ABA represses TOR and root meristem activity through nuclear exit of the SnRK1 kinase

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ABSTRACT

The phytohormone abscisic acid (ABA) promotes plant tolerance to major stresses like drought, partly by modulating plant growth and development. However, the underlying mechanisms are poorly understood. Here, we show that cell proliferation in the *Arabidopsis thaliana* root meristem is controlled by the interplay between three kinases, SNF1-RELATED KINASE 2 (SnRK2), the main driver of ABA signaling, the SnRK1 energy sensor, and the growth-promoting TARGET OF RAPAMYCIN (TOR) kinase. Under favorable conditions, the SnRK1α1 catalytic subunit is enriched in the nuclei of root meristematic cells and this is accompanied by normal cell proliferation and meristem size. Depletion of SnRK2s in a *snrk2.2 snrk2.3* double mutant causes constitutive cytoplasmic localization of SnRK1α1 and a reduction in meristem size, suggesting that, under non-stress conditions, SnRK2s enable growth by retaining SnRK1α1 in the nucleus. In response to elevated ABA levels, SnRK1α1 translocates to the cytoplasm and this is accompanied by inhibition of TOR, decreased cell proliferation and meristem size. Blocking nuclear export with leptomycin B abrogates ABA-driven SnRK1α1 relocalization to the cytoplasm and the inhibition of TOR. Fusion of SnRK1α1 to an SV40 nuclear localization signal leads to defective TOR repression in response to ABA, demonstrating that SnRK1α1 nuclear exit is a premise for this repression. Finally, the SnRK2-dependent changes in SnRK1α1 subcellular localization are specific to the proliferation zone of the meristem, underscoring the relevance of this mechanism for growth regulation.

42 The phytohormone abscisic acid (ABA) plays major roles in plant stress responses. ABA signals
43 are transduced through a well-established pathway whose main effectors in *Arabidopsis* are SNF1-
44 RELATED PROTEIN KINASE 2.2 (SnRK2.2), SnRK2.3, and SnRK2.6 (1). ABA promotes plant
45 adaptation partly by modifying developmental programs, having a major impact e.g. on root
46 architecture (2). ABA modulates primary root (PR) and lateral root (LR) growth through
47 interactions with other hormones, ultimately affecting cell division and elongation by poorly
48 understood mechanisms (2). We recently uncovered an intimate connection between ABA and
49 SnRK1 signalling that is crucial for shaping root architecture in a TARGET OF RAPAMYCIN
50 (TOR)-dependent manner (3). TOR is a protein kinase complex that promotes cell proliferation
51 (4). It is highly enriched in the meristems and its inactivation causes reduced root meristem size
52 and defective PR growth (4). SnRK1 is an evolutionarily conserved protein kinase complex that is
53 activated when energy levels decline during stress, conferring protection partly by limiting growth
54 (5). SnRK1 is also activated by ABA, enabling plants to repress growth when water is scarce (3).
55 Under favorable conditions, the main catalytic subunit SnRK1α1 is sequestered by SnRK2-
56 containing repressor complexes, allowing TOR to be active. In response to ABA, these complexes
57 dissociate, releasing SnRK2 and SnRK1α1, which inhibits TOR and growth (3). Consistent with
58 this model, the *snrk2.2 snrk2.3* mutant (*snrk2d*) shows markedly defective PR growth under
59 favorable conditions due to aberrant repression of TOR activity (3). This defect is fully rescued by
60 the *snrk1α1* mutation, demonstrating it is SnRK1α1-dependent (3).

61 To investigate further how TOR and root growth are controlled by SnRK2 and SnRK1, we
62 examined the root meristems of Col-0 control seedlings, the *snrk2d* mutant, and its cross with
63 *snrk1α1* (*snrk2d/1α1*) (Fig. 1*A*). As previously reported (6, 7), ABA treatment reduced the number
64 of root meristematic cells, leading to smaller meristems in Col-0. In contrast, *snrk2d* showed a
65 reduction in meristem size and cell number already under mock conditions, and this was fully
66 rescued by the *snrk1α1* mutation. Consistent with the ABA hyposensitivity of *snrk2d* and
67 *snrk2d/1α1* (3), the addition of ABA did not further decrease meristem size or cell number in these
68 mutants (Fig. 1*A*). These meristem phenotypes correlate well with the PR length previously
69 observed in these mutants and conditions (3) and suggest that reduced cell proliferation contributes
70 to the reduced PR length of *snrk2d* in mock, mimicking the situation of Col-0 plants under ABA.

71 We next hypothesized that regulation of SnRK1 and growth by ABA and SnRK2s could
72 involve changes in SnRK1α1 subcellular localization. Firstly, SnRK1 and SnRK2 are highly
73 enriched in the nuclei of root meristematic cells (3). Secondly, SnRK2-harboring SnRK1 repressor
74 complexes localize to the nucleus in *Nicotiana benthamiana* epidermal cells (3). Thirdly, *in planta,*
75 the TOR complex subunit RAPTOR1B interacts with SnRK1α1 (3, 8) in the cytosol (9). Fourthly,
76 the subcellular localization of SnRK1α1 is central to its function (10). We therefore monitored
77 SnRK1α1 subcellular localization in mock- or ABA-treated seedlings in two independent lines
78 expressing SnRK1α1-GFP from its own regulatory regions (*SnRK1α1-GFP#1* and *SnRK1α1-*
79 *GFP#2*). SnRK1α1 was detected in the meristematic, elongating and differentiating zones of the
80 PR both in mock and ABA, displaying a known nuclear and cytoplasmic localization (11) (Fig.
81 1*B*). In mock, SnRK1α1 exhibited a ring-shaped pattern characteristic of nuclear proteins that are
82 absent from the nucleolus in meristematic cells (12) (Fig. 1*B*). In ABA, the nuclear signal appeared
83 to decline specifically in the meristematic zone (Fig. 1*B*). Electron micrographs confirmed that a
84 ring-shaped nuclear region surrounds a large-sized nucleolus in these cells (Fig. 1*C*). Closer
85 examination and GFP quantification in the meristematic zone revealed overall similar SnRK1α1-
86 GFP levels in mock- and ABA-treated roots but a reduction in the nucleus to cytoplasm ratio (N/C)
87 from 1.19 (line #1) and 1.2 (line #2) in mock to 0.73 (line #1) and 0.71 (line #2) in ABA (Fig. 1*D*),
88 suggesting that ABA induces SnRK1α1 relocalization from the nucleus to the cytoplasm.
89 Accordingly, the ABA effect was fully abolished by the nuclear export inhibitor Leptomycin B
90 (LMB; N/C ratio=1.36 and 1.23 in lines #1 and #2; Fig. 1*D*).

91 Given the marked reduction in meristem size (Fig. 1*A*) and PR length (3) of the *snrk2d*
92 mutant, we investigated the role of SnRK2s in SnRK1α1 localization using the cross of *snrk2d*
93 with the *SnRK1α1-GFP#2* line (Fig. 1*E*). In mock, SnRK1α1-GFP was barely present in the nuclei
94 of *snrk2d* meristematic cells, with most of the GFP signal being cytoplasmatic and yielding a N/C
95 ratio of 0.75 (Fig. 1*E*), comparable to the ABA-treated *SnRK1α1-GFP#2* control (Fig. 1*D*).
96 SnRK2.2-GFP suffered from overall protein degradation, but there was no change in its subcellular
97 localization (Fig. 1*F*). Nuclear exit of SnRK1α1 could be observed in other highly proliferating
98 regions of the root, such as the LR tip (Fig. 1*G*). However, in differentiated root cells, ABA
99 treatment and SnRK2 depletion did not alter SnRK1α1 subcellular localization (Fig. 1*H*).
100 Furthermore, the ABA-triggered changes in SnRK1α1 localization remained undetected in
101 nucleus/cytoplasm fractions of whole roots (Fig. 1*I*). Altogether, these results indicate that the

102 SnRK2-dependent localization of SnRK1α1 and its ABA-triggered nuclear exit is specific to
103 meristematic cells, consistent with reports that SnRK1α1 subcellular localization is controlled in
104 a cell type-specific manner (13, 14).

105 Given that SnRK1α1 is required for the inhibitory effect of ABA on TOR and growth (3),
106 we wondered whether SnRK1α1 nuclear exit is a premise for such inhibition. Indeed, pre-treatment
107 with LMB blocked the ABA-triggered repression of TOR activity (Fig. 2*A*), measured as
108 phosphorylation of ribosomal protein S6 [$RPS6^{S240}$; (3)]. Moreover, as compared to control plants
109 expressing wild-type SnRK1α1 (*control-α1*), the repression of TOR by ABA was also defective
110 when SnRK1α1 was fused to an SV40 nuclear localization sequence (NLS) that favors its presence
111 in the nucleus (*NLS-α1*; Fig. 2*B*). This demonstrates that SnRK1α1 nuclear exit is necessary for
112 inhibiting TOR in response to ABA. Furthermore, the fact that nuclear export is crucial for
113 repressing TOR (Fig. 2*A*) but only SnRK1α1 (Fig. 1*D*), and not SnRK2.2, translocates to the
114 cytoplasm in response to the hormone (Fig. 1*F*), suggests that the previously reported role of
115 SnRK2s in this process (3, 15) may be indirect *via* SnRK1α1 regulation.

116 We conclude that root growth is modulated by ABA through changes in SnRK1α1
117 subcellular localization, allowing control of TOR activity and cell proliferation in the root
118 meristem in accordance to *e.g.* water availability (Fig. 2*C*). When conditions are favorable,
119 SnRK1α1 is sequestered in the nucleus by SnRK2-containing repressor complexes. Dissociation
120 of these complexes in response to ABA releases SnRK1α1, which translocates to the cytoplasm
121 and inhibits TOR activity and growth. This mode of regulation appears to be specific to
122 meristematic cells, where TOR is highly enriched and where active cell proliferation takes place
123 (4). Future work will address whether such specificity stems from the exclusive ability of
124 meristematic cells to form SnRK2-SnRK1α1 complexes, to export SnRK1α1 from the nucleus or
125 both.

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FIGURE LEGENDS

Figure 1. Impact of ABA and SnRK2s on SnRK1α1 subcellular localization and cell proliferation in the root apical meristem. (*A*) Meristems of Col-0, *snrk2d* and *snrk2d/1α1* 7d-old seedlings with or without ABA treatment (50 µM, 48h). Arrowheads: region for cortical cell number and meristem length quantifications (violin plots). n=23-24; $p < 0.05$, one-way ANOVA with Tukey's HSD test. Scale bar: 50 µm. (*B*) Root apices of 4d-old *SnRK1α1-GFP#1* seedlings with or without ABA treatment (50 µM, 3h). Scale bar: 30 µm. Arrowheads: same position between upper and bottom photographs. (*C*) Electron micrograph of meristematic epidermal cells

174 with magnification (*right panel*) showing the cellular ultrastructure. C: cytoplasm; N: nucleus; No:
175 nucleolus. Scale bars: left, 5 µm; right, 1 µm. (*D-E*) SnRK1α1-GFP subcellular localization in
176 epidermal cells of 4d-old root meristems. Scale bar: 10 µm. Dotted lines: nuclear boundary.
177 Quantification of SnRK1α1-GFP mean cellular fluorescence and nucleus-to-cytosol (N/C) ratios.
178 *n*=5-6 (*D*) or 8 (*E*); one-way ANOVA with Dunett´s test. (*D*) Two independent SnRK1α1-GFP
179 lines (#1 and #2) with or without ABA (50 µM, 3h) or LMB (2.5 µM, 1h prior to ABA addition)
180 treatment. (*E*) Impact of the presence (control, *SnRK1α1-GFP#2)* or absence (*SnRK1α1-GFP#2;*
181 *snrk2d*) of SnRK2s. (*F*) Expression and subcellular localization of SnRK2.2-GFP in epidermal
182 cells of 4d-old root meristems treated and quantified as in (*D*). Scale bar: 10 µm. *n*=5; two-tailed
183 Student t-test. (*G-H*) SnRK1α1-GFP subcellular localization in LRs (*G*) and the PR differentiation
184 zone (*H*) in seedlings (Line #1 and/or #2) with or without ABA treatment (50 µM, 3h) and in
185 absence (*SnRK1α1-GFP#2; snrk2d*) of SnRK2s. Scale bar: 30 µm. (*G*) Nine-day old seedlings.
186 (*H*) Four-day old seedlings**.** N/C ratios of differentiated root cells. *n*=5, two-tailed Student t-test
187 (line #1) or one-way ANOVA (line #2). *PI*, propidium iodide. *ns*, non-significant. (*I*)
188 Representative SnRK1α1 immunoblot from nuclear and cytoplasmic fractions of 14d-old Col-0
189 whole roots with or without ABA (50 µM ABA, 3h). H3, Histone 3 marker for nuclear fractions.

190 **Figure 2. Impact of SnRK1α1 subcellular localization on TOR signaling and root growth.** (*A-*
191 *B*) Representative immunoblots and quantification of $RPS6^{S240}$ phosphorylation (phospho-
192 RPS6/total-RPS6) in Col-0 (*A*) or *control-α1* and *NLS-α1* (*B*) seedlings with or without ABA (50
193 µM, 3h) or LMB (2.5 µM, 1h prior to ABA addition). Images from the same gel were cropped for
194 showing *α1* and *NLS-α1* contiguously. (*A*) *n*=5; (*B*) *n*= 3; error bars, SEM; two-tailed Student *t-*
195 test. (*C*) Under favorable conditions, SnRK1α1 is sequestered in the nucleus by repressor
196 complexes containing SnRK2 [and a PP2C (3)], enabling TOR activity in the cytoplasm,
197 meristematic cell proliferation and root growth. Dissociation of these complexes in ABA by the
198 hormone-bound PYR/PYL/RCAR receptors releases SnRK1α1 which exits the nucleus and
199 inhibits TOR and growth. *TORC1*, TOR Complex 1; *N*, nucleus; *C*, cytoplasm. Created with
200 BioRender.com.

201 **Materials and Methods**

202 Experimental details are provided in SI Appendix.

All study data are included in the paper.

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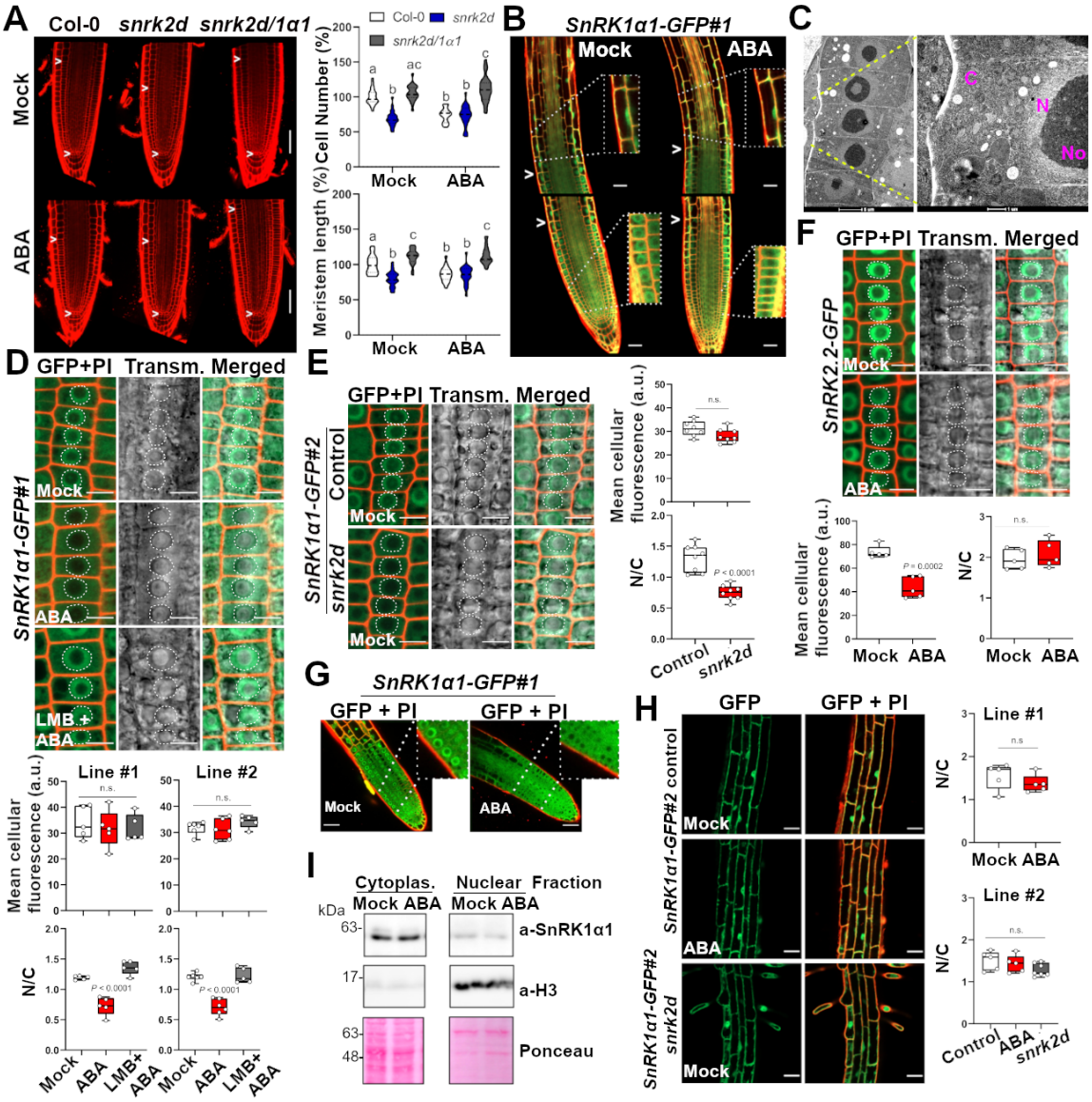
Author contributions

BBP performed experiments and analyzed data. MC performed the root meristem assays and analyzed the data. TB and FR contributed tools and expertise and supported conceptual work. EBG and BBP conceived the project, designed experiments, analyzed and interpreted data and wrote the manuscript.

Competing interests

The authors declare no competing interests.



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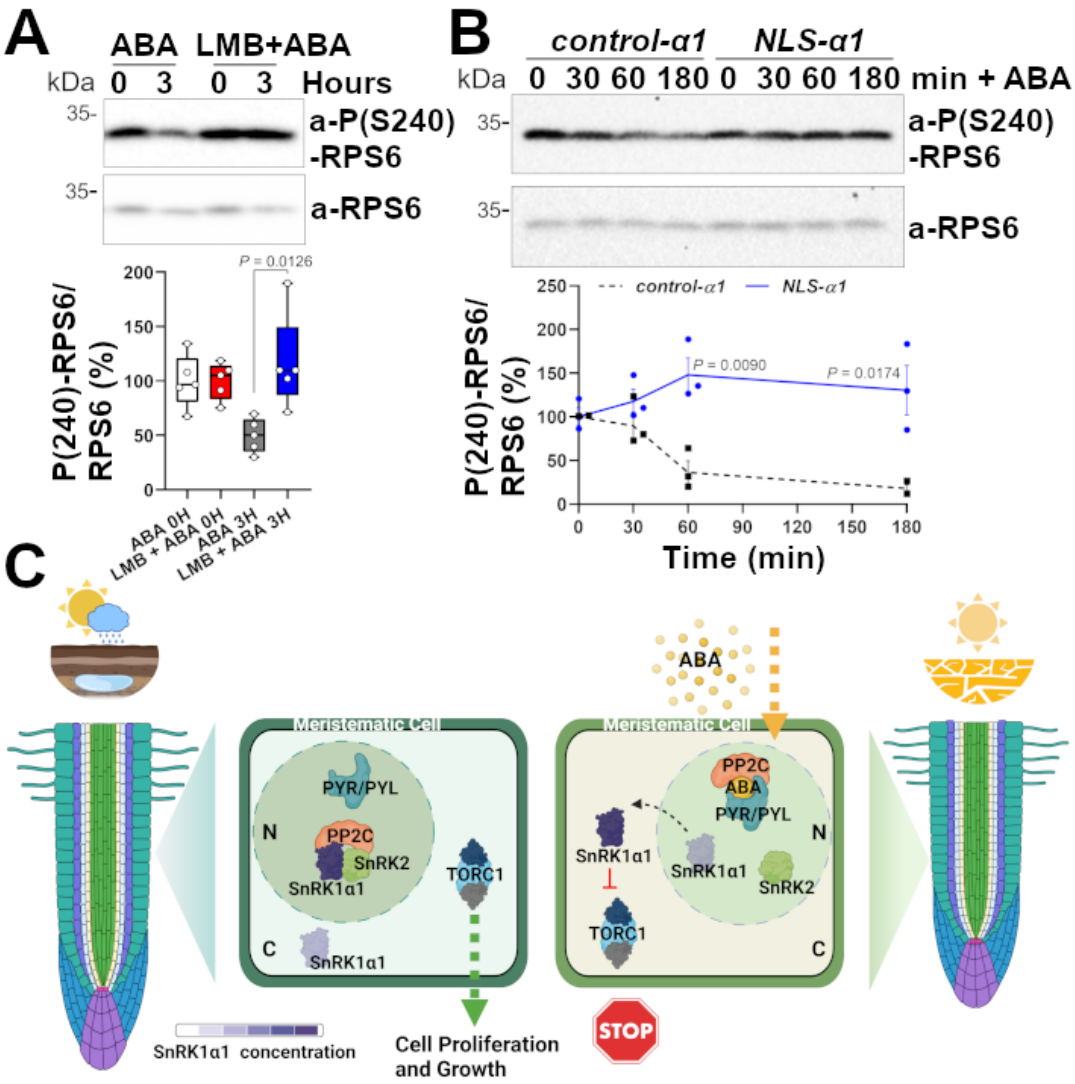

Figure 2. Impact of SnRK1α1 subcellular localization on TOR signaling and root growth. (*A-B*) Representative immunoblots and quantification of RPS6^{S240} phosphorylation (phospho-RPS6/total-RPS6) in Col-0 (*A*) or *control-α1* and *NLS-α1* (*B*) seedlings with or without ABA (50 µM, 3h) or LMB (2.5 µM, 1h prior to ABA addition). Images from the same gel were cropped for showing *α1* and *NLS-α1* contiguously. (*A*) *n*=5; (*B*) *n*= 3; error bars, SEM; two-tailed Student *t*-test. (*C*) Under favorable conditions, SnRK1α1 is sequestered in the nucleus by repressor complexes containing SnRK2 [and a PP2C (3)], enabling TOR activity in the cytoplasm, meristematic cell proliferation and root growth. Dissociation of these complexes in ABA by the hormone-bound PYR/PYL/RCAR receptors releases SnRK1α1 which exits the nucleus and inhibits TOR and growth. *TORC1*, TOR Complex 1; *N*, nucleus; *C*, cytoplasm. Created with BioRender.com.