Signs of change: hormone receptors that regulate plant development

Anthony Bishopp1,*, Ari Pekka Mähönen1,† and Ykä Helariutta1,2,3,†

Hormonal signalling plays a pivotal role in almost every aspect of plant development, and of high priority has been to identify the receptors that perceive these hormones. In the past seven months, the receptors for the plant hormones auxin, gibberellins and abscisic acid have been identified. These join the receptors that have previously been identified for ethylene, brassinosteroids and cytokinins. This review therefore comes at an exciting time for plant developmental biology, as the new findings shed light on our current understanding of the structure and function of the various hormone receptors, their related signalling pathways and their role in regulating plant development.

Introduction

Plant hormones (phytohormones) are small organic molecules that affect diverse developmental processes. Alterations in hormone responses have been responsible for several important agricultural advances, such as the breeding of semi-dwarf varieties and increased grain production (Ashikari et al., 2005; Silverstone and Sun, 2000). Unlike animal hormones, which are produced in specific organs, phytohormones are typically produced throughout the plant (see Box 1). Virtually every aspect of plant development from embryogenesis to senescence is under hormonal control. In general, this developmental control is exerted by controlling cell division, expansion, differentiation and cell death. In this manner, diverse developmental processes can be controlled, including formation of the apical-basal and radial pattern, seed germination, determination of plant architecture, flowering, fruit ripening and shedding.

In this review, we consider six classes of phytohormones that are key developmental regulators and for which the receptors are known (Table 1). This review will centre on the model plant Arabidopsis thaliana, as it is in this species that we best understand the receptors and processes involved in hormonal perception.

 Auxin signalling: regulation via destruction

Indole-3-acetic acid (IAA), the major auxin in plants, was isolated in the 1920s from oat coleoptiles as a growth-promoting factor. More recently, auxin has been shown to act as a signal for cell division, cell elongation and cell differentiation, both during embryogenesis and in the mature plant (Buchanan et al., 2000). During embryogenesis, auxin signaling is instrumental in establishing apical-basal polarity, and in the formation of the root and shoot apical meristems and hypocotyls (Jenik and Barton, 2005). However, it was only last year that the F-box protein TRANSPORT INHIBITOR RESPONSE1 (TIR1) was identified as being an auxin receptor (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005), and this finally gave a clear picture of auxin signal transduction.

Most of the components of the auxin signalling pathway have been identified from genetic screens in Arabidopsis (for a review, see Woodward and Bartel, 2005) and TIR1 was no exception. It was originally identified as a series of semi-dominant alleles from a screen based on tolerance to auxin transport inhibitors in a root elongation assay (Ruegger et al., 1997). It was quickly observed that tir1 mutations affect auxin response rather than auxin transport, as sensitivity to auxin was compromised in mutant plants while polar auxin transport appeared normal (Ruegger et al., 1998). TIR1 encodes an F-box domain protein with leucine-rich repeats (LRRs) that is similar to the S-phase kinase-associated protein (SKP2) from yeast (Ruegger et al., 1998). Like its yeast counterpart, TIR1 interacts with the scaffold protein cullin (AtCUL1), with one of the SKP1-like proteins (ASK1/ASK2), and with the ring-domain protein RBX1, to form an SCF-type ubiquitin-protein ligase complex. Such complexes ubiquitinate their substrates to target them for subsequent degradation by the 26S proteasome (see Box 2). The core cullin, SKP1-like and RBX1 proteins provide the catalytic

Box 1. Plant hormones versus animal hormones

<table>
<thead>
<tr>
<th>Plant hormones</th>
<th>Animal hormones</th>
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<tr>
<td>• Small organic molecules</td>
<td>• Peptides/proteins/small organic molecules</td>
</tr>
<tr>
<td>• Synthesised throughout the plant</td>
<td>• Synthesised in specialised glands</td>
</tr>
<tr>
<td>• Affect local cells and tissues, or can be transported through the vascular system</td>
<td>• Affect distant targets (often transported in the circulatory system)</td>
</tr>
<tr>
<td>• Wide-ranging effects</td>
<td>• Specialised effects</td>
</tr>
<tr>
<td>• ‘Decentralised’ regulation</td>
<td>• Regulated by the central nervous system</td>
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</table>

Box 2. Degradation via ubiquitination

Underlying many developmental processes is the fundamental requirement to selectively remove short-lived regulatory proteins. One way to achieve this is through the marking of proteins destined for destruction with ubiquitin (ubiquitination), followed by the recognition and catabolism of the ubiquitinated proteins by the 26S proteasome (Smalle and Vierstra, 2004). Free ubiquitins are attached to the appropriate target proteins via a three-step cascade (E1–E3), and it is at the last step, E3, where the target specificity is determined. The SCF complex is one of four E3-type ubiquitination complexes that have been identified in plants to date.

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1Plant Molecular Biology Laboratory, Institute of Biotechnology, POB 56, FI-00014, University of Helsinki, Finland. 2Department of Biology, FI-20014, University of Turku, Finland. 3Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-901 83, Umeå, Sweden.

*These authors contributed equally to this work

†Author for correspondence (e-mail: yhelariu@mappi.helsinki.fi)
Table 1. Phytohormone receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Organism*</th>
<th>Mutation available</th>
<th>Conserved domains</th>
<th>Tissue specificity</th>
<th>Subcellular localisation</th>
<th>Hormone bind†</th>
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<td>AUFIN</td>
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<tr>
<td>TIR1</td>
<td>Arabidopsis At3g62980</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>Throughout plant (Dharmasiri et al., 2005b; Gray et al., 1999; Ruegger et al., 1998)</td>
<td>Nuclear (Dharmasiri et al., 2005b; Gray et al., 1999)</td>
<td>Yes (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005)</td>
</tr>
<tr>
<td>AFB1/LRF1</td>
<td>Arabidopsis At4g03190</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
</tr>
<tr>
<td>AFB2/LRF2</td>
<td>Arabidopsis At3g26810</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
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<tr>
<td>AFB3</td>
<td>Arabidopsis At1g12820</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
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<tr>
<td>GIBBERELLIN</td>
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<td>GID1</td>
<td>Oryza sativa (Rice)</td>
<td>LOE OE</td>
<td>HSL-like</td>
<td>?</td>
<td>Primarily nuclear (Ueguchi-Tanaka et al., 2005)</td>
<td>Yes (Ueguchi-Tanaka et al., 2005)</td>
</tr>
<tr>
<td>BRASSINOSTEROID</td>
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<tr>
<td>BRI1</td>
<td>Arabidopsis At1g19350</td>
<td>LOE OE</td>
<td>LRR receptor-like kinase</td>
<td>BRI1 ubiquitous in dividing cells, BRL1 and 3 in vascular cells (Cano-Delgado et al., 2004; Li and Chory, 1997)</td>
<td>PM (Cano-Delgado et al., 2004; Li and Chory, 1997)</td>
<td>Yes (Wang et al., 2001; Kinoshita et al., 2005)</td>
</tr>
<tr>
<td>BRL1</td>
<td>Arabidopsis At1g55610</td>
<td>LOF</td>
<td>LRR receptor-like kinase</td>
<td>PM?</td>
<td>Yes (Cano-Delgado et al., 2004)</td>
<td>Yes (Cano-Delgado et al., 2004)</td>
</tr>
<tr>
<td>BRL3</td>
<td>Arabidopsis At3g13380</td>
<td>LOF</td>
<td>LRR receptor-like kinase</td>
<td>PM?</td>
<td>Yes (Cano-Delgado et al., 2004)</td>
<td>Yes (Cano-Delgado et al., 2004)</td>
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<tr>
<td>CYTOKININ</td>
<td></td>
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<tr>
<td>CRE1/WOL/AHK4</td>
<td>Arabidopsis At2g01830</td>
<td>LOF</td>
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<tr>
<td>AHK3</td>
<td>Arabidopsis At1g27320</td>
<td>LOE GOF</td>
<td>CHASE, His kinase, receiver</td>
<td>Meristems and vascular tissue, AHK3 throughout plant (Higuchi et al., 2004; Mahonen et al., 2000; Nishimura et al., 2004)</td>
<td>PM?</td>
<td>Yes (Yamada et al., 2001)</td>
</tr>
<tr>
<td>AHK2</td>
<td>Arabidopsis At5g35750</td>
<td>LOF</td>
<td></td>
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<tr>
<td>ETHYLENE</td>
<td></td>
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<tr>
<td>ETR1</td>
<td>Arabidopsis At1g66340</td>
<td>LOE GOF</td>
<td>Hydrophobic, GAF, His kinase, receiver (not in ERS1 or in ERS2).</td>
<td>Ubiquitous, strong in young tissue (Hua et al., 1998; Sakai et al., 1998)</td>
<td>ER?</td>
<td>Yes (Schaller and Bleecker, 1995)</td>
</tr>
<tr>
<td>ETR2</td>
<td>Arabidopsis At3g23150</td>
<td>LOF</td>
<td>GOF</td>
<td></td>
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<tr>
<td>ERS1</td>
<td>Arabidopsis At2g49040</td>
<td>LOF</td>
<td>GOF</td>
<td></td>
<td></td>
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<tr>
<td>ERS2</td>
<td>Arabidopsis At1g04310</td>
<td>LOF</td>
<td>GOF</td>
<td></td>
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<tr>
<td>EIN4</td>
<td>Arabidopsis At3g04580</td>
<td>LOF</td>
<td>GOF</td>
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<td>ABSICIC ACID</td>
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<tr>
<td>FCA</td>
<td>Arabidopsis At4G16280</td>
<td>LOE OE</td>
<td>Two RRM and WW domains</td>
<td>Apices, vasculature, flowers (Macknight et al., 2002)</td>
<td>Nuclear (Quesada et al., 2003)</td>
<td>Yes (Razem et al., 2006)</td>
</tr>
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</table>

†Accession number in the Arabidopsis Genome Initiative database.
†Hormone binding demonstrated in vitro.
CHASE, Cyclase/Histidine kinase-associated Sensing Extracellular; ER, endoplasmic reticulum; GOF, gain of function; His, Histidine; HSL, hormone-sensitive lipase; LOF, loss of function; LRR, leucine rich repeat; OE, overexpression; PM, plasma membrane; RRM, RNA-recognition motif; WW, tryptophan-tryptophan protein-interaction domain.
promotes Aux/IAA ubiquitination by the SCFTIR1 complex (see 1999b). Thus, for some years, a model has existed in which auxin transcriptional activators (Ulmasov et al., 1999a; Ulmasov et al., 1999b). RESPONSE FACTORS (ARFs), which typically act as by repressing the transcriptional activities of a group of AUXIN proteins cannot bind DNA directly but exert their regulatory activity (Dharmasiri and Estelle, 2004; Ulmasov et al., 1997). These gene family encoding short-lived, primary auxin-response proteins regulators, the Aux/IAAs (Gray et al., 2001). The Aux/IAAs are a degradation of, a group of auxin-dependent transcriptional transcription via its direct interaction with, and subsequent protein (Gray et al., 2001).

This SCFTIR1 complex is able to rapidly control gene transcription via its direct interaction with, and subsequent degradation of, a group of auxin-dependent transcriptional regulators, the Aux/IAAs (Gray et al., 2001). The Aux/IAAs are a gene family encoding short-lived, primary auxin-response proteins (Dharmasiri and Estelle, 2004; Ulmasov et al., 1997). These proteins cannot bind DNA directly but exert their regulatory activity by repressing the transcriptional activities of a group of AUXIN RESPONSE FACTORS (ARFs), which typically act as transcriptional activators (Ulmasov et al., 1999a; Ulmasov et al., 1999b). Thus, for some years, a model has existed in which auxin promotes Aux/IAA ubiquitination by the SCFTIR1 complex (see activity necessary for the transfer of the activated ubiquitin to the target protein, whilst target specificity is conferred by the F-box protein (Gray et al., 2001).

The problem of how the presence of auxin phytohormones controlled this process was solved only when TIR1 was identified as being a receptor for auxin. Both Ottoline Leyser’s and Mark Estelle’s groups showed, by immunoprecipitation of TIR1, that an auxin receptor co-purifies with TIR1, and by using a protein pull-down with tagged Aux/IAAs, that the interaction between SCFTIR1 and Aux/IAAs involves direct auxin binding (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005). Importantly, this auxin receptor activity was retained in in vitro assays in which TIR1 was expressed in heterologous animal systems, and auxin binding in these assays was dependent on the presence of TIR1 (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005). Taken together with previous work, these data indicate that auxin promotes SCFTIR1 – Aux/IAA interaction, by binding directly to TIR1, which leads to Aux/IAA degradation and, consequently, to the de-repression of ARF-dependent auxin responses (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005) (Fig. 1A,B).

There are over 700 F-box proteins in Arabidopsis (Gagne et al., 2002), although TIR1 belongs to a small subfamily of seven related genes (Dharmasiri et al., 2005b). As strong loss-of-function tir1 mutants have only modest alterations in auxin response and development, it is probable that genetic redundancy has disguised strong phenotypes (Dharmasiri et al., 2005b; Ruegger et al., 1998). The quadruple mutant in which TIR1 and the three most closely related genes, AUXIN SIGNALING F-BOX PROTEINS 1, 2 and 3 (AFB1-3), are mutated has a more severe phenotype, and this mutant combination is auxin insensitive. Additionally, each AFB protein is able to interact with Aux/IAAs in an auxin-dependent manner, indicating that auxin binding is collectively mediated by TIR1 and the AFB proteins (Dharmasiri et al., 2005b). This quadruple mutant represents a near complete loss of auxin response, and it is therefore possible to assess the effect of severely diminished auxin perception on plant development. The most severe quadruple

Fig. 1. Auxin and gibberellin signalling pathways. (A) Under low auxin concentrations, the transcription of auxin-response genes via the ARFs is blocked by the Aux/IAA transcriptional repressor proteins. (B) In Arabidopsis, auxin is bound by the F-box protein TIR1, or by other AFB proteins that comprise the SCFTIR1 complex (RBX1-Cullin-ASK1-TIR1 in the figure). The binding of auxin stimulates the interaction of Aux/IAAs with SCFTIR1 and so promotes the ubiquitination of Aux/IAAs proteins, targeting them for destruction by the 26S proteosome and releasing the ARFs from their inhibitory chaperone proteins. (C,D) In rice, gibberellin (GA) signalling involves a similar process, whereby transcription of the gibberellin-response genes is regulated by the GA-dependent degradation of the DELLA protein SLR1. (C) Under low gibberellin concentrations, SLR1 represses gibberellin responses. (D) Under high gibberellin concentrations, GA binds to the GID1 protein directly and initiates a GA-dependent interaction with SLR1. The SCFGID2 complex is then recruited to ubiquitinate SLR1, leading to its degradation. It is unclear whether GID1-GA induces stable conformational changes to SLR1 that lead to the recruitment of the SCFGID2 complex, or whether the GA-GID1-SLR1 is targeted by SCFGID2 as a whole. GA-TF, gibberellin-dependent transcription factors; +mod, unknown modification; NM, nuclear membrane; OsSKP15, Oryza sativa Cullin homologue 1; OsCUL1, Oryza sativa ASK1 homologue 15; +ub, ubiquitination.
Gibberellins are tetracyclic diterpenoid phytohormones. They were first identified in the 1930s as compounds that are produced by a fungus and that cause excessive shoot elongation and reduced seed production in plants; they have subsequently been implicated in several other processes, such as promoting flowering and seed germination (Buchanan et al., 2000). Unlike the cytokinin, ethylene, brassinosteroid, abscisic acid and auxin receptors, which were all identified first in Arabidopsis, the gibberelin (GA) receptor was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GIBBERELLIN INSENSITIVE DWARF1 (GID1) protein was shown to bind radio-labelled gibberelin in in vitro assays (Ueguchi-Tanaka et al., 2005). GID1 shares homology with the hormone-sensitive lipase (HSL) family and is encoded by a single gene in rice. However, in Arabidopsis, it is represented by three, as yet, uncharacterised genes. Although the receptor bears little resemblance to TIR1, the mechanism of gibberelin perception and the response of a plant to it is somewhat similar to that of auxin, in that it also targets proteins for destruction. GID1 binds the SLENDER RICE (SLR1) protein (Ikeda et al., 2001), a member of the DELLA class of transcriptional repressors, directly in a gibberellin-dependent manner (Ueguchi-Tanaka et al., 2005). These DELLA proteins may directly block the gibberellin-dependent transcription of target genes, potentially in a similar way to the blocking of auxin action by the Aux/IAAs (Fig. 1C,D). The identification of GA-related transcription factors will allow this hypothesis to be tested. As a consequence of GID1 binding, SLR1 is then targeted for degradation. This depends on the presence of a functional GID1 protein (Ueguchi-Tanaka et al., 2005) and most likely occurs through the recruitment of an SCF$^{GID2}$ ubiquitin ligase complex, involving the F-box protein GID2 (Fig. 1C,D) (Gomi et al., 2004; Sasaki et al., 2003).

The rice gid1 mutant is inherited in a recessive manner, confirming the biochemical data that the receptor is a positive regulator of gibberellin signalling. The homozygous plant is unresponsive to gibberellins, it is unable to produce fertile flowers and displays a severe dwarf phenotype with wide, dark-green leaf blades (Ueguchi-Tanaka et al., 2005) (Fig. 2C), similar to other GA-insensitive mutants (Sakamoto et al., 2004). gid1 mutants also accumulate GA1, a gibberelin, at about 95 times the level of wild-type plants, indicating that a feed-back mechanism is involved in GA synthesis. Overexpression of GID1 produces tall plants with long leaves, consistent with the phenotype of plants that are given an overdose of gibberellins (Ueguchi-Tanaka et al., 2005). It will be interesting to compare the phenotype of Arabidopsis plants carrying mutations in all three GID1 homologues, as well as GID1-overexpressing lines. Although GID1 homologues have only been characterised in rice, gain-of-function mutants in Arabidopsis that affect the DELLA genes are reported to have similar phenotypes to those observed in gain-of-function rice str1 mutants (Ikeda et al., 2001; Peng et al., 1997). Similar to the gain-of-function rice str1 mutants (Ikeda et al., 2001), gai gain-of-function mutants of Arabidopsis lead to small dwarfish plants that cannot be rescued by the addition of GA (Peng et al., 1997). Whereas in rice, SLR1 is the only DELLA protein, in Arabidopsis, the DELLA family is represented by five members (Bolle, 2004). Genetic redundancy is likely to be responsible for the fact that Arabidopsis DELLA loss-
of-function mutants have only modest phenotypes compared with those in rice. The rice slr1 mutant is tall and thin, and resembles plants saturated with gibberellins (Ikeda et al., 2001). GID2 homologues have been characterised in Arabidopsis, and like loss-of-function mutations in GID2 of rice, mutations in the Arabidopsis GID2 homologue sleepy1 (sly1) result in gibberellin-insensitive dwarf phenotypes and in the accumulation of DELLA proteins. There is also biochemical evidence to support the assembly of an Arabidopsis SCF<sup>SLY1</sup> complex (Dill et al., 2004; Fu et al., 2004; McGinnis et al., 2003).

**Brassinosteroid receptors perceive plant steroids**

Since brassinolide (BL), the most active brassinosteroid (BR), was isolated in 1979, as a substance that promoted stem elongation, BRs have been implicated in a range of biological processes, including seed germination, stem elongation, leaf expansion and xylem differentiation (Buchanan et al., 2000; Cano-Delgado et al., 2004). BRASSINOSTEROID INSENSITIVE1 (BRI1) was identified as a putative BR receptor from a collection of alleles obtained through a genetic screen for BR-insensitive Arabidopsis mutants in Joanne Chory’s laboratory (Li and Chory, 1997). These alleles conferred a dwarf phenotype, similar to loss-of-function mutants for BR biosynthesis, indicating that BRI1 is a positive regulator of brassinosteroid signalling. BRI1 is a leucine-rich-repeat (LRR) receptor-like kinase that has 24 LRRs separated by a 70-amino acid island in its extracellular domain, a transmembrane domain and a functional cytoplasmic serine/threonine kinase domain (Fig. 3A) (Friedrichsen et al., 2000; Li and Chory, 1997). Binding of a BR to BRI1 was demonstrated by the in vitro co-immunoprecipitation of BL with BRI1 (Wang et al., 2001). Later it was shown that BL binds directly to the 70-amino acid island in the extracellular domain between LRR21 and LRR22 of BRI1 (Kinoshita et al., 2005).

A second protein, BRI1-ASSOCIATED RECEPTOR KINASE (BAK1), which can heterodimerize with BRI1, is also required for brassinosteroid signalling. BAK1 was identified simultaneously in a genetic screen for constitutively expressing lines that suppress the weak <sup>bri1-5</sup> allele (Li et al., 2002) and as a BRI1-interacting protein in a yeast two-hybrid screen (Nam and Li, 2002). Although in one study, the heterodimerization of BRI1 and BAK1 was significantly affected by BR levels (Wang et al., 2005), ligand-independent heterodimerization of these two proteins almost certainly occurs, as BRI1 and BAK1 have been shown to dimerize in yeast cells without plant steroids, as well as in cowpea protoplasts (Nam and Li, 2002; Russinova et al., 2004). Genetic analysis has also demonstrated a positive role for BAK1 in BR signalling, as its overexpression can suppress a weak <sup>bri1</sup> mutation. Similarly, <sup>bak1</sup> mutants resemble weak <sup>bri1</sup> mutants and enhance this phenotype in the double mutant (Li et al., 2002; Nam and Li, 2002). It is unclear how this BRI1-BAK1 dimerization affects BR signalling. However, BRs do stimulate the phosphorylation of both BRI1 and BAK1 (Wang et al., 2005; Wang et al., 2001), and this not only activates the kinase activity, but also may provide interaction sites for downstream molecules.

The activation of the BRI1 and BAK1 receptor kinases that is stimulated by BR binding leads to the dephosphorylation and accumulation of the nuclear BR-response proteins BZR1 and BES1 (Wang et al., 2002; Yin et al., 2002), possibly by the inhibition of BIN2, a negative regulator of the BR signalling pathway (Li and Nam, 2002) (Fig. 4). BIN2 is protein kinase and a major signalling component in the brassinosteroid pathway; semi-dominant <sup>bin2</sup> mutants resemble <sup>bri1</sup> mutants in many aspects of development (Li et al., 2001). In the absence of BR, BIN2 phosphorylates BZR1 and

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**Fig. 3. Composition of the transmembrane phytohormone receptors.** (A) A diagram of the structure of brassinosteroid (BR) receptors. The number of leucine-rich repeats (LRR) is indicated. (B, C) Structure of ethylene (B) and (C) cytokinin receptors. These diagrams are not to scale.
BES1, thereby targeting them for degradation (Fig. 4) (He et al., 2002; Zhao et al., 2002). BZR1 binds to specific DNA sequences and represses the transcription of BR biosynthesis genes (He et al., 2005). BES1 also binds specific DNA sequences in association with the BIM protein and acts as a transcriptional activator for BR response genes (Fig. 4) (Yin et al., 2005). The system may be further fine-tuned by the dephosphorylation of BES1 and BZR1 by the phosphatase BSU1, in a manner that is antagonistic to BIN2 (Mora-Garcia et al., 2004).

br1 mutants resemble BR-deficient mutants, are dwarfed, and have reduced apical dominance and fertility (Clouse et al., 1996; Li et al., 1996) (Fig. 5A). In Arabidopsis, BRI1 is represented by a small gene family comprising four members, BRI1 and BRL1-BRL3 (Fig. 3A). BRL1 and BRL3, but not BRL2, encode functional BR receptors that can bind BL and rescue the br1 phenotype when ectopically expressed (Cano-Delgado et al., 2004). br1 mutants display increased phloem and reduced xylem differentiation. BRL1 and BRL3 have vascular-specific expression patterns, and when mutations in all three receptors are combined, the br1 br1 brl3 triple mutant shows enhanced dwarfism, as well as an enhanced vascular phenotype that is more severe than that of any of the single mutants (Cano-Delgado et al., 2004).

**Ethylene receptors: negative regulators of ethylene signalling**

The phytohormone ethylene is a simple, gaseous hydrocarbon molecule that was identified 100 years ago as the active component that inhibits hypocotyl elongation in dark-grown pea seedlings. In the following decades, ethylene was shown to affect other developmental processes, such as the triggering of abscission, fruit ripening, and the relaying of responses to external stress factors, such as pathogen responses (Buchanan et al., 2000). In 1993, Elliot Meyerowitz’s laboratory cloned an ethylene receptor, ETHYLENE RESPONSE 1 (ETR1), that shares similarities with the histidine kinases that are common in prokaryotic signal transduction (Box 3). It was the first plant hormone receptor to be identified (Chang et al., 1993; Schaller and Bleecker, 1995). Subsequent genetic studies in Arabidopsis revealed that ETR1 and four related ethylene receptors, ETR2, ERS1 (ETHYLENE RESPONSE SENSOR), ERS2 and EIN4 (ETHYLENE INSENSITIVE), operate as negative regulators of ethylene signalling (Hua and Meyerowitz, 1998).

Dark-grown Arabidopsis seedlings respond to ethylene by inhibiting hypocotyl and root elongation, and by thickening the hypocotyl and exaggerating the apical hook of the shoot; together these constitute to the so-called triple response (Fig. 5B). Many genes encoding ethylene signalling components, including ETR1 (Bleecker et al., 1988; Chang et al., 1993), ETR2 (Sakai et al., 1998) and EIN4 (Hua et al., 1998; Roman et al., 1995), have been identified from genetic screens based on altered effects to the triple response (Fig. 5B). Mutations in these loci result in plants with increased leaf size (largely due to cell enlargement), and confer resistance to ethylene-induced leaf senescence. In all cases, these mutations were dominant and mapped to a hydrophobic ethylene-binding pocket of the receptor (Schaller and Bleecker, 1995). Some of these mutations abolished ethylene binding in yeast, suggesting that the mutant receptors operate as dominant-negative regulators of ethylene signalling in the absence of ethylene. Upon ethylene binding, the negative activity of the receptors would be inactivated. Decisive evidence for the negative regulatory role for these receptors on ethylene signalling was obtained from the analysis of loss-of-function mutants (Hua and Meyerowitz, 1998). Single loss-of-
function mutations had little or no effect on seedling growth, indicating the existence of functional redundancy among the receptors. However, double, triple and quadruple loss-of-function mutations exhibit constitutive activation of ethylene responses, and the quadruple mutant showed reduced leaf size due to a reduction in epidermal cell size.

The binding of ethylene to the receptors ETR1, ETR2, ERS1, ERS2 and EIN4 was demonstrated when the N-terminal ethylene-binding domain of the receptors was expressed in yeast cells and these cells bound ethylene. Ethylene binding was abolished when the dominant etr1-1 mutation was introduced into the binding pocket of the ETR1 receptor (Hall et al., 2000; O’Malley et al., 2005; Schaller and Bleecker, 1995). In addition to ethylene binding, the N-terminal hydrophobic domain of ETR1, and presumably of the other receptors, is needed for targeting to the endoplasmic reticulum (ER) (Chen et al., 2002). Adjacent to the hydrophobic domain is a GAF domain of unknown function (Fig. 3B). GAF domains in other organisms have been shown to act as cGMP-specific phosphodiesterases in a variety of signalling components (Aravind and Ponting, 1997). The C-terminal part of the ethylene receptors is involved in signal output, and it shares some similarities with bacterial two-component histidine kinases (Box 3). Only ETR1 and ERS1 contain all of the conserved residues that are required for histidine kinase activity (Fig. 3B) (Chang et al., 1993; Hua et al., 1995). Additionally, ETR1, ETR2 and EIN4 contain a receiver domain at the most C-terminal part of the receptor. A conserved aspartate residue within the receiver domain can putatively receive a phosphoryl group from a histidine kinase.

Downstream of the ethylene receptors is CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1), which contains a catalytic domain similar to Raf, a member of the MAP kinase kinase (MAPKKK) family (Kieber et al., 1993). The histidine kinase and receiver domains of ETR1 can interact with CTR1 (Clark et al., 1998; Gao et al., 2003; Huang et al., 2003); however, the mechanism by which CTR1 and the ethylene receptors communicate remains to be identified. Loss-of-function mutations in CTR1 result in constitutive ethylene responses, indicating that CTR1 is a negative regulator of ethylene signalling (Kieber et al., 1993). The severity of the loss-of-function mutant phenotypes suggests that most of the ethylene responses are mediated through CTR1. It is presumed that CTR1 is part of a MAP kinase cascade that inhibits downstream responses to ethylene (Fig. 6A,B). It has been proposed that CTR1 can inhibit the MAP kinase cascade (Ouaked et al., 2003), but this is controversial (Ecker, 2004). Further downstream in the ethylene signalling pathway is a membrane-localised protein, EIN2, which

**Box 3. Two-component signalling**

Signalling via the transfer of a phosphoryl group from a conserved histidine (His) residue of a histidine kinase domain to a conserved aspartate (Asp) residue of a receiver domain is called the two-component system (Klumpp and Krieglstein, 2002). Two-component systems are common in bacteria, and have also been identified in plants and fungi (Saito, 2001). It now seems probable that only animal cells lack the two-component system. Prototypical bacterial two-component systems consist of two proteins, the sensor histidine kinase and the response regulator, the latter of which contains the receiver domain. Some prokaryotic and all eukaryotic systems consist of more than two components, and in these cases signalling via this multistep (His→Asp→His→Asp) system is referred to as phosphorelay signalling. In plants, cytokinins and ethylene are perceived by histidine kinase receptors.
acts as a positive regulator of ethylene signalling (Alonso et al., 1999). Downstream from EIN2 is EIN3, which is a key transcription factor that mediates responses to ethylene (Chao et al., 1997). In the absence of ethylene, EIN3 is ubiquitinated by the SCFEBF1/2 complex, which targets it for degradation by the 26S proteasome (Fig. 6A) (Guo and Ecker, 2003; Potuschak et al., 2003). In the presence of ethylene, signalling through EIN2 prevents EIN3 from being ubiquitinated by SCFEBF1/2, leading to EIN3 accumulation and to the activation of ethylene-responsive gene expression (Fig. 6B) (Guo and Ecker, 2003; Potuschak et al., 2003).

**Cytokinin receptors initiate phosphorelay**

The cytokinin phytohormones are adenine derivatives, and they were identified in the 1950s as compounds that, together with auxin, promote cell division and de novo shoot formation in tobacco tissue culture. Later on, they were shown to induce chloroplast development, promote seed germination, release buds from apical dominance, stimulate leaf expansion, delay senescence and regulate vascular development in several plant species (Buchanan et al., 2000; Mahonen et al., 2006). During the past few years, it has become evident that cytokinins are perceived in Arabidopsis by three related receptor histidine kinases, CRE1/WOL/AHK4, AHK3 and AHK2 (Higuchi et al., 2004; Inoue et al., 2001; Nishimura et al., 2004; Riefler et al., 2006; Suzuki et al., 2001; Ueguchi et al., 2001b). The loss-of-function mutant, cytokinin response1 (cre1) was isolated from a screen for mutants with impaired cytokinin responsiveness in tissue culture by Tatsuo Kakimoto’s laboratory (Inoue et al., 2001). Mapping and complementation analysis revealed that cre1 encodes a putative histidine kinase (Inoue et al., 2001), which is identical to WOODEN LEG (WOL) and to ARABIDOPSIS HISTIDINE KINASE4 (AHK4) (Mahonen et al., 2000; Ueguchi et al., 2001b). CRE1 belongs to a protein family that contains three highly homologous hybrid sensor histidine kinases: AHK2, AHK3 and CRE1/WOL/AHK4 (Mahonen et al., 2000;
Ueguchi et al., 2001a). The histidine kinase and receiver domains of these CRE-family receptors are similar to the respective domains in the ethylene receptors. Unlike many ethylene receptors, all three CRE-family receptors contain the conserved amino acid residues required for the function of the histidine kinase and the receiver domains, as well as a highly homologous extracellular domain in the N-terminal region (Fig. 3C). This N-terminal region resembles the ligand-binding domain that is found in diverse receptors of prokaryotes, plants and the amoeba *Dictyostelium discoideum*. It is called the Cyclase/Histidine kinase-Associated Sensing Extracellular (CHASE) domain, and is bound by a diverse set of low molecular weight ligands (Anantharaman and Aravind, 2001; Mougel and Zhulin, 2001). Three laboratories have independently demonstrated that CRE1/WOL/AHK4 (hereafter referred to as CRE1) is a cytokinin receptor, by carrying out assays in which yeast and bacterial histidine kinase mutants were complemented by CRE1 in a cytokinin-dependent manner (Inoue et al., 2001; Suzuki et al., 2001; Ueguchi et al., 2001b). Two other family members, AHK2 and AHK3, also exhibit similar cytokinin-dependent activity (M. Higuchi and T. Kakimoto, personal communication) (Yamada et al., 2001). The binding of cytokinin to CRE1 was demonstrated by using isolated yeast membranes that express CRE1 (Yamada et al., 2001).

The wol allele of CRE1 exhibits cytokinin insensitivity due to a single nucleotide mutation in the CHASE domain (Mahonen et al., 2000) (A.P.M., M. Higuchi, Y.H. and T. Kakimoto, unpublished). This mutation abolishes the binding of cytokinins in yeast, indicating that the CHASE domain senses cytokinins (Yamada et al., 2001). In vitro, CRE1 has been shown to phosphorylate the conserved His residues of downstream components called the ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEINS (AHPs) in a cytokinin-dependent manner, demonstrating that, following cytokinin binding, CRE1 initiates phosphorylation (Fig. 6C) (M. Higuchi and T. Kakimoto, personal communication). From AHPs, the phosphoryl group is believed to be transferred to the conserved Asp residue within a receiver domain of type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs), which act as transcription factors (Fig. 6C) (Hwang and Sheen, 2001; Sakai et al., 2000; Sakai et al., 2001). Type-B ARRs activate the transcription of cytokinin primary response genes, including type-A ARRs (Brandstatter and Kieber, 1998; Hwang and Sheen, 2001; Rashotte et al., 2003; Sakai et al., 2001; Taniguchi et al., 1998).

Recently, the analysis of Arabidopsis plants carrying single, double and triple mutations of the cytokinin receptors has demonstrated that the CRE-family receptors are positive regulators of cytokinin signalling (Higuchi et al., 2004; Nishimura et al., 2004; Riefler et al., 2006). The triple mutants do not respond to cytokinins in various physiological assays nor induce cytokinin primary-response genes, suggesting that the CRE-family members are the only cytokinin receptors in Arabidopsis. These triple mutants are small, mainly as a result of reduced cell proliferation in the shoot and the root apical meristems, yet they possess all of the basic organs (Fig. 5C). Therefore, either cytokinins are not required for the formation of a basic plant body plan, or, alternatively, there may still be another type of cytokinin receptor that is required during embryogenesis. Various physiological and molecular analyses, as well as expression studies, have revealed that these three receptors have overlapping, yet distinct, roles in cytokinin signalling that mediate various developmental and physiological processes (Higuchi et al., 2004; Nishimura et al., 2004; Riefler et al., 2006).

Plant vascular tissue consists of three tissue types: the transporting tissues, xylem and phloem; and the meristematic tissue, procambium (or cambium), from which the transporting tissues originate. Both the CRE-family triple mutants and the wol mutant contain fewer vascular cell lineages in the root, and all of these lineages differentiate as protoxylem, an early differentiating xylem cell type (Fig. 5D) (Mahonen et al., 2006; Scheres et al., 1995). A similar, all-protoxylem, phenotype is observed in transgenic lines when cytokinin is degraded from the root vascular bundle. Ectopic protoxylem appears soon after the induction of a cytokinin-depleting enzyme called CYTOKININ OXIDASE 1, indicating that cytokinin signalling primarily inhibits protoxylem specification and promotes procambial cell identity (Mahonen et al., 2006). Further analysis of the wol mutation has indicated that in the absence of cytokinin, CRE1 preferentially dephosphorylates AHPs, indicating that phosphorylation in plants can be bidirectional (M. Higuchi and T. Kakimoto, personal communication).

A gain-of-function mutation in AHK3 results in delayed senescence, whereas a loss-of-function mutant exhibits reduced sensitivity for cytokinin-mediated inhibition of leaf senescence (Kim et al., 2006). Because AHK3, but not the other two cytokinin receptors, affect this process, these data indicate that the long-known effect of cytokinin on delaying senescence operates specifically through AHK3 (Kim et al., 2006).

**Abscisic acid and post-transcriptional RNA processing**

Abscisic acid (ABA) was identified by two groups independently in the 1960s as a compound that promotes the shedding of cotton fruit and induces dormancy in sycamore seeds, but since then it has been studied more in the context of adaptation to environmental stress (Buchanan et al., 2000). However, the recent discovery that an ABA receptor is a key regulator of the transition between vegetative and reproductive growth established a clear link between ABA and plant development (Razem et al., 2006). Unlike the other receptors discussed so far, which were identified as components of the relevant signalling pathways through genetic screens, the ABA receptor was discovered using a biochemical approach. By screening a translated barley cDNA library for proteins that bind to ABA in vitro, Robert Hill’s laboratory identified the ABAP1 protein (Razem et al., 2004). ABAP1 is a hydrophobic molecule that has a tryptophan-tryptophan (WW) interaction domain similar to that in the Arabidopsis floral repressor FCA. FCA was subsequently shown to bind ABA in in vitro co-immunoprecipitation assays (Razem et al., 2006).

FCA was originally discovered through a genetic screen for late-flowering Arabidopsis mutants (Koornneef et al., 1991). Loss-of-function fca mutants flower substantially later than wild-type Arabidopsis under most conditions (Fig. 5E), whereas overexpression of FCA leads to an early-flowering phenotype (Macknight et al., 1997). FCA is a member of the autonomous group of floral regulators that exert an internal developmental control over flowering. Like other autonomous components, delayed flowering in fca is caused directly by elevated levels of FLC mRNA (Michaels and Amasino, 2001; Sheldon et al., 2000). FLC is a MADS box transcription factor that inhibits flowering by negatively regulating the expression of the flowering pathway integrator genes SOC1 and FT (Michaels and Amasino, 1999; Samach et al., 2000). FCA activity requires the presence of a second autonomous pathway protein, FY, which contains an RNA 3'-end processing factor (Simpson et al., 2003). The two proteins interact through the WW domain of FCA to regulate gene expression post-translationally by promoting the premature cleavage and polyadenylation of target precursor mRNA (pre-mRNA), at least in the context of the autoregulation of FCA (Macknight et al., 2002; Quesada et al., 2003). However, in the presence of ABA, this interaction between
FCA and FY proteins are severely inhibited in in vitro pull-down assays (Razem et al., 2006), although it remains to be investigated whether ABA disrupts the FCA-FY interaction in vivo (Fig. 7). The significance of this ABA-mediated inhibition was demonstrated in a study in which plants treated with ABA phenocopied fca and fy mutants; they were late flowering, had accumulated levels of FLC mRNA, and failed to autoregulate FCA by cleavage of its own pre-mRNA (Macknight et al., 2002; Quesada et al., 2003; Razem et al., 2006). An earlier report had also suggested a link between ABA signalling, FLC regulation and the control of flowering (Bezerra et al., 2004).

The identity of the RNA-binding protein FCA as a receptor for ABA demonstrates a new level of hormonal signalling that involves post-transcriptional regulation. Current evidence strongly suggests that FCA is not the only ABA receptor, as fca mutants do not show effects in other processes controlled by ABA, such as stomatal opening and seed germination (Razem et al., 2006). Additionally, mutations in ABA downstream response genes that affect stomatal opening, such as in ABI1 and ABI2 (Chak et al., 2000), do not show impaired ABA responsiveness in terms of delayed flowering and cleavage of FCA pre-mRNA (Razem et al., 2006), indicating that not only do additional ABA receptor(s) exist, but also possibly distinct signalling pathways.

Convergence of hormonal signals downstream of receptors

So far we have discussed the phytohormone receptors and their related signal transduction pathways individually. However, there is substantial evidence that these regulatory pathways interact to control plant development. These interactions can occur at multiple levels, for example, by controlling the biosynthesis of other hormones or the expression of components in other signal transduction pathways. Another emerging theme is that of crosstalk at the level of signal transduction intermediates directly downstream of receptors. Recently, Nemhauser et al. (Nemhauser et al., 2004) and Goda et al. (Goda et al., 2004) have provided evidence that brassinosteroid and auxin signalling converge at the level of transcriptional regulation in regulating hypocotyl elongation in Arabidopsis. There seems to be a subset of genes that contain regulatory elements that are commonly regulated by the two hormones. Nemhauser et al. suggest that the activity of some of the auxin signalling transcription factors, ARFs, is modulated by the formation of specific transcriptional complexes, involving input from both auxin and brassinosteroid signalling pathways. Furthermore, Nakamura et al. provided evidence that the activity of certain Aux/IAA proteins could be jointly regulated by auxin and brassinosteroids (Nakamura et al., 2006). Analogously, Fu and Harberd have provided evidence that auxin modulates gibberellin response in controlling root elongation (Fu and Harberd, 2003). They indicate that proper auxin signalling is required for GA-induced proteolysis of the DELLA growth repressing proteins, although the exact mode of this remains to be investigated.

There is also genetic evidence that signals from the GA and autonomous pathways (including ABA) may integrate at the promoter of SOC1 (Moon et al., 2003). Reduced abundance of FLC mRNA (e.g. by low levels of ABA) alone is insufficient to activate SOC1, and requires additional positive factors. Under short-day conditions, gibberellin signalling could provide such factors, as GA-biosynthetic and GA-signalling mutants flower extremely late; this correlates with reduced SOC1 expression. The exact mode of this convergence remains to be identified.

All three cytokinin receptors and some ethylene receptors contain the conserved residues that are required for histidine kinase activity, and for phosphorelay via the receiver domain (Fig. 3B,C). Therefore, both cytokinin and ethylene receptors have the potential to phosphorylate or dephosphorylate the same downstream components, the AHPs, enabling crosstalk between these two signalling pathways (Fig. 6). This potential convergence could occur at the AHPs, as specificity between the AHPs and various Arabidopsis histidine kinases does not seem to be strict (Tanaka et al., 2004; Urao et al., 2000). However, no compelling evidence to support this hypothesis has been presented.

Concluding remarks

Although considerable progress has recently been made in understanding hormone perception in plants, we are just beginning to comprehend the whole picture of developmental control following phytohormone perception. Receptors for all of the known hormones regulating plant development have been identified; however, for some phytohormones, such as abscisic acid, additional receptor(s) remain to be discovered. Considering the number of receptor kinases, RNA binding proteins and F-box proteins in plants, it is possible that some of them might act as receptors for other phytohormones, potentially including ones not yet identified. Also, there are still many interesting biochemical aspects of hormone receptors yet to be uncovered, such as substrate specificity in the case of multiple gibberellin, brassinosteroid and cytokinin ligands, multimerization, and the desensitization of a signal following ligand binding.

Similarly, our understanding of the signalling pathways downstream of the receptors remains preliminary. In each signalling pathway, there are still multiple gaps in our knowledge, especially...
concerning the specificity of certain hormone responses in a given developmental context, i.e. how a certain response is regulated spatially and temporally in individual species. New high-throughput gene expression analysis techniques and system-wide approaches will be important in investigating these questions.

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