Transcriptional regulation of development by SMAX1-LIKE proteins, targets of strigolactone and karrikin/KAI2 ligand signaling

Sun Hyun Chang[1], Wesley George[1], David C. Nelson[1]*

[1]Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

* corresponding author, david.nelson@ucr.edu

Highlight

This review synthesizes recent discoveries of how SMAX1-LIKE proteins control different aspects of plant development and responses to the environment.

Abstract

SUPPRESSOR OF MAX2 1 (SMAX1) and SMAX1-LIKE (SMXL) proteins comprise a family of plant growth regulators that includes downstream targets of the karrikin (KAR)/KAI2 ligand (KL) and strigolactone (SL) signaling pathways. Following the perception of KAR/KL or SL signals by α/β hydrolases, some types of SMXL proteins are polyubiquitinated by an E3 ubiquitin ligase complex containing the F-box protein MORE AXILLARY GROWTH2 (MAX2)/DWARF3 (D3), and proteolyzed. Because SMXL proteins interact with TOPLESS (TPL) and TPL-related (TPR) transcriptional corepressors, SMXL degradation initiates changes in gene expression. This simplified model of SMXL regulation and function in plants must now be revised in light of recent discoveries. It has become apparent that SMXL abundance is not regulated by KAR/KL or SL alone, and that some SMXL proteins are not regulated by MAX2/D3 at all. Therefore, SMXL proteins should be considered signaling hubs that integrate multiple cues. Here we review the current knowledge of how SMXL proteins impose transcriptional regulation of plant development and environmental responses. SMXL proteins can bind DNA directly and interact with transcriptional regulators from several protein families. Multiple mechanisms of downstream genetic control by SMXL proteins have been identified recently that do not involve the recruitment of TPL/TPR, expanding the paradigm of SMXL function.

Keywords: gene regulation, hormone signaling, plant development, transcription, strigolactones, karrikins

Introduction

SMXL proteins have diverse roles in plants

SUPPRESSOR OF MAX2 1 (SMAX1)-LIKE (SMXL) proteins are transcriptional regulators that control many aspects of plant development and responses to the environment. The SMXL family in flowering plants comprises four clades termed aSMAX1 (angiosperm SMAX1), SMXL39, aSMXL4 (angiosperm SMXL4), and SMXL78 (Walker *et al.*, 2019) (Figure 1). The functions of aSMAX1 clade proteins (e.g. SMAX1 and SMXL2 in *Arabidopsis thaliana*) in various species include regulation of seed germination, seedling photomorphogenesis, mesocotyl elongation in darkness, root hair density and elongation, abiotic stress tolerance (e.g. drought), immune responses, and the capacity for beneficial symbiotic interactions between roots and arbuscular mycorrhizal fungi (Stanga *et al.*, 2013, 2016; Villaécija-Aguilar *et al.*, 2019, Preprint, 2022; Bunsick *et al.*, 2020; Carbonnel *et al.*, 2020*a*; Choi *et al.*, 2020; Villaécija-Aguilar and Gutjahr, 2020; Zheng *et al.*, 2020, 2023; Bursch *et al.*, 2021; Feng *et al.*, 2022; Kim *et al.*, 2022; Li *et al.*, 2022*b*; Meng *et al.*, 2022; Kamran *et al.*, 2024). The SMXL39 and aSMXL4 clades (e.g., respectively, SMXL3; SMXL4 and SMXL5 in *Arabidopsis*) control phloem development, which also impacts primary root elongation (Wallner *et al.*, 2017, 2020, Preprint, 2023; Cho *et al.*, 2018; Hardtke, 2023). Finally, the SMXL78 clade (e.g. SMXL6, SMXL7, and SMXL8 in *Arabidopsis*, and DWARF53 (D53) in grasses) regulates shoot branching or tillering, lateral and adventitious root growth, cambial growth, drought tolerance, herbivore defense, and putatively most, if not all, other strigolactone-associated traits such as senescence (Snowden *et al.*, 2005; Agusti *et al.*, 2011; Kohlen *et al.*, 2012; Rasmussen *et al.*, 2012; Jiang *et al.*, 2013; Zhou *et al.*, 2013; Yamada *et al.*, 2014; Soundappan *et al.*, 2015; Ueda and Kusaba, 2015; Wang *et al.*, 2015; Waters *et al.*, 2017; Li *et al.*, 2020*a*,*b*; Yang *et al.*, 2020*a*; Lian *et al.*, 2023). SMXL proteins are found in all land plants, but there are fewer types of SMXL proteins in bryophytes, lycophytes, monilophytes, and gymnosperms than in angiosperms (Walker *et al.*, 2019).

SMXL proteins are signaling hubs regulated by multiple factors

SMXL proteins have received substantial attention for their role as downstream targets of strigolactone and karrikin/KAI2 ligand signaling. Strigolactones (SLs) are carotenoid-derived plant hormones that are perceived by the □/β hydrolase DWARF14 (D14)/DECREASED APICAL DOMINANCE2 (DAD2) (Hamiaux *et al.*, 2012; Yao *et al.*, 2016). Upon activation - an unresolved event that occurs during SL binding or hydrolysis - D14 interacts with the F-box

protein MORE AXILLARY GROWTH2 (MAX2)/DWARF3 (D3) and SMXL78 clade proteins. MAX2 participates in a SCF-type (Skp1, Cullin, F-box) E3 ubiquitin ligase complex that polyubiquitinates SMXL78 proteins, which are then rapidly degraded by the 26S proteasome (Stirnberg *et al.*, 2007; Zhao *et al.*, 2014; Waters *et al.*, 2017). This putatively initiates downstream responses through the relief of transcriptional repression by SMXL proteins.

A very similar mechanism mediates perception of karrikins (KARs), a class of plant growth regulators identified in smoke from burning plant material (Waters and Nelson, 2022). KAR, or more likely a karrikin metabolite, are perceived by KARRIKIN INSENSITIVE2 (KAI2)/HYPOSENSITIVE TO LIGHT (HTL), which is a homolog of D14. This causes KAI2 to interact with MAX2 and aSMAX1 clade proteins, targeting them for polyubiquitination and degradation (Khosla *et al.*, 2020; Wang *et al.*, 2020*b*; Zheng *et al.*, 2020). Several conformations of this signaling complex have been captured through cryogenic electron microscopy, revealing dynamic protein-protein interactions that underlie the SMAX1 ubiquitination process (Arold *et al.*, 2024). In addition to KAR metabolite(s), KAI2 is thought to perceive an endogenous signal, KAI2 ligand (KL), that remains undiscovered (Waters and Nelson, 2022). Proteins in the SMXL78 clade are specifically regulated by D14-SCF^{MAX2} (Jiang *et al.*, 2013; Zhou *et al.*, 2013; Soundappan *et al.*, 2015; Wang *et al.*, 2015). In contrast, regulation of aSMAX1 clade proteins is primarily mediated by KAI2-SCF^{MAX2}, but, in cases where exogenous SLs are applied or endogenous SLs are adequately high, aSMAX1 clade proteins may also be targeted by D14-SCF^{MAX2} (Khosla *et al.*, 2020; Wang *et al.*, 2020*b*; Li *et al.*, 2022*a*)

This signaling relationship makes it tempting to think of SMXL proteins as repressors of SL or KAR/KL responses and much of the literature in this field, including our own work, has promoted this idea. However, in light of new evidence, we assert that it is now more accurate to think of SMXL proteins as growth regulating hubs that integrate multiple signals. This concept is analogous to the function of DELLA proteins, which were initially considered repressors of gibberellin responses but are now known to integrate several signaling cues (Peng *et al.*, 1999; Davière and Achard, 2016; Van De Velde *et al.*, 2017; Briones-Moreno *et al.*, 2023).

One reason for this viewpoint is that SMXL protein stability is not only regulated by KAR/KL and SL signaling. In Arabidopsis seedlings, the abundance of SMAX1-GFP fusion protein declines under warm (28°C) temperatures (Park *et al.*, 2022). SMAX1-GFP abundance also declines in

seedlings within a few hours of transfer to darkness in a mostly proteasome-independent manner (Kim *et al.*, 2022). In younger Arabidopsis seedlings, however, an opposite effect of light has been observed; eYFP-SMAX1 is detectable in dark but not light growth conditions (Hountalas *et al.*, 2024). A SMAX1 reporter also declines in Arabidopsis seedlings during osmotic stress, although this is putatively via SL signaling (Li *et al.*, 2022*a*). The abundance of SMXL78 clade proteins (e.g. D53) is reduced by nitrate treatment relative to an ammonium control, and SL-induced degradation of D53 is inhibited by sucrose (Sun *et al.*, 2021*a*; Patil *et al.*, 2022). How cues such as temperature, light, and nutrient abundance influence SMXL stability is not yet understood, but it does not necessarily involve SCF^{MAX2}.

A second reason to avoid thinking of SMXL proteins as KAR/KL or SL signaling repressors is that MAX2-dependent signaling is not the only way that SMXL proteins are regulated in plants. For example, two other ubiquitin ligases have recently been reported to target SMXL78 clade proteins (Lian *et al.*, 2023; An *et al.*, 2024). In Arabidopsis, DDB1-BINDING WD-REPEAT DOMAIN HYPERSENSITIVE TO ABA DEFICIENT1 (DWA1) confers substrate specificity to a Cullin4 (CUL4)-type E3 ubiquitin ligase. DWA1 was identified as a candidate interactor in yeast two-hybrid screens of an Arabidopsis cDNA library with SMXL6, SMXL7, and SMXL8 bait proteins. These interactions were validated by pull-down assays and bimolecular fluorescence complementation (BiFC) assays *in vivo*. *In vitro* experiments suggested that degradation of SMXL6, SMXL7, and SMXL8 is reduced in *dwa1* protein extracts, and translational reporters of these proteins accumulated to higher levels in *dwa1* roots than in wild-type. Furthermore, the *smxl6,7,8* triple mutant had opposite and epistatic effects to *dwa1* on drought tolerance (Lian *et al.*, 2023).

Altogether, this supports the existence of at least two E3 ligase-mediated mechanisms for the regulation of SMXL78 clade protein abundance. It remains to be determined whether the DWA1 and MAX2 mechanisms operate in overlapping or independent spatiotemporal contexts. It will also be useful to investigate how DWA1-mediated targeting of SMXL78 clade proteins is controlled; for example, is *DWA1* expression regulated by a specific signal or is there post-translational regulation of DWA1-SMXL interactions? Notably, D14 is slowly degraded after SL perception by MAX2-dependent and MAX2-independent mechanism(s) (Chevalier *et al.*, 2014; Sánchez Martín-Fontecha *et al.*, 2024). Perhaps DWA1 contributes to MAX2-independent degradation of D14 that is in complex with SMXL78 clade proteins. However, this would not explain the putative proteasome-independent mechanism for D14 turnover (Sánchez Martín-

Fontecha *et al.*, 2024). An unidentified mechanism for MAX2-independent turnover of SMAX1 has been suggested (Khosla *et al.*, 2020). It will be intriguing to determine whether DWA1 facilitates this.

In apple (*Malus × domestica*), the E3 ubiquitin ligase PROTEOLYSIS1 (MdPRT1) physically interacts with MdSMXL8, targeting it for polyubiquitination and proteasomal degradation (An *et al.*, 2024). Because *MdPRT1* expression is induced within 30 minutes of treatment with a SL analog, this provides an alternative mechanism to D14-SCF^{MAX2} for SL control of MdSMXL8 abundance. At this time, it is unclear whether MdPRT1 acts independently or cooperatively with MdMAX2 to trigger MdSMXL8 degradation (An *et al.*, 2024).

It is further noteworthy that some SMXL proteins are not targeted for degradation by SCF^{MAX2} at all. The SMXL39 and aSMXL4 clade proteins are distinguished from other angiosperm SMXL proteins by the lack of a well-conserved Arg-Gly-Lys-Thr (RGKT) motif (also referred to as a phosphate-binding loop, or P-loop motif) in the C-terminal D2 domain (Walker *et al.*, 2019). The first mutant allele of *D53*, a gain-of-function mutation discovered in rice, showed insensitivity to SL that arose from deletion of the RGKT motif. This rendered the d53 mutant protein resistant to SL-induced degradation (Jiang *et al.*, 2013; Zhou *et al.*, 2013). Supporting what was observed in rice, similar RGKT deletions have stabilized aSMAX1 and SMXL78 clade proteins from Arabidopsis, *Lotus japonicus*, pea (*Pisum sativum*), and maize (*Zea mays*), as well as the SMXL protein in the bryophyte *Marchantia polymorpha* (Soundappan *et al.*, 2015; Wang *et al.*, 2015, 2020*b*; Liang *et al.*, 2016; Carbonnel *et al.*, 2020*a*; Khosla *et al.*, 2020; Kerr *et al.*, 2021; Liu *et al.*, 2021; Mizuno *et al.*, 2021). The RGKT motif, in particular the Arg residue, helps stabilize the ASK1-MAX2-ShHTL7/KAI2-SMAX1 signaling complex through ionic and hydrogen bonds with MAX2 residues (Arold *et al.*, 2024). Therefore, SMXL39 and aSMXL4 clade proteins are expected to be unaffected by SCF^{MAX2}. Indeed, none of these proteins are targeted for degradation following SL or KAR treatment in Arabidopsis (Wallner *et al.*, 2017). In addition to being untethered from regulation by MAX2, the stabilized SMXL39 and aSMXL4 clade proteins may influence MAX2-dependent signaling of other SMXL proteins. For example, SMXL5 attenuates SL responses by reducing SL-induced degradation of SMXL7 (Li *et al.*, 2024). The mechanism of SMXL7 protection by SMXL5 remains uncertain, but this aspect of SMXL5 function appears to be dependent on an Ethylene-responsive element binding factor-associated Amphiphilic Repression (EAR) motif and may relate to the formation of heteromeric SMXL complexes (Li *et al.*, 2024).

Other, MAX2-independent mechanisms regulate the abundance of aSMXL4 clade proteins. One mechanism, which was shown in Arabidopsis and tomato, involves translational repression by JULGI zinc-finger proteins that bind to the 5' UTRs of *SMXL4* and *SMXL5* transcripts (Cho *et al.*, 2018; Nam *et al.*, 2022). Another potential mechanism involves post-transcriptional gene silencing. In Arabidopsis, the DICER-LIKE (DCL) family ribonucleases DCL4 and DCL2 process putatively aberrant *SMXL4* and *SMXL5* transcripts through the RNA quality control pathway (Wu *et al.*, 2017). Normally DCL4 activity predominates, generating 21-nt siRNAs that induce cleavage of complementary target mRNAs but do not have a substantial effect on *SMXL4/5* transcript abundance. In the absence of *DCL4*, however, DCL2 activity produces 22-nt siRNAs that are more effective at stimulating transitive post-transcriptional gene silencing, in which many secondary siRNAs are produced from a transcript targeted by a primary siRNA. Amplification of these siRNAs leads to gene silencing, in this case of *SMXL4* and *SMXL5*, rather than RNA decay. At the moment, it is unclear whether this DCL2-based mechanism is used to regulate *SMXL4/5* expression, such as during viral infections or other stress responses, or whether it is only revealed by genetic defects in RNA processing (Wu *et al.*, 2017).

In summary, a variety of mechanisms regulate SMXL protein abundance, not just KAR/KL and SL signaling via SCF^{MAX2}. Although not discussed here, regulation of *SMXL* transcription is also a potential way for different signaling pathways to modulate SMXL activity; for example, tissue-specific differences in *SMXL* expression have been observed in Arabidopsis (Stanga *et al.*, 2013; Wallner *et al.*, 2017). Because SMXL proteins integrate multiple environmental and developmental signals in the control of plant growth, we argue that they should no longer be described as repressors of KAR/KL and SL responses. For the remainder of this review, we turn our attention to how SMXL proteins control plant growth and development.

Main text

SMXL proteins are direct and indirect regulators of transcription

SMXL proteins are distantly related to ClpB HSP100 proteins, a class of AAA+ ATPases that have chaperonin activity in bacteria, protozoa, fungi, and plants (Kędzierska-Mieszkowska and Zolkiewski, 2021). SMXL and HSP100 proteins share a similar domain organization consisting of a double Clp N-terminal domain (N), an ATPase domain (D1), a middle domain (M), and a

second ATPase domain (D2) (Temmerman *et al.*, 2022). The SMXL D1 and M domains have been found to mediate interactions with D14 or KAI2, while the D2 domain helps stabilize the tripartite receptor-SMXL-MAX2 complex, contains the above-mentioned RGKT motif, and putatively mediates SMXL-SMXL interactions (Shabek *et al.*, 2018; Khosla *et al.*, 2020; Liu *et al.*, 2021). Recent structural evidence provided by cryogenic electron microscopy supports the role of the D2 domain in stabilizing interactions with KAI2 and/or MAX2. Unexpectedly, the N domain also contributes to the signaling complex through interactions with MAX2 and the Skp1 component of the SCF E3 ubiquitin ligase complex (Arold *et al.*, 2024). The D1 and M domains were not resolved through this approach, however, so the nature of any potential direct associations with D14 or KAI2 remain unknown. The Walker A and B motifs, which mediate nucleotide-binding and -hydrolysis in NTPases (Gottesman *et al.*, 1990; Schirmer *et al.*, 1996), of the SMXL D1 and D2 ATPase domains are not well conserved. ATPase activity has been reported at least for Arabidopsis SMXL4 (Yang *et al.*, 2015), however, there is no evidence yet that SMXL proteins, which are specific to land plants, have chaperonin functions.

Instead, SMXL proteins are likely to act as transcriptional regulators, for example as repressors that bind DNA directly and/or as corepressors that interact with DNA indirectly via partner proteins. This hypothesis initially arose from the observation that an EAR motif in the D2 domain is conserved in all types of SMXL proteins (Jiang *et al.*, 2013; Zhou *et al.*, 2013; Soundappan *et al.*, 2015; Wang *et al.*, 2015; Walker *et al.*, 2019). EAR motifs are well-known as mediators of protein-protein interactions with TOPLESS (TPL) and TOPLESS-RELATED (TPR) transcriptional corepressors from the Groucho/Tup1 family (Long *et al.*, 2006; Causier *et al.*, 2012; Ke *et al.*, 2015). Consistent with this, SMXL proteins from rice and Arabidopsis interact with multiple TPL/TPR proteins in an EAR-dependent manner *in vivo* as well as *in vitro* and in heterologous assays (e.g. yeast two-hybrid) (Jiang *et al.*, 2013; Soundappan *et al.*, 2015; Wang *et al.*, 2015; Ma *et al.*, 2017). TPL/TPR proteins can repress transcription in multiple ways, including forming complexes with histones, binding to Mediator subunits, and recruiting histone deacetylases (Long *et al.*, 2006; Ma *et al.*, 2017; Collins *et al.*, 2019; Leydon *et al.*, 2021). Much, although far from all, of SMXL functions in plant development and regulation of downstream gene expression are dependent on the EAR motif (Liang *et al.*, 2016; Wang *et al.*, 2020*a*; Chang *et al.*, 2024*b*; Li *et al.*, 2024). Consistent with this, histone deacetylases influence some plant responses to racemic GR24 (*rac*-GR24), a synthetic dual agonist of KAI2 and D14 (Temmerman *et al.*, 2023). This implies that the corepressor functions conferred by interacting TPL/TPR proteins are important components of SMXL activity. However, it should also be

considered that TPL/TPR proteins may have a structural role that affects SMXL activity by facilitating the formation or stabilization of SMXL-SMXL protein complexes (Ma *et al.*, 2017; Temmerman *et al.*, 2022; Li *et al.*, 2024).

Further evidence that SMXL proteins are transcriptional regulators comes from observations that SMXL proteins interact with transcription factors, which will be detailed below, and that, surprisingly, SMXL proteins can bind DNA directly. SMXL6 from Arabidopsis was first shown to bind its own promoter directly as well as the promoters of *SMXL7* and *SMXL8*. SMXL6 recognizes the DNA motif 5'-ATAACAA-3' and/or its reverse complement (Wang *et al.*, 2020*a*). Similarly, Arabidopsis SMAX1 binds its own promoter, putatively by recognizing the same motif (Xu *et al.*, 2023). However, in many cases this motif may be insufficient for SMAX1-binding, as SMAX1 does not associate with *SMXL6*, *SMXL7*, or *SMXL8* promoters *in vitro* (Xu *et al.*, 2023). Other proteins may influence SMXL affinity or specificity during DNA-binding *in vivo*. The ATAACAA motif is also bound by SMXL78 clade proteins in cotton (*Gossypium hirsutum*), suggesting a conserved DNA recognition sequence, although SMXL transcriptional autoregulation appears to be absent (Sun *et al.*, 2024). It is notable that this particular motif is not always involved in SMXL DNA-binding interactions; for example, SMXL78 clade proteins putatively bind directly to the promoters of *SnRK2.3* and *SnRK2.6*, which lack an ATAACAA motif (Lian *et al.*, 2023).

These studies cumulatively suggest that SMXL proteins regulate gene expression through the recruitment of TPL/TPR corepressors to genomic loci through direct and indirect interactions with DNA. However, a substantial proportion of genes are regulated, by SMAX1 in Arabidopsis seedlings for example, in an EAR motif-independent manner (Chang *et al.*, 2024*b*). Multiple mechanisms for EAR motif-independent regulation of gene expression can be imagined, such as competitive binding of SMXL proteins to transcriptional regulator proteins and/or *cis*-regulatory DNA sequences.

An example of the former idea is found in interactions between SMXL proteins and light signaling proteins, which will be discussed further below. In Arabidopsis, aSMAX1 clade proteins interact with the transcription factors PHYTOCHROME INTERACTING FACTOR4 (PIF4) and PIF5, but do not directly influence their transcriptional activity (Chang et al., 2024b). Instead, SMAX1 and SMXL2 stabilize PIF4 and PIF5 proteins by protecting them from degradation induced by the red and far-red light photoreceptor phytochrome B (phyB). SMAX1

and SMXL2 physically interact with phyB protein as well as the PIF proteins, which interferes with protein-protein interactions between phyB and PIF4 or PIF5 (Park *et al.*, 2022; Chang *et al.*, 2024*b*).

Similarly, SMXL78 clade proteins in cotton bind and protect the DELLA protein SLENDER RICE1 (GhSLR1) from gibberellic acid (GA)-induced degradation. This occurs through competitive protein-protein interactions that inhibit association of the F-box protein GIBBERELLIN INSENSITIVE DWARF2 (GID2) with GhSLR1 (Sun *et al.*, 2024). D53 also binds SLR1 and protects it from SL-induced degradation in rice by interfering with D14-SLR1 interactions (Sun *et al.*, 2023). The ability of SMXL proteins to modulate the stability or availability of their protein interaction partners could help to explain how SLs and KARs can influence the abundance of PIN-FORMED (PIN) auxin efflux carriers independently of transcriptional changes or *de novo* protein synthesis (Shinohara *et al.*, 2013; Hamon-Josse *et al.*, 2022).

Finally, another way in which SMXL protein-protein interactions can influence gene expression is by preventing transcriptional regulators from binding their DNA targets. This mode of regulation has been observed in D53 interactions with the transcription factor GROWTH-REGULATING FACTOR4 (GRF4) in rice, and in GhSMXL7 interactions with the transcription factor GhHOX3 in cotton (Sun *et al.*, 2023, 2024).

What genes are regulated by SMXL proteins?

Many studies have investigated the genome-wide transcriptional changes that occur in response to perturbation of KAR/KL and SL signaling in a diverse range of plant species, tissue types, and environmental conditions. This approach ideally has the potential to reveal gene regulatory networks that are regulated by SMXL proteins, providing clues to how downstream responses occur. While interpreting or designing such experiments, however, it is critical to consider the specificity of the chemical treatments and genetic backgrounds that are used (Box 1). The size and composition of differentially expressed gene sets (DEGs) that have been reported in studies of KAR/KL and SL responses vary widely. These differences may be due to the nature of the transcript profiling method, the analytical methods and criteria for differential expression, the duration and concentration of chemical treatments, the environmental conditions under which plants were grown, the time of day at harvest, and the tissues that were surveyed.

A major difficulty lies in distinguishing the direct targets of SMXL regulation from downstream layers of a transcriptional cascade. High-resolution, short-term time-courses of transcriptional responses to KAR/KL or SL analogs can help identify early response genes that are putatively more likely to be direct SMXL targets (Yin *et al.*, 2023; Chang *et al.*, 2024*b*; Humphreys *et al.*, 2024), but even then the initial abundance, turnover rate, and synthesis rate of transcripts will influence when significant changes in expression can be detected for a given gene. Furthermore, it is possible that some direct SMXL targets may have an altered expression potential that only becomes apparent with the inclusion of additional stimuli (i.e. SMXL proteins may gate or potentiate gene expression). For example, changes in chromatin after *rac*-GR24 treatment are not always associated with differential expression (Humphreys *et al.*, 2024). Only a few studies, which were conducted in Arabidopsis, have used ChIP-seq (chromatin immunoprecipitation sequencing) to examine the direct binding of SMXL proteins to DNA, or ATAC-seq (assay for transposase-accessible chromatin with sequencing) to profile changes to chromatin accessibility following *rac*-GR24 treatment or in *smxl* mutant backgrounds (Wang *et al.*, 2020*a*; Wallner *et al.*, 2023; Humphreys *et al.*, 2024). These approaches, however, provide important complementary data that can help resolve the limitations of transcriptome analyses for identifying the genomic targets of SMXL proteins. Comparisons of putative SMXL targets to TPL/TPR chromatin targets may also prove useful for understanding the EAR-motif mediated aspect of gene regulation by this family (Griebel *et al.*, 2023).

Several genes are frequently used as markers of SL and KAR/KL signaling, including *BRANCHED1* (*BRC1*/*TCP18*), *Aux/IAA* genes, *D14-LIKE2* (*DLK2*), *KARRIKIN UPREGULATED F-BOX1* (*KUF1*), *B-BOX DOMAIN PROTEIN20* (*BBX20*)/*SALT TOLERANCE HOMOLOG7* (*STH7*)/*bzr1-1D SUPPRESSOR1* (*BZS1*), and *SMXL* genes themselves. Notably, SMXL-regulated genes in Arabidopsis are distinguished by EAR motif-dependent regulation (e.g. *KUF1*, *BRC1*, *SMXL6*) and EAR motif-independent regulation (e.g. *IAA29*) (Wang *et al.*, 2020*a*; Chang *et al.*, 2024*b*). To identify additional robust transcriptional markers of SL and KAR/KL response, we performed a meta-analysis of DEGs reported in 10 transcriptomic studies of Arabidopsis (Table S1). We also compared these DEGs to a genome-wide analysis of SMXL6 binding sites (Wang *et al.*, 2020*a*). In Table 1, we list several of the DEGs most frequently observed across these studies, which may be useful as additional molecular readouts of KAR/KL and SL signaling, regardless of whether they are regulated by SMXL proteins directly.

How is gene expression regulated by SMXL proteins?

Chromatin remodeling is one way in which SMXL proteins influence gene expression. An ATAC-seq analysis of *rac*-GR24-treated Arabidopsis protoplasts, conducted over a time course of 5 to 45 minutes, revealed 1447 differentially accessible regions associated with 1298 genes (Humphreys *et al.*, 2024). Both increased and decreased chromatin accessibility were observed. The SWITCH/SUCROSE NON-FERMENTABLE (SWI/SNF) chromatin remodeling ATPase SPLAYED (SYD) is critical for this response, as it was found to be required for 97% of the *rac*-GR24-induced changes in chromatin accessibility. 339 of the differentially accessible genes also showed differential expression within a three-hour time course of *rac*-GR24 treatment (among 3669 differentially expressed genes), usually after the appearance of nearby chromatin changes at an earlier time point. This indicates that chromatin remodeling precedes transcriptional responses to *rac*-GR24 for many genes, but in many other cases chromatin changes are not required or may have a non-immediate, priming effect on gene expression (Humphreys *et al.*, 2024).

Histone deacetylases also influence some responses to *rac*-GR24, such as germination in Arabidopsis (Temmerman *et al.*, 2023). However, it is not yet clear if this occurs through deacetylation of histones, which causes chromatin compaction and transcriptional repression, or deacetylation of TPL/TPR proteins. This posttranslational modification weakens the association of TPL/TPR with NOVEL INTERACTOR OF JAZ (NINJA) during jasmonate signaling repression, suggesting that other TPL/TPR protein-protein interactions might also be affected (An *et al.*, 2022; Temmerman *et al.*, 2023).

Further evidence for the role of chromatin remodeling in SMXL function comes from the discovery that OBERON3 (OBE3) works with SMXL3, SMXL4, and SMXL5 during phloem development (Wallner *et al.*, 2023). OBERON proteins contain plant homeodomain (PHD) finger motifs that have been associated with binding epigenetically modified histone H3 tails and recruiting chromatin remodeling complexes (Mouriz *et al.*, 2015). SMXL5 and OBE3 physically interact and are co-localized in nuclear subdomains of phloem cells. While other OBE proteins can interact with SMXL5, genetic analysis demonstrating synthetic enhancement among *obe3* and *smxl* mutants has pinpointed OBE3 as the critical partner of SMXL3/4/5. ATAC-seq experiments comparing phloem and non-phloem cells from wild-type, *smxl5*, *smxl4 smxl5*, and

smxl5 obe3 plants further demonstrated that SMXL3/4/5 and OBE3 cooperate to establish phloem-specific chromatin signatures (Wallner *et al.*, 2023).

These studies exemplify how SMXL proteins can collaborate with chromatin modifiers to execute their developmental functions. However, epigenetic regulation is only one component of how SMXL proteins work. Another important component comes from interactions between SMXL proteins and transcriptional regulators, which add specificity to SMXL regulation of gene expression.

What are the downstream signaling partners of SMXL proteins?

To better understand how SMXL proteins work, there has been substantial interest in identifying proteins that interact with SMXLs or act during the early phases of signal transduction following SMXL degradation. Many proteins that might interact with SMXLs or other components of SCF^{MAX2} signaling complexes have been identified through immunoprecipitation/affinity purification-mass spectrometry (IP-MS or AP-MS) or yeast two-hybrid screens (Struk *et al.*, 2018, 2021; Fan *et al.*, 2023; Lian *et al.*, 2023; Wallner *et al.*, 2023; Yuan *et al.*, 2023; An *et al.*, 2024; Chang *et al.*, 2024*a*,*b*; Sun *et al.*, 2024). A number of transcription factors that may be important in downstream responses to *rac*-GR24 have also been identified through constructing gene regulatory networks from coexpression analysis of transcriptome time-courses (Yin *et al.*, 2023; Humphreys *et al.*, 2024). Most of these potential signaling relationships have not yet been evaluated, however. Below, we highlight several of the currently established signaling partners that mediate transcriptional regulation by SMXL proteins (Table 2).

DELLA proteins

Several SMXL protein interactions with DELLA proteins have been identified, suggesting a mechanism for integrating signals such as KAR, SL, GA, and light during germination, seedling establishment, and other developmental processes (Kim *et al.*, 2022; Xu *et al.*, 2023). In Arabidopsis, SMAX1 interacts with the DELLA proteins RGA, GAI, RGL1, RGL3, while conflicting results have been observed for potential SMAX1-RGL2 interactions. These protein-protein interactions involve the N-domain and putatively another domain of SMAX1 and, based on RGL1, the N-terminal DELLA domain of DELLA proteins (Kim *et al.*, 2022; Xu *et al.*, 2023; Chang *et al.*, 2024*a*). Interactions between SMXL78 clade proteins and DELLA proteins have

been demonstrated in rice, apple, and cotton (Sun *et al.*, 2023, 2024; An *et al.*, 2024). Similarly, in Arabidopsis, SMXL7 may interact with RGL1 and RGL3 (Chang *et al.*, 2024*a*).

DELLA proteins are signaling hubs that interact with a wide range of transcription factors (TFs). Yeast two-hybrid assays using N-terminally truncated versions of RGA and GAI as baits showed that RGA and GAI interact with at least 244 and 243 TFs, respectively, that belong to 51 different TF families (Lantzouni *et al.*, 2020). Therefore, SMXL-DELLA interactions may have multiple consequences.

First, SMXL proteins may affect DELLA abundance. Low nitrogen availability promotes SL biosynthesis, which in turn activates D14-SCF^{D3}-mediated degradation of both OsD53 and OsSLR1 (Sun *et al.*, 2014, 2023). But, OsD53 appears to have a protective effect by interfering with OsD14-OsSLR1 interactions (Nakamura *et al.*, 2013; Sun *et al.*, 2023). A similar mechanism of DELLA protection occurs in cotton (Sun *et al.*, 2024). In Arabidopsis, SL-deficiency appears to have a weak effect on increasing RGA abundance (Lantzouni *et al.*, 2017). In contrast, the absence or KAR-triggered degradation of aSMAX1 clade proteins in Arabidopsis leads to increased RGA protein accumulation in the nucleus, implying that SMAX1 and SMXL2 destabilize DELLAs (Kim *et al.*, 2022).

A second possibility is that SMXL-DELLA interactions either interfere with or stabilize SMXL-TF or DELLA-TF interactions. Surprisingly, 19 of 29 potential SMAX1-interacting TFs identified by yeast two-hybrid (excluding DELLA proteins) also interact with either RGA or GAI (Lantzouni *et al.*, 2020; Chang *et al.*, 2024*a*). It may be that SMAX1 and DELLA proteins compete for interaction with these TFs and/or cooperatively bind to some TFs. As one example, in apple, MdRGL2a interferes with interactions between MdSMXL8 and MdAGL9. Because MdSMXL8 normally inhibits the transcriptional activity of MdAGL9, this SMXL-DELLA interaction has the effect of increasing MdAGL9-regulated transcription (An *et al.*, 2024).

Third, SMXL-DELLA interactions may affect the transcriptional regulatory activity of either protein partner. For example, coexpression of SMAX1 and protein interaction-capable RGL1 or RGL3 enhances the transcriptional suppression activity of SMAX1 on synthetic and *GIBBERELLIN 3-OXIDASE 2* (*GA3ox2*) promoters (Xu *et al.*, 2023).

It is noteworthy that, despite extensive evidence for SMXL-DELLA interactions, KAR/KL and SL do not have consistently similar effects as GA in either development or gene expression. For example, KAR/KL and GA signaling both promote Arabidopsis seed germination, but in seedlings have opposite effects on hypocotyl elongation (Nelson *et al.*, 2009, 2010; Bunsick *et al.*, 2020). Treatment of Arabidopsis seedlings with *rac*-GR24 and/or GA has largely additive effects on gene expression with relatively few cases of synergism (Lantzouni *et al.*, 2017). Therefore, other protein partners are undoubtedly important in adding specificity to SMXL and DELLA functions.

SPL proteins

Interactions between SMXL proteins and SQUAMOSA PROMOTER BINDING PROTEIN (SBP)-LIKE (SPL) family transcription factors were first reported in bread wheat (*Triticum aestivum*) and rice, providing important insights into the regulation of aboveground plant architecture by SL signaling (Liu *et al.*, 2017; Song *et al.*, 2017). In bread wheat, *TaSPL3* and *TaSPL17* are transcriptional activators of *TEOSINTE BRANCHED1*/*BRANCHED1* (*TaTB1*/*TaBRC1*) and *BARREN STALK1* (*TaBA1*/*TabHLH67*), which regulate tillering and spikelet formation. Physical interaction of TaD53, a SMXL78 clade protein, with TaSPL3 and TaSPL17 causes suppression of *TaTB1* and *TaBA1* expression. This provides a way to regulate shoot architecture that is complementary to miR156-mediated cleavage of *TaSPL3* and *TaSPL17* transcripts (Liu *et al.*, 2017). Concurrent work in rice showed that OsD53 interacts with IDEAL PLANT ARCHITECTURE1 (OsIPA1)/OsSPL14, suppressing the ability of OsIPA1 to activate expression of *OsTB1*/*OsBRC1*/*FINE CULM1* (*OsFC1*) while not interfering with its DNA-binding activity (Song *et al.*, 2017). Again, the OsD53-based mechanism to suppress OsIPA1 activity complements the miR156-induced cleavage of *OsIPA1* transcripts. Interestingly, OsIPA1 can also bind to the *OsD53* promoter, forming a negative feedback loop by which OsD53 controls its own expression. OsD53 also interacts with OsSPL17, a homolog of OsIPA1, and suppresses its transcriptional activation activity (Sun *et al.*, 2021*a*). By suppressing OsSPL14 and OsSPL17 activity, OsD53 reduces expression of the auxin efflux carrier *PIN-FORMED1b* (*OsPIN1b*), which in turn inhibits root elongation.

A similar mechanism is found in maize (*Zea mays*) and Arabidopsis (Xie *et al.*, 2020; Liu *et al.*, 2021). ZmD53 interacts with maize homologs of IPA1, UNBRANCHED3 (ZmUB3) and TASSEL SHEATH4 (ZmTSH4), repressing their transcriptional activity on *ZmTB1*. A dominant, SL-insensitive *Zmd53* mutant transgene causes increased tillering, reduced stature, and reduced

tassel branch number (Liu *et al.*, 2021). In Arabidopsis, AtSPL9 and AtSPL15, homologs of OsIPA1, interact with SMXL78 clade proteins (Xie *et al.*, 2020). As observed in rice, this interaction does not interfere with the DNA-binding activity of the SPL proteins but does inhibit their ability to activate *BRC1* transcription. That being said, analysis of Arabidopsis *spl9 spl15* double mutants has led to differing conclusions about the importance of these genes for branching control (Schwarz *et al.*, 2008; Bennett *et al.*, 2016). While Schwarz et al. (2008) reported enhanced branching, Bennett et al. (2016) observed only minor effects on shoot branching in *spl9 spl15* mutants. The source of this significant discrepancy is unknown, but might be due to differences in growth conditions (e.g. light, temperature, or nutrient availability) or the method of branching assessment.

Phytochrome B and PIF proteins

The SL and KAR/KL signaling pathways are closely intertwined with light signaling in plants. For example, in Arabidopsis, under shade conditions PHYTOCHROME-INTERACTING FACTOR (PIF) proteins accumulate and repress miR156 expression. This leads to increased SPL abundance, which provides a way to integrate light quality and SL signaling in the control of shoot architecture as described above (Xie *et al.*, 2017). KAR/KL signaling mutants in Arabidopsis have altered photomorphogenesis and many genes controlled by this pathway are also light-regulated (Shen *et al.*, 2007; Nelson *et al.*, 2010, 2011; Sun and Ni, 2011; Waters *et al.*, 2012; Stanga *et al.*, 2013, 2016; Lee *et al.*, 2018; Sepulveda *et al.*, 2022; Hountalas *et al.*, 2024). Light is not required for a number of transcriptional responses to KAR/KL signaling, and overexpression of *KAI2* or the loss of *SMAX1* and *SMXL2* can bypass a light requirement during Arabidopsis seed germination (Nelson *et al.*, 2010; Hountalas *et al.*, 2024). However, light is nonetheless important for many gene expression changes and developmental responses to KARs or *rac*-GR24 during germination and seedling growth in Arabidopsis (Nelson *et al.*, 2009, 2010). Furthermore, Arabidopsis mutants in photoreceptor genes or the transcription factor *ELONGATED HYPOCOTYL5* (*HY5*) show impaired developmental responses to KARs and *rac*-GR24 (Nelson *et al.*, 2010; Jia *et al.*, 2014; Park *et al.*, 2022; Chang *et al.*, 2024*b*). KAR and *rac*-GR24 regulate the abundance, subcellular localization, and/or activity of HY5, CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), and BBX20 proteins in Arabidopsis. Although there is strong genetic support for HY5, COP1, and BBX20 acting downstream of SMAX1 and SMXL2, there is no evidence that they interact with SMXL proteins directly (Tsuchiya *et al.*, 2010; Jia *et al.*, 2014; Wei *et al.*, 2016; Bursch *et al.*, 2021).

Instead, SMAX1 and SMXL2 physically interact with phyB protein in Arabidopsis, presumably via the SMXL N-terminal domain (Park *et al.*, 2022; Chang *et al.*, 2024*b*). IP-MS analysis also identified SMAX1 and SMXL2 interactions with PIF4 and PIF5, which were further supported by coimmunoprecipitation and pull-down assays (Chang *et al.*, 2024*b*). Although other groups have not observed interactions between SMAX1 and PIF4 in yeast two-hybrid assays, the weight of biochemical and genetic evidence strongly favors this interaction (Park *et al.*, 2022; Chang *et al.*, 2024*a*). The presence of SMAX1 or SMXL2 weakens protein-protein interactions between phyB and PIF4 or PIF5, which could be due to competitive SMXL-phyB interactions, SMXL-PIF interactions, or both (Chang *et al.*, 2024*b*). In seedlings grown under red light, the disruption of phyB-PIF4/5 interactions by SMAX1 and SMXL2 increases the stability of PIF4 and PIF5 proteins (Chang *et al.*, 2024*b*). Conversely, the loss of SMAX1 and SMXL2, either through mutation or KAI2-mediated degradation, reduces PIF4 and PIF5 stability (Chang *et al.*, 2024*b*). Under white light, however, no obvious effect of *smax1* on PIF4 abundance or PIF4 DNA-binding activity was observed at 23°C, or at a 28°C temperature that stimulates SMAX1 degradation and thermomorphogenic growth via phyB (Park *et al.*, 2022). Regardless, in both light conditions SMAX1 stimulates the transcriptional activity of PIF4. Genetic support for this model comes from observations that overexpression of a constitutively active phyB mutant protein mostly counteracts *kai2* and *max2* effects on Arabidopsis seedling elongation, and *pif4* and *pif4 pif5* mutations mostly suppress *kai2* (Park *et al.*, 2022; Chang *et al.*, 2024*a*). However, *smax1 phyB* seedlings as well as *smax1 smxl2* seedlings that overexpress *PIF4* and *PIF5* show intermediate hypocotyl elongation phenotypes that suggest the convergence of two pathways rather than epistatic interactions within a single pathway (Chang *et al.*, 2024*b*). Importantly, some downstream responses regulated by SMAX1 and SMXL2, such as cotyledonary petiole angle and the expression of many genes, are dependent on *PIF4* and *PIF5* (Chang *et al.*, 2024*b*). These responses do not require the SMXL EAR motif, suggesting that they are mediated through competitive protein-protein interactions instead of through transcriptional cosuppression by TPL/TPR.

BES1 and BZR1 proteins

Brassinosteroids (BRs) are essential steroid hormones that regulate plant growth, development, and stress responses (Sun *et al.*, 2010; Yu *et al.*, 2011; Nolan *et al.*, 2020). BR signaling is primarily mediated by the transcription factors BRASSINAZOLE RESISTANT 1 (BZR1) and *bri1*-EMS-SUPPRESSOR 1 (BES1/BZR2), which act as positive regulators of BR-responsive gene expression (He *et al.*, 2002; Yin *et al.*, 2002; Zhao *et al.*, 2002; Kim *et al.*, 2009). The

activity of BZR1 and BES1 is modulated by phosphorylation, which affects their DNA-binding affinity and nuclear accumulation (Zhao *et al.*, 2002; Kim and Wang, 2010; Wang *et al.*, 2021). The first suggestion of crosstalk between SL and BR signaling pathways emerged from a study of the gain-of-function *bes1-D* mutant in Arabidopsis, which exhibits enhanced branching (Wang *et al.*, 2013). BES1 was initially proposed to be a MAX2-interacting protein that is targeted for degradation by D14-SCF^{MAX2} (Wang *et al.*, 2013), but further genetic analysis of *BES1* contradicted this conclusion (Bennett *et al.*, 2016). Later work suggested instead that BES1 physically interacts with SMX78 clade proteins in Arabidopsis (Hu *et al.*, 2020). Similarly, OsBZR1 and OsD53 interact together, as well as with DWARF AND LOW TILLERING (OsDLT) and REDUCED LEAF ANGLE1 (OsRLA1), to regulate tillering in rice (Fang *et al.*, 2020).

Substantial overlap has been observed in differential gene expression among *Atd14*, *SMXL7-D* (a SL-insensitive, gain-of-function SMXL7 allele), and *bes1-D* mutant plants in Arabidopsis (Hu *et al.*, 2020). The shared transcriptional changes could simply reflect developmental similarities among these mutants, all of which show excess axillary branching. However, the *bes1-D* shoot branching phenotype is abolished by the addition of *smxl6,7,8* mutations, suggesting instead that *bes1-D* effects are dependent on *SMXL* function. Supporting the idea that BES1 and SMXL proteins cooperate to regulate transcription, BES1 can bind the promoter of *BRC1* but has little or no effect on its expression. Coexpression of *bes1-D* and *SMXL7-D*, however, causes stronger suppression of *BRC1* expression in transient assays than *SMXL7-D* alone. Contradicting the idea of cooperative action, disruption of BR signaling or application of BR, which influences BES1 phosphorylation and stability, has no effect on *BRC1* expression in Arabidopsis (Hu *et al.*, 2020). Thus, the functional nature of SMXL and BES1/BZR1 interactions will require further clarification.

JAZ proteins

JASMONATE ZIM-DOMAIN (JAZ) proteins act as transcriptional repressors in the jasmonate (JA) signaling pathway (Pauwels and Goossens, 2011). JAZ proteins bind a variety of transcription factors, for example MYC proteins, and regulate gene expression by inhibiting DNA-binding, recruiting TPL/TPR proteins via an EAR motif, or through interactions with the EAR motif-containing NINJA protein, which recruits TPL/TPR (Pauwels and Goossens, 2011). JAZ proteins are rapidly targeted for polyubiquitination and degradation by the E3 ubiquitin ligase SCF^{COI1} in the presence of JA-Ile, a bioactive conjugate of jasmonic acid and isoleucine.

Thus in many ways, the functions and regulation of JAZ and SMXL proteins are analogous (Blázquez *et al.*, 2020).

In *Nicotiana attenuata*, the SMXL78 clade proteins NaSMXL6 and NaSMXL7 interact with several members of the JAZ family (Li *et al.*, 2020*a*). SMXL proteins reduce NaJAZb function and increase the transcriptional activity of NaMYC2 when SL is low in two ways. First, NaSMXL6 and NaSMXL7 promote the degradation of NaJAZb. Second, they interfere with NaJAZb-NaMYC2 interactions through competitive binding of NaJAZb. This leads to increased accumulation of anthocyanin, phenolamides, and auxin, as well as decreased nicotine concentrations that make plants more susceptible to insect herbivory (Li *et al.*, 2020*a*).

WRKY6 protein

In apple (*Malus domestica* Borkh.), as seen for SMXL78 clade proteins in many other plants, MdSMXL7 inhibits the expression of *MdBRC1* (Fan *et al.*, 2023). However, MdSMXL7 does not do so through direct interaction with the *MdBRC1* promoter, implying that regulation of *MdBRC1* expression occurs via a partner protein. Yeast two-hybrid screening of a cDNA library from apple with an MdSMXL7 bait identified the transcription factor MdWRKY6 as an interacting protein. MdWRKY6 binds to the promoter of *MdBRC1* and inhibits its transcription. The presence of MdSMXL7 enhances the repression of *MdBRC1* expression by MdWRKY6, presumably due to their protein-protein interactions. Therefore, one of the downstream consequences of SL-induced degradation of MdSMXL7 is increased *MdBRC1* expression, which in turn leads to increased expression of *MdGH3.1* (an auxin-amino acid conjugating enzyme) and decreased adventitious root formation (Fan *et al.*, 2023). This mechanism may reveal how SLs inhibit adventitious root formation in other species such as Arabidopsis, pea, and tomato (Kohlen *et al.*, 2012; Rasmussen *et al.*, 2012).

GRF4 protein

Enhancing nitrogen use efficiency in crops will require a comprehensive understanding of the regulatory mechanisms that integrate growth, nitrogen (N) assimilation, and carbon fixation. In rice, the transcription factor GROWTH-REGULATING FACTOR4 (OsGRF4) and the DELLA protein OsSLR1 have antagonistic effects on these processes; OsGRF4 promotes nutrient acquisition and growth, while OsSLR1 inhibits it (Li *et al.*, 2018). The SMXL78 clade protein D53 directly interacts with OsGRF4 and inhibits its binding to DNA, while OsSLR1 interacts with

OsGRF4 to block its association with a transcriptional co-activator, OsGIF (GRF-interacting factor) (Li *et al.*, 2018; Sun *et al.*, 2023). Under low N conditions, SL biosynthesis increases, triggering OsD53 degradation via D14-SCF^{D3}. *Rac*-GR24 also promotes OsSLR1 degradation in a D14-dependent manner that is independent of GA perception. Therefore, SL perception relieves repression of OsGRF4 activity in two ways: by allowing OsGRF4 to bind to its DNA targets and to its co-activator OsGIF.

Complicating matters, OsD14 and OsD53 can each interact with OsSLR1, but the presence of OsD53 appears to interfere with OsD14-OsSLR1 interactions, helping to protect OsSLR1 from SL-induced degradation (Sun *et al.*, 2023). It is not clear whether this might be due to OsD53-OsD14 or OsD53-OsSLR1 interactions, or both, being stronger than OsD14-OsSLR1 interactions. In any case, this suggests the two modes of action are synergistic; SL-induced depletion of D53 putatively increases the SL-induced degradation of SLR1. A two-phase process might explain the different rates of D53 and SLR1 degradation. *Rac*-GR24 triggers D53 degradation within several minutes, while *rac*-GR24-induced degradation of SLR1 proceeds more slowly, typically requiring several hours (Jiang *et al.*, 2013; Zhou *et al.*, 2013; Bennett *et al.*, 2016; Struk *et al.*, 2018).

Further investigation will be needed to determine whether a similar mechanism is used in other plants. Putative interactions between SMAX1 and SMXL2 with AtGRF7 and AtGRF9 in Arabidopsis have been identified through IP-MS assays (Chang *et al.*, 2024*b*). However, in another study, interactions between SMAX1 or SMXL7 with Arabidopsis GRF family proteins were not detected by yeast two-hybrid assays (Chang *et al.*, 2024*a*).

AGL9 protein

SLs play a significant role in regulating anthocyanin biosynthesis across various plant species (Li *et al.*, 2020*b*; Wang *et al.*, 2020*a*). In apple, ELONGATED HYPOCOTYL5 (MdHY5) is a central regulator of anthocyanin biosynthesis that is also transcriptionally upregulated by the SL analog GR24^{5DS} (Shin *et al.*, 2013; Gangappa and Botto, 2016; An *et al.*, 2017, 2024; Xu, 2020). The transcription factor AGAMOUS-LIKE MADS-BOX 9 (MdAGL9) was found to bind to the *MdHY5* promoter directly and activate *MdHY5* expression following SL treatment (An *et al.*, 2024). MdSMXL8 was then discovered through IP-MS to be a physical interactor of MdAGL9. MdSMXL8 binds to MdAGL9 and inhibits its transcriptional activity (Sun *et al.*, 2021*b*; An *et al.*, 2024). This inhibition can be relieved through SL-induced degradation of MdSMXL8 via the E3

ubiquitin ligase MdPRT1 (n.b. the presumed contribution of MdD14-SCF^{MdMAX2} to MdSMXL8 degradation has not been tested) and through competitive binding of MdSMXL8 to MdRGL2a that interferes with MdSMXL8-MdAGL9 association (An *et al.*, 2024). This regulatory module illustrates an intricate mechanism to integrate light, SL, and GA signaling in the control of anthocyanin biosynthesis.

KNAT5 and OFP1 proteins

In Arabidopsis, *SMXL4*, also known as *HEAT SHOCK PROTEIN-RELATED* (*AtHSPR*), is expressed in plant vascular tissues, where it affects the size of plant organs, abiotic stress tolerance, and phloem development (Zhang *et al.*, 2014; Yang *et al.*, 2015, 2016; Wallner *et al.*, 2017). One important aspect of AtHSPR/SMXL4 function is the regulation of GA homeostasis, which in turn affects primary root growth, flowering time, and seed set. AtHSPR/SMXL4 interferes with the activity of KNOTTED1-LIKE HOMEOBOX GENE 5 (KNAT5) and OVATE FAMILY PROTEIN 1 (OFP1), transcription factors that repress the GA biosynthesis gene *GIBBERELLIN 20 OXIDASE 1* (*GA20ox1*), through physical interactions (Yang *et al.*, 2020*b*; Yuan *et al.*, 2023). KNAT5 belongs to the KNOTTED-LIKE TALE HOMEOBOX CLASS II (KNOX2) family in *Arabidopsis*, which regulates root growth (Bürglin, 1997; Truernit and Haseloff, 2007; Meng *et al.*, 2020). These nuclear-localized homeodomain proteins interact with OFPs to determine DNA binding affinity and specificity (Bellaoui *et al.*, 2001; Hackbusch *et al.*, 2005; Kanrar *et al.*, 2006). OFP1, found in the nucleus and cortical cytoskeleton, inhibits cell elongation partly by suppressing *GA20ox1* expression (Wang *et al.*, 2007; Zhang *et al.*, 2018). Interaction between AtHSPR/SMXL4 and both KNAT5 and OFP1 occurs via the region encoded by the first exon of AtHSPR/SMXL4, which includes the N domain and part of the D1 domain (Yang *et al.*, 2020*b*; Yuan *et al.*, 2023; Chang *et al.*, 2024*a*). There is strong genetic support for this interaction. Epistasis tests indicate that *KNAT5* and *OFP1* act downstream of *AtHSPR/SMXL4* in controlling primary root length. *KNAT5* and *OFP1* overexpression mimics the *Athspr* phenotype, while *knat5* and *ofp1* mutants resemble *AtHSPR* overexpression lines. Moreover, *AtHSPR* overexpression counteracts the suppression of *GA20ox1* promoter activity by KNAT5 and OFP1 (Yuan *et al.*, 2023). Notably, the positive regulation of *GA20ox1* expression by AtHSPR is contrary to the corepressor model of SMXL function, instead suggesting that AtHSPR might prevent KNAT5 and OFP1 from binding to their DNA targets.

The molecular basis of specific SMXL roles in plants

A major unresolved question about SMXL proteins is how the different types acquired their unique functions in plant growth, development, and physiology. Among bryophytes, SMXL proteins vary in their form, quantity, and regulation (Lopez-Obando *et al.*, 2016, 2018, 2021; Mizuno *et al.*, 2021; Kodama *et al.*, 2022; Guillory *et al.*, 2024). Some degree of functional conservation is present among bryophyte and angiosperm SMXL proteins, as demonstrated by the partial to full rescue of some *smxl* mutants with *SMXL* transgenes from other species (Guillory *et al.*, 2024). Likewise, some KAI2 or D14 proteins are able to function in long-separated species, implying that receptor interactions with MAX2 and/or SMXL proteins have been at least partially conserved (Drummond *et al.*, 2011; Liu *et al.*, 2014; Conn and Nelson, 2015; Waters *et al.*, 2015; Zheng *et al.*, 2016; Carbonnel *et al.*, 2020*b*; Sun *et al.*, 2020; Hu *et al.*, 2021; Lopez-Obando *et al.*, 2021; Guercio *et al.*, 2022; Kodama *et al.*, 2022; White *et al.*, 2022; Komatsu *et al.*, 2023).

The SMXL family in angiosperms is particularly interesting due to the diversification of SMXL types that far exceeds that seen in other extant plant lineages. Key to understanding the evolutionary process that led to this diversification is identifying the molecular basis of SMXL "output" specificity in angiosperms. In a recent preprint, we reported that the N domain is a critical component of output control. Promoter-swapping experiments demonstrated that *SMAX1* cannot replace the function of *SMXL7*, and *SMXL7* only replicates *SMAX1* function partially (Chang *et al.*, 2024*a*). This echoes work showing that *SMXL5* misexpression cannot rescue *smax1 smxl2* or *smxl6,7,8* mutants, although *SMAX1* and *SMXL7* can partially rescue a *smxl4,5* mutant (Li *et al.*, 2024). Therefore differential expression is not the basis of unique *SMXL* functions. Chimeric proteins consisting of swapped domains between SMAX1 and SMXL7 demonstrated that the N domain of SMAX1 confers control of germination and hypocotyl elongation and likewise the N domain of SMXL7 confers control of axillary branching. Furthermore, fusing the N domain of SMAX1 to a synthetic EAR motif, SRDX, replicates the function of the full-length protein, but not its regulation by SCF^{MAX2}-dependent signaling (Chang *et al.*, 2024*a*). The SMAX1 N domain alone was not able to rescue *smax1 smxl2*, however, which conflicts with the idea that the SMAX1 EAR motif is not necessary for regulation of hypocotyl growth in Arabidopsis (Chang *et al.*, 2024*a*,*b*). In a yeast two-hybrid screen of 158 transcription factors/regulators from Arabidopsis, 33 candidate interactors of SMAX1 or SMXL7

were identified (Chang *et al.*, 2024*a*). Almost all of these candidate interactions involved the SMXL N domain, supporting the importance of this domain for downstream control.

A more refined analysis of the SMXL N domain may yield specificity-determining residues that distinguish the functions of aSMAX1 and SMXL78 clade proteins. This will provide insights into SMXL evolution in angiosperms and facilitate genetic engineering of SMXL outputs. Some of the candidate SMXL-interacting transcription factors, for instance many proteins in the TCP (TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL FACTOR 1) transcription factor family, may also provide new leads for deepening our understanding of how SMXL proteins control different aspects of plant growth and development.

Conclusion

In summary, SMXL proteins are signaling hubs that control downstream transcriptional responses through at least five mechanisms: 1) directly binding to DNA and recruiting corepressor proteins (e.g. TPL/TPR), 2) indirectly binding to DNA through association with transcription factors and recruiting corepressor proteins, 3) interfering with the DNA-binding activity of associated transcription factors, 4) sequestering transcriptional regulators from other protein interactors, and 5) increasing or decreasing the protein stability of associated transcriptional regulators (Figure 2). While the EAR motif-mediated model of transcriptional regulation by SMXL proteins which received so much initial attention remains important, it is now apparent that SMXL protein-protein interactions that modulate the abundance of transcriptional regulators, their activity, or their availability for regulatory protein complexes are also highly relevant. Substantial progress has been made in identifying several downstream signaling partners of SMXL proteins from a diverse set of transcription factor families, and more partners likely await discovery. Likewise, SL and KAR/KL-induced degradation of SMXL proteins via SCF^{MAX2} is a prominent feature in the regulation of many, but not all, SMXL proteins. The simplified models of how SMXL proteins work and how they are regulated that have been built over the past decade must necessarily become more complex to accommodate emerging discoveries of signaling integration with other pathways.

Acknowledgements

n/a

Conflict of interest

The authors declare no conflicts of interest.

Funding

Support for this work was provided by the National Science Foundation (NSF-IOS 1856741 and 2329271) and the United States Department of Agriculture (Hatch project CA-R-BPS-5209-H).

References

Abdelrahman M, Mostofa MG, Tran CD, El-Sayed M, Li W, Sulieman S, Tanaka M, Seki M, Tran L-SP. 2023. The Karrikin Receptor Karrikin Insensitive2 Positively Regulates Heat Stress Tolerance in Arabidopsis thaliana. Plant & cell physiology **63**, 1914–1926.

Agusti J, Herold S, Schwarz M, *et al.* 2011. Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. Proceedings of the National Academy of Sciences of the United States of America **108**, 20242–20247.

An C, Deng L, Zhai H, You Y, Wu F, Zhai Q, Goossens A, Li C. 2022. Regulation of jasmonate signaling by reversible acetylation of TOPLESS in Arabidopsis. Molecular plant **15**, 1329–1346.

An J-P, Qu F-J, Yao J-F, Wang X-N, You C-X, Wang X-F, Hao Y-J. 2017. The bZIP transcription factor MdHY5 regulates anthocyanin accumulation and nitrate assimilation in apple. Horticulture research **4**, 17023.

An J-P, Zhao L, Cao Y-P, Ai D, Li M-Y, You C-X, Han Y. 2024. The SMXL8-AGL9 module mediates crosstalk between strigolactone and gibberellin to regulate strigolactone-induced anthocyanin biosynthesis in apple. The Plant cell doi: 10.1093/plcell/koae191.

Arold ST, Vancea AI, Huntington B, Steinchen W, Savva CG, Shahul Hameed UF. 2024. Mechanism of cooperative strigolactone perception by the MAX2 ubiquitin ligase-receptor-substrate complex. bioRxiv.

Bellaoui M, Pidkowich MS, Samach A, Kushalappa K, Kohalmi SE, Modrusan Z, Crosby WL, Haughn GW. 2001. The Arabidopsis BELL1 and KNOX TALE homeodomain proteins interact through a domain conserved between plants and animals. The Plant cell **13**, 2455–2470.

Bennett T, Liang Y, Seale M, Ward S, Müller D, Leyser O. 2016. Strigolactone regulates shoot development through a core signalling pathway. Biology open **5**, 1806–1820.

Blázquez MA, Nelson DC, Weijers D. 2020. Evolution of Plant Hormone Response Pathways. Annual review of plant biology **71**, 327–353.

Briones-Moreno A, Hernández-García J, Vargas-Chávez C, Blanco-Touriñán N, Phokas A, Úrbez C, Cerdán PD, Coates JC, Alabadí D, Blázquez MA. 2023. DELLA functions evolved by rewiring of associated transcriptional networks. Nature plants **9**, 535–543.

Bunsick M, Toh S, Wong C, *et al.* 2020. SMAX1-dependent seed germination bypasses GA signalling in Arabidopsis and Striga. Nature plants **6**, 646–652.

Bürglin TR. 1997. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucleic acids research **25**, 4173–4180.

Bursch K, Niemann ET, Nelson DC, Johansson H. 2021. Karrikins control seedling photomorphogenesis and anthocyanin biosynthesis through a HY5-BBX transcriptional module.

The Plant journal: for cell and molecular biology **107**, 1346–1362.

Carbonnel S, Das D, Varshney K, Kolodziej MC, Villaécija-Aguilar JA, Gutjahr C. 2020*a*. The karrikin signaling regulator SMAX1 controls Lotus japonicus root and root hair development by suppressing ethylene biosynthesis. Proceedings of the National Academy of Sciences of the United States of America **117**, 21757–21765.

Carbonnel S, Torabi S, Griesmann M, *et al.* 2020*b*. Lotus japonicus karrikin receptors display divergent ligand-binding specificities and organ-dependent redundancy. PLoS genetics **16**, e1009249.

Causier B, Ashworth M, Guo W, Davies B. 2012. The TOPLESS interactome: a framework for gene repression in Arabidopsis. Plant physiology **158**, 423–438.

Chang SH, George WJ, Nelson DC. 2024*a*. An N-terminal domain specifies developmental control by the SMAX1-LIKE family of transcriptional co-repressors in Arabidopsis thaliana. bioRxiv, 2024.05.12.593779.

Chang W, Qiao Q, Li Q, Li X, Li Y, Huang X, Wang Y, Li J, Wang B, Wang L. 2024*b*. Non-transcriptional regulatory activity of SMAX1 and SMXL2 mediates karrikin-regulated seedling response to red light in Arabidopsis. Molecular plant doi: 10.1016/j.molp.2024.05.007.

Chevalier F, Nieminen K, Sánchez-Ferrero JC, Rodríguez ML, Chagoyen M, Hardtke CS, Cubas P. 2014. Strigolactone promotes degradation of DWARF14, an α/β hydrolase essential for strigolactone signaling in Arabidopsis. The Plant cell **26**, 1134–1150.

Cho H, Cho HS, Nam H, *et al.* 2018. Translational control of phloem development by RNA G-quadruplex–JULGI determines plant sink strength. Nature Plants **4**, 376–390.

Choi J, Lee T, Cho J, *et al.* 2020. The negative regulator SMAX1 controls mycorrhizal symbiosis and strigolactone biosynthesis in rice. Nature communications **11**, 2114.

Collins J, O'Grady K, Chen S, Gurley W. 2019. The C-terminal WD40 repeats on the TOPLESS co-repressor function as a protein-protein interaction surface. Plant molecular biology **100**, 47–58.

Conn CE, Nelson DC. 2015. Evidence that KARRIKIN-INSENSITIVE2 (KAI2) Receptors may Perceive an Unknown Signal that is not Karrikin or Strigolactone. Frontiers in plant science **6**, 1219.

Davière J-M, Achard P. 2016. A Pivotal Role of DELLAs in Regulating Multiple Hormone Signals. Molecular plant **9**, 10–20.

Drummond RSM, Sheehan H, Simons JL, Martínez-Sánchez NM, Turner RM, Putterill J, Snowden KC. 2011. The expression of petunia strigolactone pathway genes is altered as part of the endogenous developmental program. Frontiers in plant science **2**, 115.

Fang Z, Ji Y, Hu J, Guo R, Sun S, Wang X. 2020. Strigolactones and Brassinosteroids Antagonistically Regulate the Stability of the D53-OsBZR1 Complex to Determine FC1 Expression in Rice Tillering. Molecular plant **13**, 586–597.

Fan X, Li Y, Deng CH, Wang S, Wang Z, Wang Y, Qiu C, Xu X, Han Z, Li W. 2023. Strigolactone regulates adventitious root formation via the MdSMXL7-MdWRKY6-MdBRC1

signaling cascade in apple. The Plant journal: for cell and molecular biology **113**, 772–786.

Feng Z, Liang X, Tian H, *et al.* 2022. SUPPRESSOR of MAX2 1 (SMAX1) and SMAX1-LIKE2 (SMXL2) Negatively Regulate Drought Resistance in Arabidopsis thaliana. Plant & cell physiology doi: 10.1093/pcp/pcac080.

Gangappa SN, Botto JF. 2016. The Multifaceted Roles of HY5 in Plant Growth and Development. Molecular plant **9**, 1353–1365.

Gottesman S, Clark WP, Maurizi MR. 1990. The ATP-dependent Clp protease of Escherichia coli. Sequence of clpA and identification of a Clp-specific substrate. The journal of biological chemistry **265**, 7886–7893.

Griebel T, Lapin D, Locci F, Kracher B, Bautor J, Concia L, Benhamed M, Parker JE. 2023. Arabidopsis Topless-related 1 mitigates physiological damage and growth penalties of induced immunity. The New phytologist **239**, 1404–1419.

Guercio AM, Torabi S, Cornu D, *et al.* 2022. Structural and functional analyses explain Pea KAI2 receptor diversity and reveal stereoselective catalysis during signal perception. Communications biology **5**, 126.

Guillory A, Lopez-Obando M, Bouchenine K, *et al.* 2024. SUPPRESSOR OF MAX2 1-LIKE (SMXL) homologs are MAX2-dependent repressors of Physcomitrium patens growth. The plant cell **36**, 1655–1672.

Hackbusch J, Richter K, Müller J, Salamini F, Uhrig JF. 2005. A central role of *Arabidopsis thaliana* ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. Proceedings of the National Academy of Sciences of the United States of America **102**, 4908–4912.

Hamiaux C, Drummond RSM, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC. 2012. DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. Current biology **22**, 2032–2036.

Hamon-Josse M, Villaécija-Aguilar JA, Ljung K, Leyser O, Gutjahr C, Bennett T. 2022. KAI2 regulates seedling development by mediating light-induced remodelling of auxin transport. The New phytologist **235**, 126–140.

Hardtke CS. 2023. Phloem development. The New phytologist **239**, 852–867.

He J-X, Gendron JM, Yang Y, Li J, Wang Z-Y. 2002. The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **99**, 10185–10190.

Hountalas JE, Bunsick M, Xu Z, Taylor AA, Pescetto G, Ly G, Boyer F-D, McErlean CSP, Lumba S. 2024. HTL/KAI2 signaling substitutes for light to control plant germination. PLoS genetics **20**, e1011447.

Hu J, Ji Y, Hu X, Sun S, Wang X. 2020. BES1 Functions as the Co-regulator of D53-like SMXLs to Inhibit BRC1 Expression in Strigolactone-Regulated Shoot Branching in Arabidopsis. Plant communications **1**, 100014.

Humphreys JL, Beveridge CA, Tanurdžić M. 2024. Strigolactone induces D14-dependent large-scale changes in gene expression requiring SWI/SNF chromatin remodellers. The Plant journal: for cell and molecular biology doi: 10.1111/tpj.16873.

Hu A, Zhao Q, Chen L, *et al.* 2021. Identification of conserved and divergent strigolactone receptors in sugarcane reveals a key residue crucial for plant branching control. Frontiers in plant science **12**, 747160.

Jia K-P, Luo Q, He S-B, Lu X-D, Yang H-Q. 2014. Strigolactone-regulated hypocotyl elongation is dependent on cryptochrome and phytochrome signaling pathways in Arabidopsis. Molecular plant **7**, 528–540.

Jiang L, Liu X, Xiong G, *et al.* 2013. DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature **504**, 401–405.

Johnson AW, Gowada G, Hassanali A, Knox J, Monaco S, Razavi Z, Rosebery G. 1981. The preparation of synthetic analogues of strigol. Journal of the Chemical Society. Perkin transactions 1 **0**, 1734–1743.

Kamran M, Melville KT, Waters MT. 2024. Karrikin signalling: impacts on plant development and abiotic stress tolerance. Journal of experimental botany **75**, 1174–1186.

Kanrar S, Onguka O, Smith HMS. 2006. Arabidopsis inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. Planta **224**, 1163–1173.

Kędzierska-Mieszkowska S, Zolkiewski M. 2021. Hsp100 Molecular Chaperone ClpB and Its Role in Virulence of Bacterial Pathogens. International journal of molecular sciences **22**.

Ke J, Ma H, Gu X, Thelen A, Brunzelle JS, Li J, Xu HE, Melcher K. 2015. Structural basis for recognition of diverse transcriptional repressors by the TOPLESS family of corepressors. Science advances **1**, e1500107.

Kerr SC, Patil SB, de Saint Germain A, *et al.* 2021. Integration of the SMXL/D53 strigolactone signalling repressors in the model of shoot branching regulation in Pisum sativum. The Plant journal: for cell and molecular biology **107**, 1756–1770.

Khosla A, Morffy N, Li Q, *et al.* 2020. Structure-Function Analysis of SMAX1 Reveals Domains That Mediate Its Karrikin-Induced Proteolysis and Interaction with the Receptor KAI2. The Plant cell **32**, 2639–2659.

Kim T-W, Guan S, Sun Y, Deng Z, Tang W, Shang J-X, Sun Y, Burlingame AL, Wang Z-Y. 2009. Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nature cell biology **11**, 1254–1260.

Kim JY, Park Y-J, Lee J-H, Park C-M. 2022. SMAX1 Integrates Karrikin and Light Signals into GA-Mediated Hypocotyl Growth during Seedling Establishment. Plant & cell physiology **63**, 932–943.

Kim T-W, Wang Z-Y. 2010. Brassinosteroid signal transduction from receptor kinases to transcription factors. Annual review of plant biology **61**, 681–704.

Kodama K, Rich MK, Yoda A, *et al.* 2022. An ancestral function of strigolactones as symbiotic rhizosphere signals. Nature communications **13**, 3974.

Kohlen W, Charnikhova T, Lammers M, *et al.* 2012. The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. The New phytologist **196**, 535–547.

Komatsu A, Kodama K, Mizuno Y, Fujibayashi M, Naramoto S, Kyozuka J. 2023. Control of vegetative reproduction in Marchantia polymorpha by the KAI2-ligand signaling pathway. Current biology: CB **33**, 1196–1210.e4.

Lantzouni O, Alkofer A, Falter-Braun P, Schwechheimer C. 2020. GROWTH-REGULATING FACTORS Interact with DELLAs and Regulate Growth in Cold Stress. The Plant cell **32**, 1018–1034.

Lantzouni O, Klermund C, Schwechheimer C. 2017. Largely additive effects of gibberellin and strigolactone on gene expression in Arabidopsis thaliana seedlings. The Plant journal: for cell and molecular biology **92**, 924–938.

Lee I, Kim K, Lee S, *et al.* 2018. A missense allele of KARRIKIN-INSENSITIVE2 impairs ligand-binding and downstream signaling in Arabidopsis thaliana. Journal of experimental botany **69**, 3609–3623.

Leydon AR, Wang W, Gala HP, Gilmour S, Juarez-Solis S, Zahler ML, Zemke JE, Zheng N, Nemhauser JL. 2021. Repression by the TOPLESS corepressor requires association with the core mediator complex. eLife **10**.

Liang Y, Ward S, Li P, Bennett T, Leyser O. 2016. SMAX1-LIKE7 Signals from the Nucleus to Regulate Shoot Development in Arabidopsis via Partially EAR Motif-Independent Mechanisms. The Plant cell **28**, 1581–1601.

Lian Y, Lian C, Wang L, Li Z, Yuan G, Xuan L, Gao H, Wu H, Yang T, Wang C. 2023. SUPPRESSOR OF MAX2 LIKE 6, 7, and 8 Interact with DDB1 BINDING WD REPEAT DOMAIN HYPERSENSITIVE TO ABA DEFICIENT 1 to Regulate the Drought Tolerance and Target to Abscisic Acid Response in. Biomolecules **13**.

Li S, Joo Y, Cao D, Li R, Lee G, Halitschke R, Baldwin G, Baldwin IT, Wang M. 2020*a*. Strigolactone signaling regulates specialized metabolism in tobacco stems and interactions with stem-feeding herbivores. PLoS biology **18**, e3000830.

Li Q, Martín-Fontecha ES, Khosla A, White ARF, Chang S, Cubas P, Nelson DC. 2022*a*. The strigolactone receptor D14 targets SMAX1 for degradation in response to GR24 treatment and osmotic stress. Plant Communications, 100303.

Li W, Nguyen KH, Chu HD, *et al.* 2017. The karrikin receptor KAI2 promotes drought resistance in Arabidopsis thaliana. PLoS genetics **13**, e1007076.

Li W, Nguyen KH, Chu HD, *et al.* 2020*b*. Comparative functional analyses of DWARF14 and KARRIKIN INSENSITIVE 2 in drought adaptation of Arabidopsis thaliana. The Plant journal: for cell and molecular biology **103**, 111–127.

Tran CD, *et al.* 2020*b*. Negative Roles of Strigolactone-Related SMXL6, 7 and 8 Proteins in Drought Resistance in Arabidopsis. Biomolecules **10**.

Li X-R, Sun J, Albinsky D, *et al.* 2022*b*. Nutrient regulation of lipochitooligosaccharide recognition in plants via NSP1 and NSP2. Nature communications **13**, 6421.

Li S, Tian Y, Wu K, *et al.* 2018. Modulating plant growth-metabolism coordination for sustainable agriculture. Nature **560**, 595–600.

Liu J, Cheng X, Liu P, Sun J. 2017. miR156-Targeted SBP-Box Transcription Factors Interact with DWARF53 to Regulate TEOSINTE BRANCHED1 and BARREN STALK1 Expression in Bread Wheat. Plant physiology **174**, 1931–1948.

Liu Y, Wu G, Zhao Y, Wang HH, Dai Z, Xue W, Yang J, Wei H, Shen R, Wang H. 2021. DWARF53 interacts with transcription factors UB2/UB3/TSH4 to regulate maize tillering and tassel branching. Plant physiology **187**, 947–962.

Liu Q, Zhang Y, Matusova R, *et al.* 2014. Striga hermonthica MAX2 restores branching but not the Very Low Fluence Response in the Arabidopsis thaliana max2 mutant. The new phytologist **202**, 531–541.

Li Q, Yu H, Chang W, *et al.* 2024. SMXL5 attenuates strigolactone signaling in Arabidopsis thaliana by inhibiting SMXL7 degradation. Molecular plant **17**, 631–647.

Long JA, Ohno C, Smith ZR, Meyerowitz EM. 2006. TOPLESS regulates apical embryonic fate in Arabidopsis. Science (New York, N.Y.) **312**, 1520–1523.

Lopez-Obando M, Conn CE, Hoffmann B, Bythell-Douglas R, Nelson DC, Rameau C, Bonhomme S. 2016. Structural modelling and transcriptional responses highlight a clade of PpKAI2-LIKE genes as candidate receptors for strigolactones in Physcomitrella patens. Planta **243**, 1441–1453.

Lopez-Obando M, Guillory A, Boyer F-D, *et al.* 2021. The Physcomitrium (Physcomitrella) patens PpKAI2L receptors for strigolactones and related compounds function via MAX2-dependent and -independent pathways. The Plant cell **33**, 3487–3512.

Lopez-Obando M, de Villiers R, Hoffmann B, *et al.* 2018. Physcomitrella patens MAX2 characterization suggests an ancient role for this F-box protein in photomorphogenesis rather than strigolactone signalling. The New phytologist **219**, 743–756.

Ma H, Duan J, Ke J, *et al.* 2017. A D53 repression motif induces oligomerization of TOPLESS corepressors and promotes assembly of a corepressor-nucleosome complex. Science advances **3**, e1601217.

Martinez SE, Conn CE, Guercio AM, Sepulveda C, Fiscus CJ, Koenig D, Shabek N, Nelson DC. 2022. A KARRIKIN INSENSITIVE2 paralog in lettuce mediates highly sensitive germination responses to karrikinolide. Plant physiology **190**, 1440–1456.

Meng L, Liu X, He C, Xu B, Li Y, Hu Y. 2020. Functional divergence and adaptive selection of KNOX gene family in plants. Open life sciences **15**, 346–363.

Meng Y, Varshney K, Incze N, *et al.* 2022. KARRIKIN INSENSITIVE2 regulates leaf development, root system architecture and arbuscular-mycorrhizal symbiosis in Brachypodium distachyon. The Plant journal: for cell and molecular biology **109**, 1559–1574.

Mizuno Y, Komatsu A, Shimazaki S, Naramoto S, Inoue K, Xie X, Ishizaki K, Kohchi T,

Kyozuka J. 2021. Major components of the KARRIKIN INSENSITIVE2-dependent signaling pathway are conserved in the liverwort Marchantia polymorpha. The plant cell **33**, 2395–2411.

Mouriz A, López-González L, Jarillo JA, Piñeiro M. 2015. PHDs govern plant development. Plant signaling & behavior **10**, e993253.

Nakamura H, Xue Y-L, Miyakawa T, *et al.* 2013. Molecular mechanism of strigolactone perception by DWARF14. Nature communications **4**, 2613.

Nam H, Gupta A, Nam H, Lee S, Cho HS, Park C, Park S, Park SJ, Hwang I. 2022. JULGI-mediated increment in phloem transport capacity relates to fruit yield in tomato. Plant biotechnology journal **20**.

Nelson DC. 2021. The mechanism of host-induced germination in root parasitic plants. Plant physiology **185**, 1353–1373.

Nelson DC, Flematti GR, Riseborough J-A, Ghisalberti EL, Dixon KW, Smith SM. 2010. Karrikins enhance light responses during germination and seedling development in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America **107**, 7095–7100.

Nelson DC, Riseborough J-A, Flematti GR, Stevens J, Ghisalberti EL, Dixon KW, Smith SM. 2009. Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. Plant physiology **149**, 863–873.

Nelson DC, Scaffidi A, Dun EA, Waters MT, Flematti GR, Dixon KW, Beveridge CA, Ghisalberti EL, Smith SM. 2011. F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America **108**, 8897–8902.

Nolan TM, Vukašinović N, Liu D, Russinova E, Yin Y. 2020. Brassinosteroids: Multidimensional Regulators of Plant Growth, Development, and Stress Responses. The Plant cell **32**, 295–318.

Park Y-J, Kim JY, Park C-M. 2022. SMAX1 potentiates phytochrome B-mediated hypocotyl thermomorphogenesis. The Plant cell **34**, 2671–2687.

Patil SB, Barbier FF, Zhao J, *et al.* 2022. Sucrose promotes D53 accumulation and tillering in rice. The New phytologist **234**, 122–136.

Pauwels L, Goossens A. 2011. The JAZ proteins: a crucial interface in the jasmonate signaling cascade. The Plant cell **23**, 3089–3100.

Peng J, Richards DE, Hartley NM, *et al.* 1999. 'Green revolution' genes encode mutant gibberellin response modulators. Nature **400**, 256–261.

Rasmussen A, Mason MG, De Cuyper C, *et al.* 2012. Strigolactones suppress adventitious rooting in Arabidopsis and pea. Plant physiology **158**, 1976–1987.

Sánchez Martín-Fontecha E, Cardinale F, Bürger M, Prandi C, Cubas P. 2024. Novel Mechanisms of Strigolactone-Induced DWARF14 Degradation in*Arabidopsis thaliana*. bioRxiv.

Scaffidi A, Waters MT, Sun YK, Skelton BW, Dixon KW, Ghisalberti EL, Flematti GR,

Smith SM. 2014. Strigolactone Hormones and Their Stereoisomers Signal through Two Related Receptor Proteins to Induce Different Physiological Responses in Arabidopsis. Plant physiology **165**, 1221–1232.

Schirmer EC, Glover JR, Singer MA, Lindquist S. 1996. HSP100/Clp proteins: a common mechanism explains diverse functions. Trends in biochemical sciences **21**, 289–296.

Schwarz S, Grande AV, Bujdoso N, Saedler H, Huijser P. 2008. The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in Arabidopsis. Plant molecular biology **67**, 183–195.

Sepulveda C, Guzmán MA, Li Q, *et al.* 2022. KARRIKIN UP-REGULATED F-BOX 1 (KUF1) imposes negative feedback regulation of karrikin and KAI2 ligand metabolism in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America **119**, e2112820119.

Shabek N, Ticchiarelli F, Mao H, Hinds TR, Leyser O, Zheng N. 2018. Structural plasticity of D3-D14 ubiquitin ligase in strigolactone signalling. Nature **563**, 652–656.

Shen H, Luong P, Huq E. 2007. The F-box protein MAX2 functions as a positive regulator of photomorphogenesis in Arabidopsis. Plant physiology **145**, 1471–1483.

Shin DH, Choi M, Kim K, Bang G, Cho M, Choi S-B, Choi G, Park Y-I. 2013. HY5 regulates anthocyanin biosynthesis by inducing the transcriptional activation of the MYB75/PAP1 transcription factor in Arabidopsis. FEBS letters **587**, 1543–1547.

Shinohara N, Taylor C, Leyser O. 2013. Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. PLoS biology **11**, e1001474.

Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ. 2005. The Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. The Plant cell **17**, 746–759.

Song X, Lu Z, Yu H, *et al.* 2017. IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice. Cell research **27**, 1128–1141.

Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson DC. 2015. SMAX1-LIKE/D53 Family Members Enable Distinct MAX2-Dependent Responses to Strigolactones and Karrikins in Arabidopsis. The Plant cell **27**, 3143–3159.

Stanga JP, Morffy N, Nelson DC. 2016. Functional redundancy in the control of seedling growth by the karrikin signaling pathway. Planta **243**, 1397–1406.

Stanga JP, Smith SM, Briggs WR, Nelson DC. 2013. SUPPRESSOR OF MORE AXILLARY GROWTH2 1 controls seed germination and seedling development in Arabidopsis. Plant physiology **163**, 318–330.

Struk S, Braem L, Matthys C, *et al.* 2022. Transcriptional Analysis in the Arabidopsis Roots Reveals New Regulators that Link rac-GR24 Treatment with Changes in Flavonol Accumulation,

Root Hair Elongation and Lateral Root Density. Plant & cell physiology **63**, 104–119.

Stirnberg P, Furner IJ, Ottoline Leyser HM. 2007. MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching: An SCFMAX2 acts at the node to suppress branching. The Plant journal: for cell and molecular biology **50**, 80–94.

Struk S, Braem L, Walton A, De Keyser A, Boyer F-D, Persiau G, De Jaeger G, Gevaert K, Goormachtig S. 2018. Quantitative Tandem Affinity Purification, an Effective Tool to Investigate Protein Complex Composition in Plant Hormone Signaling: Strigolactones in the Spotlight. Frontiers in plant science **9**, 528.

Struk S, De Cuyper C, Jacobs A, *et al.* 2021. Unraveling the MAX2 Protein Network in Arabidopsis thaliana: Identification of the Protein Phosphatase PAPP5 as a Novel MAX2 Interactor. Molecular & cellular proteomics: MCP **20**, 100040.

Sun Y, Fan X-Y, Cao D-M, *et al.* 2010. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Developmental cell **19**, 765–777.

Sun H, Guo X, Qi X, Feng F, Xie X, Zhang Y, Zhao Q. 2021*a*. SPL14/17 act downstream of strigolactone signalling to modulate rice root elongation in response to nitrate supply. The Plant journal: for cell and molecular biology **106**, 649–660.

Sun H, Guo X, Zhu X, *et al.* 2023. Strigolactone and gibberellin signaling coordinately regulate metabolic adaptations to changes in nitrogen availability in rice. Molecular plant **16**, 588–598.

Sun W, Ji X, Song L, Wang X, You C, Hao Y. 2021*b*. Functional identification of MdSMXL8.2, the homologous gene of strigolactones pathway repressor protein gene in Malus × domestica. Horticultural Plant Journal **7**, 275–285.

Sun X-D, Ni M. 2011. HYPOSENSITIVE TO LIGHT, an alpha/beta fold protein, acts downstream of ELONGATED HYPOCOTYL 5 to regulate seedling de-etiolation. Molecular plant **4**, 116–126.

Sun H, Tao J, Liu S, Huang S, Chen S, Xie X, Yoneyama K, Zhang Y, Xu G. 2014. Strigolactones are involved in phosphate- and nitrate-deficiency-induced root development and auxin transport in rice. Journal of experimental botany **65**, 6735–6746.

Sun Y, Tian Z, Zuo D, Cheng H, Wang Q, Zhang Y, Lv L, Song G. 2024. Strigolactone-induced degradation of SUPPRESSOR OF MORE AXILLARY GROWTH2-LIKE7 (SMXL7) and SMXL8 contributes to gibberellin- and auxin-mediated fiber cell elongation in cotton. The Plant cell doi: 10.1093/plcell/koae212.

Sun YK, Yao J, Scaffidi A, Melville KT, Davies SF, Bond CS, Smith SM, Flematti GR, Waters MT. 2020. Divergent receptor proteins confer responses to different karrikins in two ephemeral weeds. Nature communications **11**, 1264.

Temmerman A, De Keyser A, Boyer F-D, Struk S, Goormachtig S. 2023. Histone Deacetylases Regulate MORE AXILLARY BRANCHED 2-Dependent Germination of Arabidopsis thaliana. Plant & cell physiology **64**, 1008–1020.

Temmerman A, Guillory A, Bonhomme S, Goormachtig S, Struk S. 2022. Masks Start to

Drop: Suppressor of MAX2 1-Like Proteins Reveal Their Many Faces. Frontiers in plant science **13**, 887232.

Tian H, Watanabe Y, Nguyen KH, *et al.* 2022. KARRIKIN UPREGULATED F-BOX 1 negatively regulates drought tolerance in Arabidopsis. Plant physiology **190**, 2671–2687.

Truernit E, Haseloff J. 2007. A Role for KNAT Class II Genes in Root Development. Plant signaling & behavior **2**, 10–12.

Tsuchiya Y, Vidaurre D, Toh S, Hanada A, Nambara E, Kamiya Y, Yamaguchi S, McCourt P. 2010. A small-molecule screen identifies new functions for the plant hormone strigolactone. Nature chemical biology **6**, 741–749.

Ueda H, Kusaba M. 2015. Strigolactone Regulates Leaf Senescence in Concert with Ethylene in Arabidopsis. Plant physiology **169**, 138–147.

Van De Velde K, Ruelens P, Geuten K, Rohde A, Van Der Straeten D. 2017. Exploiting DELLA Signaling in Cereals. Trends in plant science **22**, 880–893.

Van Ha C, Leyva-González MA, Osakabe Y, *et al.* 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proceedings of the National Academy of Sciences of the United States of America **111**, 851–856.

Villaécija-Aguilar JA, Gutjahr C. 2020. The karrikin signaling regulator SMAX1 controls Lotus japonicus root and root hair development by suppressing ethylene biosynthesis. Proceedings of the *in press*.

Villaécija-Aguilar JA, Hamon-Josse M, Carbonnel S, Kretschmar A, Schmid C, Dawid C, Bennett T, Gutjahr C. 2019. SMAX1/SMXL2 regulate root and root hair development downstream of KAI2-mediated signalling in Arabidopsis.

Villaécija-Aguilar JA, Körösy C, Maisch L, Hamon-Josse M, Petrich A, Magosch S, Chapman P, Bennett T, Gutjahr C. 2022. KAI2 promotes Arabidopsis root hair elongation at low external phosphate by controlling local accumulation of AUX1 and PIN2. Current biology: CB **32**, 228–236.e3.

Walker CH, Siu-Ting K, Taylor A, O'Connell MJ, Bennett T. 2019. Strigolactone synthesis is ancestral in land plants, but canonical strigolactone signalling is a flowering plant innovation. BMC biology **17**, 70.

Wallner E-S, López-Salmerón V, Belevich I, *et al.* 2017. Strigolactone- and Karrikin-Independent SMXL Proteins Are Central Regulators of Phloem Formation. Current biology: CB **27**, 1241–1247.

Wallner E-S, Tonn N, Shi D, *et al.* 2023. OBERON3 and SUPPRESSOR OF MAX2 1-LIKE proteins form a regulatory module driving phloem development. Nature communications **14**, 2128.

Wallner E, Tonn N, Shi D, Jouannet V, Greb T. 2020. *SUPPRESSOR OF MAX2 1□LIKE 5* promotes secondary phloem formation during radial stem growth.

Wang S, Chang Y, Guo J, Chen J-G. 2007. Arabidopsis Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation. The Plant journal: for cell and

molecular biology **50**, 858–872.

Wang P, Nolan TM, Clark NM, Jiang H, Montes-Serey C, Guo H, Bassham DC, Walley JW, Yin Y. 2021. The F-box E3 ubiquitin ligase BAF1 mediates the degradation of the brassinosteroid-activated transcription factor BES1 through selective autophagy in Arabidopsis. The Plant cell **33**, 3532–3554.

Wang Y, Sun S, Zhu W, Jia K, Yang H, Wang X. 2013. Strigolactone/MAX2-induced degradation of brassinosteroid transcriptional effector BES1 regulates shoot branching. Developmental cell **27**, 681–688.

Wang L, Wang B, Jiang L, Liu X, Li X, Lu Z, Meng X, Wang Y, Smith SM, Li J. 2015. Strigolactone Signaling in Arabidopsis Regulates Shoot Development by Targeting D53-Like SMXL Repressor Proteins for Ubiquitination and Degradation. The Plant cell **27**, 3128–3142.

Wang L, Wang B, Yu H, *et al.* 2020*a*. Transcriptional regulation of strigolactone signalling in Arabidopsis. Nature **583**, 277–281.

Wang L, Xu Q, Yu H, *et al.* 2020*b*. Strigolactone and Karrikin Signaling Pathways Elicit Ubiquitination and Proteolysis of SMXL2 to Regulate Hypocotyl Elongation in Arabidopsis. The Plant cell **32**, 2251–2270.

Waters MT, Gutjahr C, Bennett T, Nelson DC. 2017. Strigolactone Signaling and Evolution. Annual review of plant biology **68**, 291–322.

Waters MT, Nelson DC. 2022. Karrikin perception and signalling. The New phytologist doi: 10.1111/nph.18598.

Waters MT, Nelson DC, Scaffidi A, Flematti GR, Sun YK, Dixon KW, Smith SM. 2012. Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. Development **139**, 1285–1295.

Waters MT, Scaffidi A, Moulin SLY, Sun YK, Flematti GR, Smith SM. 2015. A Selaginella moellendorffii Ortholog of KARRIKIN INSENSITIVE2 Functions in Arabidopsis Development but Cannot Mediate Responses to Karrikins or Strigolactones. The Plant cell **27**, 1925–1944.

Wei C-Q, Chien C-W, Ai L-F, Zhao J, Zhang Z, Li KH, Burlingame AL, Sun Y, Wang Z-Y. 2016. The Arabidopsis B-box protein BZS1/BBX20 interacts with HY5 and mediates strigolactone regulation of photomorphogenesis. Journal of genetics and genomics = Yi chuan xue bao **43**, 555–563.

White ARF, Mendez JA, Khosla A, Nelson DC. 2022. Rapid analysis of strigolactone receptor activity in a Nicotiana benthamiana dwarf14 mutant. Plant direct **6**, e389.

Wu Y-Y, Hou B-H, Lee W-C, Lu S-H, Yang C-J, Vaucheret H, Chen H-M. 2017. DCL2- and RDR6-dependent transitive silencing of SMXL4 and SMXL5 in Arabidopsis dcl4 mutants causes defective phloem transport and carbohydrate over-accumulation. The Plant journal: for cell and molecular biology **90**, 1064–1078.

Xie Y, Liu Y, Ma M, Zhou Q, Zhao Y, Zhao B, Wang B, Wei H, Wang H. 2020. Arabidopsis FHY3 and FAR1 integrate light and strigolactone signaling to regulate branching. Nature communications **11**, 1955.

Xie Y, Liu Y, Wang H, Ma X, Wang B, Wu G, Wang H. 2017. Phytochrome-interacting factors directly suppress MIR156 expression to enhance shade-avoidance syndrome in Arabidopsis. Nature communications **8**, 348.

Xu D. 2020. COP1 and BBXs-HY5-mediated light signal transduction in plants. The New phytologist **228**, 1748–1753.

Xu P, Hu J, Chen H, Cai W. 2023. SMAX1 interacts with DELLA protein to inhibit seed germination under weak light conditions via gibberellin biosynthesis in Arabidopsis. Cell reports **42**, 112740.

Yamada Y, Furusawa S, Nagasaka S, Shimomura K, Yamaguchi S, Umehara M. 2014. Strigolactone signaling regulates rice leaf senescence in response to a phosphate deficiency. Planta **240**, 399–408.

Yang T, Lian Y, Kang J, Bian Z, Xuan L, Gao Z, Wang X, Deng J, Wang C. 2020*a*. The SUPPRESSOR of MAX2 1 (SMAX1)-Like SMXL6, SMXL7 and SMXL8 Act as Negative Regulators in Response to Drought Stress in Arabidopsis. Plant & cell physiology **61**, 1477–1492.

Yang T, Sun Y, Wang Y, *et al.* 2020*b*. AtHSPR is involved in GA- and light intensity-mediated control of flowering time and seed set in Arabidopsis. Journal of experimental botany **71**, 3543–3559.

Yang T, Zhang L, Hao H, Zhang P, Zhu H, Cheng W, Wang Y, Wang X, Wang C. 2015. Nuclear-localized AtHSPR links abscisic acid-dependent salt tolerance and antioxidant defense in Arabidopsis. The Plant journal: for cell and molecular biology **84**, 1274–1294.

Yang T, Zhang P, Wang C. 2016. AtHSPR may function in salt-induced cell death and ER stress in Arabidopsis. Plant signaling & behavior **11**, e1197462.

Yao R, Ming Z, Yan L, *et al.* 2016. DWARF14 is a non-canonical hormone receptor for strigolactone. Nature **536**, 469–473.

Yao J, Scaffidi A, Meng Y, Melville KT, Komatsu A, Khosla A, Nelson DC, Kyozuka J, Flematti GR, Waters MT. 2021. Desmethyl butenolides are optimal ligands for karrikin receptor proteins. The New phytologist **230**, 1003–1016.

Yin Y, Wang ZY, Mora-Garcia S, Li J, Yoshida S, Asami T, Chory J. 2002. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell **109**, 181–191.

Yin L, Zander M, Huang S-SC, *et al.* 2023. Transcription Factor Dynamics in Cross-Regulation of Plant Hormone Signaling Pathways. bioRxiv : the preprint server for biology doi: 10.1101/2023.03.07.531630.

Yuan G, Lian Y, Wang J, Yong T, Gao H, Wu H, Yang T, Wang C. 2023. AtHSPR functions in gibberellin-mediated primary root growth by interacting with KNAT5 and OFP1 in Arabidopsis. Plant cell reports **42**, 1629–1649.

Yu X, Li L, Zola J, *et al.* 2011. A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in Arabidopsis thaliana. The Plant journal: for cell and

molecular biology **65**, 634–646.

Zhang L, Sun L, Zhang X, Zhang S, Xie D, Liang C, Huang W, Fan L, Fang Y, Chang Y. 2018. OFP1 Interaction with ATH1 Regulates Stem Growth, Flowering Time and Flower Basal Boundary Formation in Arabidopsis. Genes **9**.

Zhang L, Yang T, Li X, Hao H, Xu S, Cheng W, Sun Y, Wang C. 2014. Cloning and characterization of a novel Athspr promoter specifically active in vascular tissue. Plant physiology and biochemistry: PPB / Societe francaise de physiologie vegetale **78**, 88–96.

Zhao J, Peng P, Schmitz RJ, Decker AD, Tax FE, Li J. 2002. Two putative BIN2 substrates are nuclear components of brassinosteroid signaling. Plant physiology **130**, 1221–1229.

Zhao J, Wang T, Wang M, *et al.* 2014. DWARF3 participates in an SCF complex and associates with DWARF14 to suppress rice shoot branching. Plant & cell physiology **55**, 1096–1109.

Zheng J, Hong K, Zeng L, *et al.* 2020. Karrikin Signaling Acts Parallel to and Additively with Strigolactone Signaling to Regulate Rice Mesocotyl Elongation in Darkness. The Plant cell **32**, 2780–2805.

Zheng X, Liu F, Yang X, Li W, Chen S, Yue X, Jia Q, Sun X. 2023. The MAX2-KAI2 module promotes salicylic acid-mediated immune responses in Arabidopsis. Journal of integrative plant biology **65**, 1566–1584.

Zheng K, Wang X, Weighill DA, *et al.* 2016. Characterization of DWARF14 genes in Populus. Scientific reports **6**, 21593.

Zheng X, Yang X, Chen Z, Xie W, Yue X, Zhu H, Chen S, Sun X. 2021. Arabidopsis SMAX1 overaccumulation suppresses rosette shoot branching and promotes leaf and petiole elongation. Biochemical and biophysical research communications **553**, 44–50.

Zhou F, Lin Q, Zhu L, *et al.* 2013. D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signalling. Nature **504**, 406–410.

Tables

Table 1. Candidate transcriptional markers of KAR/KL and SL response

Simplified Combination a	Combination	AGI	Primary Gene Symbol	SMAX1 EAR motif dependance	Arabidopsis Tissues used in the assays and references
SL and KL	↓ *kai2* and ↓ *d14*	AT1G64380	*ERF61*	Independent	Seedling aerial part (Abdelrahman et al. 2023), rosette leaves (Li et al. 2017; Li et al. 2020)
		AT3G52310	*ABCG27*	Independent	
		AT3G59880	*Hypothetical protein*		
		AT3G60420	*Phosphoglycerate mutase family protein*		
		AT5G60280	*LECRK-I.8*		
SL	↑ *rac*-GR24 and ↓ *d14*	AT1G03445	*BSU1*		Whole seedling (Yin et al. 2023; Wang et al. 2020), rosette leaves (Li et al. 2020)
		AT1G03940	*HXXXD-type acyl-transferase family protein*		
		AT1G07550	*LRR kinase family protein*		
		AT1G13510	*Hypothetical protein*		
		AT1G24470	*KCR2*	Independent	
		AT1G68050	*ADO3/FKF1*		
		AT1G68250	*Hypothetical protein*		
		AT1G80555	*Isocitrate/isopropylmalate dehydrogenase family protein*		
		AT2G05510	*Glycine-rich protein family*		
		AT2G16190	*Hypothetical protein*		

		AT2G19970	*CAP52*		
		AT2G22750	*bHLH DNA-binding superfamily protein*		
		AT2G32860	*BGLU33*		
		AT2G40130	*SMXL8*	Independent	
		AT2G43010	*PIF4*	Independent	
		AT2G43860	*Pectin lyase-like superfamily protein*		
		AT2G44340	*VQ18*		
		AT2G47560	*ATL64*	Independent	
		AT3G11180	*JOX1*		
		AT3G18550	*BRC1/TCP18*	SMXL6 EAR dependant	
		AT3G46270	*Receptor like kinase protein*		
		AT3G46330	*MEE39*	Dependant	
		AT3G46400	*LRR kinase family protein*		
		AT3G53232	*RTFL1*	Independent	
		AT4G04990	*Serine/arginine repetitive matrix-like protein*		
		AT4G12550	*AIR1*		
		AT4G15393	*CYP702A5*	Dependant	
		AT4G19690	*IRT1*		
		AT4G28940	*Phosphorylase*		

			superfamily protein		
		AT4G31940	*CYP82C4*		
		AT5G06570	*CXE15*		
		AT5G07480	*KUOX1*		
		AT5G10040	*HUP9*		
		AT5G18600	*ROXY10*		
		AT5G41290	*Receptor-like protein kinase-related family protein*		
		AT5G45340	*CYP707A3*	Independent	
		AT5G49140	*Disease resistance protein*		
		AT5G52720	*Copper transport protein family*		
		AT5G56840	*myb-like transcription factor family protein*		
		AT5G64620	*ATC/VIC2*		
KL and SMAX1/SMXL2	↓ *kai2* and ↑ *smax1smxl2*	AT2G28570	*Hypothetical protein*		Seedling aerial part (Abdelrahman et al. 2023), rosette leaves (Li et al. 2017;Feng et al 2023)
		AT3G24420	*DLK2*	Dependant	
		AT3G52310	*ABCG27*	Independent	
SL and SMXL6/7/8	↑ *rac*-GR24 and ↑ *smxl6/7/8*	AT3G18550	*BRC1/TCP18*	SMXL6 EAR dependant	Whole seedling (Yin et al. 2023; Wang et al. 2020), rosette leaves (Yang et al 2020)
		AT4G21760	*BGLU47*		
		AT4G34410	*ERF109*	Independent	
		AT5G06570	*CXE15*		

		AT5G15960	*KIN1*		
		AT5G56840	*myb-like transcription factor family protein*		

Table 1. Candidate transcriptional markers of KAR/KL and SL response. RNA-seq and Microarray data on differentially expressed genes (DEGs) sourced from the table "showing RNA-seq/microarray pooling sources and plant conditions and tissues" were pooled from mutant and chemically dosed lines vs control lines of *Arabidopsis thaliana* comparison and filtered for genes with a 1.5 log2 fold change difference from control conditions, and having a corrected p-value of 0.05 or lower. This list was then put into a large array combining information from multiple sources describing up and down regulation under comparisons to control conditions. Combinations of up regulation or down regulation under particular mutant background or dose conditions are described in the combination column and genes that show up in these conditions are listed in the AGI column in the same row as the combinations listed. Additionally, information on if the gene is potentially EAR motif dependent based on a pSMAX1::SMAX1mEAR/*smax1 smxl2* background, in which the EAR motif of SMAX1 is mutated, is noted in the SMAX1-EAR dependence column based on data from (Chang *et al.*, 2024*a*). If genes stay differentially expressed in the mutant background, then it can be assumed that they might be transcriptionally regulated in a SMAX1-EAR motif dependent manner. If genes stay DEGs in *smax1 smxl2* background and are rescued to WT levels of expression by the pSMAX1::SMAX1mEAR/*smax1 smxl2* background, it can be assumed that these genes are transcriptionally independent of the SMAX1-EAR domain, otherwise genes are left blank if not regulated by SMAX1/SMXL2. In the table, ↓ and ↑ symbols indicate downregulation and upregulation of genes, respectively, and *rac*-GR24 treatment indicates 5 µM of *rac*-GR24 was treated for 2, 4, or 32 hours.

Table 2. List of reported SMXL-interacting proteins

SMXL	**Interactor(s)**	**Functions**	**Experimental evidence and reference(s)**
AtSMXL6	AtSMAX1		Y2H (Zheng *et al.*, 2021)
DELLA			
OsD53	OsSLR1	Alter TR stability	Y2H, BiFC and SLC (Sun *et al.*, 2023)
MdSMXL8.2	MdRGL2a	Alter TR regulation	Y2H, BiFC, pull-down, ubiquitination assay and Co-IP (An *et al.*, 2024)
AtSMAX1	AtRGL1/3, AtRGA, AtGAI	Alter TR regulation	Y2H (Kim *et al.*, 2022; Xu *et al.*, 2023; Chang *et al.*, 2024*a*), Co-IP, and Pull-down (Kim *et al.*, 2022; Xu *et al.*, 2023) BiFC (Xu *et al.*, 2023)
AtSMAX1	AtRGL2		Y2H, Co-IP and Pull-down (Kim *et al.*, 2022; Xu *et al.*, 2023) BiFC (Xu *et al.*, 2023)
AtSMXL2	AtRGL1/3, AtRGA, AtGAI		Y2H (Kim *et al.*, 2022)
AtSMXL7	AtRGL1/3		Y2H (Chang *et al.*, 2024*a*)
Shoot architecture and nitrogen responses			
OsD53	OsGRF4	Alter binding to DNA and TR regulation	Y2H, BiFC, pull-down and Co-IP (Sun *et al.*, 2023)
AtSMAX1, AtSMXL2	AtGRF7/9		IP-MS (Chang *et al.*, 2024*b*) *Y2H did not show the interactions (Chang *et al.*, 2024*a*)
AtSMXL6/7/8	AtSPL9/15		Y2H, SLC, pull-down and BiFC (Xie *et al.*, 2020)
OsD53	OsIPA1/SPL14, OsSPL17 (Xie *et al.*, 2020)	Alter TR regulation	Y2H, BiFC, and Co-IP (Song *et al.*, 2017; Sun *et al.*, 2021*a*)

			Pull-down (Song *et al.*, 2017)
TaD53	TaSPL3/17	Alter TR regulation	BiFC, SLC, and Y2H (Liu *et al.*, 2017)
OsD53	OsBZR1	Alter TR regulation	BiFC, Co-IP, and pull-down (Fang *et al.*, 2020)
	OsDLT		BiFC and pull-down (Fang *et al.*, 2020)
	OsRLA1		
AtSMXL6/7/8	AtBES1		BiFC and pull-down (Hu *et al.*, 2020)
ZmD53	ZmUB3		Y2H, pull-down, and BiFC (Liu *et al.*, 2021)
	ZmTSH44		
Light signaling			
AtSMAX1	AtphyB	Alter TR regulation	Y2H and Co-IP (Park *et al.*, 2022)
	AtPIF3		Y2H (Chang *et al.*, 2024*a*)
	AtPIF4/5	Alter TR regulation	IP-MS, pull-down, and Co-IP (Chang *et al.*, 2024*b*) *Y2H did not show the SMAX1-PIF4/5 interactions (Park *et al.*, 2022; Chang *et al.*, 2024*a*)
Root growth and phloem development			
AtSMXL4/HSPR	AtKNAT5/ATH1	Alter TR regulation	Y2H and pull-down (Yang *et al.*, 2020*b*) Y2H, BiFC, and genetic epistasis test (Yuan *et al.*, 2023)
	AtOFP1	Alter TR binding to DNA	
AtSMXL5	AtOBE3		Y2H screening, Y2H, Co-IP, nuclear subdomain co-localization, and FRET-FLIM (Wallner *et al.*, 2023)
	AtOBE2		Y2H (Wallner *et al.*, 2023)

TCP			
AtSMAX1	AtTCP5/7/8/9/10/13/14/16/17/18/19/21		Y2H (Chang *et al.*, 2024*a*)
AtSMXL7	AtTCP7/8/9/10/13/14/16/18/19		Y2H (Chang *et al.*, 2024*a*)
Defense responses and anthocyanin regulation			
NaSMXL6	NaJAZa/b/d/l	Alter TR regulation and stability	Y2H and Co-IP* (Li *et al.*, 2020*a*) (*Co-IP only performed for NaJAZb and NaSMXL6/7 interactions)
NaSMXL7	NaJAZa/b/d/e/j/l		
MdSMXL7	MdWRKY6		Y2H screening, Y2H, BiFC, pull-down and SLC (Fan *et al.*, 2023)
	MdbHLH93		Y2H (Fan *et al.*, 2023)
	MdRR23		
MdSMXL8	MdPRT1	Alter stability of MdSMXL8	Y2H, BiFC, pull-down, ubiquitination assay, and Co-IP (An *et al.*, 2024)
	MdAGL9	Alter TR regulation	Y2H, pull-down, BiFC, and Co-IP (An *et al.*, 2024)
Miscellaneous			
AtSMXL6/7/8	AtDWA1	Alter stability of AtSMXL6/7/8	Y2H, pull-down, and BiFC (Lian *et al.*, 2023)

SLC, Split Luciferase Complementation assay; Y2H, Yeast Two-hybrid; BiFC, Bimolecular fluorescence complementation; Co-IP, Co-Immunoprecipitation; FRET-FLIM, Förster's resonance energy transfer and Fluorescence lifetime microscopy; TR, Transcriptional regulator;

Boxes

BOX 1 - The difficulty of defining strigolactone- and karrikin-responsive genes

Caution must be exercised when labeling genes as "SL-responsive" or "KAR/KL-responsive," or as targets of a particular SMXL protein type, because many experiments have used chemical treatments or genetic backgrounds that are not sufficiently specific. Incorrect labeling of SL responses has been and continues to be a frequent problem for studies that use racemic GR24 (*rac*-GR24), which was initially developed as a synthetic analog of SLs (Johnson *et al.*, 1981). *Rac*-GR24 is commonly used because of its simpler synthesis, lower cost, and wider commercial availability compared to naturally occurring SLs. It was eventually recognized, however, that *rac*-GR24 is not a true, specific SL analog. Instead, it activates both KAR/KL and SL receptors (Scaffidi *et al.*, 2014). This is because *rac*-GR24 is a mixture of (+)-GR24 (also known as $GR24^{5DS}$), which mimics the structure and stereochemistry of the natural SL 5-deoxystrigol, and its enantiomer (–)-GR24 (also known as $GR24^{ent\text{-}5DS}$). The methyl butenolide "D-ring" of $GR24^{ent\text{-}5DS}$ has a 2'*S* configuration that has not been observed in any plant SLs, which all have 2'*R* configured D-rings. Unexpectedly, this compound activates KAI2 and, to a lesser extent, D14. In contrast, $GR24^{5DS}$ is an agonist of D14 specifically, at least in Arabidopsis (Scaffidi *et al.*, 2014). In some other species, such as *Nicotiana benthamiana* or root parasitic plants in the Orobanchaceae, however, even responses to $GR24^{5DS}$ and natural SLs are not exclusively mediated by D14 (Nelson, 2021; Li *et al.*, 2022*a*). KARs are also potentially problematic; while KARs so far appear to signal specifically through KAI2 and not D14, the putative metabolism of KARs into bioactive ligands by plants implies that the timing and intensity of KAR responses may differ from those of a direct KAI2 agonist like $GR24^{ent\text{-}5DS}$ (Waters and Nelson, 2022; Chang *et al.*, 2024*b*). In addition, selective responses to different KARs occur across species and can even vary within different organs of a single species (Nelson *et al.*, 2009; Carbonnel *et al.*, 2020*b*; Sun *et al.*, 2020; Martinez *et al.*, 2022; Waters and Nelson, 2022).

Genetic mutants, if carefully considered, can help to clarify KAR/KL and SL signaling specificity. Here it is important to remember that *max2* or *d3* mutants have defects in KAR/KL signaling as well as SL signaling (Nelson *et al.*, 2011; Soundappan *et al.*, 2015). Another point of consideration is that even D14-mediated transcriptional responses are not solely due to degradation of SMXL78 clade proteins. Because D14 can crosstalk to target aSMAX1 proteins

when exogenous SL is supplied, transcriptional responses to D14-specific agonists, even in a *kai2* mutant background, are likely to arise from a combination of aSMAX1 and SMXL78 degradation (Wang *et al.*, 2020*b*; Li *et al.*, 2022*a*).

Therefore, the use of purified SL or GR24 stereoisomers, SL-deficient mutants, and/or SL- or KAR/KL-insensitive mutants (e.g. *d14* and *kai2*, respectively) are best practices to accurately define transcriptional responses to SLs or KAR/KL, but might still be misleading. The development of more specific agonists of D14 and KAI2, such as $GR24^{4DO}$ and desmethyl-GR24, is an area of ongoing research (Wang *et al.*, 2020*a*; Yao *et al.*, 2021).

Figure Legends

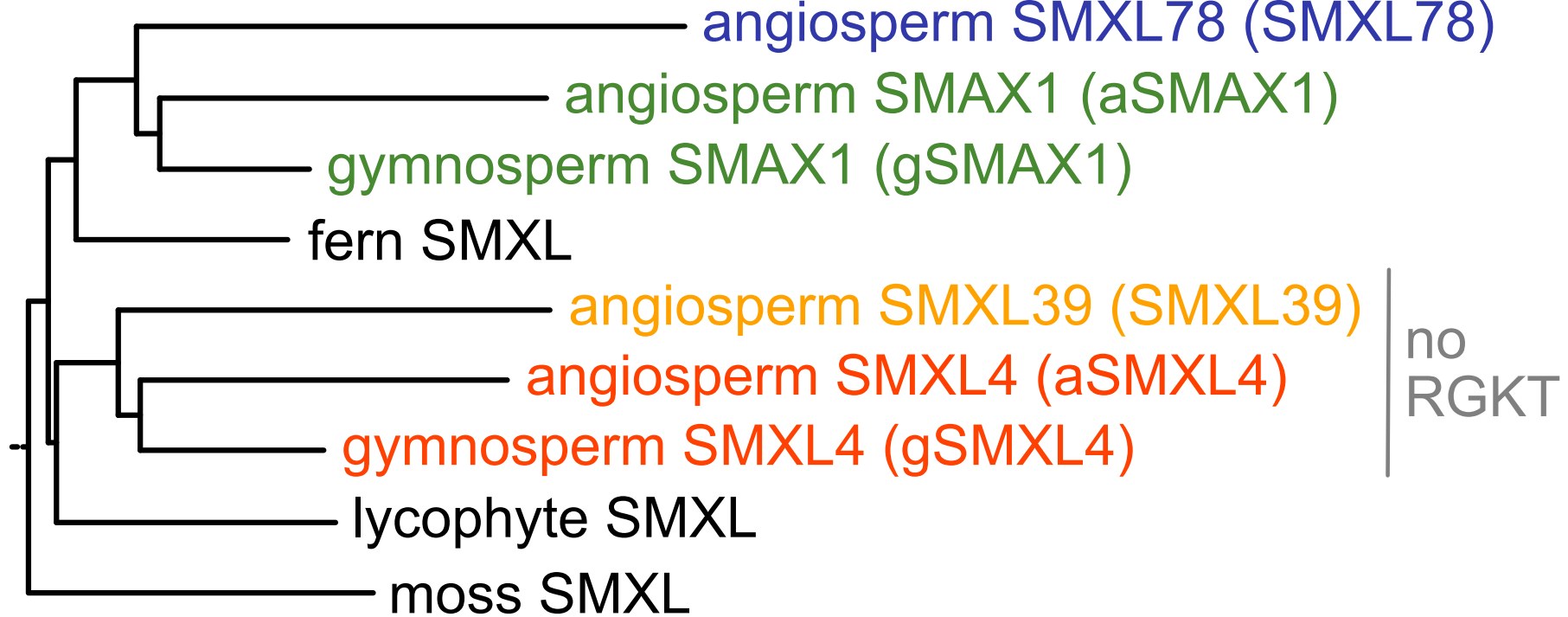
Figure 1. The four types of SMXL proteins in angiosperms.

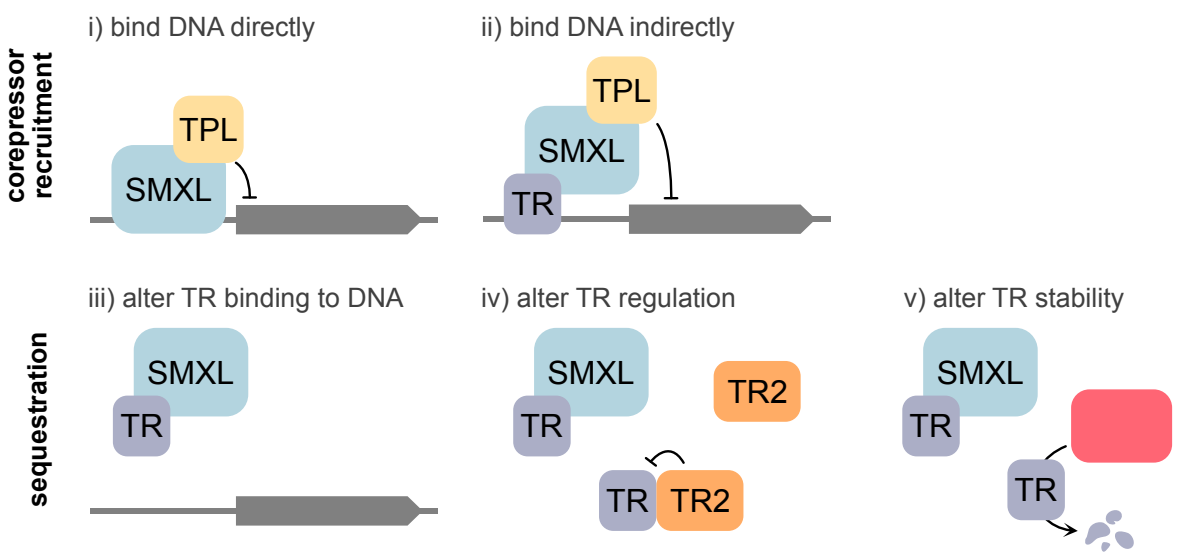
Simplified phylogeny of SMXL proteins in mosses, lycophytes, gymnosperms, and angiosperms, adapted from Walker *et al.*, 2019 (Walker *et al.*, 2019). Gymnosperms and angiosperms share SMAX1 and SMXL4 clades. SMXL39 and SMXL78 clades are specific to angiosperms. In Arabidopsis, SMAX1 and SMXL2 represent the aSMAX1 clade, SMXL3 represents the SMXL39 clade (SMXL9 was lost), SMXL4 and SMXL5 represent the aSMXL4 clade, and SMXL6, SMXL7, and SMXL8 represent the SMXL78 clade. In rice, SMAX1 represents the aSMAX1 clade and D53 represents the SMXL78 clade; other SMXL proteins in rice have not been characterized. SMXL39 and aSMXL4 clade proteins lack an RGKT motif that is critical for SCF^{MAX2}-mediated degradation in other SMXL proteins.

Figure 2. Mechanisms of SMXL protein function.

SMXL proteins use at least five mechanisms to regulate gene expression, which are not necessarily mutually exclusive. These mechanisms can be divided into those that recruit transcriptional corepressors and/or chromatin remodelers to DNA and those that involve SMXL protein-protein interactions with transcriptional regulators (e.g. sequestration). The models shown in iii), iv), and v), which respectively illustrate SMXL preventing a transcriptional regulator (TR) from binding its DNA targets, relieving repression of a TR through competitive-binding that disrupts another regulatory complex, and protecting a TR from degradation, are not the only possibilities for these protein interaction-based modes of action.

Figures


angiosperm SMXL78 (SMXL78)
angiosperm SMAX1 (aSMAX1)
gymnosperm SMAX1 (gSMAX1)
fern SMXL
angiosperm SMXL39 (SMXL39)
angiosperm SMXL4 (aSMXL4)
gymnosperm SMXL4 (gSMXL4)
lycophyte SMXL
moss SMXL
no RGKT


corepressor recruitment
sequestration
i) bind DNA directly
TPL
SMXL
ii) bind DNA indirectly
TPL
SMXL
TR
iii) alter TR binding to DNA
SMXL
TR
iv) alter TR regulation
SMXL
TR
TR2
TR
TR2
v) alter TR stability
SMXL
TR
TR