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Fellowship of the rings: a saga of strigolactones and other small signals

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Summary

Strigolactones are an important class of plant signalling molecule with both external rhizospheric and internal hormonal functions in flowering plants. The past decade has seen staggering progress in strigolactone biology, permitting highly detailed understanding of their signalling, synthesis and biological roles – or so it seems. However, phylogenetic analyses show that strigolactone signalling mediated by the D14-SCF^{MAX2}-SMXL7 complex is only one of a number of closely related signalling pathways, and is much less ubiquitous in land plants than might be expected. The existence of closely related pathways, such as the KAI2-SMAX1 module, challenges many of our assumptions about strigolactones, and in particular emphasizes how little we understand about the specificity of strigolactone signalling with respect to related signalling pathways. In this review, we examine recent advances in strigolactone signalling, taking a holistic evolutionary view to identify the ambiguities and uncertainties in our understanding. We highlight that while we now have highly detailed molecular models for the core mechanism of D14-SMXL7 signalling, we still do not understand the ligand specificity of D14, the specificity of its interaction with SMXL7, nor the specificity of SMXL7 function. Our analysis thus identifies key areas requiring further study.

Keywords: Strigolactones, KAI2-ligand, Phytohormone signalling, SMXL proteins, Evolution, D14/KAI2 family.

I. Introduction

For forty years following their first discovery, strigolactones were somewhat obscure signals, judged just to be germination stimulants for pernicious parasitic plants such as Striga species (Cook et al., 1966). However, since the twin revelations that strigolactones are both rhizospheric signals to mycorrhizal fungi (Akiyama et al., 2005; Akiyama et al., 2010) and hormonal signals within the plant body (Gomez-Roldan et al., 2008; Umehara et al., 2008), there has been huge interest in characterizing and comprehending these signals. Indeed, 'new roles for strigolactones' has become something of a scientific industry, in the same way that 'new roles for auxin' was 15 years ago. A constant stream of papers suggest novel and various functions for strigolactones in plants, but as with auxin, these functions are often based on rather hopeful interpretation of mutant phenotypes. Factor in the constant confusion caused by carefree use of max2 mutants and rac-GR24, and it sometimes seems that the more we find out about strigolactones, the less we can confidently say about them.

An evolutionary perspective can often provide clarity to complex scenarios, but a superficial view of strigolactone evolution only serves to blur our understanding of what strigolactones are. As will be discussed, they are likely only one of a broader class of related small signalling molecules in plants, and – at least in evolutionary terms – not an especially remarkable one. The strigolactone saga is really the story of how one of these signals has become pre-eminent in seed plants. If the key aim of this review it to try and reappraise what we really know about strigolactones and their signalling pathway, then the key challenge is to keep in focus that in most land plant groups, most of this simply does not apply.

II. Riddles in the dark: what are strigolactones?

Originally there was a simple molecular definition for strigolactones, based on the structure of those first identified; strigol, and the more common 5-deoxy strigol (5DS). These molecules have a characteristic structure, uniting a tri-cyclic lactone moiety (the 'ABC rings') with a butenolide moiety ('D ring') via an enol-ether bridge (Yoneyama et al., 2018) (Figure 1A). There are two key stereochemical centres in these molecules, one at the junction of the B and C rings, and the other at the junction of the D ring and the ether bridge. The first stereocentre is not critical in defining strigolactone activity, but does divide strigolactones into two sub-classes, the 5DS and 4-deoxyorobanchol (4DO) types (Figure 1B) (Yoneyama et al., 2018). Conversely, stereoisomers of 5DS and 4DO-type molecules with the 2′S configuration at the

D-ring (hereafter referred to as ‘retrolactones’) do not display strigolactone activity, and apparently do not occur naturally in plants (Scaffidi et al., 2014; Flematti et al., 2016) (Figure 1B).

The simplicity of this definition has been challenged by the growing number of molecules that display biological activity largely indistinguishable from ‘canonical’ strigolactones, and yet which do not fit the canonical structural definition (Yoneyama et al., 2018). These ‘non-canonical’ strigolactones all possess a D-ring in the 2′R configuration, linked to diverse moieties that approximate the shape and size of the ABC ring, but which do not have the tri-cyclic ABC structure (Yoneyama et al., 2018) (Figure 1C). The 2′R D-ring thus seems to be necessary for strigolactone activity, but cannot define strigolactones; there are a very large number of molecules that would not act as strigolactones if linked to a D-ring.

How then should we define strigolactones? It is likely that the biological activity displayed by these diverse molecules reflects a shared ability to bind to receptors and activate strigolactone signalling. Thus, we could circumscribe strigolactones on the basis of receptor-binding, or, in a more general way, based on their ability to trigger certain biological activities. Indeed, this is currently effectively where the field stands; non-canonical strigolactones are considered strigolactones because their bioactivity is the same as canonical strigolactones. However, this approach is problematic because strigolactones show activity in at least three separate biological systems – as hormonal signals in plants, and in parasitic plants and mycorrhizal fungi. Fundamental differences between strigolactone receptors in plants, parasites and fungi mean it is probable that not all strigolactones are bioactive in each system. Even within plants, there could be major differences in the way diverse strigolactones are perceived. This approach would thus yield different definitions depending on the exact receptor used to define strigolactones.

The best approach might therefore be based on their biosynthesis, which appears only to occur in plants themselves (reviewed in Jia et al., 2018). The core of the strigolactone synthesis pathway (Figure 1D) involves three ancient enzymes, DWARF27 (D27), CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7) and CCD8, which convert β-carotene to the universal precursor carlactone (Alder et al., 2012). As far as has been established, no naturally occurring strigolactones (whether canonical or non-canonical) can be synthesised without the activity of CCD7 and CCD8. D27 is important, but because of spontaneous isomerization of

β-carotene, is not absolutely required for strigolactone synthesis (Waters et al., 2012a). While there have previously been suggestions of non-canonical strigolactone synthesis pathways (Waldie et al., 2014), this idea was based on detection of residual strigolactones in ccd7 and ccd8 mutants that now appears to have been false-positive (Yoneyama et al., 2018). Thus, a definition based on CCD7 and CCD8 activity is likely to include all known strigolactones, irrespective of biochemistry and bioactivity in different systems. However, care is still needed, because in vitro CCD7 and CCD8 can act upon B-carotene to produce non-strigolactone products (Schwartz et al., 2004), and it cannot be ruled out that these products are also produced in planta. We thus propose that strigolactones be defined as products of CCD7 and CCD8 activity in plants that have a 2′R-configured D-ring (Figure 1C). This definition excludes other products of CCD7/CCD8 activity, non-naturally occurring strigolactone-like molecules ('strigolactone analogues', including the commonly used GR24), molecules that bind to strigolactone receptors but which do not otherwise meet these criteria ('strigolactone agonists/antagonists'), and of course, the retrolactones (Figure 1C)

III. The shadow of the past: the D14/KAI2 receptor family

The need to precisely define strigolactones becomes more apparent when considering how strigolactone perception has evolved. In flowering plants (angiosperms), α/β hydrolase DWARF14 (D14) proteins unambiguously act as strigolactone receptors (Hamiaux et al., 2012; Waters et al., 2012b), and because of the phenotypic similarity between ccd7/ccd8 mutants and d14, are widely considered to be the sole strigolactone receptor (Bennett et al., 2016). While this might well be the case in angiosperms, the evolutionary trajectory of D14 should make us cautious about this conclusion.

D14 is part of a larger family of α/β hydrolases in land plants, which also includes the KARRIKIN INSENSITIVE2 (KAI2) receptor (Waters et al., 2012b). Phylogenetic analysis demonstrates that the D14/KAI2 family in land plants consists of two ancient lineages – eu-KAI2 and DDK (D14/DLK2/KAI2), which evolved from a single KAI2-like lineage present in streptophyte algae (Bythell-Douglas et al., 2017). Eu-KAI2 Proteins have a highly conserved structure throughout land plants, which is also found in DDK lineage proteins in liverworts (Bythell-Douglas et al., 2017) (Figure 2). The ligand for the eu-KAI2 and KAI2-like receptors remains enigmatic; in angiosperms eu-KAI2 act in the perception of smoke compounds called karrikins, which promote seed germination whether species are inherently fire-following or not

(Nelson et al., 2009) (Figure 1D). However, non-seed plant eu-KAI2 proteins probably cannot act in the perception of karrikins, nor would it make sense for the eu-KAI2 pathway to have evolved to detect smoke compounds (Waters et al., 2015a; Burger et al., 2019). Instead, it is widely assumed that the primary role of eu-KAI2 is to detect a currently-unknown endogenous 'KAI2-ligand' (KL), and that karrikin perception has 'piggy-backed' onto this system once it became advantageous to detect smoke compounds (Flematti et al., 2013; Conn & Nelson, 2016; Sun et al., 2016). KAI2 receptors do not act as strigolactone receptors in angiosperms, although surprisingly one substrate they can bind with high affinity are retrolactones such as (-)-5DS and (-)-GR24 (Figure 1A). However, in parasitic plants of the Orobanchaceae, members of the eu-KAI2 lineage have 're-evolved' into specific strigolactone receptors, allowing them to detect rhizopheric strigolactone to promote their germination (Conn et al., 2015).

Strigolactones themselves are ancient signals, which have been reported across the whole land-plant (embryophyte) clade, along with their synthesis enzymes (Delaux et al., 2012). However, in non-seed plants, there are no obvious strigolactone receptors and proteins of the DDK lineage do not obviously resemble D14-type receptors (Figure 2) (Bythell-Douglas et al., 2017). Indeed, there is no clear evidence that liverworts, hornworts, lycophytes or monilophytes use strigolactones as internal hormonal signals. This suggests that strigolactones may have originally evolved as rhizospheric signals to mycorrhizal fungi, rather than as hormonal signals (Bouwmeester et al., 2007; Walker et al., 2019). Mosses certainly display sensitivity to GR24, and mutants lacking CCD7/CCD8 have developmental phenotypes, but mosses appear to use strigolactones primarily as external, rhizospheric signals rather than hormones (Proust et al., 2011; Decker et al., 2017). Mosses do not form mycorrhizal associations, and may have 're-purposed' rhizospheric strigolactones for colony density-sensing instead (Proust et al., 2011). Although it is possible there is non-D14 mediated strigolactone perception throughout non-seed plants, a strong possibility is that mosses have evolved this strigolactone perception independently.

One possibility is that proteins of the DDK lineage in mosses have evolved to perceive strigolactones (Lopez-Obando et al., 2016), but the current evidence suggests these proteins do not bind strigolactones and that strigolactone signalling in mosses does not utilize the standard MAX2-signalling complex (see below) (Lopez-Obando et al., 2018; Bythell-Douglas et al., 2017; Burger et al., 2019). Strigolactone perception in mosses thus remains enigmatic and might be completely non-canonical (Bennett & Leyser, 2014). The DDK lineage in non-seed

plants remains equally mysterious – outside liverworts, the proteins do not structurally resemble either KAI2 or D14-type receptors (Lopez-Obando et al., 2016; Burger et al., 2019), but they are well-conserved with the major land-plant clades, and retain all the important structural features of this class of receptors (Bythell-Douglas et al., 2017). Are they then receptors for another class of molecule?

At the base of the seed plant clade a duplication occurred in the DDK lineage, giving rise to two lineages. The first lineage contains the canonical D14-type strigolactones; in gymnosperms, a second duplication within the lineage gave rise to the D14-LIKE4 (DLK4) clade of proteins (Bythell-Douglas et al., 2017) (Figure 2). The second lineage contains D14-LIKE2/3 (DLK23) proteins, which are present in gymnosperms and angiosperms; in the eudicots, a further duplication gave rise to separate DLK2 and DLK3 lineages (Bythell-Douglas et al., 2017) (Figure 2). The DLK23 lineage represents a tantalizing unresolved enigma. Could these proteins also be strigolactone receptors? Or are they receptors for novel, unidentified signals? And if so, which of D14 and DLK23 represents the original pre-duplication function of the DDK lineage in seed plants? Does the DLK23 lineage conserve the function of DDK proteins from non-seed plants? Currently, functional studies have only been performed on DLK2 in Arabidopsis and are highly ambiguous. dlk2 mutants are essentially aphenotypic, possibly indicating that DLK2 acts in processes that are not easily recognized in Arabidopsis development, such as secondary growth (Waters et al., 2012b; Bennett et al., 2016; Vegh et al., 2017). Arabidopsis DLK2 protein does not show strong binding of either 2′R or 2′S stereoisomers of GR24 (Vegh et al., 2017), but this does not necessarily speak for the whole clade of proteins. Currently, evidence suggests that DLK2 and DLK3 are receptors for a novel class of signal, but it cannot be ruled out that they function as strigolactone receptors.

Taken together, the available evidence suggests that strigolactones are only one of a number of structurally-similar signalling molecules that are perceived in plants by members of the same protein family, and indeed, that strigolactones are relative latecomers to the scene. A critical goal for the field is therefore to gain better understanding of ligand-receptor specificity with the D14/KAI2 family.

IV. On the doorstep: ligand-receptor binding

Our understanding of ligand binding-specificity and receptor function in the D14/KAI2 family has benefitted enormously from structural biology approaches. D14/KAI2 proteins are easily-crystallized, and protein homology modelling has been used to predict the structure of an extensive range of other D14/KAI2 proteins based on known crystal structures (Conn et al., 2015; Lopez-Obando et al., 2016; Xu et al., 2016; Bythell-Douglas et al., 2017; Hameed et al., 2018). However, care is needed in interpreting these data: although they are extensive, they are not necessarily deep. For instance, although there are 26 crystal structures for D14 in the Protein Data Bank, these only cover six proteins from four species, only one of which (rice D14) has been used in complex with a ligand, of which only one structure used an intact strigolactone analogue. Similarly, while there are nine structures of the eu-KAI2 protein from Arabidopsis in the Protein Data Bank, there are just two structures that show the protein in complex with the karrikin KAR_1 (Guo et al., 2013; Xu et al., 2016), and no structures in complex with any stereoisomers of GR24. Thus, although precise ligand binding arrangements have been proposed for both D14 and KAI2, this is based on surprisingly little concrete evidence. Nevertheless, identified structural differences between the D14/KAI2 family members make sense in terms of the substrate preferences that have been attributed to them using techniques such as differential-scanning fluorimetry (Hamiaux et al., 2012; Waters et al., 2015b; Yao et al., 2018; Burger et al., 2019).

Based on current data, D14 and eu-KAI2 proteins themselves have a very similar overall structure, comprising a compact α/β-fold hydrolase structure of a β-sheet core flanked by α-helices (Kagiyama et al., 2013; Zhao et al., 2013; Zhao et al., 2015; Carlsson et al., 2018). The proteins contain a deep binding pocket, containing a conserved catalytic triad of serine/ histidine/aspartate, with a V-shaped cap covering the pocket. There are subtle differences between the unbound forms of D14 and KAI2 that are presumed to determine ligand specificities (Kagiyama et al., 2013; Zhao et al., 2013). In both Arabidopsis and rice, D14 has a larger binding pocket than KAI2 (Kagiyama et al., 2013; Zhao et al., 2013; Xu et al., 2016), which is also seen in Striga D14-like ShHTL proteins that perceive SL, which have consistently larger pockets than KAI2-like ShHTL proteins that perceive KAR (Xu et al., 2016; Xu et al., 2018). These differences are due to the replacement of some of the pocket-forming residues with bulkier ones, particularly within the cap, and the closer position of the cap domain relative to the protein core (Kagiyama et al., 2013; Zhao et al., 2013; Zhao et al., 2015; Xu et al., 2016;

Hameed et al., 2018; Lee et al., 2018; Xu et al., 2018; Burger et al., 2019). From the available D14-GR24 complex data, strigolactones seem to bind with the D-ring facing toward, and interacting with, the catalytic triad (Zhao et al., 2015).

These differences in pocket size between D14 and KAI2 proteins are often seen as the major determinant of ligand specificity (Kagiyama et al., 2013), but this is an over-simplification, since KAI2 can bind retrolactone molecules that are the same size as strigolactones. Rather, a combination of both pocket shape and size determines ligand-specificity; for instance, in KAI2, a leucine residue causes steric clashes with (+)-GR24 (see Figure 1), but a corresponding valine residue in D14 avoids these clashes. The orientation of the ABC ring in (+)-5DS prevents full insertion into the binding pocket of P. patens eu-KAI2 proteins due to a constriction in the middle of the pocket, but (-)-5DS (see Figure 1) complements the shape of the pocket and is fully encompassed, resulting in absolute stereoselective preference for this stereoisomer (Xu et al., 2018). The S. hermonthica KAI2-like protein HTL3 has a very low volume binding pocket (only 67% of a typical eu-KAI2 protein) and is absolutely selective for KAR_1 (Xu et al., 2016). Similarly, several of the DDK proteins from Physcomitrella patens that have notably shallower pockets and can only apparently bind KAR_1, while eu-KAI2 proteins from the same species bind retrolactones but not KAR_1 (Burger et al., 2019). In general, structural modelling of eu-KAI2 proteins from across land plants, and DDK proteins from liverworts, show no obvious differences in pocket size and shape compared to Arabidopsis KAI2 (Bythell-Douglas et al., 2017), and where tested, these proteins have a similar substrate specificity (Waters et al., 2015b). In contrast, structural modelling of moss, lycophyte and monilophyte DDK proteins suggests divergent pocket shapes and sizes, though none resemble D14 (Bythell-Douglas et al., 2017). At least one of these proteins, from Selaginella moellendorffii, can stereoselectively bind (+)-GR24 (Waters et al., 2015b), suggesting that a D14-like pocket is not needed for strigolactone binding per se.

Unfortunately it is not possible to identify ligands based on pocket shape and size, but some general conclusions can still be drawn. Firstly, the eu-KAI2 pocket is larger than needed to bind karrikins, and some eu-KAI2 proteins do not bind karrikins at all; furthermore, when required, smaller and more specific KAR-sized pockets can and have evolved (Burger et al., 2019). Thus, KL is likely a substantially larger molecule than KAR. Secondly, the existence of specifically KAR-sized binding pocket in moss DDK proteins suggests that a separate, KAR-sized molecule (presumably not KAR itself) also functions as a signal in mosses. Thirdly, the

D14 pocket is far larger than necessary to bind strigolactones, given that eu-KAI2 proteins can bind retrolactones. One possible reason for this is that the larger pocket size allows for a broader range of non-canonical strigolactones to be detected (although these are not very different in size from canonical strigolactones); but this seems to drastically diminish the selectivity of the protein, and Arabidopsis D14 binds both (+)-GR24 and (-)-GR24 (Waters et al., 2015b). If a more selective pocket was needed, it would have evolved, so the size and shape of the D14 pocket is presumably adaptive – but for what purpose?

V. Inside information: receptor-mediated hydrolysis and function

An undisputed feature of D14/KAI2 proteins is their hydrolytic activity. All members of the family possess a catalytic triad (Bythell-Douglas et al., 2017), and where tested, display hydrolytic activity against suitable substrates (Waters et al., 2015a; Waters et al., 2015b; Hameed et al., 2018; Lee et al., 2018; Yao et al., 2018; Burger et al., 2019). D14 has been shown to have potent hydrolase activity against strigolactones, which is dependent on the catalytic triad (Nakamura et al., 2013; Yao et al., 2018; Seto et al., 2019). Hydrolysis was initially proposed to proceed via nucleophilic attack on the enol-ether bridge (Mangnus & Zwanenburg, 1992), but the weight of evidence – including structural data (Kagiyama et al., 2013; Zhao et al., 2013; Yao et al., 2016) and hydrolysis of SL analogues lacking an enol-ether bridge (Takeuchi et al., 2018; Yao et al., 2018) – suggests that hydrolysis proceeds through nucleophilic attack of the D-ring carbonyl instead (Scaffidi et al., 2012). KAI2 has been shown to hydrolyse retrolactones, and the hydrolysis-deficient mutant S95A cannot bind (-)-GR24 or KAR in vitro (Lee et al., 2018) or respond to them in planta (Waters et al., 2015a). However, KAR ligands are not hydrolysed by KAI2 but are still bioactive (Guo et al., 2013), though it may be the case that KAR perception differs substantially from that of endogenous KL or (-)-GR24.

There is little question that the catalytic activity of D14 is important, but debate continues as to why exactly. It is accepted that hydrolysis is not required to produce a bioactive molecule, given the unusually slow strigolactone hydrolysis rate of D14, and that none of the hydrolysis products have in planta strigolactone-like effects (Hamiaux et al., 2012; Nakamura et al., 2013; Zhao et al., 2013). Two seminal papers proposed a model for strigolactone signalling in which D14 hydrolyses strigolactones and becomes covalently attached to the D-ring in the process (creating a covalently-linked intermediate molecule, CLIM); this triggers conformational

change of D14, allowing onward assembly of the signalling complex (de Saint Germain et al., 2016; Yao et al., 2016). A significant amount of evidence supports this model. A crystal structure of D14 in an active signalling complex (see below) showed a small molecule, identified as hydroxymethyl butenolide derived from the SL D-ring, covalently linked to the catalytic histidine (Yao et al., 2016). D14 shows a much higher rate of hydrolysis, which remains unsaturated even at high concentrations, of the poorly bioactive strigolactone analogue dYLG than with the strongly bioactive YLG, whose turnover rate is quickly saturated, presumably as result of inactivation of D14 molecules by covalent modification in the latter case (Yao et al., 2018). A strigolactone antagonist, 7′-carba-4-bromo debranone (Takeuchi et al., 2018), and a non-bioactive strigolactone analogue, GC486 (de Saint Germain et al., 2016), are rapidly hydrolysed by D14 and RMS3 (the pea orthologue of D14), respectively, but did not form a CLIM and so failed to induce the conformational changes required for signalling complex assembly. The formation of irreversibly covalently modified receptor would in turn suggest that D14 functions as a single-turnover enzyme. Consistent with this idea, the hydrolysis kinetics of RMS3 showed an initial burst followed by a plateau, the height of which was solely dependent on enzyme concentration (de Saint Germain et al., 2016). Addition of further enzyme led to a second rapid rate increase followed by another plateau, appearing to confirm the enzyme is limiting and deactivated (de Saint Germain et al., 2016).

However, there is also more recent evidence that hydrolysis and CLIM formation are not required for signal transduction, and that D14 does not function as a single-turnover enzyme. A re-evaluation of the D14 signalling complex structure in a later study (Carlsson et al., 2018) concluded the electron density in the active site was inconsistent with the CLIM model and the small molecule could not be confidently assigned, although the relatively low resolution of the structure (3.3 Å) could have accounted for this (Carlsson et al., 2018). This, combined with the observation that the previously determined slow rate of hydrolysis was inconsistent with the relatively rapid degradation of target SMXL proteins (given the proposed 1:1 stoichiometry between strigolactone cleavage and target degradation), led to a recent study which has suggested that induction of the D14 active signalling state is triggered by the binding of intact strigolactone, rather than hydrolysis or formation of a CLIM (Seto et al., 2019). Coupled time-course analyses of strigolactone binding and hydrolysis suggested that intact strigolactone molecules induce D14 signal transduction, providing evidence that D14 is activated prior to hydrolysis. These analyses also disputed the previous conclusions of D14 as a single-turnover enzyme, as all GR24 was consumed even when the hydrolysis reaction was performed with a

large excess of GR24 (Seto et al., 2019). Further, the observation that the two hydrolysis products were released at comparative rates suggests the CLIM is short-lived and so unlikely to promote recruitment of other signalling proteins within its lifetime (Seto et al., 2019). In addition, a novel D14 catalytic mutant (D218A) capable of binding strigolactones, but not their hydrolysis, was able to rescue the d14 phenotype in a strigolactone-dependent manner (Seto et al., 2019), suggesting strigolactone binding rather than hydrolysis is critical for signalling.

If these data are confirmed, then the role of hydrolysis requires further characterization. An alternative might be signal attenuation through deactivation of bioactive strigolactone following signal transmission (Seto et al., 2019). This hypothesis is supported by in planta observations of D14 mutants; R183H, capable of strigolactone hydrolysis but incapable of interacting with SMXL7 or rescuing a d14 phenotype, promoted a strigolactone-deficient phenotype when overexpressed in wild-type, while D218A, incapable of hydrolysis, resulted in increased sensitivity to GR24 when expressed in a ccd8 d14 double mutant, presumably due to decreased deactivation (Seto et al., 2019). This suggests a complete decoupling of the signalling and hydrolysis activities of D14; ligand binding for signal transduction, hydrolysis for deactivation of the bioactive hormone. However, it should be noted that whether signal transduction relies on intact strigolactone binding or CLIM formation, both hypotheses are consistent with the idea of strigolactone deactivation as a function of α/β-hydrolases.

VI. Three is company: the signalling complex

Long before D14 was identified as a strigolactone receptor, it was clear that – like auxin, gibberellin and jasmonate – strigolactone signalling must involve regulated proteolytic degradation, because the first identified signalling gene (MAX2) was an F-Box protein, and a component of an SCF-type E3 ubiquitin ligase (Stirnberg et al., 2002; Stirnberg et al., 2007). The subsequent history of strigolactone signalling can essentially be characterized as one quest to understand strigolactone perception and how this linked to SCF^{MAX2}, and a separate quest for the proteolytic target(s) of SCF^{MAX2}.

Ligand binding induces notable thermal instability in both the D14 and KAI2 protein families, determined by multiple techniques (Hamiaux et al., 2012; Nakamura et al., 2013; Abe et al., 2014; Waters et al., 2015b; Zhao et al., 2015; Yao et al., 2018), which has been linked to conformational change and structural rearrangement. However, only minor conformational

changes have been detected in the crystal structures of D14 (Nakamura et al., 2013; Zhao et al., 2013; Zhao et al., 2015) and KAI2 (Guo et al., 2013; Xu et al., 2016) in complex with ligands, compared to the apo structures. The biochemical and crystallographic data are inconsistent in this instance, but significant conformational changes of D14 after ligand binding have been observed in a complex with D3 (the rice orthologue of MAX2) and ASK1 (one of the other SCF components) (Yao et al., 2016). This suggests that assembly of the signalling complex may help stabilize structural changes in D14. In this structure, D14 is bound to the D3 leucine-rich repeat (LRR) domain, with the entrance to the binding pocket facing D3, while ASK1 is bound to the F-box domain of D3 (Figure 3). The D14 and D3 interaction occurs through a region of D14 only exposed due to a conformational shift of the cap domain to a 'closed' state, strongly suggesting the SL-induced conformational change in D14 precedes D3 binding. In addition to the subsequent recruitment and degradation of target proteins, the interactions with SCF^{MAX2} have also been implicated in D14 degradation. In rice, rac-GR24 treatment causes rapid (~4hr) D14 destabilisation in planta; D14 was polyubiquitinated and degraded following GR24 treatment, but this was impaired in d3 mutants (Hu et al., 2017). The same was observed in Arabidopsis, with rapid (~4hr) MAX2- and proteasome-dependent degradation of D14 induced by rac-GR24 treatment (Chevalier et al., 2014; Wang et al., 2015).

In terms of proteolytic targets of the D14-SCF^{MAX2} signalling complex, various proteins have been proposed. BRI1-EMS-SUPPRESSOR 1 (BES1), a component of brassinosteroid signalling that acts as a positive downstream regulator of BR-responses, and SLR1, a DELLA protein that is the target of gibberellin signalling, were proposed to be targets in Arabidopsis and rice respectively (Nakamura et al., 2013; Wang et al., 2013), but there is no convincing genetic or physiological evidence for either protein (de Saint Germain et al., 2013; Bennett et al., 2016). Rather, all available evidence suggests that proteins of the SMAX1-LIKE (SMXL) family are the principal, and indeed only, targets of D14-SCF^{MAX2}. Firstly, members of the SMXL7 clade of proteins show rapid degradation (~10mins-1hr) in response to strigolactone treatment, in a D14-, MAX2- and proteasome-dependent manner and secondly, mutation of a specific 'degron' motif in SMXL7 (Arabidopsis) or DWARF53 (D53; rice) prevents their strigolactone-responsive degradation, and phenocopies d14 mutants in planta (Zhou et al., 2013; Soundappan et al., 2015; Wang et al., 2015; Liang et al., 2016). Thirdly, loss-of-function in the Arabidopsis redundant co-orthologues SMXL6, SMXL7 and SMXL8, completely suppresses all known strigolactone-related phenotypes in d14 and max2 (Soundappan et al., 2015; Wang et al., 2015).

SMXL7/D53 proteins are exclusively localised to the nucleus, and co-localise in the nucleus with both D14 and MAX2/D3 (although neither of these proteins is exclusively nuclear). Physical and in planta interactions have been reported between SMXL7/D53 and both D14 and MAX2/D3, both in vitro and in planta (Zhao et al., 2014; Wang et al., 2015; Liang et al., 2016). In vitro, D53 has been reported to interact with D3 in a strigolactone-independent manner (Jiang et al., 2013; Wang et al., 2015), but no direct interaction was observed between SMXL7 and MAX2 in planta, even in the presence of strigolactone (Liang et al., 2016). Either way, polyubiquitination and degradation of D53 by the SCF^{D3} complex requires activated D14 (Jiang et al., 2013; Zhou et al., 2013; Chevalier et al., 2014; Wang et al., 2015). SMXL7/D53 and strigolactone-activated D14 have also been shown to interact, independent of MAX2/D3 (Jiang et al., 2013; Zhou et al., 2013; Zhao et al., 2014). Consistent with this, a signalling-deficient D14 mutant with disrupted MAX2 binding is still capable of binding SMXL6 (Yao et al., 2016), suggesting the MAX2-induced conformational change is not required for D14-SMXL interaction.

A recent study has provided new insights into the formation of the rice D14-D53-SCF^{D3} complex (Shabek et al., 2018). The crystal structure of the D3-ASK1 complex revealed the C-terminal α-helix (CTH) of D3 is dynamic and exists in two forms, dislodged from, or engaged with the D3-ASK1 complex (Shabek et al., 2018). This D3-CTH peptide was shown to directly interact with D14 in vitro and in planta in a GR24-dependent manner (Shabek et al., 2018). D3-CTH uses common residues to interact with both D3-ASK1 and D14, suggesting it binds to D14 when dislodged from D3-ASK1 (Shabek et al., 2018). Further, the c-terminal part of D53, which was suggested to be sufficient for D14 binding, was found to do so in a co-operative manner dependent on D3-CTH. Ligand hydrolysis by D14 was found to be inhibited by the D3-CTH peptide, but was re-activated by addition of D53 (Shabek et al., 2018). These observations have led to a new model for complex formation (Figure 3), in which the dislodged CTH-state of D3 allows binding of open-conformation, strigolactone-bound D14. This complex, which is competent for D53 binding, prevents strigolactone hydrolysis until D53 recruitment occurs. After D53 recruitment, D14-mediated SL hydrolysis occurs, stabilising the closed-conformation D14 and switching D3-CTH to its engaged state. This in turn results in the polyubiquitination and degradation of D14, feeding unbound D3 back in to the system (Shabek et al., 2018). However, it remains unclear what determines the engaged or dislodged state of D3, when D53 polyubiquitination occurs relative to SL hydrolysis, and exactly what

role is played by the CLIM; whether it is merely an intermediate of SL processing as has been separately suggested (Seto et al., 2019), or whether CLIM-bound D14 is an active component in the signalling complex.

KAI2 is widely assumed to act through SCF^{MAX2} to trigger degradation of SMXL proteins in the SMAX1 clade. MAX2 is also required for karrikin perception in Arabidopsis, and max2 mutants have possess all the phenotypes seen in kai2 mutants (Stanga et al., 2013; Soundappan et al., 2015). Furthermore, the kai2-related phenotypes in max2 are completely suppressed by loss-of-function mutations in the redundant SMAX1 and SMXL2 proteins in Arabidopsis. However, there has been no direct evidence of formation of a KAI2-SMAX1-SCF^{MAX2} complex so far. Limited biochemical evidence supports interaction between KAI2 and MAX2; yeast two-hybrid suggests KAI2 interacts with MAX2 in a GR24-dependent manner, although KAI2 has shown autoactivation within the yeast two-hybrid system, so these results are not conclusive (Toh et al., 2014; Yao et al., 2018). SMAX1 is degraded in response to rac-GR24 treatment in Arabidopsis (Wallner et al., 2017), also broadly supporting this model. KAI2 also undergoes degradation during signalling, but unlike D14, this is not due to polyubiquitination and proteasomal degradation, and seems completely independent of MAX2 (Waters et al., 2015a). Ligand binding-deficient and hydrolysis-deficient KAI2 signalling mutants are not degraded on ligand treatment, and as such, KAI2 degradation has been suggested to occur following ligand hydrolysis and to be triggered by these events, rather than by the activation of SCF^{MAX2} (Yao et al., 2018).

VII. Many meetings: a suite of receptor-SMXL complexes

Given the clear evidence that strigolactone binding to D14 triggers MAX2-mediated degradation of SMXL7/D53 proteins, and the circumstantial evidence that KL binding to KAI2 triggers MAX2-mediated degradation of SMAX1 proteins, it is intuitive to assume that other members of the D14/KAI2 family also target SMXL proteins for degradation. Analysis of the SMXL protein family supports this idea in most cases, but also suggests there are some intriguing exceptions to this rule. As a group, SMXL proteins can be found in all land plant taxa, but have not thus far been identified in charophyte algae (Moturu et al., 2018 Walker et al., 2019) (Figure 4). In each non-seed plant group, there is usually a single clade of SMXL proteins, while in gymnosperms, there are two clades, equivalent to SMAX1 and SMXL4, and in all angiosperms there are 4 major clades, increasing to 6 in the eudicots (SMAX1, SMXL7, SMXL8, SMXL4, SMXL3 and SMXL9; Figure 5A) (Moturu et al., 2018; Walker et al., 2019).

SMXL proteins have a large, multi-domain protein structure which is generally highly conserved across land plants; the degree of conservation varies between the different domains (A-I), few of which (Figure 5a,b). Only a few of these domains have characterized functions. Domain A is an exceptionally well-conserved 'double Clp' domain, (Stanga et al., 2013), which contains a nuclear localization signal (Liang et al., 2016). Domain C and F have strong homology to p-loop NTPases and contain one and two Walker motifs respectively. Various studies have treated domains F-I as a single large NTPase domain (e.g. Zhou et al., 2013; Shabek et al., 2018), but sequence conservation suggests this is unlikely (Figure 5b). The function of these two NTPase domains is currently not clear, but domain F contains the previously defined FRGKT motif required for SL-induced degradation of SMXL7/D53 proteins (Jiang et al., 2013; Zhou et al., 2013; Soundappan et al., 2015). This motif is not required for recruitment by the SL receptor complex, but rather for degradation of the complex through the 26S proteasome (Jiang et al., 2013), and is present in all SMXL proteins with the exception of the flowering plant SMXL4, SMXL3 and SMXL9 clades (Figure 5a,b). Domain H contains a short ERF-associated amphiphilic repression (EAR) motif present in all SMXL proteins (Jiang et al., 2013; Zhou et al., 2013; Moturu et al., 2018; Walker et al., 2019). The final domain (I) of SMXL proteins is also highly conserved, but intriguingly, this domain is absent in flowering plant SMXL3 or SMXL9 proteins, and in monilophyte SMXLJ proteins (Figure 5a,b).

Both the first NTPase (domain C) and second NTPase (domain F or domain F-I) domains have been suggested as the D14/MAX2-interacting part of SMXL proteins, based on different approaches, with more recent structural work focusing on the second domain (Zhou et al., 2013; Shabek et al., 2018). However, more work is needed to assess to definitively show this, and it is not clear that either domain is necessary or sufficient for in planta interactions with D14/MAX2; indeed, it is not unfeasible that both domains play a role.

Most non-seed plants possess eu-KAI2 and DDK receptor proteins, but only one SMXL protein, suggesting that irrespective of their ligand-binding specificities, both receptors target the same SMXL protein for degradation via SCF^{MAX2} (Bythell-Douglas et al., 2017) (Figure 5c). A possible exception is in monilophytes, where SMXL proteins lack domain I, and in which the DDK lineage receptor lacks key residues for MAX2-interaction (Bythell-Douglas et al., 2017) (Figure 5a). In angiosperms, the D14-SMXL7 pair is well supported by genetic and biochemical data, and the D14-SMXL8 and KAI2-SMAX1 pairs are well supported by genetic

data, as discussed above (Figure 5c). However, in gymnosperms, while there are clearly distinct D14/DLK4 and KAI2 proteins, there is no SMXL7/8-like protein, suggesting SL and KL signalling may both target SMAX1 for degradation (Figure 5c). Elsewhere, the origin of the DLK23 lineage matches the origin of the SMXL4 lineage; while DLK2/DLK3 in angiosperms have lost key residues for MAX2 interaction, and SMXL4 in angiosperms has lost the degron motif (Bythell-Douglas et al., 2017; Wallner et al., 2017; Moturu et al., 2018; Walker et al., 2019). Although this could be coincidence, the strong likelihood is that DLK23 receptors interact with SMXL4 proteins in seed plants, possibly inhibiting their activity without degradation (Figure 5c). Consistent with this, SMXL5 in Arabidopsis does not show rac-GR24-induced protein turnover, (Wallner et al., 2017), although this would not necessarily be expected since SMXL5 is not known to associate with D14 or KAI2. Furthermore, if the C-terminal part of SMXL proteins is indeed the key region for interaction with D14/KAI2 (e.g. Shabek et al, 2018), then the lack of domain I in SMXL3 and SMXL9 proteins might suggest that these are 'unpaired' SMXLs that are not regulated by ligand-receptor pairs at all (Figure 5c).

The current model of strigolactone and KL signalling has D14-SMXL7-SCF^{MAX2} and KAI2-SMAX1-SCF^{MAX2} complexes acting separately in each pathway (Soundappan et al., 2015; Stanga et al., 2016). However, this idea has been challenged, primarily based on data from Arabidopsis root development (Waters et al., 2017; Swarbreck et al., 2019). For instance, Swarbeck et al. (2019) suggested that KAI2 signalling might act through degradation of both SMAX1/SMXL2 and SMXL6/SMXL7/SMXL8 to regulate root skewing. This idea is based on phenotypic analysis of strongly epistatic smxl mutants in the max2 background, and while the data could be consistent with KAI2 acting through both sets of proteins, they are not in themselves positive evidence for the idea. The majority of the evidence from angiosperms supports the idea of separate, specific signalling complexes, but as highlighted here, in non-angiosperms, multiple receptors might target the same SMXL protein for degradation. It is thus plausible that the receptor-target pairs are not rigid in angiosperms either, and more work is needed to examine this crucial specificity issue.

VIII. A journey in the dark: SMXL protein function

Degradation of SMXL proteins is – at least currently – the sole identified purpose of strigolactone signalling in plants (Bennett et al., 2016; Waters et al., 2017), and is likely the sole purpose of KAI2 signalling. SMXL proteins are often characterized as 'repressors' of SL/KL signalling; proteins which must be removed before downstream signalling can occur. However, this is not consistent with the strong phenotypes seen in smxl mutants that are diametrically opposed to those seen in SL/KL mutants; nor with the complete epistasis of smxl mutations over d14/kai2 mutants. (Soundappan et al., 2015). Rather, SMXL proteins should be viewed as the key regulators of development, which are repressed in response to strigolactones and other small signals (Soundappan et al., 2015; Waters et al., 2017). As has previously been observed, the situation is comparable to gibberellin signalling and DELLA proteins (Wallner et al., 2016).

The exact molecular function of SMXL proteins remains unresolved. Most attention has focused on the highly-conserved EAR-motif, which is generally viewed as the hallmark of protein-protein interaction with the TOPLESS-RELATED (TPR) transcriptional co-repressor family (Causier et al., 2012; Martin-Arevalillo et al., 2017). Indeed, EAR-dependent, nuclear protein interactions between SMXLs and TPR2 have been shown in rice, Arabidopsis, and barley (Soundappan et al., 2015; Wang et al., 2015; Liu et al., 2017; Ma et al., 2017). More recently Ma et al. (2017) also demonstrated the rice D53 protein binds to different tetradimerisation (TPD) domains of TPR2 in an EAR-dependent manner. TPDs are specific domains of TPR proteins allowing their tetramerisation to form complexes with nucleosomes and regulate chromatin compaction (Ma et al., 2017; Martin-Arevalillo et al., 2017). This suggests that SMXL proteins might act by promoting the stabilization of TPD-nucleosome complexes and formation of repressive chromatin structures (Figure 6). However, these SMXL-TPR interactions are often weak, and their relevance has not been functionally validated in planta. At a functional level, experiments performed with SMXL7 variants lacking the EAR-motif demonstrate that it is important in some but not all roles of SMLX7 in Arabidopsis (Liang et al., 2016). This has led to suggestions that SMXL proteins have multiple functional outputs, both EAR-dependent and -independent (Liang et al., 2016). Care must be taken interpreting these data; the functional importance of the EAR motif is not a validation of the importance of TPR interactions. Furthermore, there are at least 5 families of protein in angiosperms besides TPR that also possess a CTLH domain, allowing them to interact with EAR-motifs

(Tomastikova et al., 2013)). Thus, although it remains likely that TPR proteins are interaction partners of SMXL proteins, nothing should be assumed, and more data are needed to prove the role of TPR proteins.

Irrespective of their interaction with TPR proteins, it remains an obvious hypothesis that SMXL proteins regulate transcription. A long-standing problem with this hypothesis has been the minimal transcriptional changes that occur upon strigolactone (or karrikin) treatment, which seem insufficient to explain the broad developmental roles of these signals (Bennett & Leyser, 2014). However, firm evidence that SMXL proteins act transcriptionally in at least some cases now exists. The BRANCHED1 (BRC1) class of TCP-domain transcription factor is the clearest candidate, given reduced BRC1 expression in strigolactone signalling mutants, increased BRC1 expression in smxl6 smxl7 smxl8 triple mutants, and rapid transcriptional responses to rac-GR24 (Braun et al., 2012; Dun et al., 2012; Soundappan et al., 2015; Seale et al., 2017). Data from rice and wheat now provide a molecular link connecting SMXL proteins and BRC1-class proteins. IDEAL PLANT ARCHITECHTURE 1 (IPA1) is a rice gene that encodes OsSPL14, which regulates multiple growth and developmental parameters. Mutants in IPA1 exhibit increased shoot branching whereas overexpression of IPA1 results in decreased branching (Jiao et al., 2010; Miura et al., 2010; Lu et al., 2013; Wang & Zhang, 2017). IPA1 binds to the promoter of OsTB1 (a homologue of BRC1), promoting its transcription, and thereby negatively regulating shoot branching. Recent work showed that the N-terminal part of IPA1 can directly interact with D53/SMXL7 in vivo and in vitro without intervention of any other protein, and that as a result, D53 binds to IPA1 and blocks its activation of OsTB1 (Song et al., 2017) (Figure 6). Similar results have been reported in wheat (Liu et al., 2017) where TaD53 can physically associate not only with a TPR co-repressor, but also directly interacts with SPL3/SPL17 (homologues of IPA1/SPL14), and the whole complex represses TaTB1 transcriptional activation.

SMXL7 has also previously been shown to affect the abundance of PIN proteins in Arabidopsis. PIN proteins are efflux carriers that are required for auxin transport, and changes in their abundance would explain many of the developmental effects of SMXL proteins, including regulation of shoot branching (Bennett & Leyser, 2014). PIN1 abundance at the basal plasma membrane of vascular-associated cells in the stem is strongly increased in strigolactone-deficient mutants (Shinohara et al., 2013), and is decreased in smxl6 smxl7 smxl8 mutants (Soundappan et al., 2015). SMXL7 is co-expressed with PIN1, and stabilization of

SMXL7 is sufficient to both increase PIN1 levels in stems and alter shoot branching (Liang et al., 2016). However, exactly how these effects on PIN1 are mediated is unclear. It has previously been suggested that they are non-transcriptional, since cycloheximide does not block the effect (Shinohara et al., 2013), but given the nuclear localization of SMXLs and the plasma membrane localization of PIN1, it is difficult to understand exactly how changes in SMXL levels can affect PIN1. More work is thus needed to understand the potentially very important regulation of PIN proteins by SMXLs.

IX. Flotsam and jetsam: SMXL proteins and plant development

SMXL7/D53 are the best-studied SMXL proteins, and their roles in development and physiology (together with strigolactone signalling as a whole) have been characterized in a range of species. The roles of KL signalling and SMAX1/SMXL2 in Arabidopsis have also received significant attention. The roles of these proteins have been recently reviewed elsewhere (Waters et al., 2017; Rameau et al., 2019) and will not be discussed in detail here, but are summarized in Figure 7. Excitingly, developmental roles for the SMXL3 and SMXL4/SMXL5 proteins in Arabidopsis have recently been uncovered, with a clear role for these proteins in phloem development and root growth (Wallner et al., 2017) (Figure 6). Given the wide roles that SMXL7 and SMAX1 clade proteins play in development, it is likely that further roles will be identified for SMXL3, SMXL9 and SMXL4 clade proteins from angiosperms. Non-angiosperm SMXL proteins have yet to be characterized, but given their high level of structural conservation, it seems highly likely that all SMXL proteins operate by the same mechanism – even if it is not yet clear what this mechanism is.

It is noteworthy that all eight SMXL proteins from Arabidopsis appear to play roles in root development (Figure 7), highlighting an area where the roles of each signalling pathway remain incompletely resolved. Careful interpretation of available data has long suggested that both SL and KL signalling regulate root development, but this has not been demonstrated in a systematic way, and many of the papers relating to root development have used *max2* mutants and rac-GR24 – precluding clear assignment of roles to each pathway (Soundappan et al., 2015; Waters et al., 2017). However, two recent reports have clarified at least some of these issues – while also identifying new ambiguities. Swarbreck et al. (2019) show that KL but not SL signalling regulates root skewing in Arabidopsis – but that both SMAX1/SMXL2 and SMXL6/SMXL7/SMXL8 proteins regulate root skewing. As discussed above, this might be

evidence for cross-regulation of SMXL678 by KAI2, and is certainly evidence for the overlapping role of SMXL proteins in root development. This analysis is supported by Villaecija Aguilar et al. (2019), who also show that KL signalling regulates a suite of root developmental responses previously attributed to strigolactone signalling. Intriguingly, this report also shows that both SMAX1/SMXL2 and SMXL678 proteins regulate lateral root development in Arabidopsis, again emphasizing the overlapping roles of SMXL proteins in developmental processes (Villaecija Aguilar et al., 2019). It has also recently been suggested that both KL and SL signalling both regulate drought responses in Arabidopsis (Bu et al., 2014; Ha et al., 2014; Li et al., 2017), so these overlapping SMXL roles may be more common than has been thus far appreciated.

This in turn raises the intriguing question of whether SMXL proteins are be functionally interchangeable in terms of their downstream effects. Currently, there is only limited evidence – SMAX1 can complement smxl5 mutations when expressed under the SMXL5 promoter (Wallner et al., 2017) – but it is consistent with the idea that SMXL proteins may indeed have generic downstream functions. This would also be consistent with the observation that in non-angiosperms, multiple D14/KAI2 proteins are likely to target the same SMXL protein for degradation (see above), with generic downstream effects on development. However, if their functions are generic, why do angiosperms maintain so many different SMXL proteins? A plausible answer might be that different SMXL proteins specialized to interact with different D14/KAI2-family proteins, making their degradation, and corresponding downstream developmental effects specific to a single upstream signal.

X. Conclusion

In itself, the strigolactone saga is a story of the eventual evolution of a plant perception pathway for a sometime soil-borne signal. But it is also a story about specificity, and of how a single pathway has become sub- and neo-functionalized into a series of signalling pathways, differing in nature across land plants, that control a wide range of developmental effects. Three major points of innovation in the evolutionary trajectory of the D14/KAI2-SMXL signalling complexes can be envisaged, as a single original pathway gradually split into several. Firstly, changes in the binding pocket of D14/KAI2 proteins allowed plants to perceive new ligands to control their development. Secondly, new SMXL proteins allowed the separation of receptor-target pairs into more specific complexes. And thirdly – perhaps – different SMXL proteins

specialized for different downstream functions. While we can clearly observe that there is specificity in these signalling pathways, every aspect of this specificity throws up puzzling new challenges. As highlighted above, it is not even clear that D14 proteins are really specific strigolactone receptors; we do not know whether and how D14 targets specific SMXL proteins for degradation; we do not know whether the SMXL proteins have specific functions. Thus, even though strigolactone study sometimes seems saturated, there is a huge amount still to find out about this epic signalling family.

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FIGURE LEGENDS

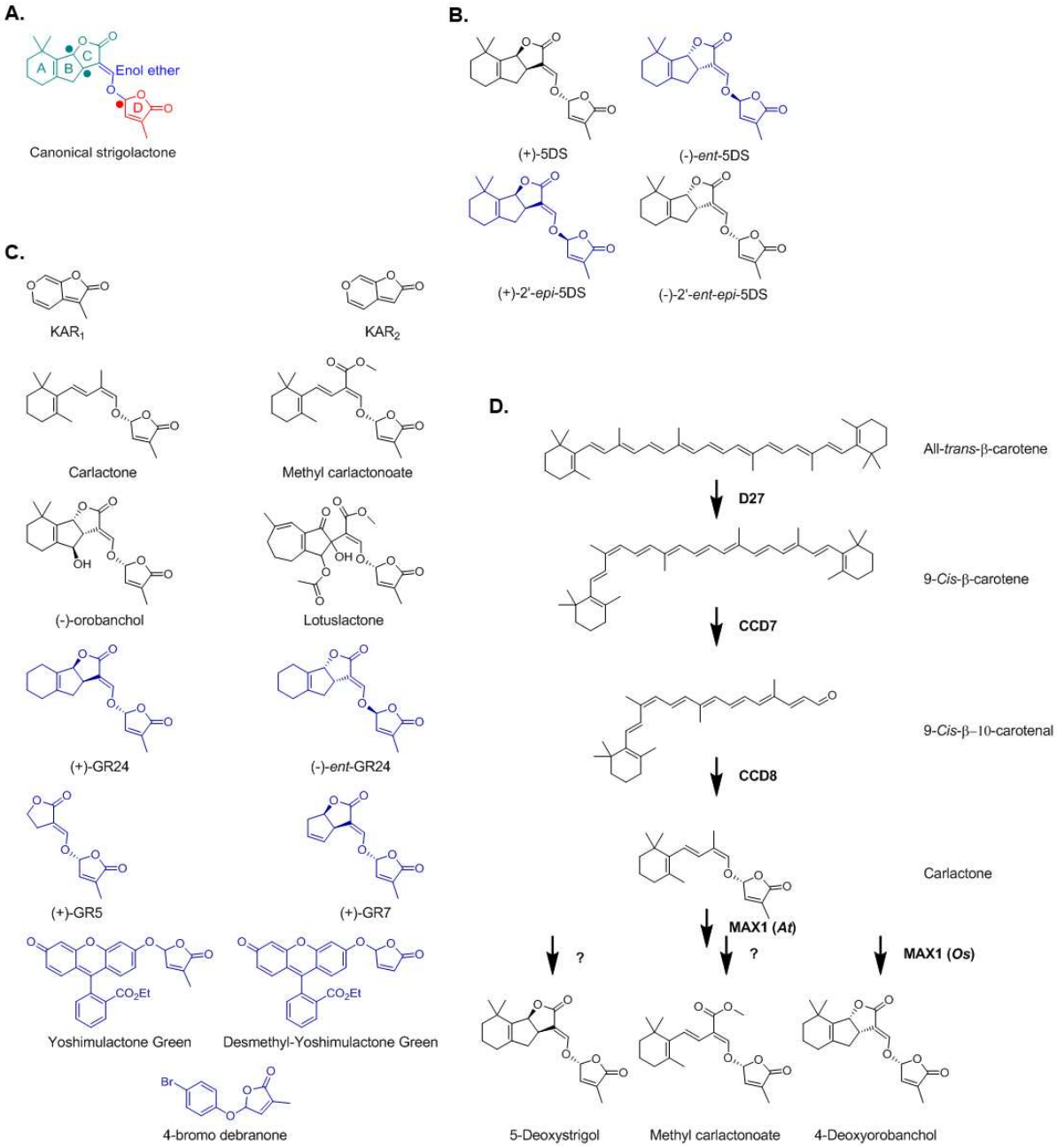

Figure 1: Strigolactone structure and synthesis

A) Basic strigolactone structure, showing ABC (green) and D ring (red) structures, linked by enol-ether bridge (blue). Solid dots indicate stereochemical sites in the structure.

B) The four stereoisomeric forms of 5-deoxystrigol. (+)-5-deoxystrigol is a naturally occurring strigolactone, while (-)-2'-ent-epi-5-deoxystrigol is not, but would display strigolactone activity as a 4DO (4-deoxyorobanchol) type molecule. Conversely, (-)-5-deoxystrigol and (+)-

2-epi-5-deoxystrigol are neither naturally occurring, nor would display strigolactone activity; instead, they are ‘retrolactones’.

C) Diversity of small molecules signalling through D14/KAI2 proteins. KAR1 and KAR2 are smoke-derived karrikin molecules that signal through KAI2. Carlactone, methyl carlactonoate, and lotuslactone are non-canonical strigolactones, lacking the ABC ring structure, but still displaying strigolactone activity. (-)-Orobanchol is a naturally occurring 4DO-type strigolactone. GR24 is the most commonly used synthetic strigolactone analogue, but is typically supplied as a racemic preparation of (+)-GR24 (strigolactone) and (-)-ent-GR24 (retrolactone). GR5, GR7, Yoshimulactone green and 4-bromo debranone are other non-naturally occurring strigolactone analogues and agonists, while desmethyl-Yoshimulactone green is a non-active analogue of Yoshimulactone green.

D) The core strigolactone signalling pathway. Sequential activity of the DWARF27 isomerase and CAROTENOID CLEAVAGE DIOXYGENASE7 (CCD7) and CCD8 enzymes converts β-carotene to the universal strigolactone precursor carlactone. The incompletely characterized activity of other enzymes including the cytochrome P450s of the MORE AXILLARY BRANCHING1 family converts carlactone to the diverse molecules observed in plants; this activity differs between Arabidopsis thaliana (At) and rice (Os).

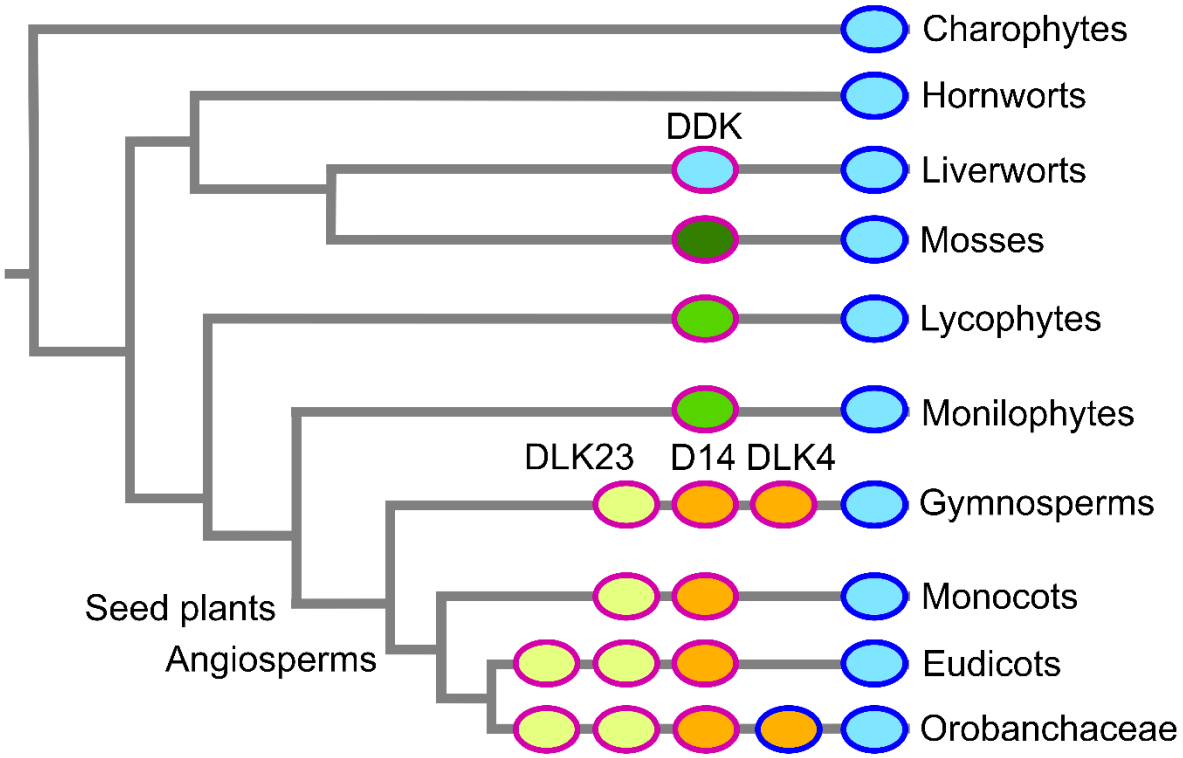

Figure 2: Evolution of D14/KAI2 proteins

Schematic diagram showing the phylogeny of land plants, with D14/KAI2 family members present in the eu-KAI2, DDK lineage indicated. In seed plants, the DDK lineage is further divided into the D14, DLK23 and DLK4 sub-lineages. Each oval represents a protein type. The border colour represents the lineage within the family (blue = eu-KAI2, pink = DDK), and the fill colour represents the probable ligand specificity (light blue = KL, orange = strigolactone, yellows/greens = unknown related ligands)

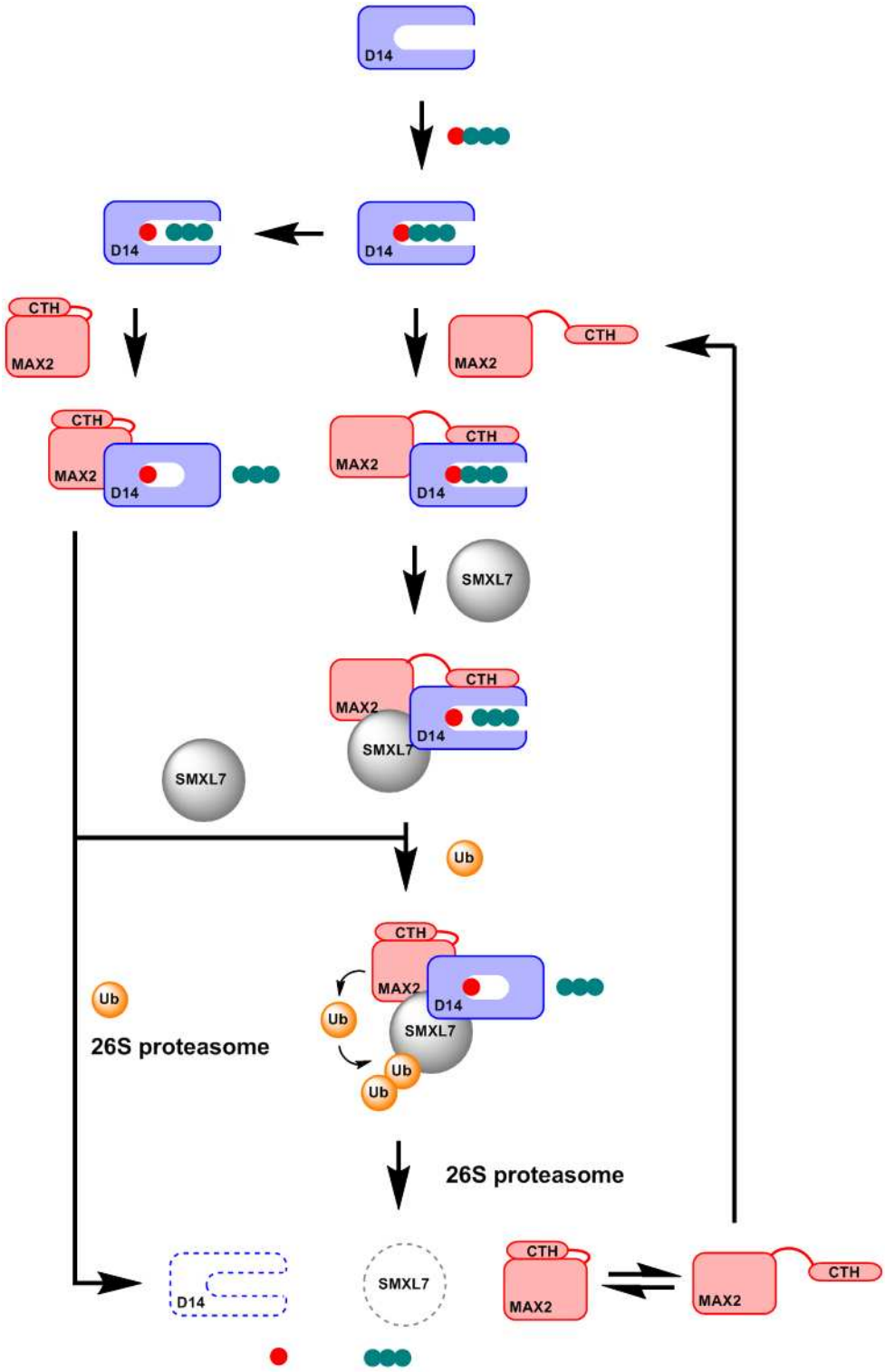

Figure 3: The core strigolactone signalling mechanism

The α/β-hydrolase D14 acts as a strigolactone receptor, and binds strigolactones (shown as 3 green and 1 red dot) with the D-ring (red dot) at the bottom of the ligand binding pocket, interacting with the catalytic triad (Top). Strigolactone-bound D14 can interact with the dislodged C-terminal alpha-helix (CTH) of D3/MAX2, which is part of an SCF-type E3 ubiquitin ligase complex (not shown for clarity). Binding of the proteolytic targets of

$SCF^{D3/MAX2}$, proteins of the SMXL family (here exemplified by SMXL7) triggers hydrolysis of the bound strigolactone, and ubiquitination of D14 and SMXL7. Alternatively, hydrolysis of strigolactones by D14 enables it to adopt a 'closed' conformation, and interact with D3 with its CTH engaged (left hand side). Binding of SMXL7 by this complex then triggers the ubiquitination of D14 and SMXL7. Poly-ubiquitinated D14 and SMXL7are degraded by the 26S proteasome, freeing SCF^{MAX2} for further signalling.

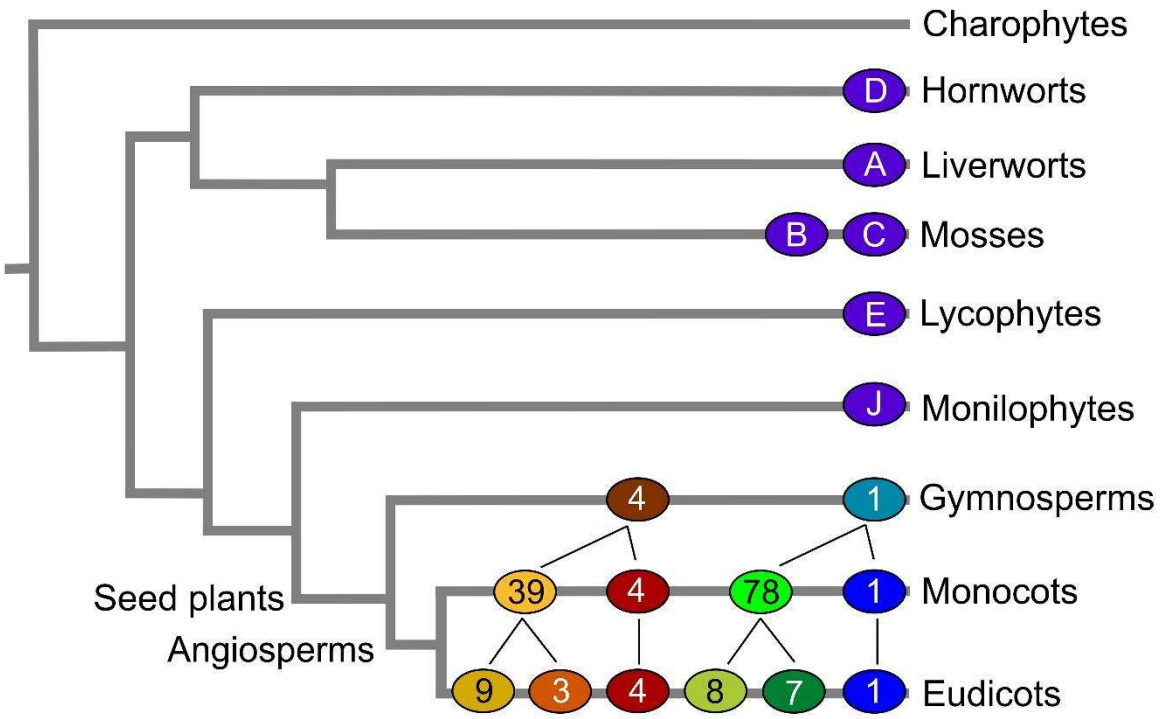

Figure 4: Evolution of SMXL proteins

Schematic diagram showing the phylogeny of land plants, with SMXL family members present in each land plant group indicated. Each oval represents a protein type, as designated by the number or letter inside. The fill colours match those in Figure 5. The phylogenetic relationship between SMXL proteins in gymnosperms and angiosperms are indicated by thin black lines.

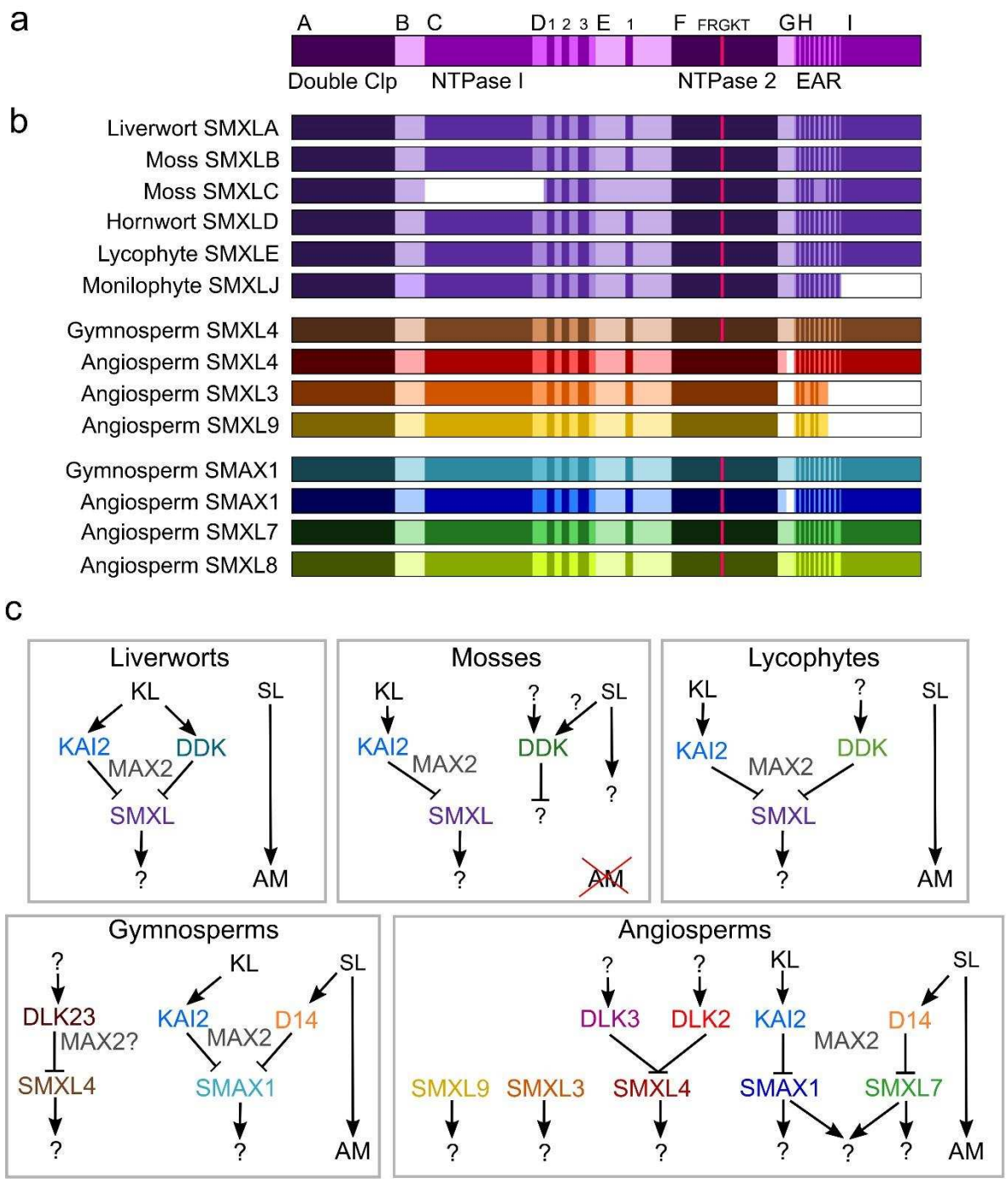

Figure 5: SMXL protein structure, diversity and interactions

A) SMXL protein structure. SMXL proteins have a highly conserved, multi-domain structure (domains A-I). The degree of sequence conservation across the family is indicated by shading; darker colours indicate more conserved domains. The FRGKT motif is indicated in pink. The position of the NTPase 1, NTPase 2, Double Clp and EAR motifs is indicates.

B) SMXL protein diversity. The structure of different SMXL protein types from across land plants is indicate in relation to the general structure. Data based on publically available sequences.

C) Known and hypothetical signalling complexes between D14/KAI2 family members (see Figure 2) and SMXL family members across land plants. SL = strigolactone, KL = KAI2-ligand, AM = arbuscular mycorrhizal fungi.

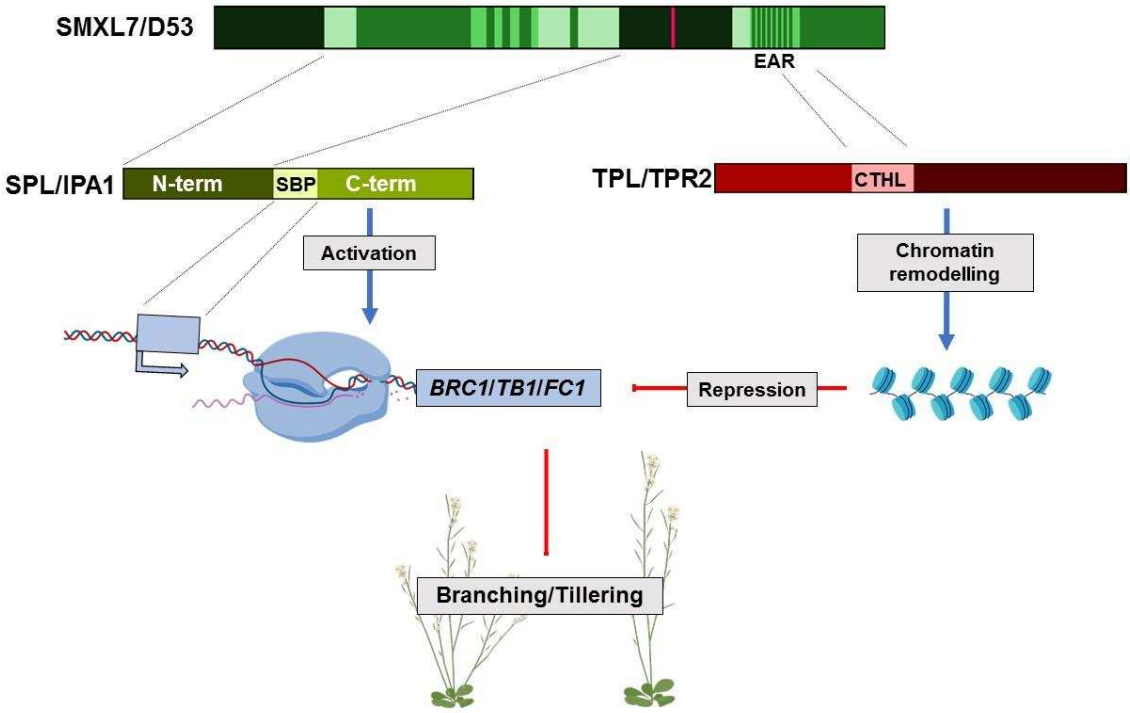

Figure 6: SMXL protein function

Model for SMXL7/D53 function in the control of shoot branching. TCP transcription factors of the BRC1-class (including BRANCHED1 (BRC1), TEOSINTE BRANCHED1 (TB1) and FINE CULM1 (FC1)) repress branching/tillering in plants by acting on as yet undefined targets. SPL transcription factors of the IPA1 (IDEAL PLANT ARCHITECTURE 1) class bind to the promoters of BRC1 genes via their SBP-box, activating the transcription of BRC1 proteins. SMXL proteins of the SMXL7/D53 class bind to both SPL proteins (via their N-terminus), and to TPL/TPR co-repressor proteins via compatible EAR-CTLH motif interactions, resulting in assembly of repressive chromatin structures that turn off BRC1 transcription – and thereby promoting branching.

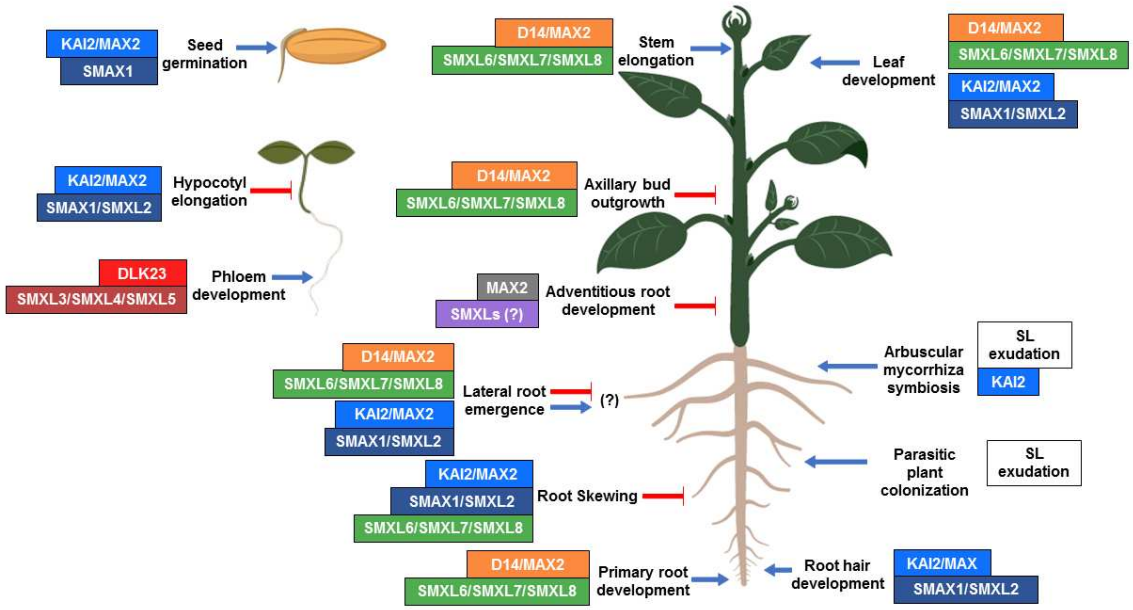

Figure 7: D14/KAI2 and SMXL protein roles in flowering plant development

Summary of known functions of strigolactones and D14/KAI2 and SMXL protein family members at different stages of flowering plant development. Nomenclature is based on Arabidopsis.