Journal of Plant Biology, June 2007, 50(3) : 383-385 **REPORT**

Overexpression of the Acidic C-Terminus Region of HvRAF from Barley Confers Enhanced Cadmium Tolerance in Yeast

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The barley ERF-type transcription factor, HvRAF, has an acidic C-terminus domain that contains a hemopexin domain signature-like sequence. As an initial study to investigate the functioning of this domain, we cloned the partial *HvRAF* gene (*pHvRAF*) corresponding to the domain into a yeast/*E. coli* shuttle vector, pYES2. We then introduced the pYES2::*pHvRAF* recombinant plasmid into yeast (*Saccharomyces cerevisiae* strain INVSc1). Here, we report that yeast transformants harboring pYES2::*pHvRAF* show significant tolerance to cadmium stress.

Keywords: cadmium stress, ERF-type transcription factor, *Hordeum vulgare*, *Saccharomyces cerevisiae*

Cadmium is extremely toxic in natural environments. When accumulated in excess within plant tissues, it can inhibit enzyme activity, membrane transport, photosynthesis, lipid peroxidation, etc. (Keck, 1978; Clijstersw and van Assche, 1985; Somashekaraiah et al., 1992; Krupa et al., 1993). In yeasts and higher plants, several components are involved in the cellular mechanism for cadmium tolerance, including phytochelatins, metallothioneins, glutathione, and an ATP-binding cassette (ABC) transporter (Li et al., 1996; Zenk, 1996; Lu et al., 1997; Cobbett et al., 1998; Lee et al., 2004). As an enzymatically synthesized peptide, phytochelatin binds heavy metals with high affinity, being sequestered to the vacuole. Therefore, the genes required for its synthesis play an important role in detoxifying cadmium. This involvement of phytochelatin in cellular defenses against cadmium stress has been well studied using two heterologous systems -- yeast and *Arabidopsis*. For example, overexpression of phytochelatin synthase genes from *Arabidopsis* and tobacco suppresses the cadmium-sensitive *yap*1 and *ycf*1 mutations in yeast (Clemens et al., 1999; Ha et al., 1999; Vatamaniuk et al., 1999; Kim et al., 2005). The yeast *YAP1* gene encodes a transcription factor required for the expression of *YCF1*, an ABC transporter gene responsible for vacuolar sequestration of the complexes of cadmium with glutathione in yeast (Li et al., 1996). In addition, *Arabidopsis* over-expressing *YCF1* shows enhanced resistance to cadmium and lead (Song et al., 2003).

Transcription factors play crucial roles as the final regulatory components in signaling pathways for defense responses against diverse environmental stresses, including heavy metal contamination. A number of transcription factors identified in higher plants contain various domains, e.g., ERF, bZIP, and WRKY (Chen et al., 2002). Previously, we reported the cloning and characterization of *HvRAF* in barley (<u>H</u>*ordeum* <u>v</u>*ulgare* <u>R</u>*oot* <u>A</u>*bundant* <u>F</u>*actor*, Genbank no. DQ102383), which encodes an ERF-type transcription factor (Jung et al., 2007). Its overexpression not only activates a variety of stress-inducible genes, but also confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*. The open reading frame of this gene, AAZ14068, encodes a

Figure 1. DNA sequence and deduced amino acids of the partial *HvRAF*. Mammalian hemopexin domain signature-like sequence is indicated by solid underline (**A**). Alignment of hemopexin domain signature-like sequences of HvRAF and several other AP2 transcription factors. Identical amino acid residues are shown in black (**B**).

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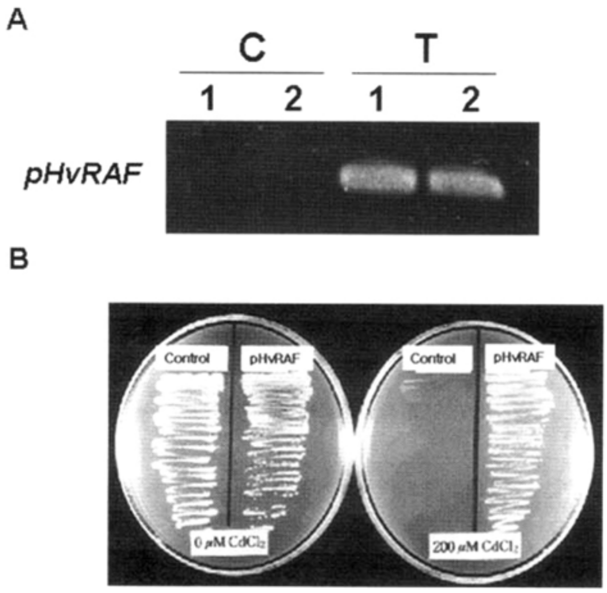

Figure 2. Cadmium tolerance by yeast transformants over-expressing *pHvRAF*. RT-PCR analyses of *pHvRAF* were performed for untransformed yeast *Saccharomyces cerevisiae* strain INVSc1 (C) and yeast transformed with pYES2::*pHvRAF* (T) (**A**). Untransformed yeasts (control) and yeast transformants over-expressing *pHvRAF* (pHvRAF) were grown on SD/-Ura media in absence (left) and presence (right) of 200 μM $CdCl_2$ (**B**).

polypeptide of 328 amino acids, containing a putative nuclear localization sequence at the N-terminus, a highly conserved ERF domain, and an acidic (pI = 3.2) C-terminus domain with a novel hemopexin domain signature-like sequence (Jung et al., 2007). Hemopexin is a mammalian serum glycoprotein that binds heme and transports it to the liver (Tolosano and Altruda, 2002). Similar sequences have been found in the C-terminus domain of several AP2 domain-containing proteins, including CBF1-4 (Fig. 1B) (Haake et al., 2002; Jung et al., 2007).

As an initial characterization of the biological functioning of the C-terminus domain in HvRAF, we constructed the pYES2::*pHvRAF* (partial *HvRAF*) recombinant plasmid and transformed it into yeast (*S. cerevisiae* strain INVSc1). This was performed according to the modified lithium acetate-mediated method (Gietz et al., 1992). YPD and SD-Ura media were used as the yeast growth substrates. The partial *HvRAF* contained a C-terminal open reading frame of 113 amino acids (pI = 3.32), including the hemopexin domain signature-like sequence, as well as 361 nucleotides within the 3'-UTR (Fig. 1A). Expression in our yeast transformants was confirmed by RT-PCR analyses (Fig. 2A), using forward (5'-ACACGATGCCGAGGGTCGA-3') and reverse (5'-TCAGAAAATGGCGCTGTCCA-3') primers. Afterward, we conducted a cadmium-tolerance test of those yeast transformants that indeed harbored pYES2::*pHvRAF*. In SD-Ura media containing 200 μM $CdCl_2$, the transformants showed enhanced tolerance to cadmium stress compared with the non-transformants (Fig. 2B). Therefore, we can conclude that the exogenously overexpressed C-terminus region of the HvRAF confers enhanced tolerance to cadmium toxicity in yeast. It remains to be studied how this is accomplished, and whether the same region will confer such tolerance in higher plants. However, it is tempting to speculate that the hemopexin domain signature-like sequence might be somehow involved in this tolerance mechanism, especially because we have previously demonstrated that 26 amino acids corresponding to this sequence act as a transactivation domain in yeast (Jung et al., 2007).

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