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Oxidation Pathways and Plant Development: Crosstalk between Thioredoxin and Glutaredoxin Pathways

Intracellular redox status is recognized as a critical parameter determining cell fate and cellular responses in eukaryotes (Foyer and Noctor, 2005; Fujino et al., 2006). Thioredoxins (TRXs) and glutaredoxins (GRXs) are small ubiquitous redox proteins involved in the regulation of numerous target proteins via thiol/disulfide exchanges and therefore play key roles in the maintenance of cellular redox homeostasis through the sensing and transfer of reducing equivalents. It is increasingly recognized that TRX plays an important role as a signaling intermediate that senses the redox state and transmits this information to other signaling molecules in chloroplasts, mitochondria, and the cytosol (Fujino et al., 2006).

In contrast with animal systems, plants contain a large family of TRX and TRX-like proteins, which are divided into a number of subgroups (Hisabori et al., 2007). Redox signaling within the chloroplast has long been recognized as a key component of photosynthesis, and chloroplast TRXs are known to regulate numerous photosynthetic enzymes, including glyceraldehyde 3-phosphate dehydrogenase, fructose 1,6-bis phosphatase, sedoheptulose 1,7-bis phosphatase, phosphoribulokinase, and Rubisco activase (reviewed in Hisabori et al., 2007). In *Arabidopsis*, ~40 genes encoding TRX and TRX-related proteins have been identified. Although the chloroplastic TRX system has been extensively studied in plants, genetic approaches aimed at identifying functions of cytosolic TRXs have been limited by the absence of phenotypes in single mutants, presumably resulting from functional redundancies among TRX family members or with GRX members (Schürmann and Jacquot, 2000).

An alternative approach is to focus on TRX reductases. Typically, chloroplastic TRXs are reduced in the light by ferredoxin-dependent heterodimeric thioredoxin reductases (FTR) (Dai et al., 2000). The main reductors of cytosolic and mitochondrial TRX in *Arabidopsis* are NADPH-dependent thioredoxin reductases (NTR) encoded by *NTRA* and *NTRB* (Reichheld et al., 2005), both of which encode cytosolic and mitochondrial isoforms. Interestingly, expression analysis suggested that NTRA represents the major cytosolic isoform, and NTRB is the major mitochondrial isoform, indicating that these two recently duplicated genes may be evolving toward specific functions (Reichheld et al., 2005). Historically, it has been thought that there are a limited number of TRX targets. However, in recent years, the list of TRX targets has been growing and now includes a large number of thiol-regulated proteins (reviewed in Meyer et al., 2005). In addition, emerging evidence suggests complex interplay between TRX and GRX systems in plants (Michelet et al., 2006). For example, most of the 94 putative plant GRX targets identified in a study by Rouhier et al. (2005) had previously been identified as possible TRX targets.

In this issue of *The Plant Cell*, **Reichheld et al. (pages 1851–1865)** characterize T-DNA insertion mutants of *NTRA* and *NTRB* in *Arabidopsis* and show that they play important roles in several plant development programs, including pollen fitness, seed development, and cell proliferation. In addition, investigation of the glutathione status in the mutants and construction of a triple mutant harboring homozygous *ntra ntrb* mutations together with *rootmeristemless1* (*rml1*), which is distrupted in glutathione biosynthesis, provided genetic evidence of crosstalk between the TRX and glutathione pathways in setting up postembryonic meristematic activities.

Previous work by Reichheld et al. (2005) showed that *NTRA* and *NTRB* likely have redundant or overlapping function because single loss-of-function mutants of either gene do not exhibit noticeable phenotypic differences from the wild type. In the current study, the authors confirm that both genes are expressed in all organs examined, and they show similar expression profiles. Expression of promoter:β-glucuronidase gene fusions showed that the genes exhibit only minor differences in expression. The authors therefore isolated homozygous *ntra ntrb* mutants by crossing homozygous *ntra* and *ntrb* plants.

The *ntra ntrb* double mutant plants were found to have slower growth and delayed bolting compare with the wild type (see figure). Although mutant plants were fully fertile, they had reduced pollen fitness and modified seed shape. Interestingly, double mutant seedlings were not hypersensitive to several abiotic and biotic oxidative stress conditions, including growth in medium containing reagents known to induce oxidative stress, high light, and infection with virulent and avirulent stains of *Pseudomonas syringae*. This is in marked contrast with mammalian systems. In mouse, disruption of the unique gene encoding the cytosolic TRX leads to early embryonic lethality (Matsui et al., 1996), and disruption of the gene encoding the mitochondrial NTR impairs embryo development due to severe retardation of growth and/or cardiac development (Jakupoglu et al., 2005). These data suggested that NTR activity in plants might be redundant with other redox pathways, for example, those involving ascorbate or glutathione.

To assess the role of ascorbate, the authors used the ascorbate biosynthesis inhibitor lycorine to alter the pool of ascorbate. Both wild-type and *ntra ntrb* double mutant plants showed similar growth kinetics when treated with different concentrations of lycorine, suggesting that ascorbate does not compensate for the inactivation of NTRs. On the other hand, glutathione was found to play a major role in compensating for NTR inactivation in the double mutants.

The role of glutathione was assessed by growing the plants on media containing L-buthionine-(*S*,*R*)-sulfoximine (BSO), which is a nontoxic and highly specific inhibitor of

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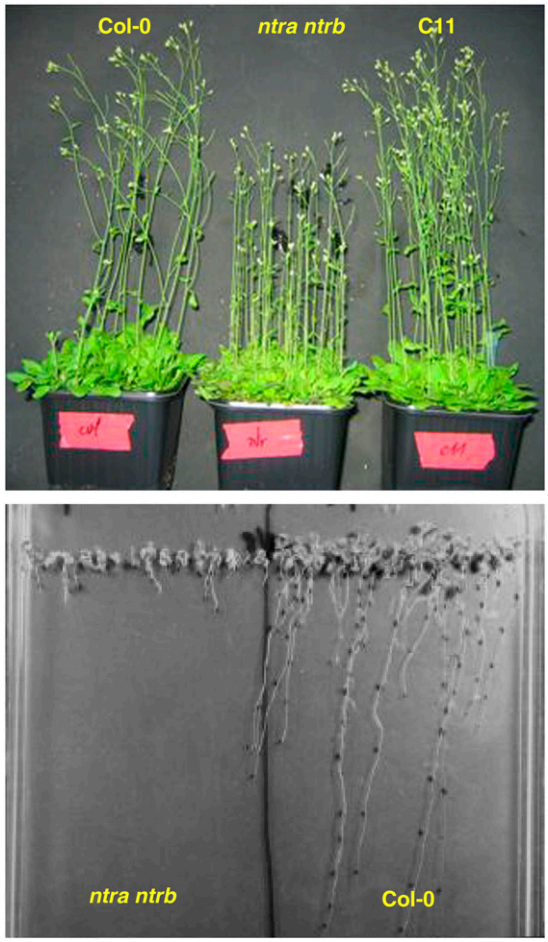

Phenotype of the *ntra ntrb* Double Mutant.

The top panel shows delayed bolting and slower growth of the double mutant, which was complemented by expression of *NTRB* in the double mutant background (C11). The bottom panel shows hypersensitivity of the *ntra ntrb* mutant to treatment with the glutathione biosynthesis inhibitor BSO. Figure reproduced from Reichheld et al. (2007).

the first enzyme of GSH biosynthesis. Since depletion of glutathione has been shown to affect the root meristem, the authors compared root growth of wild-type and *ntr* mutants subjected to a low concentration of BSO treatment. Whereas wild-type and single *ntra* or *ntrb* mutant plants were largely unaffected by this BSO treatment, BSO drastically inhibited root growth of the double mutant (see figure). Assessment of glutathione status showed that levels of reduced (GSH) and oxidized (GSSG) glutathione were both slightly lower in the double mutant compared with wild-type plants, and most glutathione was present in the reduced form in both. The authors concluded that the inactivation of the NTRs did not strongly influence glutathione pools or glutathione status, although the glutathione pathway nonetheless plays an important role in compensating for reduced NTR activity in the double mutant.

Next, Reichheld et al. sought to confirm the role of glutathione in the *ntra ntrb* double mutant by creating a triple mutant of *ntra ntrb* with *rml1*, which harbors a mutation in the first enzyme of glutathione biosynthesis, resulting in ~97% reduction of extractible GSH. The *rml1* mutant lacks an active postembryonic meristem in the root apex due to abolished cell division in the root, but the shoot meristem is unaffected (Vernoux et al., 2000). Surprisingly, the *ntra ntrb rml1* triple mutant plants did not show any meristematic activity in the root or shoot, leading the authors to conclude that either NTR or glutathione is required to activate shoot postembryonic meristematic activities. Furthermore, meristematic activities of most *ntra ntrb rml1* plants were rescued when plants were transferred to medium containing GSH, indicating that the triple *ntra ntrb rml* mutation does not alter the potential of the meristematic cells. The germination rate of the *ntra ntrb rml1* seeds was also perturbed, an effect that was partially rescued by treatment with GSH, indicating that NTS and glutathione pathways are involved in regulating germination.

Since the primary role of NTR is reduction of TRX, Reichheld et al. next sought to determine whether glutathione or GRX functions in maintaining the redox state of TRXs by examining the status of TRXh3, the most abundant cytosolic TRX in *Arabidopsis.* The redox state of TRXh3 was assessed by immunoblot analysis of proteins separated by nonreducing SDS-PAGE gel chromatography, whereupon the reduced form resolves as a larger band than the oxidized form. The data suggested that the glutathione pathway is involved in the alternative reduction of TRXh3 in mutant plants with reduced NTR activity and that plants respond to perturbation of the GSH pool (i.e., after treatment with BSO) by increasing the pool of TRXh3. In vitro biochemical experiments suggested that TRXh3 cannot be reduced directly by GSH, but rather, GTR is responsible for TRHh3 reduction. The role of GSH is therefore likely an indirect effect on maintaining the redox state of GRX.

The work of Reichheld et al. represents a significant contribution to our understanding of the TRX system in plants. Double *ntra ntrb* mutants with no NTR function, although slow growing, are viable and fertile and are not hypersensitive to a range of oxidative stress conditions. This indicates that, in contrast with mammalian systems, neither cytosolic nor mitochondrial NTRs are essential in plants, likely due to an

alternative pathway for TRX reduction. Further experiments suggested that the glutathione pathway compensates for reduced NTR activity in the mutant plants, demonstrating crosstalk between the TRX and GRX pathways. The results also implicate the TRX pathway in the proper functioning of the meristems and indicate that at least one of the NTRs and/or the GRX pathway is required for normal root and shoot growth.

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