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A direct link between BR and SA signaling: Negative regulation of TGA4 by BIN2

It is well known that plant growth, development, and response to environmental stresses are harmonized through the action of a suite of growth and defense signaling molecules. Brassinosteroid (BR) is a steroid hormone that regulates plant growth and development. BR binds to and activates the BR-INSENSITIVE 1 (BRI1)/BRI1-ASSOCIATED KINASE 1 receptor kinase complex on the cell surface to phosphorylate the intracellular receptor-like cytoplasmic kinases BR SIGNALING KINASE 1 and CONSTITUTIVE DIFFERENTIAL GROWTH 1, which in turn phosphorylate the phosphatase BRI1 SUPPRESSOR 1. BRI1 SUPPRESSOR 1 then dephosphorylates and inhibits the glycogen synthase kinase 3-like kinase BR-INSENSITIVE 2 (BIN2) that constitutively phosphorylates and suppresses the BR-responsive transcription factors BRASSINAZOLE-RESISTANT 1 and BRI1-EMS-SUPPRESSOR 1, leading to BR-mediated transcriptional reprogramming (Wang et al., 2014). In the past several years, multiple components of the BR signaling pathway, including BRI1-ASSOCIATED KINASE 1, BR SIGNALING KINASE 1, BRASSINAZOLE-RESISTANT 1, and BRI1-EMS-SUPPRESSOR 1, have been implicated in pathogen-associated molecular pattern-triggered immunity or jasmonic-acid-mediated immune responses (Chinchilla et al., 2007; Miyaji et al., 2014; Zhao et al., 2019; Liao et al., 2020). Moreover, BR has been shown to induce the expression of the *PATHOGENESIS-RELATED GENE 1* (*PR1*) gene, a hallmark of the salicylic acid (SA) signaling pathway (Divi et al., 2010). Recently, Kim et al. (2022) showed that BIN2 phosphorylates and impedes TGACG MOTIF-BINDING FACTOR 1 (TGA1) and TGA4, two basic leucine zipper transcription factors involved in SA signaling. This result unveiled a direct link between BR and SA signaling (Figure 1).

SA is a key immune signaling molecule required for both basal immunity and systemic acquired resistance. SA is perceived by NONEXPRESSOR OF PR GENES (NPR) proteins (Fu and Dong, 2013). NPR1 is a key positive regulator of SA signaling, which functions as a co-activator and activates transcription through interaction with TGAs (Fu and Dong, 2013). In *Arabidopsis thaliana*, the TGA family has 10 members that are divided into five clades (clade I: TGA1 and TGA4; clade II: TGA2, TGA5, and TGA6; clad III: TGA3 and TGA7; clade IV: TGA9 and TGA10; and clade V: TGA8). Although members of clades I, II, and III all interact with NPR1, they function in plant immune responses through distinct mechanisms. TGA2, TGA5, and TGA6 transduce SA-induced signaling and are required for systemic acquired resistance (Zhang et al., 2003). TGA3 bridges cytokinin-promoted immune responses and plays a role in basal immunity (Kesarwani et al., 2007; Choi et al., 2010). TGA1 and TGA4 have been shown to modulate SA biosynthesis by regulating the expression of *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT one* and *CALMODULIN-BINDING PROTEIN 60g* (Sun et al., 2018). In their report, Kim et al. (2022) revealed that TGA1 and TGA4 channel BR signaling into the SA signaling pathway by acting as substrates of BIN2.

Initially, Kim et al. (2022) found that brassinolide (BL; the most active BR) and bikinin (a competitive inhibitor of BIN2) significantly increased SA-induced resistance to the hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 and greatly enhanced the induction of several SA-responsive genes. Furthermore, SA-induced resistance to *Pst* DC3000 and expression of the SA-responsive genes were significantly reduced and increased in the gain-of-function mutant *bin2-1* and the loss-of-function triple-mutant *bin2-3 bil1 bil2*, respectively, indicating that BIN2 also negatively regulates SA signaling. Subsequently, Kim et al. (2022) produced a series of results that revealed the biochemical action of BIN2 on SA signaling. First, BIN2 interacted with TGA1 and TGA4 in pull down, bimolecular fluorescence complementation, and co-immunoprecipitation assays. In agreement with this result, the *tga1 tga4* mutant showed reduced sensitivity to the BIN2 inhibitor bikinin. Second, BIN2 phosphorylated TGA1 and TGA4 *in vitro*, and bikinin suppressed TGA1 and TGA4 phosphorylation *in vivo*. Third, phosphorylation of TGA4 by BIN2 inhibited TGA4 from interaction with NPR1, whereas bikinin significantly enhanced the TGA4–NPR1 interaction. Fourth, BIN2 phosphorylated the Ser-202 residue of TGA4, and the TGA4–NPR1 interaction was weakened by the S202D (phospho-mimicking) mutation of TGA4 but not by the S202A (phospho-deficient) mutation. Fifth, the S202D mutation abolished NPR1-promoted DNA binding of TGA4, while co-treatment of SA and bikinin significantly increased TGA4 binding to the SA-responsive gene promoters. And finally, BL- and bikinin-induced enhancement of resistance to *Pst* DC3000 and expression of the SA-responsive genes were abolished in the *npr1* mutant. These results together demonstrate that BR promotes SA signaling by relieving the BIN2-driven suppression of TGA4 for interaction with NPR1.

To trace the fate of the phosphorylated TGA4 protein, Kim et al. (2022) characterized transgenic lines accumulating similar levels of *TGA4–YFP*, *TGA4–S202A–YFP*, or *TGA4–S202D–YFP* transcripts. It was found that the TGA4–YFP protein level was slightly induced by SA treatment, and the induction was dramatically increased with the addition of bikinin but suppressed by overexpression of BIN2 or the *bin2-1* mutation. Furthermore, the TGA4–S202A–YFP protein level was greatly increased by SA treatment alone, while the TGA4–S202D–YFP protein level was not significantly increased by SA and bikinin

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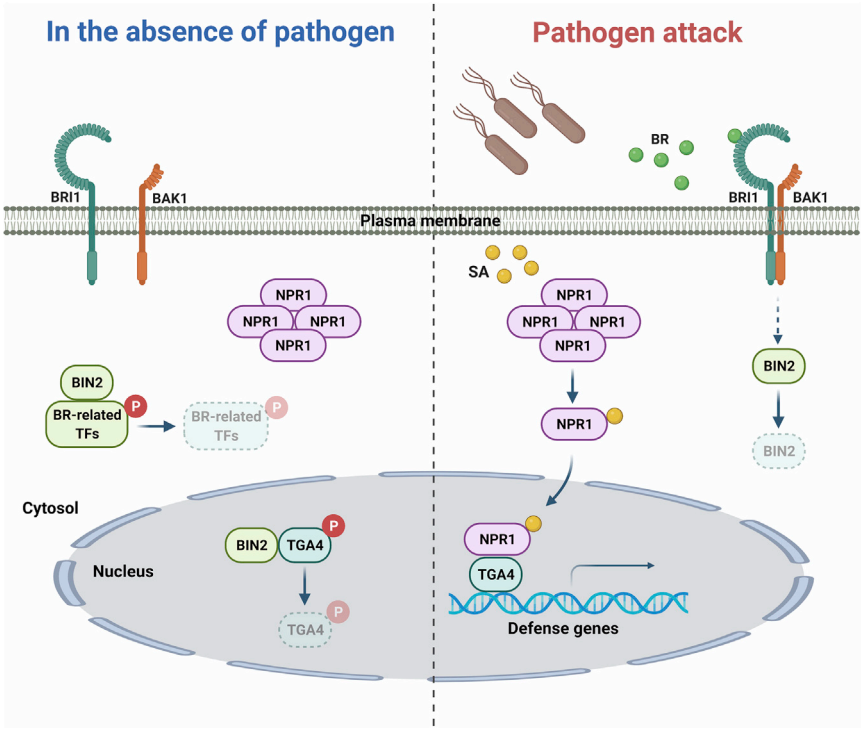

Figure 1. A working model illustrating the crosstalk between BR and SA signaling in *A. thaliana*

Without pathogen infection, BR level is low, and BIN2 phosphorylates and destabilizes TGA4, inhibiting NPR1–TGA4 interaction. Upon pathogen infection, SA and BR levels increase. SA-induced NPR1 activation and BR-mediated BIN2 inhibition synergistically promote NPR1–TGA4 interaction, leading to enhanced defense gene expression and pathogen resistance. P, phosphate group; TF, transcription factor. The figure was created using the BioRender online tool (https://biorender.com/).

co-treatment. Consistent with these results, the TGA4–S202A–YFP protein had a significantly longer half-life than TGA4–YFP in cycloheximide chase assays. To prove the biological relevance of BIN2-mediated TGA4 phosphorylation, Kim et al. (2022) evaluated SA-induced immune responses in the transgenic lines with or without addition of bikinin. Compared with the wild-type Col-0, the *TGA4–YFP* plants, but not the *TGA4–S202D–YFP* plants, showed significantly enhanced resistance to *Pst* DC3000 and increased expression of the SA-responsive genes upon SA and bikinin co-treatment, and the *TGA4–S202A–YFP* plants displayed even stronger resistance to *Pst* DC3000 and higher expression of the SA-responsive genes than the *TGA4–YFP* plants when treated with SA alone. These results indicate that BIN2 destabilizes TGA4 by phosphorylating the Ser-202 residue of TGA4 and that BR and bikinin inhibit BIN2-mediated phosphorylation of TGA4 to stabilize the protein, leading to promotion of SA signaling. This conclusion is consistent with the previous result that BR treatment induces 26S proteasome-mediated degradation of BIN2 (Peng et al., 2008).

The work of Kim et al. (2022) clearly provides a mechanistic understanding of the BR–SA crosstalk. Considering the disease susceptibility phenotypes of the mutants of multiple BR pathway components (Chinchilla et al., 2007; Miyaji et al., 2014; Zhao et al., 2019; Liao et al., 2020), it is not difficult to appreciate the roles of basal BR in modulating plant immune responses. However, results from exogenously added BR or inhibitors should be taken with caution. Kim et al. (2022) found that the levels of 6-deoxocastasterone and castasterone, two direct biosynthetic precursors of BL, were significantly increased at 36 h after *Pst* DC3000 inoculation. While this result is encouraging, further detailed time course determination of BR and SA levels is desired. It would be very informative to investigate the tempo-spatial distribution of BR and SA in plants during pathogen infection.

Recently, the crystal structure of the NPR1–TGA3 complex was resolved (Kumar et al., 2022). Most of the amino acid residues of TGA3 that are involved in the NPR1–TGA3 interaction are conserved in other TGAs, including TGA1 and TGA4. Thus, the NPR1–TGA4 complex might be formed in a similar manner. If that is the case, investigating how the Ser-202 phosphorylation of TGA4 inhibits the interaction of NPR1 and TGA4 will shed more light on the molecular mechanism of the NPR1–TGA-complex-mediated plant immune responses.

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