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A *Triticum tauschii* protein kinase related to wheat PKABA1 is associated with ABA signaling and is distributed between the nucleus and cytosol

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Abstract

A new member of the cereal PKABA1 subfamily of protein kinases, TtPK1, was isolated from *Triticum tauschii*, a diploid progenitor of hexaploid wheat, *Triticum aestivum*. The full-length *TtPK1* cDNA was cloned from a library derived from vegetative tissues from 26 d old light grown *T. tauschii* seedlings. *TtPK1* cDNA hybridizes to transcripts that are upregulated in dehydrated leaves and are abundant in coleoptile tissue of 7 d old *T. aestivum* seedlings. *TtPK1* mRNA has nucleotide identities of 82 and 79% to *PKABA1* and *TaPK3*, respectively, and deduced amino acid sequence identities of 84 and 83% to *PKABA1* and *TaPK3*, respectively. TtPK1 is similar to members of the SnRK2 subfamily of protein kinases in that it contains a unique acidic domain at the carboxyl terminus, and all twelve of the conserved subdomains found in serine/threonine protein kinases. Functional analyses of TtPK1 transiently over-expressed by bombarding barley half-grains showed that TtPK1 could suppress gibberellic acid inducible *alpha*-amylase gene expression, a suppressive activity similar to that of both PKABA1 and ABA. When transiently expressed in barley aleurone protoplasts, TtPK1-GFP accumulates in the nucleus and cytosol while a mutant TtPK1-GFP was localized only to the cytoplasm and vacuoles.

Keywords: *Triticum tauschii*; Protein kinase; SNRK; GFP; PKABA1; ABA; Dehydration

1. Introduction

Protein kinases in the AMP-activated protein kinase (AMPK) family of mammals, and the related *s*ucrose *n*on-*f*ermenting *k*inase (SNF1) family of yeast, have critical roles in the perception and transduction of cellular responses to nutrient and environmental stresses (Halford and Hardie, 1998; Kemp et al., 2003). When mammalian cells are subjected to stress associated with cellular energy changes, e.g. nutrient limitation, heat shock, hypoxia, or exercise, AMPK is activated by phosphorylation and allosterically by AMP to promote ATP production over its consumption (Hardie, 2003). AMPKs are heterotrimeric (α,β,γ)

Abbreviations: AAPK, ABA-activated protein kinase; ABA, abscicic acid; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; EST, expressed sequence tag; GA, gibberellic acid; GFP, green fluorescent protein; GUS, β-glucuronidase; NBT-BCIP, OST, open stomata; *PKABA*, *Protein kinase Abscicic acid(ABA)*; PCR, polymerase chain reaction; PSV, protein storage vacuole; PVDF, poyvinylidene fluoride; SDVAP, streptavidin alkaline phosphate; SnRK, SNF1-related protein kinases; SNF, sucrose *n*on-*f*ermenting kinase; *TaPK*, Triticum aestivum *protein kinase*; *WPK*, *wheat protein kinase*.

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complexes with the α catalytic subunit isoforms (α1 and α2) exhibiting different tissue distributions. Subunit α2, a member of the SNF1 subfamily, exhibits preferential nuclear localization (Salt et al., 1998).

SNF1 is activated by phosphorylation when glucose concentrations are low (Halford and Hardie, 1998). Activated SNF1 controls such processes as metabolic adaptation to glucose deprivation, growth on alternative carbon sources, and the activity of enzymes of fatty acid and glycogen metabolism. The subcellular localization and activity of SNF1 α-subunit is determined by its interaction with specific β-subunits. Translational fusion proteins of SNF1 β-subunits with green fluorescent protein (GFP) show distinct localization patterns to the nucleus, vacuole, or cytoplasm. Migration of these β-subunits between these locations depends on their interactions with each other, and on their phosphorylated state (Vincent et al., 2001). SNF1 activity in glucose signaling in yeast is thus partly determined by where in the cell SNF1 resides.

SNF1-related protein kinases (SnRKs) have been identified in various plant species and more recently in human and mice, suggesting that these kinases may have evolutionarily conserved roles (Gardner et al., 2000; Halford and Hardie, 1998; Hrabak et al., 2003; Kertesz et al., 2002; Yoshida et al., 2002). In Arabidopsis, 38 SnRKs have been identified based on its sequenced genome and gene prediction analyses (Hrabak et al., 2003). These kinases fall into three subfamilies designated SnRK1, SnRK2, and SnRK3. The multiplicity of these kinases may provide specific as well as redundant levels of regulation. Members of the plant SnRK1 group can functionally complement the *snf1* mutant of yeast suggesting a function in plant cells similar to that in yeast (Rolland et al., 2002).

SnRK2s are implicated in sensing changes in the environment (Halford and Hardie, 1998; Holappa and Walker-Simmons, 1995; Shen et al., 2001; Yoon et al., 1997). Wheat *Protein kinase Abscisic acid (ABA) responsive (PKABA1)* was the first member of the SnRK2 subfamily to be identified from plants. *PKABA1* is transcriptionally up-regulated in response to environmental stress via changes in ABA concentration in vegetative tissues and developing embryos of wheat (Anderberg and Walker-Simmons, 1992; Holappa and Walker-Simmons, 1995). *PKABA1*-like genes have been identified in many plant genomes and include *Triticum aestivum protein kinase 3* (*TaPK3*), soybean *SPK3*, and Arabidopsis *SNRK2E* genes (Holappa and Walker-Simmons, 1997; Yoon et al., 1997; Yoshida et al., 2002). On the other hand, SnRK3 kinases such as *wheat protein kinase 4* (*WPK4*) and its Arabidopsis homologue (*AtSR1*) are responsive to white light (Nozawa et al., 2001).

Few SnRK genes have been shown to have a role in hormone signaling, but some SnRK2 genes have roles in ABA signaling. PKABA1 acts as a key intermediate in the signal transduction pathway leading to the suppression of gibberellic acid (GA)-inducible gene expression in barley aleurone (Gomez-Cadenas et al., 1999). Esi47, a *PKABA1* homologue isolated from roots of wild wheat grass (*Lophopyrum elongatum*), is ABA and salt-inducible, and can also suppress GA-responsive promoters (Shen et al., 2001). The ABA-activated protein kinase (AAPK) regulates ABA-induced stomatal closure in *Vicia faba* (Li et al., 2000), as does its homologue, open stomata 1 (OST1) in Arabidopsis guard cells (Mustilli et al., 2002).

The subcellular location of most plant SnRKs is unknown. In *V. faba*, ABA-activated protein kinase-green fluorescent protein (AAPK-GFP) translational fusions have shown that AAPK resides in the cytoplasm and nuclei of guard cells. AAPK interacts with heteronuclear RNA binding protein (AKIP1) that is also present in the nucleus. Interestingly, ABA also affects the subnuclear partitioning of AKIP1 (Li et al., 2000).

We are interested in the functional roles of SnRKs with respect to environmental and ABA signaling pathways in cereals (Gomez-Cadenas et al., 1999; Holappa and Walker-Simmons, 1995, 1997). We hypothesize that the redundancy in the PKABA1-like kinases contributes to the plethora of responses in cereals to altered environmental, metabolic, and nutrient conditions. In this study, we present evidence that *TtPK1* mRNA is upregulated in dehydrated wheat leaves, is abundant in coleoptiles, and is present at lower levels in crown and root tissues. We also show that *TtPK1* has highest sequence identities to expressed sequence tags (EST) from wheat, barley, and rice that have not been functionally assessed. We show that the predicted *TtPK1* polypeptide belongs to the SnRK2 subfamily, and we present evidence that the *TtPK1* cDNA can suppress transcription from a GA-inducible *alpha*-amylase promoter. We also demonstrate that TtPK1-GFP accumulation in the nucleus is dependent on a nucleotide binding site in the N-terminus of the protein.

2. Experimental

2.1. Plant material

Winter wheat (*Triticum aestivum* L, cv Brevor) grain was obtained from field plots at Spillman Farm near Pullman, WA. Barley (*Hordeum vulgare*, L, cv Himalaya) grain was from the Department of Agronomy, Washington State University, Pullman). Seeds were germinated in covered Petri dishes with water in a growth chamber in darkness at 22 °C. At 2 d covers were removed from the Petri dishes. To maintain fully hydrated conditions, all seedlings were grown so roots were bathed in water in a growth chamber with a 16-h photoperiod (100 μE/m^2/s) at 100% RH. For dehydration treatments, 30 shoots for each time point were excised (4 cm from each tip) 7 d after the beginning of imbibition, weighed for fresh weight (FW), and placed in a beaker inside a chamber equilibrated with 25% (v/v) glycerol to 85% RH.