SCARECROW Has a SHORT-ROOT-Independent Role in Modulating the Sugar Response[1][W][OA]

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Sugar is essential for all cellular activities, but at high levels it inhibits growth and development. How plants balance the tradeoffs between the need for sugars and their growth inhibitory effects is poorly understood. SHORT-ROOT (SHR) and SCARECROW (SCR) are key regulators of stem cell renewal and radial patterning in the root of Arabidopsis (*Arabidopsis thaliana*). Recently, we identified direct targets of SHR at the genome scale. Intriguingly, among the top-ranked list, we found a number of genes that are involved in stress responses. By chromatin immunoprecipitation-polymerase chain reaction (PCR), we showed that SHR and SCR regulate a similar but not identical set of stress response genes. Consistent with this, *scr* and *shr* were found to be hypersensitive to abscisic acid (ABA). We further showed that both mutants were hypersensitive to high levels of glucose (Glc) but responded normally to high salinity and osmoticum. The endogenous levels of sucrose, Glc, and fructose were also elevated in *shr* and *scr*. Intriguingly, although *shr* had sugar content and developmental defects similar to those of *scr*, it was much less sensitive to Glc. Chromatin immunoprecipitation-PCR and reverse transcription-PCR assays as well as transgenic studies with an *ABA-INSENSITIVE2* (*ABI4*)-*β-glucuronidase* reporter construct revealed that in root, SCR, but not SHR, repressed *ABI4* and *ABI5* directly and specifically in the apical meristem. When combined with *abi4*, *scr* became much more tolerant of high Glc. Finally, transgenic plants expressing ABI4 under the control of the *SCR* promoter manifested a short-root phenotype. These results together suggest that SCR has a SHR-independent role in mitigating the sugar response and that this role of SCR is important for root growth.

In both animals and plants, sugar is critically important for all cellular activities, but at high concentrations, it becomes inhibitory to growth and development (Rolland et al., 2006). Although soluble sugar concentration may be low on average in plants, it can reach an inhibitory level in some organs or cell types, particularly when the rate of photosynthesis is high. Sugar is made in photosynthetic organs, fully expanded leaves in particular, and transported to nonphotosynthetic organs such as root, shoot apical meristem, and developing embryo. In most plant species, Suc is the form that is transported over long distances, but Glc is the sugar that cells can directly utilize. At the site of sugar production, Glc is converted to Suc, which is in turn loaded into the phloem for long-distance transport. Phloem companion cells are known to facilitate this process by concentrating Suc through an active mechanism, but the bundle sheath cells also play a role in sugar loading (Leegood, 2008). In organs that import sugar, Suc is converted to Glc by locally expressed Suc synthase or invertase (Koch, 2004). In root, for example, Suc invertase and synthase are expressed preferentially in the meristem and early elongation zone (Duke et al., 1991; Barratt et al., 2009). Consequently, sugar concentration is high in cells that are involved in uploading and downloading. To ensure optimal growth, plants must monitor and regulate the sugar level closely and suppress the harmful effects of exposure to high sugar.

How plants balance the tradeoffs between the need for sugars and their growth inhibitory effects is poorly understood, but presumably, this involves regulation of the sugar-signaling pathways. Sugar signaling is a conserved mechanism in eukaryotic organisms (Rolland et al., 2006). In yeast, sugar availability is sensed by HEXOKINASE2 (HXK2) and the signal is relayed to SUCROSE NONFERMENTATION1 (SNF1), which in turn alters the expression of sugar-responsive genes (Rolland et al., 2006). In plants, HXK1 and SNF1-RELATED PROTEIN KINASE1 (SnRK1) are the major players in sugar signaling (Moore et al., 2003), with ABA-INSENSITIVE4 (ABI4) acting downstream in the regulation of nuclear genes involved in the sugar response (Arenas-Huertero et al., 2000; Koussevitzky et al., 2007). In addition to HXK1 and HXK2, which are localized in the mitochondria and nucleus (Cho et al., 2006), plants possess a HXK in the chloroplasts that also acts as a Glc sensor (Zhang et al., 2010). Evidence suggest that other sugar-signaling pathways exist (Xiao et al., 2000), although little is known about these HXK-independent pathways.

A potential mechanism for plants to orchestrate the sugar response and development is to regulate both processes with the same factors. Several genes have been identified that appear to play such a role. PLEIOTROPIC REGULATORY LOCUS1 (PRL1), for example, is known to negatively regulate Glc signaling by interacting with SnRK1 kinase (Bhalerao et al., 1999). The *prl1* mutant is hypersensitive to high levels of Glc, but only under light (Németh et al., 1998), suggesting that PRL1 plays an important role in coordinating light and sugar signaling. STIMPY/WOX9, on the other hand, seems to be a positive coordinator between sugar signaling and plant growth, as the *stip* mutant is compromised in shoot apical meristem development but this defect can be rescued by exogenous Suc (Wu et al., 2005). Other proteins that play dual roles in sugar signaling and plant development include HYPERSENESCENCE1, which was initially found to be a player in the defense response (Aki et al., 2007); LOW-LEVEL BETA-AMYLASE1, a UPF1 RNA helicase (Yoine et al., 2006); and HIGH SUGAR RESPONSE8, which is involved in Ara biosynthesis (Li et al., 2007b).

SHORT-ROOT (SHR) and SCARECROW (SCR) are key regulators of root growth and development (Di Laurenzio et al., 1996; Helariutta et al., 2000). They are both essential for the maintenance of the stem cell niche, which is a group of pluripotent cells surrounding the quiescent center (QC) cells, with the QC acting as the organizing center (Supplemental Fig. S1). In *shr* and *scr*, the stem cell niche is depleted early, resulting in plants with substantially shorter roots than the wild type (Benfey et al., 1993). SHR and SCR also play an important role in radial patterning. In wild-type primary root, the ground tissue consists of two cell layers, the cortex and endodermis, which lie between the epidermis and the central vasculature or stele (Supplemental Fig. S1). The cortex and endodermis are derived from a common progenitor cell called the cortex/endodermis initial daughter cell (CEID) through a longitudinal asymmetric cell division (Supplemental Fig. S1). In *shr* and *scr* mutants, the asymmetric cell division does not occur, resulting in a ground tissue consisting of only a single cell layer.

Although SHR and SCR play a similar role in ground tissue patterning, they are expressed in different domains. SCR is expressed in the QC and endodermis and is required for the longitudinal asymmetric cell division in the CEID that gives rise to the cortex and endodermis lineages (Di Laurenzio et al., 1996; Cui et al., 2007; Cui and Benfey, 2009). In contrast, SHR is expressed in the stele, but the protein moves to the adjacent cell layer including the endodermis, CEID, and QC, where it physically interacts with SCR to activate a positive feedback loop for SCR transcription and a feed-forward loop for other factors that appear also to play a role in radial patterning (Helariutta et al., 2000; Cui et al., 2007). Independent of SCR, SHR activates genes that are involved in endodermis cell fate specification. A role for SHR in stele develop-

ment has also been identified (Levesque et al., 2006; Carlsbecker et al., 2010; Yu et al., 2010). SCR expression in root apical meristem is largely dependent on SHR, placing SHR upstream in the mechanism that determines radial patterning. However, there is evidence that SCR expression is also regulated by a SHR-independent mechanism. *SCR* is still expressed in *shr*, albeit at a reduced level, and its expression pattern is similar to that in the wild type (Cui et al., 2007). Thus, it appears that SHR and SCR control root development through both common and distinct mechanisms.

SHR and SCR belong to the GRAS family of transcriptional regulators (Pysh et al., 1999), and we have shown that they regulate downstream genes directly (Levesque et al., 2006; Cui et al., 2007; Sozzani et al., 2010). To better understand how they control root growth and development, recently we have determined the genome-wide locations of SHR binding sites using chromatin immunoprecipitation ChIP followed by analysis on microarrays (ChIP-chip; Sozzani et al., 2010). In further analysis of the ChIP-chip data, we uncovered a role for SHR in vascular tissue patterning through the regulation of cytokinin homeostasis (Cui et al., 2011). Intriguingly, among the top-ranked list of SHR direct targets, we noticed a number of genes that are associated with stress responses in addition to those that presumably play a role in development. We showed that both SHR and SCR directly regulate these stress response genes. This preliminary observation has led us to the findings that SCR, but not SHR, suppresses the sugar response through direct repression of ABI4 in the root apical meristem and that this role appears to be important for normal root growth.

RESULTS

SHR and SCR Control Genes Involved in Both Development and Stress Responses

Listed in Supplemental Table S1 are the 25 top-ranked SHR targets that we recently have identified by ChIP-chip (Cui et al., 2011). In addition to genes that are known to play a role in development, such as *MAGPIE* (Welch et al., 2007), *NUTCRACKER* (Welch et al., 2007), *SCARECROW-LIKE3* (Heo et al., 2011), and microRNA166B (Carlsbecker et al., 2010), we noticed that some of these genes are associated with stress responses, such as *ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR* (*ATAIB*; Li et al., 2007a), *TWO OR MORE ABRES-CONTAINING GENE2* (*TMAC2*; Huang and Wu, 2007), and *ARABIDOPSIS THALIANA HOMEOBOX12* (*ATHB12*; Olsson et al., 2004). To determine whether these genes are true SHR targets, we performed ChIP-PCR and confirmed binding by SHR (Fig. 1, A–E). We also confirmed binding by SHR to the promoters of At1g68670, a MYB domain transcription factor, and At1g24120, which appear to be involved in stress responses as
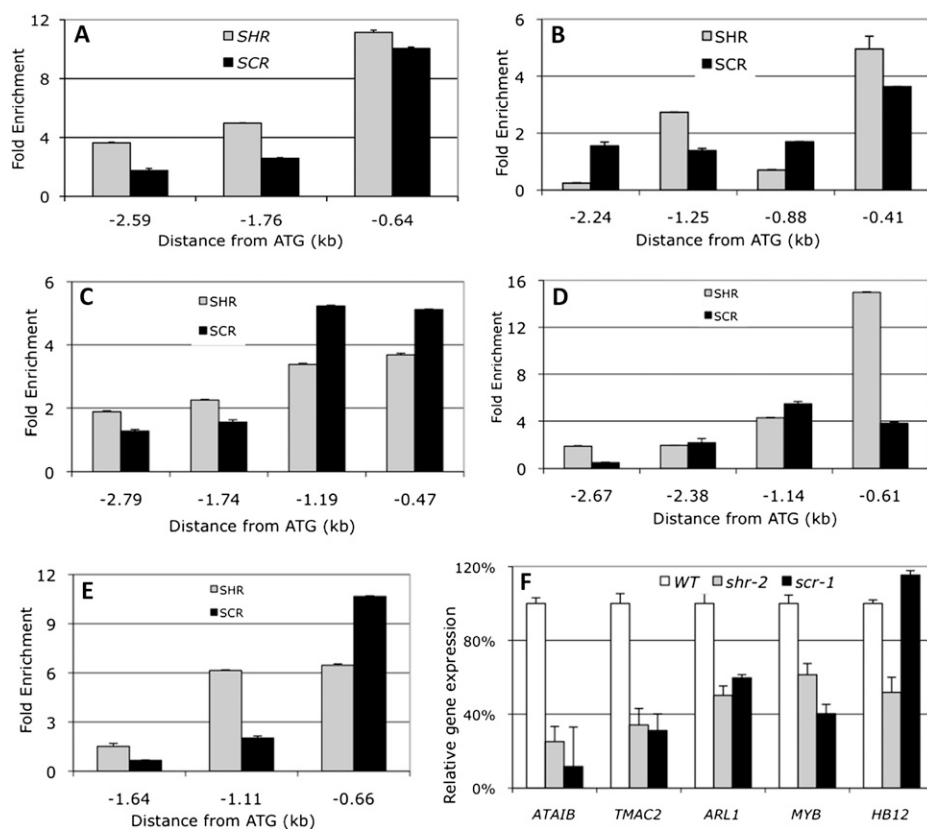
Figure 1. SHR and SCR directly control stress response genes. A to E, ChIP-PCR assay showing binding of SHR and SCR to the promoters of *ATAIB* (A), *TMAC2* (B), *ATHB12* (C), At1g68670, a *MYB* transcription factor (D), and *ARL1* (At1g24120; E). The *x* axis indicates the distance (kb) from the translation start site. F, Real-time RT-PCR assay showing the transcript level for the above genes in wild-type (WT), *shr-2*, and *scr-1* root. Error bars represent SD from technical replicates. Note the different *y* axis scales.

well on the basis of our analysis of T-DNA knockout mutants (Supplemental Fig. S2). We next compared their transcript levels in *shr* and the wild type by reverse transcription (RT)-PCR and found that all these stress-associated genes were reduced in expression in the *shr-2* mutant (Fig. 1F).

Previously, we demonstrated that SHR and SCR work as a heterodimer in radial patterning (Cui et al., 2007), so SCR probably also directly regulates these stress-associated genes. ChIP-PCR and RT-PCR assays showed that, indeed, SCR binds to the promoters of the same set of genes (Fig. 1, A–E) and that their transcript levels decreased in the *scr* mutant (Fig. 1F), except for *ATHB12*, whose expression does not seem to be altered.

SCR Plays a Role in the Sugar Response

The observation that SHR and SCR directly regulate stress-associated genes suggests that these two proteins are involved in stress responses. To test this hypothesis, we treated *shr* and *scr* mutants with the plant hormone abscisic acid (ABA), because ABA accumulates in response to various stresses, and we reasoned that exogenous application would mimic stress. The *shr-2* and *scr-4* alleles were used in this test. Although *shr-2* and *scr-4* have significantly shorter roots than the wild type, when grown on normal growth (Murashige and Skoog [MS]) medium their

shoots are of similar size during early stages of seedling development (Fig. 2A, top row). In contrast, on MS medium containing ABA, the shoots of both mutants were much smaller than when grown on MS medium alone, and root growth was severely retarded (Fig. 2A, bottom row). These results extend our recent finding that SCR mediates developmental processes and stress responses (Iyer-Pascuzzi et al., 2011) but also suggest that SHR plays a role in stress responses too.

ABA signaling is involved in numerous stress responses. To identify the specific response in which SHR and SCR are involved, we challenged *shr-2*, *scr-4*, and the wild type with a number of stresses. When grown under conditions of high salinity (NaCl) or high osmoticum (mannitol), the *shr-2* and *scr-4* mutants responded similarly to the wild type (Fig. 2, B and C). We also tested their response to Glc, as *GBF5*, a key component in the Glc-signaling pathway (Rolland et al., 2006), is also among the top-ranked putative SHR direct targets. Interestingly, we found that both mutants were hypersensitive to Glc at a concentration of 4% and above, as indicated by pigment accumulation and retarded growth, but *scr-4* was apparently much more sensitive (Fig. 2D). Because the two mutants (*scr-4* and *shr-2*) are in different ecotype backgrounds (Wassilewskija [Ws] and Columbia [Col], respectively), we next tested the response of different *scr* and *shr* alleles to Glc (*shr-2*, *shr-3*, *shr-5*, and *scr-3* are

Figure 2. *shr* and *scr* are hypersensitive to high Glc but not salt and mannitol. A, Wild-type (WT), *shr-2*, and *scr-4* seedlings on MS medium (top row) or MS medium containing 1 μM ABA (bottom row), 10 d after germination. B, The same three seedling types grown on MS medium containing 50, 100, and 150 mM NaCl, 2 weeks after germination. C, The same three seedling types grown on MS medium containing 50, 100, and 200 mM mannitol (Mann), 2 weeks after germination. D, The same three seedling types grown on MS medium (top row) or MS medium containing 4% Glc (bottom row), 10 d after germination. E and F, *shr-2* and *scr-1* as well as wild-type Col and Ws seedlings growing on MS medium with (F) or without (E) 4% Glc, 10 d after germination. The numbers represent root length in millimeters (means ± SD) from measurements of 15 seedlings. In A to D, only Col is shown as the wild type, as Col and Ws behaved similarly under the conditions tested.
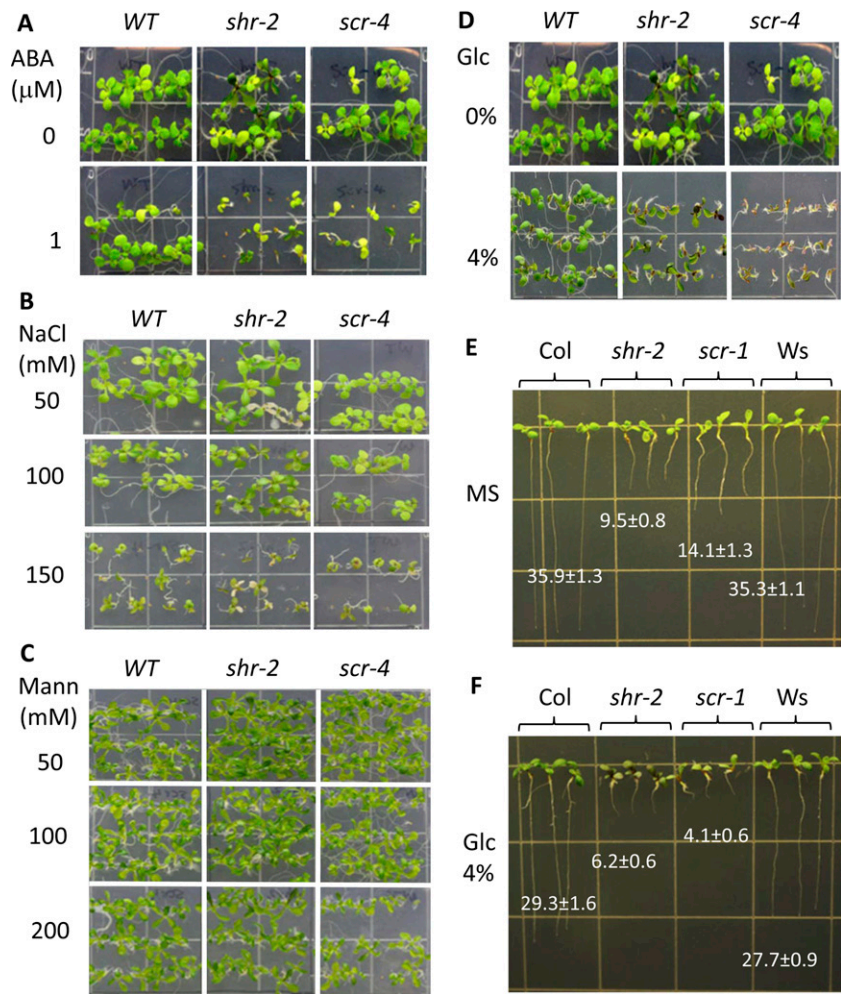
in Col; *scr-1*, *scr-2*, and *scr-4* are in Ws). All *shr* and *scr* alleles examined showed Glc hypersensitivity (Supplemental Fig. S3). Regardless of their genetic background, all *scr* alleles consistently showed a much more severe Glc hypersensitivity phenotype than the *shr* alleles: *scr* mutant plants were very small and their leaves were bleached, whereas *shr* seedlings were much larger and the leaves were largely green despite the accumulation of purple pigments. Consistent with this result, *scr* root growth was also inhibited to a greater extent than *shr* when they were grown vertically (Fig. 2, E and F). Because *shr* and *scr* have similar defects in root growth and ground tissue patterning, the sugar hypersensitivity of *scr* is unlikely to be a secondary effect from its developmental defect. These results suggest that SCR plays a major role in the sugar response.

Sugar Levels Are Elevated in *scr*

The *scr* mutant should become hypersensitive to high Glc if sugar metabolism or signaling is altered. To investigate this possibility, we measured Suc, Glc, and Fru content in *scr* and wild-type (Ws) plants. The concentrations of all three water-soluble sugars were significantly higher in both the root and shoot of *scr* than in the wild type when plants were grown on MS medium (Supplemental Table S2). An even larger difference in sugar content was apparent when the plants were grown in soil (Fig. 3A). To determine whether the increase in sugar content in *scr* results from defects in starch biosynthesis, we next compared starch content in *scr* and the wild type by Lugol staining. As shown in Figure 3B (left panel), at the end of the illumination period, starch accumulated to similar levels in *scr* and the wild type, indicating normal starch biosynthesis in *scr*. At the end of the dark period, all starch had been consumed in the wild type, but its level was still high in *scr* (Fig. 3B, right panel). These results suggest that the Glc hypersensitivity of *scr* is due to a defect in sugar homeostasis.

ABI4 Is Dramatically Up-Regulated in *scr* but Not in *shr*

Because *shr* was much less sensitive to high Glc than *scr*, we reasoned that *shr* should have lower sugar levels. Surprisingly, we found that the concentrations
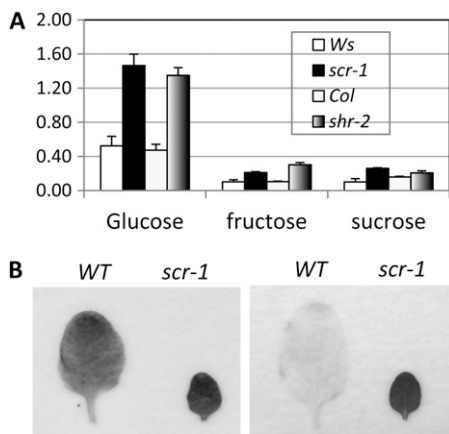
Figure 3. Sugar homeostasis is compromised in *scr* and *shr*. A, Glc, Fru, and Suc contents (mg g^{-1} fresh weight) in leaves of 10-d-old wild-type (Ws and Col), *scr-1 and shr-2* plants, measured by HPLC. B, Starch content of wild-type (WT) and *scr-1* leaves, as revealed by Lugol staining. Left, samples taken in the evening; right, samples taken in the morning.

of Suc, Glc, and Fru in *shr* were as high as those in *scr* (Fig. 3A; Supplemental Table S2). One explanation for this difference is that other stress responses were also altered in *scr* and that this would cause sugar hypersensitivity as a consequence of cross talk between different signaling pathways. An alternative explanation is that the sugar response is somehow suppressed in *shr*. To determine whether a second stress response is altered in *scr* but not in *shr*, we examined the transcript level of some key ABA signaling regulators in root, including *ABI3*, *ABI4*, and *ABI5* in *shr-2* and *scr-1*, as these *ABI* genes are involved in different stress responses (Arroyo et al., 2003). For example, *ABI3* is primarily involved in responses to cold (Tamminen et al., 2001), desiccation (Tamminen et al., 2001), and nutrient deficiency (Walker and Connolly, 2008), whereas *ABI4* plays a major role in sugar signaling and plastid-derived retrograde signaling (Arenas-Huertero et al., 2000; Koussevitzky et al., 2007). *ABI5* is required for seedling growth, but it also plays a role in sugar signaling (Finkelstein and Lynch, 2000; Lopez-Molina et al., 2001). *ABI1* and *ABI2* were also included in this comparison. As shown in Figure 4A, the *scr* mutation caused a dramatic increase in the transcript level of *ABI4* and some increase in *ABI5* but had no apparent effect on RNA levels of *ABI1* and *ABI2*. The transcript level of *ABI3* was too low to be determined by quantitative RT-PCR. In contrast, the *ABI4* transcript level in the *shr* mutant remained almost unchanged, and the *ABI5* transcript level was only moderately increased. Because SCR and SHR are also expressed in the shoot apical meristem, leaf primordium, and young leaves (Wysocka-Diller et al., 2000; Dhondt et al., 2010; Gardiner et al., 2010) and *shr* and *scr* leaves showed obvious differences in sugar hypersensitivity, we also analyzed these *ABI* genes in the shoot. A similar trend was observed for *ABI4* and *ABI5* (Supplemental Fig.

S4). *ABI3* transcript can be reliably quantified, but no difference in its level was detected between the wild type, *scr*, and *shr*. Because *ABI4* is a key regulator of Glc-signaling pathways (Rolland et al., 2006), these results suggest that an elevated level of *ABI4* in *scr* is a major cause of its sugar hypersensitivity.

SCR Modulates the Sugar Response Directly and Specifically in the Root Apical Meristem through *ABI4*

The results described above also suggest that, in contrast to the case of radial patterning, SHR and SCR do not act as a heterodimer in the regulation of the sugar response. To determine whether SCR regulates *ABI4* and *ABI5* independently of SHR, we examined SHR and SCR binding to the *ABI4* and *ABI5* promoters by ChIP-PCR. Interestingly, both the *ABI4* and *ABI5* promoters were bound by SCR but not by SHR (Fig. 4, B and C). This result demonstrated that SCR has a SHR-independent role in transcriptional regulation but also provides evidence that SCR modulates the sugar response directly.

Along the longitudinal axis of a root, three developmental stages with distinct mitotic activity and morphology can be recognized: the apical meristem, the elongation zone, and the maturation zone (Ishikawa and Evans, 1995). To determine the developmental stage when SCR is required to regulate *ABI4* and *ABI5*, we examined their transcript levels in the three zones in *scr* and wild-type roots by quantitative RT-PCR. As shown in Figure 4D, *ABI4* and *ABI5* transcripts were very low in all developmental stages but were much higher in the meristematic zone in *scr*. Although *ABI4* and *ABI5* transcript levels were also elevated in the meristem zone in *shr-2*, this was to a much lesser extent (Fig. 4D). To validate this result, we next generated transgenic plants expressing the *GUS* reporter gene under the control of a 2.6-kb sequence upstream of the *ABI4* coding region and examined its expression in the wild type, *scr*, and *shr*. As reported previously (Arroyo et al., 2003), in the wild type the *pABI4::GUS* construct was expressed in the root tip during early postembryonic growth (less than 3 d after germination), but its expression level decreased rapidly (Fig. 4E, top panel). Seven days after germination, only weak GUS staining was visible in some of the root cap cells, and by day 9, GUS activity was no longer detectable even after prolonged staining (Fig. 4E). In *scr*, however, *pABI4::GUS* was still expressed strongly in the apical meristem, with the maximum in the QC at 7 d after germination (Supplemental Fig. S5), and remained expressed in the QC at 9 d after germination (Fig. 4E, bottom row). In contrast, in *shr*, no GUS activity was visible 9 d after germination, similar to the case in the wild type. Together, these results suggest that in root, SCR suppresses the sugar response specifically in the apical meristem.

To determine whether ABI4 misexpression indeed causes sugar hypersensitivity in *scr*, we generated the *scr-4 abi4-104* double mutant and compared its

Figure 4. SCR, but not SHR, regulates *ABI4* and *ABI5* directly and specifically in the root apical meristem. A, Real-time RT-PCR assay of the transcript level of *ABI* genes in the root of *shr-2*, *scr-4*, and wild-type Col (WT). B, ChIP-PCR assay for SHR and SCR binding to the *ABI4* promoter, with primer pairs that tile the promoter sequence. C, ChIP-PCR assay of SHR and SCR binding to the *ABI5* promoter. D, RT-PCR assay of *ABI4* and *ABI5* transcripts in the root meristem, the elongation zone, and the maturation zone in wild-type and *scr-1* roots. Error bars represent SD from technical replicates. E, *pABI4::GUS* expression in the root of the wild type at different times after germination and in *scr-1* and *shr-2* at 9 d after germination. Bars = 50 μm.
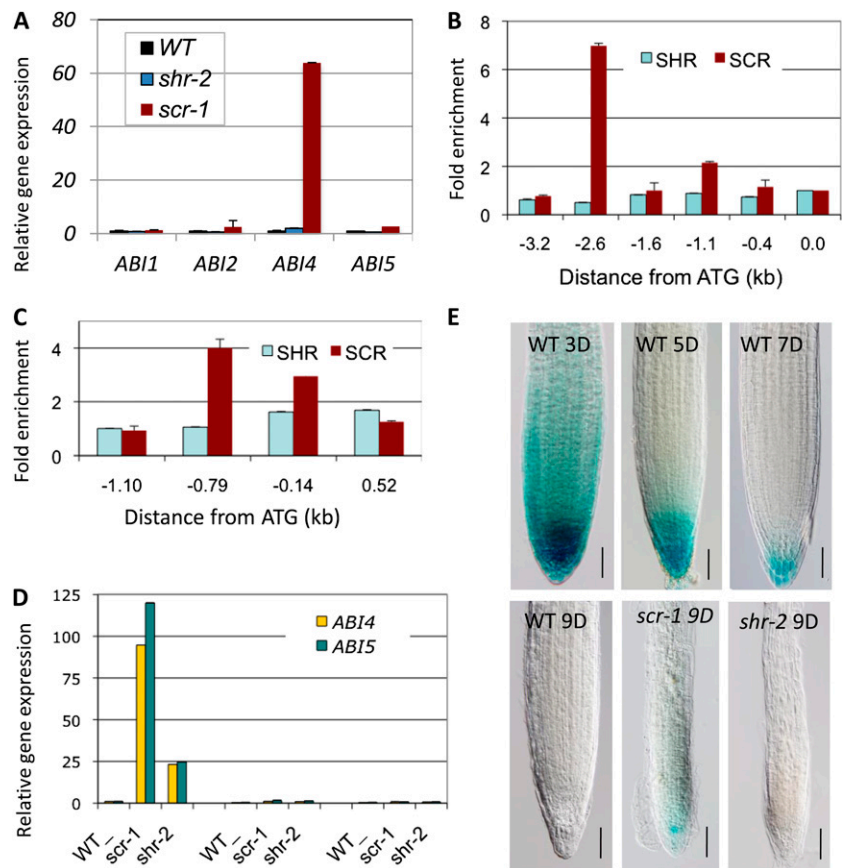
response to 4% Glc with that of the *scr-4* and *abi4-104* single mutants. Because *scr-4* and *abi4-104* are in different ecotype backgrounds (Ws and Col, respectively), to minimize ecotype-related variability, we analyzed seeds derived from the same cross but with different genotypes. As shown in Figure 5A, the *scr-4 abi4-104* double mutant was much more tolerant to high Glc than the *scr-4* single mutant, although it was still slightly more sensitive to sugar than the *abi4-104* mutant, probably because of the presence of a higher level of *ABI5*. This result supports the notion that SCR suppresses the sugar response mainly by repressing *ABI4*.

ABI4 Has a Dual Role in Root Growth

The elevated level of *ABI4* transcript in *scr* could also be a cause of its root growth defect under normal growth conditions. To test this hypothesis, we expressed *ABI4* as a fusion protein to GFP in the SCR expression domain using the *SCR* promoter. This strategy not only permits ABI4 overexpression in a cell type-specific manner but also allows us to monitor ABI4 expression. As shown in Figure 5B, the ABI4-GFP fusion protein was clearly expressed in the nucleus. As shown in Figure 5C, all transgenic plants that showed detectable levels of ABI4-GFP expression had significantly shorter roots than the wild type. To examine further the function of ABI4 in root develop-

ment, we compared root lengths of *scr*, *abi4*, and the *scr abi4* double mutant that are derived from the same cross. Interestingly, on MS medium, the *scr-4 abi41-104* double mutants had even shorter roots than the *scr-4* mutant (Fig. 5D; $P < 0.001$, $n = 30$, t test). Compared with the wild type, the *abi4-104* single mutant also had shorter roots. These results suggest that ABI4 also has a positive role in root growth. Because ABI4 is mainly expressed in the meristem zone in primary roots, we reasoned that at low levels, ABI4 probably promotes meristematic activity. Therefore, we measured the size of the apical meristem in the wild type, *scr-4*, *abi4-104*, and the *scr-4 abi4-104* double mutant. As expected, the *abi4* mutation reduced the size of the root apical meristem in both the wild type and *scr* backgrounds (128 versus 109 μm in the wild type and *abi4-104*, respectively; 108 versus 90 μm in *scr-4* and *scr-4 abi4-104*, respectively; $P < 0.05$, $n = 12$, t test). These results together suggest that ABI4 regulates root growth positively or negatively, depending on its level of expression; they further suggest that ABI4 levels must be strictly regulated to ensure root growth and that SCR plays this important role.

DISCUSSION

Plants are sessile; therefore, to ensure optimal growth and development in an ever-changing envi-
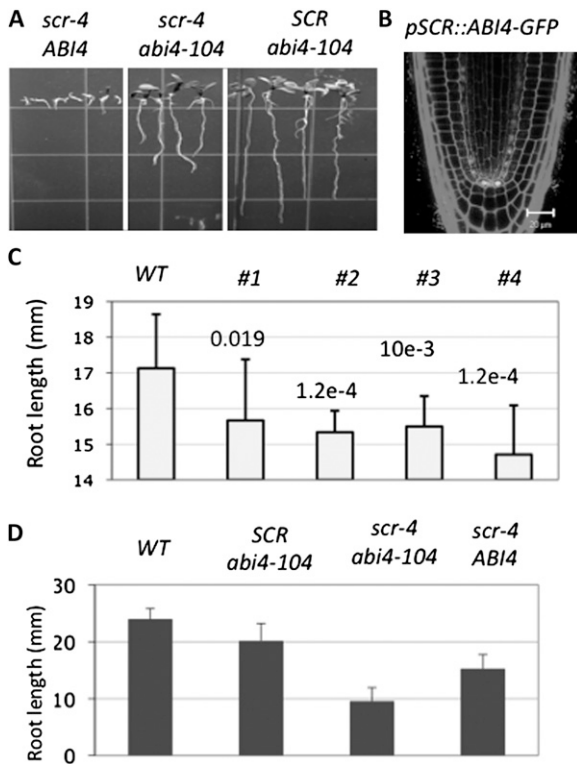
Figure 5. ABI4 regulates root growth under high-sugar and normal growth conditions. A, *scr-4 ABI4*, *scr-4 abi4-104*, and *SCR abi4-104* seedlings grown on MS medium containing 4% Glc, 10 d after germination, showing that mutation in *ABI4* confers *scr* tolerance to high Glc. B, Confocal microscopy image showing the expression of the ABI4-GFP fusion protein in transgenic plants harboring the *pSCR::ABI4-GFP* construct. Bar = 20 μm. C, Root length of wild-type Col (WT) and *pSCR::ABI4-GFP* transgenic plants on MS medium, 7 d after germination. Numbers 1 to 4 indicate independent lines, and the numbers above each bar are the *P* values for pairwise comparison between the wild type and transgenic lines (*t* test, *n* = 20). D, Root length of wild-type Col, *SCR abi4-104*, *scr-4 abi4-104*, and *scr-4 ABI4* seedlings grown on MS medium, 7 d after germination. Error bars represent sɒ from technical replicates.

ronment, they must be able not only to respond appropriately to various stimuli but also to overcome the deleterious effects from stress responses. Although recent studies have shed significant light on the mechanisms by which SHR and SCR control radial patterning, how they regulate root meristematic activity remains unclear. Our findings that SCR modulates the sugar response in the root apical meristem through direct repression of *ABI4* and *ABI5* provides an example of a mechanism that plants use to coordinate developmental programs with stress response mechanisms. Because high levels of ABI4 inhibit root growth, we suggest that SCR promotes root growth by suppressing the ABI4-mediated sugar response. Although SHR and SCR act in the same pathway regulating root stem cell renewal and radial patterning, we showed that SHR and SCR function differently

in the sugar response. This study also revealed a dual role for ABI4 in regulating root growth in response to environmental cues.

SCR Modulates the Sugar Response Directly

Evidence for a role of SCR in the sugar response comes from the observations that *scr* was hypersensitive to high Glc and that endogenous levels of Suc and Glc in *scr* were elevated. The higher sugar content in *scr* does not seem to be the sole cause of sugar hypersensitivity, however, because *shr* also had higher levels of water-soluble sugars but was much less sensitive to high Glc. The similarity of the developmental defects in *shr* and *scr* argues against the possibility that the sugar hypersensitivity in *scr* is a secondary effect. The sugar hypersensitivity in *scr* is not due to a general stress response either, because *scr* responded normally to high concentrations of NaCl and mannitol. By performing the sugar-sensitivity assay on MS medium in a closed environment (petri dish), we also minimized the effect of the root growth defects in *shr* and *scr* on nutrient uptake, which is evidenced by the observation that the shoots of *scr* and *shr* were similar in size to those of wild-type plants. In further studies, we found that SCR directly regulates *ABI4* and *ABI5*. Because *ABI4* is a key component in sugar-signaling pathways and *ABI5* also has a role in the sugar response, these results lend strong support to the conclusion that SCR modulates the sugar response directly. In line with this argument, we showed that mutation in *ABI4*, which positively regulates sugar signaling, alleviated the sugar hypersensitivity phenotype in *scr*.

The Role of SCR in the Sugar Response Is SHR Independent

Although *SCR* transcription is largely dependent on the presence of SHR, the role of SCR in the sugar response does not appear to require SHR. First, although both *shr* and *scr* were hypersensitive to high Glc, the *scr* mutant had a much more severe phenotype. Second, by ChIP-PCR, we showed that SHR did not bind to the promoters of *ABI4* and *ABI5*. Consistent with this result, by RT-PCR we found that the transcript level of *ABI4* and *ABI5* was dramatically elevated in *scr* but remained almost unchanged in *shr*, except in the meristem zone, where the basal level of SCR is lowest (Cui et al., 2007). The distinct roles for SHR and SCR in the regulation of ABI4 expression was further confirmed by transgenic analysis with the *pABI4::GUS* reporter construct.

The SHR-independent role of SCR in the sugar response is not unexpected, because we have previously shown that, in addition to an overlapping set of targets, SHR and SCR control other genes differently (Cui et al., 2007; Cui and Benfey, 2009; Sozzani et al.,

2010). Due to a positive feedback loop for SCR transcription, which is dependent on the SHR/SCR complex (Cui et al., 2007), SCR would accumulate to a level much higher than that of SHR, and consequently, some SCR protein is predicted not to form a complex with SHR. Our hypothesis is that at least two distinct pools of SCR are present, one in a complex with SHR and the other acting alone or interacting with other proteins to regulate a set of genes different from those controlled by the SHR/SCR complex. *ABI4* and *ABI5* are among the genes that are regulated only by SCR. Downregulation of *ABI4* and *ABI5* by SCR is an example of SCR's action as a transcriptional repressor. In the *shr* mutant background, SCR is still expressed because of a SHR-independent mechanism, which could explain the much less severe Glc hypersensitivity phenotype in *shr*.

The QC-specific regulation of *ABI4* by SCR is somewhat surprising, however, because SCR is expressed not only in the QC but also in the endodermis, and mutation in SCR is expected to relieve the repression on *ABI4* in both the QC and endodermis. Intriguingly, a cell type- or developmental stage-specific expression pattern has been reported for other SCR targets. *WOX7*, for example, is expressed specifically in the cortex/endodermis initial cell and its daughter cell (Cui et al., 2011), and several genes, including *MAGPIE* and *NUTCRACKER*, are not expressed even in the QC, although they are expressed in the meristem zone (Cui et al., 2007; Welch et al., 2007). These data suggest that the regulatory mechanism by SCR is complex. Although SCR is a transcriptional regulator, it does not appear to bind to DNA directly (Ogasawara et al., 2011). It is conceivable, therefore, that the expression pattern of SCR targets is actually determined by DNA-binding factors that are expressed in a cell type- or developmental stage-specific manner and recruit SCR to target promoters through protein-protein interaction, which warrants further investigation.

SCR Is Probably Also Involved in Other Stress Responses

The sugar response is unlikely to be the only stress condition in which SCR is involved, as most of its direct targets confirmed in this study do not appear to play a role in the sugar response. For example, several of its top-ranked direct targets, including *ATAIB*, *TMAC2*, and *HB12*, are known to be involved in drought tolerance (Olsson et al., 2004; Huang and Wu, 2007; Li et al., 2007a), suggesting that SCR also plays a role in drought tolerance. Because these stress-associated genes are also directly regulated by SHR, the role of SCR in other stress responses presumably depends on the SHR/SCR complex. The observation that both *shr* and *scr* were sensitive to the stress hormone ABA supports this possibility. Further elucidation of the mechanism by which SCR regulates plant growth and development will require the identification of additional stress conditions in which SCR is involved.

SCR May Have a Similar Function in the Shoot

SCR is expressed not only in root but also in shoot organs such as the apical meristem, leaf primordium, and bundle sheath cells in fully expanded leaves (Wysocka-Diller et al., 2000), but its function in these tissues and cell types remains unclear. *scr* has been shown, though, to have growth defects in the shoot (smaller leaves and shoot), but these are generally attributed to its root growth defects. By means of grafting experiments, a recent study demonstrated that SCR regulates leaf growth independently of its function in the root (Dhondt et al., 2010). This study showed that the cell cycle in *scr* leaf primordia is prolonged, entailing less frequent cell divisions and ultimately a smaller number of cells in mature leaves (Dhondt et al., 2010), but the restricted expression of SCR in only a subset of leaf cells suggests that SCR does not regulate the mitotic cycle in the whole leaf directly. Although sugar accumulation in *scr* leaves may result from low mitotic activity and therefore reduced sugar utilization, it is equally likely to be the cause of the leaf growth defect in *scr*. Further studies will be needed to determine whether SCR also promotes meristematic activity in the shoot by suppressing the sugar response.

ABI4 Regulates Root Growth in a Dosage-Dependent Manner

An essential role for ABI4 in sugar signaling and seed development has long been established, but recent studies show that ABI4 is also involved in other processes. Shkolnik-Inbar and Bar-Zvi (2010) showed that the *abi4* mutant produced more lateral roots than the wild type at a similar developmental stage, suggesting an inhibitory role for ABI4 in lateral root formation. Consistent with this observation, they showed that ABI4 is expressed in the maturation zone of the root. Surprisingly, they found that ABI4 expression is regulated not only by ABA but also by cytokinin and auxin and that ABI4 also modulates cytokinin and auxin responses. Consistent with this, lateral root formation in *abi4* is no longer affected by exogenous cytokinin. ABI4 appears to be also required for lipid biosynthesis in seedlings during nitrogen deficiency (Yang et al., 2011). In our study here, we showed that ABI4 plays an important role in regulating root growth. In agreement with other reports (Dekkers et al., 2008; Shkolnik-Inbar and Bar-Zvi, 2010), we showed that ectopic expression of ABI4 causes retardation in root growth. Our study, however, also reveals a positive role for ABI4 in root growth under normal conditions, as the *abi4* mutant had a shorter root and a smaller apical meristem than the wild type. How ABI4 promotes root growth is currently unknown, but probably this involves a complex interaction between ABI4 and several hormone-signaling pathways. Because ABI4 is stress inducible and it inhibits root growth at elevated levels but is also required for root growth

under normal growth conditions, we suggest that ABI4 is an important component in the mechanism that orchestrates plant growth with stress responses.

MATERIALS AND METHODS

Plant Materials and Treatments

All the Arabidopsis (*Arabidopsis thaliana*) seedlings used in this study were grown on MS agar plates containing 1% Suc (w/v), which were positioned vertically in a Percival growth chamber at 22°C with 16 h of daily illumination. One percent Suc was added because without it, the *shr* and *scr* mutants do not grow well. For Glc-sensitivity assays, Glc was added to the standard MS medium to a final concentration of 4% to 6%. Concentrations for other chemicals tested were 0.5, 1, and 2 μM ABA; 50, 100, and 150 mM NaCl; and 50, 100, and 200 mM mannitol.

All the *shr* and *scr* mutant alleles have been described previously: *scr-1* and *scr-2* by Di Laurenzio et al. (1996), *scr-3* and *scr-4* by Fukaki et al. (1998), *shr-2* and *shr-3* by Helariutta et al. (2000), and *shr-5* by Gallagher et al. (2004). The *aba2-1* and *abi4-104* mutants were obtained from the Arabidopsis Stock Center. T-DNA insertion lines (FLAG_587G11 for ARL1 (AT1G24120) and FLAG_532D03 for *MYB* [AT1G68670]) were obtained from the INRA (http://www-ijpb.versailles.inra.fr/en/sgap/equipes/fichiers/FST_information.htm).

Soluble Sugar Measurement

Plants used for sugar measurement were 1-week-old seedlings grown in MS medium or 3-week-old plants grown in soil. Water-soluble sugars were extracted by grinding of fresh tissues in water (3 mL g^{-1} fresh tissue) on ice. The mixture was centrifuged at 12,000 rpm, 4°C, for 10 min. The supernatant was further cleared by filtering through a Microcon centrifugal filter column with a molecular weight cutoff of 10,000 (Millipore). Suc, Glc, and Fru were then separated by HPLC on a Hamilton RCX10 ion-exchange chromatography column with 150 mM NaOH for elution and quantified according to sugar standards.

Other Methods

ChIP-PCR and RT-PCR assays were performed as described previously (Cui et al., 2007), except that a StepOnePlus real-time PCR system (Applied Biosystems) was used. For cloning of the *pSCR::ABI4* construct, RT was first performed with root total RNA; the *ABI4* cDNA was then amplified with the high-fidelity Phusion DNA polymerase (New England Biolabs) and cloned into the pDONR221 vector. The *pSCR::ABI4* construct was obtained by subcloning the ABI4 cDNA clone and the SCR promoter clone into a destination vector (dpGreenBarT) with the Multi-Site Gateway system. The *pABI4::GUS* construct was cloned similarly with the same Gateway system. Briefly, a 2.6-kb fragment upstream of the start codon of the *ABI4* gene was first amplified using the primer pair attB4_pABI4_FW and attB1_pABI4_RV and was then cloned into the pDONR-P4-P1R vector. The pEnter-pABI4 entry clone was then subcloned together with the pEnter-GUS entry clone into dpGreenBarT, yielding the *pABI4::GUS* construct. After confirmation by restriction analysis, the final constructs were transformed into wild-type Arabidopsis (ecotype Col) by the *Agrobacterium tumefaciens*-mediated method. The *pSCR::ABI4-GFP* and *pABI4::GUS* transgenic lines were selected by epifluorescence microscopy and GUS staining, respectively.

The primers used for the ChIP-PCR and RT-PCR assays as well as for cloning and genotyping are listed in Supplemental Table S3.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Schematic of the radial pattern in wild type and *shr* and *scr* roots.

Supplemental Figure S2. *ARL1* and the MYB factor, at1g68670, are probably involved in stress response too.

Supplemental Figure S3. The sugar hypersensitivity in *scr* and *shr* is independent of their genetic backgrounds.

Supplemental Figure S4. *ABI4* transcript level is dramatically elevated in the shoot of *scr*, but not that of *shr*.

Supplemental Figure S5. *pABI4::GUS* expression pattern in wild type and *scr-1* root, 7 d after germination.

Supplemental Table S1. Top-ranked direct SHR targets identified by ChIP-chip.

Supplemental Table S2. Soluble sugar content in wild-type, *scr-1*, and *shr-2* seedlings grown on MS medium.

Supplemental Table S3. Primers used for ChIP-PCR, RT-PCR, genotyping, and cloning.

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