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Research article

TaSK5, an abiotic stress-inducible GSK3/shaggy-like kinase from wheat, confers salt and drought tolerance in transgenic *Arabidopsis*

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ABSTRACT

A novel cold-inducible GSK3/shaggy-like kinase, TaSK5, was isolated from winter wheat using a macroarray-based differential screening approach. TaSK5 showed high similarity to *Arabidopsis* subgroup I GSK3/shaggy-like kinases ASK-alpha, AtSK-gamma and ASK-epsilon. RNA gel blot analyses revealed *TaSK5* induction by cold and NaCl treatments and to a lesser extent by drought treatment. *TaSK5* functionally complemented the cold- and salt-sensitive phenotypes of a yeast GSK3/shaggy-like kinase mutant, △*mck1*. Transgenic *Arabidopsis* plants overexpressing *TaSK5* cDNA showed enhanced tolerance to salt and drought stresses. By contrast, the tolerance of the transgenic plants to freezing stress was not altered. Microarray analysis revealed that a number of abiotic stress-inducible genes were constitutively induced in the transgenic *Arabidopsis* plants, suggesting that TaSK5 may function in a novel signal transduction pathway that appears to be unrelated to DREB1/CBF regulon and may involve crosstalk between abiotic and hormonal signals.

1. Introduction

Glycogen synthase kinase 3 (GSK3) is a cytoplasmic serine/threonine kinase that phosphorylates and thereby inhibits glycogen synthase, the enzyme that catalyzes the last step of glycogen biosynthesis (Plyte et al., 1992). GSK3 also directly phosphorylates more than 18 different substrates that are involved in diverse physiological responses, including gene expression as well as protein biosynthesis, subcellular targeting, and degradation in mammalian cells [for review see (Cohen and Frame, 2001)].

The genome of the yeast *Saccharomyces cerevisiae* contains four GSK3/shaggy-like (SGG) genes, *MCK1*, *MDS1*/*RIM11*, *MRK1*, and *YOL128c*. MCK1 plays a role in the mitotic chromosomal segregation that is specific to CDEIII function (Shero and Hieter, 1991). MCK1 also acts in the transcription of IME1 at the beginning of meiosis (Neigeborn and Mitchell, 1991) and is important for inducing cell cycle delay in response to Ca^{2+} (Mizunuma et al., 2001). Thus, *S. cerevisiae* GSK3 seems to play important roles in both meiosis and mitosis. In addition, MCK1 has functions relevant to stress signal transduction: a deletion mutant of *MCK1* displays cold-, salt- and heat-sensitive phenotypes (Piao et al., 1999; Puziss et al., 1994; Shero and Hieter, 1991). MCK1 modulates the binding of the transcription factor MSN2 to the stress-responsive element within promoters of downstream genes and activates stress responses (Hirata et al., 2003).

In contrast to the small number of GSK3 genes that are present in animals and microbes, an expansion of the GSK3/SGG kinase gene family has apparently occurred in plants. GSK3/SGG kinases have been identified in a number of plant species and classified into 4 subgroups (I-IV) based on phylogenetic analyses (Dornelas et al., 2000; Jonak and Hirt, 2002; Sun and Allen, 2005). The GSK3/SGG gene family in *Arabidopsis thaliana* comprises ten members. One of the best understood functions of plant GSK3/SGG kinases pertains to the brassinosteroid (BR) signaling

Abbreviations: ABA, abscisic acid; CA, cold acclimated; G6PD, glucose-6-phosphate dehydrogenase; GSK3, glycogen synthase kinase 3; LD, long day; NA, non acclimated; ROS, reactive oxygen species; SGG, shaggy-like.

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pathway (Li and Nam, 2002). A member of the subgroup II *Arabidopsis* GSK3/SGG kinase family named BIN2 (AtSK-etha/AtSK21) plays a central role in this pathway. To date at least seven out of the ten AtSKs have been implicated in BR signaling (Saidi et al., 2012). Besides their redundant involvement in BR signaling, substrate specificity for other pathways, including environmental stress responses and developmental regulation, has been shown for some other AtSKs. Two subgroup I GSK3/SGG genes, *AtSK11* and *AtSK12*, are involved in *Arabidopsis* flower development (Dornelas et al., 1999). In addition, AtSK11 regulates the oxidative stress response and its overexpression confers reduced ROS levels and salt tolerance in transgenic *Arabidopsis* (Dal Santo et al., 2012). Expression of a salt-induced *Arabidopsis* subgroup II GSK3/shaggy homolog, *AtGSK1*/*AtSK22*, in *S. cerevisae* complemented the salt and heat sensitivity of the yeast Δ*mck1* mutant (Piao et al., 1999). Constitutive over-expression of *AtGSK1* conferred resistance to high salinity in transgenic *Arabidopsis* plants (Piao et al., 2001). A member of subgroup I, AtSK13, is induced by osmotic and salinity stresses, whereas AtSK31 (subgroup III) is flower specific and responsive to osmotic changes and darkness (Charrier et al., 2002). Research in several other plant species has also implicated GSK3/SGG kinases in abiotic stress responses. The kinase activity of an alfalfa (*Medicago sativa* L.) plastid-localized subgroup IV GSK3/SGG homolog, MsK4, was rapidly increased under hyperosmotic conditions and its overexpression in *Arabidopsis* improved salt tolerance through changes in carbohydrate metabolism (Kempa et al., 2007). A rice (*Oryza sativa* L.) mutant with a T-DNA insertion in OsGSK1, a subgroup II GSK3/SGG kinase, showed elevated expression of specific stress-responsive genes and strong tolerance of high salt and drought treatments (Koh et al., 2007). It was recently reported that overexpression of TaGSK1, a wheat (*Triticum aestivum* L.) homolog of *Arabidopsis* salt stress-inducible subgroup I GSK3/SGG, confers salt and osmotic stress tolerance (He et al., 2012).

In the present study, we report cDNA cloning and functional characterization of a wheat subgroup I GSK3/SGG kinase (*TaSK5*) that is up-regulated during cold acclimation and abiotic stresses. Our data suggested that TaSK5 is involved in abiotic stress signaling.

2. Materials and methods

2.1. Plant materials, growth conditions, and stress treatment

Surface-sterilized seeds of winter wheat (*T. aestivum* L., cv. Chihokukomugi) were germinated on wet paper and planted in commercial soil mix. Plants were grown at 22 °C/18 °C (16 h light/8 h dark) in a growth chamber for 14 d prior to the initiation of cold acclimation. Cold acclimation was performed at 6 °C/2 °C (8 h light/16 h dark) for an additional 2 weeks. Plants were subsequently deacclimated at 22 °C/18 °C (16 h light/8 h dark) for 3 d.

For the stress treatments, germinated wheat seeds were cultivated hydroponically with tap water on plastic mesh grids that were suspended just above the water line in plastic containers. Seedlings were grown at 22 °C in a growth chamber under continuous illumination for 7 d. Plants were subsequently subjected to different abiotic stresses (cold, drought, or salt) by transferring the plants on grids into plastic containers with the following conditions: cold water (4 °C), without water (drought) or 200 mM NaCl, respectively. Plants were further grown at 22 °C, except for the cold stress treatment (4 °C), in a growth chamber under continuous illumination. Shoot and root tissues were collected before treatment (0 h) and at 1, 2, 6, 10, 24, 48 h of stress treatments. Samples were frozen with liquid nitrogen and stored at −80 °C until use for RNA extraction.

Arabidopsis thaliana ecotype Col-0 was grown in a mixed soil (2:1 mixture of Jiffy mix and vermiculite) in a growth chamber maintained at 22 °C under a 16-h light/8-h dark photoperiod from cool-white fluorescent lights (approximately 100 μmol m^{-2} s^{-1}).

2.2. cDNA isolation and sequence analysis

A cDNA library from 14-d cold-acclimated crown tissue of winter wheat was constructed using the ZAP Express cDNA Gigapack® Gold cloning kit (Stratagene, USA), according to the manufacturer's instructions. Approximately 10^7 plaque-forming units (pfu) of the library were *in vivo* excised in *E. coli* strain XLOLR to give pBK-CMV cDNA phagemids. Screening for cold-regulated cDNA clones in the library was performed using a macroarray-based differential screening method following the protocol described in our previous paper (Christova et al., 2006).

DNA sequencing was performed with a DNA sequencer 373A (Applied Biosystem, San Jose, CA) using a Big Dye Terminator Cycle Sequencing Kit v 1.1 (Applied Biosystems). Sequencing data analyses were performed with STADEN PACKAGE software and sequence alignments were made with Clustal W 2.0 (Larkin et al., 2007). The sequence data were deposited to DDBJ (DDBJ/EMBL/GenBank ID: AB281487). Phylogenetic trees were produced from the Clustal alignment using the Neighbor-Joining method (Saitou and Nei, 1987) as implemented in MEGA version 6.0 (Tamura et al., 2013). The optimal tree was computed by bootstrap test (500 replicates; Felsenstein, 1985).

2.3. Total RNA extraction and RNA gel blot analysis

Total RNA was isolated from wheat shoot, root and crown tissues (5–8 mm basal portion of shoot, including apical tissue and minimal leaf sheath tissue) using TRIzol reagent (Invitrogen). Total RNA (10 μg) was separated on a 1.2% agarose gel containing formaldehyde and transferred onto Hybond N^+ membrane (Amersham Biosciences). Hybridization was performed with a 3′-UTR fragment of *TaSK5* cDNA that was amplified with the following PCR primers: 5′-AAAGCAGGGCTCCCATGCGT-3′ and 5′-ACATTCATACTTCTCTAGAACAT-3′. The PCR fragment was labeled with α-^{32}P CTP by the random primer method. Hybridization and washing steps were performed according to a published protocol (Church and Gilbert, 1984).

For RNA gel blot analyses of the *35S:TaSK5 Arabidopsis* lines, total RNA from rosette leaves bulked from fifteen plants (3 weeks old) was extracted using TRIzol reagent. Ten μg RNA was loaded and hybridized overnight with a full-length *TaSK5* cDNA probe that was labeled with the ECL Kit (Amersham Biosciences) according to the manufacturer's instructions. Detection and quantification of the signals were carried out using a LAS 3000 image analyzer and Multi Gauge 2.02 software (Fuji Film, Tokyo, Japan).

2.4. Gene disruption and complementation of yeast cells

Yeast *S. cerevisiae* YPH499 (*MATa*, *ura3-52, lys2-81, ade2-101, trpΔ63, his3Δ200, leu2Δ1*) was used as the parental strain for gene disruption. PCR-based gene disruption of the yeast *MCK1* was carried out by the method previously described (Nikawa and Kawabata, 1998). Amplification of upstream and downstream *MCK1* gene fragments was carried out with the UF/UR and DF/DR primer pairs, respectively. The primer sequences were: UF-MCK1, 5′-GAGTTAAGCCCAAGAC-3′; UR-MCK1, 5′-GCATTCCATGGCAAGG-3′; DF-MCK1, 5′-GCTTATCGGCAAAGCC-3′; DR-MCK1, 5′-ACAGCGGATCAAAGGTG-3′. The T-tailed *HIS3* gene and *HIS3* primers, pT-HIS3-f (5′-CATTCAACGTTTCCCAT-3′) and pT-HIS3-r (5′-CGTTCAGAATGACACGT-3′) were kind gifts from Dr. Nikawa at the Kyushu