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Dual regulation of SnRK2 signaling by Raf-like MAPKKKs

Plants must adapt to unfavorable growth conditions such as drought, salinity, or extreme temperature and, in addition, need the flexibility to resume growth when conditions become favorable. Sensing of extracellular environmental stress and signal transduction rely on pathways that in many cases use phosphorylation of target proteins to amplify and transmit the extracellular signal. MAPK signaling has been intensively studied in yeasts, humans, and plants, and represents a signal transduction module that transmits extracellular signals to generate different cellular responses (Jonak et al., 2002). Specifically, for osmotic stress signaling in plants, both ABA (abscisic acid)-dependent and ABA-independent sucrose nonfermenting1-related protein kinases (SnRK2s) play a key role in plant adaptation (reviewed by Lozano-Juste et al., 2020). The unexpected connection in the plant field between mitogen-activated protein kinase kinase kinases (MAPKKKs) and SnRK2s was a recent breakthrough, and new findings have emerged recently that add further complexity to this signaling node (Kamiyama et al., 2021; Sun et al., 2022).

MAPK signaling is a versatile pathway that is activated in response to hormones, plant pathogens, and osmotic stress. For example, MAPK signaling is crucial in plants in the ethylene pathway, in which CTR1 is a MAPKKK that acts to negatively regulate ethylene signaling, acting upstream of MKK9–MPK3/6 proteins (Yoo et al., 2008). The original concept of the MAPK signaling cascade has evolved in plants, and the traditional MAPK cascade composed of three kinase components, i.e., MAPKKK, MAPKK, and MAPK, does not function always in a sequential manner in plant cells. For example, members of the MAPKKKs show direct interaction with SnRK2s without the need for mediatory MAPKKs. MAPKKKs include 80 members in *Arabidopsis*, distributed as 21 MEKK-like, 11 ZIK, and 48 Raf-like protein kinases (Jonak et al., 2002). Members of the *Arabidopsis* B-type RAFs are key regulators of the osmotic stress response, and direct interactions among B-type and C-type RAFs and SnRK2s have been reported (Figure 1A). A positive interaction was described between B2-, B3-, and B4-type RAFs and SnRK2s that leads to activation of ABA-dependent and ABA-independent SnRK2s (Lozano-Juste et al., 2020). In this case, RAFs function as upstream activating kinases of SnRK2s, which are substrates of RAFs. Thus, B2-, B3-, and B4-type RAFs positively regulate the SnRK2s by phosphorylation, restoring their activity after the previous inactivation of SnRK2s by phosphatases (Lozano-Juste et al., 2020).

A twist has been recently provided by C-type RAFs that negatively regulate ABA-activated SnRK2s under favorable growth conditions, whereas under stress conditions, these RAFs are SnRK2 substrates that are inactivated by SnRK2-dependent phosphorylation (Kamiyama et al., 2021; Sun et al., 2022). The first reports on C-type RAFs illuminated the role of these kinases in phototropin- and low CO_2-mediated stomatal opening (Hashimoto et al., 2006; Hiyama et al., 2017). For example, HT1/RAF19, which belongs to the C5-type branch, regulates CO_2 signaling in stomata together with other components, including SnRK2.6/OST1/SRK2E, and HT1 seems to function independent of ABA (Horak et al., 2016). In the field of osmotic stress response, recent reports on two C-type RAFs, i.e., RAF36 and RAF22, indicate that these enzymes act as brakes on ABA-responsive SnRK2s (Kamiyama et al., 2021; Sun et al., 2022). Thus, RAF36, which functions partially redundantly with RAF22, interacts with ABA-activated SnRK2s and negatively regulates ABA-mediated inhibition of post-germinative growth (Kamiyama et al., 2021). Recently, Sun et al. (2022) identified RAF22 as a negative regulator of drought stress response, which further supports the negative regulation of ABA signaling by C-type RAFs. The protein kinase activity of both RAF36 and RAF22 is required for this role; however, dead versions of SnRK2s are not substrates of these RAFs. Conversely, OST1 was able to phosphorylate them, and the downstream effect was unveiled by Kamiyama et al. (2021), who found that the N terminus of RAF36 is phosphorylated in Ser145 by ABA-responsive SnRK2s, and this seems to affect RAF36 stability. MAPKKKs contain the conserved kinase domain at the C-terminal part of the enzyme, whereas the N-terminal region is more variable and plays a regulatory role (Kamiyama et al., 2021) (Figure 1A).

Sun et al. (2022) have recently described a three-partner network that involves RAF22, ABI1, and OST1, which can be summarized as follows: (i) the mutual interaction of RAF22 and ABI1 leads to activation of both proteins, and phosphorylation of ABI1 by RAF22 enhances its phosphatase activity (which leads to OST1 inhibition and ABA signaling is OFF), whereas dephosphorylation of RAF22 by ABI1 enhances kinase activity of RAF22; (ii) in turn, OST1 phosphorylates RAF22 and inhibits its kinase activity (thus activating ABA signaling). This biochemical regulation leads to fine-tuning of ABA signaling during normal growth or stress conditions. From the study of Sun et al. (2022) emerges an additional mechanism to regulate ABI1 activity, which, upon RAF22-mediated phosphorylation of ABI1 S416 (at low ABA levels), likely escapes from PYR/PYL-mediated inhibition (Figure 1B). This mechanism converges with Target of Rapamycin (TOR)-mediated phosphorylation of PYLs to inhibit their association with clade A protein phosphatases type 2C (PP2Cs), such as ABI1 (Wang et al., 2018); therefore, both TOR and RAF22 prevent inhibition of PP2Cs under non-stress conditions

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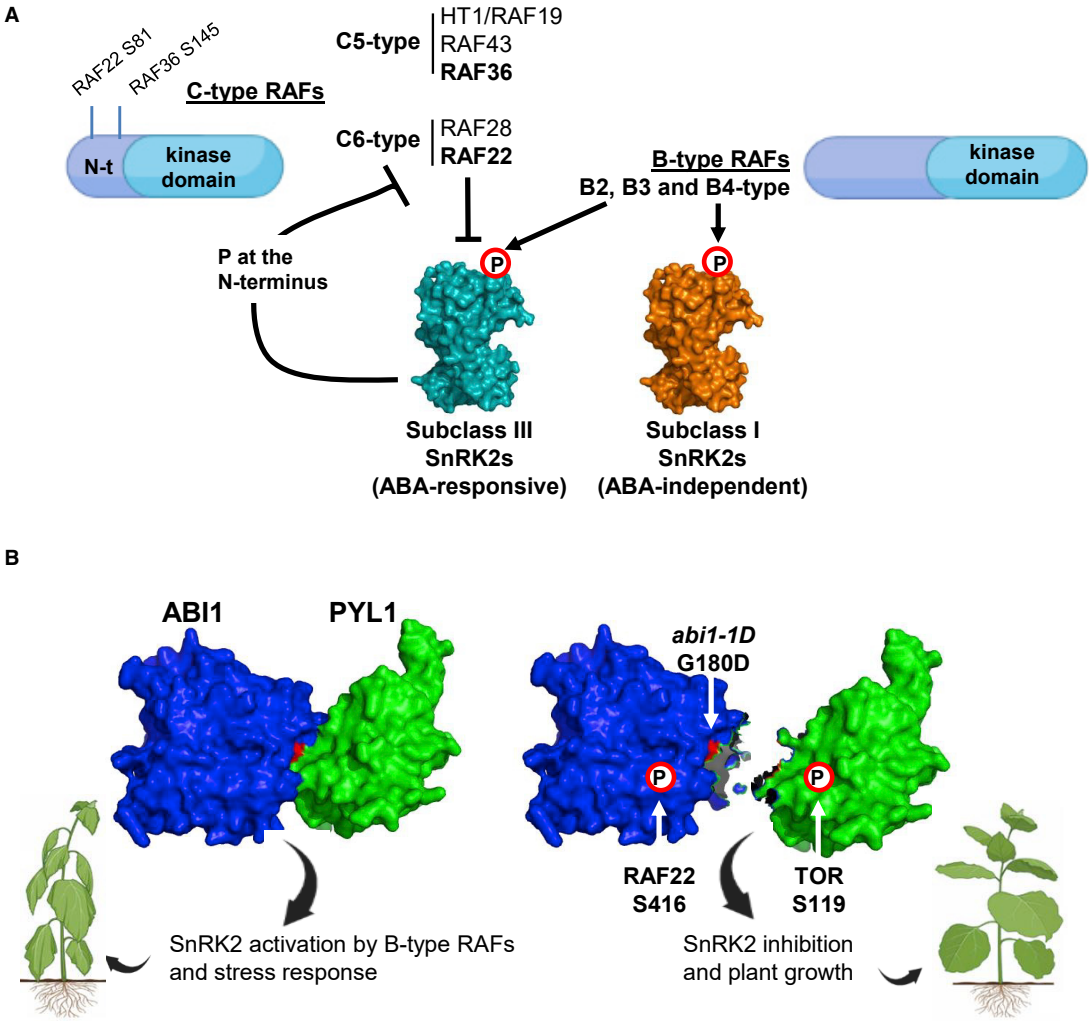

Figure 1. Molecular mechanisms that mediate positive and negative regulation of SnRK2s.

(A) C- and B-type RAFs regulate the activity of SnRK2s. The C5-type RAF36 and C6-type RAF22 negatively regulate ABA-responsive SnRK2s, although SnRK2s are not phosphorylated by RAF36/RAF22. Under stress conditions, ABA-activated SnRK2s phosphorylate the N terminus of C-type RAF36/RAF22 and promote its degradation (RAF36) or inhibit its activity (RAF22). Thus, ABA-activated SnRK2s relieve the brake of C-type RAFs. Concomitantly, B2- and B3-type RAFs act as positive regulators of subclass III SnRK2s, whereas B4-type RAFs are positive regulators of ABA-independent subclass I SnRK2s. As a model for the structure of SnRK2s, the 3UC3 code was used.

(B) Regulation of the clade A PP2C–PYL interplay determines plant response to the environment. Impairment of the interaction between the PP2C and the ABA receptor leads to inhibition of ABA-responsive SnRK2s under favorable growth conditions (right). Blockade of the ABI1–PYL interaction is achieved either by RAF22-mediated phosphorylation of ABI1 S416 or through TOR-mediated phosphorylation of PYL1 S119 (indicated by P). The ethyl methanesulfonate-induced *abi1-1D* mutation also abolishes the interaction of ABI1 with ABA receptors. Conversely, inhibition of clade A PP2Cs by ABA and ABA receptors leads to activation of SnRK2s and stress response (left). The structure of the ABI1–ABA–PYL1 complex was obtained from the 3KDJ code (Protein Data Bank). Icons were obtained from BioRender.

(Figure 1B). These results, as well as other regulatory mechanisms of the PYL–PP2C interplay, highlight that basal PP2C activity is exquisitely maintained to favor growth. Moreover, the positive interplay between RAF22 and ABI1 raised the question of how RAF22 is inhibited when ABA levels increase. Otherwise, RAF22-mediated phosphorylation of ABI1 would prevent inhibition of the phosphatase by PYR/PYLs when ABA levels increase. Inhibition of RAF22 is achieved by OST1-dependent phosphorylation (at high ABA levels), RAF22 S81 being a key target residue (Kamiyama et al., 2021; Sun et al., 2022). Thus, in the absence of the stimulatory effect of RAF22 on ABI1, and the inhibitory effect of PYR/PYLs on PP2Cs when ABA levels increase, plants adapt to drought stress (Figure 1B).

The balance between plant growth and stress response is modulated by different pathways, and SnRK2s play a central role, either as a target to be inhibited under non-stress conditions or activated under harsh conditions (reviewed by Yang et al., 2019). The TOR complex is a prominent regulator of plant growth. TOR is inhibited by energy-sensing SNF1-related protein kinase 1 (SnRK1), and sequestration of SnRK1 by SnRK2-containing complexes inhibits SnRK1 signaling, thereby allowing growth under optimal conditions (Belda-Palazon et al., 2020). This is complemented by TOR-mediated phosphorylation of a conserved Ser in the proximity of the gate loop of PYLs, which prevents PP2C inhibition and maintains repression of ABA signaling (Wang et al., 2018). Moreover, ABI1

and ABI2 dephosphorylate and inhibit BIN2, which favors brassinosteroid signaling and prevents the activation of SnRK2.2/2.3 by BIN2-dependent phosphorylation (Yang et al., 2019). The BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) further inhibits OST1 through phosphorylation of T146 (Deng et al., 2022). The cytokinin pathway is activated to favor plant growth and also leads to SnRK2 inhibition, specifically through the type B ARRs (*Arabidopsis* response regulators) that are negative regulators of ABA-responsive SnRK2s (Yang et al., 2019). In summary, the C-type RAF36/RAF22 kinases and clade A PP2Cs act in concert with the TOR, brassinosteroid, and cytokinin pathways to promote plant growth under favorable conditions, whereas B-type RAFs and PYLs are key elements to activate SnRK2s under stress conditions.

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