

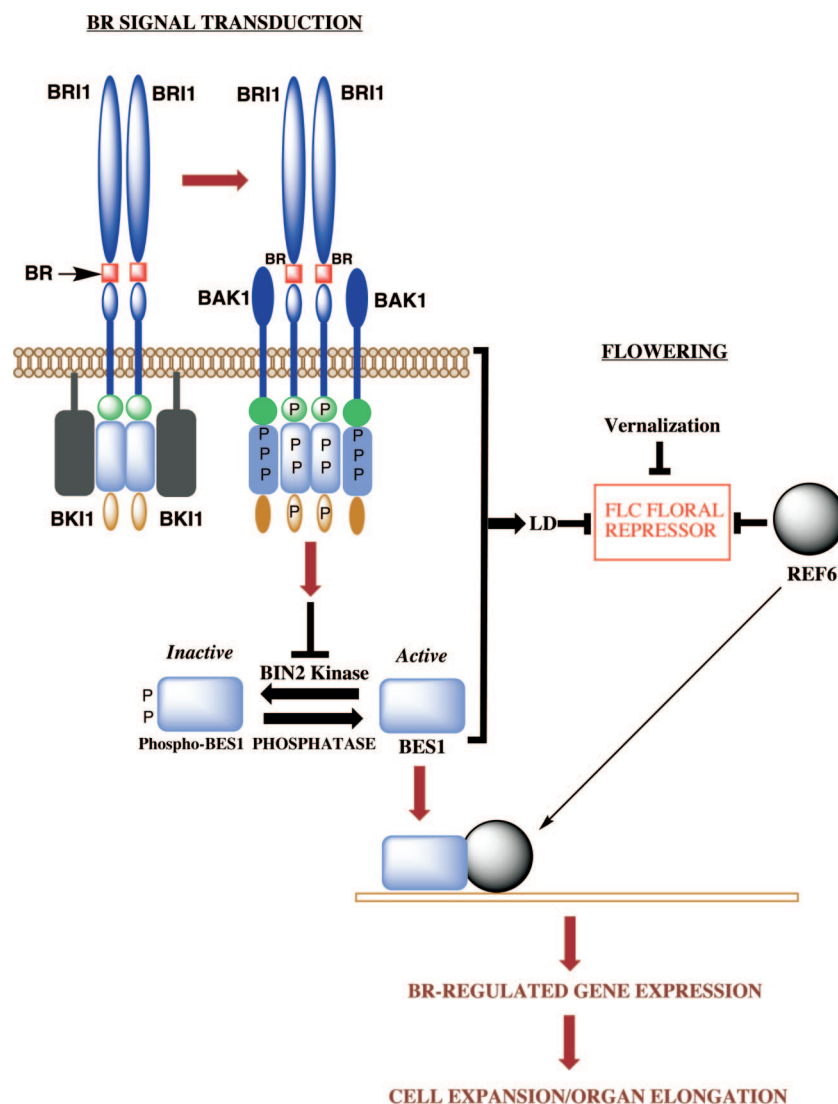
# The molecular intersection of brassinosteroid-regulated growth and flowering in *Arabidopsis*

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The transition from vegetative to reproductive growth in plants is a major developmental switch regulated by a set of complex, integrated signal transduction pathways that respond to both environmental cues and endogenous signals to alter the expression of genes associated with the initiation and development of floral organs (1). Understanding the molecular mechanisms of flowering has both intrinsic scientific interest and immense practical agricultural applications. In many plants, the timing of flowering is regulated by light quality and quantity, temperature, and the action of gibberellins (GAs), a class of plant hormones with pleiotropic effects on both vegetative and reproductive development (2). Another class of plant hormones, the brassinosteroids (BRs), also regulates multiple aspects of plant development (3), and recent evidence suggests that BRs stimulate flowering by reducing transcript levels of a potent floral repressor (4). As with many other developmental processes in plants, our understanding of molecular events underlying floral initiation has been greatly advanced by studying mutants in the model plant *Arabidopsis thaliana*. The molecular genetic analysis of *Arabidopsis* mutants exhibiting early or delayed flowering has uncovered numerous critical components of signal transduction pathways regulating response to photoperiod, hormones, and cold treatment. Similarly, analysis of mutants with defective BR perception or response has provided extensive details on the molecular components required for BR signaling (5, 6). In this issue of PNAS, Yu *et al.* (7) provide a connection between BR signal transduction and pathways controlling floral initiation by demonstrating that a critical transcription factor required for BR-dependent gene expression directly interacts with two transcription regulators previously identified as having divergent roles in modulating time of flowering in *Arabidopsis* (8).

BRs have structural similarities to animal steroid hormones and initiate a cascade of cellular events by binding to the extracellular domain of the BRASSINOSTEROID-INSENSITIVE 1 (BRI1) receptor kinase (5). This leads to phosphorylation of the BRI1 cytoplasmic kinase domain, causing disassociation from the membrane-bound BRI1



**Fig. 1.** The intersection of BR signal transduction with selected components of pathways regulating the timing of flowering in *Arabidopsis*. Details and definitions are provided in the text. P represents phosphorylation at specific Ser and Thr residues.

KINASE INHIBITOR 1 (BKI1) and oligomerization with a second receptor kinase, BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1). The active BRI1/BAK1 receptor kinase pair then propagates the signal downstream by inactivating a soluble kinase, BRASSINOSTEROID-INSENSITIVE 2 (BIN2), which is a negative regulator of BR signaling (6). Mutational analysis in *Arabidopsis* reveals that *bri1* null alleles have severe developmental defects, including extreme dwarfism, altered leaf

and vascular morphology, delayed senescence, male infertility, and moderately delayed flowering, confirming the importance of BRI1-mediated BR signaling in normal plant development (3, 6).

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BIN2 functions by phosphorylating and inactivating two novel transcription factors: BRI1-EMS-SUPPRESSOR 1 (BES1) and BRASSINAZOLE-RESISTANT 1 (BZR1). BR treatment leads to rapid dephosphorylation of BES1 and BZR1, which then accumulate in the nucleus where they bind to specific BR response elements in the promoters of BR-regulated genes, either as homodimers or heterodimers with other transcription factors (9, 10). Microarray analyses have cataloged hundreds of BR-regulated genes in functional categories ranging from wall-modifying proteins to transcription factors, and BES1 is likely to play a positive role in the expression of many of these genes (6). A summary of BR signaling is presented in Fig. 1.

The MADS box transcription factor, FLOWERING LOCUS C (FLC), quantitatively represses flowering in *Arabidopsis* and factors that reduce *FLC* transcript levels such as prolonged cold treatment (vernalization) or the action of a second transcription factor, LUMINIDEPENDENS (LD), promote flowering (11). *FLC* transcript levels are higher and flowering is later in *ld* mutants than in wild-type *Arabidopsis* grown under the same conditions. Vernalization reduces *FLC* transcript levels and promotes earlier flowering in the *ld* mutant. Interestingly, a genetic screen for enhancers of the *ld* mutant uncovered two new alleles of the BRI1 receptor kinase (4). Flowering is extremely delayed and *FLC* transcript levels remain elevated in *bri1 ld* double mutants and late flowering is suppressed by vernalization or reduction of *FLC* expression by RNAi constructs. Thus, like LD, BRI1-dependent BR signaling appears to promote flowering in wild-type *Arabidopsis* by repressing *FLC* expression, although the precise molecular mechanisms remain unclear. Chromatin remodeling may play a role, because his-

tone acetylation at the *FLC* locus is dramatically increased in the *bri1 ld* double mutant (4). Indeed, chromatin modification has been demonstrated to be critical in flowering time with histone H3 trimethylation at lysine 4 and histone acetylation activating *FLC* expression and histone deacetylation and histone H3 dimethylation at lysines 9 and 27 repressing it (12).

The exciting work of Yu *et al.* (7) has now extended the intersection of signaling pathways regulating BR response

## Yu *et al.* (7) provide a connection between BR signal transduction and pathways controlling floral initiation.

and flowering time by identifying a specific molecular interaction between components of each pathway. A genetic screen for interactors enhancing the ability of BES1 to activate transcription in yeast cells uncovered the previously identified EARLY FLOWERING 6 (ELF6) as a partner of BES1. The interaction was verified both *in vitro* and *in vivo* and a closely related protein, RELATIVE OF EARLY FLOWERING 6 (REF6), was also shown to interact directly with BES1. ELF6 is a repressor of the photoperiodic flowering pathway and *elf6* mutants exhibit early flowering, whereas REF6 is a repressor of *FLC* and *ref6* mutants accumulate *FLC* transcripts, leading to late flowering (8). While showing divergent functions, ELF6 and REF6 are highly similar structurally and both belong to the Jumonji N/C domain-containing family of transcriptional regulators that function

in chromatin modification, particularly by demethylation of histone tails (7).

By molecular genetic analysis of *elf6*, *ref6*, *elf6 ref6*, and *bri1 ref6* mutants, Yu *et al.* (7) clearly show that ELF6 and REF6 function in the BR signaling pathway by interacting with BES1 to regulate the expression of genes involved in cell wall modification and organ elongation. This is highly significant because it demonstrates that ELF6 and REF6, previously thought to be specific to floral pathway signaling, function in a vegetative development process by interacting with a pathway-specific transcription factor, BES1. Chromatin remodeling was also demonstrated to be involved in this function of ELF6 and REF6, as it was with REF6 suppression of *FLC* in the flowering pathway, although the mechanisms may be different (7, 8). Thus, REF6 may have multiple functions in chromatin modification or may be responsible for assembling different chromatin-remodeling complexes on specific promoters and in association with different pathway-specific transcription factors. A search for additional interacting partners for both ELF6 and REF6 may help define their role in vegetative and floral developmental pathways with greater precision. Moreover, the recent demonstration of BRI1-dependent suppression of *FLC* in the *ld* mutant background (4), coupled with the known REF6 suppression of *FLC* (8), suggests the possibility that BES1 might interact directly with REF6 at the *FLC* promoter to repress *FLC* expression in conjunction with LD, a hypothesis that can be tested readily.

In conclusion, the demonstration that Jumonji domain-containing transcriptional regulators interact with pathway-specific transcription factors such as BES1 provides a useful experimental platform to link distinct but interacting gene networks that control the complex events regulating plant growth and development.

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