Moonlighting SnRK2s stabilize a bZIP–bHLH switch in light-instructed plant development

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Abstract: In this issue of *Developmental Cell*, Qin et al. identify three members of the ABA-regulated kinase family SnRK2 as light quality-dependent regulators of hypocotyl elongation. SnRK2s stabilize the transcription factors HY5 and PIF4 independently of their kinase activity, revealing an abscisic acid-independent role for SnRK2s in post-translational regulation.

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Following germination light-exposed seedlings undergo photomorphogenesis. During this developmental program, seedlings undergo complex morphological changes, including a slowdown in hypocotyl elongation. These responses are controlled by signaling networks that combine environmental and internal cues. Revealing how these signals are integrated will help explain how plants adapt to both favorable and stressful environments. In this context, Qin et al.[1] identify a non-canonical role for a class of osmotic stress/abscisic acid (ABA)-regulated SNF1-related protein kinase 2s (SnRK2s) during photomorphogenesis.

In Arabidopsis, the SnRK2 family comprises 10 members grouped into subclasses I, II, and III[2]. Subclass III proteins (SnRK2.2/SRK2D, SnRK2.3/SRK2I, and SnRK2.6/SRK2E/OST1) show the strongest ABA-dependent activation. Far-red (FR)–enriched environments, such as canopy shade, are photosynthetically unfavorable and may act as stress signals. Under FR conditions s*nrk2.2/2.3/2.6* triple mutants displayed increased hypocotyl elongation compared with the wild type, despite no osmotic or nutritional/energy stress - inducing agents were applied to the growth media. No elongation defects were observed in darkness, while shorter hypocotyls were observed under red (R), blue, white light, and moderate shade. These phenotypes were specific to *snrk2.2/2.3/2.6*, as neither single, double mutant combinations of *snrk2s*, nor complementation of *snrk2.2/2.3/2.6* with *SnRK2.6* displayed the mutant phenotypes. Therefore, SnRK2s suppress hypocotyl elongation under FR-rich conditions but promote elongation under other light environments.

Photoreceptors phytochromes (phy) A and B mediate FR and R - induced responses, respectively. Light-activated phys interact with PHYTOCHROME INTERACTING FACTORs (PIFs), encoding a group of basic helix loop helix transcription factors that promote hypocotyl elongation and suppress photomorphogenesis[3]. Phys trigger rapid

phosphorylation and proteasome-mediated degradation of the PIFs, thereby relieving their repression of photomorphogenesis. ELONGATED HYPOCOTYL 5 (HY5), a bZIP transcription factor, acts antagonistically to the PIFs by promoting photomorphogenesis, thus inhibiting hypocotyl elongation[4]. Qin et al.[1] detected clear reductions in HY5 protein accumulation in *snrk2.2/3/6* mutants under both FR and R light. PIF4 accumulation was also reduced across all light conditions and in darkness. The combination of *in vitro* and *in vivo* protein-protein interaction studies led to the conclusion that the SnRK2s bind to and promote the accumulation of the two transcription factors with opposite effects: low HY5 accumulation in *snrk2.2/3/6* mutants may account for the increased hypocotyl elongation under FR, whereas reduced PIF4 levels may explain their shorter hypocotyls under R. This idea was further supported by mutant analyses. *hy5* mutants did not enhance the long-hypocotyl phenotype of *snrk2.2/3/6* in FR. Similarly, introducing *pif4* in *snrk2.2/3/6* did not aggravate the short hypocotyl phenotype of *snrk2.2/3/6* under R light. In agreement with the different contribution of HY5 and PIF4 depending on light quality, *hy5 pif4* double mutants resembled *hy5* under FR, indicating that loss of *PIF4* cannot rescue the *hy5* long-hypocotyl phenotype. *hy5 pif4* were similar to the wild type under R, indicating that loss of *PIF4* can largely rescue the *hy5* long-hypocotyl phenotype.

A key finding of Qin et al.[1] is that the regulation of SnRK2s on HY5 and PIF4 is independent of their ABA-induced kinase activity. R or FR could not stimulate SnRK2s kinase activity. Importantly, a SnRK2 protein defective in ABA-stimulated autophosphorylation was still able to rescue the hypocotyl phenotypes of *snrk2.2/3/6* in both R and FR. Treatment with the proteasome inhibitor MG132 revealed increased ubiquitination of HY5 in *snrk2.2/3/6* mutants compared with the wild type. CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1), an E3 ubiquitin ligase and

repressor of photomorphogenesis, targets HY5 for degradation[4]. Using transient expression assays and *in vitro* interaction studies, the authors showed that SnRK2.6 could interfere with the COP1-HY5 interaction and reduce COP1-mediated HY5 degradation. Notably, kinase-dead SnRK2.6 proteins retained this function. Conversely, COP1 could stabilize PIF4 under FR and R light[1]. Although the molecular basis of this role remains unresolved, COP1 could bind to the active phytochrome B–binding motif of PIF4, the same motif that enables PIFs association with R - activated phyB. *In vitro* and transient assays data indicated that, independent of their kinase activity, SnRK2.6 could facilitate the formation of the COP1-PIF4 nuclear complex and promote PIF4 stabilization.

Qin et al.[1] reveal that SnRK2s can either cooperate with or antagonize COP1, independent of their role as ABA-regulated kinases during plant development[5] (Figure 1). The kinase domains contain specialized binding sites with scaffolding function for target-specific phosphorylation. However, non-catalytic functions of kinases have been described, including those acting as adaptors for ubiquitin ligases[6]. Homologues of subclass III SnRK2 are found in algae[7], suggesting a possible coevolution with light signaling components such as COP1, HY5, and PIFs before terrestrial colonization and recruitment into the ABA pathway. This might thus represent an ancient function of the SnRK2s. However, how does ABA-regulated SnRK2s signaling coexist with their non-catalytic function in land plants? Recent studies indicate that FR increases ABA levels, and that ABA represses hypocotyl elongation[8]. Moreover, the inhibitory effects of ABA on hypocotyl elongation are strictly light dependent[9]. Thus, non-kinase functions of SnRK2s might either reinforce (in FR) or antagonize (in R) their role in ABA signaling. SnRK2s were also shown to promote seedlings growth by sequestering another kinase, SnRK1, thereby activating Target of Rapamycin (TOR) signaling[10].

Formation of the SnRK2-SnRK1 complex requires type 2C phosphatases (PP2Cs) but not SnRK2 kinase activity. Under high ABA conditions, sequestration of PP2Cs by the ABA receptors causes dissociation of the SnRK2-SnRK1 complex and SnRK2s -mediated growth inhibition. SnRK2s thus emerge as key nodes mediating between promoting/inhibiting growth and relaying stress responses depending on ABA levels, light conditions, and protein-protein interactions. Further investigation is needed to assess the scaffolding role of SnRK2s for other kinases (or phosphatases) that could modulate HY5/PIF4 phosphorylation. In this context, PP2Cs binding to the C terminus of SnRK2s may overlap with the binding sites for HY5 and PIF4. Do PP2Cs regulate HY5 and PIF4 binding under non stress conditions? Does HY5/PIF4 binding affect SnRK2 kinase activity? Future studies will reveal the structural basis of the SnRK2-HY5/PIF4 complexes and their interface with COP1 and PP2Cs, allowing a more precise understanding of ABA dependent and independent roles of SnRK2 in plant development.

Figure 1 Graphic summary of SnRK2 functions in distinct signaling pathways and their effect on hypocotyl growth. Structures illustrate AlphaFold predictions modified with ChimeraX, based on PDB 3UJG and 8YB4.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used Copilot in order to improve clarity and refine grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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