Plant Signaling & Behavior 10:5, e1017698; May 2015; Published with license by Taylor & Francis Group, LLC

Stress defense mechanisms of NADPH-dependent thioredoxin reductases (NTRs) in plants

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Keywords: NADPH-dependent thioredoxin reductase (NTR), reactive oxygen species (ROS), stress response, Thioredoxin

Plants establish highly and systemically organized stress defense mechanisms against unfavorable living conditions. To interpret these environmental stimuli, plants possess communication tools, referred as secondary messengers, such as Ca^{2+} signature and reactive oxygen species (ROS) wave. Maintenance of ROS is an important event for whole lifespan of plants, however, in special cases, toxic ROS molecules are largely accumulated under excess stresses and diverse enzymes played as ROS scavengers. Arabidopsis and rice contain 3 NADPH-dependent thioredoxin reductases (NTRs) which transfer reducing power to Thioredoxin/Peroxiredoxin (Trx/Prx) system for scavenging ROS. However, due to functional redundancy between cytosolic and mitochondrial NTRs (NTRA and NTRB, respectively), their functional involvements under stress conditions have not been well characterized. Recently, we reported that cytosolic NTRA confers the stress tolerance against oxidative and drought stresses via regulation of ROS amounts using NTRA-overexpressing plants. With these findings, mitochondrial NTRB needs to be further elucidated.

Plants are systemically established both in normal growth condition and in cases wherein plants need to enhance tolerance against various stress conditions caused by increase of second messengers, such as Ca^{2+} and ROS. Changes in intracellular Ca^{2+} concentration defined as a Ca^{2+} signature are sensed by environmental stimuli, decoded and transmitted to downstream genes by a complex of diverse Ca^{2+}-related proteins such as calmodulins (CaMs), calcium-dependent protein kinases (CDPKs) and their interacting kinases (CIPKs).[1] Like this, ROS is also maintained in plant cells with various enzymes to produce and scavenge. However, large inductions of ROS due to extreme environmental stresses work as toxic molecules, resulting in the oxidative stress in cells and ultimately trigger the cell death.[2,3] These ROS are mainly produced by apoplast-localized NADPH oxidases (respiratory burst oxidase homologs, RBOHs). Moreover, detoxifying systems operate to scavenge ROS followed by several antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (Prx).[4-6] Prxs are a family of cysteine-dependent peroxidases, and the activity is coupled to oxidation of NADPH via thioredoxin reductase (TrxR) and thioredoxin (Trx) to reduce Prx.[7,8]

In Arabidopsis and rice, 3 NADPH-dependent TrxRs (NTRs) with disulfide reductase activity, namely NTRA, NTRB and NTRC have been reported existing in different subcellular localizations such as cytosol, mitochondria and chloroplast, respectively (**Fig. 1**).[9,10] NTRs transfer electrons from NADPH to the active-site disulfide bridge (WCG/PPC) of oxidized Trx via FAD and a redox-active disulfide.[8,11] Consistent with this, diverse Trxs (Trx f, h, m, o, x, and y) also exist in distinct subcellular compartments to couple thiol/disulfide exchange reactions of NTR/Trx systems.[8] All NTRs possess a redox active CXXC dithiol motif.[11] While Arabidopsis NTRA and NTRB share 82% sequence similarity, chloroplast localized NTRC is atypical due to additional Trx domain in C-terminus of NTRC which is absent in NTRA and NTRB.[12] Compared to typical NTRA and NTRB, NTRC has been largely identified from biochemical reactions to physiological functions in Arabidopsis.[13]

Loss-of function *NTRC* mutant in Arabidopsis showed obvious pale-green leaves and dwarf phenotypes due to defects of chloroplast biogenesis and auxin levels, respectively.[14] The *ntrc* mutant plants were also sensitive to oxidative, salt, drought and heat stresses.[9,15] NTRC protein harboring this fusion of TrxR and Trx domain directly reduces chloroplast-localized 2Cys-peroxiredoxin (2Cys Prx). Furthermore, electrons from NADPH produced in chloroplast transfer to 2Cys Prx via redox-active cysteins in TrxR and Trx domain in NTRC.[13,16,17] However, cytosolic NTRA and mitochondrial NTRB are acquired to cooperate with their relevant Trx counterparts. NTRA and NTRB also share redundant function in cytosol and mitochondria both in vitro and in vivo.[10,18,19] The single loss-of function mutants of *NTRA* and *NTRB* plants showed no phenotypic perturbations with wild-type Arabidopsis plant, however, *ntra ntrb* double mutant exhibited minor differences such as wrinkled seeds, slow plant growth and reduced fitness of pollen. It indicates that NTRA and NTRB are not essential for plant development.[19]

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Submitted: 01/22/2014; Revised: 02/04/2015; Accepted: 02/05/2015
http://dx.doi.org/10.1080/15592324.2015.1017698

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Addendum to: Cha J-Y, Kim JY, Jung IJ, Kim MR, Melencion A, Alam SS, Yun D-J, Lee SY, Kim MG, Kim W-Y. NADPH-dependent thioredoxin reductase A (NTRA) confers elevated tolerance to oxidative stress and drought. Plant Physiol Biochem 2014; 80:184–191.

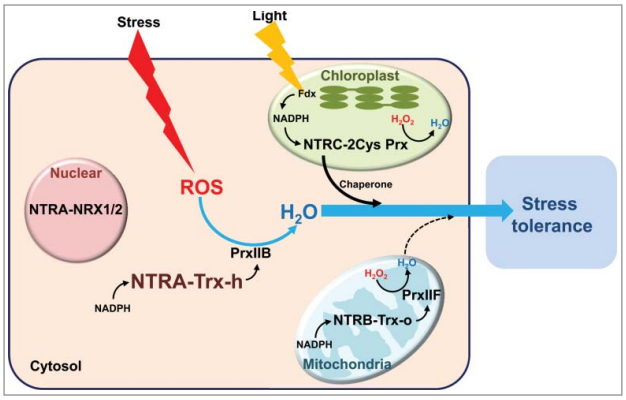

Figure 1. Model proposed for the function of NTRs in Arabidopsis. NTR/Trx systems exist in different subcellular compartments, such as cytosol (NTRA-Trx-h), mitochondria (NTRB-Trx-o) and chloroplast (NTRC), and reduce their distinct Prx which scavenges the H_2O_2. NTRA also reduces nucleus-localized thioredoxins (NRX1). Stress-induced ROS effectively reduced via the overexpression of NTRA which concomitantly confers the stress tolerance. NTR, NADPH-dependent thioredoxin reductase; Trx, thioredoxin; NRX, nucleoredoxin; ROS, reactive oxygen species; Prx, peroxiredoxin.

However, it has been reported that *ntra ntrb* double mutant plant exhibits UV-C tolerance due to high accumulation of anthocyanin.[20] Recently, nuclear Trx genes (*NRX1* and *NRX2*) have been identified in Arabidopsis, emphasizing that NRX1 was reduced by NTRA but not NRX2. Interestingly, NRX1 and NRX2 are localized in both nucleus and cytosol, while cytosolic NTRA is partially localized in the nucleus.[21] Even this finding suggests that Trx system also existed in nucleus, it is still difficult to conclude whether NTRA/NRX1 system is working in the nucleus or cytosol. Thus, due to this functional redundancy between NTRA and NTRB, their physiological functions under environmental stresses are not clearly classified and remain elusive to date.

Due to difficulties of research using single loss-of function *NTRA* mutant (*ntra-ko*), *NTRA*-overexpressing plants (NTRA-OX) in Arabidopsis were generated and functional roles of NTRA under various stress responses were investigated. As already known, cytosolic NTRA protein reduces cytosolic Trx-h proteins.[22] And Trx-h proteins are positively involved in stress responses such as pathogen and heat stress via redox regulation and chaperone function in cells.[23,24] Under oxidative stress, toxic ROS are largely accumulated in plant cells and cause damage of DNA, RNA, protein and lipid.[2,3] We have found that *NTRA* transcripts were dramatically enhanced by methyl viologen treatments which are well known to induce large accumulations of ROS and cause oxidative damage in plant cells.[25] Moreover, NTRA-OX plants showed high survival rates and retarded ROS inductions under oxidative stress whereas wild-type and *ntra-ko* plants were almost dead with high ROS contents. The phenomenon of stress tolerance and low ROS levels were consistent when plants exposed to drought stress.[25] Interestingly, transcriptional levels of drought-responsive genes (*RD29A* and *DREB2A*) were higher in NTRA-OX compared to wild-type and *ntra-ko* plants, and moreover, overexpression of *NTRA* caused induction of *CuZnSOD* and *APX1* transcripts.[25] It suggests that NTRA regulates cellular ROS levels via activated Trx systems in plant cells and protects the plants against stress.

Functional roles of mitochondrial TrxRs are well characterized under oxidative stress in yeast and human.[26,27] However, mitochondrial NTRB has been known only disulfide reductase activity for Trx proteins,[10] but its roles are still largely unknown in stress responses due to same reason such as NTRA. Thus, overexpression or dominant mutant analysis needs to be followed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by grants from Next-Generation BioGreen21 Program (SSAC, grant: PJ009615 and PJ009088), Rural Development Administration, Republic of Korea. J.-Y.C. were supported by a scholarship from the BK21Plus Program, the Ministry of Education, Korea.

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