Journal of Microbiology (2015) Vol. 53, No. 2, pp. 111–115
DOI 10.1007/s12275-015-4727-y


# Deletion analysis of LSm, FDF, and YjeF domains of *Candida albicans* Edc3 in hyphal growth and oxidative-stress response

**Eung-Chul Kim and Jinmi Kim***

*Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*

(Received Dec 23, 2014 / Revised Jan 15, 2015 / Accepted Jan 19, 2015)

***Candida albicans*** **is an opportunistic fungal pathogen whose responses to environmental changes are associated with the virulence attributes. Edc3 is known to be an enhancer of the mRNA decapping reactions and a scaffold protein of cytoplasmic processing bodies (P-bodies). Recent studies of *C. albicans* Edc3 suggested its critical roles in filamentous growth and stress-induced apoptotic cell death. The *edc3/edc3* deletion mutant strain showed increased cell survival and less ROS accumulation upon treatment with hydrogen peroxide. To investigate the diverse involvement of Edc3 in the cellular processes, deletion mutations of LSm, FDF, or YjeF domain of Edc3 were constructed. The *edc3-LSmΔ* or *edc3-YjeFΔ* mutation showed the filamentation defect, resistance to oxidative stress, and decreased ROS accumulation. In contrast, the *edc3-FDFΔ* mutation exhibited a wild-type level of filamentous growth and a mild defect in ROS accumulation. These results suggest that Lsm and YjeF domains of Edc3 are critical in hyphal growth and oxidative stress response.**

***Keywords***: Edc3, decapping activator, *Candida albicans*, hyphal growth, oxidative stress

## Introduction

*Candida albicans* is an opportunistic fungal pathogen which resides in the human body including the skin, vagina, bone marrow, and oral cavity. *C. albicans* switches rapidly among diverse morphologic forms: budding yeast, pseudohyphal, and hyphal forms in response to environmental changes (Brown and Gow, 1999; Calderone and Fonzi, 2001). The pseudohyphal and hyphal forms differ mainly at the site of septation. The pseudohyphal form has constriction, but the hyphal form has long tube-like filaments and distinction at the site of septation (Sudbery, 2011). The hyphal form is particularly associated with virulence attributes, such as passage through host tissues and defense against immune cells. Extensive virulence-associated changes in transcript or protein levels have been reported, which account the rapid response to the external signals and the yeast-to-hyphal transition (Nantel *et al*., 2002; Fernandez-Arenas *et al*., 2007).

The 5′ to 3′ decay pathway of mRNA requires effective removal of the 5′ cap and exonucleolytic turnover of the capless mRNA body. The mRNA decapping reaction is catalyzed by the Dcp2/Dcp1 enzyme, whose activity is enhanced by decapping activators such as Dhh1, Edc3, Lsm1-7, and Pat1 (Coller and Parker, 2005; Nissan *et al*., 2010). Most of these mRNA decay factors have similar cytoplasmic localization, termed as processing bodies (P-bodies). P-bodies are involved in translation and mRNA degradation and are also required for RNA silencing as well as related functions of miRNAs (Liu *et al*., 2005). Accumulation of P-bodies are induced by stress, including glucose deprivation, oxidative stress, osmotic stress, acidic stress, heat shock, and UV irradiation in yeast *Saccharomyces cerevisiae* and *C. albicans* (Teixeira *et al*., 2005). In addition, P-bodies accumulate during hyphal development in *C. albicans* (Jung and Kim, 2011).

Edc3 is known to be a decapping activator and functions as a P-body scaffold protein in yeast (Kshirsagar and Parker, 2004; Decker *et al*., 2007). Edc3 was identified in the genome sequence of *C. albicans.* Deletion mutation of *EDC3* showed a defect in filamentous growth and reduction in the P-body accumulation in *C. albicans* (Jung and Kim, 2011). A recent report suggested a function of Edc3 in the yeast apoptosis (Jung and Kim, 2014). Under apoptosis-inducing conditions such as oxidative stresses, the *edc3/edc3* deletion strain showed increased cell survival and less ROS accumulation, when compared with the wild-type. Edc3 contains three functional domains: an N-terminal LSm (Sm-like) domain, a central FDF domain, and a C-terminal YjeF-N domain (Tritschler *et al*., 2007; Fromm *et al*., 2012). The LSm domain binds Dcp2 and possesses an RNA binding site. The FDF domain interacts with Dhh1 and is enriched with polar and charged amino acids. The YjeF domain self-interacts and promotes Edc3 dimerization (Decker *et al*., 2007; Harigaya *et al*., 2010).

In this work, deletion mutations of LSm, FDF, or YjeF were constructed in *C. albicans EDC3*. LSmΔ or YjeFΔ mutation showed the phenotypes similar to those of the *edc3/edc3* mutant strain, including filamentation defect, resistance to oxidative stress, and decreased ROS accumulation. In contrast, FDFΔ mutation exhibited a mild defect in ROS accumulation, but a wild-type level of filamentous growth. These results indicate that LSm and YjeF domains are crucial to Edc3 functions in hyphal growth and oxidative stress response.

*For correspondence. E-mail: jmkim@cnu.ac.kr; Tel.: +82-42-821-6416; Fax: +82-42-822-7367

**Table 1.** Plasmids used in this study

| Plasmids | Genotype | Reference |
|---|---|---|
| pRS426 | 2μ *Ori amp*$^r$ *URA3* | Christianson *et al.* (1992) |
| pRC18 | *URA3*-marked *CaARS* vector containing pUC 18 multiple cloning site | Stoldt *et al.* (1997) |
| pJI410 | *Ori amp*$^r$ *URA3 EDC3* | Jung and Kim (2011) |
| pJI412 | *Ori amp*$^r$ *URA3 edc3-LSmΔ* | This study |
| pJI413 | *Ori amp*$^r$ *URA3 edc3-FDFΔ* | This study |
| pJI414 | *Ori amp*$^r$ *URA3 edc3-YjeFΔ* | This study |

## Materials and Methods

### Yeast strains and growth conditions

The *Candida albicans* strains used in this study are BWP17 (*ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*) and JKC81(*ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG edc3::hph/edc3::hph*) (Wilson *et al.*, 1999; Jung and Kim, 2011).

Yeast standard media were prepared according to the established procedures (Adams *et al.*, 1997). *C. albicans* strains were grown in YEPD (1% yeast extract, 2% peptone, 2% dextrose) or YNB (0.67% yeast nitrogen base without amino acid, 2% dextrose) supplemented with required amino acids. For oxidative stress, cells were treated with 10 mM $H_2O_2$ for up to 3 h.

The filamentation phenotype of the *C. albicans* cells was tested in serum-containing medium (1% yeast extract, 2% peptone, 10% new born calf serum) and spider medium (1% mannitol, 1% nutrient broth, 2% agar, 0.2% $K_2HPO_4$, pH 7.2) as described previously (Liu *et al.*, 1994). Cultures were incubated at 37°C. Observations were made using an Olympus BX51 research microscope at 400×. Colony morphologies were taken at 100×.

### Construction of domain deletion mutations of *EDC3*

Domain deletion mutations of Edc3 were constructed using Ez-cloning kit (Enzynomics). Linearized vector and two PCR-amplified fragments were ligated in a single reaction. The plasmids used in this study are listed in Table 1. For each deletion, a deletion primer set (*lsm*Δ-R/*lsm*Δ-F, *fdf*Δ-R/*fdf*Δ-F, or *yjef*Δ-R/*yjef*Δ-F) was designed in such that the deletion primer contains the upstream and downstream sequences of the domain to be deleted (Table 2). The EDC3-F or EDC3-R primer contains 5′ end or 3′ end of *EDC3* gene and 15 bp extension which is homologous to vector end. Two fragments were PCR-amplified by using primer sets, EDC3-F/*lsm*Δ-R and *lsm*Δ-F/EDC3-R, and then ligated to the *Sal*I/*Cla*I-digested pRS426. To construct pRS426-*fdf*Δ and pRS426-*yjef*Δ, the same strategy was employed by using the primer set, EDC3-F/*df*Δ-R and *fdf*Δ-F/EDC3-R, EDC3-F/*yjef*Δ-R and *yjef*Δ-F/EDC3-R. The vectors pRS426-*lsm*Δ, pRS426-*fdf*Δ, and pRS426-*yjef*Δ were confirmed by standard sequencing methods. To construct *C. albicans* plasmids, pJI412, pJI413, and pJI414, the pRS426-based deletion plasmids were digested with *Acc*65I/*Hin*dIII and ligated into the *Acc*65I/*Hin*dIII-digested pRC18.

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

| Primer name | Sequence |
|---|---|
| EDC3-F | CCCCCTCGAGGTCGGATATAACTTGTTTATTATCTA |
| EDC3-R | ATATCAAGCTTATCGAGATGTAGAAGACGAATTAC |
| *lsm*Δ-F | GTAATTCAATTACCTCCTGATTTTAAACAA |
| *lsm*Δ-R | AGGTAATTGAATTACCATTATTGAAGATATATAAAAAGAA |
| *fdf*Δ-F | CAAAAACGTGATCACACCAACATTG |
| *fdf*Δ-R | GTGATCACGTTTTTGTTTTATATCTTGAACATCAC |
| *yjef*Δ-F | ATTATACTAGCCTATAAAAATGGAACATGTCTTT |
| *yjef*Δ-R | ATAGGCTAGTATAATAGAATCGGTTGCTAATCTTT |

### Cell survival assay under oxidative stress condition

For analysis of test cell viability, cells were grown in synthetic complete (SC) liquid (YNB plus all amino acids) medium to early log phase and treated with 10 mM hydrogen peroxide ($H_2O_2$). Each sample was taken at the indicated time, diluted, and plated in duplicate on YEPD. After two days of incubation at 30°C, viability was measured by counting the number of CFU (Colony forming units) normalized to that of the sample at time zero.

### ROS staining and fluorescence microscopy

Cells were grown to early log phase (~ $2 \times 10^7$ cells/ml) in SC liquid medium. To monitor the levels of intracellular reactive oxygen species (ROS), cells were incubated for 60min at 30°C in the presence of 50 μg/ml 2′,7′-dicloroflurescin diacetate ($H_2$DCFDA) solution (Sigma-Aldrich) before treatment with hydrogen peroxide ($H_2O_2$). Cells were washed twice with distilled water and observation was made using an Olympus BX51 research microscope with a 600× objective.

## Results and Discussion

### Slow growth phenotypes caused by domain deletion mutations of *C. albicans EDC3*

The Edc3 protein contains an N-terminus LSm domain, a central FDF domain, and a C-terminal YjeF-N domain (Fig. 1A). To investigate the roles of each functional domain of Edc3, we constructed deletion mutations, *LSmΔ*, *FDFΔ*, and *YjeFΔ*. Each deletion construct was introduced into the *edc3/edc3* mutant strain as an episomal plasmid and its growth phenotype was assayed by a spot test (Fig. 1B). The *edc3/edc3* mutant strain has a slow-growth phenotype as compared with the wild-type strain (Jung and Kim, 2011). The *edc3-FDFΔ* mutation showed a growth phenotype similar to the wild-type. In contrast, *edc3-LSmΔ* or *edc3-YjeFΔ* showed the mutant phenotypes of *edc3/edc3*. These results indicate that LSm and YjeF domains are crucial to the Edc3 functions in cell growth.

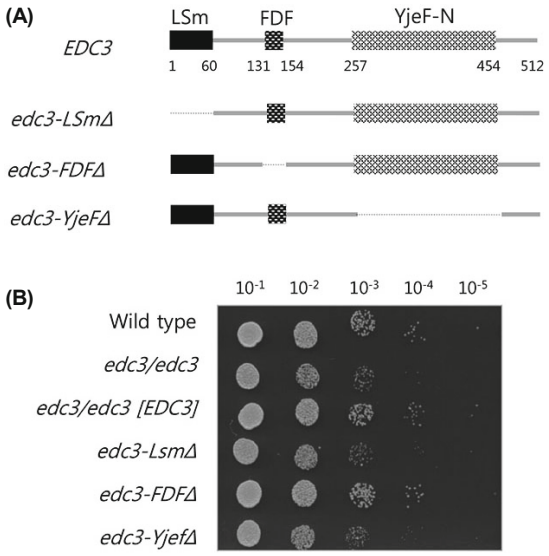


**Fig. 1. Domain deletion mutations of *C. albicans* Edc3.** (A) Schematic representation of the domain architecture of Edc3 and domain deletion constructs. The boundaries of LSm, FDF, and YjeF-N doamins are from previous studies (Jung and Kim, 2011). The deletion region is indicated as a dashed line. (B) Growth defects of domain deletion mutations of Edc3. The wild-type (BWP17), *edc3/edc3* mutant (JKC81), and *edc3/edc3* mutant transformed with *EDC3* plasmid (pJI410) or deletion construct were used. Ten-fold serial dilutions of overnight cultures were spotted onto YEPD plates. Plates were incubated at 30°C and photographed after 20 h.

### *edc3-LSmΔ* or *edc3-YjeFΔ* mutation showed a defect in hyphal growth

*C. albicans* forms hyphae under a range of environmental conditions, including the presence of serum, $CO_2$, or *N*-acetyl-D-glucosamine (GlcNAc) (Sudbery, 2011). The synthetic growth medium, such as Lee's medium and Spider medium are also useful for hyphal growth induction. The *edc3/edc3* mutant strain shows a filamentation defect, as evidenced by altered colony morphology on a solid medium and abnormal hyphal development in serum-containing liquid medium (Jung and Kim, 2011).

The colony morphologies of the wild-type and domain deletion mutations of *EDC3* were analyzed by streaking cells on the hyphal-inducing Spider medium. The mutation *edc3-LSmΔ* and *edc3-YjeFΔ* Lsm showed a filamentation defect in colony morphology, which is similar to that of *edc3/edc3* mutant strain (Fig. 2). But *edc3-FDFΔ* showed a wild-type phenotype of colony morphology. In YEP liquid medium containing 10% serum, the *edc3/edc3* mutant cells exhibit an abnormal hyphal development with prolonged incubation. About 5–10% of *edc3/edc3* cells display bipolar hyphal development. As shown in Fig. 3, *edc3-LSmΔ* and *edc3-YjeFΔ* Lsm showed the mutant hyphal phenotypes. Formation of two-headed germ tubes was apparent in these mutant cultures. The *edc3-FDFΔ* cells showed a normal hyphal growth with a mild delay. These results indicate that deletion of LSm or YjeF of Edc3 affects the hyphal formation and leads to formation of two-headed germ tubes from one cell.

The LSm and YjeF domains of Edc3 are required for P-body accumulations in *S. cerevisiae* (Decker *et al*., 2007). These domains provide the sites for protein interactions and interconnecting mRNP. It has been known that P-bodies accumulate during hyphal development in *C. albicans* (Jung and Kim, 2011). We expect that the roles of Edc3 in fila-

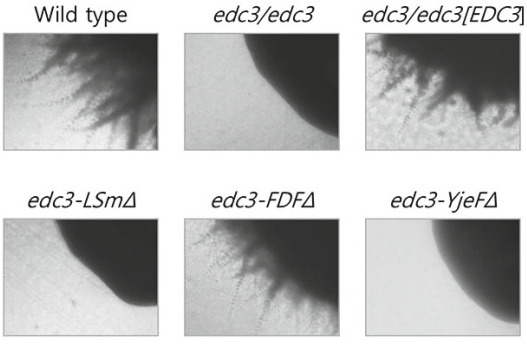


**Fig. 2. Colony morphologies of domain deletion mutations of Edc3 on filamentation-inducing medium.** The wild-type (BWP17), *edc3/edc3* mutant (JKC81), and *edc3/edc3* mutant transformed with *EDC3* plasmid (pJI410) or deletion construct were streaked on SPIDER medium. Colonies were grown at 37°C for 5 days and images were photographed at 100× magnification.

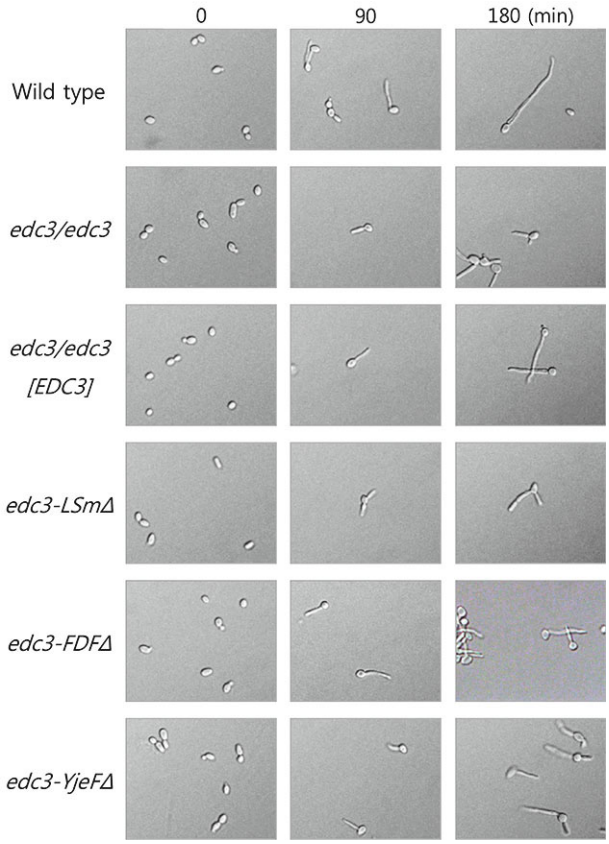


**Fig. 3. Filamentation defects of domain deletion mutations of Edc3.** The wild-type (BWP17), *edc3/edc3* mutant (JKC81), and *edc3/edc3* mutant transformed with *EDC3* plasmid (pJI410) or deletion construct were incubated in 10% serum containing medium at 37°C. Cultures were taken at the indicated time and images were photographed at 400× magnification.

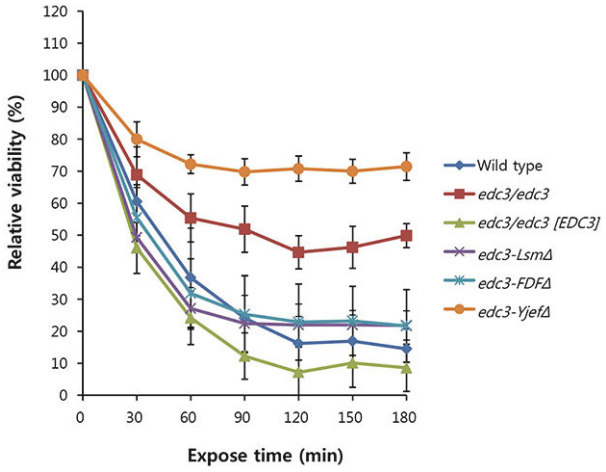


**Fig. 4. Cell viability of domain deletion mutations of Edc3 upon $H_2O_2$ treatment.** Cells were grown to early log phase in SC liquid medium at 30°C and treated with 10 mM $H_2O_2$. Culture samples taken at the indicated times were diluted and plated in duplicate onto YEPD plates. After 2 days of incubation at 30°C, viabilities were scored as a percentage of the number of colonies formed by the sample taken at time zero.

mentation might be dependent on LSm and YjeF domains of Edc3.

### *edc3-YjeFΔ* showed increased resistance to oxidative stress

In yeast *S. cerevisiae* and *C. albicans*, weak acids, oxidative stresses, or UV irradiation activate apoptotic cell death (Madeo *et al*., 1999; Frohlich and Madeo, 2000; Phillips *et al*., 2003). Treatment with hydrogen peroxide, acetic acid, or high temperature rapidly decreased the viability of *C. albicans* wild-type cells, but *edc3/edc3* cells showed increased resistance to these stress conditions (Jung and Kim, 2014). These resistant phenotypes were analyzed in domain deletion mutations of Edc3 by measuring the viability following $H_2O_2$ treatment. The *edc3-YjeFΔ* mutant strain showed increased cell survival as compared with the wild-type, and its viability was even higher than that of *edc3/edc3* cells (Fig. 4). Both *edc3-LSmΔ* and *edc3-FDFΔ* mutations showed the wild-type level of cell survival upon $H_2O_2$ treatment. These results suggest that the sensitivity to apoptosis-associated stress is mediated by the C-terminal YjeF-N domain of Edc3.

### Domain deletion mutation of Edc3 showed decreased ROS accumulation upon oxidative stress

Exposure of yeast cells to low doses of hydrogen peroxide or acetic acid increases the accumulation of reactive oxygen species (ROS) and the number of apoptotic cells (Madeo *et al*., 1999; Phillips *et al*., 2003). We examined ROS accumulation in domain deletion mutations of Edc3. Cells were incubated in the presence of the fluorescent dye 2′,7′-dichlorofluororescin diacetate ($H_2$DCFDA) before treatment with $H_2O_2$. In the case of *edc3/edc3* mutant strains, ROS accumulation decreased when compared with the wild type (Jung and Kim, 2014). Quantitative measurement of ROS-stained cells showed that *edc3-LSmΔ* and *edc3-YjeFΔ* mutations showed decreased ROS accumulation as the *edc3/edc3* mutant strains

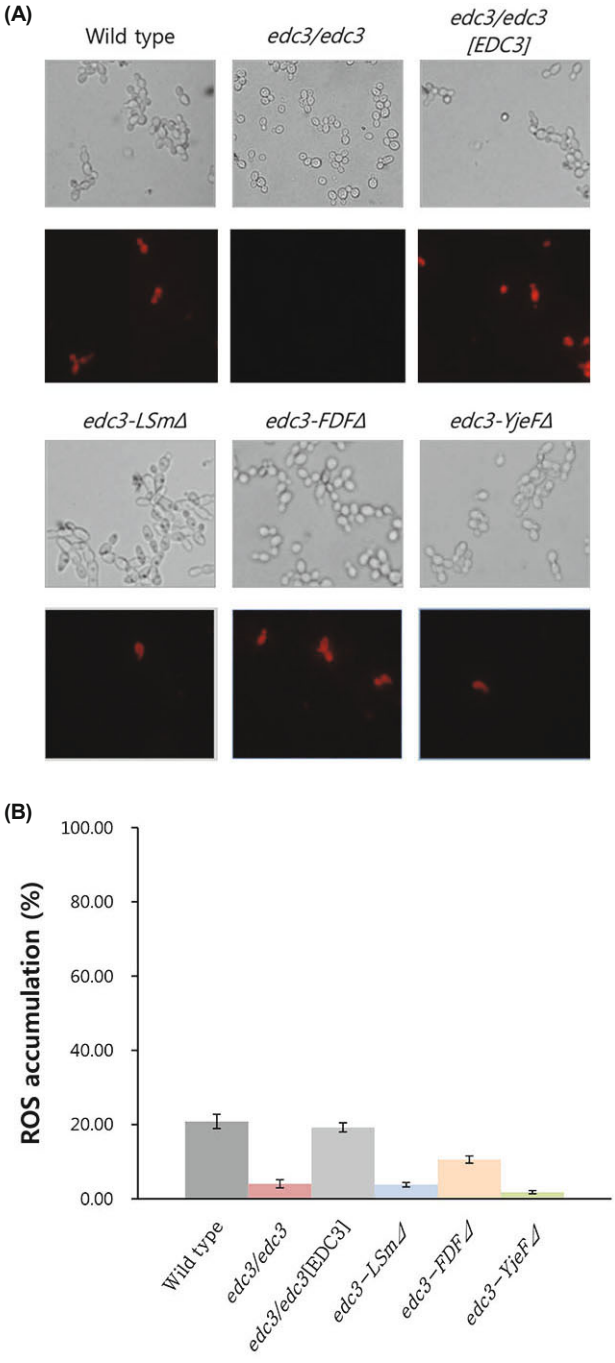


**Fig. 5. ROS accumulation in domain deletion mutations of Edc3 upon $H_2O_2$ treatment.** (A) ROS staining of the wild-type (BWP17), *edc3/edc3* mutant (JKC81), and *edc3/edc3* mutant transformed with *EDC3* plasmid (pJI410) or deletion construct. Cells in early log phase were incubated with 50 μg/ml $H_2$DCFDA for 45 min and exposed to 10 mM $H_2O_2$ for 60 min. Observations were made using an Olympus BX51 microscope with a 60× objective. (B) Quantitative analysis of ROS-stained cells following treatment with 10 mM $H_2O_2$. Percentage of stained cells was analyzed in two independent experiments, each requiring a total of 100 cells.

(Fig. 5). The *edc3-FDFΔ* mutation caused a decrease in ROS level to about half of the wild-type. It is apparent that newly constructed domain mutations of Edc3 affect the ROS ac-

cumulation at the different levels. These results suggest that each domain, LSm, FDF, and YjeF, is important to the Edc3 functions in ROS accumulation.

In a current study, we observed that deletion of LSm or YjeF domain of Edc3 affected the growth rate, filamentation phenotype, and ROS accumulation. Removal of FDF domain seemed to show the normal hyphal growth as well as the wild-type level of cell survival upon $H_2O_2$ treatment. The decreased level of ROS accumulation was observed in *edc3-FDFΔ* or *edc3-LSmΔ* mutant construct, but this level was not reflected into the cell survival phenotype. Based on our results, the YjeF domain of Edc3 seems to be critical in both hyphal growth and apoptotic stress responses.

## Acknowledgements

This research was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF), which is funded by the Ministry of Education (2012R1A1A-22007113). This work was supported in part by Chungnam National University (2014-0795-01).

## References

**Adams, A., Gottschling, D.E., Kaiser, C.A., and Stearn, T.** 1997. Methods in Yeast Genetics. Cold Spring Harbor Press, Cold Spring Harbor, N.Y., USA.

**Brown, A.J. and Gow, N.A.** 1999. Regulatory networks controlling *Candida albicans* morphogenesis. *Trends Microbiol.* **7**, 333–338.

**Calderone, R.A. and Fonzi, W.A.** 2001. Virulence factors of *Candida albicans*. *Trends Microbiol.* **9**, 327–335.

**Christianson, T.W., Sikorski, R.S., Dante, M., Shero, J.H., and Hieter, P.** 1992. Multifunctional yeast high-copy-number shuttle vectors. *Gene* **110**, 119–122.

**Coller, J. and Parker, R.** 2005. General translational repression by activators of mRNA decapping. *Cell* **122**, 875–886.

**Decker, C.J., Teixeira, D., and Parker, R**. 2007. Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in *Saccharomyces cerevisiae*. *J. Cell Biol.* **179**, 437–449.

**Fernandez-Arenas, E., Cabezon, V., Bermejo, C., Arroyo, J., Nombela, C., Diez-Orejas, R., and Gil, C.** 2007. Integrated proteomics and genomics strategies bring new insight into *Candida albicans* response upon macrophage interaction. *Mol. Cell. Proteomics.* **6,** 460–478.

**Frohlich, K.U. and Madeo, F.** 2000. Apoptosis in yeast–a monocellular organism exhibits altruistic behaviour. *FEBS Lett.* **473**, 6–9.

**Fromm, S.A., Truffault, V., Kamenz, J., Braun, J.E., Hoffmann, N.A., Izaurralde, E., and Sprangers, R.** 2012. The structural basis of Edc3- and Scd6-mediated activation of the Dcp1:Dcp2 mRNA decapping complex. *EMBO J.* **31**, 279–290.

**Harigaya, Y., Jones, B.N., Muhlrad, D., Gross, J.D., and Parker, R.** 2010. Identification and analysis of the interaction between Edc3 and Dcp2 in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **30**, 1446–1456.

**Jung, J.H. and Kim, J.** 2011. Accumulation of P-bodies in *Candida albicans* under different stress and filamentous growth conditions. *Fungal Genet. Biol.* **48**, 1116–1123.

**Jung, J.H. and Kim, J.** 2014. Roles of Edc3 in the oxidative stress response and *CaMCA1*-encoded metacaspase expression in *Candida albicans*. *FEBS J.* **281**, 4841–4851

**Kshirsagar, M. and Parker, R.** 2004. Identification of Edc3p as an enhancer of mRNA decapping in *Saccharomyces cerevisiae*. *Genetics* **166**, 729–739.

**Liu, H., Kohler, J., and Fink, G.R.** 1994. Suppression of hyphal formation in *Candida albicans* by mutation of *STE12* homolog. *Science* **266,** 1723-1726.

**Liu, J., Valencia-Sanchez, M.A., Hannon, G.J., and Parker, R.** 2005. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* **7**, 719–723.

**Madeo, F., Frohlich, E., Ligr, M., Grey, M., Sigrist, S.J., Wolf, D.H., and Frohlich, K.U.** 1999. Oxygen stress: a regulator of apoptosis in yeast. *J. Cell Biol.* **145**, 757–767.

**Nantel, A., Dignard, D., Bachewich, C., Harcus, D., Marcil, A., Bouin, A.P., Sensen, C.W., Hogues, H., van het Hoog, M., Gordon, P., *et al.*** 2002. Transcription profiling of *Candida albicans* cells undergoing the yeast-to-hyphal transition. *Mol. Biol. Cell.* **13**, 3452–3465.

**Nissan, T., Rajyaguru, P., She, M., Song, H., and Parker, R.** 2010. Decapping activators in *Saccharomyces cerevisiae* act by multiple mechanisms. *Mol. Cell* **39**, 773–783.

**Phillips, A.J., Sudbery, I., and Ramsdale, M.** 2003. Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **100**, 14327–14332.

**Stoldt, V.R., Sonneborn, A., Leuker, C.E., and Ernst, J.F.** 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *EMBO J.* **16**, 1982–1991.

**Sudbery, P.E.** 2011. Growth of *Candida albicans* hyphae. *Nat. Rev. Microbiol.* **9**, 737–748.

**Teixeira, D., Sheth, U., Valencia-Sanchez, M.A., Brengues, M., and Parker, R.** 2005. Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* **11**, 371–382.

**Tritschler, F., Eulalio, A., Truffault, V., Hartmann, M.D., Helms, S., Schmidt, S., Coles, M., Izaurralde, E., and Weichenrieder, O.** 2007. A divergent Sm fold in EDC3 proteins mediates DCP1 binding and P-body targeting. *Mol. Cell. Biol.* **27**, 8600–8611.

**Wilson, R.B., Davis, D., and Mitchell, A.P.** 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J. Bacteriol.* **181**, 1868–1874.