Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 1767–1772, February 1999
Plant Biology

An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers

AURELIO GÓMEZ-CADENAS*†, STEVEN D. VERHEY†‡, LYNN D. HOLAPPA‡, QINGXI SHEN*, TUAN-HUA DAVID HO*§, AND M. K. WALKER-SIMMONS‡§

*Department of Biology, Washington University, St. Louis, MO 63130; and ‡U.S. Department of Agriculture–Agriculture Research Service, Washington State University, Pullman, WA 99164-6420

Communicated by Jan A. D. Zeevaart, Michigan State University, East Lansing, MI, December 16, 1998 (received for review August 7, 1998)

ABSTRACT **The phytohormone abscisic acid (ABA) induces genes-encoding proteins involved in desiccation tolerance and dormancy in seeds, but ABA also suppresses gibberellin (GA)-responsive genes encoding hydrolytic enzymes essential for postgermination growth. A unique serine/threonine protein kinase, PKABA1 mRNA, up-regulated by ABA in seeds, has been identified. In this report, the effect of PKABA1 on the signal transduction pathway mediating ABA induction and suppression of genes has been determined in aleurone layers of barley seeds. Two groups of gene constructs were introduced to barley aleurone layers by using particle bombardment: the reporter constructs containing the coding sequence of β-glucuronidase gene linked to hormone-responsive promoters and the effector constructs containing the coding region of protein kinases linked to a constitutive promoter. Constitutive expression of PKABA1 drastically suppressed expression of low- and high-pI α-amylase and protease genes induced by GA. However, the presence of PKABA1 had only a small effect on the ABA induction of a gene encoding a late embryogenesis abundant protein, HVA1. Our results indicate that PKABA1 acts as a key intermediate in the signal transduction pathway leading to the suppression of GA-inducible gene expression in cereal aleurone layers.**

The phytohormone abscisic acid (ABA) is ubiquitous in plants and plays an important role in regulating many processes, including gene expression, closure of stomata, photosynthesis, and adaptation to environmental stresses, such as drought, salinity, and cold (1–4). In no other organ is ABA more important than in seeds, where ABA responses affect seed survival and reproductive capability. ABA mediates acquisition of desiccation tolerance in maturing seeds and induction of seed dormancy, essential for long-term survival, and can block germination processes by suppressing mobilization of seed reserves. Genetic evidence for ABA involvement in these processes has been provided by ABA-deficient and nonresponsive mutants (5–7).

Most physiological responses to ABA occur through ABA-mediated gene expression. As ABA levels increase in maturing seeds, a number of ABA-responsive genes are expressed during late seed development, including the *LEA* (late embryogenesis abundant protein) genes (i.e., dehydrins, group 3 *LEA*, *HVA1*) with putative functions in desiccation protection (8, 9). Not all ABA effects on gene expression are positive. During germination of cereal grains, such as barley, wheat, and rice, the embryo synthesizes and secretes another group of phytohormones, gibberellins (GAs), to the aleurone layer, where GAs induce the transcription of α-amylase and protease genes (10). Expression of these genes is suppressed by ABA during seed development or under unfavorable germination conditions. In this context, cereal aleurone layers provide a useful system for dissection of molecular mechanisms involved in hormonally regulated gene expression in germinating seeds, particularly the antagonism between GA and ABA.

The signal transduction pathway involved in ABA-regulated gene expression has been the subject of numerous research efforts (6, 7, 11). Studies of ABA-responsive promoters have revealed a G-box-type ABA-responsive element (ABRE) necessary for the ABA-inducible transcription (12, 13). ABRE together with a coupling element (CE) form an ABA response complex (ABRC) that appears to be the minimal promoter unit necessary and sufficient for specific ABA-induced gene expression (14, 15). Several trans-acting factors involved in ABA-regulated gene expression have been reported, including VP1 in maize (16), Myc- and Myb-like proteins in *Arabidopsis* (17), and EmBP-1 in wheat (18). Phospholipase D has been proposed as an intermediate in the propagation of the ABA signal (19) in barley aleurone, and two calcium-dependent protein kinases (CDPKs) are able to activate a stress/ABA-inducible promoter in maize leaf protoplasts (20). In addition, the levels of Ca^{2+} (21) and cyclic ADP ribose (22) have been shown to be central regulators in ABA signal transduction. Most of the information available to date is centered on regulatory molecules that affect ABA induction of gene expression. However, the mode of action of ABA is complex, and besides gene induction (23, 24), this phytohormone also negatively regulates GA-responsive genes, including those encoding α-amylases and proteinases in germinating cereal grains (10). Little is known about the molecular events that transmit the ABA signal and eventually trigger both up- and down-regulated gene expression.

A candidate for a component in ABA signaling in seeds is the ABA-induced protein kinase (PKABA1) in dormant seed embryos of wheat (25). This serine/threonine protein kinase has an unusual stretch of acidic amino acid residues near the carboxyl terminus distinguishing it from other types of protein kinases. PKABA1 mRNA levels increase as ABA content increases in developing seed embryos and reach high levels at seed maturity. Levels remain high in isolated embryos treated with ABA but decline in germinating seeds. PKABA1 is induced rapidly in seedlings when ABA levels increase in response to environmental stress (26). The potential function of this protein kinase is not yet known.

In light of the important role of protein kinases in many signal transduction pathways, we have examined the involvement of PKABA1 in the signal transduction pathways mediating ABA induction and suppression of gene expression in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Abbreviations: ABA, abscisic acid; ABRC, abscisic acid response complex; CDPK, calcium-dependent protein kinase; GA_3, gibberellic acid; GA(s), gibberellin(s); LEA, late embryogenesis abundant, PKABA1, ABA-responsive protein kinase; UBI, ubiquitin; GUS, β-glucuronidase.

†A.G.-C. and S.D.V. contributed equally to this project.
§To whom reprint requests should be addressed. e-mail: ho@biodec.wustl.edu or ksimmons@wsu.edu.

barley aleurone cells. Two groups of constructs were introduced into barley aleurone layers by using particle bombardment: the reporter constructs, containing the coding sequence of β-glucuronidase (GUS) gene driven by promoters of ABA- or GA-inducible genes, and the effector constructs, containing the coding region of PKABA1 driven by a constitutive promoter. Our results indicate that PKABA1 mimics ABA in suppressing GA-inducible genes, suggesting that PKABA1 acts as an intermediate in the ABA antagonism of GA-inducible gene expression in cereal aleurone layers.

MATERIALS AND METHODS

Northern Analyses. Total RNA was isolated from barley aleurone layers (27). RNA samples were a gift from Sally W. Rogers, Washington State University. Northern analysis was conducted with PKABA1 cDNA as the probe (25).

Preparation of the DNA Constructs. The reporter constructs were prepared as follows. (*i*) Amy32b-GUS was constructed by linking the promoter (up to −331 region), the entire 5′ untranslated sequence, and the first intron of the low-pI α-amylase gene, Amy 32b, to the GUS coding sequence and the 3′ untranslated region of the same α-amylase gene (28). (*ii*) ABRC3-GUS was made by ligating the 68-bp ABRC3 (ABA response complex 3, derived from *HVA1* promoter sequence), with the *Sma*I-digested MP64 progenitor (15). (*iii*) Amy46-GUS was constructed by linking the promoter of a high-pI α-amylase gene (to −400), the entire 5′ untranslated sequence, and the first intron of the α-amylase gene, Amy 6-4, to the GUS coding sequence and the 3′ untranslated region of the Amy 46 gene (obtained from J. C. Rogers, Washington State University). (*iv*) EPB1-GUS contained a 409-bp fragment of the promoter plus the 118-bp 5′ untranslated sequence of the gene encoding a GA-induced cysteine proteinase, *EPB1* (29). The GUS coding sequence and the EPB1 3′ untranslated region (positions +1204 to +1910) were fused to this fragment (M. Cercós and T.-H.D.H., unpublished data).

The effector constructs used were UBI-PKABA1, UBI-null-PKABA1, 35S-CDPK [calcium-independent (ci) and nonactivatable (na)], and 35S-PKABA1. UBI-PKABA1 was made by linking the ubiquitin (UBI) promoter from pAHC17 (30, 31) to a reconstituted PKABA1 sequence consisting of the PKABA1 cDNA (25) with additional 5′ sequence information (comprising 10 aa) from a genomic clone, TaPK3, which has high sequence homology to PKABA1 (32) and the 3′ untranslated sequence of the Agrobacterium nopaline synthase. UBI-null-PKABA1 is identical to UBI-PKABA1 but has the nucleotide-binding site (GSGNFG, amino acids 11–16) deleted. 35S-CDPK (ci and na) were made by linking the cauliflower mosaic virus 35S promoter to the coding region of two different mutants, na and ci, of the *Arabidopsis CDPK* gene (ref. 33; J. F. Harper, unpublished observations), and the 3′ untranslated sequence Nos-T. 35S-PKABA1 was made by substituting the ubiquitin promoter in the UBI-PKABA1 for the 35S promoter used in the 35S-CDPK construct.

Particle Bombardment and Transient Expression Assays. The detailed procedure of transient expression studies with the barley (*Hordeum vulgare* L., var Himalaya, 1991 crop) aleurone system and the particle bombardment technique have been published before (24, 28). Briefly, the mixture (in a 1:0.5 molar ratio) of a reporter construct and a maize UBI-luciferase internal control construct, pAHC18 (30), was bombarded into barley embryoless half-seeds (four replicates per test construct).

To test the effect of regulatory molecules such as PKABA1, effector constructs were included at the ratio indicated in each figure. After incubation in the presence or absence of 20 μM ABA or 1 μM gibberellic acid (GA_3) for 24 h, the bombarded half-seeds in sets of four were homogenized with aleurone layers still attached to the starchy endosperm in 800 μl grinding buffer (24). After centrifugation at 12,000 × *g* for 10 min at 6°C, 100 μl of the supernatant was assayed for luciferase activity by using a luminometer (Model Moonlight 2010, Analytical Luminescence Laboratory, San Diego). For GUS assays, 50 μl of supernatant was diluted with 200 μl of GUS assay buffer (24) and incubated at 37°C for 20 h. Fifty microliters of the reaction mixture was diluted in 2 ml of 0.2 M Na_2CO_3, and the resulting fluorescence was measured in a Sequoia–Turner model 450 fluorometer (Unipath, Mountain View, CA) that was adjusted to have a reading of 1,000 units for 1 μM 4-methylumbelliferone, the reaction product of GUS. The GUS activities of all samples were normalized against the luciferase internal control. The normalized GUS activity represents the total number of fluorescence units from an aliquot of extract that contained 2,000,000 relative light units of luciferase activity.

RESULTS

A Homolog of the Wheat PKABA1 Is Present in Barley Aleurone Layers. The aim of this work was to investigate the role of PKABA1, an ABA-inducible protein kinase of wheat, in ABA signal transduction by using the barley aleurone layer system. Although barley and wheat are closely related phylogenetically, it was necessary to verify that a PKABA1 homolog is expressed in barley aleurone layers and confirm that the pattern of transcript accumulation was similar to that found in wheat (25, 26). Fig. 1 presents the RNA gel blot analysis of the steady-state levels of PKABA1 in barley aleurone cells treated with various combinations of hormones. Total RNA was isolated from barley aleurone layers and the RNA samples were probed with ^{32}P-labeled PKABA1 cDNA. A significant level of a 1.3-kb RNA, similar in size to wheat PKABA1 mRNA, was present when the aleurone layers were isolated initially from the half-seeds, and this transcript level was maintained in aleurone layers treated with 20 μM ABA for 24 h (data not shown). As shown in Fig. 1, after 24 h of treatment with control succinate buffer, a low basal level of the 1.3-kb RNA was present. GA_3 treatment decreased the basal level of this message (compare lanes 1 and 2 in Fig. 1). However, when ABA and GA_3 were added together, a significant level of PKABA1 mRNA was present. Similarly, the

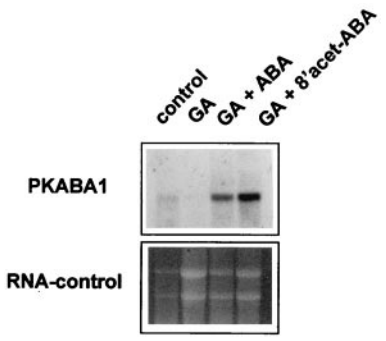

FIG. 1. Northern analysis of PKABA1 transcript levels in barley aleurone treated with GA and ABA. For mRNA analysis, barley embryoless half-seeds were imbibed in water and incubated for 4 days. Then, the aleurone layers were isolated and treated for 24 h with control buffer (20 mM succinate, pH 5, containing 10 mM $CaCl_2$) plus: GA (2 μM GA_3); GA + ABA (2 μM GA_3 + 20 μM ABA); GA + 8′-acet-ABA (2 μM GA_3 + 20 μM 8′-acetylene-ABA). Total RNA was extracted and Northern analysis was conducted with the PKABA1 cDNA clone. Ethidium bromide-stained 28S and 18S rRNA are shown as controls for loaded RNA amounts. Northern analysis results are representative of three separate experiments assessing PKABA1 mRNA levels in aleurone layers treated for 24 h with ABA and control buffers.

addition of GA_3 and 8′-acetylene-ABA, a biologically active ABA analog (34), resulted in high PKABA1 levels.

PKABA1 Suppresses the GA Induction of Low- and High-pI α-Amylases. To examine the possible role of PKABA1 in mediating the ABA induction or suppression of different genes expressed in the barley aleurone layer, we transfected half-seeds (with aleurone layers still attached to the starchy endosperm) with the PKABA1 cDNA driven by the constitutive ubiquitin gene promoter together with several reporter genes. The data (Fig. 2) show the levels of GUS activities in half-seeds bombarded simultaneously with a low-pI α-amylase-GUS construct (reporter) and the ubiquitin-PKABA1 construct (effector). The amount of reporter plasmid was always constant, whereas that of the effector varied as indicated in the *x* axis. When the low-pI α-amylase-GUS construct was transfected alone (% = 0 on the *x* axis), the treatment with 1 μM GA_3 strongly induced the expression of this gene construct. The GUS activity increased from 5.0×10^2 units in bombarded tissue incubated with no hormones to 2.8×10^4 units in tissue incubated with 1 μM GA_3, representing a more than 50-fold increase of GA induction, similar to that reported by Lanahan *et al.* (28). As expected, the treatment with both GA_3 (1 μM) and ABA (20 μM) abolished the GA_3 induction of this gene construct (28). The coexpression of PKABA1 did not affect the basal levels of expression of the low-pI α-amylase gene in the absence of GA_3. However, the expression of the low-pI α-amylase-GUS in response to GA_3 was reduced markedly by the presence of PKABA1 even at very low concentrations. When the relative amount of effector was 25% and 50%, the GUS expression was inhibited by a factor of 3 and 8, respectively. Higher amounts of effector construct virtually blocked the GA_3 induction of low-pI α-amylase-GUS, with GUS activities close to the basal level. The same pattern of suppression by PKABA1 was observed when these experiments were repeated by using a high-pI α-amylase promoter linked to GUS as a reporter construct (data not shown).

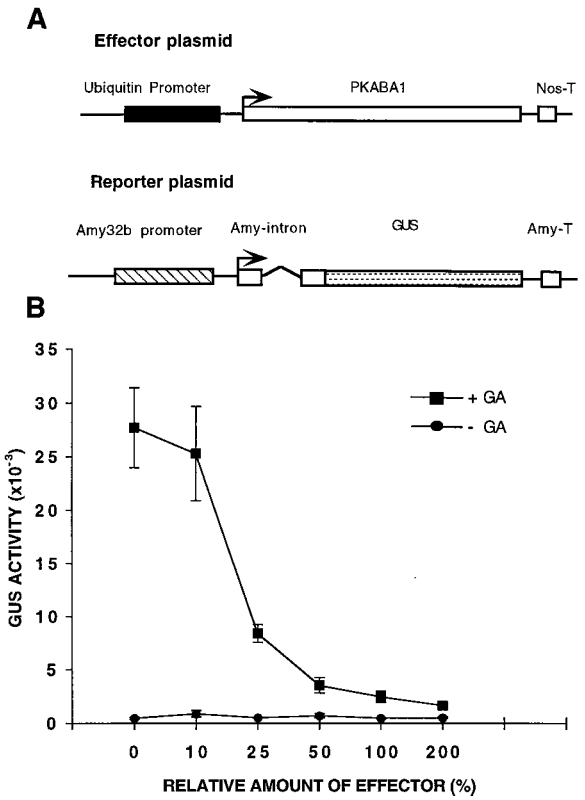

FIG. 2. PKABA1 suppresses the GA_3-induced expression of a low-pI α-amylase gene. (*A*) Schematic diagram of the effector and reporter constructs used in cobombardment experiments. (*B*) The effector construct, UBI-PKABA1, was cobombarded into barley half-seeds (with aleurone layers still attached to the starchy endosperm) along with the reporter construct (Amy32b promoter-GUS) and the internal control construct (UBI-luciferase). The amount of reporter and control plasmid DNA was always constant (1.14 μg per shot), whereas that of effector varied with respect to the reporter as shown in the *x* axis. Transfected half-seeds were incubated for 24 h with (■) or without (●) 1 μM GA_3. GUS activity was normalized in every independent transformation relative to the luciferase activity. Data are means ± SE of four replicates in a typical experiment. The experiment was repeated three times, and similar results were obtained.

PKABA1 Suppresses the GA Induction of a Cysteine Proteinase. EPB is one of the main cysteine proteinases responsible for the degradation of endosperm storage proteins in germinating barley seeds. The *EPB* gene family consists of two very similar genes, *EPB-1* and *EPB-2* (29) and, as with the α-amylases, the expression of these genes is induced by GA and suppressed by ABA. The effect of PKABA1 on the response of the cysteine proteinase (EPB-1) to GA_3 was tested. Fig. 3 shows that the coexpression of PKABA1 with EPB-1-GUS mimicked the effect of ABA in reducing the expression of *EPB-1* by approximately 60%.

PKABA1 Has a Small Effect on the Expression of HVA1 in the Absence of ABA. ABA also induces several genes in the barley aleurone layers. Shen *et al.* (14, 15) previously have defined the minimal promoter unit, named ABA response complex 3 (ABRC3), which is necessary and sufficient for ABA induction of one of these genes, *HVA1*. The ABRC3-GUS construct was used as another reporter construct. The

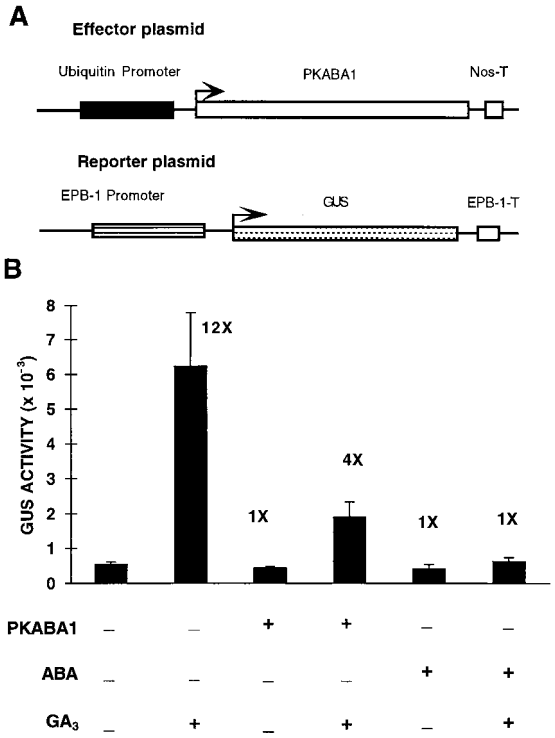

FIG. 3. PKABA1 suppresses the GA_3-induced expression of a cysteine proteinase gene. (*A*) Schematic diagram of the effector and reporter constructs used in cobombardment experiments. (*B*) The reporter construct, EPB1-GUS, and the internal control construct, UBI-luciferase, were cobombarded into barley half-seeds either with or without the effector construct (UBI-PKABA1) using the same amount of effector and reporter constructs (1.14 μg per shot). Transfected barley half-seeds were incubated for 24 h with or without 1 μM GA_3 and/or 20 μM ABA. GUS activity was normalized in every independent transformation relative to the luciferase activity. Data are means ± SE of four replicates in a typical experiment. The experiment was repeated three times, and similar results were obtained.

data (Fig. 4) indicate that the expression of PKABA1 along with ABRC3-GUS did not significantly alter the levels of the normalized GUS activity reached in response to ABA treatment. The basal level of GUS activity detected in tissues transfected with ABRC3-GUS and treated with no hormones was 8.0×10^2 units, whereas levels of GUS varied between 34.7 and 27.7×10^3 units (36- to 45-fold ABA induction) in seeds bombarded with ABRC3-GUS alone or cobombarded with UBI-PKABA1 and ABRC3-GUS and subsequently treated with ABA. On the other hand, PKABA1 had a small effect on the basal levels of GUS activity detected in the seeds transformed with ABRC3-GUS treated with no hormones; GUS activity was up to five times higher (4.1×10^3 units) in the tissues cobombarded with PKABA1 and ABRC3-GUS (200% of effector plasmid) than in that transformed only with the reporter construct (8.0×10^2 units).

Other Protein Kinases Do Not Suppress the GA Induction of a Low-pI α-Amylase. Two different sets of experiments were designed to define the specificity of the suppressory effect of PKABA1 and to determine whether the protein kinase activity is required for the functionality of the protein. Two constitutively active mutants of CDPK of *Arabidopsis* (ref. 33; J. F. Harper, unpublished observations) were used as controls. 35S-PKABA1 blocked the expression of the low-pI α-amylase gene construct as effectively as ubiquitin-PKABA1 (Fig. 5). In contrast, 35S-CDPKci did not significantly affect the expression of this α-amylase construct. The same results were obtained when another mutant, 35S-CDPKna, was used (data not shown). Heterologous expression of regulatory proteins

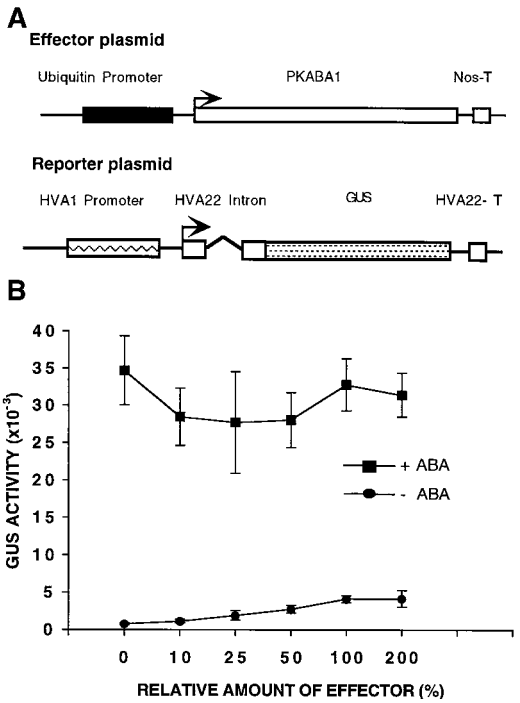

FIG. 4. PKABA1 has a small effect on the response of ABRC3 (ABA response complex 3) to ABA treatment. (*A*) Schematic diagram of the effector and reporter constructs used in cobombardment experiments. (*B*) The effector construct, UBI-PKABA1, was cobombarded into barley half-seeds along with the reporter construct (ABRC3-GUS) and the internal control construct (UBI-luciferase). The amount of reporter and control plasmid DNA was always constant (1.14 μg per shot), whereas that of effector varied with respect to the reporter as shown in the *x* axis. Transfected barley half-seeds were incubated for 24 h with (■) or without (●) 20 μM ABA. GUS activity was normalized in every independent transformation relative to the luciferase activity. Data are means ± SE of four replicates in a typical experiment. The experiment was repeated three times, and similar results were obtained.

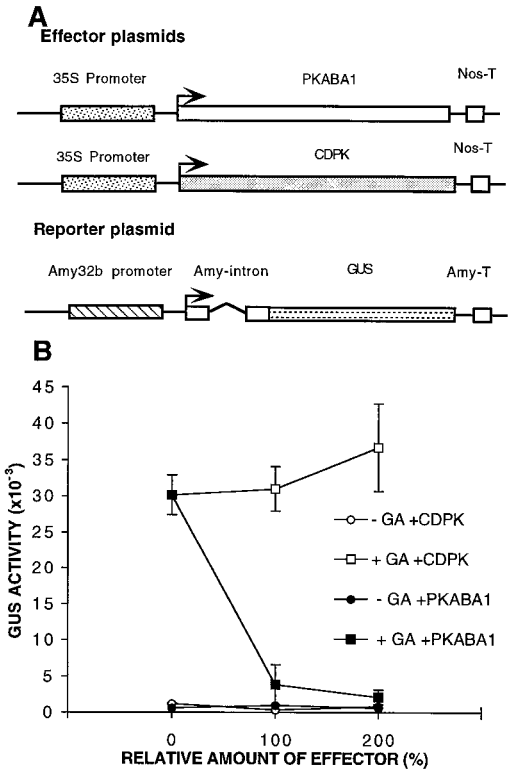

FIG. 5. CDPKci does not suppress the GA_3-induced expression of a low-pI α-amylase. (*A*) Schematic diagram of the effector and reporter constructs used in cobombardment experiments. (*B*) 35S-CDPK (open symbols) or 35S PKABA1 (solid symbols) were cobombarded into barley half-seeds along with the reporter construct (Amy32b promoter-GUS) and the internal control construct (UBI-luciferase). The amount of reporter and control plasmid DNA was always constant (1.14 μg per shot), whereas that of effector varied with respect to the reporter as shown in the *x* axis. Transfected half-seeds were incubated for 24 h with (squares) or without (circles) 1 μM GA_3. GUS activity was normalized in every independent transformation relative to the luciferase activity. Data are means ± SE of four replicates in a typical experiment. The experiment was repeated three times, and similar results were obtained.

such as protein kinases and phosphatases from *Arabidopsis* has been confirmed in maize mesophyll protoplast (20) and barley aleurone layers (Q.S. and T.-H.D.H., unpublished data).

The Nucleotide-Binding Site Is Required for the PKABA1 Suppression of Gene Expression. The glycine-rich loop is one of the most highly conserved sequence motifs in protein kinases and considered an integral part of the ATP-binding site (35). A null mutant of PKABA1 was made by deleting the glycine-rich loop (GSGNFG, amino acids 11–16), based on previous reports that mutagenesis of the glycine-rich loop of other protein kinases caused major reductions in kinase activity (35, 36). When the PKABA1 null-mutant driven by the same ubiquitin promoter was transfected along with the low-pI α-amylase-GUS construct, no significant inhibitory effect was observed. GA_3 induction was only slightly affected when the UBI-null-PKABA1 was cobombarded with the low-pI α-amylase-GUS construct (Fig. 6).

DISCUSSION

In this work, we have shown that the constitutive expression of PKABA1 strongly suppressed the GA induction of low- and high-pI α-amylase and proteinase genes but had little effect on the expression of ABA-induced *LEA* genes such as *HVA1*. We also transfected several control constructs with the reporter genes, showing that both a null mutant of PKABA1 and a

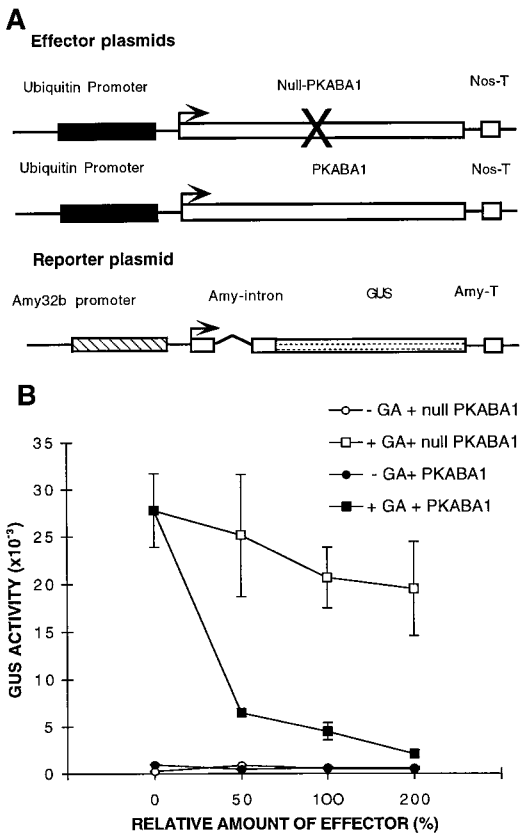

FIG. 6. The nucleotide-binding site of PKABA1 is required to suppress the GA_3-induced expression of a low pI α-amylase gene. (*A*) Schematic diagram of the effector and reporter constructs used in cobombardment experiments. (*B*) The effector constructs, UBI-Null-PKABA1 (open symbols) or UBI-PKABA1 (solid symbols), were cobombarded into barley half-seeds along with the reporter construct (Amy32b promoter-GUS) and the internal control construct (UBI-luciferase). The amount of reporter and control plasmid DNA was always constant (1.14 μg per shot), whereas that of effector varied with respect to the reporter as shown in the *x* axis. Transfected half-seeds were incubated for 24 h with (squares) or without (circles) 1 μM GA_3. GUS activity was normalized in every independent transformation relative to the luciferase activity. Data are means ± SE of four replicates in a typical experiment. The experiment was repeated three times, and similar results were obtained.

different serine/threonine protein kinase (CDPK) failed to show any significant effect on α-amylase expression. These results strongly suggest that PKABA1 acts specifically as an intermediate in the ABA suppression of GA-inducible gene expression in cereal aleurone layers. A diagram summarizing these findings is shown in Fig. 7.

Several reports that use transient expression of various gene constructs to demonstrate the involvement of several intermediates in hormone signal transduction have been published

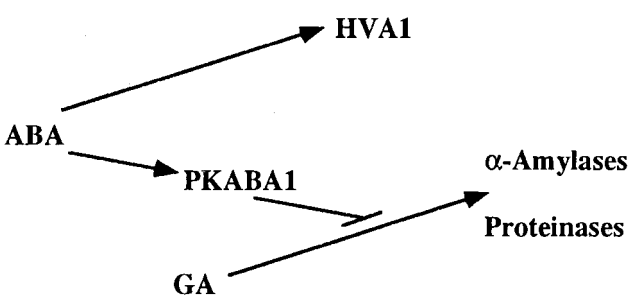

FIG. 7. Diagram summarizing the proposed role of PKABA1 in the suppression of hydrolytic enzyme expression in barley aleurone cells.

(15, 17, 20, 37–39). Previous research has established that GA and ABA tightly regulate the expression of both low- and high-pI α-amylases and a cysteine proteinase (EPB-1) (28, 29, 40, 41). Similarly, GA_3 treatment effectively induced the expression of these three genes in the aleurone system used in this work (Figs. 2 and 3), and, moreover, PKABA1 strongly inhibited their expression in response to GA, mimicking the effect of ABA. Because the expression of PKABA1 is induced by ABA, one might predict that PKABA1 also could induce the expression of ABA up-regulated genes, such as *HVA1*. However, data in Fig. 4 show that the expression of PKABA1 only slightly induced the expression of ABA-responsive construct, ABRC3 (derived from *HVA1*)-GUS. This effect appeared to be small, much less than the 40-fold induction of the same gene construct by ABA (Fig. 4). The effect of PKABA1 appears to be different from another regulatory protein, HvSPY, which affects both ABA induction and suppression of gene expression (42). It also has been reported that the expression of the *HVA1* gene is highly induced by two calcium-dependent protein kinases (CDPK1 and CDPK1a) in maize leaf protoplasts following similar coexpression approaches (20). Taken together, these data suggest the involvement of several protein kinases in ABA signal transduction and the existence of two separate pathways leading to the ABA induction or suppression of ABA-responsive genes as shown in Fig. 7. ABA suppression of hydrolytic enzymes is mediated by PKABA1, whereas CDPK1 may act only in the ABA induction of *HVA1*, although there are no data to rule out the possibility that CDPK1 mediates both pathways.

It could be argued that the constitutive expression of PKABA1 in aleurone cells produced a general and nonspecific inhibitory effect on the expression of the proteinase and α-amylase constructs. However, several lines of evidence support the specificity and physiological validity of the effect described in this report. First, the inhibitory effect of PKABA1 is observed at a very low relative amount of effector. Second, the constitutive expression of the UBI-luciferase construct (internal control) is not affected at all when PKABA1 is coexpressed (data not shown); neither is the ABA-induction of *HVA1* suppressed by the presence of PKABA1 (Fig. 4), and only a small effect on HVA1 basal level is detected when PKABA1 is coexpressed with HVA1 in the absence of ABA. Third, the coexpression of two mutants of a different protein kinase, CDPKci and CDPKna, driven by a constitutive promoter (35S) did not affect the GA-induction of the low-pI α-amylase (Fig. 5), whereas in the same experiment, PKABA1 driven by the identical promoter (35S) had a strong inhibitory effect. Finally, a null mutant of PKABA1 lacking the nucleotide-binding site (Fig. 6) failed to suppress the expression of the low-pI α-amylase, indicating that the protein kinase activity is essential for the PKABA1 effect to be observed.

Current evidence supports the notion that reversible protein phosphorylation affects gene expression in response to ABA. Protein phosphatase inhibitors interfere with both ABA and GA signal transduction in barley aleurone (43). The *ABI1* and *ABI2* genes of *Arabidopsis* that encode protein phosphatases (6) are required for ABA-responsive gene expression. Mutational analysis of *ABI1* and *AtPP2C* in ABA signaling in maize mesophyll protoplasts has been carried out (39). In addition to PKABA1, several ABA-responsive protein kinase mRNAs have been identified (11). Protein kinases and phosphatases may act in ABA signaling by regulating transcription factors for ABA-responsive genes via reversible phosphorylation. Transcription factors that affect GA-responsive gene expression also may be targets of PKABA1.

During evolution, many components of signal transduction, particularly protein kinases, have been highly conserved among plants, animals, and microorganisms (44, 45). The PKABA1 subfamily of protein kinases (32) has the highest degree of sequence identity with yeast, mammalian, and plant

kinases involved in the regulation of carbon metabolism. PKABA1 has a high degree of identity with yeast sucrose nonfermenting protein kinases (SNF1) and its plant homologs and mammalian AMP-activated protein kinase (AMPK). SNF1 protein kinase activity is required for transcriptional activation of glucose-repressed genes in yeast. Several *SNF1* homologs have been identified in plants, and a functional relationship has been shown by the complementation of the yeast *snf1* mutant with rye *RKIN1* (44, 45). When animal cells are subjected to stress such as heat shock or hypoxia, AMP levels rise and mammalian AMPK is activated. AMPK protects cells from stress by inactivating biosynthetic pathways, including fatty acid synthesis, and conserving ATP (44, 45). It can be inferred from our results that PKABA1 functions to regulate the accessibility of the germinating embryo to its primary energy resources via the breakdown of the starchy endosperm. Although other roles for PKABA1 cannot be discounted, it is likely that PKABA1 helps prevent seed embryos from germinating under unfavorable conditions. It thus appears that PKABA1, yeast and plant SNF1s, and mammalian AMPK not only share sequence similarities but also functional roles in regulating carbon metabolism and acclimating cells to stressful conditions.

We thank A. H. Christensen and P. H. Quail for providing the ubiquitin constructs, Jeff Harper for the CDPK constructs, and Sally W. Rogers for the barley aleurone RNA samples. This work was supported by a National Science Foundation grant (IBN-9408900) and a U.S. Department of Agriculture grant (NRICGP 97-35100-4228) to T.-H.D.H., a U.S. Department of Agriculture grant (NRICGP 94-37100-0313) to M.K.W.-S. and S.D.V., and a fellowship from Ministerio de Educacion y Cultura, Spain, to A.G.-C.

1. Zeevaart, J. A. D. & Creelman, R. A. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39,** 439–473.
2. Ingram, J. & Bartels, D. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47,** 377–403.
3. Bray, E. (1997) *Trends Plant Sci.* **2,** 48–54.
4. Quatrano, R. S., Bartels, D., Ho, T.-H. D. & Pages, M. (1997) *Plant Cell* **9,** 470–475.
5. Karssen, C. M., Brinkhorst-van der Swan, D. L. C., Breekland, A. E. & Koornneef, M. (1983) *Planta* **157,** 158–165.
6. Giraudat, J., Parcy, F., Bertauche, N., Gosti, F., Leung, J., Morris, P. C., Bouvier Durand, M. & Vartainiaan, N. (1994) *Plant Mol. Biol.* **26,** 1557–1577.
7. Bonetta, D. & McCourt, P. (1998) *Trends Plant Sci.* **3,** 231–235.
8. Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.-H. D. & Wu, R. (1996) *Plant Physiol.* **110,** 249–257.
9. Chandler, P. M. & Robertson, M. (1994) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45,** 113–141.
10. Jacobsen, J. V., Gubler, F. & Chandler, P. M. (1995) in *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, ed. Davies P. J. (Kluwer, Dordrecht, The Netherlands), pp. 246–271.
11. Busk, P. K. & Pages, M. (1998) *Plant Mol. Biol.* **37,** 425–435.
12. Marcotte, W. R., Jr., Russell, S. H. & Quatrano, R. S. (1989) *Plant Cell* **1,** 969–978.
13. Mundy, J., Yamaguchi-Shinozaki, K. & Chua, N.-H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1406–1410.
14. Shen, Q. & Ho, T.-H. D. (1995) *Plant Cell* **7,** 295–307.
15. Shen, Q., Zhang, P. & Ho, T.-H. D. (1996) *Plant Cell* **8,** 1107–1119.
16. McCarty, D. R., Hattori, T., Carson, C. B., Vasil, V., Lazar, M. & Vasil, I. K. (1991) *Cell* **66,** 895–905.
17. Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D. & Shinozaki, K. (1997) *Plant Cell* **9,** 1859–1868.
18. Guiltinan, M. J., Marcotte, W. R., Jr. & Quatrano, R. S. (1990) *Science* **250,** 267–271.
19. Ritchie, S. & Gilroy, S. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2697–2702.
20. Sheen, J. (1996) *Science* **274,** 1900–1902.
21. Gilroy, S. (1996) *Plant Cell* **8,** 2193–2209.
22. Wu, Y., Kuzma, J., Marechal, E., Graeff, R., Lee, H. C., Foster, R. & Chua, N.-H. (1997) *Science* **278,** 2126–2130.
23. Hong, B., Uknes, S. J. & Ho, T. H. D. (1988) *Plant Mol. Biol.* **11,** 495–506.
24. Shen, Q., Uknes, S. J. & Ho, T. H. D. (1993) *J. Biol. Chem.* **268,** 23652–23660.
25. Anderberg, R. J. & Walker-Simmons, M. K. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10183–10187.
26. Holappa, L. D. & Walker-Simmons, M. K. (1995) *Plant Physiol.* **108,** 1203–1210.
27. Rogers, J. C. (1985) *J. Biol. Chem.* **260,** 3731–3738.
28. Lanahan, M. B., Ho, T.-H. D., Rogers, S. W. & Rogers, J. C. (1992) *Plant Cell* **4,** 203–211.
29. Mikkonen, A., Porali, I., Cercos, M. & Ho, T.-H. D. (1996) *Plant Mol. Biol.* **31,** 239–254.
30. Bruce, W. B., Christensen, A. H., Klein, T., Fromm, M. & Quail, P. H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9692–9696.
31. Christensen, A. H. & Quail, P. H. (1996) *Transgenic Res.* **5,** 213–218.
32. Holappa, L. D. & Walker-Simmons, M. K. (1997) *Plant Mol. Biol.* **33,** 935–941.
33. Harper, J. F., Huang, J.-F. & Lloyd, S. J. (1994) *Biochemistry* **33,** 7267–7277.
34. Rose, P. A., Cutler, A. J., Irvine, N. M., Shaw, A. C., Squires, T. M., Lowen, M. K. & Abrams, S. R. (1997) *Bio. Med. Chem. Lett.* **7,** 2543–2546.
35. Hemmer, W., McGlone, M., Tsigelny, I. & Taylor, S. S. (1997) *J. Biol. Chem.* **272,** 16946–16954.
36. Lee, J.-H., Maeda, S., Angelos, K. L., Kamita, S. G., Ramachandran, C. & Walsh, D. A. (1992) *Biochemistry* **31,** 10616–10625.
37. Gubler, F., Kalla, R., Roberts, J. K. & Jacobsen, J. V. (1995) *Plant Cell* **7,** 1879–1891.
38. Penson, S. P., Schuurink, R. C., Fath, A., Gubler, F., Jacobsen, J. V. & Jones, R. L. (1996) *Plant Cell* **8,** 2325–2333.
39. Sheen, J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 975–980.
40. Jacobsen, J. V. & Close, T. J. (1991) *Plant Mol. Biol.* **16,** 713–724.
41. Rogers, J. C., Lanahan, M. B. & Rogers, S. W. (1994) *Plant Physiol.* **105,** 151–158.
42. Robertson, M., Swain, S. M., Chandler, P. M. & Olszewski, N. E. (1998) *Plant Cell* **10,** 995–1007.
43. Kuo, A., Cappelluti, S., Cervantes-Cervantes, M., Rodriguez, M. & Bush, D. S. (1996) *Plant Cell* **8,** 259–269.
44. Hardie, D. G., Carling, D. & Carlson, M. (1998) *Annu. Rev. Biochem.* **67,** 821–855.
45. Halford, N. G. & Hardie, D. G. (1998) *Plant Mol. Biol.* **37,** 735–748.