Lam-Son Phan Tran · Sikander Pal
Editors

Phytohormones: A Window to Metabolism, Signaling and Biotechnological Applications

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Preface

The phytohormones regulate various biological processes in plants. In the last few decades, comprehensive research efforts have displayed the existence of phytohormonal signals and their transduction in plants. Intensive molecular studies have elucidated various plant hormonal pathways, each of which consists of many signaling members, linking a specific hormone perception to the regulation of downstream genes. Among phytohormones, signal transduction pathways of auxin (Aux), abscisic acid (ABA), cytokinins (CKs), gibberellins (GAs), and ethylene (ET) have been thoroughly investigated. In the last decade, extensive research efforts have recognized brassinosteroids (BRs) as a new class of plant hormones with multiple roles in plant physiological processes. The signal transduction pathway and crucial implication of BR signaling components in execution of BR responses in plants have been recently established. Emerging evidence also supports specific signal perception and transduction pathways for salicylic acid (SA) and jasmonates (JAs). Latest research findings also support strigolactones as plant hormones.

The advanced molecular studies have demonstrated crucial implication of phytohormonal crosstalks in the regulation of key physiological events under normal and stressful conditions. For instance, the crosstalks of Aux-ABA, Aux-BRs, BRs-ABA, ET-ABA, BRs-ET, CKs-ABA, BRs-JAs, BRs-SA, and GAs-JAs have been shown to regulate a number of biological processes in plants. The phytohormonal crosstalk between two hormones can be antagonistic or synergistic or additive in action. Additionally, the signal transduction component(s) of one hormonal pathway may interplay with the signaling component(s) of other hormonal pathway(s).

The knowledge gained from the signal transduction studies of phytohormones has been practically valorized through genetic manipulation. Genetic engineering has enabled plant biologists to manipulate the signaling pathways of plant hormones for the development of crop varieties with improved yield and stress tolerance. Latest research findings have revolutionized the concept of phytohormonal studies in plants. The present book volume will describe the new facet of plant hormones; that is, not only phytohormones have been studied to understand their course of

actions in plants but also crosstalk implication of two or more hormones has become the target of plant scientists to manipulate the hormonal impact and to generate high-yielding varieties. In the preceding context, Chaps. 1–5 describe the metabolism, signaling, and genetic manipulation of classical hormones (Aux, ABA, CKs, ET, and GAs). Understanding the roles of emerging plant hormones, such as BRs, SA, JAs, and strigolactones, is of utmost significance to plant biologists. Chapters 6–9 of this book will apprise the readers about fundamentals and recent understandings of these emerging hormones. Implication of plant hormonal crosstalks under stressful conditions has just begun to be deciphered. Thus, to share the latest updates with the readers, the book will be concluding with chapters on phytohormonal crosstalks under abiotic and biotic stresses.

Overall, this volume will present our current understanding of phytohormonal signal transductions and crosstalk of phytohormones in plants as a regulation of key physiological processes. Every section will be concluded with application of biotechnological strategies based on modulation of the hormone contents or signal transduction pathway or crosstalk, enabling us to maintain agriculture in a sustainable manner.

We are grateful to the authors of various chapters of this book for writing their chapters meticulously and with great responsibility. We are extremely thankful to Dr. Kazuo Shinozaki, Director of RIKEN Center for Sustainable Resource Science, Japan; Prof. MPS Ishar, Vice-Chancellor, University of Jammu, India; and Prof. Pedro Berliner, Director and Dr. Shimon Rachmilevitch of Jacob Blaustein Institute for Desert Research, Ben-Gurion University, Israel, for providing overall support for our research and academic pursuits.

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We are quite hopeful that this book will be successful in updating the readers about the phytohormones and latest emerging trends.

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Auxin in Plant Growth and Stress Responses

Liu Liu*, Guangyan Guo*, Zhijuan Wang, Hongtao Ji, Fupeng Mu, and Xia Li

Abstract The phytohormone auxin has long been recognized for its essential role in plant growth and development. Recent advance indicated that auxin also plays critical roles in plant responses to environmental stresses. This has prompted investigation into molecular control of auxin homeostasis and plant growth in response to developmental and environmental stimuli. A simple two-step biosynthesis pathway from tryptophan to auxin has been defined. At its sites of action, three auxin receptor or co-receptor systems have been identified. Binding of auxin by ABP1 regulates ROP-GTPase-mediated gene expression and subcellular protein trafficking. Auxin perception by TIR1/AFB-Aux/IAA co-receptor and SKP2A activate auxin signaling and promote cell growth and cell division, respectively. Recent findings indicate that ABP1 functions upstream of TIR1/AFBs and negatively regulates the TIR1/AFB-Aux/IAA-mediated auxin signaling pathway, highlighting coordinate regulation of the signaling pathways mediated by different auxin receptor/co-receptors during plant growth and development. Recent advance reveals that environmental signals, such as high salinity and drought, induce modulations of auxin biosynthesis and the signaling pathway allowing for efficient cellular reprogramming of plant growth and

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development under stress. Research advance in auxin homeostatic control and response has led to success in manipulation of auxin biosynthesis and the signaling for improvement crops with desired agricultural traits.

Keywords Auxin • Biosynthesis • Auxin signaling • Abiotic stresses • Plastic development

Introduction

Growth is one of the most fundamental characteristics of living organisms. Plant growth is quite different from that of animals. Plant growth is caused by increases in both cell number and cell size, whereas growth of animals is a result of increased cell number. Another apparent difference between plant and animal growth is that plants maintain the capacity to grow throughout their life (the so-called indeterminate growth). In sharp contrast, animals have determinate growth and reach their final size before maturation. However, being multicellular organisms, plant and animal growth have a conspicuous feature in common: both plant and animal growth are regulated by hormones.

Auxin was the first plant growth hormones discovered, and their name was derived from the Greek word αυξειν (*auxein* means "to grow or to increase"). Their promoting role in plant growth was first noted by Charles Darwin and his son Francis in studying phototropism of coleoptile of canary grass (*Phalaris canariensis*) and was documented in the remarkable book entitled *The Power of Movement in Plants* published in 1888 (Darwin and Darwin 1888). The existence of auxin in the tip of oat (*Avena sativa*) that can move and regulate phototropism of coleoptile of oat was unequivocally demonstrated by Frits Went in 1926. IAA (indole-3-acetic acid), the principal of auxin in higher plants, was isolated by Kenneth V. Thimann in the 1930s (Thimann 1936). IAA and several other chemicals with similar structure and physiological activity in inducing cell elongation of stems were named as auxin in 1954 (Stowe and Thimann 1954).

In the past 80 years after auxin isolation, extensive studies have been conducted to investigate biological and physiological roles of auxin in plant growth and development. Up to date, no mutant lacking auxin has been identified. The findings have demonstrated that auxin is phytohormone that plays vital roles in plant growth and development, including leaf abscission and development of floral bud and fruit (Davies 2010). Notably, it has been proved that auxin is central regulator of root growth (Overvoorde et al. 2010). Therefore, endogenous and synthetic auxin with similar activity has been widely used in global agriculture and horticulture for more than 60 years. At the same time, numerous studies have been conducted to elucidate where auxin is synthesized, how it is transported to the sites of action, and how auxin becomes inactive after fulfilling their function (Ljung et al. 2005). Accordingly, a great deal of researches has focused on uncovering the molecular responses of plant cells to auxin.

In *Arabidopsis*, a two-step biosynthesis pathway from tryptophan to auxin has been well defined (Zhao 2012; Mashiguchi et al. 2011). A series of auxin transporters and carriers localized at the plasma membrane or the endoplasmic reticulum have been shown to be responsible for regulation of auxin homeostasis, including the location and amount of auxin, thereby the duration of auxin signaling and responses. At its sites of action, auxin is first perceived by three well-recognized receptor/co-receptor systems. Among them, TIR1/AFB-Aux/IAA (TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX-AUX/INDOLE-3-ACETIC ACID INDUCIBLE) co-receptor, the first identified and best characterized receptor system, regulates transcription of downstream auxin-responsive genes in nucleus (Villalobos et al. 2012) while newly identified auxin receptors SKP2A (S-phase Kinase-Associated Protein 2A) and ABP1 (Auxin Binding Protein 1) have been shown to mainly repress cell division during cell cycle and subcellular protein trafficking, respectively (Jurado et al. 2008a, 2010; Chen et al. 2001; Robert et al. 2010). The research advances have also highlighted the coordination of three auxin receptor systems in rapid and accurate activation of auxin signaling and responses (Chapman and Estelle 2009). Whether auxin biosynthesis, homeostasis control, and signaling pathway are conserved in various plants needs to be further characterized.

In addition to the pivotal roles of auxin during *Arabidopsis* growth and development, the functional analysis of auxin in plant response to environmental cues and plastic development have become an attractive new research area. There has been a sharp increase in deciphering the functions of auxin in plastic root development under nutrient deficiency (low nitrogen and phosphate) and abiotic stresses (e.g., salt stress) (Park et al. 2007; He et al. 2005; Gilbert et al. 2000), besides the well-known role of auxin in gravitropism and phototropism (Noh et al. 2003). These findings not only provided novel insights into the regulatory roles of auxin but also broadened the horizon of future auxin research.

Auxin Biosynthesis and Metabolism

Auxin Biosynthesis

IAA is the primary plant auxin and is predominantly synthesized in rapidly growing tissues, especially in shoot apical meristems, young leaves, and developing fruits and seeds (Ljung et al. 2001). Recently, it has been shown that root tips can also synthesize auxin that regulates root architecture together with the shoot-derived auxin (Aloni et al. 2006).

Because of the structure similarity between IAA and tryptophan (Trp), Trp has long been considered as the precursor of IAA. The compelling evidence has demonstrated that IAA is mainly converted from Trp in *Arabidopsis*, which is the so-called Trp-dependent pathway (Cohen et al. 2003). An enormous body of evidence has indicated existence of multiple pathways through which plants can convert Trp to IAA. During the past two decades, great progresses have been made in

understanding the biochemical mechanism of auxin biosynthesis, especially the mechanism of how Trp is converted to IAA. In this review, we will summarize the progress in the Trp-dependent auxin biosynthesis pathway.

A Simple Two-Step Biosynthesis Pathway for Auxin

Until very recently a complete two-step auxin biosynthesis pathway through which Trp is converted to IAA in plants was established (Mashiguchi et al. 2011; Won et al. 2011). In this pathway, the first step is that TAAl/SAV3 (TRYPTOPHAN AMINOTRANSFERASE OF *ARABIDOPSIS* 1/WEAK ETHYLENE INSENSITIVE 8/SHADE AVOIDANCE 3/CYTOKININ INDUCED ROOT CURLING1) converts Trp to indole-3-pyruvate (IPA), followed by converting IPA to IAA by the members of YUCCA (YUC) flavin monooxygenases family (Mashiguchi et al. 2011; Zhao 2012) (Fig. 1).

Conversion of Trp to IPA by TAA1

The indole-3-pyruvate (IPA) has long been considered as the most common intermediate in the Trp-dependent pathway for IAA biosynthesis (Cooney and Nonhebel 1991; Nonhebel et al. 1993). However, the role of IPA and the enzymes catalyzing the reaction from Trp to IPA in plant auxin biosynthesis are recently discovered by three independent genetic studies (Stepanova et al. 2008; Tao et al. 2008; Yamada et al. 2009). Interestingly, these studies were performed to identify the mutants with altered response of mutants to shade (*sav3*), ethylene (*wei8*), and NPA (an auxin transport inhibitor) (*tir2*) in *Arabidopsis*. However, despite of the original phenotypes in genetic screens, it turned out that these mutant phenotypes are due to mutations in a gene encoding *Arabidopsis* aminotransferase TAA1 that can convert Trp to IPA in vitro and is involved in auxin biosynthesis. The *taa1* mutants including *sav3*/*taa1*, *wei8*, and *tir2* show a decreased IAA synthesis and reduced expression of the auxin-responsive genes (Tao et al. 2008; Stepanova et al. 2008; Won et al. 2011; Yamada et al. 2009). Furthermore, it has been proved that the phenotypes of *taa1* can be partially rescued by a synthetic auxin picloram or IAA (Stepanova et al. 2008; Tao et al. 2008). Further experiments also demonstrate that simultaneous inactivation of *TAA1* and its close homologs *TAR1* and *TAR2* causes developmental defects similar to those of well-known auxin mutants (Stepanova et al. 2008). These findings provide strong evidence that the TAA1 and its close homologs play critical roles in auxin biosynthesis and plant development.

TAA1 and TARs are enzymes dependent on pyridoxal-50-phosphate (PLP) and are conserved in the plant kingdom. It is highly likely that TAA1 and its homologs act similarly to convert Trp to IPA in various plants to regulate plant growth and development. Functional analysis of TAA1 homolog genes in other species will provide novel insights into understanding the regulatory mechanisms controlling auxin biosynthesis in plants.

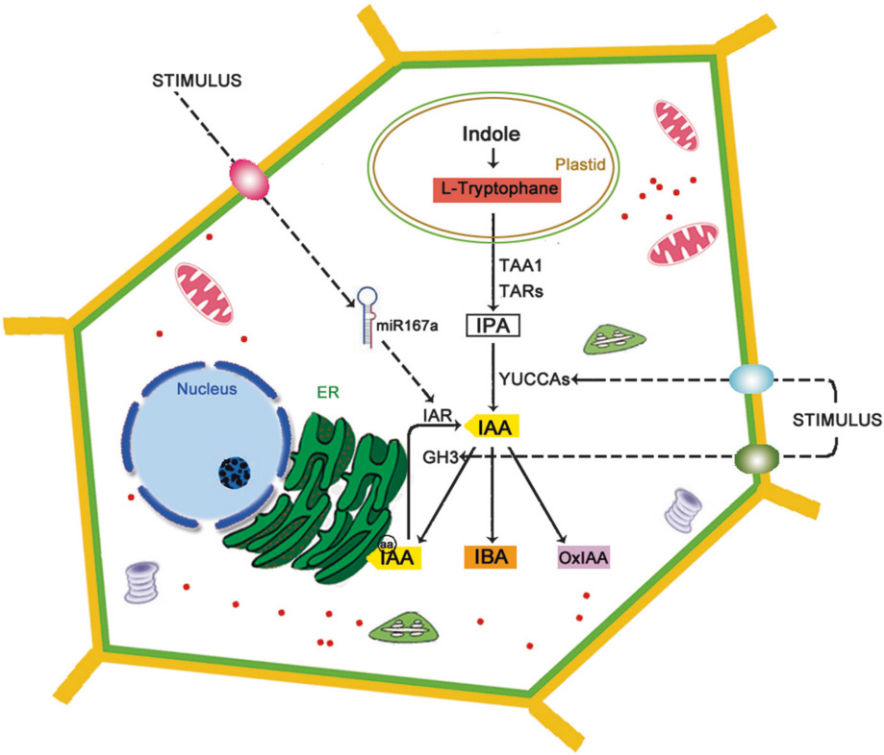

Fig. 1 Auxin synthesis and homeostasis. L-Tryptophan is the precursor of cell-synthesized indole-3-acetic acid (IAA). In the simple two-step Trp-dependent pathway, L-Trp is converted to indole-3-pyruvate (IPA) by TAA1, followed by YUCCAs converting IPA to IAA. In order to regulate IAA level, plant cells possess multiple ways to transform active IAA into inactive forms. IAA can be conjugated to other chemicals, such as sugar, amino acid, and glucan. As shown in the figure, IAA can be converted to IAA–amino acid conjugates by Gretchen Hagen 3 (GH3), which is localized to endoplasmic reticulum (ER). In addition, IAA can also be transformed into inactive indole-3-butyric acid (IBA), or be catabolized into 2-oxoindole-3-acetic acid (oxIAA). IAA level is balanced by GH3 and IAA-ALANINE RESISTANT (IAR), a gene targeted by miR167a encoding a hydrolase which can release IAA from inactive IAA–Ala form. Developmental and environmental stimuli modulate auxin homeostasis and subsequence plant growth by regulating IAA biosynthesis and catabolic pathways

The Rate-Limiting Step Catalyzed by YUC in Auxin Biosynthesis Pathway

Despite of important role of TAA1 and its homologs in the first step of auxin biosynthesis, the observation that the transgenic plants overexpressing TAA1 do not exhibit auxin overproduction phenotypes (Zhou et al. 2011) suggests that the TAA1-catalyzed step may not be a rate-limiting step in auxin biosynthesis. Indeed, the second step converting IPA to IAA catalyzed by YUC flavin

monooxygenases has been demonstrated as a rate-limiting step in a Trp-dependent auxin biosynthesis pathway (Zhao et al. 2001). Developmental defects in the *yuc* mutants can be rescued by in situ auxin production, and most importantly, overexpression of *YUC* genes encoding YUC flavin monooxygenases leads to auxin overproduction.

The role of YUC in auxin biosynthesis was first discovered in characterization of a dominant and fertile *yuc* mutant showing developmental phenotypes due to the elevated level of endogenous auxin (Zhao et al. 2001). *YUC* encodes a flavin monooxygenase (FMO)-like enzyme and is determined as a key auxin biosynthesis enzyme based on the genetic and physiological results, in particular the effect of overexpression of *YUC* in *Arabidopsis* on auxin overproduction (Zhou et al. 2011). Eleven *YUC* genes are identified in *Arabidopsis*, and genetic studies have shown that members of the *YUC* family function redundantly during plant growth and development (Cheng et al. 2006, 2007). For example, overexpression of single *YUC* gene in *Arabidopsis* and in other plant species leads to auxin overproduction and the corresponding phenotypes. Notably, loss-of-function mutation in a single *YUC* gene does not obviously influence plant development, whereas simultaneous inactivation of several *YUC* genes, such as *YUC1*, *YUC2*, *YUC4*, and *YUC6*, leads to apparent developmental defects in embryogenesis, seedling growth, floral development, etc. in *Arabidopsis* (Cheng et al. 2006, 2007), which is similar to that of well-known auxin mutants (Gälweiler et al. 1998; Dharmasiri et al. 2005a). Importantly, complementation of the developmental defects of the loss-of-function *yuc* mutants by overexpressing *iaaM*, a bacterial auxin biosynthesis gene, under the control of a *YUC* promoter demonstrates that *YUC* genes are essential for auxin biosynthesis and plant development (Cheng et al. 2006).

The very recent exciting breakthrough in auxin biosynthesis is the elucidation of biochemical mechanism of YUC in catalyzing the conversion from IPA to IAA (Dai et al. 2013). Using a recombinant *Arabidopsis* YUC6 containing FAD as a cofactor as an example, the authors provide evidence that YUC6 convert IPA to IAA through three sequential reactions using NADPH and oxygen. At the first step, the YUC6 catalyzes the reduction of the FAD cofactor to FADH(−) by NADPH. FADH(−) then forms a flavin-C4a-(hydro)peroxy intermediate by reacting with oxygen, followed by the reaction of the C4a-intermediate with IPA to produce IAA as the final chemical step. Thus, this work not only confirms the important role of YUC in auxin biosynthesis but also deciphers chemical mechanism that occurs during the flavin monooxygenase-catalyzed conversion from IPA to IAA in plants.

Genome-wide comparative analysis shows that *YUC* genes exist in all of the sequenced plant genomes. The important roles of *YUC* genes regulating auxin biosynthesis have also been experimentally validated in various plants, such as rice (Gallavotti et al. 2008). These results suggest that YUC flavin monooxygenases have a conserved role in coordinated regulation of the rate-determining step in auxin biosynthesis and subsequent plant growth and development.

A Second Pathway Converting Trp to IAA by Cytochrome P450s (IAOx Pathway)

Biochemical analyses have shown that multiple pathways from Trp to IAA exist for the auxin biosynthesis. In addition to the two-step auxin biosynthesis pathway, recent genetic studies have identified several genes, which regulate conversion from Trp to IAA through an important intermediate indole-3-acetaldoxime (IAOx). One key step in this pathway has been defined. During this step, Trp is converted to IAOx by *CYP79B2* and *CYP79B3*. The evidence for defining this reaction comes from identification and functional analysis of *CYP79B2* and *CYP79B3*, which encode two cytochrome P450s (Zhao et al. 2002). Overexpression of *CYP79B2* leads to elevated levels of free auxin and auxin overproduction phenotypes similar to the known IAA overproduction mutants such as *yuc* (Zhao et al. 2002). By contrast, the loss-of-function c*yp79b2 cyp79b3* double mutant contains reduced levels of IAA and displays the corresponding phenotypes, such as short hypocotyls and smaller stature, because of partial auxin deficiency (Zhao et al. 2002). The results show that the altered contents of auxin in the *CYP79B2* overexpression lines and c*yp79b2 cyp79b3* double mutant are due to the changes in IAOx.

Existence of the IAOx pathway is also supported by the biochemical and molecular analysis of loss-of-function mutants *sur1* and *sur2* showing similar typical auxin overproduction phenotypes (Delarue et al. 1998). SUR1 and SUR2 are involved in catalyzing the conversion from IAOx to indolic glucosinolates, a key intermediate to IAA (Delarue et al. 1998). Loss-of-function *sur2* mutant blocks the production of glucosinolates resulting in an increased IAOx flux and subsequent elevated level of IAA biosynthesis (Delarue et al. 1998). Further studies show that *SUR2* encoding the cytochrome P450 CYP83B1 has enzymatic activity of synthesizing 1-*aci*-nitro-2-indolyl-ethane from IAOx (Delarue et al. 1998; Barlier et al. 2000), thereby defining the first step in generating indolic glucosinolates from IAOx. *SUR1* encodes a C–S lyase that catalyzes the conversion of *S*-alkylthiohydroximate to thiohydroximic acid, a key reaction in indolic glucosinolate biosynthesis (Boerjan et al. 1995; Mikkelsen et al. 2004). Inactivation of *SUR1* disrupts glucosinolate production leading to the accumulation of upstream intermediates including IAOx and an increase in IAA (Boerjan et al. 1995). Taken together, these works established the catalytic role of these cytochrome P450s in converting Trp to IAOx and demonstrated existence of a parallel pathway (also termed IAOx pathway) in IAA biosynthesis (Mikkelsen et al. 2000).

Up to date, it is still not clear how IAOx is converted to IAA. Several studies have shown that IAOx is the precursor of indole-3-acetonitrile (IAN) and indole-3-acetaldehyde, which can then be used to generate IAA by nitrilases (Kobayashi et al. 1993) and aldehyde oxidases (Brumos et al. 2013), respectively. Recent biochemical analysis of the mutants suggests that indole-3-acetamide (IAM) is probably also an important intermediate in converting IAOx to IAA, but the genes and enzymes for producing IAM from IAOx are not known (Brumos et al. 2013). Although the IAOx pathway converting Trp to IAA plays a role during growth and development in *Arabidopsis*, the current results indicate that the IAOx pathway may

not be the mainly common IAA biosynthesis route in plants. The prediction comes from the observations including subtle phenotype and undetectable IAOx in *Arabidopsis cyp79b2 cyp79b3* double mutants, undetectable level of IAOx in monocots like rice and maize (Sugawara et al. 2009), and no apparent CYP79B2 and CYP79B3 orthologs found in monocots, such as rice and maize (Sugawara et al. 2009). Thus, many questions need to be answered, including how IAOx is converted to IAA, what are the key enzymes that catalyze the reactions, and whether the IAOx pathway is universal in the plant kingdom.

Auxin Conjugation and Degradation

Auxin is a hormone molecule whose activity levels are most important for its regulatory roles during plant cell, organ, and tissue development. Therefore, the precise regulation of auxin levels is an essential mechanism to fine-tune the activity of this powerful hormone during plant growth and development. After auxin is synthesized and completes it action, auxin must be attenuated to prevent overreaction. There are also two ways, conjugation with amino acids and sugars and degradation, to reduce active IAA (Normanly 2010; Barbez et al. 2012) (Fig. 1).

IAA Conjugation

Conjugation of hormone molecules with amino acids and sugars is a common mechanism to convert the active form to the inactive form. It has been shown that in many plant tissues, auxin is mainly in combination with a variety of sugars, sugar alcohols, amino acids, and proteins (Wood 1985). In this way, conjugated IAA can be stored locally or transported over long distances (Wood 1985). So far, there are basically two types of conjugated IAA found in *Arabidopsis*. One is ester-conjugated IAA, which is derived from conjugation of IAA with indole acetyl glucose, inositol, glycoproteins, glucan, or simple ester compounds, and the other is to combine IAA with amino acids, proteins, and peptides through amide connection.

Plenty of evidence shows that IAA–amino acid conjugates play an important role in auxin homeostasis. In 2005, Staswick group identified a family of *Arabidopsis GH3* (*Gretchen Hagen 3*) genes that encode an IAA-amido synthase and are responsible for production of IAA–amino acid conjugates (Hagen and Guilfoyle 1985; Wright et al. 1987; Li et al. 1991). Biochemical analysis has demonstrated that several recombinant GH3 enzymes are able to catalyze conjugation between IAA and amino acids, such as alanine (Ala), aspartic acid (Asp), phenylalanine (Phe), and tryptophan (Trp) (Staswick et al. 2005). Furthermore, loss-of-function mutants of the *GH3* genes *GH3.1*, *GH3.2*, *GH3.5*, and *GH3.17* show increased sensitivity to auxin (Staswick et al. 2005), while overexpression of a *GH3* gene reduces auxin levels in the plants resulting in a dwarfed phenotype. The results confirm that *GH3* genes are important regulators in maintaining auxin homeostasis by conjugating free IAA to amino acids (Staswick et al. 2005). IAA–amino acid conjugation is also

found in other plants. In rice, *GH3-8* gene encoding an IAA–amino acid synthetase promotes formation of IAA–Asp conjugates to reduce the auxin-induced cell wall loosening (Ding et al. 2008).

The conjugation process between IAA and sugar, glucan, and ester compounds is less understood. In *Arabidopsis*, the enzyme catalyze formation of methyl-esterified IAA (MeIAA) has been identified (Qin et al. 2005). The enzyme IAA carboxyl methyltransferase 1 (IAMT1) is a member of carboxyl methyltransferases family that can methylate the carboxyl side chain of IAA. The study has shown that overexpression of *IAMT1* gene leads to dramatic hyponastic leaf phenotypes (Qin et al. 2005). Most importantly, conjugation has been considered as an efficient pathway to rapidly regulate hormone contents because it is reversible. For example, during seed germination in maize, the IAA–inositol conjugates are transported from endosperm to the coleoptile by phloem and are then hydrolyzed to free IAA. It is noteworthy that most free IAA produced in the top of the maize coleoptile is hydrolyzed from IAA–inositol conjugates in seeds (Woodward and Bartel 2005; Ludwig-Müller 2011).

IAA Degradation

IAA levels can also be regulated by degradation, an irreversible mechanism through which the indole nuclear or chemical side chain is modified, causing auxin activity removed (Grambow and Langenbeck-Schwich 1983). The catalytic catabolism of IAA has been extensively studied. Physiological and biochemical results indicate that peroxidases are the enzymes that catalyze the catabolism of IAA into 3-methylene hydroxy indole (3-methyleneoxindole) (Meudt 1967). However, overexpression of peroxidase (POD) does not affect IAA content in *Arabidopsis* (Grambow and Langenbeck-Schwich 1983). Thus, it is possible that the peroxidase oxidation of IAA is not the main route for IAA catabolism in plants.

Recently, it has been shown that 2-oxoindole-3-acetic acid (oxIAA) and oxIAA-glucose (oxIAA-Glc) are the major degradation metabolites in rice, maize, and beans (Östin et al. 1998; Kai et al. 2007; Novák et al. 2012). OxIAA and oxIAA-Glc are induced by IAA treatment (Östin et al. 1998) or induction of IAA biosynthesis (Band et al. 2012), and the levels of oxIAA and oxIAA-Glc are markedly increased in the IAA overproduction plants (Stepanova et al. 2011; Novák et al. 2012). However, the genes involving in the IAA catabolism have not been identified, and the molecular mechanisms underlying IAA degradation still remain elusive (Fig. 1).

Auxin Homeostasis Control in Response to Environmental Stresses

Plants grow in a constantly changing environment over entire life cycle. As sessile organisms, plants regulate their growth and development according to both endogenous and environmental factors, such as high salinity, water status, and high or low temperature. During evolution, plants have evolved adaptive mechanisms to

optimize their development and survive the stress conditions. Plant hormones have been recognized as key regulators in plant adaptation. Among them, abscisic acid (ABA) is a well-recognized stress hormone that plays key roles in seed germination and plant growth in response to abiotic stresses, such as drought and salt stress (Lee and Luan 2012). During the past five decades, extensive studies have been conducted on ABA biosynthesis pathways and the regulation of ABA homeostasis and the signaling pathway under stress conditions (Verslues and Zhu 2005). Some studies have also demonstrated that ethylene is also involved in plant adaptation in response to abiotic stresses (Wang et al. 1990). Recently, accumulating evidence indicates that almost all the plant hormones, such as salicylic acid (SA), gibberellins (GAs), brassinosteroids (BR), and strigolactones, also somehow participate in regulation of plant development and adaptation to stresses (Hayat and Ahmad 2007; Davies 2010; Clouse et al. 1992; Gomez-Roldan et al. 2008). As an essential hormone molecule during plant growth and development, the roles of auxin in plant stress responses have drawn the scientists' attention focusing on the mechanisms of auxin homeostasis control and developmental plasticity under abiotic stresses, especially on salt stress, drought, and low temperature. Here we will briefly summarize the recent advances in adaptive adjustment of auxin biosynthesis and homeostasis and their roles in plant response to drought, salt stress, and low temperature.

Auxin and Plant Response to Salt Stress

Soil salinization is a global problem restricting agricultural production. High salinity causes multiple cellular stresses including osmotic stress, ion toxicity, nutritional deficiency, oxidative stress, and a series of secondary stresses, such as oxidative damage and metabolic toxicity (Hasegawa et al. 2000). As a result, salt stress causes reduced plant growth and photosynthesis, increased energy consumption, and accelerated aging and death of plants (Wang et al. 2003; Chaves et al. 2009; Zhu 2001). Most importantly, salinity has become an important environmental stress limiting crop yield in arid and semiarid areas (Pitman and Läuchli 2002). Therefore, the physiological and molecular mechanisms of plants to cope with salt stress have long been recognized as important scientific questions. However, majority of past researches focused on understanding the regulation of ion homeostasis control and osmotic stress response of plants, the regulatory roles of the individual hormones, and the interaction between growth hormones have just drawn attention.

There are still very little information on the effects of salt stress on auxin biosynthesis and the levels of auxin in the stressed plants, especially in the tissues or organs. The changes in auxin contents have been noted. However, whether auxin is increased or decreased under salt stress conditions remains controversial. A few studies reported that the increased level of IAA is correlated with the reduced plant growth (Ribaut and Pilet 1994), whereas some physiological researches show that salt stress causes great reduction in IAA in rice leaves (Prakash and Prathapasenan 1990; Nilsen and Orcutt 1996), tomato (Nilsen and Orcutt 1996), and wheat roots

(Shakirova et al. 2003). Recently, strong evidence shows that under mild salt stress, the auxin levels are maintained almost unchanged in both shoots and root tips in *Arabidopsis*. It is shown that maintenance of auxin homeostasis in these tissues of the stressed plants is regulated by the SOS (Salt Overly Sensitive) signaling pathway (Zhao et al. 2011). Research demonstrated that the auxin homeostasis in roots that is essential for lateral root formation and growth is regulated by the SOS signaling pathway. Loss-of-function mutant *sos3* shows substantially reduced auxin leading to abortion of lateral root formation and emergence and increased sensitivity to salt. Exogenous application of auxin in the growth medium containing NaCl can restore the lateral root development of *sos3* mutants under salt stress (Fig. 2). These findings confirm that maintenance of auxin homeostasis is an important adaptive mechanism for plant root growth to survive salt stress. However, whether the reduced level of auxin in *Arabidopsis* is caused by downregulation of biosynthesis pathway or stimulation of auxin catabolism remains elusive. Expression analysis of the *GH3* genes in *Sorghum bicolor* reveals that *SbGH3* is expressed at low level under normal conditions and is highly induced by salt stress (Wang et al. 2010). The result indicates that IAA conjugation may be involved in reduction of active IAA in the stressed plants. Further understanding of auxin homeostasis control in plants will provide novel insights into the molecular mechanisms of plant adaptation to saline soil.

Auxin and Plant Response to Drought Stress

Understanding the mechanism of plant response to drought and improvement of drought tolerance of crops is one of the fundamental questions in plant biology. The remarkable features of plants grown under drought conditions are stunted growth and shortened life cycle (Vinocur and Altman 2005). Therefore, it is quite apparent that auxin should participate in the adjustment of the development of plants. Genome-wide gene expression profiling shows that transcription level of auxin-responsive genes including the genes involved in auxin metabolism is changed in response to dehydration (Ghanashyam and Jain 2009). However, almost all the researches in plant drought tolerance focus on ABA. To date, only a few studies report the roles of auxin content and the auxin signaling pathway in plant responses to drought (Popko et al. 2010).

Understanding the roles of auxin comes from the results that disruption or overexpression of the genes encoding the key enzymes in auxin metabolism results in altered stress response of plants. For example, activation of *YUC7* gene elevates auxin levels and enhances drought tolerance of *Arabidopsis* (Im Kim et al. 2013; Lee and Luan 2012). Very recently, *YUC6* has also been shown to be involved in plant tolerance to drought in potato (Im Kim et al. 2013). Overexpression of *Arabidopsis YUC6* in potato causes auxin overproduction of phenotypes and enhanced drought tolerance. These results suggest that high levels of auxin are required for drought tolerance of plants, and the Trp-dependent auxin biosynthesis pathway plays critical role in the upregulation of auxin contents under water stress.

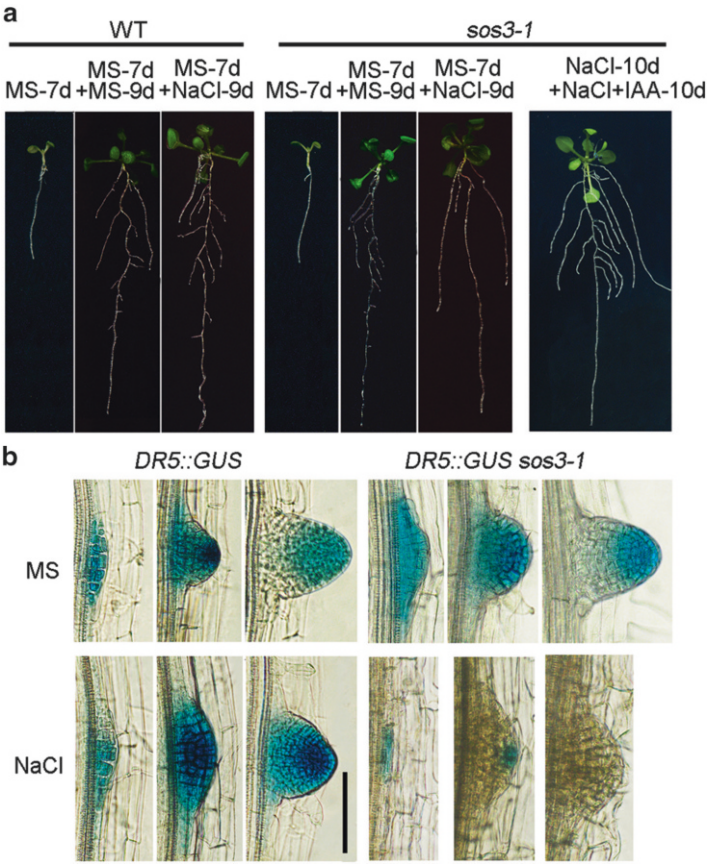

Fig. 2 Auxin is essential for lateral root development under salt stress. *sos3-1* mutant shows reduced auxin leading to abortion of lateral root formation and emergence and increased sensitivity to salt (Zhao et al. 2011). **a.** *sos3-1* mutant shows less lateral roots under 30 mM NaCl treatment. Both the wild-type and *sos3-1* seeds were sowed on the MS plates and grown for 7 days. Subsequently, the plants were transferred to the MS plates containing 30 mM NaCl, grown for an additional 9 days, and the lateral roots were compared. Exogenous application of auxin restores the lateral root development of *sos3-1* mutant under salt stress. The *sos3-1* seeds were sowed on the MS plates containing 30 mM NaCl and grown for 10 days. Subsequently, the plants were transferred to the MS plates containing 30 mM NaCl and 75 nM NAA and grown for an additional 10 days. **b.** Auxin accumulation is lower in the *sos3-1* in response to NaCl treatment. The *DR5*::*GUS* construct was analyzed in wild-type or *sos3-1* mutant for the free auxin accumulation. *DR5*::*GUS* construct shows GUS activity in sites where auxin accumulates

In addition to Trp-dependent pathway, free IAA derived from IAA conjugates also contributes to the increased levels of IAA and subsequent drought tolerance. It has been shown that overexpression of *OsGH3-2* catalyzing IAA conjugation with amino acids results in reduced free IAA level and increased sensitivity to drought (Du et al. 2012). Because alteration in *OsGH3-2* expression also changes

the level of ABA in the stressed plants, it is hypothesized that *OsGH3-2* regulates plant drought tolerance through modulating both free IAA and ABA homeostasis in rice (Du et al. 2012). It is apparent that IAA catabolism also plays an important role in maintaining IAA homeostasis when plants are subjected to water stress. Indeed, *GH3.8* and *GH3.13* also have functions in plant response to drought in rice (Ding et al. 2008; Zhang et al. 2009). Very recent report shows that *IAA-ALANINE RESISTANT 3* (*IAR3*), targeted by miR167a, encoding IAA-amido hydrolase that converts an inactive form of auxin, IAA–Ala conjugates, to free IAA is required for plant drought tolerance (Kinoshita et al. 2012). Notably, loss-of-function *iar3* mutants exhibit significantly higher sensitivity to drought than the wild type (Kinoshita et al. 2012). These results support the notion that IAA is required for plant drought tolerance. Recent works have indicated that cross talk between ABA and IAA signaling pathways modulates plant growth and survival under drought conditions (Du et al. 2013). In this aspect, the transcription factor R2R3-type MYB, MYB96, has been shown to be a molecular link that regulates the lateral root meristem activity through modulating cross talk between ABA and auxin under drought conditions (Du et al. 2013).

Plant drought tolerance is a complex trait and is unlikely controlled by single gene or single hormone. It is conceivable that there must be a complex network involving multiple hormones to fine-tune the plastic development and successive reproduction of plants under drought conditions. In the future, many questions about how the drought signal is perceived and transduced to the downstream effectors to modulate auxin contents and how ABA signaling integrates with IAA homeostasis control system still remain to be answered.

Auxin Perception, Transduction, and Attenuation

As a phytohormone molecule, auxin needs to be transported from the sites of auxin synthesis to the tissues and organs that generate appropriate responses. To do so, a perception system consisting of multiple receptor proteins has evolved to specifically recognize auxin, thereby activating a signal transduction cascade that leads to cell-type-specific responses. After providing rapid responses to developmental or environmental cues, the receptors are often rapidly attenuated in the signaling to avoid overreacting and abnormal growth.

Auxin Perception and Signaling Transduction

The word perception, derived from the Latin *perceptio*, means the organization, identification, and interpretation of sensory information. For plant hormones, perception starts with the specific binding of receptors with hormone molecules. To date, three proteins ABP1, TIR1/AFB, and SKP2A have been recognized as

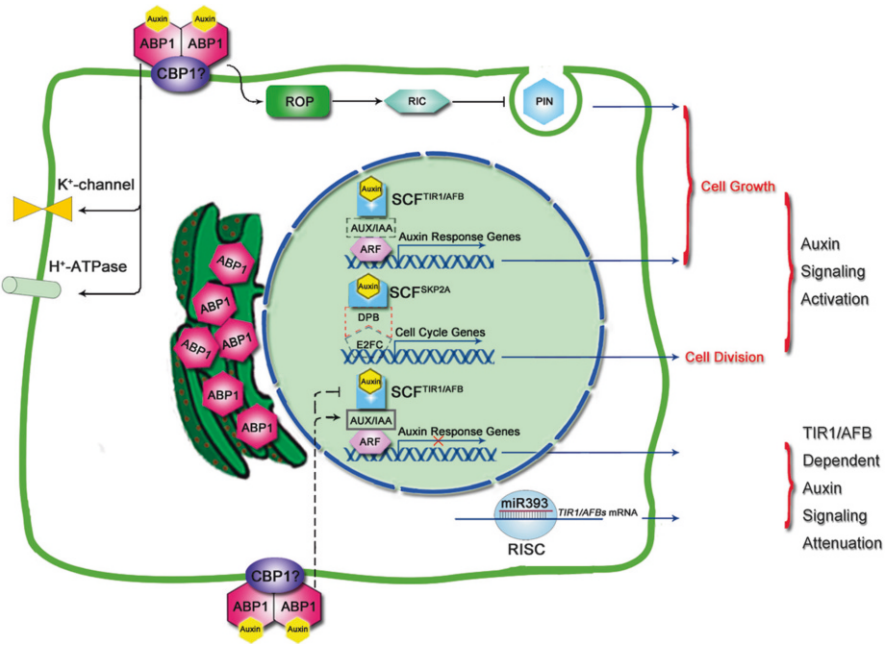

Fig. 3 A model of auxin perception, signal transduction, and attenuation. Auxin Binding Protein 1 (ABP1) is anchored by C-TERMINAL PEPTIDE-BINDING PROTEIN 1 (CBP1) with the plasma membrane. When binding to IAA, ABP1 influences the ion fluxes (such as H^+ and K^+) and inhibits clathrin-mediated PIN endocytosis through ROP-RIC (guanidine triphosphate hydrolases of plants-ROP interactive crib motif-containing proteins) pathway. Auxin can also bind to TIR1/AFB (TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX) co-receptors to regulate the expression of auxin responding genes and promote cell growth. In addition, auxin can regulate cell cycle through binding to the third receptor SKP2A (S-phase Kinase-Associated Protein 2A). The attenuation of the auxin signaling can occur at several levels. It is known that miR393 negatively regulate the expression levels of *TIR1* and *AFBs* through direct cleavage of their mRNAs and ABP1 negatively regulates the $SCF^{TIR1/AFB}$ (Skp1-Cullin-F-box) pathway through increasing the AUX/IAA stability

auxin receptors based on their strict structural and steric binding specificity with auxin (Peer 2013). Accumulating evidence has shown that each auxin receptor-mediated auxin signaling cascade plays a diverse regulatory role during plant growth and development (Fig. 3).

ABP1-Mediated Auxin Perception and Signaling Transduction

ABP1 was first identified as an auxin binding protein in maize (*Zea mays* L.) more than 40 years ago (Hertel et al. 1972). However, the ZmABP1 protein, a 22-kDa glycoprotein, was purified until 1985 (Löbler and Klämbt 1985), and the gene encoding ABP1 protein was eventually cloned 4 years later (Hesse et al. 1989;

Jones and Venis 1989). Biochemical analysis proves that ABP1 can specifically bind auxin (Jones and Venis 1989). ABP1 protein is originally detected on the endoplasmic reticulum (ER) of maize coleoptiles (Ray 1977). Indeed, a signal typical for luminal proteins of the ER consisting of the tetrapeptide ^{198}Lys-Asp-Glu-Leu (KDEL) is found at the C-terminus of the protein (Hesse et al. 1989; Inohara et al. 1989; Tillmann et al. 1989). However, the subcellular localization analysis shows that ABP1 is also localized at the plasma membrane/apoplast interface (Jones and Herman 1993; Diekmann et al. 1995). It is believed that the majority of ABP1 protein is in the ER, whereas a small fraction of ABP1 is at the plasma membrane/apoplast interface (Jones and Herman 1993). The crystal structure analysis of ABP1 protein suggests that the binding pocket of ABP1 is predominantly hydrophobic on the apoplastic side, suggesting that ABP1 binds auxin and perceives the auxin signaling outside of the plant cells (Woo et al. 2002). It is likely that the ER-localized ABP1 is transferred to the plasma membrane/apoplast interface and activates auxin signaling and response (Diekmann et al. 1995).

It has been shown that *ABP1* gene is induced by auxin in plants and activation is required for auxin-mediated responses (Hou et al. 2006). For example, overexpression of *Arabidopsis ABP1* in tobacco leaf strips results in an increase in auxin-mediated cell expansion, whereas induction of *ABP1* in intact plants leads to larger leaf cells although the leaves have normal morphology (Jones et al. 1998). *ABP1* expression is also required for auxin-mediated protoplast swelling (Steffens et al. 2001). A null mutation in *ABP1* also causes embryo lethality in *Arabidopsis* (Chen et al. 2001). However, it is noteworthy that *ABP1* plays a critical role in regulating the transition from the globular embryo to the bilaterally symmetrical structure during embryo development, because the early embryonic development is comparable to the wild-type control (Chen et al. 2001). These results support the role of ABP1 as an auxin receptor controlling plant growth and development (Jones et al. 1998).

Although ABP1 has been recognized as an auxin receptor, the further modeling studies of how ABP1 monomers bind auxin suggest ABP1 may require a co-receptor in order to effectively activate the signaling. So far, the co-receptor(s) has not been identified, but CBP1 (C-TERMINAL PEPTIDE-BINDING PROTEIN 1), which is a plasma membrane glycosylphosphatidylinositol (GPI)-anchored copper oxidase with homology to *Arabidopsis* SKEWED5 (SKU5) from maize, has been shown to participate in anchoring ABP1 to the plasma membrane (Shimomura 2006). Whether CBP1 functions as co-receptor or what ABP1 co-receptor(s) is still needs to be investigated.

Recently, the genetic and biochemical results show that ABP1 transmits the auxin signal through ROP-GTPase (guanidine triphosphate hydrolases of plants (Rho)-related GTPases of plants) and their associating RICs (ROP Interactive CRIB motif-containing proteins) (Xu et al. 2010). In the ROP-GTPase-mediated cascade, ABP1 regulates clathrin-mediated endocytosis of PIN (PIN-FORMED) auxin efflux carrier on the plasma membrane in pavement cells, guard cells, and root cells (Xu et al. 2010; Chen et al. 2012b; Lin et al. 2012). When exposed to auxin, ABP1 can rapidly activate ROPs (Murphy and Peer 2012) to inhibit ROP-RIC-mediated regulation of PIN endocytosis (Robert et al. 2010). To date, ROP2-RIC4 and

ROP6-RIC1 have been shown to function downstream of ABP1 in auxin signaling (Xu et al. 2010). In addition, ABP1 regulates clathrin-mediated endocytosis of PIN at the plasma membrane and the trans-Golgi network (Robert et al. 2010). Thus, it is widely acknowledged that ABP1 mediates non-transcriptional auxin signaling that quickly modulates cell-, tissue-, or organ-specific auxin response during growth and development. These rapid responses include auxin-mediated activation or deactivation of ion channels, transporters, and the proton pump ATPase across the plasma membrane, reflecting in response to auxin (Rück et al. 1993; Thiel et al. 1993; Zimmermann et al. 1994; Barbier-Brygoo et al. 1996). However, the molecular mechanisms underlying these rapid responses to auxin remain largely unknown. The possibility that ABP1 also mediates auxin signaling at the transcriptional level cannot be excluded, as a number of indirect evidences have already suggested the transcriptional regulatory role of ABP1 in auxin signaling and responses (Tromas et al. 2009, 2013).

TIR1-Mediated Auxin Perception and Signaling

The TIR1 is the first widely accepted auxin receptor, and the TIR1/AFB-auxin-Aux/IAA co-receptor system has been extensively characterized (Kepinski and Leyser 2005; Dharmasiri et al. 2005a; Tan et al. 2007). Interaction between the TIR1 and auxin results in degradation of Aux/IAA proteins that represses the auxin signaling, thereby activating ARF (AUXIN-RESPONSIVE FACTOR) transcription factors and the downstream signaling components (Tan et al. 2007; Mockaitis and Estelle 2008). The *TIR1* gene was first identified in a genetic screening with defects in auxin transport and/or auxin response (Ruegger et al. 1998). The *tir1* mutants show a variety of auxin-regulated growth defects including hypocotyl elongation and lateral root formation, indicating that TIR1 is required for normal response to auxin. The TIR1 protein contains 18 leucine-rich repeats (LRRs) (Tan et al. 2007) and an F-box motif with high sequence similarity to the yeast Grr1p (glucose repression-resistant 1 protein) and the human SKP2 protein which mediates the ubiquitination and subsequent proteasomal degradation of target proteins (Ruegger et al. 1998; Tan et al. 2007). The following studies demonstrate that *Arabidopsis* TIR1 forms a ubiquitin–ligase (E3) complex SCF^{TIR1} (Skp1-Cullin-F-box) with ASK (*Arabidopsis* Skp1-like protein) and AtCUL1 to degrade AUX/IAA proteins, such as AXR2/IAA7 and AXR3/IAA17 (Gray et al. 1999). In 2011, Gray et al. showed that auxin stimulates binding of SCFTIR1 to the AUX/IAA protein, resulting in the latter to be degraded. In the year of 2005, it was demonstrated separately by two papers that auxin can bind directly to SCF^{TIR1} (Dharmasiri et al. 2005a; Kepinski and Leyser 2005), thus confirming TIR1/AFB-auxin-Aux/IAA co-receptor system (Fig. 3).

There are six genes encoding TIR1 and AFB1-5 in *Arabidopsis*, which contain highly conserved sequences that bind to auxin (Lokerse and Weijers 2009; Calderon-Villalobos et al. 2010). However, they play varied roles in modulating the auxin signaling. For example, TIR1 and AFB2 are positive regulators of the auxin signaling (Dharmasiri et al. 2005b; Parry et al. 2009), while the AFB4 functions as a

negative regulator of the signaling (Greenham et al. 2011). Interestingly, a total of 29 AUX/IAA proteins are found in *Arabidopsis* (Liscum and Reed 2002). Therefore, TIR1/AFB proteins may have different binding activities to the AUX/IAA proteins at different levels of auxin, in different cells and tissues or in response to different developmental and environmental cues. The finding that the interactions between TIR1/AFB and AUX/IAA proteins and the interaction pairs are determined by the auxin concentrations (Villalobos et al. 2012) supports the above notion.

It has been well known for decades that auxin regulates expression of many genes (Abel and Theologis 1996). The compelling evidence shows that the TIR1/AFB-AUX/IAA co-receptor system is essential for activation of the auxin-responsive genes (Goda et al. 2008; Chapman and Estelle 2009). Now, it is quite clear that AUX/IAA proteins interact with ARFs to activate or repress the auxin-responsive gene expression (Weijers et al. 2005). There are 23 ARF proteins found in *Arabidopsis*, some of which are transcriptional activators (e.g., ARF5-ARF8 and ARF19), whereas others are transcriptional repressors, such as ARF2-ARF4 and ARF9 (Guilfoyle and Hagen 2007). AUX/IAA proteins interact with the ARFs at the promoters of the auxin-responsive genes to block ARF transcription activity and expression of the target genes in the absence of auxin. In the presence of auxin, binding of auxin to TIR1/ABFs promotes its interaction with AUX/IAA proteins resulting in the latter's degradation, thereby removing the repression of AUX/IAAs on the transcriptional activity of ARFs to activate the expression of the auxin-responsive genes (Ulmasov et al. 1997a, b; Kim et al. 1997).

Despite all these breakthroughs, many questions remain to be answered. The immediate questions include how three families of key proteins in the TIR1/AFB-AUX/IAA-ARFs pathway group to dynamically mediate auxin signaling and generate appropriate responses and what their specific downstream responsive genes are. Further studies using a combinatorial approach integrating application of new technology will help to decipher the molecular mechanism underlying the TIR1/AFB-AUX/IAA co-receptor system-mediated auxin signaling and plant responses.

SKP2A-Mediated Auxin Perception and Signaling

Because auxin modulates many biological processes, multiple auxin receptors are expected. Indeed, mutations of the known auxin receptors cause pleiotropic phenotypes which cannot be completely explained by these receptors and the corresponding cascade, such as cell cycle control (Gray et al. 1999; Chen et al. 2001). These observations encourage exploration of new auxin receptors. In mammals, the F-box protein SKP2 (S-phase kinase-associated protein 2) is a member of an SCF complex and plays a key role in cell cycle progression (Frescas and Pagano 2008). Thus, F-box protein SKP2A was identified in *Arabidopsis* based on sequence similarity to the human SKP2. The studies reveal that SKP2A is also a part of an SCF complex in *Arabidopsis* (del Pozo et al. 2002) and controls ubiquitin-dependent degradation of two cell division transcriptional factors, E2FC (E2 promoter

transcription factor C) and DPB (E2F dimerization partner B) (del Pozo et al. 2006). Further evidence reveals the role of SKP2A in mediating the auxin signaling. For example, the levels of nuclear protein SKP2A are reduced in the presence of auxin (Jurado et al. 2010), and accumulation of SKP2A protein is significantly reduced in the *axr2-1* and *axr3-1* mutants (Jurado et al. 2008a, b). Also, loss-of-function *skp2a* mutant exhibits auxin-tolerant phenotypes (Jurado et al. 2010). The critical evidence supporting SKP2A as an auxin receptor is its ability to directly bind auxin at the auxin binding site as predicted by comparative computational structure analysis using the TIR1 as a reference (Jurado et al. 2010; Mach 2010). Thus, SKP2A has been identified as the third auxin receptor.

SCF^{SKP2A} complex is a key regulator of the G1/S checkpoint in cell cycle progression, where some regulatory proteins need to be degraded to allow dividing cells enter the next phase. *Arabidopsis* SCF^{SKP2A} complex also positively regulates the cell cycle and functions almost in a same way to $SCF^{TIR1/AFB}$. In the absence of auxin or low auxin, transcription factors E2FC and DPB form a heterodimer that bind to the promoters of cell cycle genes and repress transcription of a subset of E2FC target genes. When auxin binds to SCF^{SKP2A}, the auxin SCF^{SKP2A} complex promotes ubiquitinylation and degradation of phosphorylated E2FC and DPB (del Pozo et al. 2006), activating transcription of cell cycle genes that function in cell cycle control. Since SKP2A is the newly discovered auxin receptor, much work is needed to be done to elucidate the entire mechanism of SKP2A in mediating the auxin signaling and cell cycle (Fig. 3).

Auxin Signaling Attenuation

After the auxin receptors transmit the signaling generating rapid responses to developmental and environmental stimuli, the signaling is often rapidly attenuated. Failure to switch the signaling off results in abnormal growth, and with attenuation, plant cells can also reset the system to prepare for the next response to a new stimulus (Peer 2013). Attenuation can occur at several levels, including removal of the stimuli, catabolism of auxin, and deactivation of receptors, and the signaling components at transcriptional or posttranscriptional levels. The mechanisms of the auxin signaling attenuation at different location within a cell/tissue/organ may vary. However, very little is known about how the auxin signaling is turned off in various auxin-mediated processes to date.

As mentioned above, auxin can be removed through the catabolism pathways, oxidation, and conjugation (Woodward and Bartel 2005; Normanly 2010). Recently, reactive oxygen species (ROS) has been shown to induce the oxidation of IAA to oxIAA (oxindole-3-acetic acid) (Peer et al. 2013). This result highlights the mechanism through which ROS regulates active auxin removal and the signaling attenuation, and the finding may be of particular importance for attenuation of auxin response under stress conditions. The control of attenuation also occurs at the level of the auxin receptors. It has been shown that microRNA (miRNA) miR393 plays an