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From signaling to function: how strigolactones regulate plant development

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Strigolactones (SLs) are a class of plant hormones first discovered based on their ability to stimulate germination of the root parasite witchweed (*Striga lutea* Lour.) and regulate symbiosis between arbuscular mycorrhiza fungi and their host plants. Recent studies have identified diverse functions of SLs in shoot branching, leaf development, root architecture, and the responses to environment stress (Gomez-Roldan et al., 2008; Umehara et al., 2008; Brewer et al., 2013). The key players in SL signaling have been well studied. In the presence of SLs, the receptor DWARF14 (D14) perceives and hydrolyzes SLs, then interacts with the F-Box protein MORE AXILLARY GROWTH2 (MAX2)/D3 to form an SCF complex. This SCF complex then recruits the transcriptional repressors SUPPRESSOR OF MAX2-LIKE (SMXL) 6, SMXL7, and SMXL8/D53 for ubiquitination and degradation (Jiang et al., 2013; Wang et al., 2015; Yao et al., 2016; Yao et al., 2018; Zhou et al., 2013). However, whether and how SLs regulate downstream genes remain unclear (Figure 1). In a recent report, Wang et al. (2020) revealed that SLs regulate shoot branching, leaf shape, and anthocyanin accumulation mainly by activating transcription of three transcription factors; most importantly, they discovered that SMXL6 autoregulation of *SMXL6* transcription maintains strigolactone signaling homeostasis.

In their report, the authors first identified a specific and highly efficient synthetic SL analogue, $GR24^{4DO}$, which triggered strong transcriptional up-regulation of *SMXL7* and *BRANCHED1* (*BCR1*) in a manner that depends on the SL receptor D14. They then treated *Arabidopsis* seedlings with $GR24^{4DO}$ and analyzed global gene expression by deep sequencing of the transcriptome (RNA-seq). They found 401 genes that were responsive to $GR24^{4DO}$ treatment, including many genes previously reported to be regulated by other SL analogs. However, the remaining genes (~360 or 90% of those identified here) were not previously described as responding to SL. They examined the differentially expressed genes (DEGs) and found that SLs function in the regulation of plant architecture, drought tolerance, crosstalk with the karrikin signaling pathway, and carotenoid and flavonoid biosynthesis. As SMXL6/7/8 were previously identified as transcriptional repressors, Wang and colleagues then focused on the $GR24^{4DO}$-induced transcription factor genes *BRC1*, *TEOSINTE BRANCHED1/CYCLOIDEA/PCF DOMAIN PROTEIN1* (*TCP1*), and *PRODUCTION OF ANTHOCYANIN PIGMENT1* (*PAP1*).

BRC1 is a key regulator that represses bud outgrowth, and SMXL6 represses *BRC1* expression in non-elongated buds (Wang et al., 2015). The authors discovered that the high-

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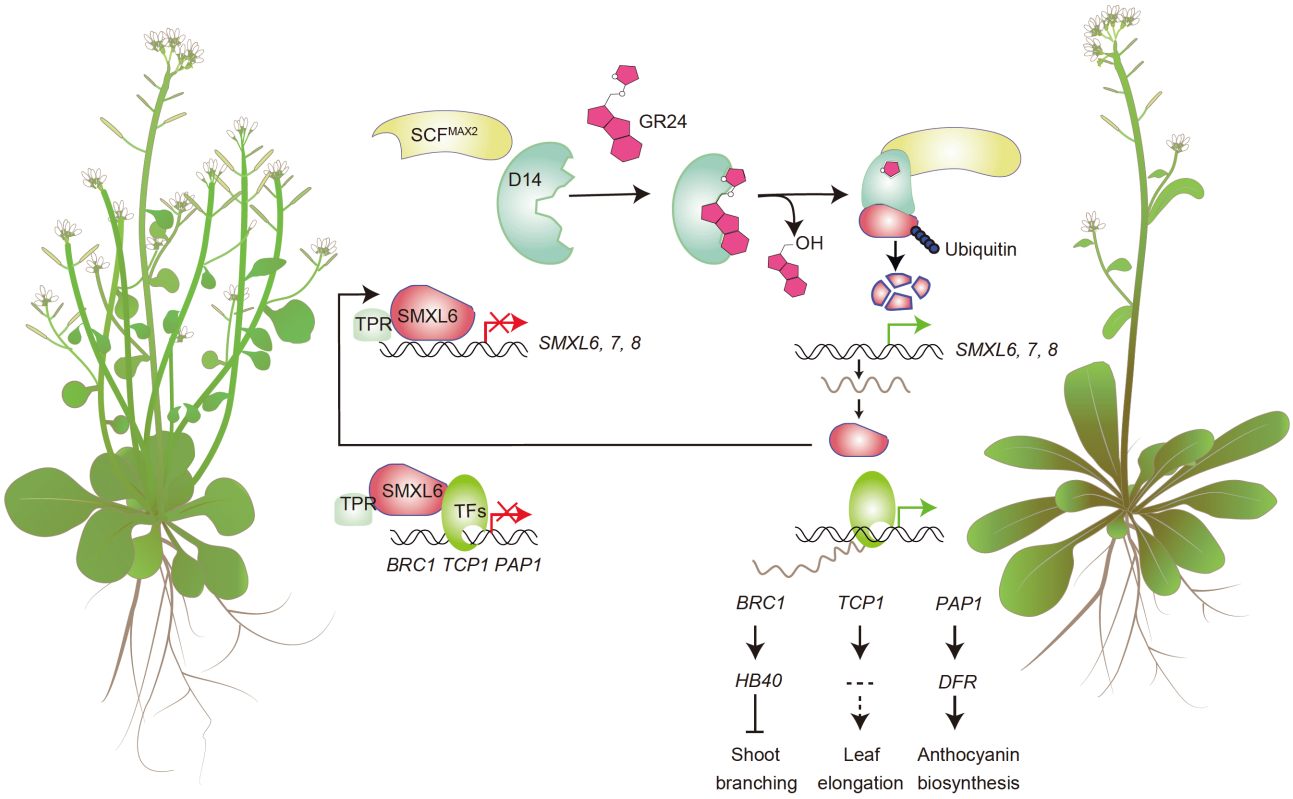

Figure 1 Diagram of how SLs regulate downstream genes. In the absence of strigolactones (SLs, left), SMXL6 binds directly to the promoters of *SMXL6*, *7*, and *8* and represses their expression. SMXL6 forms complexes with unknown transcription factors to repress expression of *BRC1*, *TCP1*, and *PAP1*. In the presence of SLs (right), receptor D14 perceives and hydrolyzes SLs to generate active hormone, then D14 interacts with SCF^{MAX2} to form a complex, which recruits SMXL6 and promotes its ubiquitination and degradation to remove the expression repress on *SMXL6*. Newly synthesized SMXL6 binds directly to promoters of *SMXL6*, *7*, *8*, which form a negative feedback loop to maintain the homeostasis of SMXL6/7/8 proteins. The degradation of SMXL6 also induces expression of *BRC1*, *TCP1*, *PAP1* and activates signaling cascades that repress shoot branching, promote leaf elongation and enhance anthocyanin biosynthesis, respectively.

branching mutant *brc1-6* reversed the attenuated branching phenotype of the *smxl6 smxl7 smxl8* triple mutant (*s678* for short), indicating that *BRC1* functions downstream of *SMXL6/7/8*. The transcript levels of *HOMEOBOX PROTEIN 40* (*HB40*), a BRC1 target gene that regulates abscisic acid (ABA) biosynthesis, also rose in response to $GR24^{4DO}$ treatment, and did so in a D14- and BRC1-dependent manner. In agreement with this observation, the SL biosynthetic mutant *max3-9* accumulated less endogenous ABA, compared with wild type, whereas the *s678* mutant with activated SL signaling accumulated more ABA. Moreover, the elevated ABA levels in *s678* were suppressed by *brc1-6*. These results suggested that SLs regulate plant branching by degrading SMXL6/7/8 and releasing the transcriptional repression of *BRC1*, thereby inducing *HB40* expression. HB40 initiates ABA biosynthesis, which then inhibits shoot branching in *Arabidopsis*.

The authors determined that $GR24^{4DO}$ treatment promoted leaf elongation via SMXL6 in an EAR repressor motif-dependent manner. They found that *TCP1*, which functions in leaf development (Koyama et al., 2010), was induced by $GR24^{4DO}$ treatment in a D14-dependent manner. They showed that *TCP1* expression was reduced in the SL-deficient mutant *max3-9* but was strongly induced in the SL signaling mutants *s678* and *max3-9 s678* (a quadruple mutant, since *s678* is a triple mutant). A dominant-negative form of TCP1 significantly reduced the leaf length-to-width ratio when overexpressed in the *s678* triple mutant, and the *s678 tcp1* quadruple mutant leaves also showed a reduced length-to-width ratio. These results suggest that SLs regulate leaf shape by promoting the degradation of SMX6/7/8 to release their transcriptional repression of *TCP1*.

The authors then showed that $GR24^{4DO}$ treatment induced the expression of the transcription factor gene *PAP1* and its homologs *PAP2*, *MYB113* and *MYB114*, in a D14-dependent manner. In turn, these transcription factors increased anthocyanin biosynthesis via the induction of *DIHYDROFLAVONOL 4-REDUCTASE* (*DFR*), *TRANSPARENT TESTA* 7 (*TT7*), and *ANTHOCYANIDIN SYNTHASE* (*ANS*). In agreement with these observations, *DFR* expression was lower in the SL-deficient *max3-9* mutant but much higher in the SL signaling mutant *s678*, relative to wild type. Likewise, the *max3-9* mutant accumulated less endogenous anthocyanins than wild type, whereas the *s678* triple mutant

and the *pap1-D* mutant, which constitutively overexpress *PAP1*, accumulated more anthocyanins. Moreover, *pap1-D* completely restored anthocyanin accumulation in the *max3-9* background. Conversely, *pap1* and *pap2* single or double mutants partially or totally reversed the strong anthocyanin accumulation seen in *s678*. Collectively, these results indicate that PAPs act downstream of SMXL6/7/8 in activating *DFR* expression to increase anthocyanin accumulation.

To understand how SMXL6/7/8 regulates transcription of downstream genes, the authors performed chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) in transgenic lines overexpressing SMXL6-HA in the *s678* background. SMXL6-HA targeted the genomic regions of 729 genes, including *SMXL6*, *SMXL7*, *SMXL8*, and *BRC1*. Furthermore, SMXL6 repressed the activity of the *SMXL6/7/8* and *BRC1* promoters in a luciferase reporter assay, but this repression was alleviated by deleting the SMXL6 EAR motif and by $GR24^{4DO}$ treatment. Notably, it is unexpected that SMXL6 and SMXL7 showed direct binding to the *SMXL6/7/8* promoters, and SMXL8 directly interacted with the *SMXL7* promoter in electrophoretic mobility shift assays (EMSAs). SMXL6 bound to the consensus motif ATAACAA, a mutated version of which disrupted SMXL6 binding *in vitro* and decreased the transcriptional repression activity of SMXL6 *in vivo*. Interestingly, SMXL6 did not directly bind to the *BRC1* promoter, suggesting that SMXL6 may work together with unknown transcription factors to repress *BRC1* expression, consistent with the notion that D53/SMXLs interact with the TOPLESS and TOPLESS-RELATED transcriptional co-repressors to function as transcriptional repressors.

In conclusion, Wang and colleagues described for the first time that SLs activate the transcription of *BRC1*, *TCP1*, and *PAP1* to control branching, leaf shape, and anthocyanin biosynthesis. More importantly, they demonstrated that SMXL6 has dual functions, repressing transcription through interaction with transcription factors, and functioning as a transcription factor that binds directly to the *SMXL6/7/8* promoters, forming a negative feedback loop to maintain the homeostasis of SMXL6/7/8 proteins. This negative feedback distinguishes SL pathway from other plant hormone pathways that also employ SCF ubiquitin ligase complexes, including the auxin, jasmonate, and gibberellin signaling pathways, thus revealing a noncanonical mechanism in phytohormone signaling. For the future, identifying and characterizing SMXL6-interacting proteins will be very helpful in precise agriculture aiming at breeding crops with special properties. For instance, rice breeding to improve yield and disease resistance has targeted IDEAL PLANT ARCHITECTURE 1, which controls rice architecture, interacts with D53, and participates in feedback regulation of strigolactone-induced *D53* (Song et al., 2017). The work of Wang and colleagues has deepened our understanding of plant hormone signaling and opened many avenues for future studies that will inform crop improvement.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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