ARTICLE ADDENDUM

VIP1 is very important/interesting protein 1 regulating touch responses of Arabidopsis

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VIP1 (VIRE2-INTERACTING PROTEIN 1) is originally identified as an Arabidopsis thaliana bZIP protein interacting with the Agrobacterium tumefaciens protein VirE2, and has been suggested to play pleiotropic roles. Previously we reported that VIP1 exists mainly in the cytosol under steady conditions but transiently accumulates in the nucleus when cells are exposed to hypo-osmotic conditions, that close homologs of VIP1 (i.e., Arabidopsis group I bZIP proteins) also exhibit such a pattern of subcellular localization, and that overexpression of a repression domain-fused form of VIP1 (VIP1-SRDX) enhances touch-induced root waving. Touch as well as the hypo-osmotic conditions seems to induce the nuclear localization of VIP1 (Fig. 1). VIP1 is a novel regulator of touch responses of Arabidopsis, and should be used to further characterize them. Here we discuss perspectives for further studies on VIP1.

What factors are involved in regulating the nuclear localization of VIP1?

Previously a treatment with a microbe associated molecular pattern, flg22, caused VIP1 to be localized to the nucleus, and a treatment with a gibberelin biosynthesis inhibitor, uniconazole-P, caused the putative tobacco VIP1 ortholog RSG (REPRESSION OF SHOOT GROWTH) to be localized to the nucleus. However, in our experiments, water itself, which was used to dilute flg22 and uniconazole-P stocks in those experiments, can induce the nuclear localization of VIP1 (ref. 10 and unpublished data), thus the effects of these chemicals may have to be re-evaluated.

VIP1 is thought to accumulate in the nucleus when its 79th serine is phosphorylated by MPK3 (MITOGEN-ACTIVATED PROTEIN KINASE 3), although this idea has been questioned. On the other hand, RSG is thought to accumulate in the nucleus when its phosphorylated 114th serine is either dephosphorylated or replaced by alanine. Although replacing the VIP1 115th serine, which corresponds to the RSG 114th serine, with alanine does not affect the subcellular localization of VIP1, our unpublished data suggest that VIP1 has multiple putative phosphorylation sites, and that these sites are dephosphorylated when VIP1 is localized to the nucleus. The RSG 114th serine is phosphorylated by the calcium-dependent protein kinase CDPK1 in tobacco, thus a CDPK1 homolog may phosphorylate VIP1 in Arabidopsis. Our unpublished data also suggest that calcium signaling regulates both the nuclear import and the nuclear export of VIP1. It should be interesting to identify protein kinases and protein phosphatases regulating VIP1 phosphorylation states, and to identify the phosphorylation sites in VIP1.

The mechanosensitive calcium channels MCA1 and MCA2 (MID-COMPLEMENTING ACTIVITY 1 and 2, respectively), the 7-transmembrane domain proteins MLO4 and MLO11 (MILDewing resistance locus O 4 and 11, respectively), and the receptor kinase FERONIA have been identified as candidate mechanical stress sensors regulating calcium signaling and root tropisms. TOUCH2 and TOUCH4, which are up-regulated by the FERONIA-mediated touch-responsive
signaling, are unlikely to be VIP1 target genes. However, it would be worth examining whether VIP1 interacts with these proteins and/or acts under the control of them. To identify novel regulators for VIP1, it would also be helpful and practical to screen for chemicals and genetic mutations that affect the subcellular localization of VIP1.

What can be done to further characterize the physiological functions of VIP1 and other Arabidopsis group I bZIP proteins?

VIP1 and its close homologs in tomato, tobacco, and rice, have been identified in different studies as a regulator of certain physiological responses. This would support the idea that such VIP1 homologs have important, pleiotropic roles. Thus far only 2 Arabidopsis group I bZIP protein genes, VIP1 and PosF21, have been associated with physiological roles. However, at least 5 of the other group I bZIP protein genes are expressed as highly as VIP1 and PosF21, and they could function redundantly. In our recent study, expressing the VIP1-GFP fusion protein suppressed the VIP1-SRDX-induced enhancement of root waving. It would be interesting to examine whether other group I bZIP proteins can also suppress the VIP1-SRDX-induced enhancement of root waving. In our preliminary experiments, the triple mutant that has T-DNA in VIP1, PosF21, and bZIP29 (another group I bZIP protein gene) was similar to the wild type in phenotypes including root waving, but knocking out various combinations of the group I bZIP protein genes should also help to elucidate the physiological roles of them.

The local auxin responses in the root tip are different between wild-type plants and VIP1-SRDX-overexpressing (VIP1-SRDXox) plants, and this may be attributed to the difference in adhesion and/or removal of the root cap cells. Expression levels of some mechanical stimulus-induced genes that should regulate cell wall properties are lower in VIP1-SRDXox plants than in the wild-type plants, and this may cause the abnormal root cap cell adhesion/removal in VIP1-SRDXox plants. On the other hand, cell death mediated by the NAC (NO APICAL MERISTEM)-family transcription factor SOMBRERO and the S1-P1 nuclease-family protein BFN1 (BIFUNCTIONAL NUCLEASE 1) is necessary for the removal of the lateral root cap cells. It would be interesting to characterize the cell wall properties and cell death in the root cap cells of VIP1-SRDXox plants, and to examine genetic and physical interactions between the group I bZIP proteins and the above regulators of cell wall properties and cell death. It would also be important to further evaluate how mechanical stimuli affect cell wall properties and cell death in root cap cells.

Disclosure of potential conflicts of interest

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