보 문

Defensive roles of Sdu1, a PPPDE superfamily member with ubiquitin C-terminal hydrolase activity, against thermal stress in *Schizosaccharomyces pombe*

Hee Han[1], Tae Young Heo[1], In Wang Ryu[2], Kyunghoon Kim[2], and Chang-Jin Lim[1]*

[1]*Department of Biochemistry, Kangwon National University, Chucheon 24341, Republic of Korea*
[2]*Department of Biological Sciences, Kangwon National University, Chucheon 24341, Republic of Korea*

카르복시 말단 유비퀴틴 가수분해 효소 활성 보유 PPPDE superfamily member인 *Schizosaccharomyces pombe* Sdu1의 열 스트레스에 대한 방어적 역할

한희[1] · 허태영[1] · 류인왕[2] · 김경훈[2] · 임창진[1]*

[1]강원대학교 자연과학대학 생화학과, [2]강원대학교 자연과학대학 생명과학과

ABSTRACT: The *sdu1*[+] gene encodes Sdu1, a PPPDE superfamily member of deubiquitinating enzymes (DUBs) in *Schizosaccharomyces pombe*. Sdu1 was previously shown to contain an actual ubiquitin C-terminal hydrolase (UCH) activity using the recombinant plasmid pYSTP which harbors the *sdu1*[+] gene. This work was designed to assess a thermotolerant role of Sdu1 against high incubation temperatures. In the temperature-shift experiments, the *S. pombe* cells harboring pYSTP grew much better after the shifts to 37°C and 42°C, when compared with the vector control cells. After being shifted to 37°C and 42°C for 6 h, the *S. pombe* cells harboring pYSTP contained lower reactive oxygen species (ROS) levels, compared with the vector control cells. The nitric oxide (NO) levels of the *S. pombe* cells harboring pYSTP were slightly lower than those of the vector control cells in the absence or presence of the temperature shifting. The total glutathione (GSH) levels of the *S. pombe* cells harboring pYSTP were significantly higher than those of the vector control cells. Total superoxide dismutase (SOD) and GSH peroxidase activities were also higher in the *S. pombe* cells harboring pYSTP after the temperature shifts than in the vector control cells. In brief, the *S. pombe* Sdu1 plays a thermotolerant role against high incubation temperature through the down-regulation of ROS and NO and the up-regulation of total GSH content, total SOD and GSH peroxidase activities.

Key words: *Schizosaccharomyces pombe*, glutathione, reactive oxygen species, Sdu1, thermal stress

Deubiquitination or deubiquitylation, which is the reversible removal of ubiquitin from conjugated proteins, is catalyzed by a large group of deubiquitinating enzymes (DUBs). DUBs participate in various major events, including activating and processing of ubiquitin proproteins into mature ubiquitin monomers, recycling the ubiquitin molecules utilized during the ubiquitination process, antagonizing ubiquitinated proteins by reversing the process of ubiquitination or ubiquitin like modification of proteins, regenerating monoubiquitin molecules from the polyubiquitin chains synthesized or released during the ubiquitination process, remodeling polyubiquitin chains on target proteins and removing ubiquitin molecules that are not associated with proteasomal degradation (Ramakrishna *et al.*, 2011). In addition to the maintenance of steady state levels of monoubiquitin, they regulate various crucial cellular processes

---

*For correspondence. E-mail: cjlim@kangwon.ac.kr;
Tel.: +82-33-250-8514; Fax: +82-33-259-5664

such as regulation of substrate degradation at the proteasome, chromatin remodeling through histone deubiquitination, gene expression, cell cycle regulation, DNA repair, activation of kinases and other enzymes, apoptosis, microbial pathogenesis, localization and degradation of signaling intermediates, and endocytosis (Reyes-Turcu *et al.*, 2009; Ramakrishna *et al.*, 2011).

Based upon their ubiquitin-protease domains, DUBs are originally categorized into five families, such as ubiquitin-specific proteases (USPs, also referred to as ubiquitin processing proteases), ubiquitin C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs, also known as otubain proteases), Machado-Joseph disease proteases (MJD, also referred to as Josephins), and JAB1/MPN/MOV34 motif proteases (JAMMs) (Komander *et al.*, 2009; Tse *et al.*, 2009). The USP, UCH, OTU, and MJD family members are cysteine proteases, whereas the JAMM family members are zinc-dependent metalloproteinases (Komander *et al.*, 2009). The two additional families, such as PPPDE (after Permuted Papain fold Peptidases of DsRNA viruses and Eukaryotes) superfamily and Wss1-like metalloproteases, were afterward identified based on bioinformatics studies (Iyer *et al.*, 2004).

UCHs, one of the best characterized DUBs, principally participate in the processing and recycling of ubiquitin, but their specific functions remain largely unknown. UCHs, structurally characterized to have active-site crossover loop, act on small conjugates produced as byproducts of proteasomal or lysosomal degradation, the short C-terminal extensions of polymeric ubiquitin precursors, and the termini of unfolded larger substrates (Larsen *et al.*, 1998; Komander *et al.*, 2009).

The three representative isoforms of UCHs, such as UCH L1, UCH L3, and UCH L5/UCH37, have been better understood. UCH L1, a key protease of the ubiquitin-proteasome system (UPS), is associated with neurodegenerative diseases and cancer. UCH L1 expression was identified to be enhanced in podocytes in patients with membranous nephropathy, which resulted in increased ubiquitin content (Meyer-Schwesinger *et al.*, 2011). Similarly, stable UCH L1 overexpression in cultured podocytes causes an accumulation of monoubiquitin and polyubiquitin proteins, while stable knockdown of UCH L1 diminishes monoubiquitin and polyubiquitin proteins and significantly enhances proteasomal activity (Meyer-Schwesinger *et al.*,

2011). These findings demonstrate that UCH L1 activity is involved in polyubiquitin accumulation, proteasome inhibition, and disease aggravation in experimental models of membranous nephropathy (Meyer-Schwesinger *et al.*, 2011). UCH L3, expressed in various tissues, is involved in learning and working memory in mice, and related with muscular and retinal degeneration, potentially due to oxidative stress (Ristic *et al.*, 2014). UCH L3 enhances osteoblast differentiation through the stabilization of Smad1 signaling in murine skeletal muscle C2C12 myoblasts (Kim *et al.*, 2011). It was also suggested to be an epithelial-to-mesenchymal transition regulator in prostate cells and a potential target for preventing metastatic prostate cancer (Song *et al.*, 2014). UCH37 reversibly associates with the 19S component of the proteasome and deubiquinates proteasome substrates, and regulates transforming growth factor-β signaling in non-neural tissue (Wicks *et al.*, 2005; Ristic *et al.*, 2014). UCH37 is a major DUB associated with *Schizosaccharomyces pombe* 26S proteasome, but its disruption does not interfere with cell viability without showing obvious signs of impaired ubiquitin-dependent proteolysis (Stone *et al.*, 2004).

In the previous work, the *sdu1*$^+$ gene encoding Sdu1, belonging to the PPPDE superfamily, was cloned from *S. pombe*, and its gene product was shown to contain the UCH activity which is involved in the defense against oxidative and nitrosative stresses in *S. pombe* (Kim *et al.*, 2013). In the present work, we further demonstrate that Sdu1 plays a defensive role against thermal stress in *S. pombe*.

Materials and Methods

Chemicals

Bovine serum albumin, Bradford reagent, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), sodium nitrite, Griess reagent, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione reductase (GR), reduced glutathione (GSH), NADPH, cytochrome c, catalase, xanthine, xanthine oxidase, and *t*-butyl hydroperoxide were obtained from Sigma-Aldrich Chemical Co. Yeast extract, peptone and agar were obtained from Amersham Life Science. All other chemicals used were of the highest grade commercially available.

Strain and growth condition

S. pombe KP1 (*h⁺ leu1-32 ura4-294*), a derivative of *S. pombe* heterothallic haploid strain 975*h⁺*, was used in the present work. The Sdu1-overexpressing recombinant plasmid pYSTP was previously constructed using a yeast-*E. coli* shuttle plasmid vector pRS316 (Myers *et al.*, 1986). The yeast cells were grown in YEPD medium (pH 6.5) which contains 1% yeast extract, 2% peptone, and 1% glucose. The yeast cells were incubated with shaking at 30°C prior to transferring to higher incubation temperatures and their growth was monitored by measuring the absorbance at 600 nm. Yeast cells used in the experiments were obtained preferentially from the early exponential growth phase.

Preparation of cellular extracts

The desired number of the yeast cells was obtained by centrifugation. They were re-suspended in 20 mM Tris buffer (pH 8.0) with 2 mM EDTA and disrupted using glass beads and ultrasonication. The cellular extracts, taken after centrifugation, were used for total GSH, SOD and glutathione peroxidase activities, and protein determinations detailed below. Protein content in cellular extracts was determined by Bradford's procedure (1976) with bovine serum albumin as a standard.

Quantitation of intracellular ROS

To determine intracellular ROS levels, the redox-sensitive fluorescent probe DCFH-DA was used as described previously (Royall and Ischiropoulos, 1993). When DCFH-DA enters the cells, its diacetate group is cleaved by an esterase, leaving a non-fluorescent molecule, which is amenable to oxidization to fluorescent dichlorofluorescein (DCF) in the presence of ROS (Kiani-Esfahani *et al.*, 2012). Yeast cells were incubated with 5 μM DCFH-DA for 30 min at 30°C. The treated cells were analyzed immediately using a microplate fluorometer.

Determination of nitrite concentration

Accumulated nitrite (NO_2^-), as an index of nitric oxide (NO), in conditioned medium was determined using a colorimetric assay based on the Griess reaction (Sherman *et al.*, 1993). Conditioned medium (100 μl) were reacted with a 100 μl Griess reagent (6 mg/ml) at room temperature for 10 min, and then

NO_2^- concentration was determined by measuring the absorbance at 540 nm. The standard curve was constructed using the known concentrations (0–160 μM) of sodium nitrite.

Quantitation of total GSH

As previously described (Nakagawa *et al.*, 1990), the total GSH content in cellular extracts was determined using an enzymatic recycling assay based on GR. The reaction mixture (200 μl) contained 175 mM KH_2PO_4, 6.3 mM EDTA, 0.21 mM NADPH, 0.6 mM DTNB, 0.5 units/ml GR, and cellular extract at 25°C. The absorbance at 412 nm was monitored using a microplate reader. The total GSH content was represented as μg/mg protein.

Determination of total SOD activity

As previously described (Lee *et al.*, 2002), the total SOD activity in cellular extracts was spectrophotometrically determined as reduced cytochrome c with the xanthine/xanthine oxidase system. The reaction mixture (200 μl) contained 50 mM phosphate buffer (pH 7.4), 0.01 units/ml xanthine oxidase, 0.1 mM EDTA, 1 μM catalase, 0.05 mM xanthine, 20 μM cytochrome c, and cellular extract. A change in absorbance was monitored at 550 nm.

Determination of glutathione peroxidase activity

As previously described (Lee *et al.*, 2002), glutathione peroxidase activity in cellular extracts was spectrophotometrically determined. The reaction mixture (1.2 ml) contained a 0.07 M phosphate buffer (pH 7.0), 0.24 units GR, 0.8 mM GSH, 0.13 mM NADPH, and cellular extract. The hydroperoxide-independent consumption of NADPH was preceded for 10 min. The overall reaction was started by adding 100 μl of prewarmed t-butyl hydroperoxide solution (1.5 mM). The decrease in absorption at 340 nm was monitored.

Statistical analysis

The results are reported as mean ± standard deviation (SD). Statistical comparisons between experimental groups were performed using unpaired Student's *t*-test. A *P* value less than 0.05 was considered to be statistically significant.

Results

Cellular growth under thermal stress

Participation of the *S. pombe* Sdu1 in the yeast growth under thermal stress was examined using temperature-shift experiments. The same numbers of the *S. pombe* cells harboring pRS316 or pYSTP, grown to the early exponential phase in rich medium at 30°C, were shifted into fresh rich medium and continued to be shaken at higher incubation temperatures, such as 37°C and 42°C. As a control, the *S. pombe* cells harboring pRS316 or pYSTP continued to be cultured also at 30°C. The yeast growths, detected by measuring absorbance at 600 nm, at 3 and 6 h after the shifts were compared in Fig. 1. At an incubation temperature of 30°C, the *S. pombe* cells harboring pYSTP and the vector control cells exhibited no significant difference between their growth patterns after the shift (Fig. 1A). However, after the shifts to 37°C and 42°C, the *S. pombe* cells harboring pYSTP appeared to grow better than the vector control cells (Fig. 1B and C). Especially at 42°C, although the growth of the vector control cells was almost arrested, the *S. pombe* cells harboring pYSTP could reasonably grow albeit delayed (Fig. 1C). When the growth rates after the shifts were compared, the
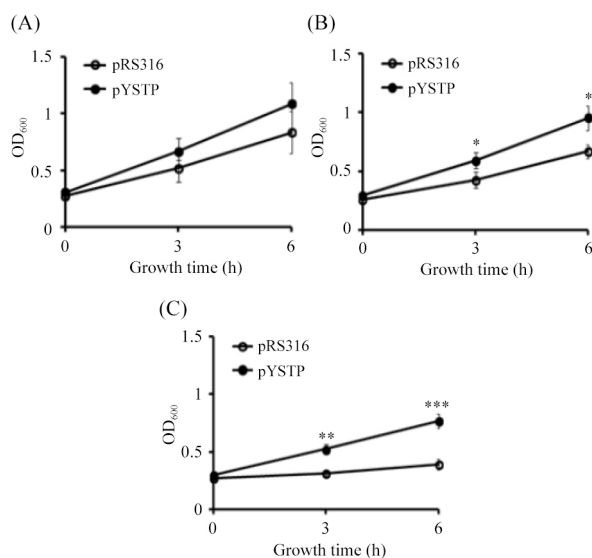

Fig. 2. Effect of Sdu1 on the growth rates of *S. pombe* cells under thermal stress. The *S. pombe* cells harboring pRS316 or pYSTP, grown to early exponential phase at 30°C in rich growth medium, were shifted to 30°C, 37°C or 42°C. The yeast growth was monitored by absorbance (OD_{600}) at 3 and 6 h following the shift, and the growth rates were obtained as ΔOD_{600}/h. Each point shows the mean ± SD of the three independent experiments. * $P < 0.05$; ** $P < 0.01$ versus corresponding pRS316-containing cells.

enhanced growths of the *S. pombe* cells harboring pYSTP at both 37°C and 42°C were convinced, compared with those of the vector control cells (Fig. 2). Taken together, Sdu1 participates in the growth of *S. pombe* cells at the high temperatures.

Reactive oxygen species (ROS)

The intracellular ROS levels are markedly elevated in presence of oxidative stress-inducing agents, including thermal stress, which sequentially threatens the integrity of various biomolecules. Cells are equipped with various defense mechanisms, including antioxidant enzymes and free radical scavengers, to protect against oxidative damages. If the cellular defense systems do not sufficiently cope with stressful agents, the cells undergo oxidative stress. Since the intracellular ROS levels increase under various kinds of stresses, especially oxidative stress, they have been considered as one of cellular markers which are related with the stress levels inside cells.

The ROS levels were determined in the *S. pombe* cells harboring pRS316 or pYSTP after shifting the same numbers of the exponentially grown yeast cells to 37°C and 42°C for 6 h. As the incubation temperature went up, the intracellular ROS levels of the vector control cells also became higher (Fig. 3). The vector control cells were found to contain the ROS levels 1.5- and 2.4-fold higher at 37°C and 42°C, respectively, than at

Fig. 1. Enhancing effect of Sdu1 on *S. pombe* growth under thermal stress. *S. pombe* cells harboring pRS316 or pYSTP, grown to early exponential phase at 30°C in rich growth medium, were shifted to 30°C (Control, A), 37°C (B) or 42°C (C). The yeast growth was monitored by absorbance (OD_{600}) at 3 and 6 h following the shifts. Each point shows the mean ± SD of the three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus corresponding pRS316-containing cells.
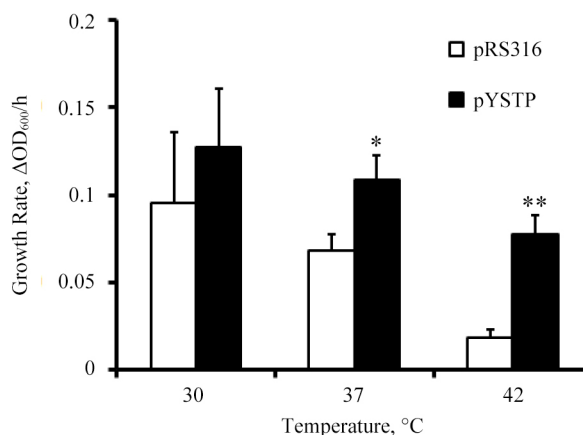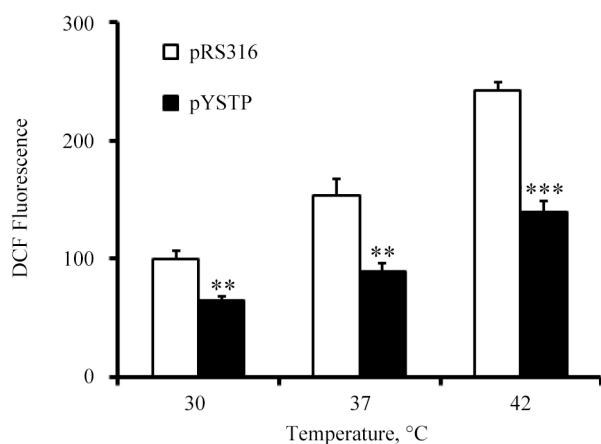
Fig. 3. Effect of Sdu1 on the reactive oxygen species (ROS) levels of the *S. pombe* **cells grown under thermal stress.** The yeast cells harboring pRS316 or pYSTP in the early exponential phase at 30°C were subjected to 30°C, 37°C or 42°C for 6 h. The ROS levels were relatively represented as DCF fluorescence, an arbitrary unit. Each point shows the mean ± SD of the three independent experiments. ** $P < 0.01$; *** $P < 0.001$ versus corresponding pRS316-containing cells.

Fig. 4. Effect of Sdu1 on the nitric oxide (NO) levels of *S. pombe* **cells grown under thermal stress.** The yeast cells harboring pRS316 or pYSTP in the early exponential phase were subjected to 30°C, 37°C or 42°C for 6 h. The levels of nitrite, an index of NO, in supernatant fractions were determined based upon Griess reaction. Each point shows the mean ± SD of the three independent experiments. ** $P < 0.01$ versus corresponding pRS316-containing cells.

30°C, convincing that the yeast cells undergo oxidative stress when grown at 37°C and 42°C (Fig. 3). The ROS levels of the *S. pombe* cells harboring pYSTP were found to drop to 64.3%, 58.3%, and 57.4% at 30°C, 37°C and 42°C, respectively, of those from the corresponding vector control cells (Fig. 3). These results indicate that the ROS-diminishing effect of Sdu1 is evident at higher incubation temperature. Collectively, Sdu1 plays a scavenging role in *S. pombe* cells exposed to high temperatures.

Nitric oxide (NO)

Nitric oxide (NO•, NO) is a free radical synthesized from L-arginine by nitric oxide synthase (NOS). Although it acts as a normal physiological regulator when produced in minute quantities by constitutive NOSs, it shows pathologic effects when produced in excessive quantities by inducible NOSs. NO can react directly with its physiological targets, while its indirect effects are mediated by reactive nitrogen species (RNS) derived from NO metabolism (Wink *et al.*, 2001). Although RNS play crucial roles in cellular signaling, they can subject cells to nitrosative stress at high concentrations, ultimately leading to cell death. Constitutive NOS, identified by western blotting using mouse monoclonal anti-neuronal NOS, is present in the budding yeast *Saccharomyces cerevisiae*, which is activated by
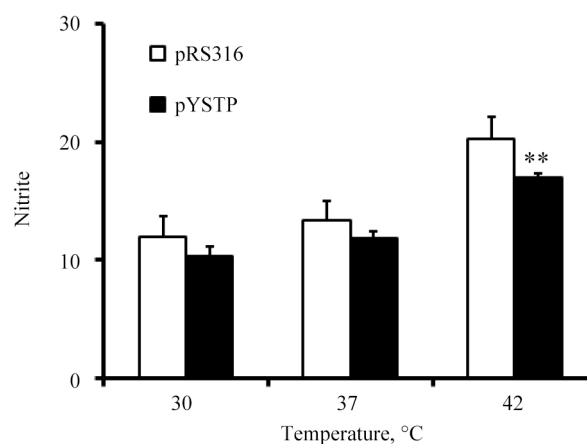
calmodulin and arginine (Kanadia *et al.*, 1998). NO, produced by an NOS-like activity in *S. pombe*, acts as a signaling molecule which can induce both transcriptional and physiological changes (Kig and Temizkan, 2009).

As shown in Fig. 4, the vector control cells were found to contain higher content of nitrite, an index of NO, at 37°C and 42°C than at 30°C. This finding implies that NO also elevates in addition to ROS in *S. pombe* cells exposed to high temperatures. The *S. pombe* cells harboring pYSTP also exhibited enhanced nitrite contents at 37°C and 42°C than at 30°C (Fig. 4). They maintained lower nitrite contents at the same temperature than the corresponding vector control cells (Fig. 4). A difference in the nitrite contents between the two yeast cultures was statistically significant (Fig. 4). Collectively, Sdu1 tends to play a diminishing role on NO levels in *S. pombe* cells grown under high incubation temperature.

Total glutathione (GSH)

GSH, one of principal non-enzymatic antioxidants, plays a major role in the defense against many kinds of stressful agents. Accordingly, its cellular levels are believed to be one of important factors to maintain redox homeostasis. The total GSH content became significantly lower in *S. pombe* cells as the incubation temperature went up (Fig. 5). As shown in Fig. 5, the
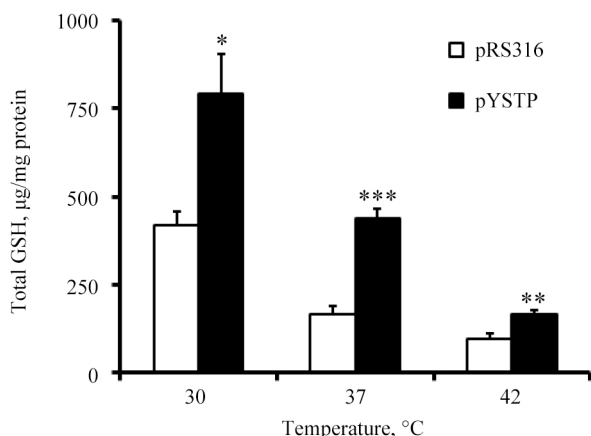
Fig. 5. Effect of Sdu1 on the total glutathione (GSH) contents in *S. pombe* cells grown under thermal stress. The yeast cells harboring pRS316 or pYSTP in the early exponential phase were subjected to 30°C, 37°C or 42°C for 6 h. The total GSH levels were represented as μg/mg protein. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus corresponding pRS316-containing cells.

total GSH contents in the vector control cells dropped to 38.9% and 22.5%, respectively, at 37°C and 42°C, compared to those at 30°C. The total GSH contents in *S. pombe* cells harboring pYSTP were 1.9-, 2.6-, and 1.8-fold higher at 30°C, 37°C, and 42°C, respectively, than those in the corresponding vector control cells (Fig. 5). In brief, Sdu1 plays an enhancing role in *S. pombe* cells at the higher incubation temperatures as well as 30°C.

Total superoxide dismutase (SOD) and peroxidase activities

In addition to total GSH, antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, and peroxiredoxin are also principal components of the cellular defense systems against RNS as well as ROS. As shown in Fig. 6A, total SOD activities in the vector control cells gradually became lower at the higher incubation temperatures than at 30°C. Although total SOD activities in the *S. pombe* cells harboring pYSTP gradually became lower at the higher incubation temperatures than at 30°C, their levels were maintained to be higher than those in the vector control cells. The SOD activity levels in the *S. pombe* cells harboring pYSTP were 1.3-, 1.3-, and 1.5-fold higher at 30°C, 37°C, and 42°C, respectively, than those in the corresponding vector control cells (Fig. 6A).

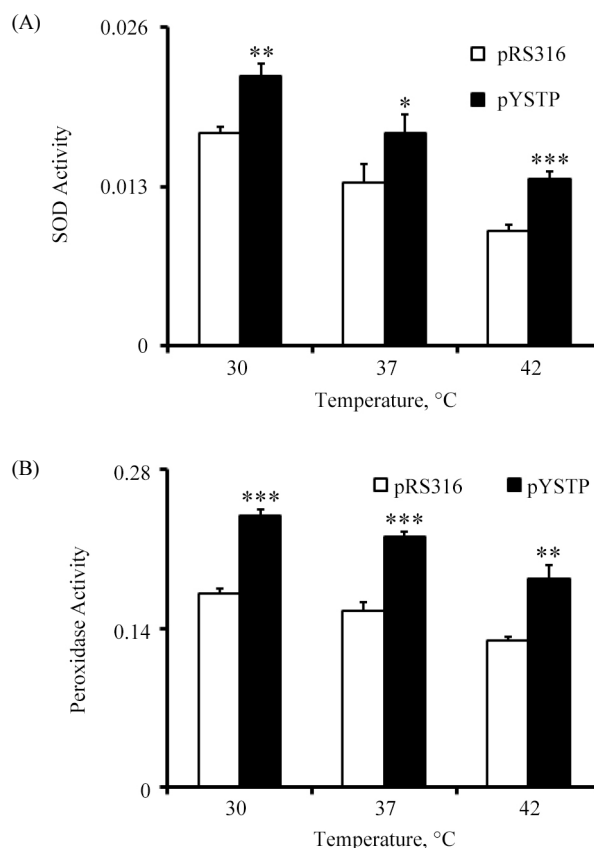Likewise, GSH peroxidase activities in both the vector

Fig. 6. Effect of Sdu1 on total superoxide dismutase (SOD, A) and glutathione peroxidase (B) activities in *S. pombe* cells grown under thermal stress. The yeast cells harboring pRS316 or pYSTP in the early exponential phase were subjected to 30°C, 37°C or 42°C for 6 h. Total SOD activity was represented as Δ_{550}/min/mg protein, and peroxidase activity was expressed as Δ_{340}/min/mg protein. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus corresponding pRS316-containing cells.

control cells and the *S. pombe* cells harboring pYSTP gradually became lower as their incubation temperatures were getting higher. However, the peroxidase activities in the *S. pombe* cells harboring pYSTP were 1.4-, 1.4-, and 1.4-fold higher than those in the vector control cells. This result suggests that Sdu1 has an enhancing activity on peroxidase in *S. pombe* cells at the higher incubation temperatures as well as 30°C. Collectively, Sdu1 is capable of enhancing activities of antioxidant enzymes, such as total SOD and glutathione peroxidase, in *S. pombe*.

Discussion

Microbial cells can be easily exposed to various kinds of stresses and respond in diverse ways ranging from activation of

survival pathways to the induction of cell death which leads to the elimination of damaged cells. They are equipped with various defense mechanisms, and one of the cellular defense mechanisms is currently known to be linked with ubiquitin homeostasis. Under certain stress conditions, monoubiquitin is in demand to cope with the accumulation of misfolded proteins which require proteasomal degradation, and one example to bolster monoubiquitin levels was shown to be the down-regulation of Rfu1 (regulator of free ubiquitin chains 1), an inhibitor of the DUB Doa4 in *Saccharomyces cerevisiae* (Wolf and Petroski, 2009). Rapid loss of free ubiquitin chains upon heat shock, a condition in which more proteins require ubiquitin conjugation, is mediated by Doa4 and Rfu1 in *S. cerevisiae*, suggesting that the regulation of ubiquitin homeostasis is controlled by a balance between DUBs and their inhibitors (Kimura *et al.*, 2009). Kimura *et al.* (2009) also demonstrated that free ubiquitin chains function as an ubiquitin reservoir which allows maintenance of monomeric ubiquitin at adequate levels under normal conditions and rapid supply for substrate conjugation under stress conditions. In the present work, when the yeast cells, grown to the early exponential phase at 30°C, were shifted to 37°C or 42°C, the overexpressed Sdu1 UCH was identified to significantly help the growth of the yeast cells at the higher temperatures. Especially at 42°C, the overexpressed Sdu1 UCH in the *S. pombe* cells harboring pYSTP could make the yeast cells grow reasonably although the growth of the vector control cells were almost arrested. This preliminary finding implies that Sdu1 UCH, recently identified as a PPPDE superfamily member, plays a protective role against thermal stress in *S. pombe*.

Various DUBs were already shown to positively or negatively participate in the defense against diverse stresses in very different ways. USP9X, as a binding partner of ASK1 mediating oxidative stress-induced cell death through activation of JNK and p38 MAPK pathways, interacts with oxidative stress-activated ASK1 and prevents it from undergoing ubiquitin-dependent degradation, demonstrating that USP9X-dependent stabilization of activated ASK1 plays an important role in oxidative stress-induced cell death (Nagai *et al.*, 2009). USP10 participates in the assembly of *S. pombe* stress granules rapidly induced under environmental stress conditions but not under favorable conditions, which interacts with GTPase activating protein SH3 domain binding protein, a member of Ras signaling

pathway (Wang *et al.*, 2012a). Inhibition of a proteasome-associated USP14 by a selective small-molecule inhibitor accelerates the degradation of oxidized proteins and enhances resistance to oxidative stress, which is based upon a strategy to diminish the levels of intracellular aberrant proteins under proteotoxic stress (Lee *et al.*, 2010). A *ubp10* mutant transcriptome displays the induction of genes whose products play as scavengers in the enzymatic defense against oxidizing agents that potentially damage proteins and nucleic acids, such as *GRX1* encoding cytoplasmic glutaredoxin, *GPX1* encoding glutathione peroxidase, *SOD2* encoding mitochondrial Mn-SOD, *CCP1* encoding cytoplasmic c peroxidase, *CTT1* encoding cytoplasmic catalase T, and *GTT1* encoding glutathione transferase (Orlandi *et al.*, 2004). These changes are accompanied by intracellular accumulation of ROS as well as by DNA fragmentation and phosphatidylserine externalization, two markers of apoptosis (Orlandi *et al.*, 2004). The increase of ROS linked to absence of Ubp10 is also verified by an increase in protein carbonylation, one of the most studied biomarkers resulting from oxidative stress (Orlandi *et al.*, 2010). USP36, an USP primarily localized to the nucleoli and involved in maintain normal nucleolar structure, was reported to regulate the protein stability of SOD2 through diminishing the ubiquitination level of SOD2 and subsequently extending its half-life by preventing it from protein degradation (Kim *et al.*, 2011). Ataxin-3, a polyglutamine-containing DUB belonging to the Josephin family, promotes the recruitment of mutant SOD1 to the aggresome, and the formation of SOD1 aggresome depends on the DUB activity of ataxin-3 and is mediated by ataxin-3 editing of K-63-linked polyubiquitin chains on mutant SOD1 proteins (Wang *et al.*, 2012b).

USP1, known as a key regulator of genomic stability, is reversibly inactivated through the oxidation of catalytic cysteine residue by ROS, proposing that DUBs of the cysteine protease family act as ROS sensors and that ROS-mediated DUB inactivation is a crucial mechanism for fine-tuning stress-activated signaling pathways (Cotto-Rios *et al.*, 2012). USP2a, specifically overexpressed in prostate adenocarcinomas, induces drug resistance in immortalized and transformed prostate cells, and its overexpression protects from drug-induced oxidative stress by reducing ROS production and stabilizing the mitochondrial potential through enhancing intracellular GSH

content (Benassi *et al.*, 2013). These findings propose that at least the USP family members can have opposite effects on the ROS levels. Like USP2a, the *S. pombe* Sdu1 UCH could reduce the intracellular ROS levels enhanced under thermal stress. This ROS-scavenging activity of Sdu1 UCH might be one supportive reason on its growth-enhancing activity under thermal stress. However, the precise mechanism of the ROS-scavenging activity remains largely unknown.

Some USP family DUBs were found to be involved in the response against thermal stress using deletion mutants. Loss of Ubp10, a USP family member of *S. cerevisiae*, causes growth defects at different temperatures exacerbated when Ubp10 is inactivated in strains with auxotrophic markers (Amerik *et al.*, 2000). A *ubp5* mutant exhibits elevated sensitivity to several external stressors such as high temperature, oxidative and nitrosative stresses, high salts, and antifungal agents, suggesting that Ubp5 is a major DUB for stress response in *Cryptococcus neoformans* (Fang *et al.*, 2012).

Until recently, a very limited number of reports on the defensive roles of UCH activities have been documented. UCH L1 possesses essential functional and anti-apoptotic roles in pancreatic beta cells under stress conditions associated with lipotoxicity (Chu *et al.*, 2012). In the early *Caenorhabditis elegans* embryo, the *cyk-3* gene encoding a UCH plays a crucial role in cellular osmoregulation by deubiquitinating certain substrates, which indirectly participates in cytokinesis and embryonic polarity (Kaitna *et al.*, 2002). This work offers an example on the participation of UCH activities in the response against thermal stress.

In conclusion, the *S. pombe* cells harboring pYSTP which contain the higher levels of Sdu1 UCH grow better under thermal stress than the vector control cells. They contain lower levels of ROS and NO levels under thermal stress than the vector control cells. However, the *S. pombe* cells harboring pYSTP have the higher levels of total GSH contents, total SOD and glutathione peroxidase activities under thermal stress than the vector control cells. In brief, the Sdu1 UCH of *S. pombe*, belonging to the PPPDE superfamily, plays a defensive role against thermal stress probably through modulating some antioxidant components.

적 요

Schizosaccharomyces pombe sdu1⁺ 유전자는 PPPDE superfamily member에 속하는 탈유비퀴틴화 효소인 Sdu1을 엔코딩한다. 이전 연구에서, *sdu1*⁺ 유전자 포함 재조합 플라스미드 pYSTP를 사용하여 Sdu1이 카르복시 말단 유비퀴틴 가수분해 활성을 보유한다는 사실이 입증된 바 있다. 본 연구에서는, 높은 배양 온도에 대한 Sdu1의 열내성적 역할이 검토되었다. 온도 천이 실험에서, pYSTP 포함 *S. pombe* 세포들이 37도나 42도로 천이한 후에 벡터 대조 세포들보다 훨씬 더 잘 성장하였다. 37도나 42도로 천이 한 후 6시간 배양한 pYSTP 포함 *S. pombe* 세포들이 벡터 대조 세포들보다 낮은 활성산소종 수준을 나타내었다. pYSTP 포함 *S. pombe* 세포들이 온도 천이와 관계없이 벡터 대조 세포들보다 다소 낮은 일산화질소 수준을 나타내었다. pYSTP 포함 *S. pombe* 세포들이 벡터 대조 세포들보다 훨씬 높은 총 글루타치온 수준을 나타내었다. 온도 천이 후의 총 수퍼옥시드 디스무타아제 및 글루타치온 과산화효소 활성들이 pYSTP 포함 *S. pombe* 세포들에서 더 높은 것으로 측정되었다. 요약하면, *S. pombe* Sdu1은 활성산소종과 일산화질소 수준은 낮추고, 총 글루타치온, 총 수퍼옥시드 디스무타아제 및 글루타치온 과산화효소 수준들은 증가시킴으로써 높은 배양 온도에 대한 열내성적 역할을 나타낸다.

References

Amerik, A.Y., Li, S.J., and Hochstrasser, M. 2000. Analysis of the deubiquitinating enzymes of the yeast *Saccharomyces cerevisiae*. *Biol. Chem.* **381**, 981–992.

Benassi, B., Marani, M., Loda, M., and Blandino, G. 2013. USP2a alters chemotherapeutic response by modulating redox. *Cell Death Dis.* **4**, e812.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Chu, K.Y., Li, H., Wada, K., and Johnson, J.D. 2012. Ubiquitin C-terminal hydrolase L1 is required for pancreatic beta cell

survival and function in lipotoxic conditions. *Diabetologia* **55**, 128‒140.

Cotto-Rios, X.M., Békés, M., Chapman, J., Ueberheide, B., and Huang, T.T. 2012. Deubiquitinases as a signaling target of oxidative stress. *Cell Rep*. **2**, 1475‒1484.

Fang, W., Price, M.S., Toffaletti, D.L., Tenor, J., Betancourt-Quiroz, M., Price, J.L., Pan, W.H., Liao, W.Q., and Perfect, J.R. 2012. Pleiotropic effects of deubiquitinating enzyme Ubp5 on growth and pathogenesis of *Cryptococcus neoformans*. *PLoS One* **7**, e38326.

Iyer, L.M., Koonin, E.V., and Aravind, L. 2004. Novel predicted peptidases with a potential role in the ubiquitin signaling pathway. *Cell Cycle* **3**, 1440‒1450.

Kaitna, S., Schnabel, H., Schnabel, R., Hyman, A.A., and Glotzer, M. 2002. A ubiquitin C-terminal hydrolase is required to maintain osmotic balance and execute actin-dependent processes in the early *C. elegans* embryo. *J. Cell Sci.* **115(Pt 11)**, 2293‒2302.

Kanadia, R.N., Kuo, W.N., Mcnabb, M., and Botchway, A. 1998. Constitutive nitric oxide synthase in *Saccharomyces cerevisiae*. *Biochem. Mol. Biol. Int.* **45**, 1081‒1087.

Kiani-Esfahani, A., Tavalaee, M., Deemeh, M.R., Hamiditabar, M., and Nasr-Esfahani, M.H. 2012. DHR123: an alternative probe for assessment of ROS in human spermatozoa. *Syst. Biol. Reprod. Med.* **58**, 168‒174.

Kig, C. and Temizkan, G. 2009. Nitric oxide as a signaling molecule in the fission yeast *Schizosaccharomyces pombe*. *Protoplasma* **238**, 59‒66.

Kim, Y., Jo, H., and Lim, C.J. 2013. Deubiquitinating activity of Sdu1, a putative member of the PPPDE peptidase family, in *Schizosaccharomyces pombe*. *Can. J. Microbiol.* **59**, 789‒796.

Kim, J.Y., Lee, J.M., and Cho, J.Y. 2011. Ubiquitin C-terminal hydrolase-L3 regulates Smad1 ubiquitination and osteoblast differentiation. *FEBS Lett.* **585**, 1121‒1126.

Kim, M.S., Ramakrishna, S., Lim, K.H., Kim, J.H., and Baek, K.H. 2011. Protein stability of mitochondrial superoxide dismutase SOD2 is regulated by USP36. *J. Cell. Biochem.* **112**, 498‒508.

Kimura, Y., Yashiroda, H., Kudo, T., Koitabashi, S., Murata, S., Kakizuka, A., and Tanaka, K. 2009. An inhibitor of a deubiquitinating enzyme regulates ubiquitin homeostasis. *Cell* **137**, 549‒559.

Komander, D., Clague, M.J., and Urbé, S. 2009. Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell. Biol.* **10**, 550‒563.

Larsen, C.N., Krantz, B.A., and Wilkinson, K.D. 1998. Substrate specificity of deubiquitinating enzymes: ubiquitin C-terminal hydrolases. *Biochemistry* **37**, 3358‒3368.

Lee, Y.Y., Kim, H.G., Jung, H.I., Shin, Y.H., Hong, S.M., Park, E.H., Sa, J.H., and Lim, C.J. 2002. Activities of antioxidant and redox enzymes in human normal hepatic and hepatoma cell lines. *Mol. Cells* **14**, 305‒311.

Lee, B.H., Lee, M.J., Park, S., Oh, D.C., Elsasser, S., Chen, P.C., Gartner, C., Dimova, N., Hanna, J., Gygi, S.P., *et al*. 2010.

Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179‒184.

Meyer-Schwesinger, C., Meyer, T.N., Sievert, H., Hoxha, E., Sachs, M., Klupp, E.M., Münster, S., Balabanov, S., Carrier, L., Helmchen, U., *et al*. 2011. Ubiquitin C-terminal hydrolase-l1 activity induces polyubiquitin accumulation in podocytes and increases proteinuria in rat membranous nephropathy. *Am. J. Pathol.* **178**, 2044‒2057.

Myers, A.M., Tzagoloff, A., Kinney, D.M., and Lusty, C.J. 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. *Gene* **45**, 299‒310.

Nagai, H., Noguchi, T., Homma, K., Katagiri, K., Takeda, K., Matsuzawa, A., and Ichijo, H. 2009. Ubiquitin-like sequence in ASK1 plays critical roles in the recognition and stabilization by USP9X and oxidative stress-induced cell death. *Mol. Cell* **36**, 805‒818.

Nakagawa, K., Saijo, N., Tsuchida, S., Sakai, M., Tsunokawa, Y., Yokota, J., Muramatsu, M., Sato, K., Terada, M., and Tew, K.D. 1990. Glutathione-S-transferase pi as a determinant of drug resistance in transfectant cell lines. *J. Biol. Chem.* **265**, 4296‒4301.

Orlandi, I., Bettiga, M., Alberghina, L., Nyström, T., and Vai, M. 2010. Sir2-dependent asymmetric segregation of damaged proteins in ubp10 null mutants is independent of genomic silencing. *Biochim. Biophys. Acta.* **1803**, 630‒638.

Orlandi, I., Bettiga, M., Alberghina, L., and Vai, M. 2004. Transcriptional profiling of ubp10 null mutant reveals altered subtelomeric gene expression and insurgence of oxidative stress response. *J. Biol. Chem*. **279**, 6414‒6425.

Ramakrishna, S., Suresh, B., and Baek, K.H. 2011. The role of deubiquitinating enzymes in apoptosis. *Cell Mol. Life Sci.* **68**, 15‒26.

Reyes-Turcu, F.E., Ventii, K.H., and Wilkinson, K.D. 2009. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu. Rev. Biochem.* **78**, 363‒397.

Ristic, G., Tsou, W.L., and Todi, S.V. 2014. An optimal ubiquitin-proteasome pathway in the nervous system: the role of deubiquitinating enzymes. *Front. Mol. Neurosci.* **7**, 72.

Royall, J.A. and Ischiropoulos, H. 1993. Evaluation of 2',7'-dichlorofluorescin and dihydrorhodamine 123 as fluorescent probes for intracellular H_2O_2 in cultured endothelial cells. *Arch. Biochem. Biophys.* **302**, 348‒355.

Sherman, M.P., Aeberhard, E.E., Wong, V.Z., Griscavage, J.M., and Ignarro, L.J. 1993. Pyrrolidine dithiocarbamate inhibits induction of nitric oxide synthase activity in rat alveolar macrophages. *Biochem. Biophys. Res. Commun.* **191**, 1301‒1308.

Song, H.M., Lee, J.E., and Kim, J.H. 2014. Ubiquitin C-terminal hydrolase-L3 regulates EMT process and cancer metastasis in prostate cell lines. *Biochem. Biophys. Res. Commun.* **452**, 722‒727.

Stone, M., Hartmann-Petersen, R., Seeger, M., Bech-Otschir, D., Wallace, M., and Gordon, C. 2004. Uch2/Uch37 is the major

deubiquitinating enzyme associated with the 26S proteasome in fission yeast. *J. Mol. Biol.* **344**, 697‒706.

Tse, W.K., Eisenhaber, B., Ho, S.H., Ng, Q., Eisenhaber, F., and Jiang, Y.J. 2009. Genome-wide loss-of-function analysis of deubiquitylating enzymes for zebrafish development. *BMC Genomics* **10**, 637.

Wang, C.Y., Wen, W.L., Nilsson, D., Sunnerhagen, P., Chang, T.H., and Wang, S.W. 2012a. Analysis of stress granule assembly in *Schizosaccharomyces pombe*. *RNA* **18**, 694‒703.

Wang, H., Ying, Z., and Wang, G. 2012b. Ataxin-3 regulates aggresome formation of copper-zinc superoxide dismutase (SOD1) by

editing K63-linked polyubiquitin chains. *J. Biol. Chem.* **287**, 28576‒28585.

Wicks, S.J., Haros, K., Maillard, M., Song, L., Cohen, R.E., Dijke, P.T., and Chantry, A. 2005. The deubiquitinating enzyme UCH37 interacts with Smads and regulates TGF-beta signalling. *Oncogene* **24**, 8080‒8084.

Wink, D.A., Miranda, K.M., and Espey, M.G. 2001. Cytotoxicity related to oxidative and nitrosative stress by nitric oxide. *Exp. Biol. Med.* **226**, 621‒623.

Wolf, D.A. and Petroski, M.D. 2009. Rfu1: stimulus for the ubiquitin economy. *Cell* **137**, 397‒398.