Evolution of plant hormone response pathways

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ABSTRACT

This review focuses on the evolution of plant hormone signalling pathways. Like the chemical nature of the hormones themselves, the signalling pathways are diverse. Therefore, we will focus on a group of hormones whose primary perception mechanism involves an Skp1/Cullin/F-box (SCF)-type ubiquitin ligase: auxin, jasmonic acid (JA), gibberellic acid (GA), and strigolactone (SL). We begin with a comparison of the core signaling pathways of these four hormones, which have been established through studies conducted in model organisms in the Angiosperms. With the advent of next-generation sequencing and advanced tools for genetic manipulation, the door to understanding the origins of hormone signaling mechanisms in plants beyond these few model systems has opened. For example, in-depth phylogenetic analyses of hormone signaling components are now being complemented by genetic studies in early diverging land plants. Here we discuss recent investigations of how basal land plants make and sense hormones. Finally, we propose connections between the emergence of hormone signaling complexity and major developmental transitions in plant evolution.

SCF-MEDIATED HORMONE SIGNALING: KNOWLEDGE FROM MODEL PLANTS

Auxin, Jasmonic Acid (JA), Gibberellin (GA), and Strigolactone (SL) signaling mechanisms all involve hormone-activated targeting of a transcriptional regulator(s) for degradation. How the targeting is activated and the nature of the targets themselves vary across these pathways and are detailed below (Figure 1). However, a few common features emerge. First, proteolytic targeting is mediated by SCF-type E3 ubiquitin ligase complexes, which consist of an Skp1 adaptor protein, a Cullin scaffold protein, an F-box protein, and a Ring-box (RBX) protein that binds the E2 ubiquitin-conjugating enzyme (124). The F-box protein confers substrate specificity to the complex. Upon association with a substrate protein, or "target," by the F-box subunit, the SCF complex attaches monoubiquitin or polyubiquitin chains to the substrate. The latter modification marks the target for rapid degradation by the 26S proteasome. A second common theme is that the proteolyzed targets of these hormone response pathways are transcriptional regulators that do not directly bind DNA. Instead, the targets function through association with DNA-binding transcription factors. A third common property is that the targets of the auxin, JA, and SL pathways either have an Ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif, or associate directly with a protein that has an EAR motif. The EAR motif enables binding to proteins with C-terminal LisH (CTLH) domains, most prominently the TOPLESS (TPL) and TOPLESS-RELATED (TPR) transcriptional co-repressors (22). Thus activation of transcriptional programs in response to these three hormones is due to relief of inhibition.

Auxin

Auxins regulate a very broad range of plant growth and developmental processes. These processes, which include cell division, cell growth, and cell differentiation, underlie many kinds of organogenesis and growth responses (76). The naturally occurring auxin family is composed of several small aromatic molecules with carboxylic acid moieties: indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA) (80). IAA, the most abundant and biologically significant auxin found in plants, is derived primarily from metabolism of Tryptophan (84). There are several storage forms of IAA, including IBA, and sugar or amino acid conjugates to IAA, but among these metabolites IAA itself may be considered the only endogenous molecule that directly activates auxin signaling (129, 130). The perception of auxin occurs through the association of an F-box protein in the TIR1/AFB family with proteins in the AUXIN/INDOLE ACETIC ACID (Aux/IAA) family (36, 70, 134). This leads to polyubiquitination and proteolysis of the bound Aux/IAA (49). The functional role of Aux/IAA is to inhibit the activity of B3-type DNA-binding domain transcription factors in the AUXIN RESPONSE FACTOR (ARF) family (136). Aux/IAAs form heterodimers with ARFs (71, 136) and also recruit TPL/TPR co-repressors through an EAR motif (132), leading to stable gene repression in the absence of auxin.

Notably, TIR1 does not undergo a conformational change after binding auxin. Rather, TIR1/AFB and Aux/IAAs function as co-receptors whose physical interaction is stabilized by IAA binding (134). This signaling mechanism, in which auxin acts as a "molecular glue," is in contrast to allosteric signaling

mechanisms, in which a receptor undergoes a conformational change in response to ligand-binding that activates downstream signaling events. The strength of the glue, so to speak, is not only determined by the auxin ligand, but also by different affinities of TIR1/AFBs for auxin and by divergence in the Aux/IAA domain that mediates interaction with TIR1/AFBs and auxin (20). In Arabidopsis, six TIR1/AFBs and 29 Aux/IAAs (107) allow many possible co-receptor complexes that have different auxin affinities.

ARFs are phylogenetically classified into A, B and C groups (154). All ARFs share a B3-type DNA binding domain, followed by a variable middle region and a C-terminal PB1 interaction domain (109). ARF classes differ most strongly in their middle region, which is thought to direct gene activation or repression. By releasing ARF transcription factors from inhibition by Aux/IAAs, auxin controls the activation or repression of their target genes.

Jasmonic Acid

Jasmonates are a class of hormones synthesized from C_{18} fatty acids that have roles in wounding responses, defense against necrotrophic pathogens, and development (150). These hormones include jasmonic acid, methyl jasmonate (MeJA), and 12-oxophytodienoate (OPDA), but the bioactive hormone in *Arabidopsis thaliana* is an isoleucine conjugate of JA, Jasmonyl-Ile (JA-Ile) (150). In terms of having a very limited complement of bioactive signals, JA and auxin signaling are quite similar. The perception of JA-Ile is also strikingly similar to that of auxin, as it involves a co-receptor complex with an F-box protein in which JA-Ile functions as a "molecular glue." JA-Ile is bound by a single F-box protein, CORONATINE INSENSITIVE1 (COI1), creating a high-affinity binding site for transcriptional regulators in the Jasmonate ZIM domain (JAZ) family (24, 119, 135). Formation of the COI1-JA-Ile-JAZ complex triggers rapid polyubiquitination and degradation of JAZ proteins. In the absence of JA-Ile, JAZ interacts with MYC1 to MYC4, a subfamily of the large bHLH transcription factor family (40), and also with the EAR-motif-containing protein Novel Interactor of JAZ (NINJA) (102). Thus JAZ proteins indirectly recruit TPL/TPR via NINJA to repress transcriptional activation by MYC. Upon JAZ degradation, transcriptional responses to JA can occur.

Gibberellic Acid

Gibberellins are diterpenoid compounds produced not only in plants, but also in fungi and bacteria (82). In most plants, GAs have been shown to promote cell expansion and division, which results in an overall control of plant size (3). Their roles in development include the differentiation of pollen in angiosperms and male organ formation in ferns (123, 133), and the promotion of developmental phase transitions such as seed germination, the acquisition of maturity traits during vegetative growth, the entrance into the reproductive phase, and early fruit development (13, 37, 39, 144). GAs have also been implicated in the modulation of responses triggered by biotic and abiotic cues, such as pathogen infection (95) or cold, salt and osmotic stress (26).

There is strong evidence that GAs perform their roles mostly through transcriptional regulation, which involves the degradation of DELLA proteins. DELLA proteins belong to the larger GAI/RGA/SCR (GRAS) family, and are nuclear proteins that interact with and modify the activity of a diverse group of transcription factors and other transcriptional regulators (29, 83). DELLA proteins harbor two distinct regions: the N-terminal "DELLA" domain, and the C-terminal "GRAS" domain. While all the interactions with transcription factors occur through the GRAS domain, the DELLA domain is responsible for interaction with GIBBERELLIN INSENSITIVE DWARF1 (GID1), the GA nuclear receptor. Upon binding to GAs, GID1 undergoes a conformational change that exposes a movable lid with a surface able to interact with the DELLA proteins. This GID1-DELLA interaction triggers recruitment of the F-box protein GIBBERELLIN INSENSITIVE DWARF2/SLEEPY1 (GID2/SLY1), followed by polyubiquitination and degradation of DELLAs.

The current model for GA signaling implies that DELLAs optimize the balance of growth and defence responses by coordinately modulating multiple genetic circuits that can each function independently of DELLA (25). For instance, DELLAs redirect plant resources upon biotic or abiotic stress towards an adaptive response that impairs growth. The way in which this mechanism would relay environmental information is based on the sensitivity of GA metabolism to cues like light quality, light intensity, and ambient temperature, which in turn would alter DELLA stability.

Strigolactones

Strigolactones (SLs) are a class of carotenoid-derived plant hormones that regulate axillary shoot growth, leaf senescence, secondary (cambial) growth, and root architecture (4, 48, 66, 110, 140, 143, 162). SLs are also exuded by roots into the soil, where they promote beneficial symbiotic interactions with arbuscular mycorrhizal (AM) fungi (5, 113). Obligate root parasitic plants in the Orobanchaceae, including *Striga* spp., have evolved the ability to germinate after sensing SLs in soil, which indicate the nearby presence of a host (151, 159).

The core mechanism of SL perception and signal transduction that has emerged over the past decade is familiar and yet unusual (151). SLs are perceived by DWARF14 (D14)/DECREASED APICAL DOMINANCE2 (DAD2), an α/β-Hydrolase protein with a strictly conserved Ser-His-Asp catalytic triad. Unlike the GA receptors, which are also members of the α/β-Hydrolase superfamily, D14/DAD2 has slow hydrolytic activity on SLs that is important for SL signal transduction (52). Nucleophilic attack by the catalytic Ser residue cleaves a butenolide ring from SL that is passed to and covalently bound by the catalytic His residue (34, 164). What exactly constitutes activation of D14 is currently under debate. Although modification of the His residue is well supported, the evidence for a proposed covalently linked intermediate molecule bridging the catalytic Ser and His has been challenged (21). Furthermore, the imperfect correlation of the biological activity and hydrolyzability of SL analogs, as well as a SL-hypersensitive, catalytically inactive *d14* mutant have been pointed to as evidence that hydrolysis is not essential for signaling (117). Regardless, SL induces a conformational change in D14 that promotes its interaction with the F-box protein

MORE AXILLARY GROWTH2 (MAX2)/DWARF3 (D3) (164). Recent work indicates that the C-terminus of MAX2 can switch between two conformations that associate with different interfaces of D14 in the receptor's SL-bound and SL-hydrolyzed states, regulating the enzymatic activity and protein interactions of D14 (118). SL also promotes interactions between D14 and a subset of proteins in the SUPPRESSOR OF MAX2 1-like (SMXL) family known as DWARF53 (D53) in rice or SMXL6, SMXL7, and SMXL8 in Arabidopsis (64, 126, 148, 174). This triggers polyubiquitination and rapid degradation of D53/SMXL6/7/8. Direct interactions between MAX2 and SMXL7 are comparably weak or do not occur (77), implying that D14 functions as a SL-activated bridge that brings together SCF^{MAX2} and its targets.

It is still unclear how SMXL proteins regulate plant growth. SMXL proteins have at least one EAR motif that enables interactions with TPL/TPR proteins (64, 126, 148). Therefore, the prevailing hypothesis has been that SMXLs function as transcriptional co-repressors, similar to the targets of auxin and jasmonate signaling. IDEAL PLANT ARCHITECTURE (IPA1) in rice and several other SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins in wheat are reported to interact directly with the SMXL protein D53, enabling transcriptional control of downstream genes (78, 125). However, genetic analysis of the orthologs SPL9 and SPL15 suggests that SPL transcription factors might not function as SMXL partners in control of branching, at least in *Arabidopsis* (12). SMXL proteins also appear to impose transcription-independent control of growth, as not all developmental processes regulated by SMXL7 require conservation of the EAR motif (77). Furthermore, plasma membrane-localization of the auxin efflux carrier PIN-FORMED1 (PIN1) is rapidly inhibited by SL, even in the presence of cycloheximide (121).

The core SL signaling pathway is highly similar to that of karrikins (KARs), a class of small butenolide molecules found in smoke that can promote seed germination and influence seedling growth. KAR signaling is also MAX2-dependent and involves a receptor and putative proteolytic targets that are ancient paralogs of D14 and SMXL6/7/8. In Arabidopsis, KAR responses require the a/b-Hydrolase protein KARRIKIN INSENSITIVE2 (KAI2)/HYPOSENSITIVE TO LIGHT (HTL) (152). KAI2 can bind KARs in vitro and two crystal structures of KAI2-KAR_1 complexes have been resolved, albeit with different orientations of the ligand (50, 161). Intriguingly, *kai2* and *max2* mutants share high seed dormancy and reduced light sensitivity phenotypes that are not found in SL biosynthesis mutants and are opposite to the effects of KAR treatment (98, 152). This and other observations have led to the hypothesis that KAI2 may perceive an as-yet-unknown endogenous signal, known as KAI2 ligand (KL), that is not derived from the SL biosynthetic pathway (28). It may be that KARs, which have not been reported in plants and therefore should not be considered hormones, can act as chemical analogs of KL for some species. Indeed, KAI2 demonstrates some flexibility in its ligand range, as it can hydrolyze and confer responses to SL analogs that have an unnatural stereochemical configuration (41, 115). Based on genetic suppressor data and homology to the SL pathway, the likely targets of SCF^{MAX2}-KAI2 are SMAX1, the founding member of the SMXL gene family, and SMXL2 (127, 128). There is some evidence of ligand-activated KAI2-MAX2 and KAI2-SMAX1 interactions from yeast two-hybrid or *in vitro* pulldowns, but in depth biochemical characterization of SMAX1 degradation and the KAR signaling mechanism is still lacking (138, 165).

Common themes in SCF-based hormone signaling

There are interesting parallels in the SCF-based hormone signaling pathways outlined above that have been derived from studies of angiosperm model species. First, TIR1/AFBs, COI1, and MAX2 are all members of a subfamily of F-box proteins with C-terminal leucine-rich repeats (LRRs). Out of approximately 700 F-box protein-encoding genes in Arabidopsis, only 23 are members of this subfamily (160). Remarkably, EBF1/2 proteins, which are involved in ethylene responses, are also within this group. It is unclear why this particular subfamily of F-box proteins features so prominently in plant hormone response pathways, but it unlikely to be only because LRRs can form small molecule-docking sites, as neither MAX2 nor EBF1/2 directly bind the hormone. Second, the logic of some pathways is conserved despite the involvement of different molecular components. In the case of auxin and JA, co-receptors recognize a very limited repertoire of ligands. Closely related F-box proteins bind different substrates (Aux/IAAs and JAZ proteins) that do not share motifs or domains. These substrates each inhibit different classes of transcription factors through recruiting the same co-repressor. In the case of GA and SL/KAR, the families of bioactive molecules are large and diverse. The receptors are both members of the α/β-Hydrolase superfamily. Although only the SL receptor has retained catalytic activity, both use allosteric signaling mechanisms. Like auxin and JA, the targets of SL and KAR signaling are likely to function at least partially through a co-repressor mechanism. Third, some of these signaling components have shared origins and evolved to serve different hormone pathways. As we discuss below, TIR1/AFBs and COI1 are ancient paralogs, as are D14 and KAI2, and SMXL proteins. Finally, all mechanisms feature fast responses, in which target protein degradation occurs within minutes of hormone treatment (73, 171, 174).

"NOTHING IN BIOLOGY MAKES SENSE EXCEPT IN THE LIGHT OF EVOLUTION"

SCF-based plant hormone pathways control many developmental and physiological responses. Comparative analysis of hormone signaling pathways in many species beyond model plants points to important differences in the functions played by these hormones. A key question is to what degree the emergence of these signaling pathways has contributed to life history trait evolution. In this section, we will briefly review the major transitions in land plant evolution, and then discuss the origin of these signaling systems within this context.

Major transitions in the evolution of land plants

The evolution of the plant lineage involved several major transitions in life history and habitat. The first of these can definitely be considered the most dramatic of transitions: algae occupying shallow, freshwater habitats developed the capacity to settle on land. This change in environment required a large number of adaptations, presumably in a stepwise manner. As no fossils are available that help reconstruct the

anatomical adaptations and their relative order, and because no extant species appear to represent the evolutionary intermediates along this transition, one can only form deductions from extant early-diverging land plants and late sister groups within the Charophycean algal lineage. The change from aquatic to terrestrial habitats went along with a switch from filamentous, or two-dimensional, growth to three-dimensional morphologies. Also, while many algae have a floating or suspended habit, land plants are anchored to the ground surface through rhizoids or roots. As the exposure to atmosphere is very different from being suspended in water, another key adaptation must have been the development of a protective surface (cuticle) and selective gas-exchange pores. Furthermore, moving to the terrestrial surface, early land plants would likely have been exposed to a new range of microbes that were beneficial, pathogenic, or even symbiotic.

Following the conquest of land, a next major transition in plant life history involved the appearance of active vascular transport systems (69). Not only did these systems mechanically support plant structures and allow increases in height, the vascular system also allowed long-distance transport. In turn, increased size and long-distance transport have been major elements of functional partitioning between light-harvesting, energy storage, and water- and nutrient-harvesting tissues. Long-distance transport and functional partitioning, however, also necessitated the use of proxies for light conditions and other environmental factors, such as soil nutrient availability, to be communicated towards distant tissues.

A next, defining modification to plant life history was the development of seeds. All land plants (by definition) form embryos following fertilization. In Bryophytes, the embryo/sporophyte forms a minimal part of the life cycle, and culminates in the production of spores after meiosis in sporophytic cells. In Lycophytes and ferns, there is an extended sporophytic stage, but there is no interruption between embryogenesis and following sporophyte development. The interruption of development at the end of embryogenesis evolved in the ancestor of seed plants (Gymnosperms and Angiosperms). This interruption naturally allowed control over the timing of development – germination of seeds – and in many cases the integration of environmental signals in this decision.

Lastly, an important transition in plant life history evolution was the establishment of flowers (92). These reproductive structures have helped attract pollinators to aid in pollination, while encapsulation in fruits have helped establish new strategies for seed dispersal. Clearly, all the developmental and physiological aspects of flower and fruit development require tight molecular control.

Origins of SCF-mediated hormone signaling in plants

Recent evidence has established that the different SCF-mediated hormone signaling pathways emerged at different points along the evolution of the plant lineage, and in some cases a fairly precise description of the ancestral states has been achieved. While the origin of auxin and JA signaling coincides with the emergence of land plants, GA-dependent degradation of DELLA proteins is linked to the appearance of vasculature, and canonical SL signaling has only been confirmed in seed plants (Figure 2).

Various studies have used genome-based phylogenetics approaches to reconstruct evolutionary histories of the three dedicated components in auxin response: TIR1/AFB, Aux/IAA and ARF proteins (35, 74, 94). These studies showed that the complexity of auxin signaling observed in model Angiosperms is found across flowering plants, and can be traced at least to the common ancestor of all seed plants, including Gymnosperms (35, 74, 94). Sampling of species for genome analysis in earlier diverging species is sparse, and until recently only included the Lycophyte *Selaginella moellendorffii*, the moss *Physcomitrella patens* and some Chlorophycean green algae. Based on such comparisons, it was not clear what the ancestral state of the auxin response system was: no components were found in Chlorophytes (35), while Physcomitrella had several copies of each component (105, 108) and Selaginella appeared to have fewer copies (9). Given that no species is representative of the ancestral state at which the lineage diverged, the only solution to establishing more accurate reconstruction is to include more species, particularly in Charophycean algae and early land plants. Indeed, the genome of the liverwort *Marchantia polymorpha* (15) has helped establish that the early liverwort lineage has a minimal auxin response system, with a single TIR1/AFB orthologue, a single Aux/IAA protein and three ARFs. These ARFs represent orthologues of each of the three subtypes found across land plants: A, B and C-class (42, 67). Again, however, it is unclear if *Marchantia* represents the ancestral state, or whether it has a contracted auxin response system.

Although genome resources are still limiting, there is a wealth of transcriptome information that can be leveraged to derive ancestral states. The 1KP (One Thousand Plants; **Sidebar 1**) initiative has generated RNA-seq-based transcriptome assemblies of many plant species, including tens of species each in the Charophyte, liverwort, hornwort, moss, Lycophyte, and fern range of early diverging species (87). Mining this resource revealed the number of auxin response components at each node in plant evolution (94). An important finding was that auxin response appears to have evolved to regulate a pre-existing proto-ARF transcription factor that is present in Charophycean algae. Furthermore, the simplicity of the auxin response system represents the ancestral state, and Marchantia is representative of the system complexity in Bryophytes and Lycophytes. The increased number of ARF and Aux/IAA copies in Physcomitrella is specific to this species, and is potentially associated with the relatively fast genome evolution found in mosses (72). Increases in auxin signaling system complexity are not found until the first ferns, and there are several further increases in complexity at the base of the flowering plants (94).

Several of the morphological adaptations to the terrestrial habitat, particularly rhizoid development (62, 111) and organized three-dimensional growth (42, 67, 105), prominently involve auxin responses in land plants. Auxin treatment dramatically promotes rhizoid formation in Marchantia (42, 67), much as it influences homologous root hair development in flowering plants (86). Likewise, inhibition of auxin response leads to defects in cell division orientation in flowering plants such as Arabidopsis (170), and loss of auxin response causes severe distortion of organized three-dimensional growth in Marchantia (67). Thus, the emergence of the auxin response system aligns well with the emergence of morphological processes controlled by auxin. It is quite possible that other adaptations to land correlate with auxin response: auxin-

insensitive Physcomitrella mutants displayed strong growth defects, accompanied by misexpression of many genes involved in photosynthesis and light response (75).

The origin and evolution of components in the JA signaling has not been studied in as much detail as is the case for auxin. The most striking observation is that the co-receptors for auxin and JA, TIR1/AFB and COI1 share a single ancestral copy in Charophytes (94). This proto-TIR1/AFB/COI1 protein shows properties of both TIR1/AFB and COI1, yet lacks hormone-binding residues and is not expected to bind either auxin or JA. Thus, a duplication in this gene in the ancestor of all land plants likely gave rise to both auxin and JA co-receptors, a process that must have included modifications in the hormone-interacting protein surface in both duplicated copies. What selection pressures shaped such modifications are unknown. Since no clear algal JAZ orthologues have been identified (99), it is unclear to what degree the JA response system was pre-adapted. Like in the case of auxin, the JA response system appears first in the land plant lineage, with Marchantia having single copies of COI1, JAZ, MYC and NINJA (15). As Marchantia is representative for the bryophytes, and thus for the earliest land plants, with regards to auxin signaling, it is likely that this degree of simplicity is also an intrinsic property of the earliest JA signaling pathway. Genetic analysis of the JA response pathway in Marchantia has now shown the system operates similarly to the flowering plant system, but with an interesting twist: the active compound facilitating MpCOI1-MpJAZ interaction is not JA-Ile – like in flowering plants – but instead is dinor-OPDA, a derivative of a metabolic precursor of JA (90). Thus, while the system, in its simplicity, operates in a manner identical to the complex flowering plant JA response system, evolution has acted on the chemical nature of the hormone itself, likely involving co-evolution with MpCOI1 and MpJAZ. An important question is whether dinor-OPDA represents the ancestral hormone, or if this is a specific modification in Marchantia.

JA in flowering plants is associated with wound-, UV- and drought responses, and defense against pathogens, prominently to necrotrophic microbes and herbivorous insects (58). It is very unlikely that this latter function – defense against insects – is part of the ancestral function of JA, given that the first airborne insects did not evolve until tens of millions of years after the emergence of the first land plants (38). However, with the transition to land, plants were likely exposed to a different suite of microbial pathogens, and the changes in organismal complexity and morphology may have encouraged novel strategies to deal with pathogen attack. Some JA metabolites are volatile, and their use as a plant-to-plant communication signal will be limited to terrestrial plants. Other JA metabolites, including the most active JA-Ile, as well as the dinor-OPDA that acts as the COI1 ligand in Marchantia, are water-soluble (150), and would thus be compatible with being a signaling molecule in both aquatic and terrestrial species. The roles of JA response in adapting to high UV (33), drought (68) and wounding (10), in land plants are very clearly connected to the terrestrial lifestyle: UV light intensity in air is higher than in water, and clearly the exposure to air causes dessication if not protected against. Finally, in the absence of the dampening effect of water, the impact of colliding particles, and thus the chance of wounding, on land is also larger. Thus, one can rationalize how the roles of JA signaling may have contributed to early land adaptation.

From an evolutionary point of view, there is increasing evidence that DELLAs predate the emergence of GA metabolism and perception. First, active GA metabolism seems to be missing in non-vascular land plants, beyond the production of the precursors ent-kaurene and kaurenoic acid (KA) (88). Second, there is no evidence so far for GID1 receptors in the genomes of non-vascular land plants (169). The structural similarity between GID1 and carboxylesterases (CXEs) that hydrolyze short-chain fatty acid esters suggests a common origin, and key changes in the CXE active site would have abolished catalytic activity and modified the movable lid to allow binding of GA molecules (141, 169). Third, *DELLA* genes are present in all the land plant genomes sequenced so far, including *M. polymorpha* (15), but in spite of the sequence features necessary for the interaction with GID1 being conserved in most non-vascular plants, they do not allow the interaction with GID1 (54) (55, 166). Although there is no information on their functions in non-vascular land plants, the activity of the N-terminal domain of DELLA proteins as a transcriptional coactivator has been conserved at least in mosses and liverworts (54). Expression of *P. patens* DELLAs rescues to some extent the loss of two of the five *DELLA* genes in Arabidopsis (166), but does not promote dwarfism in rice plants (55), indicating that there may be at least a partial degree of conservation in the capacity to interact with partner TFs in early-diverging land plants. Therefore, it is likely that DELLAs would be transcriptional regulators in the common ancestor of Embryophyta, and the GA synthesis and perception module recruited DELLAs through the conserved N-terminal domain to exert GA-dependent control over transcriptional programs during the establishment of the vascular plant lineage (54).

It is striking that the GA response system emerged first in the vascular plants. GA has many activities in vascular plants, many of which are related to controlling the extent and rate of growth. Intrinsic to GA activity seems to be its tight integration with other signaling pathways, for example auxin or light signaling (6). The DELLA proteins, effectors of GA action, appear to constitute hubs in transcriptional regulation, interacting with components of many signaling pathways (30, 83). Examples of these are interactions with PIF, ARF and brassinosteroid response proteins to control growth-regulating genes (32, 45, 100). Thus, GA may have emerged as a facile long-distance signal to coordinate local growth with information on environmental inputs from distant organs. Strikingly, both the DELLAs and many of their interaction partners, predate the emergence of the GA signaling pathways, and it is thus possible that DELLAs represent an ancient transcription hub, that was subverted to GA regulation with the establishment of vascular plants.

Phylogenetic analyses have suggested that the SL signaling mechanism found in angiosperms is derived from the KAR/KL pathway. KAI2 is present in Bryophytes, but D14 emerges only in the seed plants (19). Similarly, SMXL genes are present in Bryophytes, but diversification into SMAX1 is not apparent until the Gymnosperms, and D53-type SMXLs (e.g. SMXL6/7/8) are an even more recent innovation of Angiosperms (91, 146). In contrast, MAX2 is present throughout land plants, and is typically maintained in genomes as a single-copy gene (23).

Despite the lack of clearly recognizable SL receptors and targets prior to the Angiosperm lineage, several lines of evidence suggest that SL perception evolved much earlier. First, canonical and non-

canonical SLs have been detected in Bryophytes and putatively in green algae in the Charales (31, 168). The core enzymes involved in SL biosynthesis from carotenoids are found in most Bryophytes, although *Physcomitrella patens* and *Marchantia polymorpha* appear to lack at least one of the these (146). Second, deletion of CAROTENOID CLEAVAGE DIOXYGENASE 8 (CCD8), which carries out a key step of SL biosynthesis in angiosperms, causes a dramatic effect on protonemal growth in *P. patens* (106). This phenotype can be recovered by application of *rac*-GR24, a racemate of synthetic molecules that mimic SL and potentially KL. Low concentrations of *rac*-GR24 also influence growth of *Chara corallina* (Charales) and *Marchantia* spp (31). Third, there is a precedent for KAI2 proteins having the ability to function as SL receptors. In root parasitic plants in the Orobanchaceae, KAI2 has undergone an atypical degree of gene duplication. A subset of the KAI2 paralogs found in these parasites evolved into SL receptors, enabling SL-activated germination responses (Conn et al. 2015, Toh et al. 2015, Tsuchiya et al. 2015). Similar, but independent, diversification of KAI2 is evident in Bryophytes, leading to the hypothesis that some of the KAI2 paralogs might recognize SLs (Bythell-Douglas et al. 2017, Lopez-Obando et al. 2016). If so, most of the sequence divergence that distinguishes D14 from KAI2 may be due to coevolution with D53-type SMXLs rather than acquisition of SL perception (19).

Countering this hypothesis, three *P. patens* KAI2-like proteins in the clade most closely related to angiosperm KAI2 bind, hydrolyze, and are structurally destabilized by (-)-5-deoxystrigol, which has a stereochemical configuration not found in any known SLs (18). Three other PpKAI2-like proteins bind KAR_1 *in vitro*, and a fourth does not bind either class of chemicals (18). Because responses to natural SLs or the SL precursor carlactone were not found among these proteins, it raises questions of whether one of the remaining four PpKAI2-like proteins, which were not amenable to purification, functions as a SL receptor, whether PpCCD8 produces an atypical carlactone stereoisomer, or whether there is an alternative mechanism for SL perception in moss. To resolve this it would be useful to determine whether the phenotype of *Ppccd8* mutants is restored by enantiomerically pure SLs with natural or unnatural configurations, and whether knockout of any *PpKAI2-like* genes reproduces a *Ppccd8* phenotype.

Another challenge to an angiosperm-like SL signaling model in moss is that *max2* mutants in *P. patens* have phenotypes that are very different or potentially opposite to SL-deficient *ccd8* mutants. While this might be explained as a complex phenotype resulting from the loss of both KL and SL signaling, as found in Arabidopsis *max2* plants, it is striking that the *Ppmax2* mutant retains responses to *rac*-GR24 (81). This may indicate that MAX2 does not carry out SL-induced turnover of SMXL proteins, or that its role in this process is only auxiliary.

The contribution of KARs to land adaptation is less intuitive. The capacity to respond to smoke, which encompasses KARs as well as other chemical stimulants, is widespread among the Angiosperms (97). However, for many species this may reflect the ability of KARs to substitute for an endogenous KAI2 ligand (KL) more than an adaptation for post-fire regrowth, as KAR responses are found among plants that are not considered fire followers. KAR perception is not apparent in the Bryophytes, suggesting that their KAI2 are more KL-specific. This is seen in *P. patens*, which has not shown any obvious responses to KAR_1

treatments, despite having KAI2-like proteins that bind KAR_1 in vitro (18, 56). Also, a *KAI2* gene from *Selaginella moellendorfii* is able to partially or fully rescue some phenotypes of an Arabidopsis *kai2* mutant, and yet is not responsive to KARs or GR24 (153). Therefore, a better question may be how KL (or at least KAI2 activity) contributed to land adaptation? KAI2 has functions in seed germination, seedling photomorphogenesis, leaf shape, drought resistance, and abiotic stress tolerance in Arabidopsis (79, 126, 149, 152). As the Physcomitrella *max2* mutant has photomorphogenesis defects, it is possible that KAI2 has a conserved role in light regulated growth throughout land plants (81). Another interesting possibility is that an ancestral function of KAI2 was to enable interactions with AM fungi, as KAI2 is required for AM symbiosis in rice (51). This symbiosis is used by more than 80% of land plants, including Bryophytes, and it is conceivable that KAI2-dependent processes have contributed to its adoption and establishment.

EVOLUTIONARY TRENDS AND DIVERSIFICATION IN SCF-MEDIATED HORMONE SIGNALING

The origin of the different SCF-based hormone response pathways can be intuitively linked to transitions in plant life history, although causality is unclear. A prominent property of each of the hormone pathways discussed here is the diverse functions that are controlled by each hormone in flowering plants. A key question is how this divergence came to be. Here, we focus on the steps in pathway evolution that led to increases in response diversity.

In the auxin response system, all diversity seems to be contained within the TIR1/AFB, Aux/IAA and ARF protein families. An important question is whether the complexity of the auxin response system, as derived from genome/transcriptome information, is predictive of a species' response properties. Many species, even beyond the green lineage, were shown to physiologically respond to auxin (7, 14, 35, 74). Surprisingly, two studies showed that, despite the absence of a full nuclear auxin response system, two Charophycean algal species readily respond to auxin (94, 101), both activating and repressing hundreds of genes within an hour (94). Thus, while specificity of such responses to the chemical structure are not clear and may represent a general response to amino acid derivatives, there are clearly unexplained aspects of auxin response that are revealed by studies in early plant lineages. In a landmark study, all three Aux/IAA genes in Physcomitrella (105) were deleted, and transcriptome analysis demonstrated that no transcriptional auxin response could be detected in such mutants (75). Thus, the widespread Aux/IAA-independent auxin-regulated transcription in Charophytes was clearly lost in land plants, along with the gain of a specific response system.

Among land plant species that have a nuclear auxin response system, there are clear differences in system complexity. Duplications within each response component family increased the complement of response components during land plant evolution. Significant expansion occurred both in the ancestor of vascular plants and in the ancestor of flowering plants. A prediction would be that complexity of the auxin response system determines the output both qualitatively and quantitatively. Comparing each species' genomic auxin response component complement with auxin response output (number of genes, activation

versus repression, amplitude of regulation) across four land plant species revealed that indeed, ARF number positively correlates with the number of auxin-regulated genes. Furthermore, a switch from dominant gene repression to gene activation is correlated with a gene duplication in the A-class ARF subfamily (94). Finally, the high amplitude of gene regulation that is typical for many auxin-regulated genes in flowering plants (1, 142) seems to be associated with more efficient repression in the absence of auxin, rather than more activation in the presence of auxin, and correlated with duplications of Aux/IAA and TIR1/AFB genes (94). Thus, comparisons among species reveal the design principles of the auxin response system.

Given the deep origin of the auxin response system in the ancestor of all land plants and the regulation of similar cellular processes, a key question is whether the same genes are regulated by the system across land plants. The amount of transcriptome data is limited, even within the flowering plants. A comparative transcriptomic experiment including three Bryophyte species and a fern, however, revealed that all of these species share a small set of auxin-regulated genes that appear to have acted in a module since the origin of land plants. These genes include both activated and repressed ones, and both encompass developmental regulators (WIP and HD-ZIP transcription factors, Expansin) and feedback regulation of auxin levels (YUC enzyme) (94).

An interesting aspect of deep evolutionary analysis is that progressive steps towards later diverging plants such as angiosperms have not only led to increases in complexity, but also to losses of ancestral components. Indeed, there are several clades in the ARF and TIR1/AFB families that were lost in Brassicaceae, and are thus not present in the Arabidopsis genome (94). In addition, however, there is a non-canonical member of the ARF family (ncARF) which lacks a DNA-binding domain that arose in the Bryophyte ancestor and was lost in the common ancestor of ferns. This ncARF has a positive role in auxin response and auxin-dependent gene regulation in Marchantia (43, 94), and thus represents a relevant component in these species that would not have been predicted from the angiosperm-based knowledge.

The overall trajectory of JA response system evolution may have closely followed the one described for auxin. The DNA-binding transcription factor controlled by JAZ, MYC, belongs to the bHLH family, which itself is ancient and not limited to plants (85). Genes with close homology to MYC are found in algal genomes, although it remains to be seen if the JAZ-interacting domain is likely to allow JA-regulation (57, 99). Nonetheless, it seems that also in this case, JA signaling evolved to regulate a pre-existing transcription factor. In this pathway, expansion mostly occurred in the JAZ family, while COI1 seems to be present as a single copy gene in all genomes surveyed. The MYC transcription factor has undergone limited duplications (40), and thus the diversity of JA responses is likely driven by diversification of JAZ proteins. There has not yet been a systematic analysis of JAZ gene function and functional diversification, but the presence of substantial primary sequence differences within the angiosperm JAZ family (8) suggests a role in mediating specific JA responses.

How has the evolution of the different GA signaling elements shaped the pervasive functions of this hormone in plant development and defense? Although a full answer is still missing, comparative analysis

of GID1 and DELLA activity in different plants has provided a few clues. While functional diversity of auxin and JA has been associated with the presence of multiple paralogs of signaling elements performing different roles, this does not seem to be the case with DELLAs because the actual number of *DELLA* genes per genome is not associated to different levels of functional diversification. For instance, the *Solanum lycopersicum* and *Oryza sativa* genomes harbor only one copy of a *DELLA* gene (*PROCERA* and *SLENDER RICE*, respectively) (60, 63), while Brassicaceae tend to have five members (*GAI*, *RGA*, *RGL1*, *RGL2* and *RGL3* in Arabidopsis) to perform the same functions as in tomato and rice (103, 122). Multiplication of *DELLA* genes in Arabidopsis has resulted in subfunctionalization linked to the expression patterns of the particular genes, and not to differential ability of corresponding DELLAs to recognize partner TFs (46). A similar logic can be applied to GID1, with only one copy in monocot genomes, and three in Arabidopsis (61, 141, 157). In this case, the different DELLAs in Arabidopsis display differential affinities for the three GID1 receptors (131), providing a mechanism by which certain changes in GA levels might preferentially affect the stability of only a subset of DELLA proteins. Given that this regulation would not explain the multiplicity of processes regulated by GAs, functional diversification must rely on the promiscuous capacity of DELLAs to interact with large sets of TFs that serve different functions. In other words, GAs would regulate as many processes as those under the control of DELLA partners. This is for instance supported by the species-specific interactions found between DELLAs and legume-specific nodulation TFs (44, 65, 104). On the other hand, although there is no experimental evidence that confirms that the conservation of DELLA promiscuity is a beneficial trait selected for during plant evolution, comparative *in silico* network analysis of putative DELLA targets in species with GA-regulated DELLAs (Arabidopsis and *S. lycopersicum*), GA-independent DELLAs (*P. patens*), and without DELLAs (*Chlamydomonas reinhardtii*) suggests that DELLAs and GAs have gradually increased the coordination of expression between transcriptional circuits (17).

Diversification of MAX2-associated signaling has occurred through its receptor partners and proteolytic targets, as *MAX2* itself is typically present as a single copy in land plants. *KAI2* is prone to gene duplication, unlike *D14*, which is usually maintained as a single copy in seed plant genomes. Although Arabidopsis only has one *KAI2*, it is common to observe two or more copies of *KAI2* in angiosperm genomes, with lineage-specific amplifications often occurring (19, 27). Whether various *KAI2* paralogs have functional differences largely remains to be determined but, as noted above, a dramatic expansion of the *KAI2* family in the parasitic Orobanchaceae has given rise to changes in ligand specificities for these receptors (27). Other homologs of *KAI2* and *D14*, such as *D14-LIKE2* (*DLK2*), are found in seed plants, but their roles are unclear. *DLK2* has a very weak ability to hydrolyze an unnatural stereoisomer of the SL analog GR24, and does not have a conserved MAX2 interface (145). A phenotype for *dlk2* loss-of-function mutants has remained elusive, although overexpression of *DLK2* can influence seedling growth (145, 152).

The seed plant lineage began with two *SMXL* types, *SMAX1* and *SMXL4*. Expansion at the base of the angiosperms gave rise to the *SMXL7/8* and *SMXL3/9* subclades, which are derived from *SMAX1* and *SMXL4*, respectively. These four groups are found in all angiosperms, and further divergence at the base

of the eudicot lineage led to the *SMXL7* and *SMXL8* subclades and *SMXL3* and *SMXL9* subclades. Duplications of *SMAX1*, *SMXL7*, and *SMXL4*, and loss of *SMXL9* within the Brassicaceae have produced the complement of eight *SMXL* genes found in *Arabidopsis thaliana* (146). *SMAX1* and *SMXL2* are partially redundant regulators of KAR/KL responses, with *SMAX1* playing a more substantial role in germination and seedling growth (127, 128). *SMXL6*, *SMXL7*, and *SMXL8* all contribute to branching regulation, but *SMXL7* and *SMXL6* account for most of this activity (126, 148). There is no evidence that SMXL3, SMXL4, or SMXL5 are regulated by either KAR/KL or SL pathways, and indeed these proteins lack a motif that has been associated with MAX2-dependent proteolysis. These three genes have partially redundant functions in phloem formation, and the triple mutant is lethal after the seedling stage (147). Beyond this, it is unclear how diversified SMXL functions may be within each of these major groups.

How has chemical diversity contributed to hormone signaling evolution?

Studies in plants and animals have provided evidence for the co-evolution of hormone metabolism and signaling networks and have hinted to its biological relevance (155). In the case of SCF-based hormone signaling, the hormones involved display striking differences in the degree of complexity of the biologically active molecules. For instance, while chemical diversity among auxins is limited to four relatively similar compounds whose synthesis involves only a few steps, around 100 GA molecules and at least 23 canonical SLs are found in plants. Therefore, several questions deserve some attention from an evolutionary perspective.

Is chemical diversity associated to the number or type of functions exerted by the different hormones? It does not seem to be the case, given that auxin is involved in as many developmental stages as GAs. As in the case of auxin, functional diversification in GA action relies on the molecular features of their signaling pathway, rather than each GA molecule triggering differential responses. First, only a small fraction of the known GA molecules are biologically active, and second, there is no clear evidence for any given response to GAs that cannot be induced by either one of the known active molecules. A similar situation is found for SLs, in which chemical modifications of the core structure, e.g. hydroxylation or acetoxylation, impact their activity on parasitic weed germination and branching of arbuscular mycorrhizal fungi.

Is chemical diversity associated to specific features of the hormone perception modules? It is likely that the higher degree of chemical diversity in GA and SL reflects evolutionary constraints in auxin and JA metabolism imposed by the mechanistic nature of the perception module – a co-receptor in the case of auxin and JA, while GA and SL perception depends on single receptor proteins. In support of this idea is also the observation that the complexity in GA and SL metabolism has only increased during evolution. GAs are divided into two classes based on the number of carbon atoms: C20-GAs and C19-GAs, in which C20 has been replaced by a gamma-lactone ring. GA biosynthesis occurs in three steps: (i) formation of ent-kaurene and kaurenoic acid in the proplastids; (ii) formation of GA_{12} in the endoplasmic reticulum by kaurenoic acid (KA) oxidase; and (iii) formation of active GA in the cytosol by successive oxidation steps.

Non-vascular land plants lack bioactive GAs and there are no homologs of KA oxidase in *P. patens* (53, 89). Although *ent*-kaurene-deficient *P. patens* mutants (*Ppcps/ks*) showed limited protonemal cell differentiation of chloronemata to caulonemata –which are fast-growing cells that produce gametophores–, the application of KA, but, not of GA, rescued the phenotype. Subsequent work has identified the nature of the actual KA-derived bioactive molecule in moss, as 3β-hydroxy-kaurenoic acid (3OH-KA) (88). However, the lack of GID1 receptor homologs in moss makes it unlikely that this compound acts through the canonical GID1-DELLA pathway operating in vascular plants.

The emergence of GID1 GA receptors required the recruitment of a specific Y residue in the active center of ancestral carboxylesterase, to establish the interaction with hydroxylated C3 in bioactive GAs (93, 120), and the discrimination between active and inactive GAs gradually increased during evolution, as indicated by the observation that the Lycophyte GID1 receptor binds both the active GA_4 and the inactive GA_{34} or GA with similar efficiencies (169). Therefore, evolution has operated towards more specific discrimination between active and inactive GAs, but additional GA-evolution mechanisms have developed in specific taxa, such as the lack of the C13-OH related GA molecules in Lycophytes (55) or the spatially separated synthesis of the GA precursor antheridiogen (Methyl-GA9) and the bioactive GA4 moiety (133).

A similar case is postulated for SLs. Canonical SLs share a tricyclic ABC-ring structure connected by an enol-ether bond to a butenolide D-ring in a 2'*R* configuration. More than 23 canonical SLs have been discovered. These molecules vary in the side chain configurations of the ABC-ring and are divided into strigol- and orobanchol-type classes based on the stereochemical configuration of the BC-ring (167). In addition, several non-canonical SLs have been identified that lack the conventional ABC-ring structure but retain the D-ring and SL-like activity. These include the biosynthetic precursor of SLs, carlactone (CL), and its derivatives, carlactonoic acid (CLA) and methyl carlactonoic acid (MeCLA) (2, 16).

Plants vary in the types and amounts of SLs they synthesize. Tobacco, for example, produces 11 canonical SLs of both stereochemical types (158). Some species, such as *Physcomitrella patens* and *Arabidopsis thaliana*, neither of which are mycotrophic, probably produce only non-canonical SLs (167). Remarkably, the composition of even major SL types can vary within a single family. Among the Poaceae, for example, Sorghum normally produces strigol-type SLs, rice makes only orobanchol-type SLs, and maize produces non-canonical SLs.

The evolutionary history of SL diversification remains murky, in part because the biosynthetic pathways have not been fully defined. *Selaginella moellendorfii* makes orobanchol-type SLs, as do many of the Angiosperms tested so far, but it is not clear that orobanchol-type SLs are an ancestral molecule from which strigol-type SLs evolved (167). A cytochrome P450, MAX1, plays an important role in SL biosynthesis steps after CL. Typically, MAX1 converts CL to CLA. In some species, such as rice and *S. moellendorfii*, MAX1 paralogs are also able to catalyze reactions that produce the canonical SLs 4-deoxyorobanchol or orobanchol, while in others, such as tomato, unknown enzymes must carry out these final steps (167, 172, 173). A sulfotransferase, LOW GERMINATION STIMULANT 1 (LGS1), has recently been implicated in

strigol production in Sorghum, but it remains to be determined how it does so and whether this function is evolutionarily conserved (47).

The biological significance of the diversified SL family is also unclear. In angiosperms the SL receptor D14 is typically maintained as a single copy gene under strong purifying selection (27). While it is possible that many SLs with different affinities for D14 have emerged as a way to fine-tune SL signaling activity, much simpler evolutionary paths to achieve the same regulatory effect can be imagined. Instead, SL diversification may have been driven by selective pressures imposed by AM fungi and parasitic plants. Most canonical SLs have been identified in exudates from phosphate-starved roots, usually through parasite germination bioassays, and it is not known which of these molecules have significant roles as internal hormones. It is possible that non-canonical SLs such as CLA, which appears to be found broadly in land plants, serve the hormonal role (168). Canonical SLs, which have higher stability and lower diffusibility in soil, might act primarily as rhizosphere signals to AM fungi (168). The very low abundance of SLs in plant tissues makes evaluation of this hypothesis difficult to achieve. However, a highly sensitive *in vivo* assay for SL activity has demonstrated that D14 in Arabidopsis has selective responses to applied canonical SLs (112). It would be very interesting to use this assay to compare how D14 responds to the non-canonical SLs that Arabidopsis actually makes, or to examine the ligand preferences of D14 in species that make both canonical and non-canonical SLs.

The diverse SL profiles of host plants seem likely to have driven expansion of the clade of KAI2 receptors for SL that evolved in parasitic plants. Limited sampling of the Orobanchaceae has so far shown that specialist parasites tend to have fewer KAI2d, while generalists such as *Striga* spp. have many (27). This correlation may indicate a link between KAI2d diversity and the ability to detect different SLs. Supporting this idea, a range of affinities for different SLs has been demonstrated for several KAI2d from *Striga hermonthica* (137, 139).

SUMMARY POINTS

- Building upon major contributions from genetic and biochemical approaches, plant hormone biology has now entered the era of bioinformatics and evolutionary biology.

- While pathways mediating responses to several plant hormones are based upon an SCF complex receptor, both the origin and diversification patterns in each pathway are different

- Diversification in the Gibberellic Acid and Strigolactone pathways occurs at the level of the chemistry of the hormone itself, but in the case of Auxin and Jasmonic Acid, protein signaling intermediates are the subject of diversity

- Expansion of hormone signaling capacity has been a driver of innovations in plant life history and anatomical traits

- While the use of model species has been instrumental in understanding the generic workings of hormone response pathways, caution must be exercised in inferring evolutionary histories based on limited numbers of species.

FUTURE ISSUES

- Obtaining genomic data from many plant lineages is not a limiting step anymore: future efforts should focus on developing broadly accessible analytical tools that can extract the information relevant to evolutionary analysis.

- Hormone signaling evolution studies need to advance beyond phylogenomics and incorporate structural and (bio-)chemical approaches in order to understand genetic, as well as chemical, evolution.

- More efforts are needed in establishing experimental tools for species in critical clades that allow hypothesis testing using molecular genetics. Examples are Charophycean algae, hornworts, Lycophytes, ferns and early diverging Angiosperms.

- A central question is to what extent increased complexity of plant architecture follows from increased complexity of hormone signaling.

- Pathways have accumulated complexity, but it needs to be determined how much of that is irreducible and intrinsic to hormone signaling.

- Future studies should bridge plant hormone evolution with ecology to address how plant-plant and plant-microbe interactions shape innovations through the different hormone signaling pathways.

GLOSSARY

Ubiquitin - a highly conserved 76 aa eukaryotic protein that is covalently attached to other proteins as monoubiquitin or polyubiquitin chains

Co-receptor - a protein that requires another protein partner to bind a ligand and transduce a signal

Phylogenetics - an analytical approach used to infer evolutionary relationships between organisms or genes based on morphological traits or genetic sequences

Necrotrophic - an organism that kills cells of its host and feeds off of them

bHLH - basic helix-loop helix, a structural motif found in proteins that typically function as dimeric transcription factors

transcription factor - a protein that influences the transcription of genes by binding to specific cis-regulatory DNA sequences

Paralogs - homologous genes that are derived from a gene duplication event

Orthologs - homologous genes in different lineages that are derived from the same ancestral gene

F-box - an ~50 aa domain that enables interaction with Skp1 protein and recruitment into SCF E3 ubiquitin ligase complexes

Life history trait – A trait that contributes to the major changes occurring an organism during its lifetime

Extant - currently in existence

sister groups - the closest relatives within an evolutionary tree, e.g. two branches split from the same node in a phylogeny

Sporophytic - the life cycle phase following egg cell fertilization and prior to meiosis, in which chromosome numbers are double that of the gametes

transcriptome assembly - a method to produce, or collection of, the putative sequences of RNA transcripts found within a sample from alignment of short, next-generation sequencing reads

RNA-seq - RNA sequencing, a method to survey the amounts and types of RNAs present in a sample through next-generation shotgun sequencing of fragmented cDNAs

ancestral state - The inferred gene set at the time of divergence of a group of related species

Rhizoid - an outgrowth of early diverged plants with similar structure and function as roots, e.g. anchoring and nutrient/water uptake

Pre-adaptation – A case where pre-existing components are compatible with a new function that will appear only later during evolution

Sidebar 1: The OneKP initiative

Inferring evolutionary histories, conservation, and diversification patterns requires sequence information from species sampled across the plant phylogeny. Given that no single species' genome is representative of the time it departed from the common ancestor of later-evolving lineages, studies on individual model species can be deceiving. Clade-specific gene losses and gain will have shaped that species' genome, but may be different from even closely related species. To overcome this problem, the international One Thousand Plants (OneKP) consortium (onekp.com) has generated RNA-seq-based transcriptomes for more than a thousand species broadly sampled from the plant phylogeny. While the quality of individual transcriptomes is perhaps limited, and despite the fact that not all genes will be expressed, the sampling of multiple species per group (e.g. 21 Lycophytes, 421 Mosses, 10 Hornworts, 27 Liverworts) allows one to infer the "archetype" of a gene family at the time of divergence. The RNA-seq data have been publicly released (http://www.onekp.com/public_read_data.html), and have been used to analyze plant phylogeny placement models (156), as well as specific gene families such as LEAFY (114), PINs (11), auxin response (94) and SL and KAR response (Bythell-Douglas et al., 2017; Walker and Bennett, 2018) components.

Sidebar 2: F-box proteins in hormone signaling evolution

The prominent role of F-box proteins in several hormone signaling mechanisms raises questions about why proteolysis-based signaling mechanisms were favored during plant evolution. One possibility may be that extensive duplications of F-box genes have provided ample opportunities for neofunctionalization. The F-box protein superfamily varies dramatically in size across land plants. Whereas 241 are found in *Physcomitrella patens* and the grape genome only has 156 F-box proteins, Arabidopsis, rice, and Medicago genomes contain from ~700 to ~900 F-box proteins (59, 160, 163). The high degree of variability in F-box protein numbers in different plants is one sign of the lineage-specific expansions that have occurred for many F-box protein clades. The F-box protein superfamily can be divided into different groups based upon the C-terminal domain. Among the most prominent families are those with C-terminal Kelch repeats, or FBA domains that appear to have been derived from Kelch repeats. TIR1 and the five AFBs, COI1, MAX2, and the ethylene regulators EBF1 and EBF2 are all members of a 23-gene (in Arabidopsis) subfamily of proteins with C-terminal leucine-rich repeats (LRR_7), which is well conserved throughout land plants (160). The presence of signs of purifying selection in F-box domains and signatures of adaptive selection in portions of the C-terminal domain suggest a means by which new protein targets may be acquired (96, 116).

Figure Legends

Figure 1: SCF-based plant hormone response systems

Cartoons describing the mode of response to four plant hormones (Auxin [Indole-3-Acetic Acid], Jasmonic Acid, Gibberellic Acid [GA_3], Strigolactone [Orobanchol]) and Karrikin [KAR_1] of which structures are shown on the top. Uninduced situation is shown on the top in red, hormone-induced situation is shown in green in the bottom. SCF complex subunits are in grey shade, F-box proteins in yellow, receptors in blue, unless receptors are F-box proteins, in which case they are yellow/blue striped. In both Auxin and Jasmonic Acid cases, the hormone (black hexagon) binds F-box protein and degradation target (red) to promote degradation, thus releasing the TPL-mediated inhibition of the transcription factor (green). In the case of Gibberellic Acids, Strigolactone and Karrikin, the ligand changes conformation of the receptor, which facilitates binding of the degradation target to the F-box protein. This allows transcriptional regulation by the transcription factor. For Gibberellic Acid, the DELLA degradation target may act both as a positive and as negative regulator of transcription factor activity. In the case of Strigolactone and Karrikin, there may be non-transcriptional effects through yet unknown interactors of the SMXL degradation target.

Figure 2: Evolutionary history of SCF-based plant hormone pathways

Top: lineages of extant plants in the order they appeared in evolution (from left to right). The major transitions in life history traits that emerged with each extant group are indicated above each group name. The tables indicate when components in each hormone response pathway (as well as the hormone itself) emerged. If multiple copies of each component are shown, this indicates duplications that are associated with the emergence of that group. The Target TF for SL and KAR is shown in dashed line because its identity is not yet entirely clear. The different shades of brown for Target TF in the GA pathway signify the multiple TF families that are targeted by DELLA proteins.

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