Nafees A. Khan · Rahat Nazar
Noushina Iqbal · Naser A. Anjum *Editors*

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Preface

Plants are exposed to rapid and various unpredicted disturbances in the environment resulting in stressful conditions. Abiotic stress is the negative impact of nonliving factors on the living organisms in a specific environment and constitutes a major limitation to agricultural production. The adverse environmental conditions that plants encounter during their life cycle disturb metabolic reactions and adversely affect growth and development at cellular and whole plant level. Under abiotic stress, plants integrate multiple external stress cues to bring about a coordinated response and establish mechanism to mitigate the stress by triggering a cascade of events leading to enhanced tolerance. Responses to stress are complicated integrated circuits involving multiple pathways and specific cellular compartments, and the interaction of additional cofactors and/or signaling molecules coordinates a specified response to a given stimulus. Stress signal is first perceived by the receptors present on the membrane of the plant cells. The signal information is then transduced downstream resulting in the activation of various stress-responsive genes. The products of these stress genes ultimately lead to stress tolerance response or plant adaptation and help the plant to survive and surpass the unfavorable conditions. Abiotic stress conditions lead to production of signaling molecule(s) that induce the synthesis of several metabolites, including phytohormones for stress tolerance. Phytohormones are chemical compounds produced in one part and exert effect in another part and influence physiological and biochemical processes. Phytohormones are critical for plant growth and development and play an important role in integrating various stress signals and controlling downstream stress responses and interact in coordination with each other for defense signal networking to fine-tune defense. The adaptive process of plants response imposed by abiotic stresses such as salt, cold, drought, and wounding is mainly controlled by the phytohormones. Stress conditions activate phytohormones signaling pathways that are thought to mediate adaptive responses at extremely low concentration. Thus, an understanding of the phytohormones homeostasis and signaling is essential for improving plant performance under optimal and stressful environments.

Traditionally five major classes of plant hormones have been recognized: auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Recently, other signaling molecules that play roles in plant metabolism and abiotic stress tolerance have also been identified, including brassinosteroids, jasmonic acid, salicylic acid, and nitric oxide. Besides, more active molecules are being found and new families of regulators are emerging such as polyamines, plant peptides, and karrikins. Several biological effects of phytohormones are induced by cooperation of more than one phytohormone. Substantial progress has been made in understanding individual aspects of phytohormones perception, signal transduction, homeostasis, or influence on gene expression. However, the physiological, biochemical, and molecular mechanisms induced by phytohormones through which plants integrate adaptive responses under abiotic stress are largely unknown. This book updates the current knowledge on the role of phytohormones in the control of plant growth and development, explores the mechanism responsible for the perception and signal transduction of phytohormones, and also provides a further understanding of the complexity of signal crosstalk and controlling downstream stress responses. There is next to none any book that provides update information on the phytohormones significance in tolerance to abiotic stress in plants.

We extend our gratitude to all those who have contributed in making this book possible. Simultaneously, we would like to apologize unreservedly for any mistakes or failure to acknowledge fully.

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Contents

Chapter 1
Signal Transduction of Phytohormones Under Abiotic Stresses

F. Eyidogan, M. T. Oz, M. Yucel, and H. A. Oktem

Abstract Growth and productivity of higher plants are adversely affected by various environmental stresses which are of two main types, biotic and abiotic, depending on the source of stress. Broad range of abiotic stresses includes osmotic stress caused by drought, salinity, high or low temperatures, freezing, or flooding, as well as ionic, nutrient, or metal stresses, and others caused by mechanical factors, light, or radiation. Plants contrary to animals cannot escape from these environmental constraints, and over the course of evolution, they have developed some physiological, biochemical, or molecular mechanisms to overcome effects of stress. Phytohormones such as auxin, cytokinin, abscisic acid, jasmonic acid, ethylene, salicylic acid, gibberellic acid, and few others, besides their functions during germination, growth, development, and flowering, play key roles and coordinate various signal transduction pathways in plants during responses to environmental stresses. Complex networks of gene regulation by these phytohormones under abiotic stresses involve various *cis*- or *trans*-acting elements. Some of the transcription factors regulated by phytohormones include ARF, AREB/ABF, DREB, MYC/MYB, NAC, and others. Changes in gene expression, protein synthesis, modification, or degradation initiated by or coupled to these transcription factors and their corresponding *cis*-acting elements are briefly summarized in this work. Moreover, crosstalk between signal transduction pathways involving phytohormones is explained in regard to transcriptional or translational regulation under abiotic stresses.

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1.1 Introduction

Plants have successfully evolved to integrate diverse environmental cues into their developmental programs. Since they cannot escape from adverse constraints, they have been forced to counteract by eliciting various physiological, biochemical, and molecular responses. These responses include or lead to changes in gene expression, regulation of protein amount or activity, alteration of cellular metabolite levels, and changes in homeostasis of ions. Gene regulation at the level of transcription is one of the major control points in biological processes, and transcription factors and regulators play key roles in this process. Phytohormones are a collection of trace amount growth regulators, comprising auxin, cytokinin, gibberellic acid (GA), abscisic acid (ABA), jasmonic acid (JA), ethylene, salicylic acid (SA), and few others (Tuteja and Sopory 2008). Hormone responses are fundamental to the development and plastic growth of plants. Besides their regulatory functions during development, they play key roles and coordinate various signal transduction pathways during responses to environmental stresses (Wolters and Jürgens 2009).

A range of stress signaling pathways have been elucidated through molecular genetic studies. Research on mutants, particularly of *Arabidopsis*, with defects in these and other processes have contributed substantially to the current understanding of hormone perception and signal transduction. Plant hormones, such as ABA, JA, ethylene, and SA, mediate various abiotic and biotic stress responses. Although auxins, GAs, and cytokinins have been implicated primarily in developmental processes in plants, they regulate responses to stress or coordinate growth under stress conditions. The list of phytohormones is growing and now includes brassinosteroids (BR), nitric oxide (NO), polyamines, and the recently identified branching hormone strigolactone (Gray 2004).

Treatment of plants with exogenous hormones rapidly and transiently alters genome-wide transcript profiles (Chapman and Estelle 2009). In *Arabidopsis*, hormone treatment for short periods ($<$1 h) alters expression of 10–300 genes, with roughly equal numbers of genes repressed and activated (Goda et al. 2008; Nemhauser et al. 2006; Paponov et al. 2008). Not surprisingly, longer exposure to most hormones ($\geq$1 h) alters expression of larger numbers of genes. Complex networks of gene regulation by phytohormones under abiotic stresses involve various *cis*- or *trans*-acting elements. Some of the transcription factors, regulators, and key components functioning in signaling pathways of phytohormones under abiotic stresses are described in this work. Moreover, changes in gene expression, protein synthesis, modification, or degradation initiated by or coupled to plant hormones are briefly summarized.

1.2 Auxins

Application of auxin to plant tissues brings out various responses including electrophysiological and transcriptional responses, and changes in cell division, expansion, and differentiation. Rapid accumulation of transcripts of a large number of genes which are known as primary auxin response genes occurs with auxin. Auxin gene families include the regulator of auxin response genes, auxin response factors (ARFs), and the early response genes, auxin/indole-3-acetic acid (Aux/IAA), GH3, small auxin-up RNAs (SAURs), and LBD (Abel et al. 1994; Abel and Theologis 1996; Guilfoyle and Hagen 2007; Hagen and Guilfoyle 2002; Iwakawa et al. 2002; Yang et al. 2006). Although the roles of these factors in specific developmental processes are not fully understood yet, it was suggested that many members of these gene families are also involved in stress or defense responses (Jain and Khurana 2009).

When auxin-treated cells were examined, it was proposed that part of the auxin response is mediated by modification of gene expression and that it does not require de novo protein synthesis. It was identified that three main families (Aux/IAA, GH3, and SAUR) of early auxin response genes were expressed within 5–60 min after auxin treatment (Tromas and Perrot-Rechenmann 2010).

With the tight cooperation of these genes, plants can properly respond to auxin signals and environmental stresses, as well as maintain natural growth and development. The DNA-binding domains of ARFs bind to auxin response elements (AuxREs) (TGTCTC) of auxin-responsive genes and regulate their expression (Fig. 1.1). ARFs bind with specificity to AuxRE in promoters of auxin response genes and function in combination with Aux/IAA repressors, which dimerize with ARF activators in an auxin-regulated manner. It was suggested that differences in AuxRE sequences and abundance may serve as the first level of complexity in the transcriptional regulation of auxin-responsive genes (Szemenyei et al. 2008).

Northern and reverse transcriptase PCR (RT-PCR) analyses suggested that ARF genes are transcribed in different tissues and organs in *Arabidopsis* and rice plants (Okushima et al. 2005; Wang et al. 2007a). Most ARFs have a DNA-binding domain at the N-terminal. ARFs are transcription factors involved in the regulation of early auxin response genes. It was proposed that ARFs act as activators if they contain a glutamine/serine/leucine-rich (QSL-rich) middle region or as repressors if they contain a serine or serine/proline/glycine-rich middle domain (Tromas and Perrot-Rechenmann 2010).

In the literature, it was shown that the expression of ARF genes responds to environmental or hormonal signals. ARF2, 7, and 19 transcripts increased to some level, and ARF1 transcripts decreased slightly in response to dark-induced senescence in leaves (Ellis et al. 2005). Responses of ARF genes to environmental factors were indicated to be small or negligible; therefore, it was suggested that unidentified factors should play a key role in regulating expression of these genes or regulation by environmental factors is highly specific to selected tissue type (Guilfoyle and Hagen 2007).

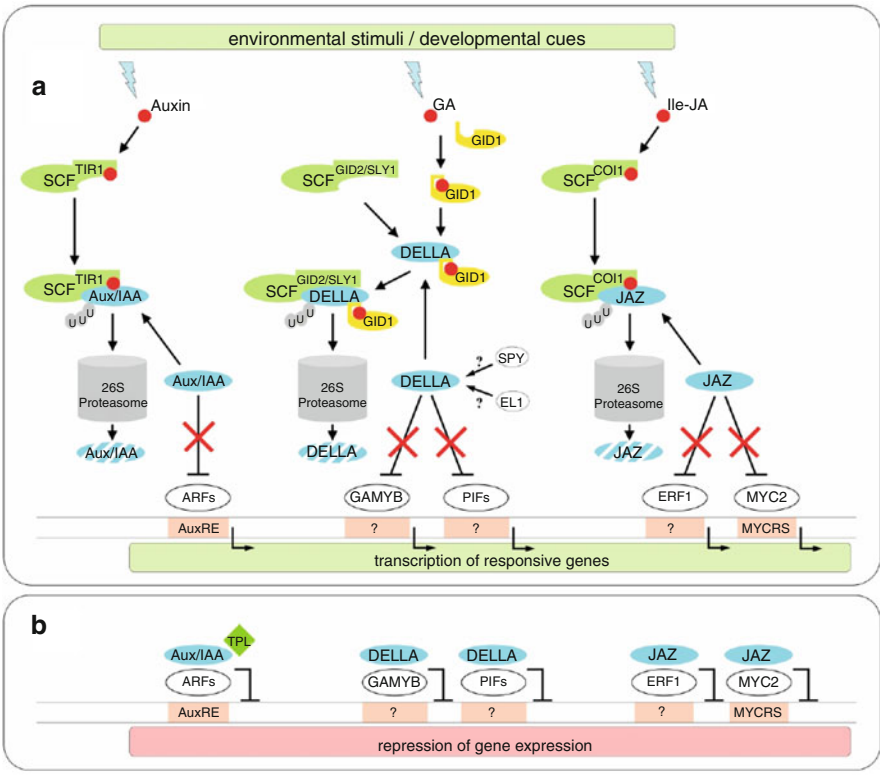

Fig. 1.1 Models for signal transduction pathways of auxin, gibberellic acid (GA), and jasmonoyl isoleucine (Ile–JA). (**a**) Upon phytohormone accumulation in a plant cell, repression on expression of responsive genes is relieved by degradation of transcriptional regulator. (**b**) In the absence or low levels of phytohormones, transcriptional regulators bind to certain transcription factors and repress gene expression. *Arrows* and *T-bars* indicate activation and inhibition, respectively

The Aux/IAA genes comprise a large class of auxin-inducible transcripts and have been identified in many plants. They encode short-lived nuclear proteins and act as repressors of auxin-regulated transcriptional activation (Berleth et al. 2004). Genetic and molecular studies showed that these proteins function as negatively acting transcription regulators that repress auxin response (Fig. 1.1). Aux/IAA proteins do not bind to AuxREs directly, but they regulate auxin-mediated gene expression by controlling the activity of ARFs. Aux/IAA proteins negatively regulate auxin-mediated transcription activity by binding ARFs through conserved domains (domains III and IV) found in both types of proteins (Ulmasov et al. 1997; Tiwari et al. 2003; Kim et al. 1997).

The Aux/IAA transcription factor has no DNA-binding domain, but together with ARF, it coregulates the transcription of auxin-responsive genes (Gray et al. 2001). With interactions between ARF and Aux/IAA proteins, the specific response

to auxin is generated. Yeast two-hybrid and other physical assays in vivo have confirmed a number of interactions, such as the ARF–Aux/IAA interactions and the AtIAA1, 6, 12, 13, and 14 interactions with ARF5 or ARF7 (Hamann et al. 2004; Fukaki et al. 2005; Weijers et al. 2005; Wang et al. 2010). It was also reported that the domain I of Aux/IAA recruits topless (TPL), which acts as a transcriptional corepressor for ARF–Aux/IAA-mediated gene regulation during the auxin response (Szemenyei et al. 2008).

Derepression of auxin responses occurs after an increase in the intracellular auxin level. When auxin levels increase in nucleus, the targeted degradation of the Aux/IAA repressors by the 26 S proteasome is promoted (Fig. 1.1). Auxin increases the interaction of the domain II of Aux/IAAs with transport inhibitor response 1/auxin-related F-Box (TIR1/AFBs), F-box proteins of the E3 ubiquitin ligase complex Skp1/Cullin1/F-box-TIR1/AFBs ($SCF^{TIR1/AFBs}$). There is limited information about relative affinity of interaction between various Aux/IAAs and the different TIR1/AFBs F-box proteins. With the presence of Aux/IAA peptides, auxin binds to TIR1, but the mechanism is not clear.

The $SCF^{TIR1/AFBs}$ auxin signaling pathway is short and controls the auxin-induced changes of gene expression by targeting the degradation of transcriptional repressors. It was shown that multiple signaling components such as MAP kinases (Kovtun et al. 1998), IBR5 protein phosphatase (Strader et al. 2008), or RAC GTPases (Tao et al. 2002) participate in the regulation of early auxin response genes. Therefore, it is not clear whether the $SCF^{TIR1/AFBs}$ pathway is sufficient to tightly regulate auxin-regulated gene expression.

It was also shown that two additional proteins were involved in the regulation of auxin-responsive gene expression. First is the long-standing auxin-binding protein 1 (ABP1) receptor involved in very early auxin-mediated responses at the plasma membrane in *Arabidopsis* (Braun et al. 2008). Since TIR1/AFBs and Aux/IAAs are mainly located in the nucleus, physical interaction with ABP1 is highly unlikely. Second is the indole-3-butyric acid response 5 (IBR5) phosphatase which promotes auxin responses through a pathway different from TIR1-mediated repressor degradation (Strader et al. 2008).

The transcription of LBD genes is enhanced in response to exogenous auxin, indicating that the LBD gene family may act as a target of ARF (Lee et al. 2009). The LBD genes encode proteins harboring a conserved lateral organ boundaries (LOB) domain, which constitute a novel plant-specific class of DNA-binding transcription factors, indicative of its function in plant-specific processes (Husbands et al. 2007; Iwakawa et al. 2002).

It was reported that the transcription of GH3 genes is also related to ARF proteins. AtGH3-6/DFL1, AtGH3a, and At1g28130 expression was reduced in a T-DNA insertion line (*arf8-1*) and increased in overexpression lines of AtARF8. This indicates that the three GH3 genes are targets of AtARF8 transcriptional control. The control of free IAA level by AtARF8 in a negative feedback fashion might occur by regulating GH3 gene expression (Tian et al. 2004). In the *atarf7* or *atarf7*/*atarf19* mutants, downregulation of AtGH3-6/DFL1 and in rice, downregulation of OsGH3-9 and OsGH3-11 levels under IAA treatment was

observed (Okushima et al. 2005; Terol et al. 2006). It was shown that multiple auxin-inducible elements were found in promoters of the GH3 gene family. This result confers auxin inducibility to the GH3 genes (Liu et al. 1994). GH3 genes were not only regulated by ARFs but also modulated by plant hormones, biotic and abiotic stresses, and other transcriptional regulators. Auxin-induced transcription is also modulated by tobacco bZIP transcription factor, BZI-1, which binds to the GH3 promoter (Heinekamp et al. 2004). A GH3-like gene, CcGH3, is regulated by both auxin and ethylene in *Capsicum chinense* L. (Liu et al. 2005). The upregulation of the GH3 genes in response to Cd was shown in *Brassica juncea* L. (Minglin et al. 2005). A GH3-5 gene in *Arabidopsis*, WES1, was shown to be induced by various stress conditions like cold, heat, high salt, or drought and by SA and ABA (Park et al. 2007). Auxin metabolism was induced by GH3 genes via R2R3-type MYB transcription factor, MYB96, and optimization of root growth was observed under drought conditions in *Arabidopsis* (Seo and Park 2009). Therefore, GH3-mediated auxin homeostasis is important in auxin actions which regulate stress adaptation responses (Park et al. 2007).

Accumulation of small auxin-up RNAs (SAURs) occurs rapidly and transiently with auxin in many plants (Woodward and Bartel 2005). The short half-lives of SAUR mRNAs appear to be conferred by downstream elements in the 3′ untranslated region of the messages (Sullivan and Green 1996). *Arabidopsis* mutants that stabilize downstream element-containing RNAs, and thus stabilize SAUR transcripts, have no reported morphological phenotype (Johnson et al. 2000), and although their function is not clearly established, they have been proposed to act as calmodulin-binding proteins. As in GH3 and Aux/IAA genes, most SAUR genes share a common sequence in their upstream regulatory regions, TGTCTC or variants, which was first identified from the promoter region of the pea PS-IAA4/5 gene (Ballas et al. 1993).

A wide variety of abiotic stresses have an impact on various aspects of auxin homeostasis, including altered auxin distribution and metabolism. Two possible molecular mechanisms have been suggested for altered distribution of auxin: first, altered expression of PIN genes, which mediate polar auxin transport; and second, inhibition of polar auxin transport by phenolic compounds accumulated in response to stress exposure (Potters et al. 2009). On the other hand, auxin metabolism is modulated by oxidative degradation of IAA catalyzed by peroxidases (Gazarian et al. 1998), which, in turn, are induced by different stress conditions. Furthermore, it has been shown that reactive oxygen species generated in response to various environmental stresses may influence the auxin response (Kovtun et al. 2000; Schopfer et al. 2002). Although these observations provide some clues, the exact mechanism of auxin-mediated stress responses still remains to be elucidated.

To address whether auxin-responsive genes were also involved in stress response in rice plants, their expression profile was investigated by microarray analysis under desiccation, cold, and salt stress. It was indicated that at least 154 auxin-induced and 50 auxin-repressed probe sets were identified that were differentially expressed, under one or more of the stress conditions analyzed. Among the 154 auxin-induced genes, 116 and 27 genes were upregulated and downregulated,

respectively, under abiotic stress conditions. Similarly, among the 50 auxin-repressed genes, 6 and 41 genes were upregulated and downregulated, respectively. Moreover, 41 members of auxin-related gene families were found to be differentially expressed under at least one abiotic stress condition. Among these, 18 (two GH3, seven Aux/IAA, seven SAUR, and two ARF) were upregulated and 18 (one GH3, five Aux/IAA, eight SAUR, and four ARF) were downregulated under one or more abiotic stress conditions. However, another five genes (OsGH3-2, OsIAA4, OsSAUR22, OsSAUR48, and OsSAUR54) were upregulated under one or more abiotic stress conditions and downregulated under other stress conditions. Interestingly, among the 206 auxin-responsive (154 auxin-induced and 50 auxin-repressed) genes and 41 members of auxin-related gene families that were differentially expressed under at least one abiotic stress condition, only 51 and 3 genes, respectively, were differentially expressed under all three stress conditions (Jain and Khurana 2009).

It was indicated that the expression of Aux/IAA and ARF gene family members was altered during cold acclimation in *Arabidopsis* (Hannah et al. 2005). Molecular genetic analysis of the auxin and ABA response pathways provided evidence for auxin–ABA interaction (Suzuki et al. 2001; Brady et al. 2003). The role of IBR5, a dual-specificity phosphatase-like protein, supported the link between auxin and ABA signaling pathways (Monroe-Augustus et al. 2003).

Promoters of the auxin-responsive genes and members of auxin-related gene families differentially expressed under various abiotic stress conditions were analyzed to identify *cis*-acting regulatory elements linked to specific abiotic stress conditions. Although no specific *cis*-acting regulatory elements could be linked to a specific stress condition analyzed, several ABA and other stress-responsive elements were identified. The presence of these elements further confirms the stress responsiveness of auxin-responsive genes. The results indicated the existence of a complex system, including several auxin-responsive genes, that is operative during stress signaling in rice. The results of study suggested that auxin could also act as a stress hormone, directly or indirectly, that alters the expression of several stress-responsive genes (Jain and Khurana 2009).

It was shown that genes belonging to auxin-responsive SAUR and Aux/IAA family, ARFs and auxin transporter-like proteins are downregulated in the grapevine leaves exposed to low UV-B (Pontin et al. 2010). Similar results were also found in the study of pathogen resistance responses, where a number of auxin-responsive genes (including genes encoding SAUR, Aux/IAA, auxin importer AUX1, auxin exporter PIN7) were significantly repressed (Wang et al. 2007b), supporting the idea that downregulation of auxin signaling contributes to induction of immune responses in plants (Bari and Jones 2009).

Some of the plant glutathione *S*-transferases (GSTs) are induced by plant hormones auxins and cytokinins. The transcript level of GST genes was induced very rapidly in the presence of auxin. OsGSTU5 and OsGSTU37 were preferentially expressed in root and were also upregulated by auxin and various stress conditions (Jain et al. 2010).

1.3 Gibberellins

Gibberellins (GAs) are a large family of tetracyclic, diterpenoid phytohormones, which regulate plant growth. Bioactive GAs influence various developmental processes such as seed germination, stem elongation, pollen maturation, and transition from vegetative growth to flowering (Olszewski et al. 2002). Growth and stress are often opposed, and a retardation of development is generally observed under environmental stress conditions. Therefore, components of GA signaling are candidates for putative integrator of growth and stress signals. Moreover, crosstalk of GA signaling with various phytohormone signaling events, which function in response to stress, bestows an important role on GA under stress conditions. Crosstalk could potentially occur by altering expression levels of GA-signaling components or modulating their protein activity or stability (Fu and Harberd 2003; Achard et al. 2003, 2006).

Mutants of rice (*Oryza sativa*) and *Arabidopsis* deficient in GA biosynthesis or signaling were utilized to identify proteins that are essential for GA perception and signaling. The current model of GA signaling suggests binding of GA to a soluble GA-insensitive dwarf 1 (GID1) receptor (Ueguchi-Tanaka et al. 2005) (Fig. 1.1). The GID1–GA complex then interacts with DELLA repressor proteins, resulting in degradation of DELLA protein through a ubiquitin–proteasome pathway initiated by SCF (Skip/Cullin/F-box) complex (Sun 2011). The GA-specific F-box proteins, GID2 in rice (Sasaki et al. 2003), and sleepy1 (SLY1) and sneezy (SNE) in *Arabidopsis* (McGinnis et al. 2003; Strader et al. 2004) confer specificity to the SCF-type E3 ubiquitin ligase, $SCF^{GID2/SLY1}$, toward the DELLA–GID1–GA complex. $SCF^{GID2/SLY1}$ adds a polyubiquitin chain to the DELLA protein and hence induces its degradation by the 26 S proteasome complex (Fig. 1.1). The degradation of DELLA repressors by the 26 S proteasome activates GA action (Ueguchi-Tanaka et al. 2007).

The GID1 receptor, which encodes a soluble protein with similarity to hormone-sensitive lipases, was first identified in rice (Ueguchi-Tanaka et al. 2005). Its homologs GID1a, GID1b, and GID1c were identified and characterized as the major GA receptors in *Arabidopsis* (Nakajima et al. 2006; Griffiths et al. 2006). Subsequently, GA receptors in various plants such as cotton, barley, and fern have been identified (Aleman et al. 2008; Chandler et al. 2008; Yasumura et al. 2007). GID1 is a soluble nuclear-enriched receptor which interacts with DELLA proteins in a GA-dependent manner (Willige et al. 2007). Structural analysis of GID1 has revealed basis for GID1–GA and DELLA–GID1–GA interactions as well as evolutionary aspects of the GA receptor (Shimada et al. 2008; Murase et al. 2008; Ueguchi-Tanaka and Matsuoka 2010).

DELLA repressors are the key regulators of GA signaling (Schwechheimer 2008). Five DELLA proteins, namely, GA-insensitive (GAI), repressor of *ga1*-3 (RGA), RGA-like 1 (RGL1), RGL2, and RGL3, have been identified in *Arabidopsis* (Bolle 2004). On the other hand, single DELLA protein genes present in rice and barley genomes are slender rice1 (SLR1) (Ogawa et al. 2000; Ikeda et al. 2001) and

slender 1 (SLN1) (Chandler et al. 2002; Fu et al. 2002), respectively. DELLA repressor loss-of-function mutants are taller than the wild-type plants and flower early, whereas transgenic plants overexpressing a DELLA protein are dwarf and flower late (Fu et al. 2002; Peng et al. 1997). The N-terminal domains of these repressors containing the DELLA motif play a regulatory role in GA-signal perception and GA-induced degradation (Dill et al. 2001). The absence of a typical basic DNA-binding domain suggests that DELLA proteins are more likely to function as transcriptional regulators instead of as transcription factors (Hussain and Peng 2003) (Fig. 1.1). Molecular studies showed that dwarf wheat varieties adopted during "green revolution" are affected in components of GA-signaling pathways, specifically orthologs of GAI (Peng et al. 1999).

Repressor activity of DELLA proteins might be controlled by mechanisms such as posttranslational modifications. Though initial studies had indicated phosphorylation of DELLA repressors as a prerequisite for GA-dependent degradation (Sasaki et al. 2003; Gomi et al. 2004), later studies have shown that DELLA proteins are phosphorylated in a GA-independent manner and phosphorylated as well as nonphosphorylated DELLA proteins are degraded in response to GA (Itoh et al. 2005). Requirement of DELLA dephosphorylation for subsequent degradation has been suggested in an *Arabidopsis* cell-free assay system and in tobacco BY2 cells (Wang et al. 2009; Hussain et al. 2005). Moreover, it was reported that phosphorylation of SLR1 by early flowering1 (EL1), encoding a serine/threonine protein kinase, might be critical for DELLA protein activity (Dai and Xue 2010). The *Arabidopsis* spindly (SPY) protein, which is an *O*-linked *N*-acetylglucosamine (GlcNAc) transferase, may function as a negative regulator of GA response. Though evidence of direct modification is lacking, it was suggested that SPY increases the activity of DELLA proteins, by adding a GlcNAc monosaccharide to serine/threonine residues (Silverstone et al. 2007). Thus, posttranslational modifications are clearly important for proper functioning or stability of the DELLA proteins, although the identities of the factors responsible for these modifications and modes of regulation remain to be determined.

Several putative direct targets of DELLA in *Arabidopsis* were identified by expression microarrays (Zentella et al. 2007; Hou et al. 2008). DELLA has induced expression of upstream GA biosynthetic genes and GA receptor genes, suggesting direct involvement of DELLA in maintaining GA homeostasis via a feedback mechanism. Other DELLA-induced target genes encode transcription factors/regulators like basic helix-loop-helix (bHLH), MYB-like, and WRKY family proteins. Among DELLA targets were RING-type E3 ubiquitin ligases including XERICO which is important for ABA accumulation. Thus, DELLA inhibits GA-mediated responses in part by upregulating ABA levels through XERICO. This revealed a role of DELLA in mediating interaction between GA and ABA signaling pathways (Zentella et al. 2007). Recently, it was reported that in *Arabidopsis* scarecrow-like3 (SCL3) and DELLA antagonize each other in controlling both downstream GA responses and upstream GA biosynthetic genes (Zhang et al. 2011).

DELLA stability is indirectly affected by other phytohormone pathways or environmental cues through alteration of GA metabolism and bioactive GA levels.

Auxin induces root and stem elongation, at least in part, by upregulating GA biosynthetic genes and downregulating GA catabolism genes (Sun 2010). During cold and salt stresses, AP2 transcription factors such as CBF1 and dwarf delayed-flowering 1 (DDF1) induce expression of GA catabolism genes (Magome et al. 2004). Similarly, stabilization of DELLA by ABA treatment is achieved by reduction of GA accumulation (Sun 2010). Integrative role of DELLA repressors in salt stress, ABA, and ethylene responses was described, and it was stated that salinity activates ABA and ethylene signaling, two independent pathways whose effects are integrated at the level of DELLA function (Achard et al. 2006). Growth restraint conferred by DELLA proteins extends the duration of the vegetative phase and promotes survival under adverse conditions.

DELLA proteins play critical roles in protein–protein interactions within various environmental and phytohormone signaling pathways. They are involved in many aspects of plant growth, development, and adaptation to stresses (Feng et al. 2008; Harberd et al. 2009; Arnaud et al. 2010; Hou et al. 2010). It was hypothesized that GA signaling or DELLA proteins enable flowering plants to maintain transient growth arrest, giving them the flexibility to survive periods of adversity (Harberd et al. 2009). The binding of DELLA proteins to the phytochrome-interacting factor (PIF) proteins integrates light and GA-signaling pathways (Fig. 1.1). This binding prevents PIFs from functioning as positive transcriptional regulators of growth in the dark. Since PIFs are degraded in light, they can only function in the combined absence of light and presence of GA (Hartweck 2008). DELLA inhibits hypocotyl elongation by binding directly to PIF3 and PIF4 and preventing expression of PIF3/PIF4 target genes (Feng et al. 2008). The transcription factor PIF3-like5 (PIL5) directly promotes the transcription of the GAI and RGA DELLA protein genes before germination and thereby controls repressor protein abundance. In response to light, PIL5 is degraded, and the transcription of GAI and RGA is reduced, relieving the restraint on germination (Oh et al. 2007). In barley, activation of α-amylase expression is induced by GAMYB (Gubler et al. 1999). It has been demonstrated that GA response mediated through GAMYB is dependent on the DELLA proteins SLN1 and SLR1, in barley and rice, respectively (Gubler et al. 2002), in which the DELLA proteins act as negative regulators of GAMYB-mediated gene expression.

Recently, two homologous GATA-type transcription factors from *Arabidopsis*, namely, GNC (GATA, nitrate-inducible, carbon-metabolism involved) and GNL/CGA1 (GNC-Like/cytokinin-responsive GATA factor 1), were identified as GA-regulated genes. It was indicated that GNC and GNL/CGA1 are important downstream targets of DELLA proteins and PIF transcription factors and that they might be direct PIF targets (Richter et al. 2010). In another recent study, role of DELLA as a transcriptional activator has been revealed. It was shown that the jasmonic acid (JA) ZIM-domain 1 (JAZ1) protein, a key repressor of JA signaling, interacts in vivo with DELLA proteins. JAZ proteins inhibit the activity of MYC2, which regulate target genes including some of JA-responsive genes. Binding of DELLA to JAZ removes the repression on MYC2 and JA-responsive genes (Hou et al. 2010). In *Arabidopsis*, DELLA proteins were implicated in JA signaling or perception, and

a role of DELLA in the regulation of plant–pathogen interactions was suggested (Navarro et al. 2008). Consequently, function of DELLA proteins as transcriptional repressors or activators grants these regulatory proteins a critical role at the crossroads of phytohormone signaling pathways during development or under various environmental conditions.

It is essential to identify the genes that are the final targets of GA-signaling pathway. GA function and GA-induced gene transcription in cereal aleurone cells have been reviewed (Olszewski et al. 2002; Sun and Gubler 2004). DNA microarrays have been utilized to dissect the transcriptional changes that promote GA-induced seed germination in *Arabidopsis*. Identified GA-responsive genes included the ones encoding for expansins, xyloglucan endotransglycosylase/hydrolases (XETs), aquaporins, a D-type cyclin, and a replication protein A, which are implicated in cell elongation and cell division (Ogawa et al. 2003). A cDNA microarray was employed to understand the molecular mechanisms by which GA and BRs regulate the growth and development in rice seedlings. Increased expression of XETs and downregulation of stress-related genes were observed after exogenous application of GA (Yang et al. 2004). In citrus, effects of GAs on internode transcriptome were investigated using a cDNA microarray. An overall upregulation of genes encoding proteins of the photosystems and chlorophyll-binding proteins, as well as of genes of the carbon fixation pathway, was observed (Huerta et al. 2008). In maize, transcriptional profiles of immature ears and tassels were investigated with microarrays at early stage of water stress. Transcripts upregulated in both organs included those involved in protective functions, detoxification of reactive oxygen species, nitrogen metabolism, and GA metabolism (Zhuang et al. 2008).

1.4 Cytokinins

Cytokinin signaling is similar to the two-component signal transduction pathways present in most bacteria and fungi. Hybrid histidine kinase (HK) receptors bind to cytokinin and then are autophosphorylated. Then phosphate group is transferred to histidine phosphotransfer proteins (HPs) (Fig. 1.2). The *Arabidopsis* HPs (AHPs) are a small family of proteins that act as intermediates in cytokinin signaling. The AHPs interact directly with various sensor HKs and type A and type B response regulators (RRs) in yeast two-hybrid assay. It was found that there were 23 *Arabidopsis* response regulators (ARRs) and nine related proteins (APRRs) in *Arabidopsis* (Schaller et al. 2002). The type B or transcription factor-type class also has 11 members. Each type B protein is composed of an N-terminal receiver domain and a long C-terminal part containing a single-repeat MYB-type DNA-binding domain (Sakai et al. 1998) called a GARP domain (Riechmann et al. 2000) and the proline- and glutamine-rich region frequently observed in eukaryotic transactivating domains (Tjian and Maniatis 1994). The ARRs are classified according to their C-terminal domains. Type A and type C have short C-termini, while type B ARRs have longer C-termini.

Transcription of type A ARRs is rapidly elevated by exogenous cytokinin (Brandstatter and Kieber 1998; Jain et al. 2006). In addition to transcriptional regulation, cytokinin treatment also results in an increase in the half-life of a subset of type A ARR proteins (To et al. 2007). Type A ARRs which are direct targets of the type B ARR transcription factors are negative regulators of cytokinin signaling. Consistent with their role as transcription factors, type B ARRs localize to the nucleus (Hwang and Sheen 2001; Asakura et al. 2003; Mason et al. 2005). Genetic and molecular analyses indicate that the type B ARRs are redundant positive elements in cytokinin signaling and are the immediate upstream activators of type A ARR gene expression (Hwang and Sheen 2001; Mason et al. 2005; Argyros et al. 2008). It was shown that type B ARRs are positive elements in cytokinin signaling (Ishida et al. 2008; Mason et al. 2005; Argyros et al. 2008) (Fig. 1.2).

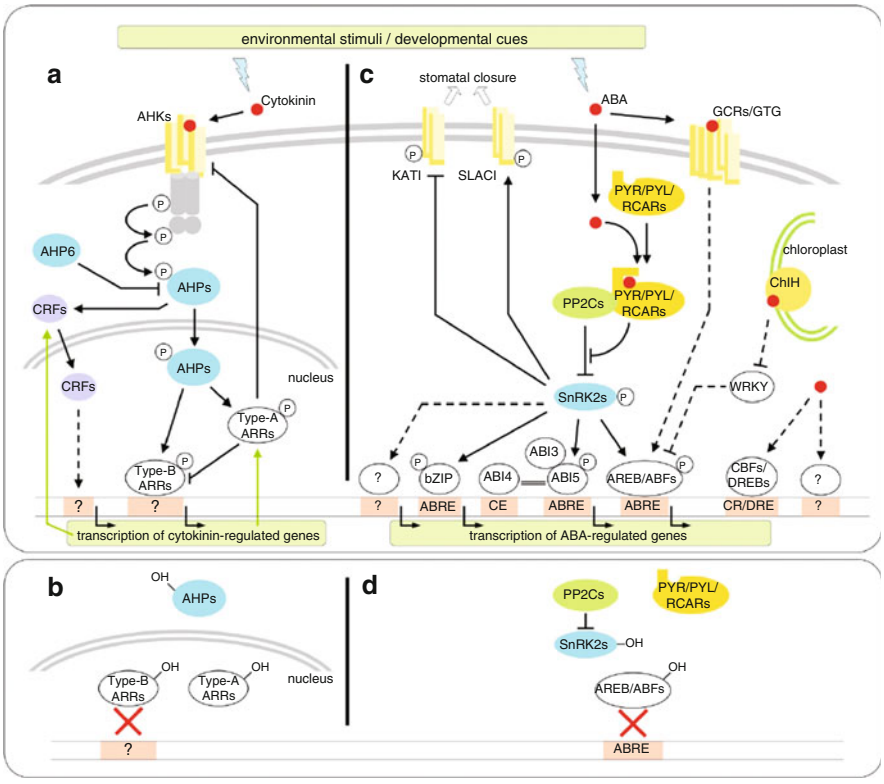

Fig. 1.2 Models for signal transduction pathways of cytokinin and abscisic acid (ABA). (**a**, **c**) Accumulation of phytohormones triggered by environmental stimuli or developmental cues initiates cascades of events involving phosphatases or kinases to induce expression of responsive genes. (**b**, **d**) In the absence or low levels of phytohormones, inactive transcription factors cannot induce gene expression. *Arrows* and *T-bars* indicate activation and inhibition, respectively. *Dashed arrows* or *T-bars* indicate possible interactions

To determine the target genes of the cytokinin-regulated transcriptional network, microarray analyses have been performed by different groups (Brenner et al. 2005; Rashotte et al. 2003; Rashotte et al. 2006). In addition to the type B ARRs, there are several other transcription factors that have been implicated by microarray analyses in the response to cytokinin. The cytokinin response factors (CRFs) act, along with the type B ARRs, to mediate the transcriptional response to cytokinin (Fig. 1.2). The CRFs have six family members, which are a subset of the AP2-like superfamily. Three of CRFs are transcriptionally upregulated by cytokinin in a type B ARR-dependent manner (Rashotte et al. 2006). Microarray analysis of cytokinin-regulated genes in a multiple *crf* mutant revealed that many genes regulated by type B ARRs are also regulated by CRFs.

It was indicated that the functions of the cytokinin-regulated genes reflect processes known to be targets of cytokinin signaling, including genes involved in cell expansion, other phytohormone pathways (auxin, ethylene, and GA), responses to pathogens, and regulation by light. Other, more directed approaches have identified individual genes regulated by cytokinin, including cyclinD3 (Riou-Khamlichi et al. 1999), which provides a mechanistic link between cytokinin and the regulation of the cell cycle. Additionally, other clusters of genes suggest unsuspected targets of cytokinin, including genes involved in trehalose-6-phosphate metabolism and potential effects in the redox state of the cell. Undoubtedly, there are many additional targets that remain to be identified. Moreover, the transcription factors responsible for the regulation of these targets and how they interact remain to be determined (Argueso et al. 2010).

It was also known that cytokinin function has been linked to a variety of abiotic stresses (Hare et al. 1997). When public microarray expression data was examined, it was revealed that the genes encoding proteins in the cytokinin signaling pathway were differentially affected by various abiotic stresses. For example, it was shown that cold stress appears to rapidly upregulate the expression of multiple type A ARRs and conversely to downregulate the expression of all three cytokinin receptors. Although there are no reports linking cytokinin to a rapid response to cold stress, these results can suggest a role for cytokinin in the response to cold stress (Argueso et al. 2009). After dehydration, the expression of the AHK2 and AHK3 genes was found to be induced (Tran et al. 2007), which was shown in the analysis of public microarray data (Argueso et al. 2009). Exposure of plants to drought results in a decrease in the level of cytokinins in the xylem sap (Bano et al. 1994; Shashidhar et al. 1996). A recent study has confirmed that isoprene-type cytokinins (zeatin and zeatin riboside) are decreased in the xylem in response to drought stress. Surprisingly, in the same study, it was found that the level of the aromatic cytokinin 6-benzylaminopurine (BAP) was elevated (Alvarez et al. 2008).

It was found that the expression of *Agrobacterium* isopentenyl transferase (IPT), rate-limiting enzyme in cytokinin biosynthesis, downstream of a drought/maturation-induced promoter resulted in a remarkable tolerance to extreme drought conditions in tobacco (Rivero et al. 2007). While wild-type plants died, transgenic plants had complete recovery after drought conditions. In addition to this, under water restriction, there was no yield loss (Rivero et al. 2007). This result was

consistent with the notion that elevated cytokinin levels may promote survival in drought conditions. Similar results were obtained in another study, which suggested that endogenous cytokinin may play a role in conferring drought tolerance (Alvarez et al. 2008).

Especially in roots, the expressions of several of the CRF genes were down-regulated in response to salt stress. It was suggested that these genes may play an important role in mediating the input of cytokinin into the salt stress response pathway (Rashotte et al. 2006). In another study, one out of ten recently described rice RR genes had shown to be upregulated in seedlings exposed to a high concentration of salt (Jain et al. 2006). In developing kernels where the cytokinin role in response to water stress was previously studied (Brugiere et al. 2003), only specific genes for de novo biosynthesis (e.g., IPT2), degradation (e.g., CKX1, CKX4), and signal response (e.g., RR3) were active.

Cytokinins control many aspects of development and responses to the environment. Recent research highlighted the importance of cytokinin-regulated transcriptional networks in the regulation of these processes. As well as type B ARRS, additional classes of transcription factors take role in the control of cytokinin-regulated gene expression in shoot development (e.g., STM, WUS, GL1) and root development (e.g., SHY2, SCR, PLT1) (Argueso et al. 2010). Thus, it was suggested that crosstalk between cytokinin and other plant hormones at the transcriptional level is widespread.

1.5 Abscisic Acid

Abscisic acid (ABA) is a major phytohormone that regulates a broad range of events during development and adaptive stress responses in plants. It plays crucial roles in responses of vegetative tissues to abiotic stresses such as drought and high salinity (Zhu 2002). It accumulates in cells under osmotic stress, promotes stomatal closure, and regulates the expression of various protective or adaptive genes. ABA and coordinated action of different hormonal signaling pathways control maintenance of root growth, regulation of stress-responsive gene expression, accumulation of osmocompatible solutes, and synthesis of dehydrins and late embryogenesis abundant (LEA) proteins under environmental stress (Zhu 2002; Sharp et al. 2004; Verslues et al. 2006). Recently, role of ABA in response to biotic stress has been reviewed as well (Ton et al. 2009). ABA might be providing resistance to pathogens and disease via inhibition of pathogen entry through stomata or via increasing susceptibility by crosstalk with other signaling pathways.

Mutants altered in phytohormone sensitivity have led to identification of physiological receptors for auxin (Dharmasiri et al. 2005; Kepinski and Leyser 2005), gibberellins (Ueguchi-Tanaka et al. 2005), and other phytohormones. However, similar genetic screens for mutants have not directly yielded ABA receptors. On the other hand, ABA perception and signal transduction have been studied extensively. Microinjection into cytosol or treatment with ABA or its analogs has suggested

multiple ABA receptors at various locations including cytosol and plasma membrane. Though controversy exists, flowering time control protein FCA (Razem et al. 2006), G-protein-coupled receptor 2 (GCR2) (Liu et al. 2007), GCR-type G-protein 1 (GTG1) and GTG2 (Pandey et al. 2009), and Mg-chelatase H subunit (ChlH) (Shen et al. 2006) were identified as ABA receptors. Among these putative receptors, FCA was later shown to be not binding ABA (Risk et al. 2008). It was indicated that the filter-based ligand-binding assays employed in receptor studies are prone to artifacts because of incomplete removal of nonprotein-bound ABA. Similar concerns were raised for ABA-binding ability of ChlH and GCR2 (Risk et al. 2009; Guo et al. 2008). Alternative techniques like affinity chromatography were employed to reinforce the hypothesis that ChlH can bind to ABA in *Arabidopsis thaliana* (Wu et al. 2009). Although GCRs and ChlH were proposed to play important roles in ABA responses, their physiological and molecular connections to well-known signaling factors such as type 2C protein phosphatases (PP2C) and sucrose nonfermenting (SNF) 1-related protein kinase 2 (SnRK2) remained unclear.

Negative regulatory system employed in ABA signaling cascade is composed of PP2C phosphatases and SnRK2 kinases which act as negative and positive regulators, respectively (Fig. 1.2). Mutants of *Arabidopsis*, insensitive to ABA, were used for identification of two genes, ABA-insensitive1 (ABI1) and ABI2, encoding group A PP2Cs (Leung et al. 1994, 1997; Meyer et al. 1994). Discovery of these phosphatases has led to isolation or characterization of various other regulators of ABA signaling including protein kinases. Members of SnRK2 family such as ABA-activated protein kinase (AAPK) from *Vicia faba* (Li et al. 2000) and *Arabidopsis* SRK2E/Open stomata 1 (OST1)/SnRK2.6 (Mustilli et al. 2002; Yoshida et al. 2002) were determined as positive regulators in ABA signaling. Gene encoding ABA-induced protein kinase 1 (PKABA1), which is a serine–threonine type protein kinase, was isolated from wheat (Anderberg and Walker-Simmons 1992). In the absence of ABA, PP2C inactivates SnRK2 by direct dephosphorylation. On the other hand, in response to environmental or developmental cues, ABA promotes inhibition of PP2C and accumulation of phosphorylated SnRK2. Active SnRK2 subsequently phosphorylates ABA-responsive element (ABRE)-binding factors (AREBs/ABFs) and initiates ABA-regulated gene expression.

ABA signaling model was updated with the discovery of pyrabactin resistance 1/pyrabactin resistance 1-like/regulatory component of ABA Receptor (PYR/PYL/RCAR) proteins as a new type of soluble ABA receptor (Ma et al. 2009; Park et al. 2009). Furthermore, protein phosphatase–kinase complexes (PP2C–SnRK2) were identified as downstream components of PYR/PYL/RCARs (Umezawa et al. 2009; Vlad et al. 2009). After these major findings, several studies offered a double-negative regulatory system for ABA signaling which consists of four components: ABA receptors (PYR/PYL/RCAR), protein phosphatases (PP2C), protein kinases (SnRK2), and their downstream targets (Fujii et al. 2009; Umezawa et al. 2009) (Fig. 1.2). In the presence of ABA, interaction of PYR/PYL/RCAR and PP2C is promoted, resulting in PP2C inhibition and SnRK2 activation. Besides direct

interactions between PYR/PYL/RCARs, PP2Cs, and SnRK2s, the interaction between other ABA-binding receptors (e.g., GCRs, GTGs, and ChlH) and any component of signaling (e.g., PP2Cs, SnRK2s, and AREBs/ABFs) is unknown.

The double-negative regulatory system provided by signaling complex of PYR/PYL/RCARs, group A PP2Cs, and subclass III SnRK2s is very simple yet sophisticated. The system probably varies widely in plant cells, tissues, and organs at various developmental stages. There are 14 PYR/PYL/RCARs, 9 PP2Cs, 3 SnRK2s, and 9 AREB/ABFs in *A. thaliana* alone to regulate transcription (Ma et al. 2009; Park et al. 2009; Umezawa et al. 2009; Uno et al. 2000), increasing number of possible combinations of the signaling complex to more than 3,000 (Umezawa et al. 2010). Fine tuning of ABA responses in plant cells is probably provided by multiple determinants, like spatial or temporal limitations, stress-responsive gene expression patterns, subcellular localization, and preferences in protein–protein interactions (Umezawa et al. 2010).

Downstream targets of the PYR/PYL/RCAR–PP2C–SnRK2 complex should be determined to clarify the details of ABA signaling. These include proteins that interact with PP2C and SnRK2. Several bZIP transcription factors (AREBs/ABFs) and some membrane proteins have been identified as substrates for SnRK2 phosphorylation. In guard cells SRK2E/OST1/SnRK2.6, homologue of SRK2D/SnRK2.2 and SRK2I/SnRK2.3 acts as positive regulator of stomatal closure (Mustilli et al. 2002). It activates anion channel SLAC1 and inhibits cation channel KAT1 which is essential for K^+ uptake during stomatal opening (Geiger et al. 2009; Raghavendra et al. 2010). ABA- and PYR/PYL/RCAR-mediated inactivation of PP2C allows activation of SLAC1 which has a central role in guard cells (Fig. 1.2).

It is well known that abiotic stress conditions like drought and salinity activate ABA-dependent gene expression systems involving various transcription factors like AREBs/ABFs, MYC/MYB, C-repeat binding factors (CBFs)/drought-responsive element (DRE)-binding proteins (DREBs), and NAC family proteins. On the other hand, cold stress regulates gene expression in an ABA-independent manner through some CBFs/DREBs (Agarwal and Jha 2010). Large-scale transcriptome analyses, which provided valuable information on ABA-mediated regulation of transcription, have shown that ABA dramatically alters genomic expression (Hoth et al. 2002; Seki et al. 2002). These genome-wide expression studies not only revealed key components of ABA signaling but also contributed in identification of novel downstream target genes. Key regulators of ABA-mediated gene expression are AREBs/ABFs with ABI5 as a typical representative. Several SnRK2s regulate AREB/ABFs in ABA signaling in response to water stress (Fujii and Zhu 2009). Wheat SnRK2 ortholog, PKABA1, phosphorylates the wheat AREB1 ortholog, TaABF, and the rice SnRK2 orthologs, SAPK8, SAPK9, and SAPK10, phosphorylate the AREB1 ortholog TRAB1, in vitro (Johnson et al. 2002; Kagaya et al. 2002; Kobayashi et al. 2005). OsABI5 from rice showed transcript upregulation by ABA and high salinity and downregulation by drought and cold. Its overexpression enhanced salinity tolerance (Zou et al. 2008).

The AREBs/ABFs encode bZIP transcription factors and belong to the group A subfamily, which is composed of nine homologs in the *Arabidopsis* genome

(Jakoby et al. 2002). The AREBs/ABFs were isolated by using ABRE sequences as bait in yeast one-hybrid screening method (Choi et al. 2000). The bZIP transcription factors interact as dimers with ABREs (PyACGTGGC), which are ACGT containing G-box-like *cis*-elements in promoter regions. ABA response usually requires a combination of an ABRE with a coupling element (CE), which is similar to an ABRE or a DRE (Himmelbach et al. 2003). ABRE-binding AREBs/ABFs, DRE-binding AP2-type transcription factors, and other transcriptional regulators such as viviparous1 (VP1)/ABI3 also contribute to ABA-mediated gene expression. ABI3 binds to ABI5 and enhances its action. ABI4, an AP2-type transcription factor, and a number of additional *trans*-acting factors including MYC/MYB family proteins act as positive ABA response regulators (Yamaguchi-Shinozaki and Shinozaki 2006). ZmABI4 interacts specifically with CE and functions in ABA signaling during germination and in sugar sensing in maize (Niu et al. 2002).

Among the group A bZIP subfamily, AREB1/ABF2, AREB2/ABF4, and ABF3 are induced by dehydration, high salinity, and ABA treatment in vegetative tissues (Uno et al. 2000; Kim et al. 2004; Fujita et al. 2005). In *Arabidopsis*, four cDNA sequences of ABFs (ABF1, ABF2, ABF3, and ABF4) similar to AREB1 and AREB2 were identified. ABF1 expression was induced by cold, ABF2 and ABF3 by high salt and ABF4 by cold, drought, and high salt (Choi et al. 2000). Recently, an *areb1*/*areb2*/*abf3* triple mutant was generated (Yoshida et al. 2010). Transcriptome analysis of triple mutant revealed novel AREB/ABF downstream genes in response to water stress, including many LEA class and group A PP2C genes and transcription factors. These results indicate that AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent gene expression in ABA signaling under stress conditions (Yoshida et al. 2010). Various bZIP transcription factor genes of different groups were identified from soybean (*Glycine max*). It was found that GmbZIP44, GmbZIP62, and GmbZIP78 belonging to subgroup S, C, and G, respectively, were also involved in salt and freezing stresses. These proteins bind to ABRE and couple of other *cis*-acting elements with differential affinity and improve stress tolerance in transgenic *Arabidopsis* by upregulating ERF5, KIN1, COR15A, COR78A, and P5CS1 and downregulating DREB2A and COR47 (Liao et al. 2008).

Orthologs of AREBs/ABFs have also been reported in barley (Casaretto and Ho 2003) and rice (Lu et al. 2009; Amir Hossain et al. 2010). OsbZIP72 was shown to be an ABRE-binding factor in rice using the yeast hybrid systems. Transgenic rice overexpressing OsbZIP72 was hypersensitive to ABA and showed elevated levels of expression of ABA response genes such as LEAs. Transgenic rice plants displayed an enhanced ability of drought tolerance (Lu et al. 2009). Expression of OsABF1 was found to be induced by various abiotic stress treatments such as anoxia, salinity, drought, oxidative stress, and cold (Amir Hossain et al. 2010). In cultivated tomato (*Solanum lycopersicum*), two members of AREBs/ABFs, namely, SlAREB1 and SlAREB2, were identified. Expression of SlAREB1 and SlAREB2 was induced by drought and salinity in both leaves and root tissues. Microarray and cDNA-amplified fragment length polymorphism (AFLP) analyses were employed in order to identify SlAREB1 target genes responsible for the

enhanced tolerance in SlAREB1-overexpressing lines. Genes encoding oxidative stress-related proteins, lipid-transfer proteins (LTPs), transcription regulators, and LEA proteins were found among the upregulated genes in transgenic lines (Orellana et al. 2010). ABA regulation of gene expression in *Arabidopsis* guard cells was investigated using microarrays. Global transcriptomes of guard cells were compared to gene expression in leaves and other tissues, and approximately 300 genes showing ABA regulation unique to guard cells were determined (Wang et al. 2011).

1.6 Jasmonic Acid

Lipid-derived jasmonic acid (JA) and its metabolites, collectively known as jasmonates, are important plant signaling molecules that mediate plant responses to environmental stress and function in various aspects of growth and development (Wasternack 2007; Balbi and Devoto 2008). Phytohormones regulate development not via linear pathways, but through complex interconnections between different signaling pathways. Extensive crosstalk occurs between JAs and salicylic acid (SA), another signaling molecule with an important function in plant defense responses (Beckers and Spoel 2006). In higher plants, after synthesis via the octadecanoid pathway, JA can be conjugated to amino acids, preferentially to isoleucine (Ile) to form Ile–JA or converted to methyl jasmonate (Me–JA) or other metabolites (Wasternack 2007). Ile–JA has been proposed to be the active form of the hormone. Pathogens, mechanical wounding, water deficit, and some other abiotic stresses trigger a rapid increase in JA levels. In general, JAs help to modulate the competitive allocation of energy to defense or growth.

Mutants of *Arabidopsis* were utilized for determination of key components of JA-signaling pathway. Central roles of an F-box protein, coronatine-insensitive 1 (COI1) (Xie et al. 1998), and negative transcriptional regulators, Jasmonate ZIM-domain (JAZ) (Thines et al. 2007; Chini et al. 2007) proteins, were defined in *Arabidopsis*. Taken together, these results suggested SCF^{COI1}-dependent degradation of JAZ repressors via 26 S proteasome following perception of Ile–JA. As in the case of GA and auxin signaling pathways, Ile–JA, active hormone, relieves the repression by JAZ, transcriptional regulator (Fig. 1.1). Moreover, coronatine, which is a phytotoxin that is structurally related to JA, binds to COI1–JAZ complexes with high affinity, which strongly suggests that COI1 functions as a receptor (Melotto et al. 2008; Katsir et al. 2008; Gfeller et al. 2010). However, direct binding of Ile–JA to COI1 has not been shown yet. Thus, crystal structure analyses of COI–JAZ complexes, identification of new JAZ targets, and determination of JA-responsive genes will help to clarify the JA-signaling pathway.

JAZ proteins directly interact with MYC2, repressing its activity in the absence of Ile–JA (Fig. 1.1). MYC2 encodes a bHLH transcription factor and induces JA-mediated responses such as wounding, inhibition of root growth, JA biosynthesis, oxidative stress adaptation, and anthocyanin biosynthesis (Boter et al. 2004).

MYC2 binds to the G-box (CACGTG) or T/G-box (AACGTG) in the promoters of JA-regulated genes (Chini et al. 2007). Ethylene response factor 1 (ERF1) and other ERFs integrate JA and ethylene signals and regulate some of the MYC2-modulated responses in an opposite fashion (Lorenzo et al. 2003). Recently, involvement of additional transcriptional factors belonging to different families such as NAC (e.g., ANAC019, ANAC055) and WRKY (e.g., WRKY70, WRKY18) have been reported (Bu et al. 2008; Xu et al. 2006; Fonseca et al. 2009).

Research has been concentrated on role of JA and its metabolites in defense response against biotic stresses. Response often implies changes in the content of several phytohormones, which correlate with changes in the expression of genes involved in their biosynthesis and the responses they regulate. Local or systemic responses at the level of gene expression have been investigated using high-density microarrays (López-Ráez et al. 2010; Schlink 2010; Lewsey et al. 2010). On the other hand, JA was reported to take part in responses to some abiotic stresses such as salinity, drought, and boron toxicity. Desiccation response was shown to involve the regulation of JA-responsive genes in barley leaf segments (Lehmann et al. 1995). Exogenous application of JA to salt-stressed rice seedlings improved recovery, suggesting a role for JA during response to salinity stress (Kang et al. 2005). In barley, induction of genes involved in JA biosynthesis or known as JA-responsive genes was reported as a key feature of response to salinity (Walia et al. 2006). JA was hypothesized to be involved in the adaptation of barley to salt stress. Treatment with JA before salt application partially alleviated photosynthetic inhibition caused by salinity stress. Expression profiling after a short-term exposure to salinity stress indicated a considerable overlap between genes regulated by salinity stress and JA application. It was suggested that three JA-regulated genes, arginine decarboxylase, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase, and apoplastic invertase, were possibly involved in salinity tolerance mediated by JA (Walia et al. 2007). In a global transcriptome analysis of response to boron toxicity using microarrays, it was shown that high concentrations of boric acid treatment resulted in upregulation of JA-biosynthetic and JA-induced genes in barley leaves. Induction of JA-related genes was found to be an important late response to boron toxicity (Öz et al. 2009). In maize developing kernels, expression patterns of some genes in several stress response-associated pathways, including ABA and JA, were examined, and these specific genes were responsive to drought stress positively (Luo et al. 2010).

1.7 Ethylene

When key components of ethylene signaling from membrane receptors to nuclear activators were investigated in *Arabidopsis*, five membrane receptors, ethylene response 1 (ETR1), ETR2, ethylene response sensor 1 (ERS1), ERS2, and ethylene insensitive 4 (EIN4), were determined. These receptors act as negative regulators through genetically identified negative regulator, constitutive triple response

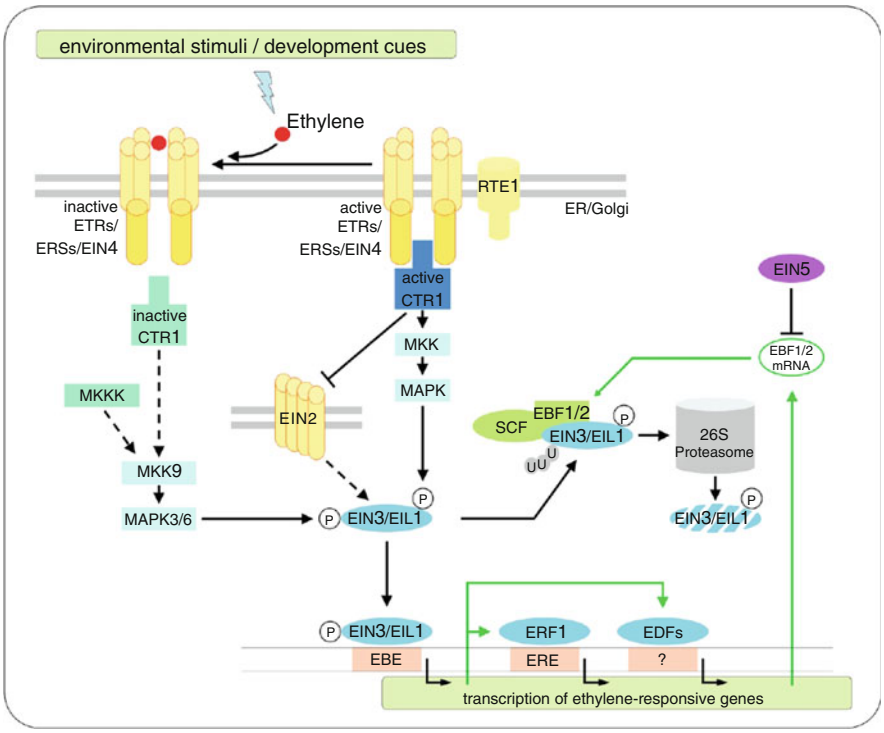

Fig. 1.3 Model for ethylene signal transduction pathway. Accumulation of ethylene triggers cellular events involving kinases or phosphatases to induce transcription of ethylene-responsive genes. *Arrows* and *T-bars* indicate activation and inhibition, respectively. *Dashed arrows* or *T-bars* indicate possible interactions

(CTR1), encoding a putative Raf-like MAPK kinase kinase (MKKK) (Kieber et al. 1993). Another membrane protein EIN2 has a pivotal role by regulating the availability of key transcription factor, EIN3, in ethylene signaling downstream of CTR1 (Fig. 1.3). The mechanism how EIN2 regulated the EIN3 is still unknown.

EIN3 is a plant-specific transcription factor mediating ethylene-regulated gene expression (Chao et al. 1997). It belongs to a multigene family in *Arabidopsis*, including EIN3, EIN3-Like 1 (EIL1), EIL2, EIL3, EIL4, and EIL5, in which EIN3 and EIL1 are the most closely related homologs. It is supposed that EIN3 and EIL1 are the major transcription factors in mediating ethylene responses.

Biochemical studies showed that EIN3 and EIL1 can directly bind to the promoter of ERF1 (ethylene response factor 1), which belongs to the EREBP (ethylene-responsive element-binding protein) family of transcription factors (Solano et al. 1998).

Ethylene response factors (ERFs), the first member of which was identified in tobacco, act at the last step of ethylene signaling pathways (Ohme-Takagi and Shinshi 1995). To date, in different plant species, ERFs have been found to be

involved not only in growth, development, and regulation of metabolism (van der Fits and Memelink 2000; van der Graaff et al. 2000; Banno et al. 2001) but also in the response to biotic and abiotic stresses (Stockinger et al. 1997; Liu et al. 1998; Yamamoto et al. 1999; Fujimoto et al. 2000; Gu et al. 2000; Berrocal-Lobo et al. 2002; Gu et al. 2002; Dubouzet et al. 2003; Aharoni et al. 2004; Broun 2004; Zhang et al. 2005).

ERF4, *Arabidopsis* ERF1, ERF5, CBF1, DREB1, and DREB2, periwinkle ORCA2 and ORCA3, and tomato Pti4, Pti5, Tsi1, and JERF3 act as transcriptional activators that, when overexpressed, lead to the activation of downstream genes (Stockinger et al. 1997; Zhou et al. 1997; Liu et al. 1998; Solano et al. 1998; Menke et al. 1999; Fujimoto et al. 2000; Ohta et al. 2000; van der Fits and Memelink 2000; Park et al. 2001; Wang et al. 2004). Ethylene affects the expression of a group of genes related to pathogen attack, wounding, extreme temperatures, and drought stress.

It was indicated that overexpression of ERF1 rescued only a subset of *ein3* phenotypes. This result suggested that EIN3 regulates additional target genes in mediating distinct ethylene responses (Solano et al. 1998). Since mRNA levels were rapidly accumulated upon ethylene treatment, and knockout mutants resulted in partial ethylene insensitivity, four novel transcription factors EDF1–4 (ethylene-responsive DNA-binding factors) were suggested as potential target genes of EIN3 (Alonso et al. 2003). Collectively, a transcriptional cascade from EIN3/EIL1 to ERF1 and EDF is involved in the ethylene response pathway (Fig. 1.3).

It was demonstrated that EBF1 and EBF2 play a negative role in ethylene signaling by targeting EIN3 for degradation (Fig. 1.3). Interestingly, ethylene treatment results in an increase in the transcription level of EBF2, suggesting that there exists a negative feedback mechanism in ethylene signaling (Guo and Ecker 2003; Potuschak et al. 2003). When the ethylene signal is enhanced, the EIN3 protein becomes stabilized, which, in turn, induces the expression of EBF2. The accumulation of EBF2 is likely to suppress the high level of EIN3 protein to its basal level, thus restoring plant responsiveness to ethylene again (Guo and Ecker 2003; Cho and Yoo 2009).

It was shown that EIN2, EIN5, and EIN6 are positive regulators of EIN3 action (Li and Guo 2007). It was shown that *ein5* and *ein6* mutants were weakened in ethylene-induced EIN3 accumulation, but in *ein2* mutants, EIN3 accumulation was inhibited (Guo and Ecker 2003).

AP2/EREBP (APETALA2/ethylene-responsive element-binding protein) is a large family of transcription factor genes. The AP2/EREBP gene family has been divided into four subfamilies: AP2, RAV (related to ABI3/VP1), dehydration-responsive element-binding protein (DREB), and ERF (Sakuma et al. 2002). After identification of the ERF domain as a conserved motif in four DNA-binding proteins from tobacco (Ohme-Takagi and Shinshi 1995), many ERF-like genes have been identified from various plant species, such as *Arabidopsis* and rice (Nakano et al. 2006), tomato (Gu et al. 2000), soybean (Zhang et al. 2008), sugarcane (Trujillo et al. 2008), and two fruit crops, apple (Wang et al. 2007c) and plum (El-Sharkawy et al. 2009). To date, different members of plant ERF genes