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LEAD ARTICLE

Plant Hormone Signalling: Current Perspectives on Perception and Mechanisms of Action

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ABSTRACT

Plant growth and development are regulated by a chemically and structurally diverse group of hormones. During the last few decades many advances have been made in understanding the perception and mechanisms of action of these plant hormones. While certain hormone responses are not necessarily related to gene regulation, all these hormones are involved in modulating gene expression by controlling either the abundance of transcriptional factors or repressors, or their activities through post-translational modifications. In this regard, ubiquitin mediated protein degradation has become a central theme in many plant hormone signalling pathways. Additionally, a multitude of novel signalling mechanisms have been uncovered for several other plant hormones during the past decade. This review discusses the recent findings related to these hormonal signalling pathways highlighting the mechanisms of hormone perception and subsequent signalling pathways leading to the regulation of gene expression.

Key words: growth and development, hormones, ubiquitin, protein degradation, E3 ligases, gene expression.

INTRODUCTION

Being sessile organisms, plants should be extremely sensitive to their surrounding and able to respond to constant changes in the environment. Execution of growth and developmental programs in response to these external as well as internal parameters are mediated by a group of structurally and chemically diverse molecules, commonly known as plant hormones. Early efforts by several leading research groups initially identified five plant hormones, which are considered as classical phytohormones. These include auxin, cytokinin, gibberellins (GA), abscisic acid (ABA) and ethylene. Recently, this list of phytohormones has been expanded to include several more chemicals such as brassionosteroids (BR), jasmonic acid (JA), salicylic acid (SA), polyamines, strigolactones (SL), nitric oxide (NO) and peptide hormones (Santner *et al*., 2009). It would not be surprising if this list continues to grow to include many more new compounds. While previous studies mainly focused on physiological responses, biosynthetic pathways and transport mechanisms of phytohormones, recent research has focused intensively on the mechanisms of hormone action. Owing to the recent advances on plant genetic, molecular and biochemical techniques, our knowledge on phytohormones has advanced to a new level. Many genes involved in hormone perception and signalling pathways have been identified, and their precise molecular functions have been characterized in detail. Responses to hormones are diverse, and involve mechanisms that may or may not require gene regulation. Although, there are non-gene regulatory hormonal responses, so far they have not been characterized extensively. Many known growth and developmental responses to hormones are due to modulation of gene expression, and these responses are among the best characterized to date (Santner *et al*., 2009). This review focuses on recent advances in molecular mechanisms of perception and signalling of several major phytohormones, emphasizing on the modulation of gene expression. As discussed in this review, hormones control the expression of genes by regulating the abundance of two types of gene regulatory proteins, transcriptional factors (TFs) and transcriptional repressors through regulated protein degradation, or by modifying their activities through post-translational modifications.

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Ubiquitin-mediated protein degradation is a common theme in several plant hormone response pathways. Ubiquitin (Ub) is a small protein that is ubiquitously found in all eukaryotes. It is specifically and covalently attached to other proteins through a process called ubiquitination which is essential either to change properties of proteins or to destroy the Ub-tagged proteins through 26S proteasome (Glickman and Ciechanover, 2002). Ub mediated protein degradation is vital to many cellular processes such as cell cycle control, transcriptional regulation, hormone signalling, pathogenesis, photomorphogenesis, chromatin structure regulation, responses to environmental challenges (Vierstra, 2009), self-incompatibility (Zhang *et al*., 2009), control of shoot apical meristem (Di Giacomo *et al*., 2013), and chloroplast biogenesis (Huang *et al*., 2013). The ubiquitination process involves three sequential enzymes, Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase (E3) (Dharmasiri and Estelle, 2002). First, E1 activates Ub by coupling with ATP to form E1-Ub in which C- terminal glycine of Ub is linked through a thiolester bond to a cysteine of E1. Ub is then transferred to a conserved cysteine in E2, and is finally transferred to the target protein through E3 Ub-ligase which recruits the target protein (Smalle and Vierstra, 2004). In *Arabidopsis*, there are two E1s, 37 E2s and more than 1400 E3s (Vierstra, 2009).

There are several groups of E3 ubiquitin ligases in plants, namely RING (REALLY INTERESTING NEW GENE), U-box, HECT (HOMOLOGY TO E6-AP C TERMINAL), CUL3-BTB (CULLIN3-BROAD-COMPLEX/ TRAMTRACK/BRIC-A-BRAC), CUL4-DDB1 (CULLIN4-DNA DAMAGE BINDING1), SCF (SKP1CULLIN1 F-BOX) and APC (ANAPHASE PROMOTING COMPLEX) (Santner and Estelle, 2010). Of these, SCF, RING and CUL3-BTB E3 ligases play a direct role in plant hormone responses. Additionally, E3 ligases are also involved in strigolactone response and biosynthetic pathways of some hormones (Santner and Estelle, 2010), but those mechanisms will not be discussed in this review.

As the name implies, SCF type E3 ligase is a multi subunit protein complex made up of SKP1, CULLIN1 (or CULLIN2), an F-box protein and RBX1 (Dharmasiri and Estelle, 2004). Target proteins to be ubiquitinated interact with the F-box protein while RBX1 protein recruits the Ub-conjugated E2 enzyme (Ub-E2). RBX1, CUL1, and SKP1 are shared by many different SCF complexes while the F-box protein is specific for each SCF complex thus giving the specificity to the complex (Figure 1a). As shown by several groups during the last few decades, several plant hormones such as auxin, JA, GA and ethylene use SCF dependent ubiquitin-proteasome pathway to regulate the expression of genes specific to each hormone (Santner and Estelle, 2009; Santner and Estelle, 2010). Of these, auxin and JA work through a very similar mechanism while GA works in a slightly different way. Ethylene on the other hand functions through a different mechanism but still utilizes the SCF E3 ligase system (see below and Figure 2). At least some ABA responses are likely to be regulated through ubiquitin-proteasome pathway using RING E3 ligases. Unlike the SCF complex, RING E3 ligases are composed of a single protein that recruits both Ub-E2 enzyme and the target protein. In this system, RING finger motif recruits the Ub-E2 (Santner and Estelle, 2010) (Figure 1b). SA on the other hand, uses CUL3-BTB type E3 ligase to regulate the abundance of a regulatory protein (Boatwright and Pajerowskaâ Mukhtar, 2013). Similar to SCF E3 ligases, CUL3-BTB E3s are multi-subunit complexes, but use CULLIN3a or 3b as the scaffold protein while BTB protein recruits the target protein (Santner and Estelle, 2010) (Figure 1c).

Perception and transduction of auxin signal

Among all the plant hormones, whether known for over a century or for just a few years, auxin (Indole 3-acetic acid, IAA) is still regarded as the most studied, and one of the most important plant hormones. Effects of Auxin on plants are evident from embryogenesis to senescence, and from root tip to shoot tip (Woodward and Bartel, 2005). The fact that auxin is involved in almost all aspects of plant growth and development underscores the magnitude of research conducted, and the volume of knowledge accumulated by many groups around the world for almost a century to elucidate the mechanism of auxin perception (Abel and Theologis, 2010).

Latest findings suggest that auxin signal may be perceived inside the nucleus, extra-cellular matrix, and ER, thus determining numerous downstream responses, mainly, the rapid regulation of gene expression and cell expansion (Friml and Jones, 2010). The role of ABP1 (AUXIN BINDING PROTEIN1) as a receptor in auxin signalling either on ER or on the outer surface of the plasma membrane has been linked to rapid cell expansion, epidermal cell pattern formation and auxin transport (Effendi and Scherer, 2011).

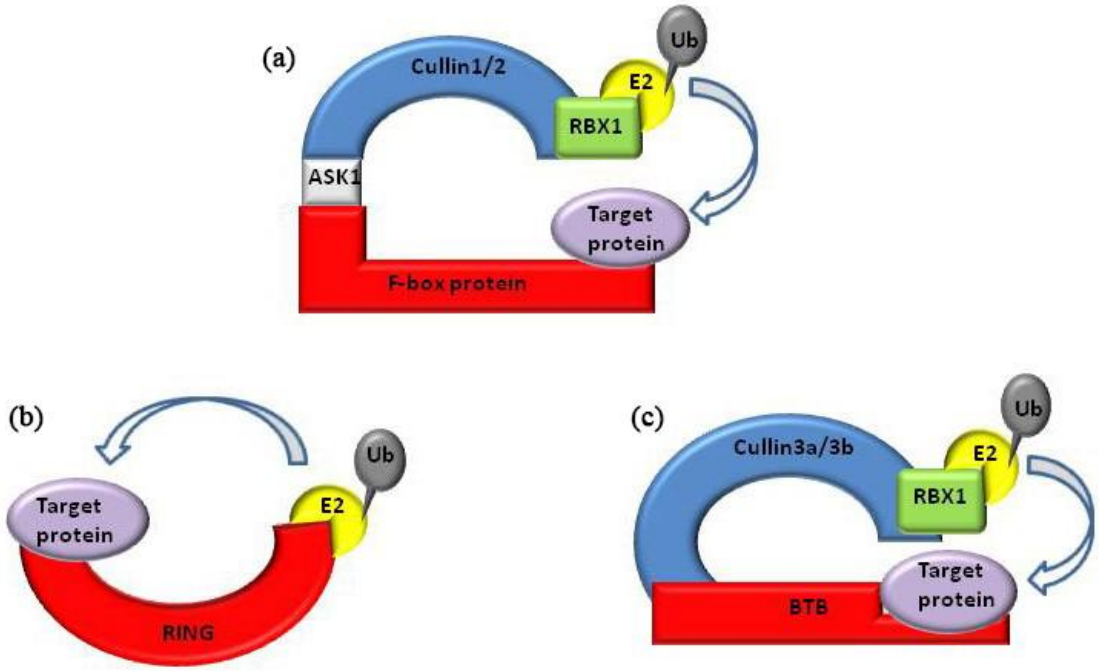

Figure 1. Schematic diagram of E3 ligases involved in ubiquitination of either transcriptional repressors or transcriptional factors. Generalized diagrams of (a) SCF, (b) RING, and (c) CUL3-BTB E3 ligases.

However, the best-elucidated auxin perception mechanism directly leading to transcriptional regulation of auxin responsive genes happens inside the nucleus. Therefore, this review will mainly focus on auxin perception in the nucleus.

Auxin signalling is a classical example for the involvement of SCF E3 ligase complex in perception of a phytohormone. When auxin is absent, auxin responsive gene expression is inhibited by Aux/IAA (Auxin/Indole-3-acetic acid) proteins which function as transcriptional repressors of auxin responsive genes. Aux/IAA proteins bind to Auxin Response Factors (ARFs) via the scaffolding function of TOPLESS1 (TPL1) protein and block the transcription of auxin responsive genes (Szemenyei *et al*., 2008). In the presence of auxin, the F-box protein TIR1 (TRANSPORTER INHIBITOR RESPONSE1) of the SCF^{TIR1} complex interacts with Aux/IAA transcriptional repressors leading to their ubiquitination (Gray *et al*., 2001) thereby promoting the ubiquitination and degradation of Aux/IAAs. Destruction of Aux/IAA releases the repression on ARF transcription factors allowing the transcription of auxin responsive genes (Figure 2a) (Gray *et al*., 2001).

How does auxin enhance the interaction between the F-box protein and Aux/IAAs? Ending a century-long search for an auxin receptor, in 2005, the F-box protein TIR1 in the model plant *Arabidopsis* was identified as an auxin receptor (Dharmasiri *et al*., 2005a; Kepinski and Leyser, 2005). Contrary to the initial belief that auxin directly or indirectly promotes the modification of Aux/IAAs or an interacting protein (Kepinski and Leyser, 2004), auxin binds directly to the TIR1 protein and enhances rapid degradation of Aux/IAAs. This direct binding of auxin to TIR1 explains the rapid transcriptional induction of auxin responsive genes. Further investigations reveal that five other members of the TIR1 protein family (AFB1-5, AUXIN SIGNALING F-BOX1-5) also function as auxin receptors (Greenham *et al*., 2011). Interestingly, structural studies as well as *in vivo* studies in yeast and *in-vitro* assays using purified proteins indicate that auxin signal is perceived together by TIR1/AFB and Aux/IAA proteins, functioning as co-receptors (Tan *et al*., 2007; Villalobos *et al*., 2012).

Further studies using x-ray crystallography reveals that auxin serves as molecular glue, enhancing the interaction between TIR1/AFBs and Aux/IAA proteins (Tan *et al*., 2007; Villalobos *et al*., 2012). Using the natural auxin IAA, as well as two synthetic auxins, 1-NAA and 2,4-D, Tan *et. al*., (2007), demonstrated that the

hydrophobic interactions between TIR1 and Aux/IAAs at the bottom of the binding pocket are enhanced by the presence of auxin, thereby increasing the affinity between TIR1 and Aux/IAAs. This finding presents a historical landmark in plant hormone research, where crystal structure of a receptor is uncovered for the first time.

Perception and transduction of jasmonate signal

Striking similarities exist between the mechanisms of perception and signal transduction of auxin and jasmonate. The co-receptor complex forms between the F-Box protein COI1 (CORONATINE INSENSITIVE1) and the transcriptional repressor JAZ (JASMONATE-ZIM DOMAIN) in the presence of jasmonate-Ile, the physiologically active form of jasmonate (Chini *et al*., 2007; Thines *et al*., 2007). COI1 is the most distant, and the sole additional member of the phylogenetic clade that consists of TIR1/AFB 1-5, and provides the substrate specificity to SCF^{COI1} E3 ligase complex (Xie *et al*., 1998). Similar to Aux/IAAs, JAZ proteins serve as transcriptional repressors by binding to general transcription factors MYB/MYC (Qi *et al*., 2011; Song *et al*., 2011), and this interaction of JAZs with transcription factors requires the association with the common co-repressor TPL. While Aux/IAA proteins interact directly with TPL, JAZ proteins interact with TPL via the adaptor protein NINJA (NOVEL INTERACTOR OF JAZ) (Pauwels *et al*., 2010). Interaction of JAZ with SCF^{COI1} leads to ubiquitination and subsequent degradation of JAZs by 26S proteasome complex, thereby releasing the MYB/MYC transcription factors for induction of jasmonate sensitive gene transcription (Perez and Goossens, 2013) (Figure 2b).

Perception and transduction of Gibberellic acid signal

GAs are composed of a large family of structurally related tetracyclic diterpenoids. This class of hormones also regulates a diverse array of developmental processes such as seed development and germination, organ elongation and control of flowering time (Peter and Stephen, 2012). GA is perceived by a nuclear localized soluble receptor, GID1 (GIBBERELLIN INSENSITIVE DWARF1). While GID1 was first identified in rice (Ueguchi-Tanaka *et al*., 2005), there are three orthologs (GID1a, GID1b and GID1c) in *Arabidopsis* (Griffiths *et al*., 2006; Nakajima *et al*., 2006). GID1 is a member of the hormone–sensitive lipase (HSL) family (Ueguchi-Tanaka *et al*., 2005). Rice gid1 and *Arabidopsis* gid triple mutants are insensitive to GA indicating that functional GID1 is required for GA responses (Griffiths *et al*., 2006).

Rice GID1 has been shown to bind with biologically active GA. Structural analysis of GID1 shows that it contains a deep binding pocket with a flexible lid (Murase *et al*., 2008; Shimada *et al*., 2008). Interaction of GA with the binding pocket of GID1 probably causes the lid to fold back, allowing it to interact with another group of proteins called DELLAs. These proteins contain an N-terminal DELLA domain and a conserved C-terminal GRAS domain, and function as transcriptional repressors of gene expression (Schwechheimer and Willige, 2009). While rice genome encodes a single DELLA protein called SLR1 (SLENDER RICE1), *Arabidopsis* genome encodes five DELLA proteins, GAI (GA-INSENSITIVE), RGA (REPRESSOR OF GA1-3), RGL1 (RGA-LIKE1), RGL2, and RGL3 (Achard and Genschik, 2009). DELLA domain of these proteins is essential for interaction with GID1 receptor as deletions in this domain of GAI and RGA results in abolishing the interaction with GID1 (Griffiths *et al*., 2006; Willige *et al*., 2007).

How DELLA proteins function as transcriptional repressors was not clear until recently. Although a previous study using chromatin immunoprecipitation (ChIP) has shown that RGA is one of the DELLA proteins, which interacts with target gene promoters (Zentella *et al*., 2007), DELLA proteins do not contain a known DNA binding domain suggesting that DELLA may repress gene expression indirectly by binding with other DNA interacting proteins. For example, light inhibits hypocotyl cell elongation while GA enhances it. This process is regulated by a group of transcriptional factors known as PIFs (PHYTOCHROME INTERACTING FACTORS), members of a subfamily related to bHLH (BASIC HELIX–LOOP–HELIX) transcription factors (Leivar *et al*., 2008). In the absence of GA, DELLA proteins interact with the bHLH DNA binding domains of PIFs thereby preventing the PIFs' interaction with the G-box elements in the promoters of light regulated genes. In the presence of GA, DELLA proteins are degraded through ubiquitin-proteasome pathway allowing PIFs to interact with corresponding G-box elements (de Lucas *et al*., 2008; Feng *et al*., 2008). Thus, GA induced gene expression shares similarities with both auxin and JA, as all these hormones degrade respective transcriptional repressors through ubiquitin-proteasome pathway.

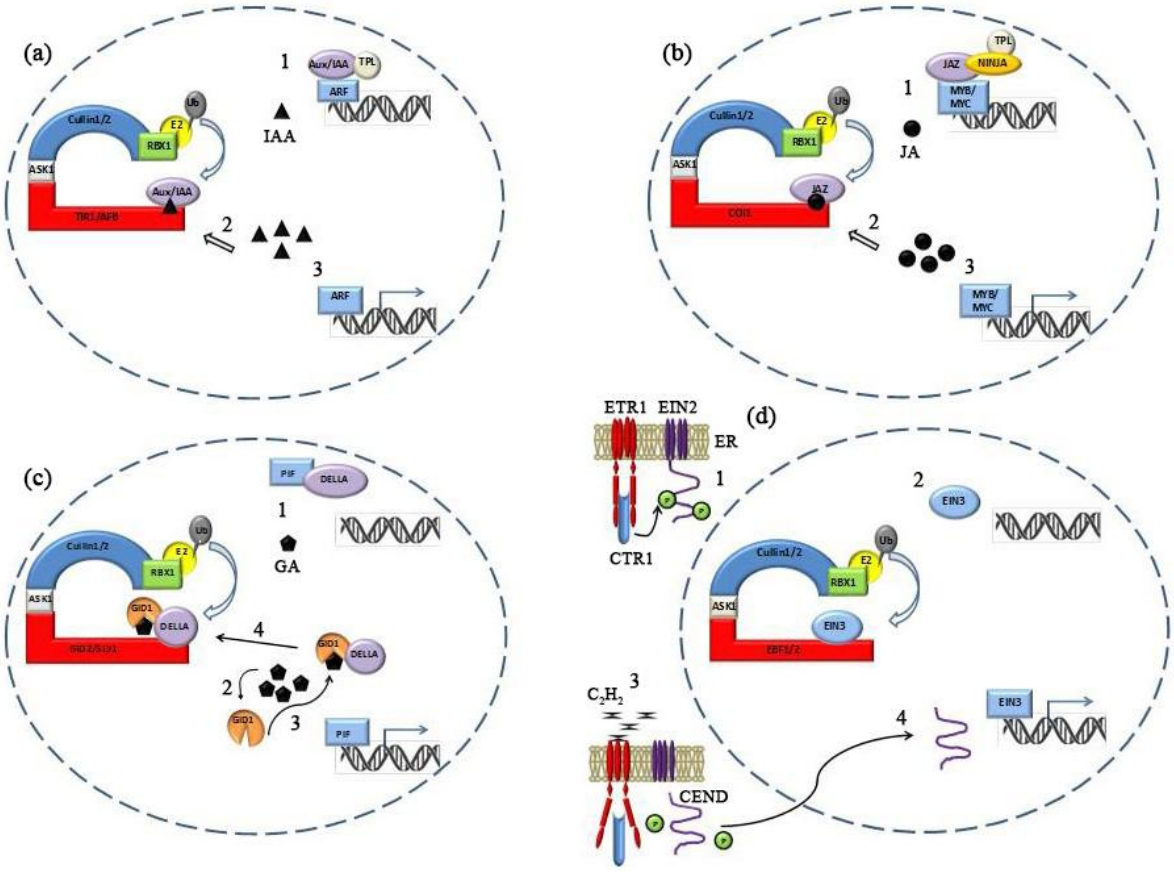

Figure 2. Schematic diagrams of hormonal signaling pathways that use SCF E3 ligases. Broken line indicates the nuclear membrane.
(a) 1. At low level of auxin, Aux/IAA and TPL co-repressor intetracts with transcriptional factor ARF repressing the gene transcription. 2. At high levels, auxin functions as molecular glue to promote the interaction between Aux/IAA and the TIR1/AFB proteins enhancing the ubiquitination of Aux/IAA repressors. 3. Ubiquitinated Aux/IAAs degrade through 26S proteasome allowing transcription of auxin inducible genes.
(b)1. At low level of JA, JAZ repressor along with TPL and NINJA bind to MYB/MYC transcriptional factors inhibiting gene transcription. 2. At high level, JA promotes interaction between JAZ protein and the F-box protein COI1 enhancing the ubiquitination of JAZ repressors. 3. Ubiquitinated JAZ proteins degrade through 26S proteasome relieving the repression on MYB/MYC transcriptional factors.
(c) 1. Al low level of GA, DELLA repressor interacts with DNA binding domain of PIF transcriptional factor. 2. At high levels, GA binds to its receptor GID1 probably changing its conformation. 3. This conformational change allows the GA-GID1 complex to interact with DELLA repressor. 4. GA-GID1-DELLA complex interacts with its F-box protein GID2/SLY1 allowing the ubiquitination of DELLA protein.
(d) 1. Ethylene receptor ETR1and EIN2 are localized to the ER memberane. When there is no ethylene, C-terminal end of EIN2 (EIN2-CEND) is phosphorylated through CTR1 and activated ETR1. 2) This allows EIN3 transcriptional factor to interact with F-box proteins EBF1/EBF2 through an unknown mechanism enhancing the EIN3 ubiquitination. 3. When ethylene binds, ETR1 deactivates and loses interaction with CTR1 inhibiting the phosphorylation of EIN2-CEND. This results in dephosphorylation of CEND and proteolytic clevage. 4. CEND is translocated into the nucleus inhibiting the ubiquitination of EIN3. Accumulation of EIN3 results in ethylene induced gene transcription.

According to the current model, in response to GA, DELLAs interact with GID1 and the complete GID1/GA/DELLA complex interacts with corresponding $SCF^{GID2/SLY1}$ complex in which GID2 (GIBBERELLIN INSENSITIVE DWARF2) and SLY1 (SLEEPY1) are F-box proteins found in rice (Sasaki *et al*., 2003) and *Arabidopsis* (McGinnis *et al*., 2003) respectively (Figure 2c). What causes DELLAs to interact with GID2/SLY1 in response to GA is not clear, but the interaction of DELLA domain with GID1/GA may cause a conformational change in the C-terminal GRAS domain of DELLA proteins allowing them to interact with GID2/SLY1 (Murase *et al*., 2008), and subsequently get ubiquitinated and degraded by 26S proteasome (Hedden, 2008). Nevertheless, another study indicates that ubiquitination-independent down regulation of DELLA proteins may also exist in GA signalling (Ariizumi *et al*., 2008).

Perception and transduction of ethylene signal

Ethylene, a gaseous hormone that was earlier known for fruit ripening, is involved in many other growth and developmental processes such as seed germination, senescence, abscission and abiotic and biotic responses (Kendrick and Chang, 2008). Unlike other plant hormones that use SCF type E3 ligases, ethylene perception occurs through membrane bound receptors located in the endoplasmic reticulum (ER). In *Arabidopsis*, there are five members of ethylene receptor proteins known as ETR1 (ETHYLENE RESPONSE1), ETR2, ERS1 (ETHYLENE RESPONSE SENSOR1), ERS2 and EIN4 (ETHYLENE INSENSITIVE4). These five members show relatedness to bacterial two component histidine kinase (HK) sensors, and bind to ethylene through their N-terminal domains that are embedded in the ER (Yoo *et al*., 2009). They are divided into two sub families (I and II) depending on the number of transmembrane domains (TMD). Members of the subfamily-I have three TMDs, and are represented by ETR1 and ERS1 in *Arabidopsis*. These two receptors possess histidine kinase activity. The subfamily-II consists of ETR2, ERS2 and EIN4 and possesses serine/threonine kinase activity (Kendrick and Chang, 2008). Genetic studies indicate that unlike other plant hormone receptors that function as positive regulators, ethylene receptors function as negative regulators of ethylene signalling (Hua and Meyerowitz, 1998; Qu *et al*., 2007).

All the members of the ethylene receptor family physically interact with each other to form a large multimeric signalling complex at the ER (Ju and Chang, 2012). Nevertheless, the basic functional unit appears to be a homodimer linked with a disulfide bond, but these receptors probably assemble into higher order multimeric complexes for signal amplification and cross-talk, as is the case with prokaryotic histidine-kinase linked chemoreceptors (Hall *et al.,* 2000; Gao *et al*., 2008).

CTR1 (CONSTITUTIVE TRIPLE RESPONSE1) is known to function just immediately downstream of ethylene receptors (Kieber *et al*. 1993). CTR1 physically interacts with all five ethylene receptors to varying levels. For example, CTR1 interacts more efficiently with ETR1 and ERS1 (subfamily I) receptors compared to ETR2 that belongs to the subfamily II (Clark *et al*., 1998; Cancel and Larsen, 2002). CTR1 is a Mitogen Activated Protein Kinase Kinase Kinase (MAPKKK) related to Raf protein kinase suggesting that ethylene signalling is regulated by MAPKinase (MAPK) pathway (Ouaked *et al*., 2003). Nevertheless, new evidence points to the direct contact of CTR1 with the next downstream signalling component known as EIN2 (ETHYLENE INSENSITIVE2) (Ju and Chang, 2012).

The loss-of-function *ein2* mutation is insensitive to all tested ethylene responses suggesting that EIN2 plays a central role in ethylene responses (Alonso *et al*., 1999). EIN2 is a transmembrane protein with a hydrophilic carboxyl-terminus. Similar to ethylene receptors and CTR1, EIN2 is also localized to the ER membrane, with the hydrophilic C-terminus facing the cytoplasmic side. EIN2 is required for the stabilization of transcriptional factors, EIN3 (ETHYLENE INSENSITIVE3) and EIL1 (ETHYLENE INSENSITIVE3-LIKE1) that are necessary for ethylene induced gene transcription (Guo and Ecker, 2003). Recent studies indicate that hydrophilic C-terminal end of EIN2 (EIN2-CEND) is cleaved in response to ethylene and then translocated to the nucleus connecting ER-originated ethylene signal to the subsequent events in the nucleus (Ji and Guo, 2013). In the nucleus, EIN2-CEND involves in regulating the abundance of transcriptional factors, EIN3 and EIL1.These proteins are degraded through ubiquitin proteasome pathway involving $SCF^{EBF1/EBF2}$ in which EIN3 BINDING F-BOX PROTEIN1 (EBF1) and EBF2 function as ethylene specific F-box proteins (Guo and Ecker, 2003).

According to the current model, in the absence of ethylene, activated ethylene receptors interact

with CTR1 leading to the phosphorylation of EIN2. This will prevent the cleavage of EIN2-CEND and its subsequent translocation to the nucleus. As a result, EIN3 and EIL1 interact with EBF1/EBF2 of the $SCF^{EBF1/2}$ leading to ubiquitination of EIN3/EIL1 and their subsequent degradation through 26S proteasome. Thus, in the absence of ethylene, due to the low abundance of EIN3/EIL1, ethylene induced gene expression is inhibited.

Binding of ethylene to receptors inhibits the phosphorylation of CTR1, resulting in dephosphorylation of EIN2, thus enhancing its CEND cleavage and subsequent transport of CEND to the nucleus. The EIN2- CEND interferes with EIN3/EIL1 ubiquitination and degradation perhaps by either down regulating EBF1 and EBF2 expression (An *et al*., 2010), or through an unknown mechanism, thus leading to the accumulation of EIN3/EIL1 transcription factors resulting in ethylene induced gene transcription (Figure 2d).

Perception and transduction of ABA signal

ABA is one of the important plant hormones that helps plants to adapt to environmental challenges (Finkelstein *et al*., 2002). High salinity, drought, low temperature and pathogen attacks enhance ABA responses in plants leading to physiological changes required to survive such adverse conditions (Lee and Luan, 2012). ABA is involved not only in stress responses but also in regulating leaf size, inter-node length, seed dormancy, bud dormancy, embryo and seed development and reproduction. Early studies suggest that ABA may be perceived by multiple receptors at various cellular locations (Guo *et al*., 2011). Confirming those observations, three ABA receptors have been identified. ChlH/ABAR (H SUBUNIT OF THE CHLOROPLAST Mg^{2+}-CHELATASE)/(ABA RECEPTOR) is a chloroplast envelope localized ABA receptor (Shen *et al*., 2006). ABA can also bind to GTG1/GTG2 (GPCR-TYPE G PROTEIN1 AND 2) plasma membrane localized ABA receptors (Pandey *et al*., 2009). PYR/PYL/RCARs (PYRABACTIN RESISTANCE/PYRABACTIN RESISTANCE LIKE/REGULATORY COMPONENT OF ABA RECEPTORS) were identified as nucleo-cytoplasmic ABA receptors (Ma *et al*., 2009; Park *et al*., 2009). ChlH/ABAR binds with ABA (Shen *et al*., 2006) and promotes its interaction with WRKY transcriptional factors, WRKY40, 18 and 60 (Shang *et al*., 2010). At low ABA levels, WRKY40 represses key ABA responsive genes. Therefore, the binding of ChlH/ABAR with WRKYs retains them in the cytoplasm relieving the repression of ABA responsive genes such as ABI5 and DREB2A (Shang *et al*., 2010).

GTG1 and GTG2, a class of GPRCs (G-PROTEIN COUPLED RECEPTORS) have also been identified as ABA receptors (Pandey *et al*., 2009). They can bind with sole *Arabidopsis* canonical heterotrimeric GPA1 (G-PROTEIN α-SUBUNIT1). GTGs have GTPase activity and contain a nucleotide binding site. Binding of GDP to GTGs enhances the ABA binding ability, thus proposed as the active form.GPA1 interaction abolishes GTGs' GTPase activity (Pandey *et al*., 2009). It has been suggested that GTGs regulate the ABA response via regulation of ion channel activity (Geiger *et al*., 2009). However, the downstream signalling components of this pathway remain elusive.

Currently, the best established ABA perception happens via PYR/PYL/RCAR receptors (Figure 3a). Binding of ABA to PYR/PYL/RCAR changes its conformation to facilitate the binding of PP2C (TYPE 2C PROTEIN PHOSPHATASE) phosphatise, and inactivates it (Melcher *et al*., 2009; Miyazono *et al*., 2009; Park *et al*., 2009). When the cellular ABA levels are low, PP2Cs de-phosphorylate SnRK2s (SNF1-RELATED PROTEIN KINASES) (Fujii *et al*., 2009; Umezawa *et al*., 2009), and prevent SnRK2s from phosphorylating its target proteins such as b-ZIP transcription factors and ion channels, which leads to repressed ABA signalling (Kobayashi *et al*., 2005; Geiger *et al*., 2009). The b-ZIP transcription factors are ABA responsive element (ABRE) binding factors (AREBs) such as AREB1, 2, 3 and ABI5 (Uno *et al*., 2000; Furihata *et al*., 2006). When PP2Cs are inactivated in the presence of ABA, SnRKs get activated by autophosphorylion and lead to phophorylation of b-ZIP transcription factors to regulate ABA responsive gene activation (Fujii *et al*. 2009) (Figure 3A). SLAC1 and KAT1 are examples of two ion channels regulated by SnRK2s where both are involved in ABA regulated stomatal movements (Pilot *et al*., 2001; Vahisalu *et al*. 2008).

Although it may not play a central role, ubiquitin proteasome mediated protein degradation has been implicated at least in some ABA responses. Of several RING E3 ligases linked to ABA responses, AIP2 (ABI3 INTERACTING PROTEIN2) and KEG (KEEP ON GOING) RING E3 ligases regulate the abundance of ABI3 and ABI5 transcriptional factors, respectively, in response to ABA.

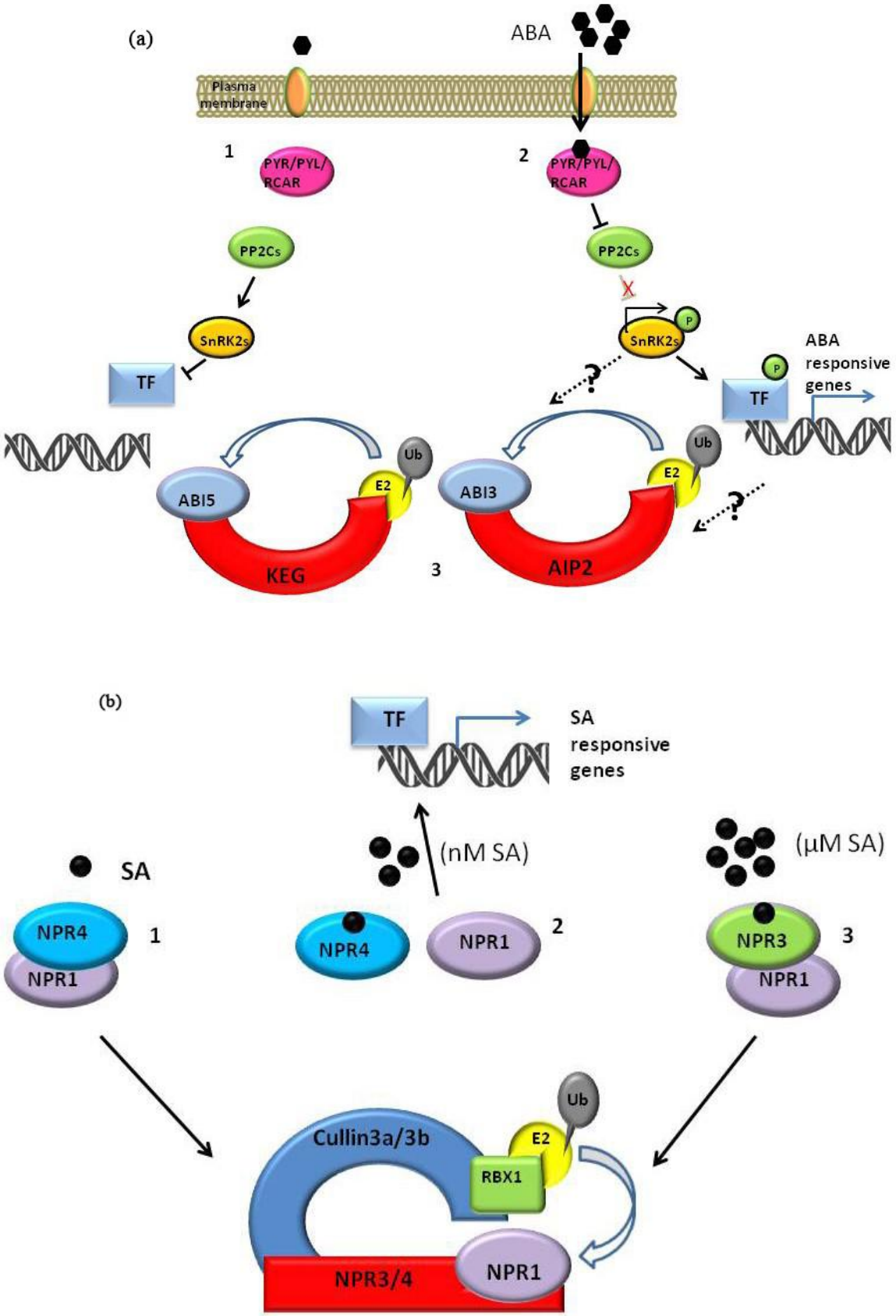

Figure 3. Schematic diagram of (a) PYR/PYL/RCAR – ABA and (b) SA signalling pathways (continued in page 9).

Figure 3 (contd.). (a) PYR/PYL/RCAR – ABA: 1. At low levels of ABA, active PP2C does not interact with the ABA receptor, PYR/PYL/RCAR. Thus, PP2C dephosphorylates SnRK2. Dephosphorylated SnRK2 is unable to phosphorylate TFs necessary for gene activation. 2. At high ABA levels, ABA interacts with the receptor, PYR/PYL/RCAR, allowing it to bind and inactivate PP2C. As a result, SnRK2 autophosphorylates and subsequently phosphorylate TFs involved in ABA induced gene expression. 3. ABA enhances the ubiquitination and degradation of ABI3 transcriptional factor probably by enhancing the expression of AIP2. Conversely, ABA prevents ABI5 from ubiquitination and degradation through KEG E3 ligase. Intermediate signalling components that connect KEG, AIP2 E3 ligases to ABA perception are not yet known.
(b) SA signalling pathways: 1. NPR1 regulates the expression of SA induced genes. At very low levels of SA, NPR1 interacts with NPR4 and recruits to the CUL3-NPR4 E3 ligase enhancing the ubiquitination and degradation of NPR1. 2. In response to pathogen attack, when SA level is slightly elevated (at nano molar levels), SA binds with NPR4 dissociating NPR1. Free NPR1 induces the pathogenesis related gene expression. 3. At very high SA levels (at micro molar levels), NPR3 binds with SA and recruits NPR1 to the CUL3-NPR3 E3 ligase enhancing the ubiquitination and degradation of NPR1.

While ABA protects ABI5 from KEG mediated ubiquitination and destruction through an unknown mechanism, ABA enhances AIP2 mediated ubiquitination and degradation of ABI3 probably by enhancing the AIP2 expression (Santner and Estelle, 2010) (Figure 3a). Nevertheless, intermediate steps between ABA perception and these E3 ligases are not yet clear.

Perception and transduction of Salicylic acid signal

Although salicylic acid (SA) is known to regulate many physiological processes such as cell growth, stomatal aperture, respiration, seed germination, seedling development, thermotolerance, fruit yield, nodulation in legumes and the expression of senescence-related genes, it is best known for its central role in plant defence responses. Several *Arabidopsis* mutants deficient in SA synthesis are more susceptible to pathogens. (Boatwright and Pajerowska Mukhtar 2013). While many genes in SA response pathway have been identified, NPR1 (NONEXPRESSOR OF PATHEGENESIS RELAETD PROTEIN1) is known to play a central role as it controls 95% of SA-regulated genes (Wang and Yang, 2006).NPR1 is a member of the BTB domain family of proteins. BTB proteins interact with CUL3a (CULLIN3a) and CUL3b to form BTB-E3 ligases (Gingerich *et al*., 2005) (Figure 1c). In fact, studies have shown that NPR1 is ubiquitinated through CUL3-E3 ligase and subsequently degraded by 26S proteasome (Spoel *et al*., 2009). Additionally, NPR1 functions as a cofactor along with TGA transcription factors to regulate the expression of PR (PATHOGENESIS RELATED) genes (Boyle *et al*., 2009).

A recent study indicates that NPR1 related proteins, NPR3 and NPR4 function as SA receptors. While NPR3 has a low affinity, NPR4 has high affinity toSA (Fu *et al*., 2012). Though one study proposes that NPR1 is also an SA receptor (Wu *et al*., 2012), according to Fu *et al*., (2012), NPR1 does not bind to SA. Therefore, NPR1's function as an SA receptor is controversial. According to the current model, NPR3 and NPR4 help to recruit NPR1 to CUL3 E3 ligase to enhance NPR1 degradation. SA functions either to enhance or diminish NPR1 destruction depending on the SA concentration. At very low SA levels, NPR1 interacts with NPR4 increasing NPR1 recruitment to CUL3 E3 ligase, thereby degrading NPR1 through ubiquitination. At nano-molar range, SA interacts with NPR4, disrupting NPR1-NPR4 interaction and making NPR1 less susceptible toCUL3 E3 ligase dependent destruction. At very high SA levels, NPR3 interacts with SA promoting the NPR1-NPR3 interaction and thereby enhancing the NPR1 degradation (Fu *et al*., 2012; Moreau *et al*., 2012) (Figure 3b). In fact, the current model explains SA responses competently, as NPR1 is necessary for basal level defense gene expression, but proteasome mediated degradation of NPR1is necessary for effector triggered immunity (ETI) (Moreau *et al.*, 2012).

Perception and transduction of BR signal

The steroid hormone, Brassinosteroid (BR) triggers the signalling cascade by binding to plasma membrane localized LRR (LEUCINE-RICH REPEAT) receptor like kinase BRI1 (BRASSINOSTEROID INSENSITIVE1) (Kim *et al*., 2010). While BRI1 functions as the major BR receptor, its homologs BRL1 (BRI1 LIKE1) and BRL3 can also perceive BR (Yang *et al*., 2011). BKI1 (BRI1 KINASE INHIBITOR1) inhibits the kinase activity of BRI1 at low levels of cellular BRs by binding to the C-terminal region of BRI1 (Wang *et al*., 2001).

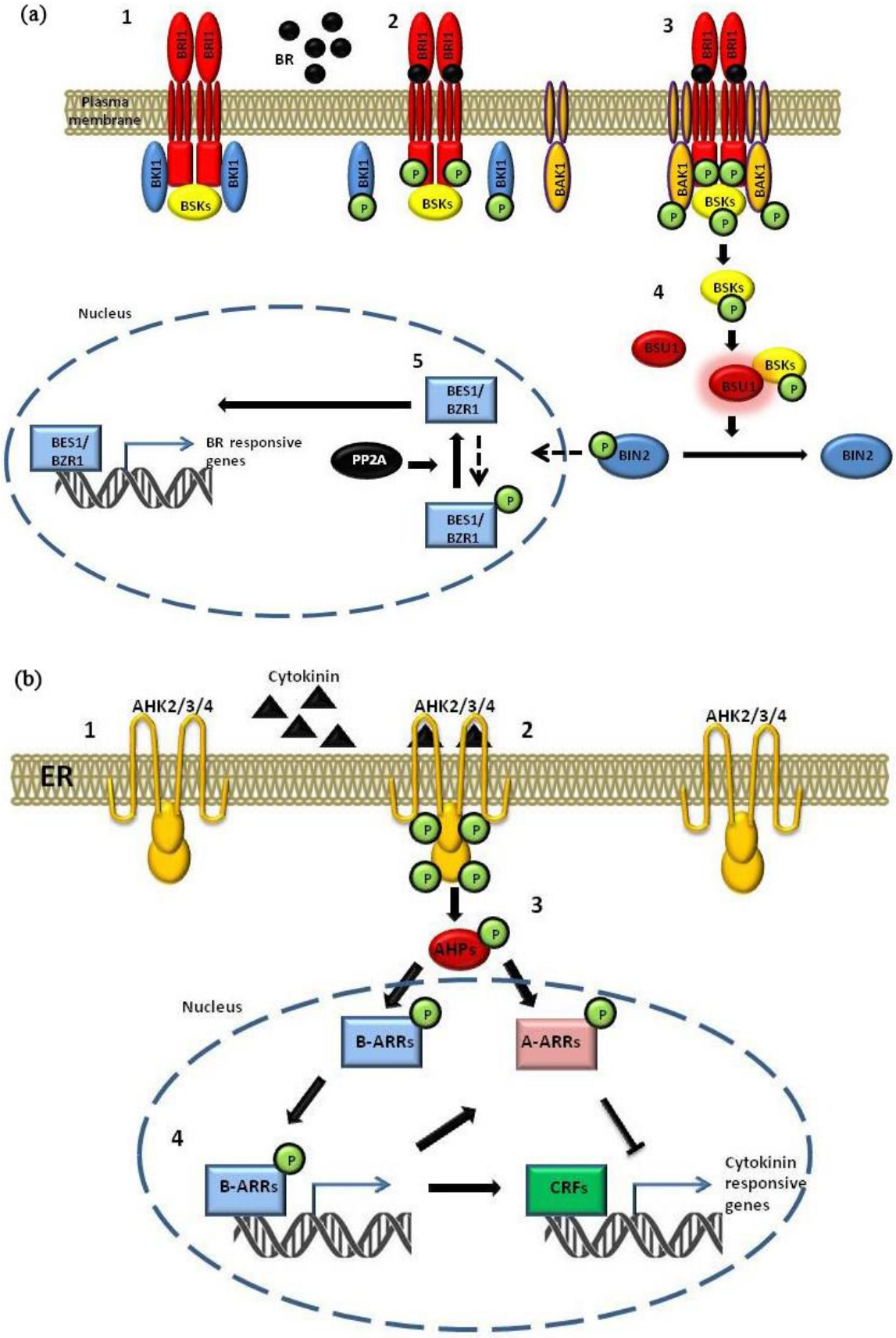

Figure 4. Overview of BR and Cytokinin perception and signal transduction (continued in page 11).

Figure 4 (contd.). Overview of BR and Cytokinin perception and signal transduction.
(a). Brassinosteroid signal transduction. 1. In the absence of BR, BKI1 interacts with BRI1 and prevents it from interacting with BAK1. Inactive BRI1 bound BSKs are also kept inactive. 2. Binding of BR to BRI1 induces dissociation of BKI1 and association with BAK1. 3. Transphosphorylation of BRI1 and BAK1 activates the BRI1 and subsequently phosphorylates BSKs. 4. Phosphorylated BSKs release from BRI1 and bind with BSU1. Activated BSU1 inactivates BIN2 kinase by dephosphorylation. 5. The active, unphosphorylated BEZ1 and BZR1 accumulate in the nucleus and induce the transcription of BR responsive genes.
(b). Cytokinin signalling pathway. 1. Cytokinin is perceived by ER localized AHK2/3/4 receptors. 2. Cytokinin binding activates autophosphorylation of AHKs. 3. Phosphoryl group in receiver domain of AHKs is then transferred to AHPs. Phosphorylated AHPs translocate into nucleus to phosphorylate Type-A and Type-B ARRs. 4. Activated Type-B ARRs induce the transcription of cytokinin regulated genes including Type-A ARRs and CRFs. While CRFs act to induce the transcription of cytokinin responsive genes, Type-A ARRs down regulate cytokinin induced transcription as a feedback mechanism.

At high levels of BR, binding of BR causes conformational changes in BRI1 that dissociates BKI1 and undergoes autophosphorylation (Wang *et al.*, 2001; Jaillais *et al*., 2011). This autophosphorylation promotes the hetero-oligomerization of BRI1 with BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE1) and transphosphorylation of BAK1 and BRI1 (Kim *et al*., 2010). BAK1 belongs to a small subfamily of receptor-like kinases called SERKs (SOMATIC EMBRYOGENESIS RECEPTOR KINASES). Among them, two SERKs, SERK1 and BKK1 (BAK1-LIKE1/SERK4) act redundantly with BAK1 (Kim *et al*., 2009). The perceived BR signal is transduced through phosphorylation of BSKs (BR SIGNALING KINASES) by BRI1 (Clouse, 2011). Phosphorylated BSKs then bind and activate protein phosphatase BSU1 (BRI1 SUPPRESSOR1) (Kim *et al*., 2009). The activated BSU1 dephosphorylates and inactivates a GSK3/Shaggy-like kinase, BIN2 (BRASSINOSTEROID INSENSITIVE2). This results in the accumulation of unphosphorylated, active BR transcription factors, BES1 (BRI1-EMS SUPPRESSOR1) and BZR1 (BRASSINAZOLE RESISTANT1) (Kim *et al*., 2009). BES1 and BZR1 are bHLH (BASIC HELIX-LOOP-HELIX) like transcription factors. BZR1 and BES1 bind to promoters containing BR-response elements (BRREs) and E-BOX motifs, and regulate their transcription (Clouse, 2011; Yu *et al*., 2011) (Figure 4a).

Perception and transduction of Cytokinin signal

Similar to BR signalling, cytokinin signalling also utilizes a phosphorelay mechanism. In *Arabidopsis*, cytokinin is perceived by endoplasmic reticulum localized AHK (ARABIDOPSIS HISTIDINE KINASE) receptors (Figure 4b). Three AHK receptors have been identified in *Arabidopsis*, AHK2, AHK3 and AHK4/WOL1 (WOODENLEG1)/ CRE1 (CYTOKININ RESPONSE1) (Hwang *et al*., 2012). Cytokinin receptors have an extracellular cytokinin-binding CHASE (CYCLASE/HIS KINASE-ASSOCIATED SENSING EXTRACELLULAR) domain, HIS KINASE domain and cytoplasmic receiver domain (To and Kieber, 2008). Upon cytokinin binding to CHASE domain, AHKs autophosphorylate the conserved His residue in the HIS KINASE domain. Subsequently, the phosphate group is transferred to a conserved Asp residue in the receiver domain of AHKs, and then transferred to AHPs (HISTIDINE PHOSPHOTRANSFER PROTEINS) in the cytoplasm (Argueso *et al*., 2010; Perilli *et al*., 2010; Hwang *et al*., 2012). Five AHPs (AHP1-5) involved in cytokinin signalling (Hutchison *et al*., 2006) in *Arabidopsis* can actively shuttle between nucleus and cytoplasm independent of their phosphorylation status and cellular cytokinin level (Punwani *et al*., 2010). In the nucleus, phosphorylated AHPs can phosphorylate a conserved Asp residue in a group of transcription factors called ARRs (ARABIDOPSIS RESPONSE REGULATORS) (To and Kieber, 2008; Kieber and Schaller, 2010). There are three types of ARRs classified according to their C-terminal domains. Type-A and Type-C ARRs possess short C-termini while Type-B ARRs contain long, DNA binding C-termini. N terminal receiver domain of the unphosphorylated Type-B ARRs represses the DNA binding (Argueso *et al*., 2010; Perilli *et al*., 2010). Phosphorylated Type-B ARRs activate the transcription of cytokinin responsive genes including Type-A ARRs and CRFs (CYTOKININ RESPONSE FACTORS) (Rashotte *et al*., 2006; To and Kieber, 2008) (Figure 4b). Phosphorylation stabilizes Type-A ARRs and activates a negative feedback loop in

cytokinin signalling (To *et al*., 2007). Type-A ARR functions are also implicated in circadian regulation and meristem development (Salome *et al*., 2006; Buechel *et al*., 2010).

Conclusions and future prospects

During the last few decades, there has been a steady progress in molecular mechanisms of plant hormone responses. Receptors of several hormones such as auxin, JA, GA, ABA, BR, SA, cytokinin and ethylene have been identified and the mechanisms of perception as well as some details of signal transduction have been well characterized. Nevertheless, there are many unanswered questions. While receptors for many known phytohormones have been identified, receptors for some hormones such as strigolactone, nitric oxide, and polyamine are not yet known. Even for the hormones with characterized receptors, have we identified all the potential receptors, or are there many more to discover? There are many gaps in our knowledge on hormonal signalling pathways, and these gaps should be filled to get a better understanding of the hormonal control of plant growth and development. Additionally, these phytohormones coordinate their actions regulating the signalling pathways of each other. Though recent efforts have shed light on hormone integration, much work is needed in this area. Therefore, during the next few years we can anticipate to see a better understanding of the coordinated regulation of plant growth and development by hormones. Availability of high throughput advance technologies will certainly play a major role in our quest for dissecting the hormonal network in plants.

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