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 signalling 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 (Deshaies, 1999; Gray et al., 1999; Moon et al., 2004). 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
### 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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<tbody>
<tr>
<td><strong>AUXIN</strong></td>
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<tr>
<td>TIR1</td>
<td>Arabidopsis At3g62980</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>Through 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>
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<tr>
<td>AFB1/LRF1</td>
<td>Arabidopsis At4g03190</td>
<td>LOF</td>
<td>F-box, LRRs</td>
<td>-</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>-</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>-</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
<td>Indirectly (Dharmasiri et al., 2005b)</td>
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**GIBBERELLIN**

| GID1 | Oryza sativa (Rice) | LOE OE | HSL-like | ? | Primarily nuclear (Ueguchi-Tanaka et al., 2005) | Yes (Ueguchi-Tanaka et al., 2005) |

**BRASSINOSTEROID**

| BRI1 | Arabidopsis At1g19350 | LOE OE | LRR receptor-like kinase | BRI1 ubiquitous in dividing cells, BRL1 and 3 in vascular cells (Cano-Delgado et al., 2004; Li and Chory, 1997) | PM (Cano-Delgado et al., 2004; Li and Chory, 1997) | Yes (Wang et al., 2001; Kinoshita et al., 2005) |
| BRL1 | Arabidopsis At1g55610 | LOF | LRR receptor-like kinase | - | PM (Cano-Delgado et al., 2004) | Yes (Cano-Delgado et al., 2004) |
| BRL3 | Arabidopsis At3g13380 | LOF | LRR receptor-like kinase | - | PM (Cano-Delgado et al., 2004) | Yes (Cano-Delgado et al., 2004) |

**CYTOKININ**

| CRE1/WOL/ AHK4 | Arabidopsis At2g01830 | LOF | - | Meristems and vascular tissue, AHK3 throughout plant (Higuchi et al., 2004; Mahonen et al., 2000; Nishimura et al., 2004) | PM? | Yes (Yamada et al., 2001) |
| AHK3 | Arabidopsis At1g27320 | LOE GOF | CHASE, His kinase, receiver | - | PM (Kim et al., 2006) | Indirectly (Yamada et al., 2001) |
| AHK2 | Arabidopsis At5g35750 | LOF | - | - | PM (Kim et al., 2006) | Indirectly (M. Higuchi and T. Kakimoto, personal communication) |

**ETHYLENE**

| ETR1 | Arabidopsis At1g66340 | LOE GOF | Hydrophobic, GAF, His kinase, receiver (not in ERS1 or in ERS2). | Ubiquitous, strong in young tissue (Hua et al., 1998; Sakai et al., 1998) | ER (Chen et al., 2002) | Yes (Schaller and Bleecker, 1995) |
| ETR2 | Arabidopsis At3g23150 | LOF, GOF | - | - | ER (O’Malley et al., 2005) | Yes (O’Malley et al., 2005) |
| ERS1 | Arabidopsis At2g49840 | LOF, GOF | - | - | ER (O’Malley et al., 2005) | Yes (O’Malley et al., 2005) |
| ERS2 | Arabidopsis At1g04310 | LOF, GOF | - | - | ER (O’Malley et al., 2005) | Yes (O’Malley et al., 2005) |
| EIN4 | Arabidopsis At3g04580 | LOF, GOF | - | - | ER (O’Malley et al., 2005) | Yes (O’Malley et al., 2005) |

**ABSCISIC ACID**

| FCA | Arabidopsis At4G16280 | LOE | Two RRM and WW domains | Apices, vasculature, flowers (Macknight et al., 2002) | Nuclear (Quesada et al., 2003) | Yes (Razem et al., 2006) |

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* 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.
This SCF<sub>TIR1</sub> 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 SCF<sub>TIR1</sub> complex (see Box 2), triggering Aux/IAA degradation by the 26S proteosome and thereby releasing the ARFs from the repressive effects of the Aux/IAA complex (Fig. 1A,B).

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 SCF<sup>TIR1</sup> 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 SCF<sup>TIR1</sup> – 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 AFB proteins (Dharmasiri et al., 2005b), 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
mutants exhibit embryonic phenotypes with failure to specify the root meristem (Fig. 2B), similar to gain-of-function mutations in Aux/IAAs or certain loss-of-function mutations in ARFs (Dharmasiri et al., 2005b). The less severe individuals exhibit defective hypocotyl elongation, apical hook formation, lateral root formation, tropic responses, root hair development and meristem organisation. Later in development, the less severe quadruple mutant individuals display defective leaf morphology and inflorescence architecture, including reduced apical dominance and size (Fig. 2A). The phenotypic variability and presence of some auxin signalling in the quadruple mutant may be explained by either residual AFB activity (in the case that all the mutant alleles are not null) or by the presence of other genes that encode auxin receptors.

It is possible that the SCF^{TIR1}, the SCF complexes involving the AFB proteins, and perhaps other TIR1-like F-box proteins, do not account for all of the responses of Arabidopsis to auxin, such as plasma membrane-associated proton pumping (for a review, see Leyser, 2005). One candidate for such an extracellular receptor is the AUXIN BINDING PROTEIN1 (ABP1), as changes in the ion transport associated with the early stages of auxin-induced growth can be inhibited by extracellular treatment with anti-ABP1 antibodies (Leblanc et al., 1999).

**Dwarfed rice plants and the gibberellin receptor**

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 gibberellin (GA) receptor was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GIBBERELLIN INSENSITIVE DWARF1 (GID1) protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory. A recombinant GID1 protein was identified in rice by Makoto Matsuoka’s laboratory.

**Fig. 2. Phenotypes associated with the auxin and gibberellin receptor mutants.** All images are shown alongside wild type for comparison. (A) A wild-type Arabidopsis plant (left) and a quadruple auxin receptor mutant, tir1 afb1 afb2 afb3, which has reduced apical dominance and size (right). (B). Cell division of the hypophysis (arrows), which is necessary for root meristem specification, is strongly delayed in tir1 afb2 afb3 globular embryos (right). (C) Growth of the rice gibberellin receptor mutant gid1-1 (right) is severely reduced. Inset: higher magnification of gid1-1. Scale bars, 10 cm (1 cm in inset). [A and B are reprinted, with permission, from Dharmasiri et al. (Dharmasiri et al., 2005b). C is reprinted, with permission, from Ueguchi-Tanaka et al. (Ueguchi-Tanaka et al., 2005).]
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 sleepyl (syl1) 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 SCFSLY1 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 bri1-5 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 bri1 mutation. Similarly, bak1 mutants resemble weak bri1 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 bin2 mutants resemble bri1 mutants in many aspects of development (Li et al., 2001). In the absence of BR, BIN2 phosphorylates BZR1 and

![Fig. 3. Composition of the transmembrane phytohormone receptors.](image-url)
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).

_bril_ 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*, _BRII_ is represented by a small gene family comprising four members, _BRII_ and _BRL1-BRL3_ (Fig. 3A). _BRL1_ and _BRL3_, but not _BRL2_, encode functional BR receptors that can bind BL and rescue the _bril_ phenotype when ectopically expressed (Cano-Delgado et al., 2004). _bril_ 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 _bril brl1 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-
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-response 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;
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 phosphorelay (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 phosphorelay 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 bound 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 interaction domain similar to that in the Arabidopsis floral repressor FCA. FCA was subsequently shown to bind ABA 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-transcriptionally 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., 2000; Quesada et al., 2003). However, in the presence of ABA, this interaction between
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

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**Fig. 7. The abscisic acid (ABA) signalling pathway that controls the regulation of flowering time.** (A) At low ABA concentrations, FCA and FY interact and together prevent the accumulation of FLC pre-mRNA. FLC is a potent inhibitor of flowering, as several pathways converge on it to block the expression of the floral integration gene SOC1 by directly binding to its promoter (Hepworth et al., 2002). FCA binds to the C-terminal part of FCA, close to its interaction site with FY, disrupting the association of these two proteins in vitro and leading to the accumulation of FLC in vivo, which delays flowering. Transcription of SOC1 only occurs in plants with low FLC levels; additional cues are required for the transition to flowering.

**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
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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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