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1 VIP1 is Very Important/Interesting Protein 1 regulating touch responses of Arabidopsis

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13	
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22 Abstract

VIP1 (VIRE2-INTERACTING PROTEIN 1) is a bZIP transcription factor in Arabidopsis 2324thaliana. VIP1 and its close homologs (i.e., Arabidopsis group I bZIP proteins) are present in the cytoplasm under steady conditions, but are transiently localized to the nucleus when cells 25are exposed to hypo-osmotic conditions, which mimic mechanical stimuli such as touch. 2627Recently we have reported that overexpression of a repression domain-fused form of VIP1 represses the expression of some touch-responsive genes, changes structures and/or local 28auxin responses of the root cap cells, and enhances the touch-induced root waving. This 29raises the possibility that VIP1 suppresses touch-induced responses. VIP1 should be useful to 30 further characterize touch responses of plants. Here we discuss two seemingly interesting 31 32perspectives about VIP1: (1) What factors are involved in regulating the nuclear localization of VIP1?; (2) What can be done to further characterize the physiological functions of VIP1 33 and other Arabidopsis group I bZIP proteins? 3435TEXT 36

VIP1 (VIRE2-INTERACTING PROTEIN 1) was originally identified as an *Arabidopsis thaliana* bZIP protein interacting with the *Agrobacterium tumefaciens* protein VirE2,¹ and has
been suggested to play pleiotropic roles.¹⁻¹² Previously we reported that VIP1 exists mainly in
the cytosol under steady conditions but transiently accumulates in the nucleus when cells are

41	exposed to hypo-osmotic conditions, ⁹ that close homologs of VIP1 (i.e., Arabidopsis group I
42	bZIP proteins ¹³) also exhibit such a pattern of subcellular localization, ¹¹ and that overexpression
43	of a repression domain-fused form of VIP1 (VIP1-SRDX) enhances touch-induced root
44	waving. ¹⁴ Touch as well as the hypo-osmotic conditions seems to induce the nuclear
45	localization of VIP1 (Fig. 1). VIP1 is a novel regulator of touch responses of Arabidopsis,
46	and should be useful to further characterize them. Here we discuss perspectives for further
47	studies on VIP1.

48

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

⁵⁰ Previously a treatment with a microbe associated molecular pattern, flg22, caused VIP1 to be ⁵¹ localized to the nucleus,⁶ and a treatment with a gibberellin biosynthesis inhibitor, uniconazole-P, ⁵² caused the putative tobacco VIP1 ortholog RSG (REPRESSION OF SHOOT GROWTH) to ⁵³ be localized to the nucleus.¹⁵ However, in our experiments, water itself, which was used to ⁵⁴ dilute flg22 and uniconazole-P stocks in those experiments, can induce the nuclear localization of ⁵⁵ VIP1 (ref. 10 and unpublished data), thus the effects of these chemicals may have to be ⁵⁶ re-evaluated.

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VIP1 is thought to accumulate in the nucleus when its 79th serine is phosphorylated by
 MPK3 (MITOGEN-ACTIVATED PROTEIN KINASE 3),⁶ although this idea has been

60	questioned. ¹¹ On the other hand, RSG is thought to accumulate in the nucleus when its
61	phosphorylated 114th serine is either dephosphorylated or replaced by alanine. ¹⁵ Although
62	replacing the VIP1 115th serine, which corresponds to the RSG 114th serine, with alanine
63	does not affect the subcellular localization of VIP1, ¹¹ our unpublished data suggest that VIP1
64	has multiple putative phosphorylation sites, and that these sites are dephosphorylated when
65	VIP1 is localized to the nucleus. The RSG 114th serine is phosphorylated by the
66	calcium-dependent protein kinase CDPK1 in tobacco, ¹⁶ thus a CDPK1 homolog may
67	phosphorylate VIP1 in Arabidopsis. Our unpublished data also suggest that calcium signaling
68	regulates both the nuclear import and the nuclear export of VIP1. It should be interesting to
69	identify protein kinases and protein phosphatases regulating VIP1 phosphorylation states, and
70	to identify the phosphorylation sites in VIP1.
71	
72	The mechanosensitive calcium channels MCA1 and MCA2 (MID-COMPLEMENTING
73	ACTIVITY 1 and 2, respectively), the seven-transmembrane domain proteins MLO4 and
74	MLO11 (MILDEW RESISTANCE LOCUS O 4 and 11, respectively), and the receptor
75	kinase FERONIA have been identified as candidate mechanical stress sensors regulating
76	calcium signaling and root tropisms. ¹⁷⁻²⁰ TOUCH2 and TOUCH4, which are up-regulated by
77	the FERONIA-mediated touch-responsive signaling, ²⁰ are unlikely to be VIP1 target genes. ¹⁴
78	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.

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

VIP1 and its close homologs in tomato, tobacco, and rice, have been identified in different 85 studies as a regulator of certain physiological responses.^{15,16,21-26} This would support the idea 86 87 that such VIP1 homologs have important, pleiotropic roles. Thus far only two Arabidopsis group I bZIP protein genes, VIP1 and PosF21, have been associated with physiological 88 roles.^{1-12,14,27} However, at least five of the other group I bZIP protein genes are expressed as 89 highly as *VIP1* and *PosF21*,¹¹ and they could function redundantly. In our recent study, 90 expressing the VIP1-GFP fusion protein suppressed the VIP1-SRDX-induced enhancement 91of root waving.¹⁴ It would be interesting to examine whether other group I bZIP proteins can 92 also suppress the VIP1-SRDX-induced enhancement of root waving. In our preliminary 93experiments, the triple mutant that has T-DNA in VIP1, PosF21, and bZIP29 (another group I 94 bZIP protein gene) was similar to the wild type in phenotypes including root waving, but 95 knocking out various combinations of the group I bZIP protein genes should also help to 96 elucidate the physiological roles of them. 97

98	The local auxin responses in the root tip are different between wild-type plants and
99	VIP1-SRDX-overexpressing (VIP1-SRDXox) plants, and this may be attributed to the
100	difference in adhesion and/or removal of the root cap cells. Expression levels of some
101	mechanical stimulus-induced genes that should regulate cell wall properties are lower in
102	VIP1-SRDXox plants than in the wild-type plants, and this may cause the abnormal root cap
103	cell adhesion/removal in VIP1-SRDXox plants. ¹⁴ On the other hand, cell death mediated by
104	the NAC (NO APICAL MERISTEM)-family transcription factor SOMBRERO and the S1-P1
105	nuclease-family protein BFN1 (BIFUNCTIONAL NUCLEASE 1) is necessary for the
106	removal of the lateral root cap cells. ^{28,29} It would be interesting to characterize the cell wall
107	properties and cell death in the root cap cells of VIP1-SRDXox plants, and to examine
108	genetic and physical interactions between the group I bZIP proteins and the above regulators
109	of cell wall properties and cell death. It would also be important to further evaluate how
110	mechanical stimuli affect cell wall properties and cell death in root cap cells.
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197	
198	Figure Legend
199	Figure 1.
200	Touch-induced nuclear localization of VIP1.
201	Transgenic Arabidopsis plants expressing the VIP1-GFP fusion protein were grown for 3 days in
202	darkness. VIP1-GFP signals were observed approximately 3 minutes after their hypocotyls
203	were touched by a needle tip. More than five plants were used, and a representative result is
204	presented. Arrows indicate the approximate position where the hypocotyl was touched.
205	Scale bar = $200 \ \mu m$.

