The Regulatory Domain of SRK2E/OST1/SnRK2.6 Interacts with ABI1 and Integrates Abscisic Acid (ABA) and Osmotic Stress Signals Controlling Stomatal Closure in Arabidopsis*

Received for publication, September 7, 2005, and in revised form, December 2, 2005 Published, JBC Papers in Press, December 19, 2005, DOI 10.1074/jbc.M509820200

Riichiro Yoshida†, Taishi Umezawa†, Tsuyoshi Mizoguchi‡, Seiji Takahashi†, Fuminori Takahashi‡, and Kazuo Shinozaki‡¶

From the †Laboratory of Plant Molecular Biology, RIKEN Tsukuba Institute, 3-1-1, Koyadai, Tsukuba, Ibaraki 305-0074, Japan, ‡Plant Mutation Exploration Team, Plant Functional Genomics Research Group, RIKEN Genomic Sciences Center, 1-7-2 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan, ¶Institute of Biological Sciences, Tsukuba University, Tennodai, Tsukuba, Ibaraki, 305-8572, Japan, and §Gene Discovery Research Group, RIKEN Plant Science Center, 1-7-2 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan

AB1 and ABI2 encode PP2C-type protein phosphatases and are thought to negatively regulate many aspects of abscisic acid (ABA) signaling, including stomatal closure in Arabidopsis. In contrast, SRK2E/OST1/SnRK2.6 encodes an Arabidopsis SnRK2 protein kinase and acts as a positive regulator in the ABA-induced stomatal closure. SRK2E/OST1 is activated by osmotic stress as well as by ABA, but the independence of the two activation processes has not yet been determined. Additionally, interaction between SRK2E/OST1 and PP2C-type phosphatases (AB1 and ABI2) is not understood. In the present study, we demonstrated that the abi1–1 mutation, but not the abi2–1 mutation, strongly inhibited ABA-dependent SRK2E/OST1 activation. In contrast, osmotic stress activated SRK2E/OST1 even in abi1–1 and abi2–1 plants. The C-terminal regulatory domain of SRK2E/OST1 was required for its activation by both ABA and osmotic stress in Arabidopsis. The C-terminal domain was functionally divided into Domains I and II. Domain II was required only for the ABA-dependent activation of SRK2E/OST1, whereas Domain I was responsible for the ABA-independent activation. Full-length SRK2E/OST1 completely complemented the wilt phenotype of the srk2e mutant, but SRK2E/OST1 lacking Domain II did not. Domain II interacted with the ABI1 protein in a yeast two-hybrid assay. Our results suggested that the direct interaction between SRK2E/OST1 and ABI1 through Domain II plays a critical role in the control of stomatal closure.

Water is indispensable for plant activities such as photosynthesis, respiration, and growth. To overcome the serious problems caused by water-deficit conditions, plants have developed unique systems to prevent water loss from, and to retain water inside, their cells. Abscisic acid (ABA) is a stress-related plant hormone and plays a significant role in the adaptation to water stress (1–6). Much is known concerning the function of ABA in the adaptive process, and many factors have been identified in ABA signaling. Protein phosphorylation is one of the best characterized events involved in ABA signaling. However, to understand the major process of ABA signaling, we need to characterize the detailed functions of each signaling molecule.

Through a combination of biochemical and bioinformatics approaches, we found the ABA-specific protein kinase activities in Arabidopsis T87 cells and identified their genes as SnRK2 (SNF1-related protein kinase 2) (7). SRK2E is one of the Arabidopsis SnRK2 specifically activated by ABA (7). Loss of SRK2E function causes an ABA-insensitive phenotype in stomatal closure (7). This srk2e mutant cannot cope with a rapid humidity decrease and results in a wilty phenotype. On the other hand, a drought-sensitive mutant, open stomata 1 (ost1), was identified by a unique system using infrared thermography, and the OST1 gene was found to be identical to SRK2E (At4g33950) (8). Reactive oxygen species have been proposed to function as second messengers for ABA signaling in stomata cells and to activate Ca²⁺ channels (9). SRK2E/OST1 may act upstream of reactive oxygen species production (8). Thus, SRK2E/OST1/SnRK2.6 is now regarded as a major, positive regulator of ABA signaling in Arabidopsis.

The importance of SnRK2 in water stress signaling has been extensively studied in many species. The genes that have been described are wheat ABA-induced protein kinase 1 PKABA1 (10), fava bean ABA-activated serine-threonine protein kinase AAPK (11), soybean protein kinase SPK1 and SPK2 (12), tobacco osmotic stress-activated protein kinase NIOSAK (13, 14), rice osmotic stress/ABA-activated protein kinase SAPK (15), and those in Arabidopsis (16). The SnRK2 gene family is unique to plants and consists of 10 genes in Arabidopsis (17). Recently, we demonstrated that SRK2C/OSKL4/SnRK2.8 was activated by osmotic stress and significantly increased the drought tolerance of Arabidopsis plants through activating stress-responsive gene expression in transgenic overexpressors (18).

The manner in which stomata cells sense humidity changes to activate stomatal closure remains unknown. There are apparently at least two pathways in drought stress signaling that target stomatal closure (19). One is ABA dependent; the other is ABA independent, namely, an osmosensing pathway. Osmoregulation of inward K⁺ channels has been found to function in stomata cells, which provoked the presence of the osmosensing pathway in plant cells (20). However, little is known about the nature of the ABA-independent osmosensing pathway in stomatal closure. In the present study, we examined whether ABA-dependent or ABA-independent pathways, or both, function in the activation of SRK2E/OST1 to close stomata cells under low humidity stress. Using Arabidopsis T87 cultured cells we demonstrated that not only ABA, but...
also osmotic stress (OS), activates SRK2E/OST1 in vivo (7). An experiment using ABA-insensitive or ABA-deficient mutants revealed that the OS-dependent activation of SRK2E/OST1 was not mediated by ABA, implying that two independent pathways exist and function in SRK2E/OST1 signaling. Domain analysis of the SRK2E/OST1 protein indicated that its C-terminal region plays an important role in controlling both ABA- and OS-dependent activation of SRK2E/OST1. A yeast two-hybrid analysis showed direct physical binding between SRK2E/OST1 and ABI1. Interestingly, the binding site was located to the C-terminus of SRK2E/OST1 that was needed for ABA response. The possible mechanisms involved in the activation of SRK2E/OST1 in the response to ABA and OS were discussed in relation to stomata closure.

MATERIALS AND METHODS

Plant Growth Conditions—The Columbia and Landsberg erecta (Ler) ecotypes of Arabidopsis thaliana were used. The seeds of wild-type (Columbia, Ler), srk2e, and transgenic plants were sterilized with 70% ethanol and 1% antiformin and sown on Gamborg’s B5 medium agar plates (21) containing 1% sucrose. They were grown under 16 h of light/8 h dark conditions at 22 °C for 3 weeks.
Generation of Transgenic Cells and Plants—The transgenic plants and cells were generated by fusing each construct in-frame to green fluorescent protein (GFP) driven by the CaMV 35S promoter in pBE2113 (22) or pBiG2113 (23). The transformation vector was introduced into Agrobacterium to transform wild-type (Col) and srk2e plants and T87 cells.

Generation of 35S::2E-GFP abi1–1, 35S::2E-GFP abi2–1, and 35S::2E-GFP aba2–1 plants were treated with ABA or sorbitol, and leaves of these plants were subjected to low humidity stress. Their cell extracts were then subjected to in-gel kinase assay. A, SRK2E-GFP proteins were overexpressed in the aba2–1 background. Root tissues of 35S::2E-GFP aba2–1 plants were treated with ABA (50 μM) or sorbitol (0.8 M), and the cell extracts were subjected to in-gel kinase assay. B, water loss in abi1–1 and aba2–1 mutant seedlings (mean ± S.E., n = 3).

Protein Extraction and In-gel Kinase Assay—Protein extraction and in-gel kinase assay were performed as previously described (7).

RNA Extraction and RT-PCR Analysis—RNA extraction and RT-PCR analysis were performed as previously described (7) except that the following primers were used in rab18 expression: 5′-CGATCCAG-CACGAGTATGAC-3′ for forward and 5′-TTCAAGCTTAAAG-GCCACC-3′ for reverse.

Two-hybrid Interaction Assays—For the two-hybrid assays, each construct shown in Fig. 6 was amplified by PCR and cloned into the pGBK7 or pGADT7 vectors (Clontech). For interaction studies, plasmids containing fusion proteins were co-introduced into Saccharomyces cerevisiae AH109 and grown on medium lacking Leu, Trp, and His in the presence of 20 mM 3-AT. The α-galactosidase activity assay was performed according to the manufacturer’s instructions (Clontech).

RESULTS

Low Humidity Stress Activated SRK2E/OST1—ABA activated GFP-fused SRK2E (simply designated as 2E-GFP) in Arabidopsis root tissues in a dose-dependent manner (Fig. 1A) as previously reported (8). A low concentration of ABA (0.5 μM) was sufficient to activate 2E-GFP in our case. ABA activated two protein kinases, p44 and p42, in wild-type roots, and p44 was not detected in srk2e, indicating that p44 corresponds to SRK2E (Fig. 1B). The kinase activities were not detected in leaf tissues treated with ABA even though ABA-inducible genes were clearly expressed in the same tissues under the same conditions (Fig. 1, C and D). These results suggested that the activation of the kinases was root specific. Using the transgenic plants, we next examined whether low humidity directly activated SRK2E/OST1. 2E-GFP was clearly activated by low humidity in leaf tissues (Fig. 1E). The p44 kinase was also activated by low humidity in wild-type leaves, but not in the srk2e leaves.
To confirm whether OS activated SRK2E/OST1 in Arabidopsis, we transformed Arabidopsis T87 cells with 35S::SRK2E-GFP. Not only ABA, but also OS, such as sorbitol and NaCl, activated the 2E-GFP in Arabidopsis cells (Fig. 1G). Cold stress, however, did not activate 2E-GFP (Fig. 1G).

The ABA-dependent Activation of SRK2E/OST1 Suppressed by abi1–1 but Not by abi2–1—To investigate the roles of ABI1 and ABI2 in the activation of SRK2E/OST1 by ABA, we tested the 2E-GFP activity in root tissues of abi1–1 and abi2–1 plants, and aba2–1 was used as a control. ABA activated the 2E-GFP in wild-type plants, but not in abi1–1 plants (Fig. 2A). In contrast, abi2–1 and aba2–1 did not affect the activation of the 2E-GFP by ABA (Fig. 2A). These results indicated that two similar PP2C, ABI1 and ABI2, have distinct roles in ABA signaling in terms of regulation of SRK2E/OST1.

The abi and aba Mutations Did Not Affect the OS-dependent Activation of SRK2E/OST1—To investigate the role of ABA signaling in the OS-dependent activation of SRK2E/OST1, we tested the 2E-GFP activity in root and leaf tissues treated with low humidity and sorbitol, respectively. None of the abi1–1, abi2–1, and aba2–1 mutations significantly affected the OS-dependent activation of 2E-GFP. These results indicated that ABA signaling is not required for the OS-dependent activation of SRK2E/OST1 and that ABA and OS may independently activate kinase in Arabidopsis plants. We observed severe water loss in both abi1–1 and aba2–1 mutants (Fig. 2C), but the OS-dependent 2E-GFP
activation was normal in these mutants (Fig. 2A). These results supported the central role of the ABA-dependent pathway in SRK2E/OST1 activation in stomatal closure (7, 8).

Domain Analysis of the SRK2E/OST1 Protein—The Arabidopsis SnRK2 gene has a kinase domain that is similar to the yeast SNF1 protein kinase (24). Unlike SnRK1 and SnRK3 proteins, SnRK2 proteins do not have common regulatory motifs but seem to have an important domain(s) in their N- or C-terminal regions. We first compared the C-terminal regions of 10 Arabidopsis SnRK2 proteins and found two conserved domains; one is ~30 amino acids starting from the end of the kinase domain (Domain I), and the other is ~40 amino acids starting from the end of Domain I (Domain II) (Fig. 3A). Domain I is highly conserved in all 10 SnRK2 proteins. Domain II can be classified into two types in Arabidopsis SnRK2 proteins, as previously pointed out (24).

One group has an Asp-rich region defined as SnRK2a, and the other has a Glu-rich region defined as SnRK2b. SRK2E/OST1 belongs to the SnRK2a group together with four other SnRK2 proteins (SRK2C/OSKL4, SRK2D/OSKL3, SRK2F/OSKL5, and SRK2I/OSKL2). We therefore overexpressed SRK2D, SRK2F, and SRK2I in T87 cells as a GFP fusion protein and examined their responses to ABA and OS. Fused to GFP, these kinases were activated by both ABA and OS in a manner similar to 2E-GFP (Fig. 3B). We also tested the response to ABA and OS in SRK2G/OSKL8, which belongs to the SnRK2b group, by using 35S::SRK2G-GFP transgenic cells. The 2G-GFP was strongly activated by OS, but not by ABA (Fig. 3B), as previously reported (16).

Domain II was well conserved among three SnRK2s from different plant species, i.e. Arabidopsis SRK2E/OST1, fava bean AAPK (11), and rice SAPK8 (Fig. 3C) (15). AAPK and SAPK8 have been shown to be activated by ABA (11, 15). These results suggested that Domain II is critical for ABA-induced activation of SRK2E/OST1 and that other SnRK2s belong to the SnRK2a group. Thus, we made two types of C-terminal-deleted constructs of SRK2E/OST1 and overexpressed them as GFP fusion proteins in T87 cells to determine the role of Domain II in the activation (Fig. 4A). We also overexpressed the N-terminus-deleted SRK2E/OST1 in T87 cells. Effects of these deletions on SRK2E/OST1 activities were analyzed by in-gel kinase assay (Fig. 4B). Both ABA- and OS-dependent activities decreased but were not completely lost in N-terminal-truncated SRK2E/OST1 (2EΔN). When a C-terminal region of 319–357 amino acids (Domain II) was deleted (represented by 2EΔC1), the ABA-dependent activation was specifically inhibited, whereas the OS-dependent activation was either normal or increased. The SRK2E/OST1 kinase lacking both Domains I and II (2EΔC2) completely lost responsiveness to ABA and OS. These deletion analyses supported our idea that SRK2E/OST1 may be activated by two independent pathways, namely ABA- and OS-dependent pathways.

Essential Roles of the C-terminal Domain in the Physiological Function of SRK2E/OST1—Because the C-terminal region is indispensable for both ABA- and OS-dependent activation of SRK2E/OST1, we examined the effects of C terminus deletion on the functional complementation of the wilt phenotype in srk2e. As shown in Fig. 5A, 35S::2EAC1-GFP srk2e plants had a wilt phenotype in comparison to 35S::2EWT-GFP srk2e plants. We measured the water loss in these plants. 2EWT-GFP fully complemented the wilt phenotype of srk2e, but 2EAC1-GFP did not completely rescue the wilt phenotype (Fig. 5B). This truncated SRK2E/OST1 appeared to retain partial function of SRK2E/OST1, as the level of water loss was intermediate between that of srk2e plants and the transgenic srk2e plants complemented with full-length SRK2E/OST1. However, the level of water loss in 35S::2EAC1-GFP srk2e plants reached the same level as that in srk2e plants 60 min after the start of water depletion. To elucidate the effect of ABA on the C terminus deletion, we treated the transgenic plants with ABA prior to the water depletion experiment. A high level of water loss was still observed in the ABA-treated 35S::2EAC1-GFP srk2e plants as compared with the ABA-treated 35S::2EWT-GFP srk2e plants (Fig. 5C). The effect of low humidity on the activation of the truncated SRK2E/OST1 was examined in the transgenic plants. The in-gel kinase assay clearly demonstrated that 2EAC1 was still able to respond to the OS signal (Fig. 5D). These results suggested that low humidity or OS-activated SRK2E/OST1 can sensitize the stress signal to induce stomatal closure and that the C-terminal Domain II region may be responsible for both ABA-dependent activation of SRK2E/OST1 and complete stomatal closure.

Physical Interaction between the C-Terminal Regions of SRK2E/OST1 and ABI1—The yeast two-hybrid assay was conducted to examine the interactions between SRK2E/OST1 and well known factors identified in ABA signaling, i.e. ABI1 (25), ABI2 (26), AtRac1 (27), and GPA1 (28). Strong binding activity was observed between SRK2E/OST1 and ABI1 (Fig. 6A). The abi1–1 mutation weakened the binding to SRK2E/OST1.
relative to the wild-type ABI1 (Fig. 6B). Domain analysis indicated that the C-terminal Domain II involved in the ABA-dependent response was required and sufficient for the interaction with ABI1 (Fig. 7, A and B). ABI1 did not bind to the C-terminal corresponding region of SRK2G/OSKL8 (311–353 amino acids) that does not respond to ABA (16).

**DISCUSSION**

SRK2E/OST1/SnRK2.6 plays key roles in the responses to OS and ABA in stomatal closure. SRK2E/OST1/SnRK2.6 with deletions of the C-terminal regulatory domains were used to study the roles of this kinase in the stomatal closure triggered by low humidity in *Arabidopsis*. Biochemical analysis of the GFP fusion of the kinases with the *abi* (aba-insensitive) and *aba* (aba-deficient) mutations and the yeast two-hybrid analysis enabled us to demonstrate that the ABA-dependent and -independent pathways mediate between low humidity and stomatal closure in *Arabidopsis*. We also pointed out critical roles of Domain II in the C-terminal regulatory region of SRK2E/OST1 in the interaction with ABI1 and activation by ABA in stomata closure.

ABA-dependent and -independent Pathways Mediate between Low Humidity and Stomatal Closure in *Arabidopsis*—In *Arabidopsis* T87 culture cells, OS, such as sorbitol and NaCl, activated SRK2E/OST1 very rapidly, i.e. in 5 min, as quickly as ABA did (Fig. 1G). Because OS itself promotes ABA synthesis through 9-cis-epoxycarotenoid dioxygenase or *Arabidopsis* aldehyde oxidase gene expression (29, 30), in the present study the ABA dependence of OS-activated SRK2E/OST1 was examined using *Arabidopsis* abi and aba mutants. In the *abi1–1* mutant, OS activated SRK2E/OST1, but ABA did not (Fig. 2A). In *aba2–1* and *abi2–1*, however, both ABA and OS activated SRK2E/OST1. These results clearly showed that OS directly activates SRK2E/OST1 in *Arabidopsis* and that there are at least two independent pathways to activate SRK2E/OST1, the ABA-dependent and -independent pathways.

Not only ABA and OS, but also low humidity, activate SRK2E/OST1 and prevent rapid water loss in *Arabidopsis*. Low humidity resulted in more water loss in *abi1–1* and *aba2–1* than in wild-type plants (Fig. 2C). Although *abi1–1* blocked the ABA-dependent activation of SRK2E/OST1, kinase activation was detected even in *abi1–1* and *aba2–1* plants with the severe wilty phenotype (Fig. 2). The loss-of-function srk2e had a wilty phenotype when exposed to low humidity stress (7). These results suggested that the ABA-dependent and -independent pathways mediate cellular signals between low humidity and stomatal closure as described in Fig. 8 and discussed below.

**Distinct Roles of Domains I and II in the Activation of SRK2E/OST1**—We proposed that SRK2E/OST1 may play a critical role in ABA-dependent stomatal closure (7). Characterization of SRK2E/OST1 is important to understanding the molecular bases of this signaling and will help in identifying novel upstream factors, including specific receptor(s) of ABA. There are no conserved regulatory domains in SnRK2 genes, except for the short acidic patches in their C-terminal regions.
Using T87 cells, we found two distinct domains required for the activation of SRK2E/OST1 (Figs. 3 and 4); one is the Domain II (319–357 amino acids) region involved in ABA-dependent activation (Fig. 8, Pathway I), and the other is the Domain I (283–318 amino acids) region that functions in the ABA-independent pathway (Pathway III). The Domain II region contains a sequence conserved among all the reported SnRK2 proteins (Fig. 3A). This region may be important for the OS-dependent and ABA-independent activation of all the SnRK2 proteins.

Deletion of Domain II specifically blocked the ABA-dependent activation of SRK2E in Arabidopsis (Fig. 3). The importance of the C terminus was also demonstrated in rice SnRK2 (15). ABA activates SAPK8, but not SAPK2, in rice. A chimeric kinase made by substitution of the C terminus of SAPK8 (including both Domains I and II) for the same region of SAPK2 was activated by ABA (15). In our study we further isolated the ABA-responsive region to Domain II. Domain II is highly conserved in the ABA-activated SnRK2s in different plant species and may commonly function as an ABA-responsive domain. We also confirmed that the deletion of Domain II significantly increased the OS-dependent activation of SRK2E. This suggested that Domain II may affect Domain I and repress the OS-dependent kinase activity.

SRK2E/OST1 without Domain II only partially complemented the wilty phenotype of srk2e (Fig. 5), but the truncated form of SRK2E was activated by low humidity in a gel kinase assay. These results also supported our idea that the ABA-dependent and -independent pathways shown in Fig. 8 mediate between low humidity and stomatal closure.
Both signaling pathways may be required for complete stomatal closure in response to sudden water stress.

Roles of the Physical Interaction between SRK2E and ABI1 in the Stomatal Closure Triggered by Low Humidity in Arabidopsis—We found that SRK2E/OST1 interacted with ABI1, but not with other factors involved in ABA signaling, including a homologous PP2C ABI2 (Fig. 6). The abi1–1 mutation, but not the abi2–1 mutation, inhibited the ABA-dependent activation of SRK2E/OST1 (Fig. 2). These results indicated that ABI1 and ABI2 have distinct roles in the signaling in terms of the control of SRK2E in Arabidopsis as shown in Fig. 8. ABI1 did not bind to the C terminus of SRK2G/OSKL8 that lacked the conserved sequence in Domain II of the ABA-responsive SnRK2s, such as SRK2E/OST1, AAPK, and SAPK8 (Fig. 3A, C). ABI1 interacted with a HD-Zip transcriptional regulator, ATBH6 (31), and a SOS2-like protein kinase of PSK18 (32). Phospholipase D-derived phosphatidic acid has been shown to interact with ABI1 and inhibit its PP2C activity (33). The abi1–1 mutation weakened the binding to SRK2E/OST1, as found in the interactions between ATHB6 and ABI1 (31) or SOS2 and ABI2 (34). Members of the SnRK2 family may activate and be activated by different downstream and upstream factors, respectively, using their distinct C-terminal regions, especially Domain II.

Based on the present results, we propose that ABI1 may have two roles in the ABA-dependent pathway to control stomatal closure, as shown in Fig. 8. ABI1 and ABI2 may function as negative regulators of ABA signaling in step 5 of Pathway II. ABI1 may have a role in Pathway I of the ABA-dependent pathway as well. In this pathway, ABI1 interacts with Domain II of SRK2E/OST1. Considering the similar wilty phenotypes of srk2e and abi1–1 and the weakened binding activity of the abi1–1 protein to SRK2E, we prefer a model in which ABI1 functions as a positive regulator in the activation of SRK2E by low humidity (Fig. 8, Pathway I, step 3). If ABI1 functions as a negative regulator for SRK2E,

the weaker interaction between the abi1–1 protein and SRK2E should result in higher SRK2E activity. Domain II is required for interaction with ABI1 and activation by ABA (Pathway I, step 4). SRK2E without Domain II was activated by low humidity but only partially complemented the wilty phenotype of srk2e (Fig. 5). Both steps 5 and 9 in the ABA-dependent and -independent pathways may be required for the full complementation of the wilty phenotype of srk2e, as described earlier. An alternative explanation is that Domain II may be required for an interaction with a downstream factor in stomatal closure triggered by low humidity (Fig. 8, step 9). We obtained a loss-of-function mutant of abi1 with a T-DNA insertion and found that the ABA-dependent activation of SRK2E was not affected by the abi1 null mutation (data not shown). The abi1–1 mutation behaved as a dominant negative mutation in the ABA signaling (35) (Pathway II, step 5). The abi1–1 protein may trap a regulator protein or a downstream factor of SRK2E and function as a dominant negative inhibitor by blocking interaction of the proteins with Domain II of SRK2E.

The OS-induced activation of rice, SAPK1, and SAPK2 appeared to be regulated by protein phosphorylation (15). Therefore, protein phosphorylation may participate in the activation of SRK2E/OST1. ABI1 encodes a PP2C-type protein phosphatase (25). One possible explanation for the functional interaction between SRK2E and ABI1 is that the latter may regulate the former by de-phosphorylation. This is not likely, however, because the abi1–1 protein has a much weaker binding activity to Domain II (Fig. 6) and less phosphatase activity (35) than wild-type ABI1. The ABA-dependent activation of SRK2E was inhibited by abi1–1 (Fig. 2), supporting the idea that ABI1 may not be involved in the dephosphorylation of SRK2E. The upstream kinases that directly activate yeast SNF1 or animal AMPK, similar to the SnRK2 family members, were recently identified and characterized (36–38). Thus, to understand the molecular bases of ABA or OS signaling, it is necessary to identify the upstream factor(s) that directly activate SRK2E/OST1. We are currently trying to find the candidate genes that would bind to the SRK2E/OST1 C terminus and regulate its ABA-specific response.

Acknowledgment—We thank Hiroko Kobayashi for technical assistance.

REFERENCES

1. Bray, E. A. (1997) Trends Plant. Sci. 2, 48–53
2. Bonetta, D., and McCourt, P. (1996) Trends Plant Sci. 3, 231–235
3. Shinozaki, K., Yamaguchi–Shinozaki, K., and Seki, M. (2003) Curr. Opin. Plant Biol. 6, 410–417
4. Yamaguchi–Shinozaki, K., and Shinozaki, K. (2005) Trends Plant Sci. 10, 88–94
5. Finkelstein, R. R., Gampala, S. S. L., and Rock, C. D. (2002) Plant Cell 14 (suppl.), S15-S45
6. Zhu, J. K. (2002) Annu. Rev. Plant Physiol. Plant Mol. Biol. 53, 247–273
7. Yoshida, R., Hibi, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Alonso, J., Ecker, J. R., and Shinozaki, K. (2002) Plant Cell Physiol. 43, 1473–1483
8. Mustilli, A. C., Merlot, S., Vavasseur, A., Fenzi, F., and Giraudat, J. (2002) Plant Cell 14, 3089–3099
9. Pei, Z. M., Murata, Y., Benning, G., Thomine, S., Khusein, B., Allen, G. J., Grill, E., and Schroeder, J. I. (2000) Nature 406, 731–734
10. Anderberg, R. J., and Walker–Simmons, M. K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10183–10187
11. Li, J., Wang, X. Q., Watson, M. B., and Assmann, S. M. (2000) Science 287, 300–303
12. Monks, D. E., Aghoram, K., Courtney, P. D., DeWald, D. B., and Dewey, R. E. (2001) Plant Cell 13, 1205–1219
13. Mikolajczyk, M., Awotunde, O. S., Muszynska, G., Klesiss, D. F., and Dobrowolska, G. (2000) Plant Cell 12, 165–178
14. Kolner, A., Pekar, I., Kaczarewski, S., Muszynska, G., Hardie, D. G., and Dobrowolska, G. (2004) Plant Physiol. 136, 3255–3265
15. Kobayashi, Y., Yamamoto, S., Minami, H., Kagaya, Y., and Hottori, T. (2004) Plant Cell 16, 1163–1177
16. Boudsocq, M., Barbier-Brygoo, H., and Lauriere, C. (2004) J. Biol. Chem. 279, 41578–41766

FIGURE 8. A model to explain SRK2E/OST1 signaling in stomatal closure. When the plants sense low humidity stress, the signal may integrate into two pathways. One is ABA dependent (step 1), and the other is ABA independent (step 6). ABA produced by low humidity (step 2) may act on Domain II through Pathway I (step 3). ABI1 binds to this region and may regulate SRK2E activity (step 4). ABI1 may also activate Pathway II. In this case, ABI1 and ABI2 may function as negative regulators (step 5). On the other hand, the ABA-independent pathway specifically acts on Domain I through Pathway III (step 7) and also regulates SRK2E activity (step 8). These three pathways converge (steps 5, 9) and result in the complete closure of stomata cells.

J. Biol. Chem. 5317

FEBRUARY 24, 2006 VOLUME 281 NUMBER 8
Functional and Physical Interaction between SRK2E and ABI1

17. Hrabak, E. M., Chan, C. W. M., Gribskov, M., Harper, J. F., Choi, J. H., Halford, N., Kudia, J., Luan, S., Nimmo, H. G., Sussman, M. R., Thomas, M., Walker-Simmons, K., Zhu, J. K., and Harmon, A. C. (2003) Plant Physiol. 132, 666–680
18. Umezawa, T., Yoshida, R., Maruyama, K., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 17306–17311
19. Luan, S. (2002) Plant Cell Environ. 25, 229–237
20. Liu, K., and Luan, S. (1998) Plant Cell 10, 1957–1970
21. Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5536–5540
22. Mitsuhara, I., Ugaki, M., Hirochika, H., Ohshima, M., Murakami, T., Gotoh, Y., Katayose, Y., Nakamura, S., Honkura, R., Nishimiya, S., Ueno, K., Mochizuki, A., Tanimoto, H., Tsugawa, H., Otsuki, Y., and Ohashi, Y. (1996) Plant Cell Physiol. 37, 49–59
23. Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2002) Plant J. 29, 417–426
24. Halford, N. G., Boulj, J. P., and Thomas, M. (2000) in Plant Protein Kinases, Advances in Plant Pathology (Kreis, M., and Walker, J. C., eds) pp. 405–428, Academic Press, London
25. Leung, J., Bouvier-Durand, M., Morris, P. C., Guerrier, D., Chefdor, F., and Giraudat, J. (1994) Science 264, 1448–1452
26. Leung, J., Merlot, S., and Giraudat, J. (1997) Plant Cell 9, 759–771
27. Lemichez, E., Wu, Y., Sanchez, J. P., Mettoussi, A., Mathur, J., and Chua, N. H. (2001) Genes Dev. 15, 1808–1816
28. Wang, X. Q., Ullah, H., Jones, A. M., and Assman, S. M. (2001) Science 292, 2070–2072
29. Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kubbari, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2001) Plant J. 27, 325–333
30. Seo, M., and Koshiha, T. (2002) Trends Plant Sci. 7, 41–48
31. Himmelbach, A., Hoffmann, T., Leube, M., Hohener, B., and Grill, E. (2002) EMBO J. 21, 3029–3038
32. Zhang, W., Qin, C., Zhao, J., and Wang, X. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 9508–9513
33. Ohta, M., Guo, Y., Halfter, U., and Zhu, J. K. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 11771–11776
34. Guo, Y., Xiong, L., Song, C. P., Gong, D., Halfer, U., and Zhu, J. K. (2002) Dev. Cell 3, 233–244
35. Gosti, F., Beaudoin, N., Serizet, C., Webb, A. A. R., Vartanian, N., and Giraudat, J. (1999) Plant Cell 11, 1897–1909
36. Nath, N., McCartney, R. R., and Schmidt, M. C. (2003) Mol. Cell. Biol. 23, 3909–3917
37. Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., Schlattner, U., Wallmann, T., Carlson, M., and Carling, D. (2003) Curr. Biol. 11, 2004–2008
38. Hong, S. P., Leiper, F. C., Woods, A., Carling, D., and Carlson, M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 8839–8843