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Comparison of plant hormone signalling systems

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Abstract

Plant growth and development are controlled by nine structurally distinct small molecules termed phytohormones. Over the last 20 years, the molecular basis of their signal transduction, from receptors to transcription factors, has been dissected using mainly *Arabidopsis thaliana* and rice as model systems. Phytohormones can be broadly classified into two distinct groups on the basis of whether the subcellular localization of their receptors is in the cytoplasm or nucleus, and hence soluble, or membrane-bound, and hence insoluble. Soluble receptors, which control the responses to auxin, jasmonates, gibberellins, strigolactones and salicylic acid, signal either directly or indirectly via the destruction of regulatory proteins. Responses to abscisic acid are primarily mediated by soluble receptors that indirectly regulate the phosphorylation of targeted proteins. Insoluble receptors, which control the responses to cytokinins, brassinosteroids and ethylene, transduce their signal through protein phosphorylation. This chapter provides a comparison of the different components of these signalling systems, and discusses the similarities and differences between them.

Key words:

abscisic acid, auxin, brassinosteroids, cytokinins, ethylene, F-Box E3 ubiquitin ligases, gibberellins, jasmonic acid, phosphorylation cascade, phytohormones, salicylic acid, SCF complex, serine/threonine kinases, strigolactones, ubiquitination.

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Introduction

Plant growth and development depend on numerous signals that include endogenous molecules, such as peptides and metabolites, as well as external cues. These signals converge on small signalling molecules called phytohormones, affecting their metabolism, transport and/or signalling, and rendering them central regulators of the plant life cycle. To date, nine families of phytohormones for which receptors are known (Table 1) have been identified in plants.

Hormone signalling involves two distinct steps. First, the signal is recognized in the target cells or tissues by a receptor. Secondly, upon binding of the ligand to the receptor, a signal is transmitted downstream through a variable number of intermediates towards the final targets (hereafter termed effectors). Importantly, hormone signals not only switch genes on and off but also control other non-transcriptional responses, such as ion pump activity and clathrin-mediated endocytotic recycling. However, in this chapter we shall focus exclusively on transcriptional responses, as these have been extensively described in the literature.

Phytohormone receptors are either localized at the plasma membrane (PM), endoplasmic reticulum (ER) or endosomes, and are therefore insoluble, or are localized in the cytoplasm and/or the nucleus, and are therefore soluble (Table 1). This has important implications for the way that phytohormones transduce their signal towards the effectors. Membrane-bound receptors of cytokinins (CKs) and brassinosteroids (BRs) act as multimers that trigger a phosphorylation cascade through histidine kinases (in the case of CKs) or serine/threonine kinases (in the case of BRs). Cytosolic and/or nuclear receptors of auxin, jasmonates (JAs), gibberellins (GAs) and strigolactones (SLs) mediate their responses through the controlled degradation of proteins by the proteasome, either through a 'molecular glue' mechanism (in the case of auxin and JAs) or via an adaptor protein (in the case of GAs and SLs). Salicylic acid (SA) signalling remains elusive, although its soluble receptors have been recently identified. Abscisic acid (ABA) signalling is controlled by a family of nucleo cytoplasmic receptors that trigger the phosphorylation of a family of transcription factors (TFs), which is a unique situation among soluble receptors in plants. Finally, ethylene signalling offers a mixture of phosphotransfers, controlled by its insoluble ER-localized receptors, and protein degradation, controlled by several E3 ubiquitin ligases. In the following account we highlight the similarities and differences between the various hormonal signalling pathways.

Auxin, jasmonates (JAs), gibberellins (GAs), strigolactones (SLs), abscisic acid (ABA) and salicylic acid (SA): signalling through cytoplasmic and/or nuclear receptors

Soluble receptors of auxin and JAs are localized in the nucleus, whereas GA and SL receptors are also found in the cytoplasm. Their signalling pathways rely on protein polyubiquitination by the SCF (Skp–Cullin–F-box) complex (see below for details) and their subsequent degradation by the proteasome. ABA signal transduction starts in the cytoplasm or the nucleus, and leads to the phosphorylation of a family of TFs. SA signalling remains elusive despite the recent identification of its receptors, which are found in both the cytoplasm and the nucleus.

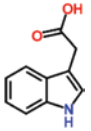
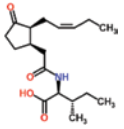
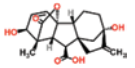
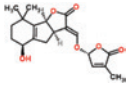
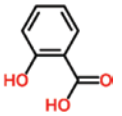
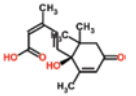
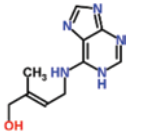
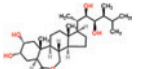
Table 1. The receptors, intermediates, effectors, signalling systems and molecular structures of the nine phytohormones

The table summarizes all of the different components and the different systems used by phytohormones to transduce their canonical transcriptional responses. Note that there are other receptors described, notably for auxin and ABA signalling. All of the gene names indicated are from *Arabidopsis* except for SL signalling, for which they come from rice. The effector of SL signalling BES1 was identified in *Arabidopsis*.

Component	Auxin	Jasmonates (JAs)	Gibberellins (GAs)	Strigolactones (SLs)	Salicylic acid (SA)	Abscisic acid (ABA)	Ethylene (ET)	Cytokinins (CKs)	Brassino-steroids (BRs)
Receptor(s)	TIR1, AFB1–AFB5	COI1	GID1a–GID1c	DWARF 14	NPR1, NPR3 and NPR4*	PYR1–PYR14	ETR1 and ETR2, EIN4, ERS1 and ERS2	AHK2–AHK4	BRI1, BRL1 and BRL3
Receptor gene family	F-Box	F-Box	Hormone-sensitive lipase	α/β-Hydrolase	BTB/POZ domain	START domain-containing protein	Histidine kinase	Histidine kinase	Serine/threonine protein kinases
Subcellular localization of the receptor(s)	Nucleus	Nucleus	Nucleus and cytoplasm	Nucleus	Nucleus and cytoplasm	Nucleus and cytoplasm	ER	Plasma membrane and ER	Plasma membrane and early endosomes
Other known receptors (subcellular localization)	ABP1 (PM, ER)					GTGs (PM), CHLH (chloroplast)			
Intermediates	Aux/IAAs†	JAZs†	SLY, SNE, DELLA	DWARF 53	NPR1	PP2Cs, SnRK2.2, SnRK2.3 and SnRK2.6	CTR1, EIN2	AHP1–AHP6	BAK1†, BKI1, BSK1/CDG1, BSU1, BIN2

(*Continued*)

Table 1. The receptors, intermediates, effectors, signalling systems and molecular structures of the nine phytohormones (*Continued*)

Component	Auxin	Jasmonates (JAs)	Gibberellins (GAs)	Strigolactones (SLs)	Salicylic acid (SA)	Abscisic acid (ABA)	Ethylene (ET)	Cytokinins (CKs)	Brassino-steroids (BRs)
Effectors	ARFs	bHLH‡ TFs (MYC2, . . .)	PIFs, . . .	BES1	bZIP‡ TFs (TGA, . . .)	bZIP‡ TFs (ABI3, ABI5, . . .)	EIN3, EILs	A and B type ARRs	BZR1, BES1
Signalling system	SCF-mediated protein degradation	SCF-mediated protein degradation	SCF-mediated protein degradation	SCF-mediated protein degradation	Protein degradation (?)	Phosphorylation	Phosphorylation and SCF-mediated protein degradation	Phosphoryl-ation	Phosphoryl-ation
Molecular structure							$H_2C{=}CH_2$		
Name	Indole-3-acetic acid	(+)-7-*iso*-jasmonoyl-L-isoleucine	Gibberellic acid A3	(+)-Strigol	Salicylic acid	Abscisic acid	Ethylene	Zeatin	Brassinolide

*The role of the SA receptors NPR1, NPR3 and NPR4 appears to be important, but their function as primary SA receptors needs to be confirmed.

†Intermediates in signalling pathways that directly interact with the phytohormone, and hence that are considered to be co-receptors.

‡Not all proteins belonging to these gene families, but only a specific subfamily, participate in hormonal responses.

Functions of the SCF complex

The SCF complex controls the polyubiquitination of proteins in eukaryotes. Ubiquitin is a polypeptide (consisting of 76 amino acids) that is added to proteins post-translationally and functions as a marker for protein degradation, translocation and many other processes. Ubiquitination is a process whereby three classes of enzymes act in concert to add one or several ubiquitin moieties to a target protein. It involves an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin-ligase enzyme that recruits the substrate for ubiquitination. F-Box proteins are a class of E3 ubiquitin ligases that work in a complex termed the SCF complex. This complex is composed of four core subunits, namely SKP1, CULLIN1, a variable F-Box E3 ubiquitin ligase and RBX1 (Figure 1). The complex forms a horseshoe-shaped structure that brings a substrate specifically recruited by the F-Box subunit into the vicinity of an E2 ubiquitin-conjugating enzyme, recruited by the RBX1 subunit. The substrate is then rapidly polyubiquitinated and degraded by the proteasome. The *Arabidopsis* genome encodes more than 700 F-Box proteins, which can theoretically bind a similar number of distinct protein families. It plays pivotal roles during multiple cellular processes, but notably in hormonal signalling, including auxin, JA, GA, SL and ethylene signalling.

Auxin and Jasmonate (JA) signalling

Auxin and JAs transduce their signal in a remarkably similar way. The bioactive molecules are indole-3-acetic acid (IAA) for auxin, and JA conjugated to the amino acid isoleucine (+)-7-*iso*-jasmonoyl-L-isoleucine or JA-Ile for JA (Table 1). These small compounds act as a 'molecular glue' between pairs of proteins that function as co-receptors, which are on one side of the F-Box E3 ubiquitin ligases, and on the other side are specific proteins that act as transcriptional repressors [1,2] (Figure 1). In *Arabidopsis* there are six nuclear-localized F-box co-receptors for auxin in the core pathway (see Table 1 and the Discussion for alternative receptors), termed TRANSPORT-INHIBITOR RESPONSE 1 (TIR1) and AUXIN SIGNALLING F-BOX PROTEIN 1 to 5 (AFB1–AFB5), whereas there is only one receptor for jasmonates, CORONATINE-INSENSITIVE 1 (COI1). Auxin repressors are encoded by a protein family called the Aux/IAAs, whereas JA repressors are members of the JASMONATE ZIM DOMAIN (JAZ) gene family [3,4]. Aux/IAAs and JAZs interact with their co-receptors through a specific domain, and this interaction is greatly stabilized in the presence of auxin and JA-Ile respectively. The term 'molecular glue' has been coined to describe the function of these hormones in mediating protein–protein interactions, and the term 'co-receptor' is used to describe the co-operative binding of the hormone in the presence of both the F-Box E3 ligase and the transcriptional repressor, as has been clearly demonstrated for auxin [5].

Aux/IAA proteins repress TFs that belong to the auxin-response factors (ARFs) family, whereas JAZ proteins repress multiple TFs that notably belong to the basic helix-loop-helix (bHLH) family (such as MYC2), but also include other TFs [6,7]. ARF proteins fall into two categories, namely those that activate and those that repress transcription [8]. In the absence of the hormones, Aux/IAA and JAZ repressors are stabilized and repress transcription through the recruitment of a transcriptional repressor called TOPLESS (TPL) [9]. Although Aux/IAAs and some JAZ proteins can recruit TPL directly through an EAR motif, several JAZ proteins require another protein termed NOVEL INTERACTOR OF JAZ (NINJA) [10]. In the presence of the

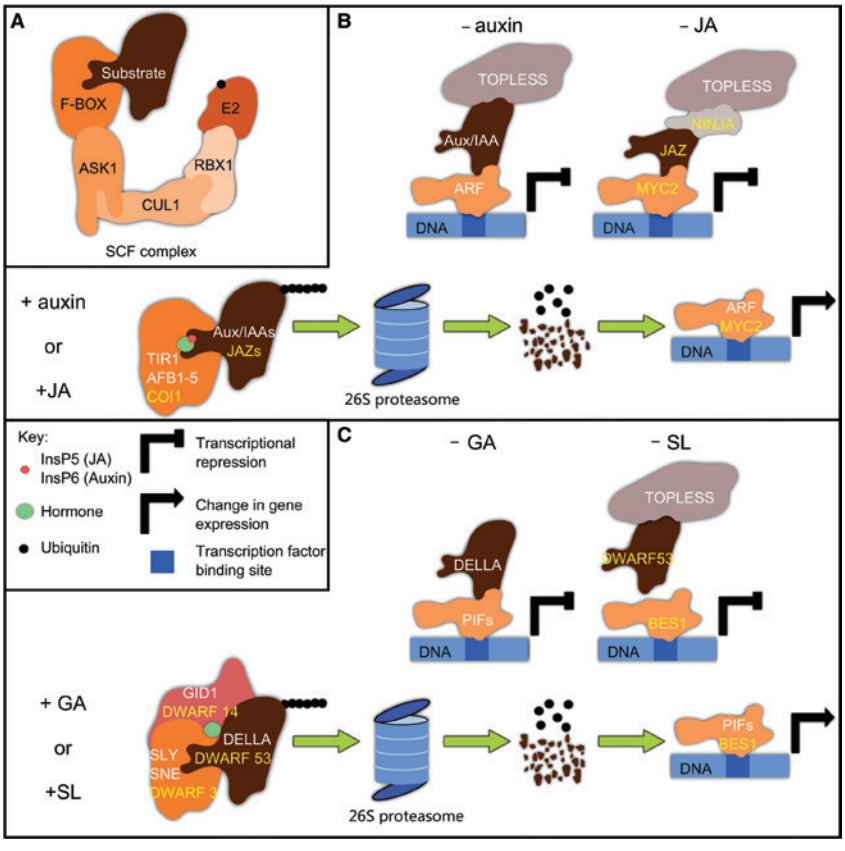


Figure 1. The SCF complex and signal transductions of auxin, JA, GA and SL

(**A**) Schematic representation of the SCF complex. (**B**) Signal transduction of auxin and JA starts in the nucleus. In the absence of these hormones, transcriptional responses are repressed through the recruitment of the TOPLESS complex in the promoter of regulated genes. In the presence of the hormones, repressors of auxin and JA signalling are degraded by the SCF complex (only the F-Box protein is shown), and transcriptional responses are triggered (either activation or repression of regulated genes). The intermediates and effectors of auxin signalling are shown in white, and those of JA signalling are shown in yellow. (**C**) Signal transduction of GA and SL starts in the nucleus and/or cytoplasm. In the absence of these hormones, transcriptional responses are repressed directly by DELLA proteins (GA) or through the recruitment of the TOPLESS complex (SL) in the promoter of regulated genes. In the presence of the hormones, repressors of GA and SL signalling are degraded by the SCF complex (only the F-Box protein is shown due to space limitations), and transcriptional responses are triggered (either activation or repression of regulated genes). The intermediates and effectors of GA signalling are shown in white, and those of SL are shown in yellow. All of the gene names indicated are from *Arabidopsis,* except for SL signalling, for which they come from rice. The effector of SL signalling, BES1, was identified in *Arabidopsis*.

hormones, Aux/IAAs and JAZ proteins are rapidly polyubiquitinated by the $SCF^{TIR1/AFB1–AFB5}$ and SCF^{COI1} complexes respectively, and subsequently degraded by the 26S proteasome [11] (Figure 1).

Gibberellin (GA) and Strigolactone (SL) signalling

Whereas auxin and JA receptors are F-Box proteins themselves, the receptors and the F-Box proteins in GA and SL signalling are uncoupled (Figure 1). Their signalling pathways involve additional protein–protein interactions, notably through adaptor proteins, that lead to the rapid

degradation of targeted proteins via the SCF complex. Most of the original research on GA and SL signalling has been undertaken on rice, and therefore the gene names cited in this section are from rice. However, the names of homologous genes in *Arabidopsis* are also provided for consistency.

The rice GA receptor, GA-INSENSITIVE DWARF 1 (GID1), belongs to the family of hormone-sensitive lipases (HSLs), whereas the only SL receptor identified so far, namely DWARF14 (D14), belongs to the family of α/β hydrolases [12,13]. In *Arabidopsis* there are three homologous GID1 genes (GID1a, GID1b and GID1c) and at least one SL-receptor-homologous gene (also termed DWARF14).

Structural studies of the binding of GA to GID1 have shown that GA triggers a conformational change which allows the direct interaction of GID1 with a DELLA protein termed SLENDER RICE 1 (SLR1), a repressor of GA signalling [14]. The GID1–GA–SLR1 complex can interact with the F-Box protein GID2, which triggers the polyubiquitination of SLR1 by the SCF^{GID2} complex and its degradation by the proteasome (Figure 1). In *Arabidopsis* there are five homologous genes for SLR, termed REPRESSOR OF *ga1-3* (RGA), GA-INSENSITIVE (GAI) and RGA-LIKE 1–3 (RGL1–RGL3), and two homologous genes for GID2 termed SLEEPY (SLY) and SNEEZY (SNE). Following the degradation of DELLA proteins, several effectors of GA signalling, such as the phytochrome-interacting factors (PIFs), can bind DNA and modulate the expression of GA target genes [15,16].

There are still large gaps in our understanding of SL signalling. However, in the same manner as GA, SL binding to its receptor D14 triggers a conformational change that allows the SL–D14 complex to interact with the F-Box E3 ligase DWARF3 (D3) and with a repressor of SL signalling that resides in the nucleus, DWARF53 (D53) [13]. This interaction leads to the polyubiquitination of D53 by the SCF^{D3} complex and its degradation by the proteasome, which in turn leads to the induction of SL-responsive genes (Figure 1) [17]. In *Arabidopsis* there eight homologous genes for SLR, termed SUPPRESSOR OF MAX2 1 (SMAX1) and SMAX1-LIKE 2–8 (SMXL2–SMXL8), and there is one homologous gene for D3 termed MORE AXILLARY BRANCHES (MAX2). Effectors of SL signalling have so far remained elusive. However, BR-INSENSITIVE 1 EMS-SUPPRESSOR 1 (BES1), which also regulates BR responses, appears to be targeted for degradation by an SCF^{D3} complex [18].

Abscisic acid (ABA) signalling

ABA perception and signalling are complex, and combine not one but several families of receptors, some of which are membrane bound, whereas others are present in the cytosol or nucleus. Here we shall only focus on the core pathway (for alternative receptors, see Table 1), which is mediated by a family of soluble receptors known as PYRABACTIN RESISTANCE (PYR)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR)/PYR-LIKE (PYL) proteins, which will be referred to as PYRs hereafter (Figure 2). In the absence of ABA, a clade of protein phosphatases 2C (PP2Cs) maintain SNF1-related kinases (SnRKs) 2.2, 2.3 and 2.6 in a dephosphorylated and hence inactive state [19]. Structural studies have shown that ABA binding to PYRs is achieved through a 'gate–latch–lock' mechanism, and is increased in the presence of PP2Cs [20,21]. However, this mechanism is different from those described for auxin and JA signalling, as there is no direct contact of PP2Cs with the hormone, and hence these are not ABA co-receptors. In the presence of ABA, PYR proteins interact with and inactivate PP2C2, which releases SnRKs to phosphorylate basic leucine zipper (bZIP) TFs, ion pumps and other effectors of ABA signalling [22] (Figure 2).

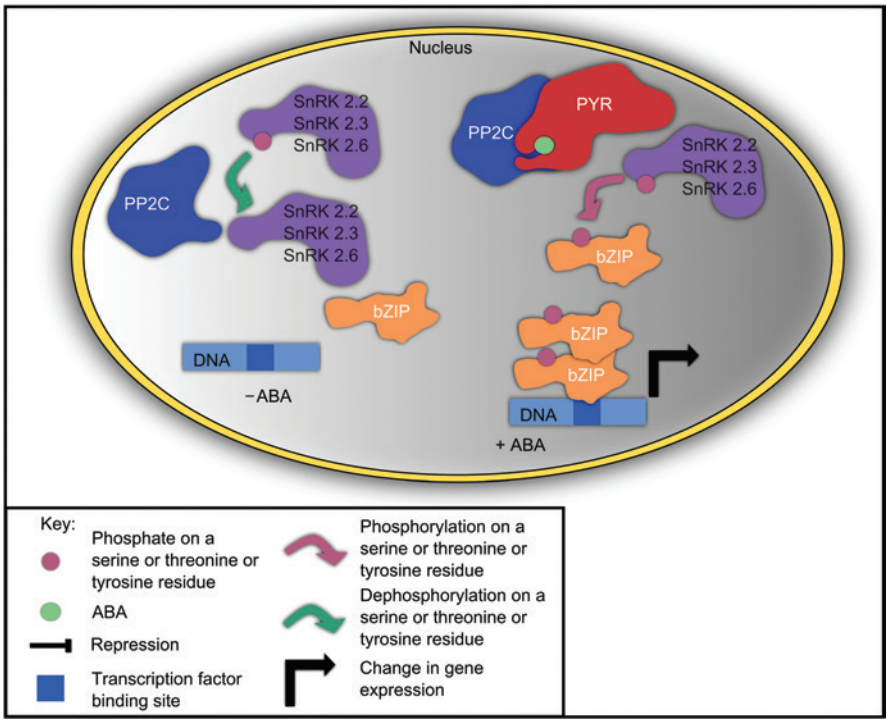


Figure 2. Signal transduction of ABA

ABA signalling starts in the cytoplasm or the nucleus. In the absence of this hormone, the level of phosphorylated effectors of ABA signalling (belonging to the bZIP family of TFs) is low, thus hindering hormonal responses. In the presence of the hormone, the receptors sequester PP2Cs phosphatase, which allows several members of a family of serine/threonine kinases, the SnRKs (SnRK2.2, SnRK2.3 and SnRK2.6) to phosphorylate effectors of ABA signalling. This in turn triggers transcriptional responses (either activation or repression of regulated genes). All of the gene names indicated are from *Arabidopsis*.

Salicylic acid (SA) signalling

Important advances have recently been made in our understanding of SA signalling. However, its signal transduction remains poorly understood. In two studies, three related proteins, NON-EXPRESSER OF PATHOGENESIS-RELATED GENES 1 1, 3 and 4 (NPR1, NPR3 and NPR4), were shown to be SA receptors in *Arabidopsis* [23,24]. However, it is unclear whether all three proteins actually bind SA. In the first study [23], it was shown that NPR3 and NPR4 control the levels of NPR1 in the nucleus through the ubiquitin–proteasome system in an SA-dependent manner. In the second study [24], NPR1 was shown to bind SA (NPR3 and NPR4 were not tested). Some results suggest that the binding of SA to NPR1 triggers a conformational change that allows NPR1 to bind DNA and regulate gene expression.

There are still many unanswered questions about how SA controls its responses, and further research is needed to elucidate whether or not the two models coexist. Notably, NPR1-mediated induction of SA responses is delayed after SA treatments, and therefore NPR1-, NPR3- and NPR4-mediated signalling appear to act as secondary responses. For this reason, no representation of its signalling system is shown, as at the time of writing no clear picture

has emerged from the literature. However, SA signalling logic is possibly unique among phytohormone signalling systems.

Cytokinins (CKs), brassinosteroids (BRs) and ethylene: signalling through transmembrane receptors

CK and BR receptors belong to two different classes of receptor kinases. Histidine kinases control CK signalling, whereas serine/threonine kinases control BR signalling. Both phytohormones trigger a reversible cascade of phosphorylation from a membrane, either the ER or the PM respectively. The cascade ends up in the nucleus where effectors of each pathway regulate the expression of target genes. Ethylene has a complex multi-layered signalling pathway, which relies on protein phosphorylation and also on the controlled degradation of specific proteins, which, as for auxin, JAs, GAs and SLs, is controlled by several F-Box proteins.

Cytokinin (CK) signalling

In *Arabidopsis*, CKs are perceived by a family of two-component histidine kinase receptors [ARABIDOPSIS HISTIDINE KINASES (AHKs) 2, 3 and 4] that are primarily localized at the ER but also found at the PM [25–27] (Figure 3). Upon binding of a bioactive CK, AHKs autophosphorylate the conserved histidine and aspartate residues in their intracellular domain, and transfer it to a conserved histidine residue on ARABIDOPSIS HISTIDINE PHOSPHOTRANSFERASE (AHP) proteins [28]. Another AHK, CYTOKININ-INDEPENDENT 1 (CKI1), also acts as an upstream regulator of the pathway and of AHP phosphorylation, but most probably in a CK-independent manner [29]. AHP1–AHP5 have the conserved histidine residue and act as positive regulators of CK signalling, whereas AHP6 lacks it, and acts as a negative regulator of CK signalling [30]. AHPs shuttle between the cytoplasm and the nucleus upon phosphorylation. In the nucleus they phosphorylate B-type ARABIDOPSIS RESPONSE REGULATORS (ARRs). Once phosphorylated, B-type ARRs control the expression of cytokinin-responsive genes that include the A-type ARRs, which are also phosphorylated by the AHPs and provide negative feedback on CK signalling [31] (Figure 3).

Brassinosteroid (BR) signalling

BR signal transduction involves a complex phosphorylation/dephosphorylation cascade (Figure 4). At first, BRs are perceived by a family of three leucine-rich repeat (LRR) receptor-like kinases that are localized at the cell surface. BRASSINOSTEROID-INSENSITIVE 1 (BRI1), the main BR receptor in *Arabidopsis*, and its homologous genes BRI1-LIKE1 and BRI1-LIKE 3 (BRL1 and BRL3), can bind brassinolide (BL), the most bioactive BR, together with one of their co-receptors, BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1), BAK1-LIKE 1 (BKK1) and SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (SERK1) [32–34]. As was seen in the example of auxin and JA, brassinolide acts as a 'molecular glue' that brings the receptor and co-receptor together [32]. Binding of BL stimulates the phosphorylation and membrane detachment of BRI1 KINASE INHIBITOR1 (BKI1), which activates the

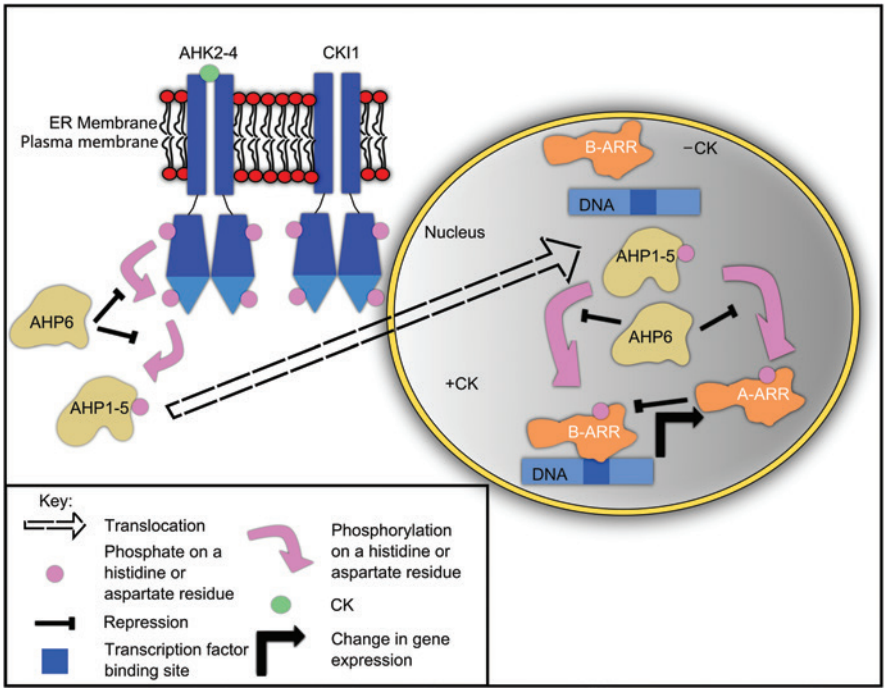


Figure 3. Signal transduction of CK

CK signalling starts from the plasma membrane or ER membrane. In the presence of the hormone, a phosphorylation cascade is activated by the receptors. CKI1 is able to promote CK responses, but it appears to be unable to bind bioactive CK. In the presence of CK, AHPs are phosphorylated, and this allows them to shuttle from the cytoplasm to the nucleus where they phosphorylate effectors of CK signalling, namely the B-type ARRs, which results in the triggering of transcriptional responses (either activation or repression of regulated genes). Among the genes so regulated, A-type ARRs, which can also be phosphorylated by the AHPs, provide direct feedback on CK responses. All of the gene names indicated are from *Arabidopsis*.

trans-phosphorylation of the receptor and co-receptor [35]. This allows BRI1 to interact with and phosphorylate two families of membrane-anchored kinases related to BR-SIGNALLING KINASE 1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1s). Phosphorylated BSKs and CDGs phosphorylate a family of cytoplasmic phosphatases related to BRI1-SUPPRESSOR 1 (BSU1) [36]. Phosphorylated BSU1 inactivates BRASSINOSTEROID-INSENSITIVE2 (BIN2), a soluble serine/threonine kinase, by dephosphorylating a conserved tyrosine residue. The level of phosphorylated BIN2 controls the activity of BR signalling effectors, the TFs BRASSINAZOLE-RESISTANT 1 and 2 (BZR1 and BZR2) [37]. Phosphorylated BIN2 phosphorylates the BZRs, which inactivates them. Conversely, inactivation of BIN2 by BSU1 triggers the dephosphorylation of BZRs by a clade of protein phosphatases 2A (PP2As). Non-phosphorylated BZRs interact with other TFs to regulate the expression of BR target genes [37] (Figure 4).

Ethylene (ET) signalling

There are five ethylene receptors encoded in the *Arabidopsis* genome, namely ETHYLENE-RESPONSE 1 and 2 (ETR1 and ETR2), ETHYLENE-RESPONSE SENSOR 1 and 2 (ERS1 and

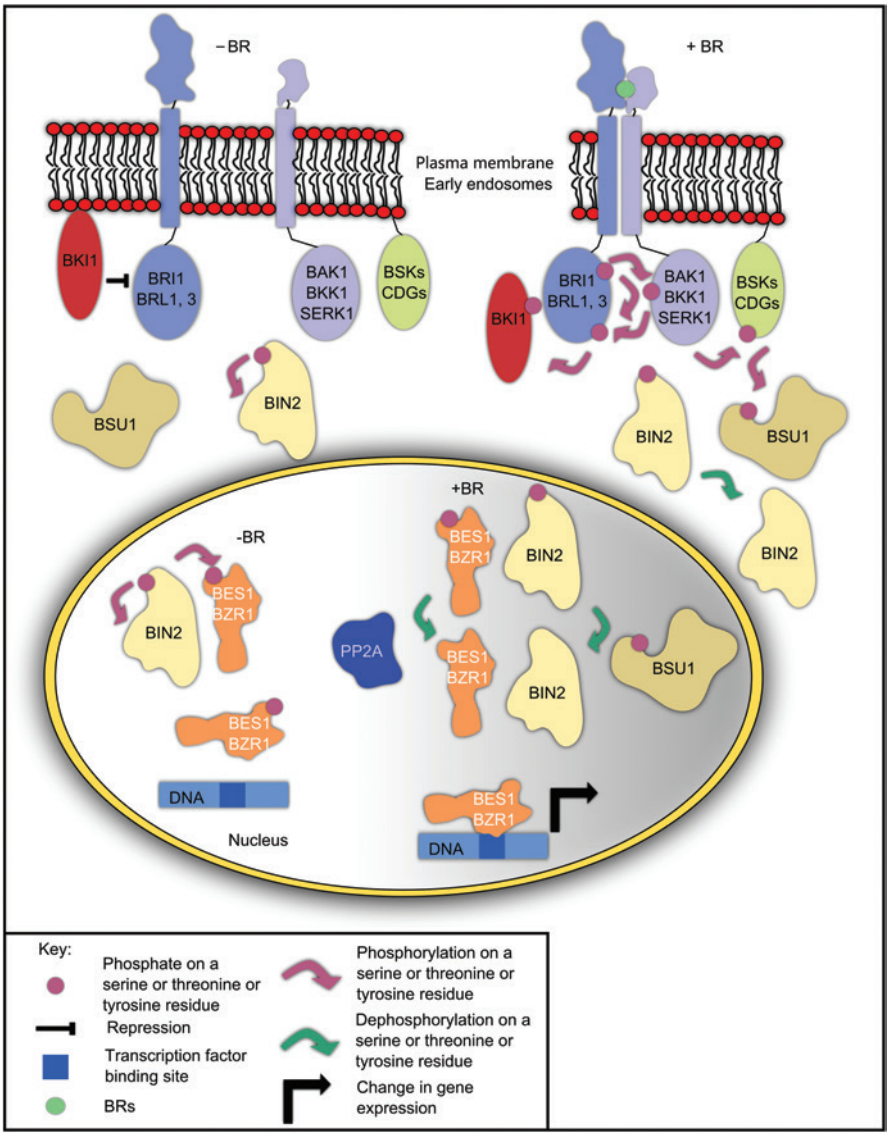


Figure 4. Signal transduction of BR

BR signalling starts from the plasma membrane or the membrane of early endosomes. In the absence of the hormone, the receptor BRI1 is kept inactive by BKI1. In the presence of the hormone, a phosphorylation cascade is activated by the receptors. The cascade leads to the inactivation of BIN2, which increases the proportion of unphosphorylated BES1 and BZR1 (collectively referred to as BZRs). Unphosphorylated BZRs trigger transcriptional responses (either activation or repression of regulated genes). All of the gene names indicated are from *Arabidopsis*.

ESR2) and ETHYLENE-INSENSITIVE 4 (EIN4), which are all localized in the ER membrane [38] (Figure 5). Like CK receptors, they share a strong homology with bacterial two-component histidine kinases. However, it is unclear whether the kinase activity is essential for their function. The receptors interact with CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a serine/threonine kinase that in the absence of ethylene phosphorylates the C-terminal end of EIN2, which is also

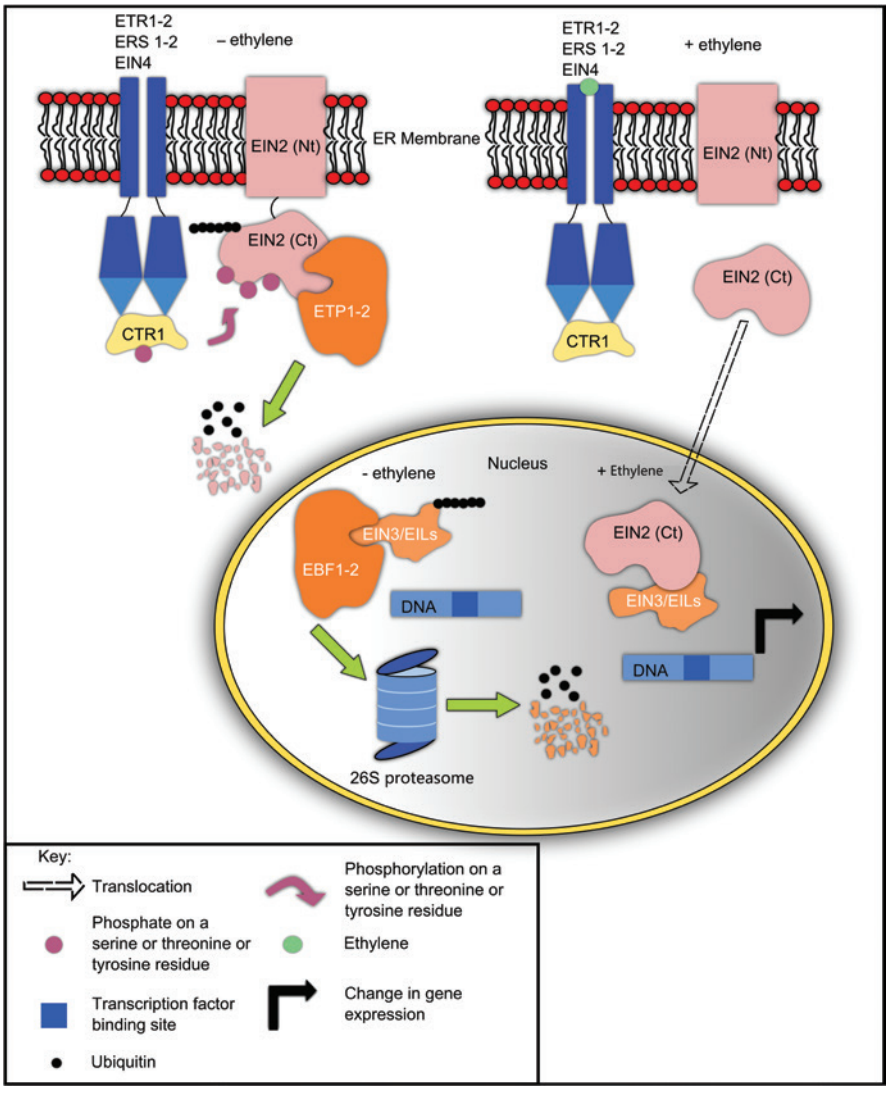


Figure 5. Signal transduction of ethylene

Ethylene signalling starts from the ER membrane. In the absence of the hormone, the receptors are constitutively active, leading to the phosphorylation of EIN2, which is the central regulator of ethylene signalling. Phosphorylated EIN2 is rapidly degraded, thus hindering ethylene responses. In the presence of ethylene, EIN2 is not phosphorylated, and this leads to the proteolytic cleavage of its C-terminus, which is translocated to the nucleus where it stabilizes effectors of ethylene signalling that belong to the EIN3/EILs family of TFs. This in turn triggers transcriptional responses (either activation or repression of regulated genes). All of the gene names indicated are from *Arabidopsis*.

located on the ER membrane [39]. In response to an ethylene signal, CTR1 is inactivated, which reduces EIN2 phosphorylation. This triggers the cleavage of EIN2, and its C-terminus is translocated to the nucleus, where it interacts with and participates in the stabilization of key effectors of ethylene signalling, namely EIN3 and EIN3-LIKE 1 to 3 (EIL1-3) [39–41].

There are two additional levels of ethylene signalling (Figure 5). First, EIN2 protein stability is regulated by two F-Box proteins, namely EIN2-TARGETING PROTEIN 1 and 2 (ETP1 and ETP2), which trigger EIN2 degradation in the absence of ethylene [42]. Secondly, EIN3 and EIL1 protein levels are also regulated by two F-Box proteins, EIN3-BINDING F-BOX 1 and 2 (EBF1 and EBF2), which also trigger EIN3 and EIL1 degradation in the absence of ethylene [43,44]. The complexity underlying these interactions is still largely a mystery. However, they probably allow the integration of multiple signals to fine-tune ethylene responses.

Discussion

As we have seen, hormones in plants use three main strategies to transduce their signals to their effectors, namely the control of protein degradation, protein phosphorylation, or a combination of both. The end result is usually reprogramming of the repertoire of genes expressed by the target cell. A key aspect of phytohormones is that both individually and in various combinations they control very diverse developmental processes.

This is primarily achieved by the redundancy at each step of their signalling pathways. For instance, CK signalling includes four receptors (the AHKs and CKI1), six intermediates (the AHPs) and 21 effectors (the ten A-type and 11 B-type ARRs). As different homologues have different biochemical properties and expression profiles, the available combinations of receptors and intermediates, of intermediates and effectors, and of effectors between them allow for a vast range of possible responses. In addition, hormonal cross-talk allows these responses to be fine-tuned. For example, the signalling pathway of both GA and BR converges on the PIF TFs, which are master regulators in the control of cell elongation and growth [45].

In this chapter we have focused on signalling systems that lead to changes in gene expression. However, there are numerous examples of hormonal signals triggering non-transcriptional responses. For instance, auxin can bind a PM- and ER-localized protein, termed AUXIN-BINDING PROTEIN 1 (ABP1). ABP1 has historically been regarded as the regulator of non-transcriptional auxin responses, and notably involved in clathrin-mediated endocytosis. However, the link between ABP1 and downstream responses has remained elusive despite its crucial function, as demonstrated by the embryonic lethality of *abp1* loss-of-function mutants. A recent study may have identified receptor-like kinase transmembrane kinases, TRANSMEMBRANE KINASE 1–4 (TMK1–TMK4), as the missing links signalling downstream of ABP1 [46]. Future research will determine the impact of ABP1 on auxin responses, and notably on their interaction with the TIR1/AFB1–AFB5 pathway [47], although the importance of this alternative auxin receptor has recently been challenged [48].

Overall, it can be noted that signalling between protein degradation and protein phosphorylation will have a direct impact on the dynamics of the response. Indeed, protein phosphorylations are reversible, thus allowing their signalling pathways to be swiftly turned on and off. In contrast, protein degradation is permanent and requires *de novo* translation to shut down a signalling pathway which, in terms of time and energy cost, is significant compared with protein phosphorylation.

Conclusions

An understanding of phytohormone signalling is essential for advances in both fundamental and applied research. Novel elite crop varieties can be selected based on particular traits related to hormone signalling. This is exemplified by the Green Revolution, which took place between the 1970s and the 1990s, and saw the emergence of novel crop cultivars that had much improved yields, which were later shown to be primarily linked to altered GA signalling.

The recent identification of the receptors for SLs and SA, which are involved in the regulation of shoot branching and the response to pathogens respectively, is a very exciting development [23,24,49]. In view of the much reduced increments in wheat production resulting from plateauing yields, these discoveries offer promising routes for exploration by plant breeders.

Finally, the deciphering of hormone signalling allows the design of specific tools for studying signal transduction, such as the novel hormone biosensors reported in the literature [50]. These will enable a much better understanding of the fundamental processes that govern hormone signalling and plant development.

Summary

- There are nine small signalling molecules that have been described in plants and for which receptors are known. These are termed phytohormones.
- Phytohormone receptors that are soluble, and hence localized in the cytoplasm and/or the nucleus, include auxin, jasmonates, gibberellins, strigolactones, salicylic acid and abscisic acid. Those that are insoluble, and hence membrane-bound, include brassinosteroids, cytokinins and ethylene.
- Their signalling pathways can be classified as protein-phosphorylation-dependent or protein-degradation-dependent, or a combination of both.
- Protein degradation through the SCF complex plays a key role in regulating the transcriptional responses to auxin, jasmonates, gibberellins, strigolactones and ethylene.
- Abscisic acid promotes the phosphorylation of bZIP TFs, which allows their binding to DNA and the regulation of target genes.
- Salicylic acid signalling remains poorly understood. However, it is expected that important discoveries will be made in the near future, as its receptors have recently been identified.
- Histidine kinase receptors control the responses to cytokinins and ethylene. The kinase activity of the receptor is required for cytokinin signalling, but it is unclear whether this is also the case for ethylene signalling.
- A phosphorylation cascade controls plant responses to brassinosteroids and cytokinins.
- Ethylene is the only phytohormone that negatively regulates its receptor, which is otherwise constitutively activated in the absence of the signal.

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