REVIEWS

Recent advances and emerging trends in plant hormone signalling

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Plant growth and development is regulated by a structurally unrelated collection of small molecules called plant hormones. During the last 15 years the number of known plant hormones has grown from five to at least ten. Furthermore, many of the proteins involved in plant hormone signalling pathways have been identified, including receptors for many of the major hormones. Strikingly, the ubiquitin–proteasome pathway plays a central part in most hormone-signalling pathways. In addition, recent studies confirm that hormone signalling is integrated at several levels during plant growth and development.

Because plants have a sessile lifestyle, they must adjust to numerous external stimuli and coordinate their growth and development accordingly. The plant hormones, a group of structurally unrelated small molecules, are central to the integration of diverse environmental cues with a plant's genetic program. The 'classical' phytohormones, identified during the first half of the twentieth century, are auxin, abscisic acid, cytokinin, gibberellin and ethylene[1]. More recently, several additional compounds have been recognized as hormones, including brassinosteroids, jasmonate, salicylic acid, nitric oxide and strigolactones[2–7] (Table 1). Plants also use several peptide hormones to regulate various growth responses, but this class of hormones is beyond our scope here[8]. With the application of genetic approaches, mainly in *Arabidopsis thaliana*, many aspects of hormone biology have been elucidated. Most hormones are involved in many different processes throughout plant growth and development[1]. This complexity is reflected by the contributions of hormone synthesis, transport and signalling pathways, as well as by the diversity of interactions among hormones to control growth responses.

Genetic screens resulted in the identification of many of the proteins involved in hormone signalling and the analysis of these proteins has contributed significantly to our current models of hormone action. One particularly exciting outcome is the recent identification of receptors for auxin[9–11], gibberellin[12], jasmonate[13–15] and abscisic acid[16] (Fig. 1). Though far from complete, our improved understanding of hormone perception and signalling has allowed for comparisons between hormones. From these it is clear that some hormones (cytokinins, ethylene and the brassinosteroids) use well-characterized signalling mechanisms[17]. On the other hand, the identification and characterization of the auxin and jasmonate receptors, as well as proteins in gibberellin signalling, have highlighted a novel mechanism for hormone perception in which the ubiquitin–proteasome pathway has a central role[9–11,15,18].

In addition to these advances, the comparison of hormone signalling pathways between evolutionarily tractable members of the plant kingdom has yielded some important insights into the conservation and evolution of hormone signalling pathways. These comparisons have been facilitated by large-scale genome-sequencing projects such as those of *Physcomitrella patens* (moss), *Selaginella* (fern), *Arabidopsis thaliana* and *Oriza sativa* (rice). For example, the moss genome (an ancient plant ancestor) encodes proteins that function in auxin, abscisic acid and cytokinin signalling, whereas the genome of green algae does not, suggesting that these pathways emerged when plants were colonizing land[19,20]. In contrast, a comparison of the moss genome with more recently diverged plant genomes suggests that signalling mechanisms for gibberellin, ethylene and the brassinosteroids probably did not evolve until after the evolutionary split of moss and vascular plants[19,20]. These observations will be expanded as additional hormone signalling components are identified and more genome sequences become available.

This is an exciting time in the field of plant hormone biology because our knowledge of hormone biosynthesis, metabolism, transport, perception, signalling and response has grown exponentially over the past few years. As a result, recent reviews have been written for individual hormones covering topics from metabolism and transport to signalling[21–28]. Here, we review some of the advances in plant hormone signalling. We focus on newly identified hormone receptors and the broad role of regulated protein turnover in plant hormone

Table 1 | Plant hormone receptors

Hormone	Receptor type	Receptors	References
Auxin	F-box protein	TIR1, AFBs	9–11
Abscisic acid	G-protein, Chelatase	GTG1, GTG2, GCR2*, CHLH*	16, 55, 58
Cytokinin	Two-component regulators	CRE1, AHK2, AHK3	Reviewed in ref. 17
Gibberellins	Hormone-sensitive lipase like	GID1	12
Ethylene	Two-component regulators	ETR1, ERS1, ETR2, EIN4, ERS2	Reviewed in ref. 17
Brassinosteroids	Leucine-rich repeat receptor-like kinases	BRI1	Reviewed in ref. 17
Jasmonic acid	F-box protein	COI1	13–15
Salicylic acid	Unknown		
Nitric oxide	Unknown		
Strigolactones	Unknown		

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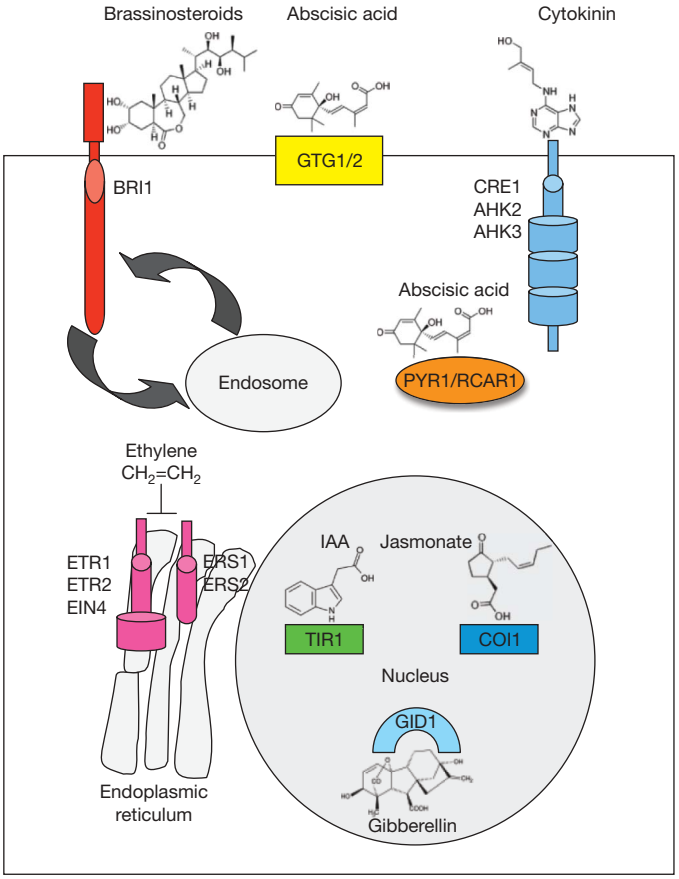

Figure 1 | Sites of plant hormone perception. BRI1 is a membrane-associated receptor that cycles between the plasma membrane and endosomal compartments. The extracellular leucine-rich repeat domain binds brassinosteroids and transduces the signal through an intracellular kinase domain. GTG1 and GTG2 are GPCR-type G proteins that bind abscisic acid. They have inherent GTPase activity but also interact with the only canonical Gα subunit in *Arabidopsis*. PYR1/RCAR1 is a soluble ABA receptor that represses PP2C phosphatases in the presence of ABA. The cytokinin receptors CRE1, AHK2 and AHK3 are plasma-membrane-associated and perceive cytokinin through their extracellular domains. Cytokinin binding triggers a phosphorylation cascade that is ultimately transmitted to response regulators in the nucleus. Like the cytokinin receptors, the known ethylene receptors are two-component regulators. All five receptors are active in the endoplasmic reticulum and transmit their signal through a common downstream component called CTR1. TIR1 and COI1 are F-box proteins that are integral components of SCF-type E3 ligases and recognize the plant hormones auxin and jasmonic acid respectively. GID1 is a nuclear-localized receptor for gibberellins. Gibberellin binding to GID1 results in the enhanced degradation of DELLA proteins.

signalling pathways. We also discuss some of the ways that hormone pathways are integrated during plant growth and development.

Auxin perception by a new class of receptor

Auxin is crucial in regulating plant growth and development from embryogenesis through maturity. As were most hormone signalling proteins identified in plants, the auxin receptors were first found through mutant screens. In this case the screen was for *Arabidopsis* seedlings with an altered response to auxin or auxin-transport inhibitors. Many of the auxin-resistant mutants identified in this way are disrupted in components of the Skp1/Cullin/F-box (SCF) ubiquitin ligases (E3) or in proteins that regulate SCF activity[29].

The E3 ligases are the last enzymes in the ubiquitin–protein-conjugation pathway and confer specificity to the pathway. In the case of SCF-type E3 ligases, the F-box protein interacts directly with the substrate and thus determines the substrate specificity of the complex[30]. SCFs were first implicated in auxin signalling with the identification of an F-box protein called TIR1. Recessive mutations in *TIR1* confer auxin resistance, implying that the protein is required for degradation of negative regulators of auxin response[31]. A key event in the characterization of the auxin-signalling pathway was the discovery that SCF^{TIR1} is directly linked to auxin-regulated transcription[32].

The auxin transcriptional response is controlled by two large families of transcription factors; the auxin/indole-3-acetic acid (Aux/IAA) proteins and the auxin response factors (ARFs) (of which *Arabidopsis* has 29 and 23 members respectively). ARFs bind the promoters of auxin-responsive genes and either activate or inhibit transcription depending on the type of ARF[33]. The Aux/IAA proteins bind to the ARFs through shared domains in both proteins called domains III and IV and repress auxin-regulated transcription[34]. Importantly, the Aux/IAA proteins are short-lived; their degradation is promoted by auxin and dependent upon TIR1. Many gain-of-function mutations in *Aux/IAA* genes have been isolated and in every case the mutations affect residues within a highly conserved region called domain II[34]. Biochemical studies demonstrated that domain II binds TIR1 and that this binding is enhanced by auxin[32,35,36].

Although these results suggested a mechanism for auxin-dependent de-repression of transcription, how auxin promotes the SCF^{TIR1}–Aux/IAA interaction remained unclear. Ultimately, TIR1 itself was shown to bind biologically active auxins directly and specifically[9,11]. Auxin binding to TIR1 increases the stability of the TIR1–Aux/IAA complex. Structural studies of TIR1 in the presence of auxin and a peptide encompassing domain II revealed how auxin promotes Aux/IAA degradation[37]. A single hydrophobic pocket on the surface of the leucine-rich repeat domain of TIR1 binds both auxin and the domain II peptide[37]. Auxin binds to residues at the base of this pocket and contributes to binding of the Aux/IAA protein[37]. Domain II of the canonical Aux/IAAs interacts with TIR1 residues directly above auxin, filling the remainder of the pocket[37]. One important implication of the structure is that both TIR1 and the Aux/IAAs appear to contribute to high-affinity binding of auxin. In this sense, it may be more appropriate to call TIR1 and the Aux/IAA protein co-receptors. If true, this also implies that different combinations of F-box protein and substrate may have unique auxin-binding characteristics.

Auxin research has a long history and the discovery that TIR1 functions as an auxin receptor was groundbreaking in several respects. The work indicates that F-box proteins, and perhaps other E3 ligases, can function as receptors for small molecules. Indeed, studies have demonstrated that this is probably true (see jasmonate signalling below). Further, the discovery that a small molecule can significantly enhance the interaction between an E3 and its substrate presents a new strategy for the development of drugs that target the ubiquitin–proteasome pathway[38]. Finally, detailed knowledge of auxin receptor function may stimulate the development of new plant growth regulators[39].

Recent results have also shed new light on the mechanism of Aux/IAA repression. Earlier studies showed that conserved domain I in the Aux/IAA proteins is required for transcriptional repression but the mechanism of repression was unclear[40]. Domain I of most Aux/IAAs contains an ethylene-response-factor-associated amphiphilic repression motif[40]. In 2008, a protein called TOPLESS (TPL) was shown to associate with domain I of the Aux/IAA protein IAA12 and to function as a transcriptional co-repressor[41]. These findings support an updated model in which the Aux/IAA proteins act as repressors of ARF-mediated transcription by recruiting TPL or related transcriptional co-repressors to the multi-protein complex[41]. Auxin de-represses transcription by promoting ubiquitination and subsequent degradation of Aux/IAA proteins through the action of SCF^{TIR1}. Without the Aux/IAA proteins, TPL is no longer associated with promoters of auxin-regulated genes (Fig. 2). Important questions about this model still remain. For example it is not known whether SCF^{TIR1} interacts with the Aux/IAA while in a complex with TPL and an ARF, with an ARF alone, or perhaps by itself. In addition, each of the relevant proteins is part of a large family (6 TIR1/AFBs, 29 Aux/IAAs, 23 ARFs, 5 TPL/TOPLESS

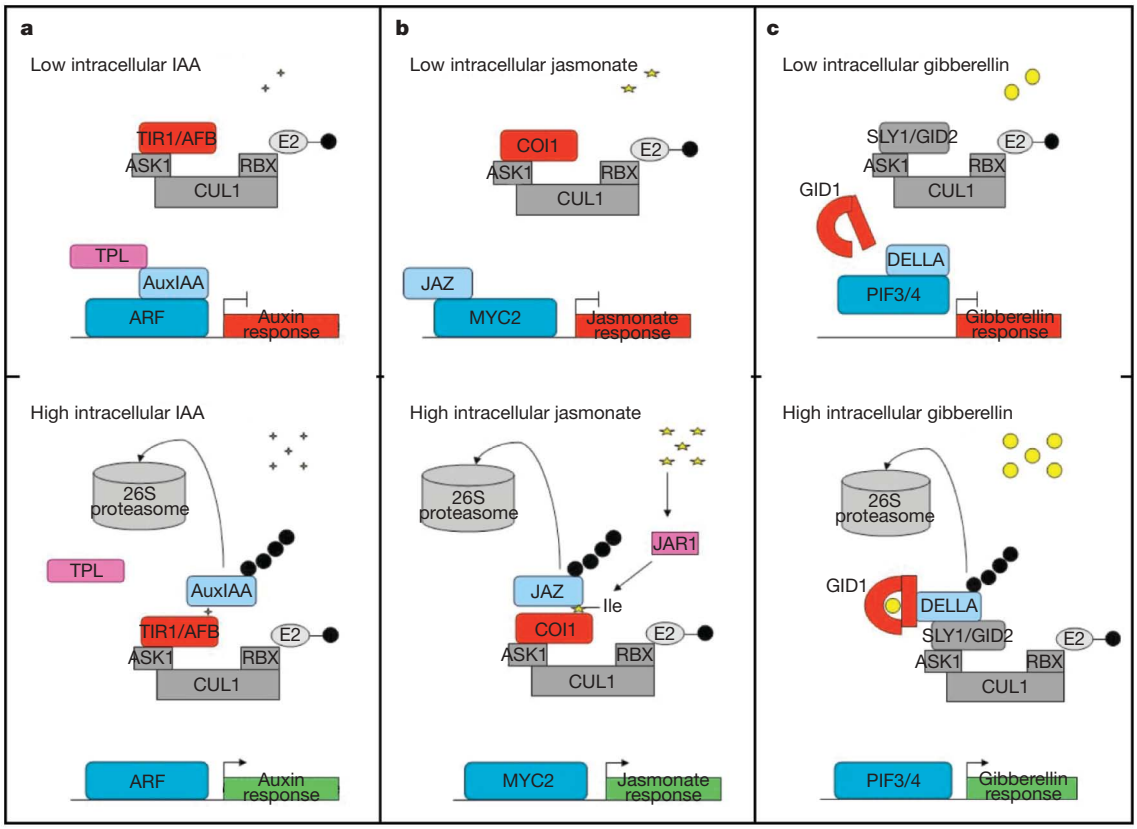

Figure 2 | SCFs are required for auxin, jasmonate and gibberellin signalling. **a**, The TIR1/AFB family of F-box proteins are auxin receptors. TIR1 is a component of the SCF complex that also consists of ASK, CUL and RBX. Auxin binding stabilizes the TIR1-AUX/IAA complex, resulting in degradation of the AUX/IAAs, which in turn releases TPL and permits ARF-dependent transcription. **b**, Binding of JA-Ile to COI1 promotes JAZ binding and ubiquitination. This results in de-repression of MYC2-dependent transcription of jasmonate-responsive genes. **c**, Gibberellin binding to the GID1 receptor promotes GID1–DELLA complex formation. GID1–DELLA binding promotes the interaction between the C terminus of the DELLA protein and $SCF^{SLY1/GID2}$. Degradation of the DELLAs promotes the release of PIFs, thus permitting DNA-binding gibberellin responses. In each panel, the hormone receptor is coloured red, the substrate protein is light blue, symbols representing hormones are yellow and components of the ubiquiting proteasome pathway are in grey. Black circles represent ubiquitin.

RELATEDs) and the potential specificity of interactions between different family members is just beginning to be explored.

Jasmonate perception is similar to that of auxin

It is now clear that TIR1 and its closest relatives the auxin-signalling F-box proteins (AFBs) serve as receptors for auxin[10]. There are roughly 700 F-box proteins encoded by the *Arabidopsis* genome. This raises the question: do any other F-box proteins function as receptors for plant hormones? Recent findings on jasmonate perception and signalling strongly suggest that jasmonate and auxin share a conserved mechanism of hormone sensing and response.

The oxylipin jasmonic acid and its metabolites, collectively known as jasmonates, are important plant signalling molecules that mediate biotic and abiotic stress responses as well as aspects of growth and development[23]. One of the mutants that helped define the role of jasmonate in plant growth is the *Arabidopsis coronatine-insensitive1* (*coi1*) mutant[42]. Coronatine is a phytotoxin that is structurally and biologically related to jasmonate. The *coi1* mutant is resistant to both coronatine and methyl jasmonate, and also confers male sterility[42]. Subsequent studies demonstrated that *coi1* mutants are perturbed in every aspect of jasmonate response, indicating that *COI1* has an essential role in jasmonate signal transduction. *COI1* encodes an F-box protein that is closely related to TIR1[43]. These results suggested that jasmonate response requires SCF^{COI1}-dependent degradation of repressors in much the same way as SCF^{TIR1} targets the Aux/IAAs. However, until 2007 the SCF^{COI1} substrates were unknown.

This changed when a novel family of transcriptional regulators called JAZ proteins (jasmonate ZIM-domain) was identified[13,14,44]. These groups showed that several full-length JAZ proteins are degraded in a proteasome-dependent manner following jasmonate treatment, but were stabilized in the *coi1-1* background, implicating SCF^{COI1} in the JAZ degradation pathway. Furthermore, members of the JAZ family were shown to interact with COI1 both *in vitro* and in a yeast two-hybrid test[13,14]. Indeed, subsequent biochemical analyses showed that radiolabelled coronatine binds to COI1-JAZ complexes with high affinity[15,45]. Interestingly, these studies also showed that jasmonate conjugated to isoleucine (JA-Ile) is the active molecule[45]. Taken together, the data suggest that SCF^{COI1} serves as a receptor for JA-Ile in order to stabilize the interaction between the F-box protein and its substrate.

The similarity between auxin signalling and jasmonate signalling does not end there. JAZ proteins do not have an obvious DNA-binding domain, suggesting that their effects on transcription could be indirect. Pull-down and yeast-two-hybrid assays indicate that the carboxy-terminal domain of JAZ3 (also known as JAI) interacts with sequences at the amino terminus of MYC2, a well-characterized transcription factor that modulates jasmonate-mediated transcription[13]. This raises the possibility that JAZ3/JAI directly blocks MYC2 function. JAZ3/JAI degradation via SCF^{COI1} in response to jasmonate would permit MYC2 to activate or repress downstream target genes in jasmonate signalling cascades. Several of the JAZ genes are themselves upregulated in response to jasmonate[13], indicating that a negative feedback mechanism may limit the response after jasmonate perception, again much like the auxin signalling pathway (Fig. 2).

Ubiquitination is a recurring theme

The newly identified receptors for auxin and jasmonic acid dramatically illustrate the importance of the ubiquitin–proteasome pathways in hormone signalling. However, it is also clear that targeted

protein turnover is an integral component in several other hormone signalling pathways.

Gibberellin signalling. Like auxin, gibberellins play a major role in diverse growth processes, including seed development, organ elongation and the control of flowering time[24]. Physiological data demonstrate that gibberellin responses are negatively regulated by DELLA proteins. (The DELLA proteins get their name from the conserved N-terminal DELLA domain[18].) Recent work exploring the integration of light and gibberellin signals during cell elongation has elucidated a model for DELLA-mediated growth regulation. DELLA proteins were shown to interact directly with the DNA-binding domain of two basic helix–loop–helix transcription factors (PIF3 and PIF4), sequestering them in inactive complexes[46,47]. Gibberellin accumulation destabilizes DELLAs, freeing PIF3 and PIF4 to activate the transcription of their target genes[46,47]. It is likely that DELLAs regulate the activity of several other transcription factors by this mechanism, as PIF3 and PIF4 are members of a larger subfamily of basic helix–loop–helix proteins that have similar DNA-binding domains.

Mutations within the DELLA domain of DELLA proteins result in a dominant gain-of-function gibberellin-insensitive phenotype[18]. Further, the DELLAs were found to accumulate in *Arabidopsis* and rice mutants defective in the F-box protein genes *sleepy1* (*sly1*) and *gibberellin-insensitive dwarf2* (*gid2*), respectively[48,49]. Thus, as observed in auxin and jasmonate signalling, gibberellin appears to regulate the abundance of a transcriptional repressor family by promoting ubiquitination through the activity of SCF-type ligases.

The gibberellin receptor was first identified in a genetic screen for signalling mutants in rice[12]. The *gibberellin-insensitive dwarf 1* (*gid1*) mutant is completely insensitive to gibberellin-treatment, suggesting a prominent role for the protein in gibberellin signalling[12]. The GID1 protein is nuclear-localized and binds biologically active gibberellins[12]. Three orthologous genes—*GID1a*, *GID1b* and *GID1c*—were identified in *Arabidopsis* and mutations in each gene were obtained and combined to study their contributions to the gibberellin response[50,51]. The *gid1a–c* triple mutant is gibberellin-insensitive, suggesting that all gibberellin responses require functional GID1 proteins in both rice and *Arabidopsis*[51].

The GID1 proteins interact with DELLA proteins in a gibberellin-dependent manner[12,51,52]. Furthermore, co-expression of the GID1 receptor enhances the interaction between DELLA proteins and the F-box proteins SLY1/GID2[51]. These data suggest that DELLAs are better able to interact with $SCF^{GID2/SLY}$ while in a complex with gibberellin-bound GID1. This interaction ultimately leads to ubiquitination and degradation of the DELLA repressor, thus promoting gibberellin-mediated transcription (Fig. 2).

The structures of rice GID1 and *Arabidopsis* GID1a have recently been reported and support this model[53,54]. GID1 has sequence and structural similarities to members of the hormone-sensitive lipase family. Like hormone-sensitive lipases, the GID1 primary structure forms a deep binding pocket whose access is controlled by an N-terminal flexible lid[53,54]. Gibberellin binding to GID1 probably induces the protein to adopt a compact form, with the N-terminal lid folding back over the gibberellin-binding pocket[53,54]. DELLA–GID1 interaction is achieved through three conserved motifs in the DELLA domain (DELLA, VHYNP and LExLE) that directly contact the N-terminal lid of GID1[53]. It is not yet clear how complex formation enhances the DELLA-$SCF^{SLY/GID2}$ interaction, but it may involve a conformational change in the C-terminal GRAS domain of the DELLA proteins[53].

Abscisic-acid signalling. Much recent work has elucidated putative mechanisms of abscisic-acid perception. A number of potential abscisic-acid receptors have recently been described but some of these studies have been controversial. One candidate, magnesium protoporphyrin-IX chelatase H subunit (CHLH), also known as GUN5 in *Arabidopsis*, was reported to have specific abscisic-acid-binding activity[55]. This group also presented data indicating that *Arabidopsis* plants with decreased CHLH levels have abscisic-acid-insensitive germination and stomatal aperture phenotypes, while plants overexpressing CHLH are hypersensitive to abscisic acid in these assays. However, GUN5 is an unconventional site of abscisic-acid perception because it is localized to chloroplasts[55,56]. Further, a recent study reported that barley CHLH did not bind abscisic acid and that mutants with reduced CHLH levels did not display an abscisic-acid phenotype, casting doubt on the idea that this protein is an abscisic-acid receptor[57].

The second candidate abscisic-acid receptor is a G-protein-coupled receptor called GCR2[58]. This suggestion is also controversial because the presence of a proposed transmembrane domain in GCR2 has been disputed[59]. Furthermore, genetic analysis of *gcr2* mutants failed to detect an abscisic-acid-related phenotype and some biochemical studies indicate that GCR2 does not bind abscisic acid after all[60–62]. At this point, it seems unlikely that GCR2 functions as an abscisic-acid receptor.

Three very recent papers show more promise. In a study from the Assman laboratory, another pair of G-protein-coupled receptors have been implicated in abscisic-acid response, GTG1 and GTG2[16]. Genetic evidence indicates that GTG1 and GTG2 act redundantly to mediate abscisic-acid responses during germination, flowering, root elongation and stomatal closure[16]. Perhaps more importantly, GTG1 and GTG2 were demonstrated to bind biologically active abscisic acid specifically in *in vitro* binding assays. Furthermore, the abscisic-acid binding dissociation constants of ~35 nM for GTG1 and ~41 nM for GTG2 fall within a physiologically relevant range.

Finally, two other papers present compelling evidence that a family of START proteins function as abscisic-acid receptors[63,64]. One of these studies began with the discovery of a selective abscisic-acid agonist called pyrabactin[63]. A genetic screen for pyrabactin targets led to the identification of the *PYRABACTIN RESISTANCE 1* (*PYR1*) gene, encoding a cyclase subfamily member in the START domain superfamily. Mutants deficient in *PYR1* and at least two additional members of the *PYR1-LIKE* (*PYL*) family are strongly abscisic-acid-resistant[63]. To learn more about the function of these proteins, the Cutler group[63] performed a yeast-2-hybrid screen using PYR1 as bait. They recovered HAB1, a member of a family of PP2C protein phosphatases known to regulate abscisic-acid signalling. The best characterized of these is ABI1. Strikingly, the interaction between PYR1 and the PP2C proteins depends on abscisic acid. Further, the PYR1 binding inhibits the phosphatase activity. At the same time, the Grill group were screening for ABI1 interacting proteins in yeast[64]. They identified a protein called RCAR1, which turned out to be identical to PYL9. Like PYR1, RCAR1 interacts with ABI1 in an abscisic-acid-dependent manner and inhibits its activity. Together, these studies suggest a fascinating model in which abscisic-acid signalling requires repression of PP2Cs through the action of PYR1/RCAR1 and related proteins[63,64]. At this point the relationship between the GTGs and PYR1/RCAR1 is unknown. However, abscisic-acid responses are diverse and it is possible that this diversity requires multiple sites of perception[65].

The intermediate signalling steps between abscisic-acid perception and response are unclear but the ubiquitin–proteasome pathway is known to be important (Fig. 3). Two RING E3 ligases, ABI3-Interacting Protein (AIP2) and Keep on Going (KEG), promote normal abscisic-acid signalling by regulating the abundance of abscisic-acid-responsive transcription factors, namely ABA-Insensitive 3 (ABI3) and ABA-Insensitive 5 (ABI5)[66,67]. ABI3 levels are reduced as abscisic-acid levels increase in cells. The detailed mechanism is unknown but there is evidence suggesting that abscisic acid increases AIP2 transcript and protein levels[66]. Increasing AIP2 levels then promotes the ubiquitination and degradation of ABI3, contributing to its accelerated degradation. Conversely, abscisic acid protects ABI5 from ubiquitin-mediated degradation, causing ABI5 levels to increase in response to the hormone. KEG has been implicated in the regulation of ABI5 and abscisic-acid signalling may prevent either

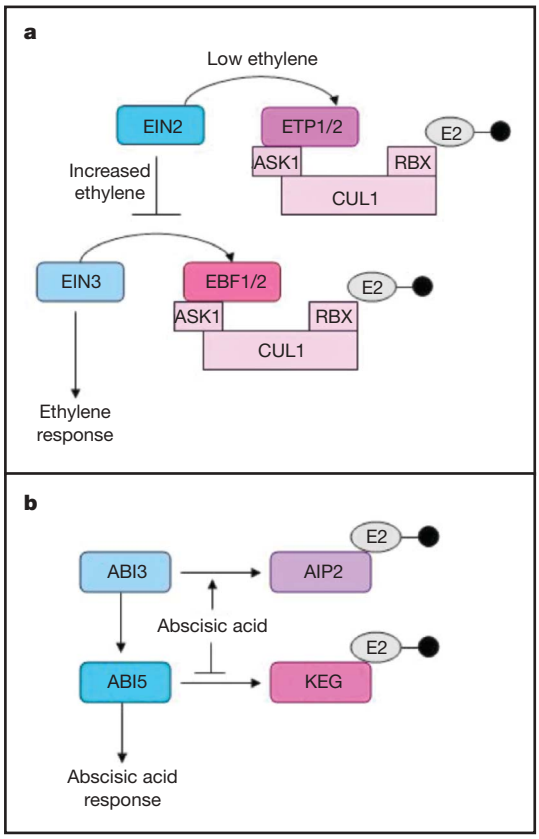

Figure 3 | E3 ligases in ethylene and abscisic-acid signalling. a, Two key proteins in the ethylene signalling pathway are regulated by SCF-type E3 ligases. In the absence of ethylene, EIN2 levels are kept low by SCF^{ETP1} and SCF^{ETP2}. As ethylene levels increase, *ETP1/2* expression is reduced and EIN2 levels increase. Concomitantly, EIN3 levels also increase, in part due to the effects of EIN2 on $SCF^{EBF1/2}$-mediated degradation of EIN3. **b**, ABI3 and ABI5 levels are controlled by the RING E3 ligases AIP2 and KEG, respectively. Abscisic acid promotes the ubiquitination of ABI3 and inhibits the ubiquitination of ABI5 to promote abscisic-acid responses. Black circles denote ubiquitin.

the recognition or the ubiquitination of ABI5 by KEG[67]. *keg* seedlings have a severe phenotype that includes growth arrest immediately following germination. Reducing ABI5 levels in *keg* mutants rescues some but not all aspects of the phenotype, suggesting a broader role for KEG in abscisic-acid signalling that may also extend to other abscisic-acid-responsive transcription factors[67].

Ethylene signalling. Ethylene signalling is also dependent on regulated protein turnover. ETHYLENE INSENSITIVE 3 (EIN3) is a positive transcriptional regulator required for transcription of *ETHYLENE RESPONSE FACTOR* genes[68]. EIN3 levels are regulated through the action of at least two related F-box proteins, EIN3-Binding F-box 1 (EBF1) and EBF2[69,70]. SCF^{EBF1} is thought to repress EIN3 levels when ethylene is low[71]. Two putative functions have been suggested for SCF^{EBF2}. SCF^{EBF2} may prevent excessive accumulation of EIN3 or perhaps remove it when ethylene levels decrease (Fig. 3). Normal ethylene responses are thought to decrease the degradation of EIN3 by SCF^{EBF1} and SCF^{EBF2}, thereby increasing EIN3 levels within responding cells[71].

More recently, another pair of F-box proteins—ETP1 and ETP2—have been shown to promote degradation of the ethylene signalling protein EIN2 in the absence of ethylene[72]. When ethylene is present, expression of *ETP1* and *ETP2* is reduced, allowing accumulation of EIN2. Thus the ubiquitin pathway is involved in ethylene signalling at multiple points in the pathway.

Strigolactone signalling. For many years auxin and cytokinin were thought to be the major mediators of shoot branching. However, studies of series of mutants including *ramosus* (*rms*) of pea, *dwarf* (*d*) of rice, *more axillary growth* (*max*) of *Arabidopsis*, and *decreased apical dominance* (*dad*) of petunia indicated that axillary bud outgrowth is also inhibited by an unidentified hormone[73]. Several affected genes were identified and found to encode CAROTENOID CLEAVAGE DIOXYGENASE 7 (*max3*, *rms5*, *d17*) or CCD8 (*max4*, *rms1*, *d10*, and *dad1*), suggesting that the hormone was related to carotenoids[73]. Very recently, two independent groups showed that levels of strigolactone, a carotenoid derivative, were reduced in rice *d* mutants and the pea *rms1* mutant[6,7]. Treating the respective rice and pea mutants with the synthetic strigolactone GR24 restored axillary bud outgrowth inhibition, suggesting that strigolactones are branching hormones[6,7]. In the context of shoot branching, it is interesting to note that an F-box protein called MAX2/RMS4 is required for response to strigolactone[74,75]. We expect to learn soon whether MAX2/RMS4 is the strigolactone receptor.

Integration of hormone signalling

Crosstalk between hormones is a very active area of research that has benefited from the recent elucidation of hormone signalling pathways. Although our knowledge of the molecular components and pathways that mediate hormone responses has improved enormously in recent years, the molecular mechanisms of hormone interaction remain poorly understood. The coming years should see a greater focus on the function of each hormone pathway in the context of larger regulatory networks.

Evidence for hormone crosstalk comes largely from the analysis of mutant phenotypes. Frequently mutants that are affected in one hormone pathway also display changes in other hormone responses. For example, auxin-related mutants such as *tir1*, *aux1* and *pin2* exhibit altered response to other hormones, including ethylene and abscisic acid[76,77]. From these studies and many others it is clear that hormone crosstalk is as complex as it is important. Almost certainly, all plant hormones interact with one or more additional hormones by affecting synthesis, transport or response. The type of interaction often depends on the tissue, developmental stage and environmental conditions. This presents a nearly endless number of possible opportunities for regulation. Even though the documented instances of crosstalk are numerous, our current knowledge of hormone interaction is quite sketchy. Here we will discuss some of the broad strategies for integrating hormone responses through examples from the recent literature.

It has become clear that diverse mechanisms have evolved to coordinate activity of hormonal pathways during development (Fig. 4). A prominent example is the regulation of hormone metabolism by an interacting hormone. This mechanism is exemplified by ethylene and auxin. Measurement of the rate of auxin biosynthesis after ethylene treatment revealed that ethylene stimulates the auxin biosynthetic pathway. Indeed, several genes required for auxin biosynthesis are under the transcriptional control of ethylene. These genes encode both the alpha and beta subunits of anthranilate synthase (*ASA1* and *ASB1*) and a newly identified family of tryptophan aminotransferases (TAA1)[78–80]. These enzymes function in tryptophan synthesis and the indole-3-pyruvic auxin biosynthetic pathway, respectively.

Auxin can influence ethylene biosynthesis as well. One of the rate-limiting enzymes in the ethylene biosynthesis pathway is 1-aminocyclopropane-1-carboxylate synthase (ACS)[81]. In *Arabidopsis*, there are nine ACS genes that can homodimerize or heterodimerize to form active enzymes[82]. Several of the ACS genes have been found to be regulated by auxin treatment at the transcriptional level[81]. Auxin has also been implicated in the production of jasmonic acid in flowers. Mutant analysis of *arf6 arf8* double mutant plants demonstrated a role in the coordination transition from immature to mature flowers[83]. During floral development, jasmonate levels peak just before anther dehiscence and then decrease[83]. Direct measurements showed that jasmonate production was reduced in *arf6 arf8*

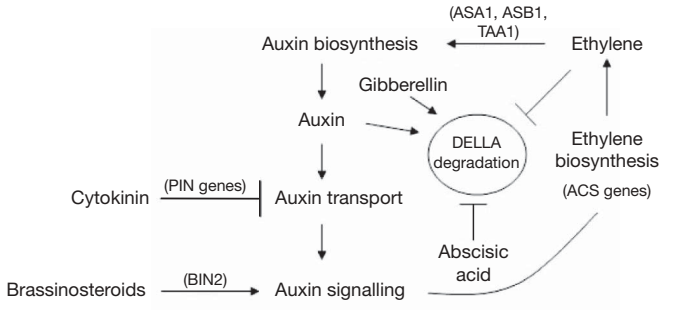

Figure 4 | Hormone integration. Auxin and ethylene maintain an important balance in cells, in part by regulating synthesis. Both hormones exert transcriptional control over critical genes in biosynthesis. A second mechanism of crosstalk is through the control of hormone transport. Cytokinin and auxin are antagonistic during lateral root initiation. To inhibit lateral root formation, cytokinin represses *PIN* expression, thereby inhibiting formation of an auxin gradient required for lateral root initiation. Brassinosteroids and auxin coordinate growth, in part by regulating an overlapping set of genes. In addition, phosphorylation of ARF2 by a brassinosteroid-regulated kinase modulates the activity of ARF2. Finally, auxin, ethylene and abscisic acid all converge on the DELLA proteins.

mutants at every stage of floral development[83]. Furthermore, gene expression studies revealed that several known jasmonate biosynthetic genes were underexpressed in *arf6 arf8* mutants, revealing a regulatory role for auxin in jasmonate production[83].

Hormone interactions also occur at the level of hormone distribution. An illustrative example of this type of crosstalk is the opposing action of auxin and cytokinin during lateral root initiation. It is widely known that polar auxin transport and the establishment of an auxin gradient is a very important determinant of plant growth and morphological patterning[84]. During root development, auxin promotes lateral root initiation while cytokinin opposes this response. One way that cytokinin exerts this effect is to influence the expression of the *PIN* auxin-efflux carrier genes[85]. At least five *PIN* genes work collectively to establish auxin gradients in roots by controlling the direction of polar-auxin transport[86]. By reducing *PIN* expression, cytokinin disrupts the local auxin gradient formation in lateral root founder cells, thereby inhibiting lateral root initiation[85].

Hormonal signalling pathways are also known to interact at the level of gene expression. For example, studies show that there is significant overlap between auxin- and brassinosteroid-responsive gene sets[87,88]. Generally, common target genes repressed by auxin are also repressed by brassinosteroids, and genes induced by auxin are induced by brassinosteroids, suggesting coordination between the signalling pathways. Furthermore, transcriptional profiling in the brassinosteroid-deficient mutant *brx* showed that very few auxin response genes responded normally to auxin[89]. Conversely, many brassinosteroid-responsive genes are mis-regulated in the *yucca* mutant that overproduces auxin[87]. Taken together, the data suggest that auxin and brassinosteroid signalling pathways often converge on a set of common target genes. A molecular mechanism for this convergence was recently elucidated in which the brassinosteroid-regulated BIN2 kinase directly regulates ARF2 activity[90]. ARF2 is an auxin-response factor that inhibits the transcription of auxin-responsive genes. The phosphorylation of ARF2 by BIN2 disrupts DNA binding, leading to inactivation of ARF2 and a subsequent increase in transcription of auxin-responsive genes[90].

Control over key components of signalling pathways by other hormone signals is another common example of cross-talk strategy. As described above, DELLA proteins are central regulators of the gibberellin-mediated signalling pathway and appear to be a common crosstalk node for several interacting hormones, including auxin, ethylene and abscisic acid[91]. Gibberellin signalling during root elongation is known to require auxin because disruption of polar auxin transport or signalling diminished the effects of gibberellin on root elongation[92]. The attenuated growth response corresponded with reduced RGA (a specific DELLA protein) degradation in root cells. These observations indicate that auxin promotes the gibberellin-induced destabilization of some of the DELLA proteins to affect gibberellin responses[92].

Similarly, ethylene may also target DELLA proteins to exert antagonistic actions with gibberellin during root growth[93]. The gibberellin-insensitive *gai/rga* mutant exhibits ethylene-insensitive root growth, indicating that ethylene regulates root growth in a DELLA-dependent manner[93]. In *Arabidopsis* roots, abscisic acid and gibberellin act antagonistically during root growth. Application of abscisic acid was demonstrated to stabilize the DELLA protein RGA and inhibit its gibberellin-induced degradation[94]. Furthermore, higher-order DELLA mutants are resistant to the effects of abscisic acid on growth inhibition[94]. DELLA proteins are not only a node for various hormone signal inputs but also mediate hormone signalling pathways in addition to gibberellin response. DELLA proteins were recently implicated as modulators of plant immune responses[95]. Genetic studies showed that DELLA proteins promote susceptibility to virulent biotrophs and resistance to necrotrophs. These observations were attributed, at least partially, to an alteration of the balance between salicylic acid and jasmonate signalling[95].

These are but a few of the common strategies used to coordinate hormone signals. The regulatory network connecting individual pathways is far more complex in that hormone interactions can result in different outcomes depending on the organ, developmental stage and environmental conditions. Continued efforts to describe the plant hormone regulatory network will be essential to understand and predict plant growth and development.

Concluding remarks

This is a very exciting time for plant hormone biologists as the rate of discovery has expanded exponentially in recent years. The number of recognized small-molecule hormones has doubled in the past 15 years. Candidate receptors for all of the 'classical' hormones and a few of the newer hormones have been identified. We now know the structure of several receptors, leading to exciting new models of hormone perception and new opportunities to synthesize novel growth regulators. Likewise, major advances have been achieved in our knowledge of downstream signalling components and some of their interactions. As we move forward, a major challenge will be to understand how hormone-signalling pathways are integrated during environmental control of plant growth. To take one example, auxin, gibberellin and the brassinosteroids are all known to promote elongation of the *Arabidopsis* hypocotyl. However, the relative contribution of each signalling pathway to growth regulation by light, temperature or the circadian clock is uncertain. Similarly, it is not known whether cell elongation in the hypocotyl requires the same set of genes, regardless of the growth signal. The answers to these and other questions will require a detailed characterization of growth responses, together with information on corresponding changes in the transcriptome and proteome, ideally at cellular resolution. Computational tools can be used to identify the gene modules associated with diverse growth responses. Ultimately, this information can be used to develop predictive models of plant growth and development that will be invaluable tools for modern agriculture.

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