Arabidopsis Monothiol Glutaredoxin, AtGRXS17, Is Critical for Temperature-dependent Postembryonic Growth and Development via Modulating Auxin Response

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Global environmental temperature changes threaten innumerable plant species. Although various signaling networks regulate plant responses to temperature fluctuations, the mechanisms unifying these diverse processes are largely unknown. Here, we demonstrate that an Arabidopsis monothiol glutaredoxin, AtGRXS17 (At4g04950), plays a critical role in redox homeostasis and hormone perception to mediate temperature-dependent postembryonic growth. AtGRXS17 expression was induced by elevated temperatures. Lines altered in AtGRXS17 expression were hypersensitive to elevated temperatures and necroplastic mutants altered in the perception of the phytohormone auxin. We show that auxin sensitivity and polar auxin transport were perturbed in these mutants, whereas auxin biosynthesis was not altered. In addition, atgrxs17 plants displayed phenotypes consistent with defects in proliferation and/or cell cycle control while accumulating higher levels of reactive oxygen species and cellular membrane damage under high temperature. Together, our findings provide a nexus between reactive oxygen species homeostasis, auxin signaling, and temperature responses.

Postembryonic growth and development in plants is drastically affected by many external factors, including light and temperature (1, 2). Plants have developed elaborate measures to sense environmental changes and adapt their growth and development accordingly (3). In particular, temperature perception and heat stress responses involve many genes and signaling pathways (4–7). For example, both hormone and reactive oxygen species (ROS) are key mediators in regulating plant responses to temperature variations. However, the identity of early molecular components in this signal transduction pathway has remained enigmatic (5, 6).

Auxin is a phytohormone that is involved in most of, if not all, aspects of plant growth and development (8–11). It has been postulated that auxin plays an essential role in stress-induced growth and morphogenic responses (2). Previous studies also indicated that elevated temperature can regulate hormone biosynthesis and subsequently alter cell growth, morphology, and flowering time (12–15). The complexity of auxin signaling often obscures efforts to integrate this seemingly ubiquitous signal with specific signaling pathways.

ROS can be formed as by-products in all oxygenic organisms during aerobic metabolism (16). Plants also actively generate ROS as signals through activation of various oxidases and peroxidases in different cellular compartments in response to internal developmental cues and/or external environmental changes (17–19). There is growing evidence that there is cross-talk between the ROS-mediated redox signal (redox homeostasis) and hormonal action and response during plant development and adaptation to stress conditions, as occurs during seed germination, root hair development, stomata closure, and root gravitropic responses (20–24). Recent work suggests that redox status directly affects auxin signaling to alter growth (25). Triple mutants of Arabidopsis altered in key components of redox signaling display phenotypes consistent with perturbed auxin transport and metabolism (25). However, in that study (25), the contribution of specific ROS gene products to auxin signaling could not be determined, and no efforts were made to establish cross-talk between these pathways and the temperature response.

The thioredoxin and Grx enzyme systems help to control cellular redox potential (26). Grxs are ubiquitous small heat-stable disulfide oxidoreductases which are conserved in both prokaryotes and eukaryotes (27). Although plant genomes contain...
tain many Grxs (28, 29), only a few have been characterized (30). A recent report indicates that an Arabidopsis Grx interacts with a transcription factor to alter defense responses (31). We previously demonstrated that both AtGRXCp (also termed AtGRXS14) and AtGRX4 (also termed AtGRXS15) play a pivotal role in protecting the cell against oxidative stress (32, 33). However, the function of plant Grxs in diverse stress responses remains to be explored.

In the present study, we have characterized an Arabidopsis monothiol Grx, AtGRXS17, and describe altered expression of AtGRXS17 at an elevated temperature. Characterization of mutant phenotypes indicated alterations in ROS signaling, auxin responses, and thermo-sensitivity. These findings offer a clue to the elaborate regulatory interplay between ROS and auxin signaling in response to a heat stress.

**EXPERIMENTAL PROCEDURES**

**Isolation of AtGRXS17-null Alleles and Creation of AtGRXS17 RNAi Lines**—To isolate atgrxs17 alleles, a T-DNA insertional mutant line, was obtained from the SALK T-DNA collection (SALK_021301) (34). Homozygous plants from the T3 generation were obtained by PCR screening using an AtGRXS17 reverse primer (5′-TAG CTC GGA TAG AGT TGC TTT-3′) and a T-DNA left border primer (5′-GGC TGG ACC GCT TGC TGC A-3′) for atgrxs17 allele; an AtGRXS17 forward primer: 5′-ATG AGC GAT CCG TGG GTG AAG GAT-3′ and the AtGRXS17 reverse primer were used for identifying the wild type. The location of the T-DNA insertion was determined by sequencing the PCR product. The atgrxs17 allele was backcrossed to wild type to remove any potential unlinked mutations. To generate AtGRXS17 RNAi lines, the AtGRXS17 cDNA was cloned into the binary vector pCHF3 with opposite orien-

**Plant Growth Conditions**—Wild type (ecotype Columbia, Col-0), atgrxs17 KO, AtGRXS17 RNAi seeds were surface-sterilized, germinated, and grown on one-half strength Murashige and Skoog (MS) medium solidified with 0.8% agar or MS supplemented with various concentrations of phytohormones, as described previously (37). For quantitative RT-PCR analysis of AtGRXS17 tissue distribution, total RNA was extracted from 5-week-old Arabidopsis wild type leaves, roots, stems, and flowers. To examine AtGRXS17 expression under heat stress, seeds were germinated and grown at 22 °C for 10 days, and seedlings were moved to 28 °C for 3, 6, 24, and 48 h, respectively, whereas control seedlings were kept at 22 °C. Root tips (~3 mm from the tip) were collected and pooled from each treatment. Total RNA was extracted and quantitative RT-PCR was performed with 18 S rRNA used for normalizing the data (38). For the root gravitropism assay, surface-sterilized wild type, atgrxs17 KO, and AtGRXS17 RNAi seeds were germinated and grown on one-half strength MS medium. All plates were sealed with 3M surgical tape and cultured vertically at 22 or at 28 °C with illumination by cool white fluorescent light under a 16 h light/8 h dark cycle for 5 days. Digital images were taken before the seedlings were gravistimulated for 24 h. (The plates were turned 90° clock-

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**Ion Leakage Measurement and Anthocyanin Determination**—Four-week-old wild type and atgrxs17 KO plants were subjected to heat stress at 38 °C in a growth chamber for 10, 20, and 40 h, respectively. During heat stress, plants were maintained at a relatively high humidity (~85%) in the growth chamber to minimize water loss from the plants. For electrolyte leakage, leaf samples were incubated in 15 ml of distilled water for 10 h to measure the initial electrolyte leakage using a conductance meter (Model 32, YSI, Inc., Yellow Springs, OH). The samples were subjected to 80 °C for 2 h to release the total electrolytes and then held at room temperature for 10 h. The final conductivity of the leachate was measured to determine the percent electrolyte leakage from the leaf samples. For anthocyanin quantitation, 0.1 g of fresh weight of wild type and atgrxs17 KO seedlings, grown on one-half strength MS medium for 2 weeks, were harvested at either 22 or 28 °C, homogenized in 1.6 ml of extraction buffer (0.6 ml of methanol-1% HCl, 0.4 ml of H2O, and 0.6 ml of chloroform), and mixed well before spinning for 2 min at 16,000 × g. One ml of supernatant was used to measure the absorbance at 535 nm. The amount of anthocyanin is expressed as cyanidin-3-glucoside equivalents (mg·g of fresh weight−1) (41).
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[13C₆]IAA and 200 ng of [13C]indoleacetonitrile (IAN) as internal standards (42). After 1 h on ice, 150 μl of the homogenate was purified through two sequential solid phase extraction (SPE) columns, anion exchange and plastic affinity, using a Gilson SPE 215 system, methylated, dried, and redissolved in ethyl acetate exactly as described previously (43). The flow-through from the amino anion exchange SPE column was collected for IAN analysis. The samples were then analyzed using gas chromatography-selected ion monitoring–mass spectrometry (GC-SIM-MS) on an Agilent 6890/5973 system. The level of free IAA was quantified by isotope dilution analysis based on the [13C₆]IAA internal standard (43). For IAN analysis, the flow-through collected from the amino SPE column was passed through a C18 SPE column (100 mg; Varian) and then washed with 3 × 0.6 ml water, eluted with 3 × 0.3 ml acetonitrile, evaporated to complete dryness, and derivatized with 50 μl of bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane at 45 °C for 45 min. The samples were analyzed using GC-SIM-MS, and the correction factor for nonenzymatic conversion of IAN to IAA was determined (42). For the rest of the homogenate, 100 μl was hydrolyzed in 1 N NaOH (1 h, room temperature) for measurement of free plus ester-linked IAA, and 50 μl was hydrolyzed in 7 N NaOH (3 h, 100 °C under nitrogen gas) for measurement of total IAA. After hydrolysis, the pH of the homogenate was adjusted to 2.7 and desalted by passing through a C18 SPE column (100 mg; Varian), washed with 3 × 0.6 ml water, eluted with 3 × 0.3 ml methanol, evaporated to dryness, and redissolved in 150 μl of homogenization buffer. The purification of IAA released from the conjugates was subsequently the same as used for the purification of free IAA. The samples were analyzed using GC-SIM-MS, and the levels of free plus ester-linked IAA and total IAA were quantified with correction for IAN hydrolysis (42).

Polar Auxin Transport in Arabidopsis Hypocotyls—The hypocotyl basipetal IAA transport assay was modified from that described previously (44, 45). Wild type, atgrxs17 KO, and AtGRXS17 RNAi line seeds were surface-sterilized and plated on one-half strength MS medium. After 3 days in the dark at 4 °C and 12 h under cool white fluorescent lights (photosynthetically active radiation = 80 μmol m⁻² s⁻¹), the seeds were grown at either 22 or 28 °C continuously in darkness for 4 days and then exposed to continuous cool white fluorescent lights (photosynthetically active radiation = 80 μmol m⁻² s⁻¹) for an additional 2 days. Six mm of the hypocotyl section directly below the shoot apex was placed on an agar plate after excision, and an auxin donor agar block of 1.5% agar containing 0.2 M MES (pH 6.5) and 10⁻² M [3H]IAA was placed in contact with the apical end of the tissue section, whereas a receiver agar block containing 0.2 M MES (pH 6.5) was placed in contact with the basal end. Receiver blocks containing 0.2 M MES (pH 6.5) and 10 μM N-1-naphthylphthalamic acid (NPA) were used as the NPA control, and the orientation of the tissue section was inverted in the acropetal control. Two strips of polyethylene film (Saran™ Original, S.C. Johnson & Sons, Inc.) were placed between the agar blocks and the support agar on the plates to avoid diffusion of [3H]IAA and thus avoid an undesirable increase in background counts. The agar plates were placed vertically with donor blocks down in a chamber with maximal humidity for 4 h, and each of the hypocotyl sections was then divided into apical and basal halves. The receiver block and each half-section of the hypocotyl were incubated individually in scintillation mixture overnight, and the radioactivity was determined using a liquid scintillation counter (LS 6500, Beckman Coulter). Data were analyzed using one- and two-way ANOVA.

ROS Production and Measurement—For hydrogen peroxide staining, wild type and atgrxs17 KO seedlings were grown at 22 and 28 °C for 10 days, respectively, and vacuum-infiltrated with 1 mg/ml 3,3′-diaminobenzidine in 50 mM Tris acetate buffer, pH 5.0. Samples were incubated for 4 h at room temperature in the dark before transferring to 96% ethanol. Thirty seedlings from each genotype and treatment were scored based on the brown-colored deposition in root tips, vascular bundles, and the root-hypocotyl junction. Data were analyzed using a chi-square test.

RESULTS

AtGRXS17 Expression in Response to High Temperature—The expression of AtGRXS17 under normal growth conditions was detected in all tissues using quantitative RT-PCR (Fig. 1A). AtGRXS17 expression appeared lower in mature leaves and higher in roots and flowers (Fig. 1A). In line with this observation, histochemical analysis of AtGRXS17-GUS transgenic plants showed expression in young cotyledons (arrowhead), growing leaves (arrow), roots, anthers, and developing embryos (Fig. 1, B–E). Interestingly, AtGRXS17 expression was induced in young seedlings exposed to elevated temperature for 24 h (Fig. 1F). These results are in agreement with public microarray data sets (Genevestigator) and suggest that AtGRXS17 is a temperature-responsive gene.

atgrxs17 KO and AtGRXS17 RNAi Lines Display Growth Defects—To understand the function of AtGRXS17 in planta, we identified a T-DNA insertion line. In the atgrxs17-1 allele, the T-DNA insertion was located in the second exon (supplemental Fig. 1A). AtGRXS17 expression was not detected in atgrxs17-1 using semi-quantitative RT-PCR (supplemental Fig. 1A). We termed atgrxs17-1 as atgrxs17 KO. We then generated >60 independent RNAi lines using an AtGRXS17 antisense RNA construct (supplemental Fig. 1B). AtGRXS17 expression levels were variable among individual RNAi lines (supplemental Fig. 1B). Three RNAi lines that showed lower levels of AtGRXS17 expression were selected for further phenotypic analyses. Both atgrxs17 KO and AtGRXS17 RNAi seeds germinated in a manner indistinguishable from wild type on one-half strength MS medium under normal growth conditions (22 °C); however, KO and RNAi seedlings had shorter primary roots (~25%) than wild type controls (Fig. 2, A and C). In addition, KO and RNAi seedlings had fewer growing leaves in comparison with wild type controls (supplemental Fig. 1, C and E). In soil at 22 °C, the mutant plants grew shorter inflorescence stems but flowered and produced seeds (Fig. 2, F and G and supplemental Fig. 1, G and H). These results suggest that AtGRXS17 plays a critical role in postembryonic growth in plants.
Atgrxs17 KO and AtGRXS17 RNAi Lines Are Hypersensitive to High Temperature—Disruption of AtGRXS17 led to growth defects at a restrictive temperature (28 °C). The length of primary roots of both KO and RNAi seedlings were reduced ~70% compared with wild type controls, and the growth of both shoots and primary roots was also reduced (Fig. 2, B and C). Atgrxs17 KO and RNAi seedlings grown at 28 °C showed pin-like shoots in comparison with the normal flower buds on wild type controls (Fig. 2, D and E). In addition, KO and RNAi plants grown at 28 °C were stunted and arrested in comparison to wild type plants (Fig. 2, D and E). When grown at 25 °C, AtGRXS17 loss-of-function plants displayed severe growth defects, such as curled leaves, leafy shoots, and malformed ovule development (supplemental Fig. 2, A–L). These results indicate that AtGRXS17 loss-of-function plants are hypersensitive to temperature changes. In agreement with those observations, biochemical analysis demonstrated that KO and RNAi plants had high ion leakage and accumulated significantly higher amount of anthocyanin compared with wild type controls at 28 °C (supplemental Fig. 3). These data suggest that AtGRXS17 loss of function leads to significant damage to lipid membranes and changes in stress responses. Notably, the growth inhibition of atgrxs17 KO and RNAi plants under high temperature was reversible because the same KO plants and RNAi lines reverted to normal growth and seed production when transferred from 28 to 22 °C (supplemental Fig. 2, M–Q). These findings indicate that AtGRXS17 is required for postembryonic growth in a temperature-dependent manner.

Auxin Sensitivity of atgrxs17 KO and AtGRXS17 RNAi Lines Is Impaired under High Temperature—The morphological phenotypes of atgrxs17 KO and RNAi plants at 28 °C (Fig. 2 and supplemental Figs. 1 and 2) were similar to those observed in auxin-related mutants (46). To test whether auxin response is altered in atgrxs17, the DR5-GUS reporter line (47) was intro-
gressed into the \textit{atgrxs17} KO plants. The DR5-GUS reporter was expressed at a similar level in both \textit{atgrxs17} and wild type controls when grown at 22 °C (Fig. 3A). DR5-GUS expression was enhanced at 28 °C in wild type, particularly in the vasculature (Fig. 3B). However, DR5-GUS expression was reduced in \textit{atgrxs17} roots under an elevated temperature (Fig. 3B), indicating that auxin response was inhibited in \textit{atgrxs17} seedlings.

To examine whether the reduced DR5-GUS expression in \textit{atgrxs17} KO plants at 28 °C was due to impaired auxin sensitivity, we tested root growth inhibition by applied auxin. The primary root elongation of wild type seedlings was inhibited by auxin in a dose-dependent manner but independent of temperature treatments (Fig. 3F and G), whereas \textit{atgrxs17} KO and RNAi seedlings were resistant to exogenous auxin under high temperature. Wild type, \textit{atgrxs17} KO, and RNAi seedlings were germinated and grown for 7 days on one-half strength MS medium with or without 2,4-Dichlorophenoxyacetic acid (2,4-D) as indicated at 22 °C (F) and 28 °C (G). The primary root length was measured (n ≈ 30). Student’s \( t \) test, *, \( p < 0.01 \); **, \( p < 0.0001 \).

Two-way ANOVA, \( p < 0.001 \). B and D, the seedlings were stained for GUS expression in primary roots at 28 °C without treatment (B) or treated with 1 mM 2,4-Dichlorophenoxyacetic acid (2,4-D) for 1 h (D). GUS expression was significantly reduced in KO seedlings compared with wild type controls. Two-way ANOVA, \( p < 0.001 \). Scale bars, 50 μm. F and G, AtGRXS17 loss-of-function seedlings were resistant to exogenous auxin under high temperature. Wild type, \textit{atgrxs17} KO, and RNAi seedlings were germinated and grown for 7 days on one-half strength MS medium with or without 2,4-Dichlorophenoxyacetic acid (2,4-D) as indicated at 22 °C (F) and 28 °C (G). The primary root length was measured (n ≈ 30). Student’s \( t \) test, *, \( p < 0.01 \); **, \( p < 0.0001 \).

FIGURE 3. Disruption of AtGRXS17 altered auxin response under high temperature. A–E, wild type/DR5-GUS and \textit{atgrxs17}/DR5-GUS seeds were germinated and grown on one-half strength MS medium at 22 °C (A, C, and E) and at 28 °C (B and D) for 7 days. A, C, and E, the seedlings were stained for GUS expression in primary roots at 22 °C without treatment (A) or treated with 1 μM 2,4-Dichlorophenoxyacetic acid (2,4-D) for 1 h (C), or with 1 mM H2O2 for 2 h (E). Two representative images from each treatment were shown (n ≈ 30). No significant difference in GUS expression was seen between wild type controls and KO seedlings within each treatment, but H2O2 treatment significantly inhibited GUS expression in both with type and KO seedlings. Two-way ANOVA, \( p < 0.001 \). B and D, the seedlings were stained for GUS expression in primary roots at 28 °C without treatment (B) or treated with 1 μM 2,4-Dichlorophenoxyacetic acid (2,4-D) for 1 h (D). GUS expression was significantly reduced in KO seedlings compared with wild type controls. Two-way ANOVA, \( p < 0.001 \). Scale bars, 50 μm. F and G, AtGRXS17 loss-of-function seedlings were resistant to exogenous auxin under high temperature. Wild type, \textit{atgrxs17} KO, and RNAi seeds were germinated and grown for 7 days on one-half strength MS medium with or without 2,4-Dichlorophenoxyacetic acid (2,4-D) as indicated at 22 °C (F) and 28 °C (G). The primary root length was measured (n ≈ 30). Student’s \( t \) test, *, \( p < 0.01 \); **, \( p < 0.0001 \).
Reduced root systems (Fig. 2) are characteristic phenotypes for Arabidopsis mutants with impaired polar auxin transport (8, 48, 49). We thus performed experiments to determine whether polar auxin transport was altered in atgrxs17 KO plants. As shown in Fig. 4D, basipetal transport of auxin in the elongating hypocotyl of atgrxs17 KO and RNAi seedlings was reduced compared with wild type controls at 22 °C, but this difference was not statistically significant. At 28 °C, basipetal transport of auxin in the elongating hypocotyls of wild type seedlings increased but not of atgrxs17 KO and RNAi seedlings. The difference between wild type seedlings and AtGRXS17 loss-of-function seedlings was significant (p < 0.001). However, there was no difference in polar auxin transport activity among atgrxs17 KO and RNAi seedlings at both temperatures, suggesting that the reduction of basipetal transport of auxin in the elongating hypocotyls of atgrxs17 KO and RNAi seedlings was independent of temperature treatment. As expected, basipetal auxin transport was greatly reduced by NPA, an inhibitor of polar auxin transport, among wild type controls, atgrxs17 KO, and AtGRXS17 RNAi seedlings (Fig. 4D). The consistently low acropetal transport indicated a low level of background in the assay (Fig. 4D). Interestingly, higher temperatures promoted polar auxin transport in wild type controls, but not in either atgrxs17 KO or RNAi seedlings (Fig. 4D), indicating that temperature-dependent promotion of polar auxin transport was lost in both atgrxs17 KO and RNAi seedlings. Polar auxin transport has been frequently linked with gravitropism (48). Consistent with the findings of polar auxin transport assays (Fig. 4D), impaired gravitropism was observed in both atgrxs17 KO and RNAi seedlings compared with wild type controls at both temperatures (Fig. 5). Together, these results demonstrate that AtGRXS17 is required for NPA-sensitive polar auxin transport under restrictive temperature and the impaired polar auxin transport at least partly, if not fully, accounts for the temperature-dependent auxin-related defects observed in AtGRXS17 loss-of-function plants.

Disruption of AtGRXS17 Alters Cell Cycle Progression—

Growth defects of atgrxs17 mutants suggest a unique role of AtGRXS17 in cell proliferation and/or cell cycle control. To clarify the mechanism of AtGRXS17 in this process, a cell cycle reporter (Cyclin B1;1::GUS, a G2 phase marker) was introduced into the atgrxs17 KO plants. Cyclin B1;1::GUS expression in primary root tips, lateral roots, and shoots was similar in atgrxs17 KO plants and wild type controls at 22 °C (Fig. 6, A–F). At restrictive temperatures, reduced cyclin B1;1::GUS activity was noted in atgrxs17 KO plants, indicating that cell proliferation in the root tips of atgrxs17 KO plants were inhibited by elevated temperature (Fig. 6, G–L). We also observed that the atgrxs17 KO roots were malformed (Fig. 6, J–L), suggesting that the cells may undergo differentiation instead of cell division.
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atgrxs17 KO Seedlings Display Increased ROS Levels—Monothiol Grxs have an antioxidant function in protecting cells against oxidative stress (32, 33, 50). Thus, we hypothesized that AtGRXS17 loss-of-function plants accumulated more ROS than controls. H$_2$O$_2$ accumulation was detected by 3,3′-diaminobenzidine staining in wild type and atgrxs17 KO seedlings. The root tips and the junction areas (between the hypocotyl and the root) displayed more intense staining in AtGRXS17 loss-of-function seedlings than the corresponding area of the wild type seedlings at 28 °C (Fig. 7). Most interestingly, under high temperature, atgrxs17 KO seedlings accumulated higher amounts of H$_2$O$_2$ in vascular bundles (Fig. 7) in comparison with wild type controls. Thus, excess ROS accumulation in particular cell types and tissues could contribute to impaired auxin transport and/or inhibit postembryonic growth at elevated temperatures.

ROS Inhibits Auxin Sensitivity and Compromises Cell Cycle Progression—Given the increased ROS levels in atgrxs17 KO plants under high temperature (Fig. 7), we hypothesized that excess ROS in AtGRXS17 loss-of-function roots accounted for the inhibition of auxin response. To test this hypothesis, we treated both wild type controls and atgrxs17 KO DR5-GUS seedlings with H$_2$O$_2$ and then measured DR5-GUS expression. Indeed, exogenous H$_2$O$_2$ blocked DR5-GUS expression in wild type seedlings (Fig. 3E). The expression of the cell cycle progression marker, cyclin B1;1-GUS was also inhibited by H$_2$O$_2$ (Fig. 6, M–R). Together, these results indicate a critical role of AtGRXS17 in the mechanistic link between ROS and auxin signaling in mediating plant growth and temperature responses.

DISCUSSION

Glutaredoxins have emerged to be key regulators in stress responses and organ development in plants (33, 51, 52). In the present study, we characterized an Arabidopsis monothiol glutaredoxin, AtGRXS17, and demonstrated that AtGRXS17 is a critical component involved in ROS accumulation, auxin signaling, and temperature-dependent postembryonic growth in plants.

AtGRXS17 expression is low in comparison with two other Arabidopsis monothiol Grxs, AtGRXcp and AtGRX4 (Fig. 1, A–E) (32, 33). But still, AtGRXS17 expression appears to be regulated in different tissues and/or organs with lower levels of expression in mature leaves and higher accumulation in flowers (Fig. 1A). In contrast to AtGRXcp and AtGRX4, AtGRXS17 expression was induced by elevated temperature (Fig. 1F), suggesting a unique role in temperature stress responses. Interestingly, time course analysis indicated that AtGRXS17 expression was induced significantly when seedlings were exposed to higher temperatures for 24 h (Fig. 1F). However, we were unable to monitor more rapid responses to elevated temperatures. This finding suggests that AtGRXS17 may not be involved in the early stages of heat responses in plants but may play a role in protecting plants against the cumulative effects of high temperatures. Alternatively, AtGRXS17 induction may be due to a secondary effect, such as ROS accumulation caused by heat stress (Fig. 7). Further studies will be required to clarify factors modulating the AtGRXS17 expression and identification of downstream targets.

Arabidopsis AtGRXS17 deletion mutants do not have any visible defects in seed germination under normal growth conditions (data not shown). However, mutant plants did display...
induced in wild type roots at 28 °C compared with that at 22 °C. The DR5-GUS expression level was increased in function plants was more profound at 28 °C than that at 22 °C, making them distinct from atgrxcp and atgrx4 mutants (Fig. 2; supplemental Figs. 1 and 2). It appears that the sensitivity of atgrxs17 mutants to high temperature is contingent on both the duration and degree of temperature treatment. For example, at 22 °C, atgrxs17 mutants displayed slight (but significant) growth defects; at 25 °C, the more severe phenotypes were observed (Fig. 2; supplemental Fig. 2, A–L); at 28 °C, the growth of atgrxs17 mutants were drastically inhibited (Fig. 2), which correlated with the high accumulation of ROS detected in the growing tissues (Fig. 7). Furthermore, cell cycle progression in meristematic tissues was blocked in the atgrxs17 mutants at high temperature (Fig. 6). It is known that excess ROS can cause plant cell cycle arrest and impaired development (58, 59). Our findings support this notion that AtGRXS17 negatively modulates ROS-mediated signaling pathways and protects cells against oxidative stress caused by high temperature.

ROS can act as signals to facilitate hormonal responses involving many physiological processes (3, 22, 23). Recent work has shown H2O2 mediates auxin-regulated gravitropic response in roots (20) and high levels of ROS (as found in oxidized environments) closely correlates with the high levels of auxin required for formation and maintenance of stem cell niches in the root quiescent center (60). In this study, the developmental defects observed in AtGRXS17 loss-of-function plants were shown to be accompanied by increased accumulation of ROS (Figs. 2 and 7), which significantly compromised auxin sensitivity. This was clearly indicated by the reduced DR5-GUS expression (Fig. 3), altered polar auxin transport (Fig. 4), and impaired gravitropic responses (Fig. 5). Thus, our findings suggest that AtGRXS17 may intersect with auxin-mediated signaling to regulate cell growth and development.

The compromised auxin sensitivity in AtGRXS17 loss-of-function plants was more profound at 28 °C than that at 22 °C (Fig. 3), indicating that auxin-related phenotypes or auxin response in AtGRXS17 loss-of-function plants was temperature-dependent. Previous studies reported that high temperature can increase the levels of endogenous IAA and promotes hypocotyl elongation (13). The DR5-GUS expression level was induced in wild type roots at 28 °C compared with that at 22 °C (Fig. 3, A and B), which is consistent with previous reports. Unexpectedly, measurement of endogenous free IAA, conjugated IAA, and total IAA revealed no difference between wild type controls and AtGRXS17 loss-of-function seedlings under both temperatures (Fig. 4, A–C). We speculate this may be due to the different growth conditions (29 °C in the previous study versus 28 °C in this study) and/or plant tissues used for IAA measurement (hypocotyls versus whole seedlings). This hypocotyl-specific increase in IAA production could be masked by the IAA measurement performed on the whole seedling (61).

AtGRXS17::GUS expression was induced by high temperature (supplemental Fig. 4A) might have a role in inhibiting auxin transport in the mutants. Furthermore, auxin responses are mediated by a vast array of auxin-induced or suppressed transcripts (65). There is a possibility that auxin-regulated transcripts at high temperature are dependent on AtGRXS17 function. In support of this notion, cyclin B1:1::GUS expression, which is induced by auxin (66, 67), was inhibited in atgrxs17 KO plants under high temperatures (Fig. 6).

Recent genetic analysis of an Arabidopsis triple mutant lacking both Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent thioredoxin reductase (A and B) and glutathione biosynthesis (CAD2) genes revealed that auxin metabolism and polar auxin transport are inhibited when the redox homeostasis was altered in the mutant plants, suggesting cross-talk among redox and auxin signaling systems in controlling plant growth and development (25). Whether AtGRXS17 is involved in this regulatory interplay is still unknown. However, Grxs could be substrates of thioredoxin reductases (68). Our work here establishes a foundation to examine the role of Grxs in redox regulatory mechanisms underlying hormonal responses and adaptation to temperature stresses.

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