Possible inhibition of Arabidopsis VIP1-mediated mechanosensory signaling by streptomycin

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Abstract

VIP1 (VIRE2-INTERACTING PROTEIN 1) and its close homologues are *Arabidopsis thaliana* bZIP proteins regulating stress responses and root tropisms. They are present in the cytoplasm under steady conditions, but transiently accumulate in the nucleus when cells are exposed to mechanical stress such as hypo-osmotic stress and touch. This pattern of changes in subcellular localization is unique to VIP1 and its close homologues, and can be useful to further characterize mechanical stress signaling in plants. A recent study showed that calcium signaling regulates this pattern of subcellular localization. Here, we show that a possible calcium channel inhibitor, streptomycin, also inhibits the nuclear accumulation of VIP1. Candidates for the specific regulators of the mechanosensitive calcium signaling are further discussed.

VIP1 (VIRE2-INTERACTING PROTEIN 1) and its close homologues are *Arabidopsis thaliana* bZIP proteins. They are members of the plant bZIP protein group I,[1] and are involved in regulating responses to biotic and abiotic stresses.[2-15] Such stresses include hypo-osmotic stress and touch.[6,9-11] Hypo-osmotic stress and touch are not identical, but both impose mechanical stress on cells and induce partially common cellular responses.[16] When either VIP1 or its close homologue bZIP29 is expressed as a repression domain-fused form in Arabidopsis, touch-induced root bending is enhanced.[10-12] At least six Arabidopsis group I bZIP proteins (VIP1, PosF21/bZIP59, bZIP69, bZIP29, bZIP30 and bZIP52) are present in the cytoplasm under steady conditions, but transiently accumulate in the nucleus when cells are exposed to hypo-osmotic stress.[6,9,10] The transient nuclear accumulation of VIP1 can also be induced by touch.[11] No other plant proteins with similar subcellular localization patterns have been identified thus far. The group I bZIP proteins can therefore be a clue to more deeply understanding mechanical stress signaling.

Phosphorylation is a factor regulating the nuclear-cytoplasmic shuttling of VIP1 and its close homologues. Under steady conditions, VIP1 is largely phosphorylated and bound by 14-3-3 proteins, which are thought to retain VIP1 in the cytoplasm. Hypo-osmotic stress causes dephosphorylation of VIP1, and this dephosphorylation can be needed for the nuclear accumulation of VIP1.[14] Calcium signaling has recently been found to be another factor regulating the nuclear-cytoplasmic shuttling of VIP1 and its close homologues. These

proteins physically interact with the calmodulin calcium-binding proteins, and both the nuclear accumulation and the cytoplasmic accumulation of VIP1, bZIP59 and bZIP29 are inhibited by either a calcium chelator (EDTA or EGTA) or a calmodulin inhibitor (chlorpromazine).[15] Calcium-dependent protein kinases and B'' subunits of the protein phosphatase 2A complex may regulate phosphorylation states of group I bZIP proteins in calcium-dependent manners,[12,17] although neither of them was co-immunoprecipitated with VIP1 in a previous study (i.e., they may not interact with VIP1 very strongly).[15] The same study also showed that the nuclear-cytoplasmic shuttling of VIP1 is not affected either by the double knockout of two calcium channels, MCA1 and MCA2, or by $GdCl_3$ or $LaCl_3$, which can inhibit other types of mechanosensitive calcium channels.[15,18,19]

Which factor initiates the calcium signaling that regulates functions of group I bZIP proteins, then? In our recent experiments, 1 mM streptomycin, which can inhibit mechanosensitive calcium channels in animals,[20,21] was found to inhibit the hypo-osmotic stress-induced nuclear accumulation of VIP1 (Fig. 1, middle panel). This effect of streptomycin was not observed when 20 mM $CaCl_2$ was present (Fig. 1, right panel). Land plants have no homologues of transient receptor potential (TRP) channels,[22] which are putative targets of streptomycin in animals,[20,21] and the major target of streptomycin is thought to be plastid 70S ribosome-mediated protein synthesis in plants.[23] However, it would be still possible that the streptomycin-sensitive proteins or processes are initiators of the

mechanosensory signaling mediated by VIP1 and its close homologues. In a similar experiment, cycloheximide, which binds to 60S ribosomes and thereby inhibits cytoplasmic protein synthesis, did not affect the nuclear-cytoplasmic shuttling of VIP1, raising the possibility that cytoplasmic protein synthesis is not required for either the calcium signaling or downstream events regulating the subcellular localization of VIP1. Two calcium ionophores, ionomycin and A23187, did not affect it, either, raising the possibility that a calcium gradient does not primarily control the nuclear cytoplasmic shuttling of VIP1. Effects of inhibitors of calcium signaling and protein synthesis on the subcellular localization of VIP1 are summarized in Table 1. These results, again, support the idea that the calcium signaling regulating the subcellular localization of VIP1 and its close homologs is generated by specific factors. It should be important to dissect specificity in cellular and physiological functions of possible regulators of calcium signaling.

Disclosure of interest

The authors report no conflict of interest

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149

150 Table 1. Summary of effects of inhibitors of calcium signaling and/or protein synthesis on the

151 subcellular localization of VIP1.

Chemical	Known role	Conc. used	Effects on the VIP1 subcellular localization
EDTA	Divalent cation chelator	2 mM	Inhibitory on both the nuclear accumulation and the cytoplasmic accumulation[15]
EGTA	Divalent cation chelator	2 mM	Inhibitory on both the nuclear accumulation and the cytoplasmic accumulation[15]
Chlorpromazine	Calmodulin inhibitor	0.5 mM	Inhibitory on both the nuclear accumulation and the cytoplasmic accumulation[15]
GdCl3	Inhibitor of mechanosensitive calcium channels	1 mM	Not detected[15]
LaCl3	Potential replacement of calcium in the cell wall	1 mM	Not detected[15]
Streptomycin	Inhibitor of 70S ribosomes and mechanosensitive calcium channel	1 mM	Inhibitory on the nuclear accumulation of VIP1 (this study)
Cycloheximide	Inhibitor of 60S ribosomes	0.5 mM	Not detected (this study)
A23187	Calcium ionophore	0.1 mM	Not detected (this study)
Ionomycin	Calcium ionophore	0.05 mM	Not detected (this study)

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154 **Figure legend**

155 Figure 1. Streptomycin inhibits the hypo-osmotic stress-induced nuclear accumulation of VIP1.

156 Arabidopsis plants overexpressing GFP-tagged VIP1[6] were grown for 10 days on half strength

157 MS medium containing 1 % (w/v) sucrose and 0.8 % (w/v) agar, incubated for 0 or 10 minutes in

158 a hypotonic solution (20 mM Tris-HCl, pH 7.5) containing 0 or 1 mM streptomycin and 0 or 20

159 mM $CaCl_2$, and subjected to epifluorescence microscopy to detect GFP signals. The presence of

160 streptomycin and $CaCl_2$ in the solution is indicated as "+ Streptomycin" and "+ $CaCl_2$". For each

161 treatment, more than five individual plants were observed, and representative results are

162 presented. Scale bars = 0.1 mm.

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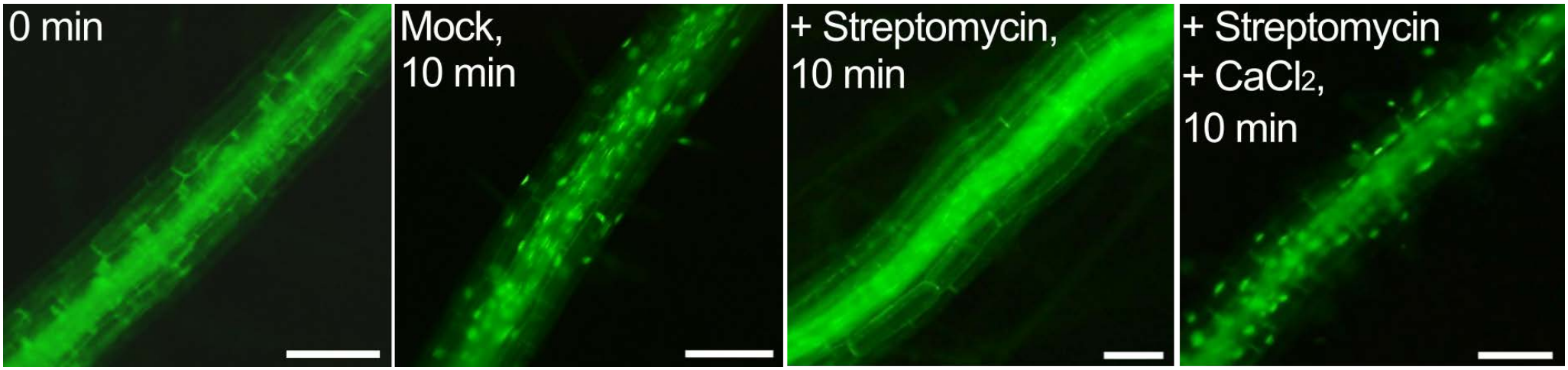
0 min
Mock,
10 min
+ Streptomycin,
10 min
+ Streptomycin
+ CaCl2,
10 min