URA3*-depleted *atg8Δ/Δ* mutant was transformed with the *URA3* cassette amplified from the plasmid pDDB57, generating the *YSY6* heterozygous mutant *atg8Δ/Δysy6/YSY6*, followed by *URA3* depletion, obtaining heterozygous *atg8Δ/Δysy6/YSY6-URA3*. This strain was then transformed again with the *URA3* cassette, generating the *YSY6* homozygous mutant *atg8Δ/Δysy6Δ/Δ*. Finally, *atg8Δ/Δysy6Δ/Δ* cells were also plated on the 5-FOA-contained SC agar, obtaining the *URA3*-depleted double mutant *atg8Δ/Δysy6Δ/Δ-URA3*. The *URA3*-depleted double mutant *atg8Δ/Δysy6Δ/Δ-URA3* was transformed with the *Nru*I-digested pDDB78-YSY6 obtaining a complemented mutant *atg8Δ/Δysy6Δ/Δ + YSY6*. Another complemented mutant *atg8Δ/Δysy6Δ/Δ + ATG8* was obtained by a similar method with pDDB78-ATG8. The Ysy6-localization strain WT + *YSY6-GFP* was constructed by transforming BWP17 with the PCR fragment amplified from the pGFP-URA3 plasmid with the primers YSY6-GFP1 and YSY6-GFP2 using the *URA3* marker. To obtain the strains with the UPR reporting system, the strains were transformed with the plasmids P_{PMT4}-GFP and P_{PRB1}-GFP using the *URA3* marker (Yu et al., 2015). To detect the ER membrane protein Sec63 with C-terminally tagged by GFP, the wide type, *ysy6Δ/Δ*, *atg8Δ/Δ* and *atg8Δ/Δysy6Δ/Δ* were transformed with the GFP-URA3 fragment amplified from the plasmid pGFP-URA3, which was inserted into the 3′ terminus of the *SEC63* gene, obtaining the strains WT + *SEC63*-GFP, *ysy6Δ/Δ + SEC63*-GFP, *atg8Δ/Δ + SEC63*-GFP and *atg8Δ/Δysy6Δ/Δ + SEC63*-GFP.

Normally, *C. albicans* strains were cultured in liquid YPD medium

Table 2
The primers in this study.

Primers	Sequence(5′-3′)
YSY6-5DR	TAATCTTTCTTCATTAGATTCTATTATTGATATAAATAAAAACATATACATAAATAGAAATTTCCCAGTCACGACGTT
YSY6-3DR	TGATTCGAAAAGCAATTTGACTTGGGAATGGGAAGTGTGGGGTAGGTAGGTAGGCAAAATTGGAATTGTGAGCGGATA
YSY6-5det	CGTGGTCATAAGAAGAAATCGCA
YSY6-3det	AGATCAAGGTCCAAGAGATGT
YSY6-GFP-1	TTTGTATTATTATTCTTAGTATGTGGTGGAGCAATTTTAGAATTAATAAGATTGATCTTTGGTGGTGGTTCTAAAGGTGAAGAATTATT
YSY6-GFP-2	GGAATGGGAAGTGTGGGGTAGGTAGGTAGGCAAAATTAAATATGAACTGTAAACTATGATCTAGAAGGACCACCTTTGATTG
HAC1-5RT	TGAGGATGAACACCAAGAAGAA
HAC1-3RT	TCAAAGTCCAACTGAAATGAT
SEC63-5GFP	AGTGAAGATGAAGAGGTGTTCACTGATATTAATACTGATACAGAAGATGAAGGAGATAATGGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC63-3GFP	TACAGAAAGGTTATGTATTTTGAGTGAATATTATTGTTATGAGGCTATAGTACTTCAATTCTAGAAGGACCACCTTTGATTG