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A cold-induced thioredoxin h of rice, OsTrx23, negatively regulates kinase activities of OsMPK3 and OsMPK6 in vitro

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ABSTRACT

Cytosolic thioredoxins are small conserved proteins that are involved in cellular redox regulation. Here, we report that a major and cold-induced thioredoxin h of rice, OsTrx23, has an inhibitory activity on stress-activated mitogen-activated protein kinases (MAPKs), OsMPK3 and OsMPK6 in vitro. This inhibition effects were redox-dependent and did not involve stable physical interaction. The data suggested a novel mechanism for redox regulation of MAPKs in plants.

Structured summary:
MINT-7234362: *MPK3* (uniprotkb:Q10N20) *phosphorylates* (MI:0217) *MBP* (uniprotkb:P02687) by *protein kinase assay* (MI:0424)
MINT-7234435: *MPK6* (uniprotkb:Q336X9) *phosphorylates* (MI:0217) *MBP* (uniprotkb:P02687) by *protein kinase assay* (MI:0424)

1. Introduction

Thioredoxin (Trx) is a small (12–14 kDa), heat-stable and ubiquitous thiol-disulfide oxidoreductase with two conserved redox-active half-cysteine residues, Cys-X–X-Cys, in an active site [1]. Two cysteine residues in the active site provide sulfhydryl groups that are involved in a reducing activity. The reduced form of thioredoxin, Trx–$(SH)_2$, reduces disulfide bonds of target proteins, while the oxidized form, Trx–S_2, contains a disulfide bond within the active site, which is reduced to Trx–$(SH)_2$ dithiol by NADPH and Trx reductases [2].

In plants, six different types (m, f, x, y, o and h) of Trxs have been described according to their function and localization within the cell. The type h Trx is assumed to be localized to the cytoplasm and mitochondria, and is reduced by mitochondrial or cytoplasmic NADPH-dependent thioredoxin reductases (NTR) [3,4]. NTRs belong to a superfamily of flavoprotein disulfide oxidoreductases [5] and transfer electrons from NADPH to the active site disulfide bonds of oxidized Trx h via FAD [6]. Plant Trx h proteins are implicated in many cellular processes, including protein folding, protein repair after damage by oxidation, sulfur metabolism, and regulation of transcription factors [2]. Trx h proteins are also involved in plant development and stress responses, such as seed germination and early seedling development [7], self-incompatibility reactions [8] and oxidative stress [9]. In rice, thirty potential thioredoxin-encoding genes (*OsTrxs*) have been identified; nine genes of which were predicted to encode Trx h [10]. Meanwhile, eleven thioredoxin h genes have been recently identified in Arabidopsis [2].

A highly conserved and ubiquitous mitogen-activated protein kinase (MAPK) pathway can transduce external stimuli into cellular responses, and has been identified as a conserved signal pathway which link, in various ways, the upstream receptors, or sensors, and downstream targets. The MAPK pathways consist of three sequential activating components: MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK) and MAPK [11,12]. Of the 20 MAPK genes in Arabidopsis, *AtMPK3* and *AtMPK6*, two members from group A, have been implicated in multiple abiotic and biotic stress tolerance [13]. Rice orthologs of *AtMPK3* and *AtMPK6*, *OsMPK3/OsMAP1/OsWIPK* and *OsMPK6/OsSIPK*, respectively, are also involved in stress responses such as low-temperature, salinity, dehydration and wounding [14,15]. Forty-eight potential substrates for AtMPK3 and 39 substrates for AtMPK6 have been identified by plant protein microarray analysis [16]. An m-type thioredoxin, Trx m1, was shown to be one of the potential common substrates for AtMPK3 and AtMPK6.

Abbreviations: NADP-MDH, NADP-Malate Dehydrogenase; GST, glutathione S-transferase; IPTG, isopropyl-β-D-thiogalactopyranoside; MAPK, Mitogen-activated protein kinase; MBP, Myelin basic protein; NTR, NADPH-dependent thioredoxin reductase; PBS, phosphate-buffered saline; TBS, Tris-buffered saline

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Here, we report that a major, cold-induced, thioredoxin h, OsTrx23, has an inhibitory function on two stress-activated MAPKs of rice, OsMPK3 and OsMPK6 in vitro. We also suggest a novel mechanism of redox regulation of MAPKs in plants.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatment

Rice variety Yukihikari seeds (*Oryza sativa* L. subsp. Japonica) were surface-sterilized in 70% ethanol for 2 min and then in 2.5% NaClO for 30 min with gentle shaking, and then were washed several times with sterile water. Sterilized seeds were soaked in distilled water for 4 d and were then set for germination at 25 °C as previously described [15]. After growing for 7 d, the seedlings were subjected to 12 °C stress; in a growth chamber a mesh grid with the seedlings were transferred into a container filled with one-day pre-chilled (12 °C) water. Shoot and root tissues of rice seedlings were collected after 0, 1, 2, 4, 6, 12, 24 and 48 h of chilling stress treatments, were immediately frozen in liquid nitrogen, and were then stored at −80 °C until used.

2.2. DNA sequencing and phylogenetic analysis

DNA sequencing was carried out by using an ABI PRISM 3100 Genetic Analyzer (PE Biosystems, Foster City, CA, USA). A BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) was used for the sequencing reaction. Multiple amino acid alignments of the thioredoxin domains, among the *Trx h* gene families from rice and Arabidopsis, were performed using the online ClustalW program provided by DDBJ (http://www.nig.ac.jp/search/clutalm-e.html). The phylogenetic tree was created using the Neighbor–Joining method.

2.3. RNA gel blot analysis

Total RNA was isolated from shoot and root tissues using TRIzol reagent (Invitrogen, USA). Ten micrograms of total RNA was then denatured in formamide and formaldehyde, separated on 1.0% agarose gels, and were then transferred onto Hybond-N^+ membranes (GE Healthcare, USA). The blots were hybridized in Rapid-Hyb Buffer (GE Healthcare, USA) at 65 °C with a ^{32}P-labeled *OsTrx23* full-length cDNA fragment, which was amplified by PCR with primers (forward primer: 5′-CCTCTCTTTGATCTCGTC-3′, reverse primer: 5′-CTACTACAACACCAGGTG-3′). The blots were washed twice with primary wash buffer (2 × SSC, 0.1% SDS) for 15 min at 65 °C, and then washed once with secondary wash buffer (0.2 × SSC, 0.1% SDS) for 15 min at 65 °C. The blots were then exposed to Hyperfilm MP X-ray film (GE Healthcare, USA) for signal detection.

2.4. Molecular cloning, recombinant protein production and purification

The ORFs of *OsSK1* (AK068737, Os01g0252100) (5′-GGATCCATGGGTTCAGTAGGGGT-3′, 5′-GAATTCTACTCCTGCATAGGAGCA-3′), *OsMPK3* (5′-GGATCCATGGACGGGCGC-3′, 5′-GAATTCATACCGGATGTTTGGGTTCAT-3′) and *OsMPK6* (5′-GGATCCATGGACGCCGGGG-3′, 5′-GAATTCCTGGTAATCAGGGTTGAA-3′) were PCR-amplified and fused in-frame to pGEX-6P-3 (GE Healthcare, USA) to produce N-terminal glutathione S-transferase (GST)-fused proteins. The ORF of *OsTrx23* cDNA (AK121423) was amplified with primers, 5′-GGATCCAATGGCCGCCGAGG-3′ and 5′-GTCGACGGCAGAAGCAGATGCA-3′, and was then cloned into pQE31 (Qiagen, USA) to produce an in-frame N-terminal 6× His-tag fusion protein. The GST-OsMPK3, GST-OsMPK6 and His-OsTrx23 recombinant proteins were produced and purified according to the manufacturer's instructions. Briefly, *E. coli* BL21 (DE3) containing pGEX-6P-OsMPK3, pGEX-6P-OsMPK6 and *E. coli* M15 containing pQE31-OsTrx23 was cultured at 37 °C in LB medium containing 50 μg/ml ampicillin until an OD_{600} of 0.40–0.60 was reached. Isopropyl-β-D-thiogalactopyranoside (IPTG) was subsequently added to a final concentration of 1 mM and the cultures were further induced at 20 °C for 10 h. *E. coli* cells were collected by centrifugation, washed with Tris-buffered saline (TBS), and resuspended in 1 × TBS. Cells were sonicated for 4 min at an interval of 15 s. The sonicated solutions were then centrifuged and the total soluble fraction (supernatant) was affinity purified with a Glutathione-Sepharose 4B (GE Healthcare, USA) or a His-band Column (Novagen, USA). Eluted GST, GST-OsMPK3, GST-OsMPK6 and His-OsTrx23 proteins were concentrated in 10 mM Tris buffer (pH 7.5).

2.5. Preparation of the reduced and oxidized forms of OsTrx23

The oxidized form of His-OsTrx23 was prepared as described by Baker et al. [17]. Briefly, the reduced form of His-OsTrx23, prepared as described above, was incubated with 5 mM diamide at room temperature for 3 h. The reaction was terminated by exchanging the buffer twice with 1× phosphate-buffered saline (PBS) using the Microcon Ultracel YM-10 (Millipore, USA). The concentration of the oxidized thioredoxin h was measured by the Bradford Protein Assay (Bio-Rad, USA).

2.6. In vitro protein kinase assay

An in vitro protein kinase assay was performed according to Zhang et al. [18] with minor modification as follows. One microgram of purified GST-OsMPK3 or GST-OsMPK6 was incubated with different concentrations of OsTrx23 or GSH in the kinase reaction buffer (50 mM Tris, pH 7.5, 25 mM ATP, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 1 mM EGTA, 1 mM DTT) with 1 μCi [γ-^{32}P]-ATP and 0.25 μg/μl Myelin basic protein (MBP) at 30 °C for 30 min. When the kinase activity was measured, using OsTrx23 as a substrate, OsTrx23 (0.25 μg/μl) was added in place of MBP. For a two-step kinase assay, GST-OsMPK3 and GST-OsMPK6 were first incubated with GST-OsMKK6 (0.5 mg) and 50 mM unlabelled ATP in the kinase reaction buffer prior to the incubation with a specific substrate. The reaction was terminated by adding an equal volume of 2× SDS loading buffer (50 mM Tris.HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue) and then boiling at 100 °C for 5 min. Kinase activities were determined by autoradiography after 15% SDS–PAGE.

3. Results

3.1. OsTrx23 is a major, cold-induced, thioredoxin h in rice

Phylogenetic analysis of rice and Arabidopsis sequences revealed a common grouping of thioredoxin h in these two plant species. Subgroup I contained three rice members, *OsTrx23*, *OsTrx26* and *OsTrx30*, as well as four Arabidopsis members, *AtTrx h1*, *AtTrx h3*, *AtTrx h4* and *AtTrx h5*. Subgroup II involved two rice members, *OsTrx10* and *OsTrx24*, as well as three Arabidopsis members, *AtTrx h2*, *AtTrx h7* and *AtTrx h8*. The subgroup III thioredoxin h proteins exhibit an unusual active site (WCXXS) and an N-terminal extension, and include four rice and four Arabidopsis members (Fig. 1).

Among the nine *Trx h* genes found in rice, we focused our research on *OsTrx23*, because *OsTr23*, together with *OsTrx10*, showed cold-induced expression in our microarray data (Kato et al. unpublished). Northern blot analysis determined detailed expression pattern of *OsTrx23* in response to chilling (12 °C) stress (Fig. 2).

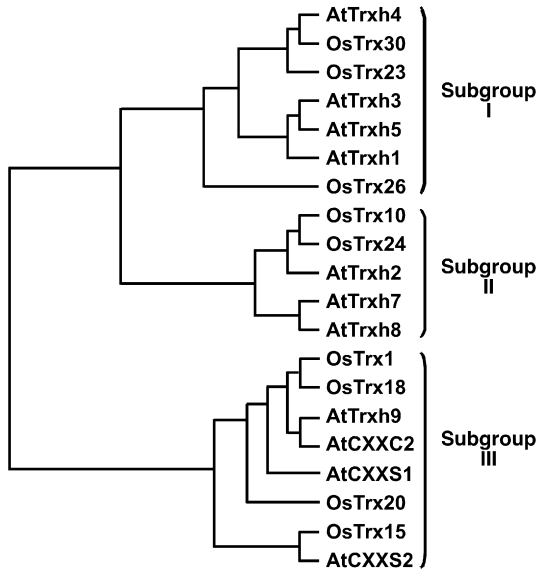

Fig. 1. Phylogenetic tree of the h-type thioredoxins identified in rice and Arabidopsis genomes. The thioredoxin domains of nine rice thioredoxin h-encoding genes, including: *OsTrx1* (Os01g07376), *OsTrx10* (Os03g58630), *OsTrx15* (Os04g53740), *OsTrx18* (Os05g07690), *OsTrx20* (Os05g40190), *OsTrx23* (Os07g08840), *OsTrx24* (Os07g09310), *OsTrx26* (Os09g23650) and *OsTrx30* (Os12g18360), and eleven Arabidopsis thioredoxin h-encoding genes, including: *AtTrx h1* (At3g51030), *AtTrx h2* (At5g39950), *AtTrx h3* (At5g42980), *AtTrx h4* (At1g19730), *AtTrx h5* (At1g45145), *AtTrx h7* (At1g59730), *AtTrx h8* (At1g69880), *AtTrx h9* (At3g08710), *AtCxxC2* (At3G56420), *AtCxxS1* (At2g40790) and *AtCxxS2* (At1g11530), respectively, were aligned with the Clustal W software.

Transcripts of *OsTrx23* in shoot and root tissues accumulated slowly and reached significant levels after 24 h of chilling stress (Fig. 2), which indicates that OsTx23 is involved in the cold stress response in a rice seedling.

3.2. OsTrx23 is not a substrate for OsMPK3 and OsMPK6 in vitro

Since it was suggested that Trx m1 is a common potential substrate for AtMPK3 and AtMPK6 [16], we first performed yeast two-hybrid and in vitro pull-down assays in order to detect an interaction between OsTrx23 and OsMPK3 or OsMPK6. However, no substantial interaction between OsTrx23 and OsMPK3 or OsMPK6 was observed in these assays (data not shown).

NetPhos 2.0 Server (http://www.cbs.dtu.dk/services/NetPhos) predicted that phosphorylation scores of Thr22 and Ser38 were 0.836 and 0.795, respectively, which indicates a high potential for phosphorylation of OsTrx23. Therefore, possible phosphorylation of OsTrx23 by OsMPK3 and OsMPK6 was tested. MBP exhibited phosphorylation catalyzed by OsMPK3 and OsMPK6, and was further enhanced by the coexistence of a putative rice MAPKK, OsMKK6. In contrast, His-OsTrx23 was not phosphorylated by either of these kinases (Fig. 3). These data suggest that OsTrx23 is not a substrate for OsMPK3 and OsMPK6 in vitro.

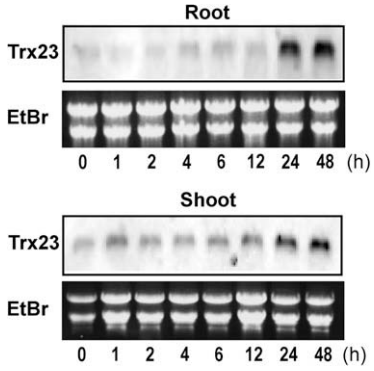

Fig. 2. Expression of *OsTrx23* in response to 12 °C chilling stresses. Total RNA (10 μg/lane) was extracted from shoot and root tissues of 7-day-old seedlings exposed to 12 °C stress for different time periods. Total RNA blots were hybridized with an OsTrx23 probe as described under Section 2.

3.3. OsTrx23 specifically inhibits in vitro kinase activities of OsMPK3 and OsMPK6

We next determined the effect of OsTrx23 on the in vitro kinase activities of OsMPK3 and OsMPK6. As shown in Fig. 4A, OsMPK3 and OsMPK6 strongly phosphorylated MBP in the absence of OsTrx23, while the kinase activities of OsMPK3 and OsMPK6 were abolished in the presence of OsTrx23. In contrast, the kinase activity of OsSK1, a shaggy-like Ser/Thr kinase, did not change irrespective of the presence of OsTrx23. In addition, other non-reductant proteins, such as BSA and IgG, had no effect on the changes in the in vitro kinase activities of OsMPK3 and OsMPK6 (Fig. 4B).

3.4. The in vitro kinase activities of OsMPK3 and OsMPK6 are redox-controlled

To further clarify the mechanism by which the kinase activities of OsMPK3 and OsMPK6 are regulated, we determined the in vitro kinase activities of OsMPK3 and OsMPK6 in the presence of the oxidized or the reduced form of OsTrx23. The inhibition effect of the reduced form of OsTrx23 was concentration-dependent and increased within the range tested up to 4 μM (Fig. 5). However, the oxidized form of OsTrx23 did not show any inhibitory activity on

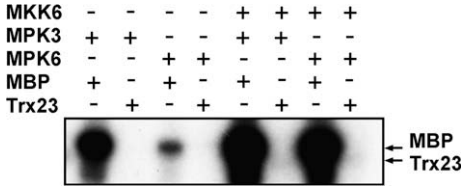

Fig. 3. Phosphorylation of OsTrx23 and Myelin basic protein (MBP) by OsMPK3 and OsMPK6. His-OsTrx23 (5 μg) or MBP (5 μg) was incubated with 1 μg of GST-OsMPK3 or GST-OsMPK6 in 20 μl kinase reaction buffer at 30 °C for 30 min. GST-OsMKK6 (0.5 μg) was added to the kinase buffer where indicated. The reaction was terminated by adding 20 μl of 2× loading buffer and then boiling. Five microliters from each reaction sample was separated by 15% SDS–PAGE and autoradiographed. CBB-stained gel image is shown in Supplementary Fig. S1.

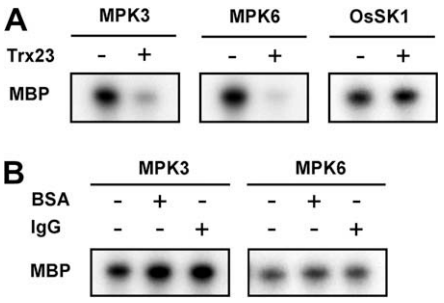

Fig. 4. Inhibitory activity of OsTrx23 against OsMPK3 and OsMPK6. (A) MBP kinase activity was measured for GST-OsMPK3 (1 μg) and GST-OsMPK6 in the presence (+) or absence (−) of His-OsTrx23 (4 μM) in the 20 μl kinase reaction buffer. GST-OsSK1 (a rice homolog of AtSK1, 1 μg) was used as a control. (B) MBP kinase activity was measured for GST-OsMPK3 (1 μg) and GST-OsMPK6 (1 μg) in the presence of BSA (4 μM) or IgG (4 μM). Five μl from each reaction sample was separated by 15% SDS–PAGE and autoradiographed. CBB-stained gel images are shown in Supplementary Fig. S2.

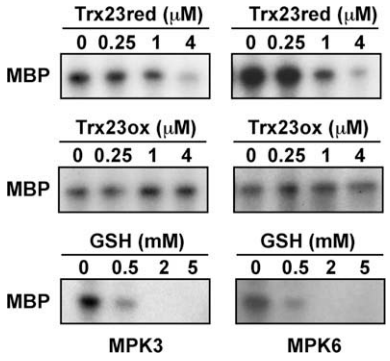

Fig. 5. Effect of redox status of OsTrx23 on in vitro kinase activities of OsMPK3 and OsMPK6. MBP kinase activity was measured for GST-OsMPK3 (1 μg) and GST-OsMPK6 (1 μg) in the presence of the indicated concentrations of reduced (red) His-OsTrx23, oxidized (ox) His-OsTrx23 or GSH in the 20 μl kinase reaction buffer. Five microliters from each reaction sample was separated by 15% SDS–PAGE and autoradiographed. CBB-stained gel images are shown in Supplementary Fig. S3.

OsMPK3 and OsMPK6. Likewise, the reduced form of glutathione inhibited the kinase activities of OsMPK3 and OsMPK6 (Fig. 5), although GSH required much higher concentrations for inhibition effects compared to OsTrx23. Together, these data suggest that the in vitro kinase activities of OsMPK3 and OsMPK6 are redox-controlled.

4. Discussion

OsTrx23 was initially identified as an abundant low molecular weight protein in rice phloem sap [19] and was predicted to be localized in cytosol because of a lack of N-terminal signal sequence [10]. In this report, we found that OsTrx23 is a potential negative regulator of two stress-related MAPKs of rice, OsMPK3 and OsMPK6.

Phylogenic analysis revealed that OsTrx23 is grouped into Subgroup I together with *AtTrx h1, h3, h4* and *h5*. All of these thioredoxin h proteins in this subgroup contain two thioredoxin domains. *AtTrx h5* is specifically involved in oxidative stress tolerance and W-box-mediated response to pathogen elicitors [3]. Similarly, the *OsTrx23* mRNA is induced by paraquat stress through a novel *cis*-element [9], and the OsTrx23 protein accumulates under glyphosate-induced oxidative stress in rice leaves [20]. We observed that OsTrx23 is one of the two cold (12 °C)-induced Trx h proteins (Kato et al., unpublished data). Therefore, it will be interesting to determine if induction of Trx23 is triggered by oxidative stress imposed by a chilling treatment.

Trxs regulate a variety of physiological processes in plants [2,7–9]. There are two action modes by which Trxs regulate the functions of target proteins. First, Trxs physically interact with, and regulate the activity of, target proteins by redox-regulated binding and releasing. For example, human Trx interacts with the N-terminal region of ASK1, an apoptosis signal regulating kinase, and negatively regulates ASK1 activity. Oxidation of Trx induces the release of Trx from ASK1 and rescues ASK1 activity [21,22]. In the second mode of action, Trxs exchange the redox state of target proteins without physical interaction. This mechanism of thiol redox control is emerging as a major regulatory mechanism in signal transduction [1]. Since no stable interactions were detected between OsTrx23 and OsMPK3 or OsMPK6, the later may be the case for OsTrx23.

In plants, many enzymes in the Calvin cycle and C4 pathway, such as glyceraldehyde 3-phosphate dehydrogenase, FBPase, sedoheptulose 1,7-bis phosphatase, phosphoribulokinase and NADP-Malate Dehydrogenase (NADP-MDH) are redox-regulated [23]. However, it is unknown if plant MAPKs are directly regulated by redox. Our data suggest that OsMPK3 and OsMPK6 are negatively regulated by OsTrx23. A mammalian thioredoxin, TRX, negatively regulates p38 MAP kinase and p38 MAPK-mediated cytokine expression through scavenging ROS by the reduced form [24]. MEKK1 is inhibited in vitro directly by GSH-interaction or glutathionylation in menadione-treated cells [25], and GSH depletion has been shown to activate p38 MAPK in the kidney and liver [26]. These previous studies have suggested that MAPK signaling pathways are redox-regulated. Here, our in vitro data suggest that MAPK can be directly regulated by a redox state.

The mechanism by which OsTrx23 regulates OsMPK3 and OsMPK6 activities during chilling stress is currently unknown. However, it is possible to propose a potential model for the action. In maize, chilling stress (14 °C) leads to an immediate increase in H_2O_2 levels, which subsequently drops after 6 h [27]. Assuming a similar transient accumulation of H_2O_2 occurs in rice during chilling stress, elevated H_2O_2 levels may activate OsMPK3 and OsMPK6 [28]. Simultaneously, reduced OsTrx23 may be oxidized by direct scavenging of H_2O_2 [29] or through peroxiredoxins [2], resulting in decreased OsTRX23 inhibitory activity against OsMPK3 and OsMPK6. As the H_2O_2 levels decrease after the initial transient induction, the reduced form of Trx23 would prevail to repress OsMPK3 and OsMPK6. In contrast to the rapid induction of OsMPK3 in response to chilling (at 12 °C) [15], induction of *OsTrx23* occurred relatively late (24 h) after initiation of the stress. The timing of *OsTrx23* induction upon chilling stress coincided with the decline in OsMPK3 expression [15] and kinase activity (Xie et al., unpublished). These results propose a possible involvement of OsTrx23 in the repression of OsMPK3 activity after transient activation in response to chilling stress.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.07.057.

References

[1] Holmgren, A. (1989) Thioredoxin and glutaredoxin systems. J. Biol. Chem. 264, 13963–13966.
[2] Meyer, Y., Siala, W., Bashandy, T., Riondet, C., Vignols, F. and Reichheld, J.P. (2008) Glutaredoxins and thioredoxins in plants. Biochim. Biophys. Acta 1783, 589–600.
[3] Laloi, C., Rayapuram, N., Chartier, Y., Grienenberger, J.M., Bonnard, G. and Meyer, Y. (2001) Identification and characterization of a mitochondrial thioredoxin system in plants. Proc. Natl. Acad. Sci. USA 98, 14144–14149.
[4] Gelhaye, E., Rouhier, N. and Jacquot, J.P. (2004) The thioredoxin h system of higher plants. Plant Physiol. Biochem. 42, 265–271.
[5] Reichheld, J.P., Meyer, E., Khafif, M., Bonnard, G. and Meyer, Y. (2005) AtNTRB is the major mitochondrial thioredoxin reductase in *Arabidopsis thaliana*. FEBS Lett. 579, 337–342.
[6] Mustacich, D. and Powis, G. (2000) Thioredoxin reductase. Biochem. J. 346 (Pt 1), 1–8.
[7] Shahpiri, A., Svensson, B. and Finnie, C. (2008) The NADPH-dependent thioredoxin reductase/thioredoxin system in germinating barley seeds: gene expression, protein profiles, and interactions between isoforms of thioredoxin h and thioredoxin reductase. Plant Physiol. 146, 789–799.
[8] Cabrillac, D., Cock, J.M., Dumas, C. and Gaude, T. (2001) The S-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. Nature 410, 220–223.
[9] Tsukamoto, S., Morita, S., Hirano, E., Yokoi, H., Masumura, T. and Tanaka, K. (2005) A novel cis-element that is responsive to oxidative stress regulates three antioxidant defense genes in rice. Plant Physiol. 137, 317–327.

[10] Nuruzzaman, M., Gupta, M., Zhang, C., Wang, L., Xie, W., Xiong, L., Zhang, Q. and Lian, X. (2008) Sequence and expression analysis of the thioredoxin protein gene family in rice. Mol. Genet. Genomics 280, 139–151.
[11] Jonak, C., Kiegerl, S., Ligterink, W., Barker, P.J., Huskisson, N.S. and Hirt, H. (1996) Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. Proc. Natl. Acad. Sci. USA 93, 11274–11279.
[12] Nakagami, H., Pitzschke, A. and Hirt, H. (2005) Emerging MAP kinase pathways in plant stress signalling. Trends Plant Sci. 10, 339–346.
[13] Moon, H. et al. (2003) NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc. Natl. Acad. Sci. USA 100, 358–363.
[14] Cho, K., Agrawal, G.K., Jwa, N.S., Kubo, A. and Rakwal, R. (2009) Rice OsSIPK and its orthologs: a ''central master switch" for stress responses. Biochem. Biophys. Res. Commun. 379, 649–653.
[15] Wen, J.Q., Oono, K. and Imai, R. (2002) Two novel mitogen-activated protein signaling components, OsMEK1 and OsMAP1, are involved in a moderate low-temperature signaling pathway in rice. Plant Physiol. 129, 1880–1891.
[16] Feilner, T. et al. (2005) High throughput identification of potential Arabidopsis mitogen-activated protein kinases substrates. Mol. Cell. Proteomics 4, 1558–1568.
[17] Baker, A., Santos, B.D. and Powis, G. (2000) Redox control of caspase-3 activity by thioredoxin and other reduced proteins. Biochem. Biophys. Res. Commun. 268, 78–81.
[18] Zhang, S., Liu, Y. and Klessig, D.F. (2000) Multiple levels of tobacco WIPK activation during the induction of cell death by fungal elicitins. Plant J. 23, 339–347.
[19] Ishiwatari, Y. et al. (1995) Thioredoxin h is one of the major proteins in rice phloem sap. Planta 195, 456–463.
[20] Ahsan, N., Lee, D.G., Lee, K.W., Alam, I., Lee, S.H., Bahk, J.D. and Lee, B.H. (2008) Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. Plant Physiol. Biochem. 46, 1062–1070.
[21] Ichijo, H. et al. (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 275, 90–94.
[22] Saitoh, M. et al. (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. 17, 2596–2606.
[23] Buchanan, B.B. (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Perspective on its discovery, present status, and future development. Arch Biochem. Biophys. 288, 1–9.
[24] Hashimoto, S. et al. (1999) Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor-alpha. Biochem. Biophys. Res. Commun. 258, 443–447.
[25] Cross, J.V. and Templeton, D.J. (2004) Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. Biochem. J. 381, 675–683.
[26] Limón-Pacheco, J.H., Hernandez, N.A., Fanjul-Moles, M.L. and Gonsebatt, M.E. (2007) Glutathione depletion activates mitogen-activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice. Free Radic. Biol. Med. 43, 1335–1347.
[27] Prasad, T.K., Anderson, M.D., Martin, B.A. and Stewart, C.R. (1994) Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6, 65–74.
[28] Lee, M.O. et al. (2008) Novel rice OsSIPK is a multiple stress responsive MAPK family member showing rhythmic expression at mRNA level. Planta 227, 981–990.
[29] Mitsui, A., Hirakawa, T. and Yodoi, J. (1992) Reactive oxygen-reducing and protein-refolding activities of adult T cell leukemia-derived factor/human thioredoxin. Biochem. Biophys. Res. Commun. 186, 1220–1226.