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INTERACTION STUDIES OF DIFFERENT *ARABIDOPSIS* 14-3-3 ISOFORMS WITH THE PLASMA MEMBRANE H⁺-ATPASE

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The eukaryotic regulatory proteins 14-3-3 are involved in many important plant cellular processes including regulation of electrochemical gradient across the plasma membrane through the stimulation of H⁺-ATPase activity. In both animals and plants, 14-3-3 proteins are present as multiple isoforms whose overall amino acid sequence is highly conserved. In Arabidopsis thaliana 12 isoforms are known to be expressed and, based on phylogenetic analysis, they are divided in two groups: epsilon and non-epsilon group. So far it is not fully clarified whether different isoforms may accomplish different functions. To verify a possible specificity of 14-3-3 isoforms towards the H⁺-ATPase, two Arabidopsis isoforms, GF140mega and GF14epsilon, characterized by a highly sequence divergence at their C-terminal domain, have been assayed for the ability to interact with the H⁺-ATPase. Results demonstrate that GF140mega has a higher affinity towards the enzyme than GF14epsilon and it is more active also in stimulating the H⁺-ATPase activity. Interaction studies performed with other 14-3-3 target proteins confirmed the higher binding ability of GF140mega. In order to understand whether the observed different binding ability of GF140mega and epsilon was limited to these isoforms or was instead shared by all isoforms belonging to epsilon and non-epsilon group, other Arabidopsis 14-3-3 isoforms were expressed and tested in binding assays with the H⁺-ATPase.