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Yeast heat-shock protein gene *HSP26* enhances freezing tolerance in *Arabidopsis*

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Summary

In the yeast *Saccharomyces cerevisiae*, the molecular chaperone Hsp26 is one component of the heat-shock response. Hsp26 has the remarkable ability to directly sense increases in temperature and switch from an inactive state to a chaperone-active state. In this study, we report a functional analysis of Hsp26 in *Arabidopsis thaliana* and its response to freezing stress. After freezing stress, the *HSP26* transgenic plants exhibited stronger growth than the wild-type plants. We found that over-expression of *HSP26* in *Arabidopsis* increased the amounts of free proline and soluble sugars, elevated the expression of stress defense genes, and enhanced *Arabidopsis* tolerance to freezing stress. Taken together, our results indicate that Hsp26 may play an important role in the response of transgenic *Arabidopsis* plants to freezing stresses.

Introduction

Heat-shock proteins (HSPs) comprise a protein family whose expression can be induced when a cell is responding to certain stimuli. Functioning as molecular chaperones, HSPs can prevent the accumulation of protein precursors, accelerate the transport of proteins, and absorb complexes of unfolded proteins to maintain their transport

Abbreviations: HSP, heat-shock protein or proteins; MDA, malondialdehyde; REC, relative electrical conductivity; sHSP, small heat-shock protein or proteins.

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abilities. HSPs can also sustain the normal folded state of proteins, degrade misfolded proteins, stabilize polypeptide strands, and prevent protein inactivity. In addition, HSPs participate in regulating the activation and function of target proteins, although they themselves are not components of the target proteins. HSPs with low molecular masses (about l5–30 KDa) are called small heat-shock proteins (sHSP) (Sugiyama et al., 2000). The sHSP family is a stress-inducible group of molecular chaperones that can prevent the polymerization of denatured proteins. In the yeast *Saccharomyces cerevisiae*, Hsp26 has the remarkable ability to directly sense increase in temperature and switch from an inactive state to a chaperone-active state (Haslbeck, 2002; Haslbeck et al., 2004; Franzmann et al., 2008).

Some direct evidence exists for the function of individual HSPs in thermotolerance in plants. Changes in the expression of HSP17.7 after heat stress caused modest changes in the growth rates of tissue culture cells and electrolyte leakage of leaves (Malik et al., 1999). The introduction of the tomato (*Lycopersicon esculentum*) MT-sHSP gene under the control of the 35S promoter into tobacco (*Nicotiana tabacum*) enhanced thermotolerance in the transformed plants (Sanmiya et al., 2004). Furthermore, *sHSP17.7* has been over-expressed in the rice cultivar 'Hoshinoyume' by *Agrobacterium*-mediated transformation, under the control of a CaMV 35S promoter. The transgenic rice plants exhibited significantly increased thermotolerance compared with untransformed plants (Murakami et al., 2004).

It has recently been recognized that the sHSPs have protective roles not only against high temperatures, but also against a variety of stresses. In response to low temperatures, the genes and gene products of HSP70 are induced in spinach (Neven et al., 1992; Anderson et al., 1994; Guy et al., 1998) and soybean (Cabané et al., 1993), and those of HSP90 are induced in *Brassica napus* (Krishna et al., 1995) and rice (Pareek et al., 1995). While all HSPs have the capacity to interact with other proteins and to act as molecular chaperones, different HSPs may have different functional properties. Further studies are necessary to determine the precise role of HSP26 in relation to freezing tolerance in plants.

Here, we show direct evidence for the anti-freezing role of the yeast heat-shock protein *HSP26* gene in *Arabidopsis thaliana* (*A. thaliana*). Our results indicate that *HSP26* may play an important role in the response of the transgenic *Arabidopsis* plants to freezing stress, and mediate the induction of certain stress-related protective genes that confer tolerance to freezing.

Materials and methods

Plant materials, growth conditions, and treatments

Seeds of *Arabidopsis thaliana* were surface sterilized with bleaching powder (5%, w/v) for 20 min, washed with sterile water three times, and placed in Petri dishes that contained MS medium (Murashige and Skoog, 1962) with 0.8% agar. The incubation and growth conditions for *Arabidopsis thaliana* (ecotype Columbia) were as described by Zhang et al. (2006). For freezing tests, whole plants were removed from agar, placed between damp paper towels, packed in moist sand in metal boxes, and placed in a temperature-regulated freezer at −10 °C for 2.5 h for temperature equilibration and ice formation. After freezing, plants were allowed to thaw overnight in the sand boxes at 4 °C. Plants were subsequently removed from the sand boxes, placed on moist paper towels in clear plastic boxes, and returned to 22 °C with a 12 h photoperiod.

Isolation and transformation of a cDNA encoding the putative HSP26

The full cDNA was cloned by polymerase chain reaction (PCR) from the yeast *Saccharomyces cerevisiae* cDNA library. PCR products were cloned into the pUCm-T vector and sequenced by the Chinese National Human Genome Center in Shanghai. One pair of special primers was used to get real full-length cDNA (the forward primer was based on the ATG start codon: 5′-GGATCCATGTCATTTAACAGTCCATTTTTTG-3′ and the reverse primer was based on the TTA stop codon: 5′-GAGCTCTTAGTTACCCCACGATTCTTGAG-3′). The *Arabidopsis thaliana* cv. Columbia was transformed with the *Agrobacterium tumefaciens* strain GV3101 using the floral dip method as described previously (Clough and Bent, 1998). The plasmid used in the transformation was derived from pYF7716 (Peng et al., 2001). The full-length HSP26 cDNA was digested with *Bam*HI and *Sac*I, and cloned into the binary vector under the control of an enhanced double CaMV 35S promoter and the tobacco mosaic virus TMV Ω sequence (Figure 1).

Reverse transcription-PCR analysis

Total RNA was digested with DNase I (Promega, Madison, WI, USA) to remove genomic DNA. The first strand of cDNA was synthesized using 5 μg of total RNA as a template with the Reverse Transcription System (Promega, Madison, WI, USA) in a