Stress Tolerance and Glucose Insensitive Phenotypes in Arabidopsis Overexpressing the \textit{CpMYB10} Transcription Factor Gene\textsuperscript{1}

Miguel Angel Villalobos, Dorothea Bartels, and Gabriel Iturriaga*

Instituto de Biotecnología-UNAM, Cuernavaca 62210, Mexico (M.A.V.); Institute of Physiology and Biotechnology of Plants, D-53115 Bonn, Germany (D.B.); and Centro de Investigación en Biotecnología-UAEM, Cuernavaca 62210, Mexico (G.I.)

The resurrection plant \textit{Craterostigma plantagineum} has the ability to survive complete dehydration. In an attempt to further understand desiccation tolerance in this plant, the \textit{CpMYB10} transcription factor gene was functionally characterized. \textit{CpMYB10} is rapidly induced by dehydration and abscisic acid (ABA) treatments in leaves and roots, but no expression was detected in fully hydrated tissues. Electrophoretic mobility shift assay experiments showed binding of r\textit{CpMYB10} to specific \textit{mybRE} elements within the LEA \textit{Cp11-24} and \textit{CpMYB10} promoters. Localization of \textit{CpMYB10} transcript by in situ reverse transcription-PCR reactions showed expression in vascular tissues, parenchyma, and epidermis both in leaves and roots in response to ABA. Transgenic Arabidopsis plants transformed with \textit{CpMYB10} promoter fused to \textit{GUS} gene showed reporter expression under ABA and stress conditions in several organs. Overexpression of \textit{CpMYB10} cDNA in Arabidopsis led to desiccation and salt tolerance of transgenics lines. Interestingly, it was found that plants overexpressing \textit{CpMYB10} exhibited Glc-insensitive and ABA hypersensitive phenotypes. Therefore, our results indicate that \textit{CpMYB10} in Arabidopsis is mediating stress tolerance and altering ABA and Glc signaling responses.

Diurnal and seasonal environmental fluctuations as well as extreme conditions have been a major selective pressure for plant evolution. Plants are sessile organisms that cannot move to escape from adverse environmental cues, thus complex metabolic and anatomical adaptations have been developed to cope with abiotic stresses. Availability of water is probably the most limiting factor for crop productivity and yield, compromising economical output and human food supply. Therefore, there is a strong need to understand plant adaptation mechanisms against adverse environmental conditions to improve stress tolerance.

Plant stress responses involve the expression of a plethora of genes with an adaptive role. Among the products of these genes are enzymes catalyzing the synthesis of osmoprotectants or antioxidants, late-embryogenesis abundant (LEA) proteins, chaperones and heat shock proteins, lipid desaturases, water channels, and ion transporters, representing some of the best characterized examples (Ingram and Bartels, 1996). Abscisic acid (ABA) plays a major role in transducing stress responses (Knight and Knight, 2001).

Rapid stress responses are in most cases ABA-independent, and there is growing evidence that ABA-dependent and independent pathways cross-talk (Shinozaki and Yamaguchi-Shinozaki, 2000). Signal transduction components include protein kinases such as calcium-dependent protein kinases (CDPK) and mitogen-activated protein (MAP) kinases, G-proteins, phosphatase 2C, and second messengers such as Ca\textsuperscript{2+} and phosphoinositides. A phospholipase D raises its activity minutes after dehydration (Frank et al., 2000). Also, within 1 min after osmotic shock, inositol 1,4,5-P\textsubscript{3}, a breakdown product of phospholipase C, dramatically increases its concentration, and rapid changes in cytosolic free Ca\textsuperscript{2+} concentrations are triggered during this process (DeWald et al., 2001). It has been claimed that nitric oxide is involved in ABA-induced response to stomatal closure and requires cGMP and cADPR (Neill et al., 2002). An Arabidopsis transmembrane His kinase functions as an osmosensor in a yeast mutant, suggesting a similar role in plants (Urao et al., 1999).

Several plant model systems have been used to study responses to water deficit, according to the severity of the stress. Upon a mild water deficit, plants reduce water loss by closing stomata, retain water by osmotic adjustment, and increase water uptake. These responses have been thoroughly studied in Arabidopsis and other mesophytes (Tabaeizadeh, 1998). A different situation occurs in the so-called resurrection plants that exhibit protoplasmic desiccation tolerance. These organisms withstand long periods with air of 0\% (v/v) relative humidity, reviving a few hours after

\textsuperscript{1} This work was supported in part by CONACYT (grant no. 27703–N [Mexico] to G.I.) and by ICGEB (grant no. CRP/MEX98-01 [Trieste] to G.I.). M.A.V. was supported by a CONACyT PhD fellowship.

* Corresponding author; e-mail iturri@cib.uaem.mx; fax 52-777-3297030.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.103.034199.