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Review

Glutaredoxins in plant development, abiotic stress response, and iron homeostasis: From model organisms to crops

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ABSTRACT

Plant growth, development, and response to environmental stress require the judicious balance of reactive oxygen species (ROS). Glutaredoxins (GRXs) are a group of oxidoreductases that participate in the control of ROS and are traditionally defined as redox regulators. New studies suggest the members of the GRX family may be involved in more biological processes than previously ascribed. While the core structure of GRX proteins are similar, localization and expression differences afford a multiplicity of functions between species and individual isoforms. Emerging evidence indicates that various plant monothiol GRXs perform diverse functions, including transcriptional regulation of defense responses, flower development, oxidative stress response, redox signaling, hormonal regulation, iron homeostasis, and environmental adaptation. This review highlights the recent progress in our understanding of the roles played by class II CGFS-type and class III CC-type GRXs in plant development, abiotic stress adaptation, iron homeostasis, and crop productivity. In particular, the abiotic stress tolerance functions of class II GRXs make them attractive targets for genetic engineering, potentially providing enhancements in salt, drought, heavy metal, and temperature stress responses.

1. Introduction

Reactive oxygen species (ROS) are formed as by-products in all oxygenic organisms during aerobic metabolism (Halliwell, 2006). In angiosperms, chloroplasts/plastids and mitochondria contribute to production of ROS during photosynthesis and carbon metabolism (Apel and Hirt, 2004). Glutaredoxins (GRXs) are disulfide oxidoreductases (thioltransferase) that catalyze reversible reduction of disulfide bonds of substrate proteins by using the reducing power of glutathione (GSH) and function in scavenging cellular ROS and regulating redox homeostasis within these organelles (Rouhier et al., 2004) (Fig. 1**A**). Since the first cloning of plant *GRX* genes in the mid-1990s, *GRX* genes have been partially characterized in angiosperms, including *Arabidopsis thaliana* (50 genes), *Populus trichocarpa* (36) and *Oryza sativa* (27) (Belin et al., 2015; Garg et al., 2010). The GRXs can be subdivided into four groups based on the active site motifs (Couturier et al., 2009). GRXs of class I and class II have CxxC/S and CGFS active site motifs, respectively, and exist in all photosynthetic organisms. GRXs of class III are specific to angiosperms and have a peculiar CCxx active site motif, which is a diversified active site. Class IV GRXs harbor an N-terminal GRX domain with a CxDC/S active site motif in angiosperms and a CPxC active site motif in green algae, which are fused to two domains of unknown functions in the C-terminal (Couturier et al., 2009). As a group, GRXs play versatile roles in plant development, abiotic stress adaptation, and iron homeostasis (Wu et al., 2012; Li, 2014; Hu et al., 2015).

Among various monothiol GRXs, recent studies showcase the breadth of physiological and cellular functions that GRXs of class II and III possess beyond ROS homeostasis, and new insights are being gleamed from both yeast and *Arabidopsis* studies (Hu et al., 2015; Knuesting et al., 2015; Liu et al., 2013; Moseler et al., 2015; Nagels Durand et al., 2016; Stroher et al., 2016; Wu et al., 2012). Further, genome mining has identified a wide diversity of GRXs while high-resolution protein structural characterization has also begun (Abdalla et al., 2016; Garg et al., 2010; Li et al., 2010; Wang et al., 2014). This review will focus on the function of monothiol class II CGFS-type and class III CC-type GRXs in plant development, iron homeostasis, and abiotic stress responses and the potential of GRX manipulation for genetic improvement of crops (Table 1).

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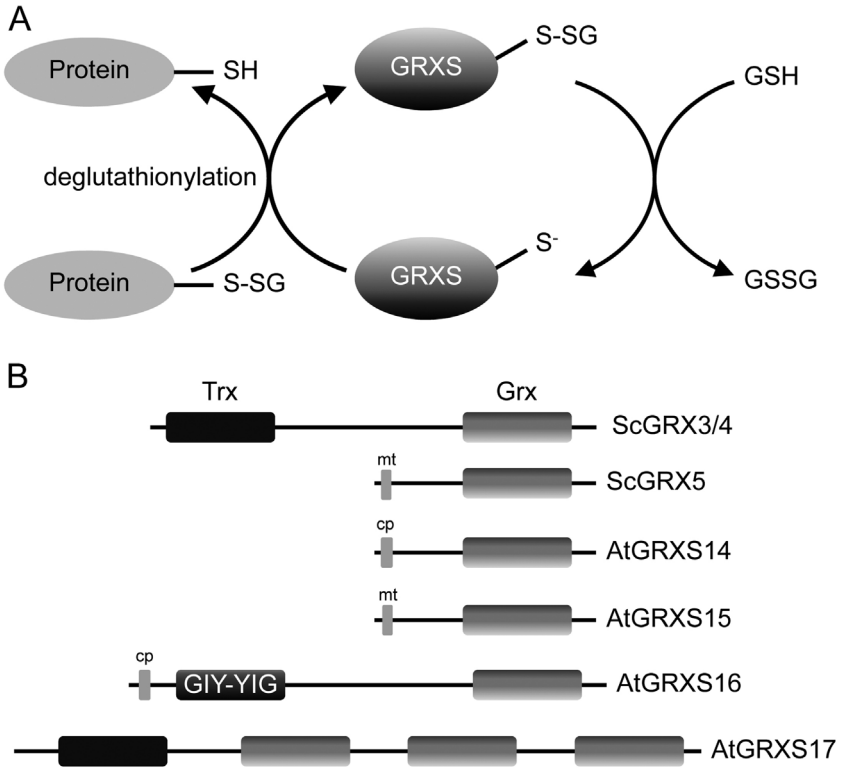

Fig. 1. Overview of plant CGFS-type monothiol glutaredoxins (GRXS). **(A)** The components of monothiol glutaredoxin system for dithiol bond reduction. **(B)** Domain organization of monothiol glutaredoxins in yeast and plants. Trx: thioredoxin module; Grx: glutaredoxin module; GIY-YIG: GlyIleTyr-TyrIleGly endonuclease motif; cp, mt: chloroplast and mitochondria signal peptide, respectively.

2. Heterologous expression in yeast and structural studies now guide plant monothiol GRXs research

GRXs of class II are conserved in all photosynthetic organisms and only reduce the mixed disulfide bond between GSH and target proteins (Lemaire, 2004). GRXs of class II have CGFS active sites and are homologous to *Escherichia coli* GRX4 and yeast *Saccharomyces cerevisiae* GRX3, GRX4 and GRX5 (Herrero and De La Torre-Ruiz, 2007). The four *Arabidopsis* class II GRXs include GRXS14, GRXS15 and GRXS16, which are low molecular weight proteins with one GRX domain, and GRXS17, which is larger with three GRX domains and an N-terminal thioredoxin (TRX)-like homology domain (Fig. 1**B**). Many studies have examined class II GRX biochemical and physiological properties, often using yeast heterologous expression and T-DNA insertion mutagenesis (Cheng et al., 2011; Sessions et al., 2002; Wu et al., 2012).

Initial characterizations of monothiol GRXs were achieved in yeast (Rodriguez-Manzaneque et al., 1999). Two subclasses of monothiol GRXs exist, those with a single GRX domain and those with a TRX-like region followed by one or more GRX domains (Herrero and De La Torre-Ruiz, 2007). The yeast monothiol ScGRX5 is located at the mitochondrial matrix and is used for iron-sulfur (Fe-S) cluster biogenesis. Yeast *grx5* mutants display loss of iron/sulfur enzyme activities and sensitivity to oxidative stress (Rodriguez-Manzaneque et al., 2002). ScGRX5 contains a single GRX domain, while two other yeast GRXs, ScGRX3 and ScGRX4, contain a TRX-like domain fused to the GRX domain (Fig. 1**B**). ScGRX3 functions in the mitochondria and nucleus, and the TRX-like domain is required for nuclear localization. Mitochondrial forms of ScGRX3 and ScGRX4 partially rescue the defects of a *grx5* null mutant. Both the TRX-like and GRX domains are needed for the mitochondrial activity of ScGRX3.

AtGRXS14 is a chloroplast/plastid-localized GRX (Cheng et al., 2006). *AtGRXS14* was initially isolated from *Arabidopsis* cDNA library in a yeast interaction assay (Cheng et al., 2003). In yeast functional assays, AtGRXS14 localizes to the mitochondria and suppresses the sensitivity of yeast *grx5* cells to H_2O_2 and protein oxidation (Cheng et al., 2006). Furthermore, AtGRXS14 can suppress iron accumulation

Table 1
Plant GRXs function in abiotic stress tolerance and iron homeostasis.

Gene	Group	Functions	Organisms used	References
AtGRXS14	II	Iron homeostasis	*in vitro*, yeast, structure, *Arabidopsis* protoplasts	Bandyopadhyay et al. (2008), Wang et al. (2014)
AtGRXS15	II	Arsenic tolerance, iron homeostasis	*in vitro, Arabidopsis*	Cheng, (2008), Moseler et al. (2015), Stroher et al. (2016)
AtGRXS16	II	Iron homeostasis	*in vitro*, yeast, *Arabidopsis* protoplast, structural study	Bandyopadhyay et al. (2008), Liu et al. (2013)
AtGRXS17	II	Heat and chilling tolerance, iron homeostasis	Yeast, *Arabidopsis*, tomato	Cheng et al. (2011), Hu et al., (2015), Inigo et al. (2016), Knuesting et al. (2015), Nagels Durand et al. (2016), Wu et al. (2012)
PvGRX5	II	Heat and arsenic tolerance	*E.Coli, Arabidopsis*	Sundaram and Rathinasabapathi, (2010); Sundaram et al. (2008), Sundaram et al. (2009)
SlGRXI	II	Salt and drought tolerance	*Arabidopsis*, tomato	Guo et al. (2010)
AtGRXS13	III	Photooxidative stress tolerance	*Arabidopsis*	Laporte et al. (2012)
OsGRX8	III	Salt and osmotic tolerance	*Arabidopsis*	Sharma et al. (2013)

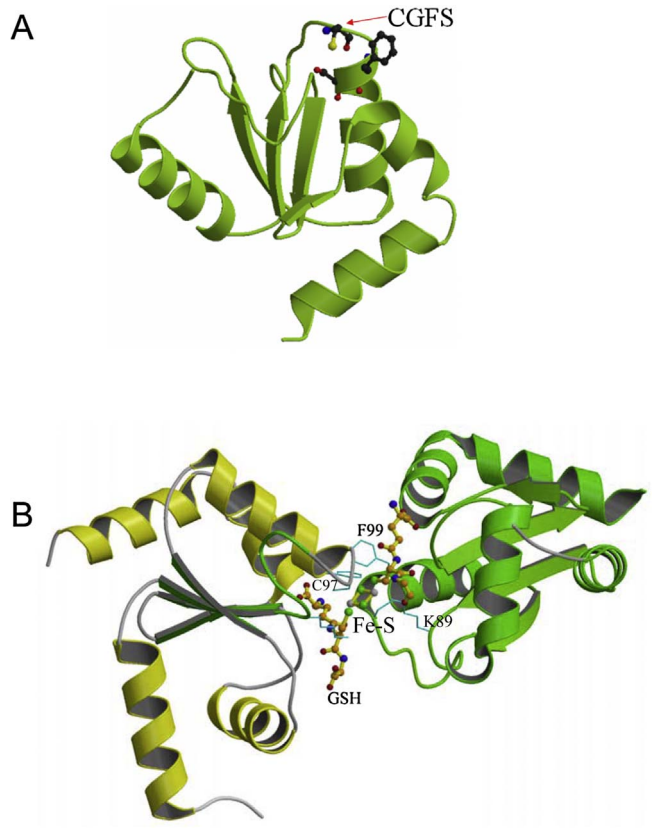

Fig. 2. Structures of plant CGFS-type monothiol glutaredoxins. **(A)** Overall structure of *Arabidopsis* AtGRXS14. The CGFS domain is indicated by red arrow. **(B)** Model of AtGRXS14-[2Fe-2S] dimer joined by 2 GSH molecules. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and partially rescue the lysine auxotrophy of yeast *grx5* cells. The crystal structure of AtGRXS14 has been determined at 2.4 Å resolution (Li et al., 2010). AtGRXS14 has a GRX/TRX-like fold with distinct structural elements that differ from those of dithiol GRXs. The structure reveals that the putative active-site motif CGFS is located on the molecular surface and that a groove ranges to both sides of the catalytic cysteine (Fig. 2**A**). Molecular modeling indicates that this active motif can bind an iron-sulfur cluster ligated by 2 GSH molecules (Fig. 2**B**). Further comparative studies suggest that a loop with five additional residues near the active-site motif may be a structural feature of monothiol GRXs (Izquierdo et al., 2008; Li et al., 2010). AtGRXS15 also has high similarity to yeast ScGRX5 and is localized to mitochondria (Moseler et al., 2015). In yeast expression assays, AtGRXS15 suppresses the sensitivity of *grx5* cells to oxidants. AtGRXS15 also reduces iron accumulation and the lysine auxotrophy (Cheng, 2008).

Like AtGRXS14 and AtGRXS15, AtGRXS16 contains a conserved C-terminal GRX domain (CTD) (Herrero and De La Torre-Ruiz, 2007). However, in yeast assays, the protein does not suppress sensitivity to oxidative stress in *grx5* cells. This inability is not based on the lack of localization, as the fusion protein is found predominately in mitochondria. AtGRXS16 is composed of two fused domains that are components of algal and plant proteins (Meheust et al., 2016). The N-terminal GIY–YIG endonuclease fold of cyanobacterial origin and a C-terminal CGFS-type monothiol GRXS of bacterial origin are negatively regulated by the formation of an intramolecular disulfide bond (Liu et al., 2013). If the negative regulatory elements are removed, yeast cells expressing the plant variant can grow on the non-permissive conditions (Liu et al., 2013). AtGRXS17 has multiple GRX domains compared with the yeast homologs. Expression of *AtGRXS17* in yeast *grx3gxr4* mutant cells can suppress their sensitivities to oxidative stress, suggesting that these GRXs share some common pathways and/or functions (Cheng et al., 2011).

3. GRXs function in abiotic stress acclimation

The heterologous expression in yeast and structural studies imply that plant monothiol GRXs play an important role in redox regulation within the plant chloroplast/plastid, mitochondria, cytoplasm and nuclei. These observations have helped guide genetic and functional studies. A common characteristic of plant responses to abiotic stresses is the production of ROS, which alters cellular redox homeostasis and produces oxidative stress (Li, 2014). While the ROS serve as important signaling molecules for initiating abiotic stress responses, over-accumulation of ROS in plant cells causes deleterious effects, such as inducing oxidative damage to lipids, proteins, and DNA (Apel and Hirt, 2004). The effects of ROS require meticulous temporal and spatial regulation in order to achieve biological responses while mitigating ROS damage to the plant (Bailey-Serres and Mittler, 2006). Plants have also evolved a versatile ROS scavenging system, which works together with ROS production regulation to tightly control ROS levels. Among them, GRXs are important players in fine-tuning the ROS levels in a variety of stress responses and participate in dynamic biological processes related to abiotic stress adaptation in plants (Hu et al., 2015; Kapoor et al., 2015; Li, 2014; Rouhier et al., 2008; Wu et al., 2012).

3.1. Roles of GRXs in plants exposed to extreme temperatures

Yeast studies suggest that monothiol CGFS-type GRXs may confer tolerance to temperature stress. The yeast *grx3grx4* double knockout mutants are more sensitive to heat shock, and introducing *AtGRXS17* into the *grx3grx4* strain improves the survival rates of heat-treated cells (Wu et al., 2012). In *Arabidopsis*, the transcription of *AtGRXS17* is induced by elevated temperatures, and knocking out *AtGRXS17* by T-DNA insertion causes hypersensitivity to heat stress (Hu et al., 2015; Wu et al., 2012). The expression of *AtGRXS17* is also regulated at the post-translational level. AtGRXS17 is a substrate of the E3 ubiquitin ligases RING DOMAIN LIGASE 3 (RGLG3) and RGLG4, and it is ubiquitinated and degraded in an RGLG3- and RGLG4-dependent manner, indicating the AtGRXS17 accumulation might need to be tightly regulated in plants (Nagels Durand et al., 2016). Ectopic expression of *AtGRXS17* in tomato plant modulates ROS accumulation and confers tolerance to heat stress without causing yield penalty (Fig. 3**A, B)** (Hu et al., 2015; Wu et al., 2012). Furthermore, a recent study has demonstrated that *AtGRXS17*-expressing tomato plants show enhanced tolerance to chilling stress, suggesting a general means of modulating both heat and chilling stresses in a variety of crops (Hu et al., 2015). The subcellular localization of *AtGRXS17* during temperature stress has been investigated. AtGRXS17:GFP fusion proteins translocate from the cytoplasm into the nucleus upon temperature stress (Wu et al., 2012). The expression level of heat shock proteins are markedly elevated in the *AtGRXS17*-expressing tomato lines, suggesting the AtGRXS17 may regulate temperature adaptation machinery via modulating certain transcription factors under stress conditions (Wu et al., 2012) (Fig. 4**)**. Similarly, AtGRXS17 in tomato, which is initially localized in the cytoplasm, also migrates into the nucleus during chilling stress and may coordinate with some transcription factors that impact gene expression to promote temperature adaptation (Hu et al., 2015; Wu et al., 2012). Moreover, expression of *AtGRXS17* enhances the activity of catalase, a target protein of Poplar GRX C1, indicating AtGRXS17 may regulate ROS accumulation via indirectly protecting ROS scavenging enzymes (Rouhier et al., 2005; Wu et al., 2012).

Individual class II GRX family members from other plant species have similar functions. For instance, *Arabidopsis* lines constitutively expressing *PvGRX5*, a fern *Pteris vittata* class II GRX with both CxxS and CGFS motifs, exhibit greater heat tolerance (Sundaram and

Fig. 3. Ectopic expression of *AtGRXS17* confers enhanced heat tolerance in tomato plants. **(A)** 30-day-old wild type and *AtGRXS17*-expressing tomato plants after being treated under 3-day at 38 °C/28 °C (day/night) followed by 8-day at 42 °C/32 °C (day/night). **(B)** Phenotypes of heat stress recovery *AtGRXS17*-expressing tomato after being transferred into normal growth condition for 2 days. The leaves of wild-type plants were severely wilted and damaged; in contrast, the leaves of *AtGRXS17*-expressing tomato plants were still green and healthy.

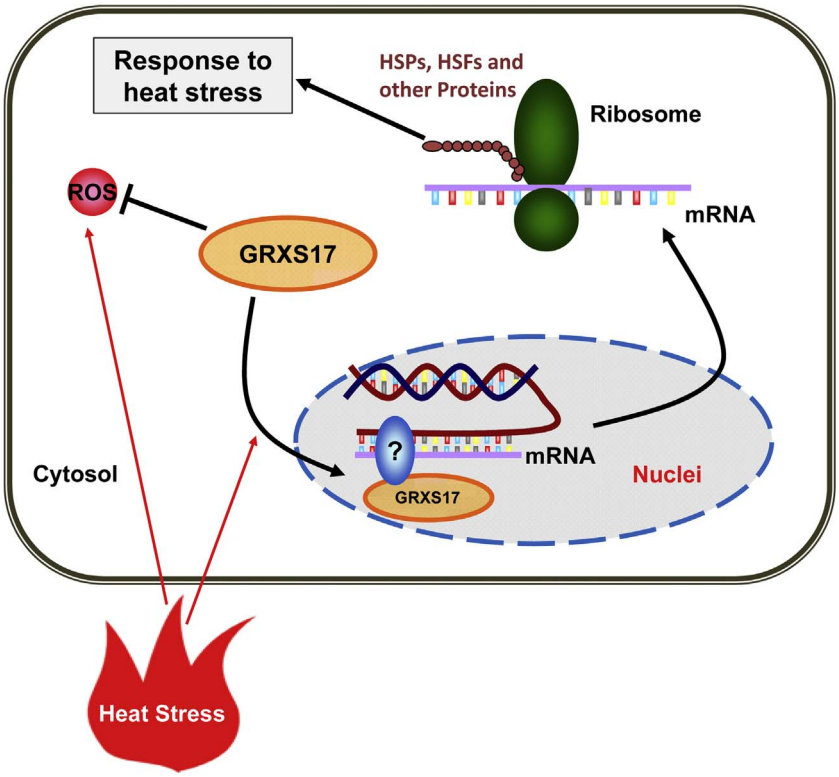

Fig. 4. A working model for the function of GRXS17 in regulating heat stress in plant cells. Heat stress triggers an increase in ROS levels, which, in turn, damage cell membranes and macromolecules. GRXS17 reduces ROS by elevation of the antioxidant network system, which is involved in ROS scavenging. Furthermore, heat stress can induce the translocation of GRXS17 into nuclei, where the protein may interact with unknown partner(s) to regulate the expression of thermotolerance genes. Candidate up-regulated genes might include heat shock transcription factors (HSFs) and heat shock proteins (HSPs), which directly mediate the damage caused by heat stress.

Rathinasabapathi, 2010). It is unknown if the GRXs from crops may have special properties that are absent in *Arabidopsis*. Therefore, it will be fascinating to study the functions of crop GRXs and to utilize genetic variability among variants to increase crop yield in suboptimal temperature conditions.

3.2. Roles of GRXs in salt, drought, photooxidative, and heavy metal stresses

Manipulation of GRXs in crops has been shown to be a promising approach to improve crop tolerance not only to heat and chilling stresses but also to other abiotic stresses. Virus-induced silencing of the class II gene *SlGRX1* in tomato leads to increased sensitivity to salt and drought stresses, whereas over-expression of *SlGRX1* in *Arabidopsis* plants improves tolerance of plants to drought and salt stresses. Both drought and salt stress response-related genes are up-regulated in *SlGRX1*-overexpressed *Arabidopsis* plants (Guo et al., 2010). A cDNA of *PvGRX5* from the arsenic hyperaccumulator fern confers arsenic resistance in bacteria (Sundaram et al., 2008). The *PvGRX5*-expressing bacteria cells have significantly lower levels of arsenite as compared with vector controls when cultured in medium containing arsenate, indicating that *PvGRX5* has a role in regulating intracellular arsenite levels (Sundaram et al., 2008). Importantly, ectopic expression of *PvGRX5* in *Arabidopsis* also reduces arsenic accumulation (Sundaram et al., 2009). Another class II GRX gene *AtGRXS15,* a gene in the same clade with *PvGRX5*, has also been shown to confer arsenic tolerance in *Arabidopsis* (Stroher et al., 2016), indicating the common functionality of this clade of GRXs in arsenic homeostasis. These findings may facilitate breeding heavy-metal excluding crops.

Intriguingly, class III GRXs, which are specific to angiosperms and have a peculiar CCxx active site, may also be important for stress tolerance. Over-expression of *OsGRX8*, a CC-type rice *GRX* gene, improves tolerance to salt and osmotic stresses in *Arabidopsis* (Sharma et al., 2013). Further, *OsGRX8* silenced rice plants exhibit increased susceptibility to multiple stresses. Again, a large number of stress-associated genes show altered expression levels in the *OsGRX8*-expressing *Arabidopsis* plants (Sharma et al., 2013). *AtGRXS13*, which codes for two CC-type GRX isoforms, also plays a role in photooxidative stress tolerance. Altered expression of *AtGRXS13* reduces tolerance to high light, whereas overexpression of the *AtGRXS13.2* variant shows increased tolerance to high light (Laporte et al., 2012), indicating that AtGRXS13 is critical for limiting photooxidative damage.

The above studies suggest that GRXs may improve abiotic stresses by either direct ROS scavenging or redox regulation of target proteins via their disulfide oxidoreductase or deglutathionylation activities. In addition, GRXs might indirectly mediate this function through interacting and regulating transcription factors that regulate gene expression (Fig. 4). However, the detailed mechanisms regarding how the GRXs modulate the expression of stress-related genes remain enigmatic. Nonetheless, the examples of using GRXs to increase stress tolerance highlight the potential of GRXs-based genetic engineering to improve stress tolerance traits in crops. Further understanding the underlying mechanisms of GRX functions in abiotic stress response through identifying the interaction partners should facilitate genetic improvement of stress adaptation traits while minimizing yield losses.

4. GRXs function in iron-sulfur cluster biogenesis and assembly

Plants have a high iron demand, especially in chloroplasts and mitochondria, to ensure proper functions of respiration and photosynthesis (Couturier et al., 2013). The involvement of GRXs in Fe-S cluster assembly and/or cellular iron homeostasis was initially described in yeast (Rodriguez-Manzaneque et al., 2002). The yeast monothiol ScGRX5 was found to be required for some mitochondrial enzyme activities and involved in Fe-S cluster biogenesis. GRXs also possess the ability to bind Fe-S clusters (Rodriguez-Manzaneque et al., 2002). In plants, the Fe-S clusters are essential for photosynthesis, metabolism and respiration as electron carriers (Balk and Schaedler, 2014). In addition, Fe-S cluster serves as a prosthetic group for many Fe-S proteins that play regulatory roles in diverse cellular processes, such as response to oxidative stresses and genome stability (Balk and Pilon, 2011). For example, mutants showing defects in the Fe-S assembly pathway can display abnormalities in the transcriptional regulation of gametophytes and abiotic stress responses in the vegetative tissues (Nakamura et al., 2013).

Yeast studies have helped elucidate the roles of *Arabidopsis* class II CGFS-type GRXs AtGRXS14, AtGRXS16, and AtGRXS17 in Fe-S cluster biogenesis and iron homeostasis (Bandyopadhyay et al., 2008; Cheng et al., 2006; Inigo et al., 2016). Furthermore, several independent groups have provided biochemical and genetic evidence to show that another CGFS-type GRX, AtGRXS15, is also essential for Fe-S cluster transfer (Bandyopadhyay et al., 2008; Cheng, 2008; Moseler et al., 2015; Stroher et al., 2016). The importance of this CGFS-type GRX is highlighted by the early embryonic lethality phenotype of mutant lines defective in this gene product. Recombinant AtGRXS15 is able to coordinate and transfer Fe-S clusters, and the process depends on reduced GSH. AtGRXS15 modeling onto the crystal structures of related non-plant proteins demonstrates amino acid residues critical for Fe-S cluster coordination. The import of these motifs was verified by plant complementation assays using AtGRXS15 variants mutagenized at these residues. For example, the mutation K83/A in AtGRXS15 causes severe developmental delays and a pronounced decrease in the activity of aconitase, a prominent Fe-S cluster containing enzyme. This work highlights the critical roles that the mitochondrial AtGRXS15 plays in the Fe-S protein maturation process and demonstrates AtGRXS15 as an essential protein in plant development.

The structural characteristics of the *Arabidopsis* chloroplastic AtGRXS16 further deepen our understanding of GRXs in Fe-S cluster assembly and maintenance (Liu et al., 2013). Similar to AtGRXS14 and AtGRXS15, AtGRXS16 consists of a conserved GRX domain at its C-terminal domain (CTD) but it also contains a putative N-terminal domain (NTD) with GIY-YIG endonuclease motif that other known GRXs lack. As mentioned previously, these functional domains are negatively regulated through the formation of an intramolecular disulfide. This intramolecular linkage exerts inhibitory effects on both endonuclease and GRX activities including Fe–S cluster binding of AtGRXS16 *in vivo* under oxidative stress. Part of AtGRXS16 exists as dimers and the NTDs are active with more efficient DNA cleavage than those of the monomeric form. Despite progress regarding the biochemical and structural characteristics of AtGRXS16, the physiological functions of AtGRXS16 have not been revealed. However, the fact that AtGRXS16-NTD has nuclease enzymatic activities in cleaving chloroplastic DNA indicates that AtGRXS16 is involved in DNA damage repair. Photosynthesis is a highly oxygenic and ROS-producing process, and this GIY–YIG motif containing endonuclease may help mediate DNA repair and promote chloroplast genome stability. Based on the capability of AtGRXS16 CTD to rescue yeast *grx5* Fe-S cluster phenotypes, recent studies propose that the monothiol GRX dimer form serves as a carrier to deliver the intact Fe–S cluster to the apoprotein or form a [2Fe–2S] cluster–ligand complex to mediate signaling events in the cell (Li and Outten, 2012). This hypothesis is consistent with emerging evidence that Fe–S clusters act as essential components of diverse nucleic acid processing machinery and their roles in DNA damage recognition. A plausible mode of action for AtGRX16, or even other GRXs such as AtGRXS15, is through regulation of Fe–S cluster assembly and/or regulation of the redox status of the target apoprotein.

Yeast ScGRX3 and ScGRX4 were first reported to be critical for iron uptake and maintenance of cellular iron homeostasis by their regulation of a transcriptional factor, *Aft1* (Herrero and De La Torre-Ruiz, 2007). ScGRX3 and ScGRX4 are also important in intracellular iron trafficking that affects Fe-S cluster biogenesis in mitochondria (Muhlenhoff et al., 2010). *Arabidopsis* AtGRXS14, 15, 16, and 17 all can bind Fe–S clusters

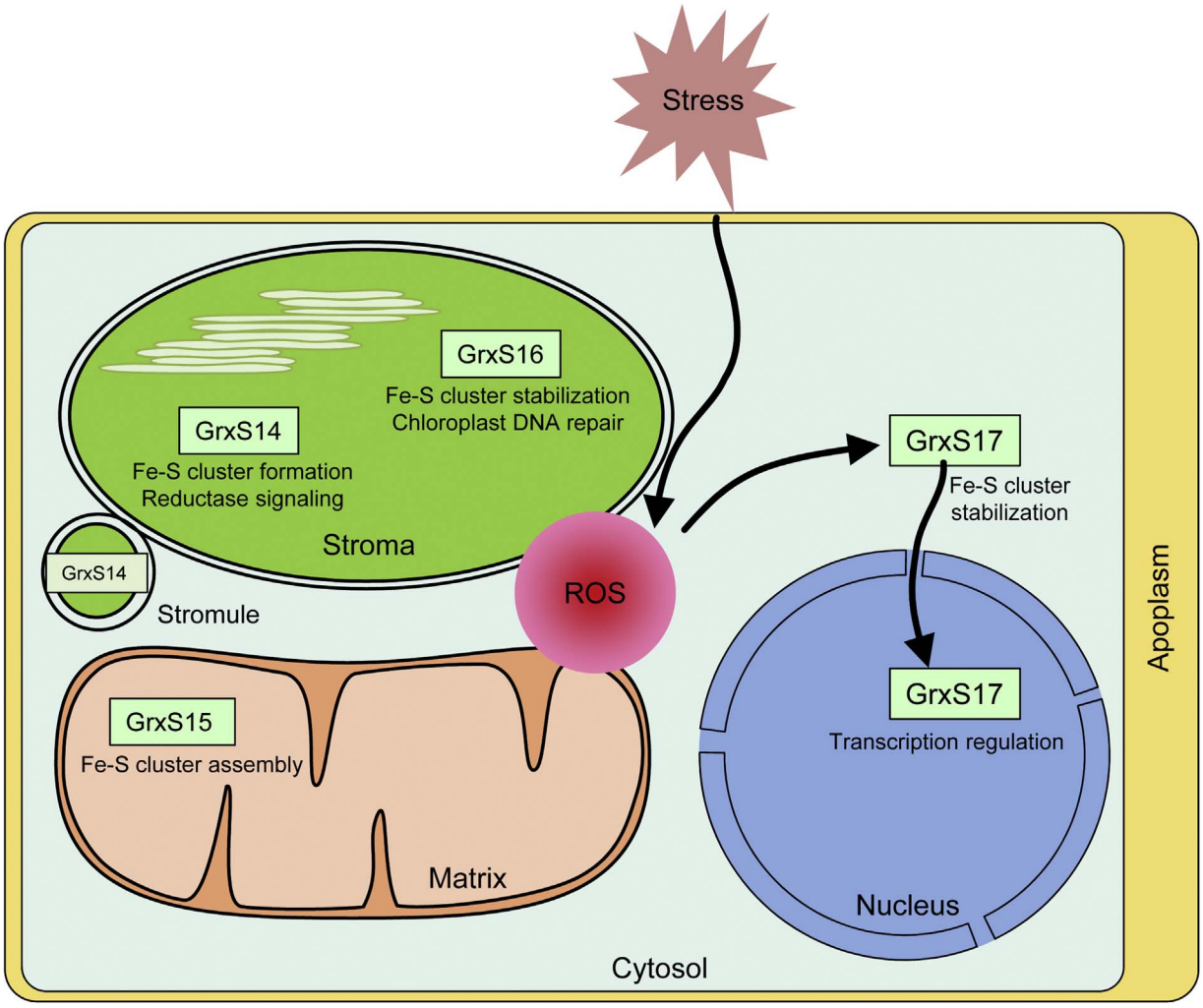

Fig. 5. Model for functions of plant monothiol glutaredoxins as stress response and Fe-S cluster regulators in various subcellular compartments.

(Fig. 5) and AtGRXS15 and AtGRXS17 have been shown to be important in Fe–S cluster assembly and/or delivery *in planta* (Inigo et al., 2016; Stroher et al., 2016). However, the physiological function of those GRXs in modulating cellular iron homeostasis is not well understood. Additional mechanistic studies are required to unravel how CGFS-type GRXs function in the Fe–S cluster assembly and iron regulatory pathways and help regulate the plethora of downstream factors that control stress responses and potentially contribute to improved crop productivity.

5. GRXs function in plant development

GRXs, along with thioredoxins (TRXs), have long been recognized to be crucial regulators of meristem development, since they control ROS status of the cells that, in turn, regulate auxin signaling and cell replicative cycles (Dietz, 2014; Eckardt, 2010). The role of GRX in plant development was first described in *Arabidopsis* upon identification of so-called *roxy1* mutants, which display reduced numbers of petal primordia and abnormalities during subsequent petal development. *ROXY1* was found to encode a class III CC-type GRX, which are specific for higher plants (Xing et al., 2005). The function of another GRX gene, *ROXY2* and the closest homolog of *ROXY1*, was found to partially overlap with *ROXY1* function. Single *roxy2* mutants do not display any obvious phenotype. However, *roxy1 roxy2* double mutants are sterile (Xing and Zachgo, 2008). Expression studies revealed that *ROXY1* and *ROXY2* also have overlapping tissue expression patterns, and the loss of *ROXY1* and *ROXY2* affects expression of a large number of anther genes (Xing and Zachgo, 2008). Further studies suggest that ROXY1 regulates flowering development through post-translational regulation of the cysteine^{340} in PERIANTHIA (PAN), a TGA transcription factor known to be involved in regulating floral organ primordium formation (Li et al., 2009). Interestingly, two other members in the TGA family, TGA9 and TGA10, also act together with ROXY1 and ROXY2 to promote anther development (Murmu et al., 2010). By dissecting the ROXY1 C-terminus, an α-helical L**LL motif immediately adjacent to the ROXY1 C-terminal eight amino acids was identified and found to be essential for the interaction with TGA transcription factors (Li et al., 2011).

The roles of ROXY in floral organ development are conserved in monocot crops. The rice orthologs, *OsROXY1* and *OsROXY2,* have similar floral expression patterns with their *Arabidopsis* counterparts, and *OsROXY1* or *OsROXY2* can rescue the floral phenotypes of *Arabidopsis roxy1* mutants (Wang et al., 2009). The close homolog of ROXY in maize, *MALE STERILE CONVERTED ANTHER1* (*MSCA1*), has been reported to play a crucial role in determining male germline fate and shoot apical meristem size (Kelliher and Walbot, 2012). Interestingly, the maize *abphyl2* mutants, caused by transposition of MASC1, switch the phyllotactic pattern from alternate to decussate (Yang et al., 2015). Similar to *Arabidopsis*, MSCA1 also binds with a TGA transcription factor known as FASCIATED EAR4 in maize and regulates meristem fate (Pautler et al., 2015).

The class II CGFS-type GRX gene *GRXS17* is also involved in plant development. Both auxin sensitivity and polar auxin transport are perturbed in the *grxs17* mutants of *Arabidopsis*, resulting in defects in cell proliferation and cell cycle control, particularly under high-

temperature conditions (Cheng et al., 2011). In addition, *grxs17* mutants exhibit smaller shoot apical meristem, altered photoperiod, and delayed bolting (Knuesting et al., 2015). A transcriptional regulator, the Nuclear Factor Y Subunit C11/Negative Cofactor 2α (NF-YC11/NC2α), has been identified as a GRXS17 interaction partner using affinity chromatography (Knuesting et al., 2015). Interestingly, the mutants deficient for NF-YC11/NC2α exhibit phenotypes similar to mutants of *grxs17* in long day conditions (Knuesting et al., 2015). These findings suggest that GRXs of class II and III play critical roles in plant development in rice and maize as well as *Arabidopsis*. Thus, manipulation of GRXs has the potential to introduce desirable traits for crops, such as altering the inflorescence architecture and flowering time.

6. Perspective

Crop plants are constantly challenged by unfavorable conditions, whether extreme temperatures, high salt, or drought, during their life cycle. Global climate change scenarios predict increased temperature and drought stress in the most regions worldwide. Improvements in each abiotic stress alone are possible by manipulating individual stress-associated traits across different species. At the same time, few genetic engineering strategies exist for tolerance to these multiple abiotic stresses. In this light, some GRX family members have been reported to have multiple functions in plants. For examples, AtGRXS17 plays roles in auxin signaling, meristem development, heat and chilling stress tolerance, PvGRX5 regulates both heat and arsenic tolerance, and AtGRXS13 functions in both pathogen and photooxidative stress responses. The overlapping involvement of the proteins may be attributable to the role played by GRX in the regulation of ROS, which participate as intermediary signals in many aspects of plant growth, development, hormone sensing, and stress tolerance. More diverse functions are emerging for GRXs, such as regulation of DNA repair and Fe-S cluster related processes. In angiosperms, chloroplasts and mitochondria are two major organelles that contribute to the production of ROS during photosynthesis and carbon metabolism. Several other features link mitochondria and plastids within the plant cell. Both organelles maintain and express genetic information, conduct electron transport functions and participate in organellar-nuclear signaling. Could the monothiol GRXs function as signaling link between subcellular stresses and plant global responses?

Further work must address monothiol GRX functions with other redox-mediated signaling pathways. Since GRX is a large protein family, the functional redundancy of GRXs remains to be explored. Interaction assays, genomic/proteomic approaches and genetic studies will be needed to confirm the role of these proteins in plant growth, iron homeostasis and stress responses. Furthermore, understanding the roles of GRXs in the nucleus, especially identifying their interaction partners such as transcription factors, will be needed. Dissecting the functions of these GRXs can inform better applications for crop improvement.

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