



Title	Possible inhibition of Arabidopsis VIP1-mediated mechanosensory signaling by streptomycin
Author(s)	Tsugama, Daisuke; Liu, Shenkui; Fujino, Kaiken et al.
Citation	Plant signaling & behavior, 13(10), e1521236 https://doi.org/10.1080/15592324.2018.1521236
Issue Date	2018-10
Doc URL	https://hdl.handle.net/2115/75477
Rights	This is an Accepted Manuscript of an article published by Taylor & Francis in Plant signaling & behavior on 20 Sep 2018, available online: https://doi.org/10.1080/15592324.2018.1521236
Type	journal article
File Information	Short_commun_PSB_r1_clean_texts Strep_PSB_r1.pdf



Possible inhibition of Arabidopsis VIP1-mediated mechanosensory signaling by streptomycin

Daisuke Tsugama^{12*}, Shengkui Liu³, Kaiken Fujino¹ and Tetsuo Takano²

¹Laboratory of Crop Physiology, Research Faculty of Agriculture, Hokkaido University
Kita 9 Nishi 9 Kita-ku, Sapporo-shi, Hokkaido 060-8589, Japan

²Asian Natural Environmental Science Center, The University of Tokyo
1-1-1 Midori-cho, Nishitokyo-shi, Tokyo 188-0002, Japan

³State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, Lin'an,
Hangzhou 311300, P.R. China

Submitted: 20 July 2018

Key words: *Arabidopsis thaliana*; bZIP transcription factor; calcium signaling; hypo-osmotic stress; mechanical stress; root tropisms

***Correspondence to:**

Daisuke Tsugama, Email: tsugama@res.agr.hokudai.ac.jp

18 **Abstract**

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

29

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

43 Phosphorylation is a factor regulating the nuclear-cytoplasmic shuttling of VIP1 and
44 its close homologues. Under steady conditions, VIP1 is largely phosphorylated and bound by
45 14-3-3 proteins, which are thought to retain VIP1 in the cytoplasm. Hypo-osmotic stress
46 causes dephosphorylation of VIP1, and this dephosphorylation can be needed for the nuclear
47 accumulation of VIP1.¹⁴ Calcium signaling has recently been found to be another factor
48 regulating the nuclear-cytoplasmic shuttling of VIP1 and its close homologues. These

49 proteins physically interact with the calmodulin calcium-binding proteins, and both the
50 nuclear accumulation and the cytoplasmic accumulation of VIP1, bZIP59 and bZIP29 are
51 inhibited by either a calcium chelator (EDTA or EGTA) or a calmodulin inhibitor
52 (chlorpromazine).¹⁵ Calcium-dependent protein kinases and B'' subunits of the protein
53 phosphatase 2A complex may regulate phosphorylation states of group I bZIP proteins in
54 calcium-dependent manners,^{12,17} although neither of them was co-immunoprecipitated with
55 VIP1 in a previous study (i.e., they may not interact with VIP1 very strongly).¹⁵ The same
56 study also showed that the nuclear-cytoplasmic shuttling of VIP1 is not affected either by the
57 double knockout of two calcium channels, MCA1 and MCA2, or by GdCl₃ or LaCl₃, which
58 can inhibit other types of mechanosensitive calcium channels.^{15,18,19}

59 Which factor initiates the calcium signaling that regulates functions of group I bZIP
60 proteins, then? In our recent experiments, 1 mM streptomycin, which can inhibit
61 mechanosensitive calcium channels in animals,^{20,21} was found to inhibit the hypo-osmotic
62 stress-induced nuclear accumulation of VIP1 (Fig. 1, middle panel). This effect of
63 streptomycin was not observed when 20 mM CaCl₂ was present (Fig. 1, right panel). Land
64 plants have no homologues of transient receptor potential (TRP) channels,²² which are
65 putative targets of streptomycin in animals,^{20,21} and the major target of streptomycin is
66 thought to be plastid 70S ribosome-mediated protein synthesis in plants.²³ However, it would
67 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

Acknowledgments

This work was supported by research grants from Tokyo Institute of Technology and Kato Memorial Bioscience Foundation.

88 **References**

- 89 1. Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T,
90 Parcy F; bZIP Research Group. bZIP transcription factors in Arabidopsis. Trends Plant
91 Sci 2002; 7:106-111.
- 92 2. Tzfira T, Vaidya M, Citovsky V. VIP1, an Arabidopsis protein that interacts with
93 Agrobacterium VirE2, is involved in VirE2 nuclear import and Agrobacterium infectivity.
94 EMBO J 2001; 20:3596-3607.
- 95 3. Tzfira T, Vaidya M, Citovsky V. Increasing plant susceptibility to Agrobacterium infection
96 by overexpression of the Arabidopsis nuclear protein VIP1. Proc Natl Acad Sci U S A
97 2002; 99:10435-10440.
- 98 4. Djamei A, Pitzschke A, Nakagami H, Rajh I, Hirt H. Trojan horse strategy in Agrobacterium
99 transformation: abusing MAPK defense signaling. Science 2007; 318:453-456.
- 100 5. Wu Y, Zhao Q, Gao L, Yu XM, Fang P, Oliver DJ, Xiang CB. Isolation and
101 characterization of low-sulphur-tolerant mutants of Arabidopsis. J Exp Bot 2010;
102 61:3407-3422.
- 103 6. Tsugama D, Liu S, Takano T. A bZIP protein, VIP1, is a regulator of osmosensory
104 signaling in Arabidopsis. Plant Physiol 2012; 159:144-155.
- 105 7. Van Oosten MJ, Sharkhuu A, Batelli G, Bressan RA, Maggio A. The *Arabidopsis thaliana*
106 mutant *air1* implicates SOS3 in the regulation of anthocyanins under salt stress. Plant

107 Mol. Biol 2013; 83:405-415

108 8. Shi Y, Lee LY, Gelvin SB. Is VIP1 important for *Agrobacterium*-mediated transformation?

109 Plant J 2014; 79:848-860.

110 9. Tsugama D, Liu S, Takano T. Analysis of functions of VIP1 and its close homologs in

111 osmosensory responses of *Arabidopsis thaliana*. PLoS ONE 2014; 9:e103930

112 10. Tsugama D, Liu S, Takano T. The bZIP protein VIP1 is involved in touch responses in

113 *Arabidopsis* roots. Plant Physiol 2016; 171:1355-1365.

114 11. Tsugama D, Liu S, Takano T. VIP1 is very important/interesting protein 1 regulating

115 touch responses of *Arabidopsis*. Plant Signal Behav 2016; 11:e1187358.

116 12. Van Leene J, Blomme J, Kulkarni SR, Cannoot B, De Winne N, Eeckhout D, et al.

117 Functional characterization of the *Arabidopsis* transcription factor bZIP29 reveals its role

118 in leaf and root development. J Exp Bot 2016; 67:5825-5840

119 13. Lapham R, Lee LY, Tsugama D, Lee S, Mengiste T, Gelvin SB. VIP1 and its homologs

120 are not required for *Agrobacterium*-mediated transformation, but play a role in *Botrytis*

121 and salt stress responses. Front Plant Sci 2018; 9:749.

122 14. Takeo K, Ito T. Subcellular localization of VIP1 is regulated by phosphorylation and

123 14-3-3 proteins. FEBS Lett 2017; 591:1972-1981

124 15. Tsugama D, Liu S, Fujino K, Takano T. Calcium signalling regulates the functions of the

125 bZIP protein VIP1 in touch responses in *Arabidopsis thaliana*. Ann Bot 2018; in press

- 126 16. Shih HW, Miller ND, Dai C, Spalding EP, Monshausen GB. The receptor-like kinase
127 FERONIA is required for mechanical signal transduction in Arabidopsis seedlings. *Curr*
128 *Biol* 2014; 24:1887-1892.
- 129 17. Ishida S, Yuasa T, Nakata M, Takahashi Y. A tobacco calcium-dependent protein kinase,
130 CDPK1, regulates the transcription factor REPRESSION OF SHOOT GROWTH in
131 response to gibberellins. *Plant Cell* 2008; 20:3273-3288.
- 132 18. Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, et al. Arabidopsis
133 plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. *Proc Natl*
134 *Acad Sci USA* 2007; 104:3639-3644.
- 135 19. Yamanaka T, Nakagawa Y, Mori K, Nakano M, Imamura T, Kataoka H, et al. MCA1 and
136 MCA2 that mediate Ca^{2+} uptake have distinct and overlapping roles in Arabidopsis. *Plant*
137 *Physiol* 2010; 152:1284-1296.
- 138 20. Brănișteanu DD, Sauciuc A, Proca B, Fuică A, Gogescu G. Mechanisms of the neuromuscular
139 blocking activity of the aminoglycoside antibiotic streptomycin. *Rev Med Chir Soc Med Nat*
140 *Iasi* 1982; 86:287-294
- 141 21. Goralczyk A, van Vijven M, Koch M, Badowski C, Yassin MS, Toh SA, Shabbir A,
142 Franco-Obregón A, Raghunath M. TRP channels in brown and white adipogenesis from
143 human progenitors: new therapeutic targets and the caveats associated with the common
144 antibiotic, streptomycin. *FASEB J* 2017; 31:3251-3266

145 22. Wheeler GL, Brownlee C. Ca^{2+} signalling in plants and green algae--changing channels.

146 Trends Plant Sci 2008; 13:506-514

147 23. Ahlert D, Ruf S, Bock R. Plastid protein synthesis is required for plant development in

148 tobacco. Proc Natl Acad Sci U S A 2003; 100:15730-15735

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
GdCl ₃	Inhibitor of mechanosensitive calcium channels	1 mM	Not detected ¹⁵
LaCl ₃	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)

152

153

154 **Figure legend**

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

156 Arabidopsis plants overexpressing GFP-tagged VIP1⁶ 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 CaCl₂, and subjected to epifluorescence microscopy to detect GFP signals. The presence of

160 streptomycin and CaCl₂ in the solution is indicated as “+ Streptomycin” and “+ CaCl₂”. For each

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

162 presented. Scale bars = 0.1 mm.

163

