Epigenetic Modifications and Plant Hormone Action

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ABSTRACT

The action of phytohormones in plants requires the spatiotemporal regulation of their accumulation and responses at various levels. Recent studies reveal an emerging relationship between the function of phytohormones and epigenetic modifications. In particular, evidence suggests that auxin biosynthesis, transport, and signal transduction is modulated by microRNAs and epigenetic factors such as histone modification, chromatin remodeling, and DNA methylation. Furthermore, some phytohormones have been shown to affect epigenetic modifications. These findings are shedding light on the mode of action of phytohormones and are opening up a new avenue of research on phytohormones as well as on the mechanisms regulating epigenetic modifications.

Key words: epigenetics, auxin, plant hormones, gene expression, chromatin regulation, DNA methylation


INTRODUCTION

Epigenetic modifications regulate mitotically or meiotically heritable gene expression without altering any changes in the genomic DNA sequences, and therefore contribute to flexible and reversible regulation of gene expression. Epigenetic modifications involve histone modification, chromatin remodeling, non-coding RNAs, and DNA methylation. Each of these modifications alone, or in combination with one another, and the interplay between different epigenetic modifications, controls gene expression patterns. Numerous studies show that genetic programming can be overridden by altering epigenetic modifications in response to environmental conditions, thus contributing to flexible survival strategies of sessile plants (Kim et al., 2008; Down et al., 2012). Intriguing underexplored aspects in this field of research include the biochemical signals that alter the epigenome and the transduction of these signals to control the downstream epigenetic pathways. An increasing number of studies suggest a tight link between epigenetic regulation and plant hormone signaling (Zhu, 2010). The plant hormone auxin is perceived by the nuclear auxin receptors TRANSPORT INHIBITOR RESPONSE1 (TIR)/AUXIN SIGNALING F BOX PROTEINS (AFBs), leading to the activation of AUXIN RESPONSE FACTORS (ARFs), the transcriptional factors that activate auxin-induced gene expression (Salehin et al., 2015). Emerging evidence indicates that the ARF-dependent induction of auxin-responsive genes is modulated by microRNAs (miRNAs) as well as by multiple epigenetic factors, such as histone modifications and the chromatin remodeling factor PICKLE (PKL) (Rhoades et al., 2002; Jones-Rhoades and Bartel, 2004; Mallory et al., 2005; Long et al., 2006; Navarro et al., 2006; Wu et al., 2006; Chen et al., 2010; Zhu, 2010; Weiste and Drège-Laser, 2014). Interestingly, auxin has also been implicated in the regulation of changes in the epigenome, suggesting an auxin-linked epigenetic regulation loop. In this review, we discuss recent literature on the interconnection between epigenetic control and phytohormone signaling, with a focus on auxin signaling.

HISTONE MODIFICATION MACHINERY AND PLANT HORMONE SIGNALING

Histone Acetylation and Plant Hormones

Eukaryotic chromatin is a highly organized complex of DNA and proteins, and is composed of the basic repeat element, the nucleosome. Each nucleosome contains two copies of the histone protein H2A, H2B, H3, and H4, and is typically enfolded by 147 bp of DNA. Modifications of histone tails such as acetylation, methylation, phosphorylation, and ubiquitination play an important role in epigenetic regulation. One major histone modification, which occurs at the ε-amino group of conserved lysine residue, is...
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acetylation mediated by the reversible activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Both histone acetylation and deacetylation play an important role in gene regulation and have been implicated in hormone signaling (Sieberer et al., 2003; Zhou et al., 2005; Long et al., 2006; Chen and Wu, 2010; Chen et al., 2010; Zhu, 2010). Acetylation neutralizes the positive charges of lysine residues on the histone N-terminal tail, thereby decreasing the interaction between histone protein and negative charged DNA, leading to a more open and loose chromatin conformation (Shahbazian and Grunstein, 2007). There are four HAT families. GCN5 (general control nonderepressible 5) belongs to the Gcn5 N-acetyltransferase (GNAT) subfamily and is the best characterized HAT in yeast, mammals, and plants (Baker and Grant, 2007; Chen and Tian, 2007; Lee and Workman, 2007). Arabidopsis GCN5 acetyltransferase and the transcription factor (TF) adaptor proteins ADA2a and ADA2b (also known as PROPORZ1) interact with each other, and are the subunits of the transcriptional adaptor complex SAGA (Sp-Ada-Gcn5-Acetyltransferase) (Servet et al., 2010). GCN5’s HAT activity is modulated by ADA2b in Arabidopsis (Mao et al., 2006).

Genome-wide analysis showed that the expression of ~5% of all genes is changed in gcn5 and ada2b/prz1 mutants (Benhamed et al., 2008). However, some reports indicate that specific genetic pathways are controlled by GCN5 or ADA2.

gcn5/hag1 mutants have a short root phenotype with defects in the columella differentiation layer and in QC marker gene expression (Vlachonasios et al., 2003; Kornet and Scheres, 2009), implicating the GCN5 complex in the maintenance of root stem cell niche in Arabidopsis. PLT1 and PLT2 genes encode AP2 domain TFs induced by auxin in an ARF-dependent manner, and play a major role in the specification of root stem cells (Aida et al., 2004; Galinha et al., 2007). Interestingly, GCN5 acts in the same genetic pathway as the PLT genes, and the short root phenotype of gcn5/hag1 mutant results from severely reduced expression of PLT genes, suggesting a chromatin modification-based mechanism that underlies the PLT-dependent stem cell specification. However, whether the GCN5 acetylase complex is recruited to the promoter of PLT genes directly to activate PLT gene expression remains obscure (Kornet and Scheres, 2009).

The prz1 (proporz1) mutant was isolated based on the phenotype of ectopic callus tissue formation in root under auxin treatment (Sieberer et al., 2003). The PRZ1 gene encodes for ADA2b, and the observed phenotype in prz1 mutant is at least partially caused by misexpression of KIP RELATED PROTEIN (KRP) family genes (Sieberer et al., 2003). Auxin treatment did appear to have an impact on histone acetylation at the whole chromatin level. However, chromatin immunoprecipitation (ChIP) experiments showed that ADA2b/PRZ1 is associated with the KRP7 locus, and auxin treatment decreased histone H3Kac9 and H3Kac14 levels in the KRP7 locus, which correlated with the reduction in expression of the KRP7 gene. Interestingly, the auxin-mediated reduction in KRP7 expression was more obvious in the prz1 mutant. Furthermore, constitutively reduced histone H3Kac9 and H3Kac14 levels were observed in the KRP7 locus in prz1 mutant. Collectively these studies support the hypothesis that auxin reduces histone acetylation level, whereas ADA2b/PRZ1 oppose the auxin-mediated suppression signal to control appropriate KRP7 expression (Anzola et al., 2010). Future areas of research will involve auxin regulation of histone acetylation at a specific locus.

Elongator was first identified as a RNA polymerase II-associated protein complex in yeast (Otero et al., 1999). This elongator protein complex consists of six subunits (ELP1–ELP6), with ELP3 containing a HAT domain (Wittsiebien et al., 1999). Some publications reported that mutations in elongator subunits cause pleiotropic phenotypes including abscisic acid (ABA), auxin, ethylene, and jasmonic acid (JA)-related phenotypes (Nelissen et al., 2005; Chen et al., 2006; Ding and Mou, 2015). ChIP experiments indicated that the SHORT HYPOCOTYL 2 (SHY2)/IAA3 and auxin influx carrier LIKE AUXIN RESISTANT 2 (LAX2) genes were direct targets of elongator HAT activity. Interestingly, SHY2/IAA3 is also a target of the GCN5 HAT (Benhamed et al., 2006), thus indicating a complex regulatory mechanism whereby two different HATs modulate SHY2/IAA3 gene expression.

HDACs and Plant Hormone Responses

Histone deacetylation has also been implicated in the regulation of hormone responses in plants. Histone deacetylation is mediated by the HDAC complex, which is composed of HDAC and other components. The Arabidopsis genome encodes 18 HDACs, and the largest and most characterized HDAC family is RPD3/HDA1, which can be divided into three classes (I–III) based on sequence similarity (Hollender and Liu, 2008; Alinsug et al., 2009). HDA6, 7, 9, and 19 belong to the class I family of RPD3/HDA1. Class II has three members, HDA5, HDA15, and HDA18. Class III comprises the plant-specific HD2A, HD2B, and HD2C (Pandey et al., 2002; Hollender and Liu, 2008). In contrast to HATs, HDACs repress transcription activity. Similarly to HATs, the recruitment of HDACs to DNA seems to occur both globally and at specific gene loci. For example, hda19 knockout and knockdown mutants show pleiotropic phenotypes, implicating HDA19 in the regulation of various developmental processes, such as seed dormancy and embryo, leaf, and flower development (Tian and Chen, 2001; Tian et al., 2003, 2005; Long et al., 2006). The observed pleiotropic effects suggest a global role for HDA19 in gene regulation. However, HDA19 is also implicated in the specific regulation of auxin signaling (more details on this point are discussed later).

Several studies suggest an important role for HDA6 and HDA19 in the regulation of plant hormone responses. The expression of HDA6 and HDA19 is induced by plant hormones ethylene and JA (Zhou et al., 2005), and knocking out HDA6 and HDA19 causes ABA hypersensitivity (Chen and Wu, 2010; Chen et al., 2010). The transcriptional repressors (JASMONE AT ZIM-DOMAIN) JAZ proteins and the TFs ETHYLENE INSENSITIVE 3 (EIN3) and its homolog EIN3-LIKE 1 (EIL1) act as master regulators for JA and ethylene signaling, respectively (Alonso et al., 2003; Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Zhong et al., 2009; An et al., 2010). JAZ inhibits the EIN3/EIL1 function, thus JAZ2 and EIN3 act at the crosstalk point of JA-inducible ethylene-regulated gene expression. HDA6 interacts with both EIN3 and JAZ proteins, and act as a repressor for EIN3-mediated transcription and JA signaling through HAT activity (Zhu et al., 2011). This evidence highlights a mechanism whereby HDAC can be
Histone Acetylation and Deacetylation Regulate TPL-EAR Repressome-Mediated Expression of Auxin-Responsive Genes

How are the HAT and HDAC complexes recruited to target gene loci in response to hormonal or environmental signal, and how is the cumulative action of histone acetylation and deacetylation controlled or integrated into the plant hormone signaling pathway? The studies of TOLESS (TPL) co-suppressor for auxin signaling have begun to unravel the answers to these interesting questions (Long et al., 2006). ARFs are TFs that bind auxin-responsive elements (AuxRE) to regulate auxin-responsive genes (Ulmasov et al., 1997; Tiwari et al., 2003). AUX/IAA proteins contain four conserved sequence motifs, domains I–IV, and each domain is presumed to have distinct interacting partners and functions. For example, the auxin receptor, TIR1, interacts with AUX/IAA through domain II, and this binding recruits AUX/IAA to the SCFTIR E3 complex for ubiquitination and subsequent degradation by the 26S proteosome (Dharmasiri et al., 2005; Ponting, 2001). Importantly, IAA12/BDL has been identified as a TPL-interacting protein, suggesting a link between TIR1-dependent auxin signaling and TPL. Moreover, double-mutant analysis showed that the tpl-1 mutation suppressed the bdl severe phenotype, such as basal patterning defects and reduction in cotyledon vasculature development. This supports the biological significance of TPL as a co-repressor of the IAA complex (Szemenyei et al., 2008). The EAR motif, defined by the consensus sequence of LXLXL, in the N terminus of IAA12/BDL, is necessary and sufficient for the interaction with TPL. The EAR motif-mediated repressosome is postulated to recruit the HDAC complex to the promoter of target genes (Song et al., 2005). In this scenario, HDAC is supposed to be recruited to the AUX/IAA repressor complex through EAR-mediated IAA-TPL interaction. Consistently, suppressor screening of the tpl-1 mutant identified the GCC5/HAG1 histone acetylase gene (Long et al., 2006). Moreover, the T-DNA insertion allele of
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*a* hda19* mutant has several *tpi-1* like phenotypes at the restrictive temperature (29°C), suggesting that TPL and HD19 act on the same target. Collectively, these studies support the model that the co-repressor TPL recruits HDA19 to the AUX/IAA repressor in an EAR motif-dependent manner, and that GCN5/HAG1 histone acetylase has a role opposing the IAA12/BDL-TPL-HDA19 repressor complex in the ARF-dependent expression of auxin-responsive genes (Figure 1) (Long et al., 2006; Szemenyei et al., 2006).

The EAR repressome is plant specific and appears to be widely used to regulate plant hormone signaling. For example, the EAR motif was identified in several proteins, including NOVEL INTERACTOR OF JAZ (NINJA), ABI-FIVE BINDING PROTEINS (AFPs), BRASSINAZOLE RESISTANT 1 (BZR1) and BR1-EMS-SUPPRESSOR 1 (BES1), and are involved in ethylene, JA, ABA, gibberellic acid (GA), and brassinosteroid (BR) signaling (Pauwels et al., 2010; Fukazawa et al., 2014; Oh et al., 2014; Ryu et al., 2014). In particular, the EAR repressome and the signaling pathways leading to the degradation of the EAR repressors are essentially paralleled in auxin and JA signaling (Pérez and Goossens, 2013). TPL and its family proteins TOPLESS-RELATED are implicated in auxin, JA, GA, and BR signaling (Pauwels et al., 2010; Fukazawa et al., 2014; Oh et al., 2014; Ryu et al., 2014). The same mechanisms involving HDACs via TPL may be employed by other plant hormone regulatory machineries. However, a direct interaction between TPL and HDA19 has not been observed (Gonzalez et al., 2007; Causier et al., 2012), suggesting an additional bridge protein is required for HDAC recruitment. To control the specificity for each hormonal signaling pathway, plants must have evolved specific partner proteins of TPL. Further studies will be necessary to reveal the mechanism for the functional specificity of TPL family proteins for EAR repressome-mediated plant hormone signaling.

ARF TFs bind AuxRE elements in the promoter of auxin-responsive genes to activate or inactivate their expression (Umasov et al., 1997, 1999; Tiwari et al., 2003; Guilfoyle and Hagen, 2007). Binding of AUX/IAA to ARF allows the TPL–HDAC complex to come into proximity with the AuxRE element of chromatin, inducing its repression status. However, how the GCN5/HAG1 histone acetylase complex is recruited to the auxin-responsive promoter to activate gene expression has yet to be elucidated. A possible mechanism involves an auxin-inducible bZIP11 TF that binds ADA2b, and the bZIP11–ADA2b complex is then targeted to the G-box-related elements (GREs) motif, the binding site for bZIP TFs (Jakoby et al., 2002). bZIP11–ADA2b is able to act as a key adaptor protein complex in the recruitment of GCN5/HAG1 acetylase to the GH3.3 promoter, inducing the activation of GH3.3 expression (Figure 1).

ADA2a showed a weaker ability to interact with bZIP proteins compared with ADA2b (Weiste and Dröge-Laser, 2014). This might explain the observation that only the *prz1/ad2b* knockout displays auxin and morphological phenotypes (Sieberer et al., 2003; Vlachonasios et al., 2003; Weiste and Dröge-Laser, 2014). However, the interaction of bZIP11 with ADA2b cannot explain all of the auxin-related phenotypes found in the *ada2b* single knockout. Most probably, the ADA2b–GCN5 complex also controls a number of auxin-responsive genes. AuxRE on the promoter of auxin-responsive gene is a well-studied motif, which is the site for recruiting ARF-IAA-TPL protein complexes. Both AuxRE and GRE motifs are presumed to be necessary for sensitive and quantitative regulation of auxin signal in response to varying developmental and environmental changes (Weiste and Dröge-Laser, 2014). To date, there is only evidence for bZIPs/ADA2b recruitment to the GREs motif in the GH3.3 gene promoter. It will be important to determine whether this attractive model can be applied to other auxin-responsive genes as a general mechanism for auxin responses.

**Histone Methylation and Plant Hormones**

Unlike acetylation, methylation does not change the charges of the histone tail. However, it increases the affinity of the histone for negatively charged DNA. The H3K4me3 or H3K27me3 modifications of histone protein are catalyzed by regulatory proteins of the Trithorax-group (Trx-G) and Polycomb-group (Pc-G) (Zhang et al., 2007). Histone methylation has been implicated in the regulation of several hormones. In particular, H3K4me3, a well-known epigenetic mark of active transcription, has been shown to mark several genes that affect hormone functions in plants. ARABIDOPSIS HOMOLOG OF TRITHORAX 1 (ATX1) directly targets the 9-cis-epoxydocosanoid dioxygenase 3 (NCED3), which plays a key role in the ABA biosynthesis pathway, and regulates its transcriptional activity. Consequently, atrx1 knockout shows various ABA-related phenotypes during dehydration stress (Ding et al., 2011).

Pc-G was shown to methylate H3K27 via its histone methyltransferase subunit to maintain the silent state of gene expression in Drosophila (Cao et al., 2002; Czermin et al., 2002). In Arabidopsis, a large number of genes appear to be regulated by H3K27me3, which is catalyzed by PRC2 proteins CURLY LEAF (CLF) and WINGER (SWN) (Lafos et al., 2011). Whole-genome tiling array analysis identified genes involved in auxin biosynthesis, transport, and signaling as targets of H3K27me3 (Lafos et al., 2011). For example, H3K27me3 targets 14 AUX/IAA genes directly. Furthermore, all previously reported miRNAs and ta-siRNAs that regulate ARF genes are H3K27me3 targets, suggesting that H3K27me3 controls ARF gene expression through miRNA loci indirectly (Lafos et al., 2011). The gene loci encoding the auxin transporter genes *PIN-FORMED 1* (*PIN1*), *PIN4*, *PIN7*, and *PIN8* are differentially methylated at H3K27 in leaves and meristems (Lafos et al., 2011). Differential H3K27me3 levels are correlated with tissue-specific *PIN1*, *PIN4*, *PIN7*, and *PIN8* expression patterns in the leaf and meristem, suggesting that H3K27me3 is one of the major determinants of tissue-specific *PIN* gene expression among the PIN gene family (Lafos et al., 2011).

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HETEROCROMATIN PROTEIN 1 (HP1) was originally named because of its protein enrichment in the heterochromatic region, and is known to be involved in the formation and maintenance of heterochromatin (Allshire et al., 1995). However, a role for HP proteins in euchromatic gene regulation has also been demonstrated in mammals and Drosophila (Piacentini et al., 2003; Crisendam et al., 2005; Yakoc et al., 2005). THRMINAL FLOWER 2 (TFL2), also known as LHP1, is the only homolog of HP1 in Arabidopsis that regulates specific genes in
Chromatin remodeling factors can directly change nucleosome structures in an ATP-dependent manner by altering histone–DNA interactions that affect the accessibility of nucleosomal DNA (Clapier and Cairns, 2009). Thus, it is anticipated that chromatin remodeling factors may regulate responses to various hormones that regulate gene expression. Genetic analysis suggested that BRAYAMA (BRM), which encodes a homolog of the conserved SWI/SNF chromatin remodeling ATPase, participates in the modulation of GA and cytokinin (CK) responses (Arackei et al., 2013; Efroni et al., 2013), and in auxin distribution by controlling the expression of several PIN genes. ChIP assays showed that PIN2, 3, 4, and 7 are direct targets of BRM (Yang et al., 2015). Furthermore, SWI3B interacts with several proteins involved in ABA responses, such as HYPERSENSITIVE TO ABA1 (HAB1), ABA INSENSITIVE 1 (ABI1), and ABI2, and modulates ABA signaling (Seae et al., 2008). Moreover, SWI3C interacts with DELLA proteins, and the knockout mutant shows altered responses to ethylene, ABA, BR, and GA (Samowska et al., 2013). These findings strongly suggest important roles of chromatin remodeling in plant hormone signaling.

Arabidopsis PICKLE (PKL), a homolog of CHD3/Mi-2 ATP-dependent chromatin remodeling factors in animals, prevents inadvertent activation of the embryonic programs in post-germination processes (Ogas et al., 1997, 1999; Eshed et al., 1999) and affects responses to several hormones in Arabidopsis. First, PKL is required for normal GA responsiveness and biosynthesis (Henderson et al., 2004). Second, ABI3 and ABI5 gene expression is upregulated in PKL knockout, leading to an ABA-hypersensitive phenotype (Perruc et al., 2007). Third, loss-of-function mutations in PKL cause a CK-hypersensitive phenotype, and enhance growth and greening of callus in detached hypocotyl independent of CK (Furuta et al., 2011). In addition, the PKL function in auxin responses has also been suggested by suppressor mutations of the solitary root (slr) mutant. slr does not produce any lateral root (LR), due to a dominant mutation in the SLR/IAA14 gene, which causes the stabilization of the SLR/IAA14 protein and blocks auxin-inducible LR formation (Fukaki et al., 2002). Interestingly, mutations in PKL abolished the auxin-insensitive phenotype for LR formation in slr mutant (Fukaki et al., 2006). In animals, HDACs are found in the Mi-2/NuRD complex, and Mi-2/NuRD is thought to act as a transcriptional repressor (Ahringer, 2000). In agreement with this, treatment with the HDAC inhibitor TSA induces LR formation in slr mutant (Fukaki et al., 2006). These effects of pkl mutations and TSA treatment for LR induction in the sr mutant require AFR7 and AFR19 functions. Therefore, it is likely that PKL controls auxin/ARF-dependent cell-fate specification and cell-cycle progression through changes in histone modifications during the LR formation process (Fukaki et al., 2006). Key aspects to be elucidated include whether the PKL-mediated chromatin organization that suppresses auxin-induced gene expression is directly regulated by auxin signaling, and whether PKL and TPL-AUX/IAA-mediated repressor complexes for auxin signaling act independently or synergistically (Figure 2A).
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proteins allow Mi2/NuRD complexes to have a large array of biological functions. MT3 is a cell-type-specific subunit of Mi2/NuRD and interacts with master regulator for cell-fate determination (Fujita et al., 2004). PKL may regulate a common molecular machinery for each plant hormonal signal, depending on the plant hormone-specific factors with which they associate (Figure 2A).

A recent publication has demonstrated a role for PKL in the regulation of gene expression mediated by GA and BRs (Zhang et al., 2014). The authors showed that PKL functions not only in repression but also in the activation of gene expression. The basic helix-loop-helix TF PHYTOCHROME INTERACTING FACTOR (PIF) family proteins accumulate in the nucleus under dark conditions to promote gene expression that is required for cell elongation (Leivar et al., 2008; Shin et al., 2009). Both BR and GA promote the cell elongation-related gene expression. After BRs are perceived by BRASSINOSTEROID INSENSITIVE 1 (BRI1), the downstream signaling cascade dephosphorylates and translocates BZR1 from the cytosol to the nucleus, allowing BZR1 to promote the expression of cell elongation-related genes, such as INDOLE-3-ACETIC ACID INDUCIBLE 19 (IAA19) and PACLOBUTRAZOL RESISTANCE 1 (PRT1) (Nakamura et al., 2003; Zhang et al., 2009; Kim and Wang, 2010; Wang et al., 2012). Arabidopsis have five DELLA family proteins, GIBBERELLIC ACID INSENSITIVE (GA), REPRESSOR OF ga1-3 (RGA), RGA-LIKE 1 (RGL1), RGL2, and RGL3, which negatively regulate cell elongation. GA promotes the degradation of DELLA proteins through proteasome activity, which results in cell elongation (Silverstone et al., 2001; Sun and Gubler, 2004; Sun, 2011). PKL negatively regulates photomorphogenesis (Jing and Lin, 2013). PKL physically interacts with PIF3 and BZR1 and represses H3K27me3 at the promoter of cell elongation-related gene, thus promoting gene expression. Conversely, DELLA proteins suppress the expression of cell elongation-related genes by inhibiting the binding between PKL and PIF3, decreasing the accumulation of the PKL protein in the promoter of these genes. Moreover, GA and BR treatments promote PKL accumulation at the promoter of cell elongation genes and reduce H3K27me3 in the promoter (Zhang et al., 2014). Taken together, these findings suggest that the interaction between PKL and different partner proteins enables the integration of different signaling pathways to control appropriate histone modification and gene expression levels (repression or activation) in response to external and internal signals. Future studies should identify specific partner proteins of PKL for each plant hormone signaling pathway in order to understand how PKL functions to coordinate multiple hormone signaling pathways (Figure 2A).

MICRORNAS MODULATE MULTIPLE REGULATORY LAYERS OF PLANT HORMONE SIGNALING

Small RNAs are commonly involved in the epigenetic regulation of gene expression. Plant miRNAs are small, endogenous, non-coding RNAs generated from the processing of local hairpin precursor structures. Mature miRNAs can target mRNAs for cleavage, leading to the destabilization of target mRNAs and thereby suppressing specific gene expression (Bartel, 2004; He and Hannon, 2004; Bologna and Voinnet, 2014). MICRORNA 393 (miR393) targets TIR1, AFB1, AFB2, and AFB3, and thus modulates auxin sensitivity in plants (Jones-Rhoades and Bartel, 2004; Navarro et al., 2006; Chen et al., 2011). miR393 negatively regulates TIR1, AFB1, AFB2, and AFB3 in response to pathogen attack. Overexpression of miR393 leads to a decrease in TIR1 transcript levels and enhances bacterial resistance (Navarro et al., 2006). miR393 targets all auxin receptor F-box genes, TIR1/AFBs, suggesting that this miRNA has evolved as a conserved mechanism to regulate auxin responses. However, miR393 might have also evolved as a mechanism to regulate specific TIR1/AFBs under certain conditions. It was shown that miR393 expression is induced by nitrate treatment, but only changes in the AFB3 transcript level are negatively correlated with miR393 expression level after nitrate treatment. It is likely that additional factors are needed to specifically destabilize the AFB3 transcripts by miR393 in response to nitrate treatments (Vidal et al., 2010), although the mechanism underlying the specificity remains unknown.

Multiple components of the auxin signaling pathway are under the control of miRNAs. In addition to the TIR/AFB family genes, ARF6 and ARF8 are targets of miR167, while RF10, ARF16, and ARF17 are targeted by miR160 (Rhoades et al., 2002; Mallory et al., 2005; Wu et al., 2006). miR160 and its target gene, ARF10, play a role for shoot regeneration from somatic culture cells that is mediated by a balance between auxin and CK. Although ARF10 overexpression does not have an impact on shoot regeneration, miR160-resistant mARF10 transgenic plants have greater shoot regeneration ability than wild-type. Thus, the abundance of ARF10 transcripts seems to be mainly controlled by miR160, indicating a precise regulatory mechanism during regeneration processes (Qiao et al., 2012; Qiao and Xiang, 2013).

 AuxRE motifs were found in the promoters of several miRNAs that might target auxin-related genes in rice, suggesting a potential role for miRNAs in the auxin signaling feedback loop (Meng et al., 2010). Feedback regulations between miRNA and TF have been demonstrated in animals (Johnston et al., 2005; Tsang et al., 2007). In Arabidopsis, positive and negative feedback regulation mechanisms were observed in miR390 and ARFs involved in trans-acting short interfering RNA (ta-siRNA)-mediated LR growth (Marin et al., 2010). ta-siRNAs are plant-specific small RNAs whose biogenesis requires the siRNA pathway. However, their production requires the cleavage of TRANS-ACTING SIRNA (TAS) transcripts by action of specific miRNA-ARGONAUTE 7 (AGO7) complex, and unlike miRNA, the activity of RNA-DEPENDENT RNA POLYMERASE 6 (RDR6) and SUPPRESSOR OF GENE SILENCING 3 (SGS3) are necessary for the subsequent production of the 21 nucleotide ta-siRNAs mediated by DICER-LIKE 4 (DCL4) (Peragine et al., 2004; Vazquez et al., 2004; Allen et al., 2005; Gasioli et al., 2005; Xie et al., 2005; Yoshikawa et al., 2005). TAS3 precursor requires miR390 to produce ta-siRNA (ta-siARF) and target ARF2, ARF3, and ARF4 for transcript cleavage. ARF2, 3, and 4 inhibit LR growth. Auxin induces miR390 production and consequently promotes LR growth by the reduction of ARF2, 3, and 4 expression through the ta-siRNA function (Adenot et al., 2006; Fahlgren et al., 2006; Garcia et al., 2006; Hunter et al., 2006; Marin et al., 2010). Interestingly, the level of auxin-inducible miR390 expression

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was significantly higher in both arf4 knockout mutant and transgenic line that carried a ta-siARF-resistant form of ARF3 gene compared with that of control, indicating that the expression levels of these ARF genes and miR390 accumulation are connected by both negative and positive feedback loops. The authors further showed exciting data that the negative feedback loops between miR390 and ARF4 play a pivotal role in spatial regulation of miR390 expression during LR growth (Marin et al., 2010).

miRNA-mediated feedback regulation was also observed between CK and its activator HD-ZIPII TF PABULOSA (PHB). The destabilization of PHB by miR165 was negatively regulated by CK in roots (Dello ioio et al., 2012). It is conceivable that feedback loop mechanisms between miRNA and TF may be conserved in plants.

One notable role of ta-siARF has been demonstrated in leaf development. ARF3 is required for abaxial fate in leaf development (Allen et al., 2005; Pekker et al., 2005). The expression of pARF3:ARF3-GUS is present mainly on the abaxial side, and ta-siARF-resistant pARF3:ARF3m-GUS is expressed ubiquitously in young leaf primordia. ARF3 mRNA expression in the adaxial side is supposed to be destabilized by ta-siARF. Moreover, the gradient of ta-siARF expression from the adaxial to abaxial sides has been observed in older leaves. The expression of essential components for ta-siARF biogenesis, AGO7 and TAS, is limited to the central region of the adaxialmost cell layers of leaves, suggesting that ta-siARF moves from the adaxial to the abaxial side to create a gradient of ARF3 expression (Chitwood et al., 2009). The moving ta-siARF provides a new regulatory layer for the auxin control of leaf polarity, which is different from well-established roles of polar auxin transport and distribution. Intercellular movement of small RNAs to regulate hormone action appears to be a common mechanism. miR165 and miR166, which target CK-regulated genes PHABULOSA (PHB) (Carlsbecker et al., 2010; Dello ioio et al., 2012), also move from the endodermis to the stele, creating the gradient of target class III homeodomain-leucine zipper (HD-ZIPIII), PHB, REVOLUTA, ATHB8, and ATHB15 expression, although the movement is limited in several cell layers in this case (Pfrigge et al., 2005; Carlsbecker et al., 2010). The study of moving small RNAs may provide a new paradigm of miRNA-mediated gradients of plant hormone signaling.

As discussed above, the studies of miRNA shed light on additional regulatory layers of hormone signaling/action. A recent report proposes a molecular basis for a new concept, “miRNA timer,” involved in regenerative capacity (Zhang et al., 2015). Plant cells from a piece of differentiated tissue have the competence for regeneration. In general, cells of young plants have higher competence for regeneration compared with cells from old plant tissues. miR156 targets SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) TFs, and thus regulates the juvenile-to-adult phase transition (Chuck et al., 2007; Wu et al., 2009; Wang et al., 2011; Bergonzi et al., 2013; Zhou et al., 2013). These authors found that miR156 expression is abundant in early leaves and targets SPL9 transcript. SPL9 protein can interact with B-type ARABIDOPSIS RESPONSE REGULATOR (ARR) protein, ARR2, and affect its transcriptional activity. Type B ARRs are positive regulators of cytokinin-regulated gene expression (Hwang and Sheen, 2001; Sakai et al., 2001; Tajima et al., 2004; Mason et al., 2005; Taniguchi et al., 2007; Yokoyama et al., 2007). Interestingly, B-type arr knockout mutants show decreased regeneration capacity. These results suggest that SPL9 protein negatively regulates the expression of CK responsible genes through its interaction with B-type ARR. Moreover, SPL9 expression becomes higher with age due to the reduction in miR156 abundance, and therefore early leaves exhibit a higher regeneration rate than late leaves (Zhang et al., 2015). An interesting question is what regulates the decrease in miR156 expression during the plant life cycle: accumulated cellular damage, deregulation of the epigenome, or specific developmental mechanism?

**DOES HORMONE SIGNALING REGULATE DNA METHYLATION OR DEMETHYLATION?**

DNA methylation is a relatively stable but reversible epigenetic mark regulating gene expression and suppressing transposon activities in plants and animals. DNA methylation occurs at the three different cytosine sequence contexts (CG, CHG, and CHH, where H is C, A, or T) in plants (Henderson and Jacobsen, 2007). DNA cytosine methylation in the CG and CHG contexts is maintained by Methyltransferase 1 (MET1), the ortholog of mammalian DNA methyltransferase Dnmt1, and the plant-specific DNA methyltransferase CHROMOMETHYLASE 3 (CMT3), respectively (Finnegan and Dennis, 1993; Jackson et al., 2002; Kankel et al., 2003; SaZe et al., 2003). The maintenance of CHH sequence contexts are controlled by DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) and CMT2 (Law and Jacobsen, 2010; Zemach et al., 2013; Stroud et al., 2014). De novo DNA cytosine methylation requires 24-nt small interfering RNAs (siRNA) that direct DRM2 to methylate all three sequence contexts via the RNA-directed DNA methylation (RdDM) pathway (Matzke et al., 2009; Zhang and Zhu, 2012). Active DNA demethylation depends on the function of the REPRESSOR OF SILENCING 1 (ROS1) family genes that encode 5-methylcytosine DNA glycosylase/lyases. ROS1 and its paralogs, DEMETER-like 2 (DML2) and DEMETER-like 3 (DML3), are required for the prevention of hypermethylation at thousands of genomic regions and play a critical role in the regulation of transgenes, transposable elements, and some endogenous gene expression (Gong et al., 2002; Penterman et al., 2007; Zhu et al., 2007; Lister et al., 2008; Qian et al., 2012; Yamamuro et al., 2014). DME is expressed in the central cell, and its function is required for active DNA demethylation of maternal allele and gene imprinting in endosperm development (Gehring et al., 2006).

Some studies suggest a potential link between the maintenance of DNA methylation and plant hormone signaling. met1 null allele embryos exhibit a wide range of developmental phenotypes, including delayed transition from vegetative phase to reproductive phase (Soppe et al., 2000; Kankel et al., 2003) and embryo abnormalities, which are reminiscent of mutants that have defects in auxin distribution (Xiao et al., 2006). Indeed the DR5::GFP transgene, which has been used to report auxin-induced gene transcription (Friml et al., 2002a, 2002b;
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Ottenschläger et al., 2003), and PIN1, which encodes an auxin efflux carrier and is required for auxin distribution in the early embryo (Friml, 2003; Weijers et al., 2005), are expressed abnormally in met1 null allele abnormal embryos. However, DNA methylation at the PIN1 gene locus was not observed in either wild-type or met1 null allele, indicating that although an auxin gradient formation in early embryos requires a proper MET1-maintained DNA methylation level, MET1 influences PIN1 expression indirectly (Xiao et al., 2006).

**WUSCHEL (WUS)** encodes a homeodomain containing TF that plays an important role as a master gene for both stem cell-fate determinations in the shoot apical meristem. Both auxin and CK affect WUS function and the stem cell niche, and the WUS gene expression is regulated by auxin and CK (Laux et al., 1996; Mayer et al., 1998; Gallois et al., 2004; Gordon et al., 2007; Chen et al., 2010; Zhao et al., 2010). WUS also plays an important role in the specification of the stem cell organizing center during de novo shoot regeneration. met1 knockout callus starts greening and regenerating faster than that of wild-type on shoot induction medium. During de novo shoot regeneration, the DNA methylation status around the WUS gene is gradually reduced, eventually leading to active transcription of WUS gene in the organizing center. WUS gene expression is also controlled by dynamic changes of histone modification levels. Epigenetic marks of active transcription, H3K4me3 and H3Kac9, were increased, whereas the repressive mark, H3K9me2, was reduced, consistent with WUS expression during de novo shoot regeneration. These observations suggest that the WUS-mediated specification of organizing centers seems to be regulated by complex epigenetic mechanisms (Li et al., 2011). In addition, it has been shown that WUS expression is controlled by several epigenetic regulators in the organizing center of the shoot apical meristem (Kaya et al., 2001; Takeda et al., 2004; Kwon et al., 2005). However, the connection between DNA methylation and these epigenetic regulators involved in the WUS expression is currently unclear.

A recent study has linked DNA demethylation to auxin-mediated chromatin opening and gene expression. PINOID (PID) gene encodes an AGC family Ser/Thr protein kinase (Christensen et al., 2000), and controls polarized localization patterns of PINs (Friml et al., 2004). The expression of PID gene is regulated by chromatin loop from PID promoter to APOLO locus that is located 5148 bp upstream of the PID. APOLO encodes a non-coding APOLO (AUXIN REGULATED PROMOTER LOOP) RNA that influences PID expression directly (Ariel et al., 2014). APOLO RNA knockdown line displayed a delayed response to gravitropism, a phenotype that is similar to the pid knockout mutant (Sukumar et al., 2009; Ariel et al., 2014). Auxin treatment can induce both PID and APOLO expression. Remarkably, the APOLO RNA directly binds to the LHP1 protein, which is associated with genes marked by H3K27me3 in Arabidopsis (Gaudin et al., 2001; Turck et al., 2007; Henning and Derkacheva, 2009), and this RNA-protein complex is physically associated with the APOLO locus through an LHP-mediated chromatin loop. Interestingly, auxin treatment rapidly reduces the chromatin loop formation and the direct binding of the APOLO RNA and LHP1 protein. Consistent with these findings, a decrease of repressive marks, H3K27me3 and H3K9me2, and accumulation of LHP1 protein at the PID-APOLO locus was observed in response to auxin. Furthermore, the authors showed that active DNA demethylation also contributes to the auxin-mediated dynamics of the chromatin loop, indicating the existence of an auxin-mediated complex regulation at the locus (Ariel et al., 2014). The rdd mutant, a triple mutant of rdd, dml2, and dml3 (Penterman et al., 2007), displayed enhanced basal loop formation. Moreover, dynamic changes in both PID and APOLO expression, and chromatin loop dynamic and DNA demethylation in response to auxin treatment were significantly altered in triple knockout of the ROS1 family genes (Ariel et al., 2014). Although the significance of auxin-inducible active DNA demethylation at the APOLO locus for PID function in the polar localization of PIN2 is still unknown, these observations show that ROS1 family gene-mediated active DNA demethylation plays a role in chromatin loop opening and PID gene expression in response to auxin. The authors proposed an attractive possibility that the chromatin loop formation may affect the accessibility of ARF TF to the promoter of PID and APOLO for proper auxin-responsive expression (Ariel et al., 2014). The function of active DNA methylation in chromatin loop opening seemed to be a very rare event. However, the response to auxin in chromatin loop opening was very quick and dynamic (Ariel et al., 2014). Given the association of auxin action with both ROS1 family-mediated active DNA demethylation and changes in chromatin structures, it would be of interest to determine whether auxin signaling directly regulates ROS1 activity and targeting to the chromatin loop.

**CONCLUSIONS**

In the past decade, extensive genetic mutant studies have revealed that epigenetic controls contribute to the regulation of plant hormonal signaling and action in various developmental and physiological processes. These findings offer us exciting prospects of considering the unexpected complex and layered but ordered regulatory mechanisms modulating hormone actions at the epigenetic levels in addition to biochemical and cellular levels of post-transcriptional controls. Remarkably, auxin signaling is modulated by all of the epigenetic modification machineries discussed herein. Moreover, some clear lines of evidence reveal the existence of an auxin-linked epigenetic loop (Figure 2B). It would be interesting to find out whether these observations can be extended to other plant hormones. In this review, we discussed the involvement of different epigenetic modifications in hormone actions; however, as we discussed in the section on DNA methylation/demethylation, these regulatory mechanisms do not exist solitarily in the regulatory network but are interconnected. In addition, given that these epigenetic modifications are reversible reactions, it is likely that these modifications happen simultaneously at the same locus, and thus may affect each other synergistically or antagonistically in response to numerous external or internal signals.

Plant hormones often display crosstalk with other plant hormones, and these signals are integrated as gene regulatory networks. We discussed that some plant hormones, such as auxin, GA, and BR, can modulate epigenomes. The competing or coordinating rewriting of epigenomes might be one crosstalk point among different plant hormones. In addition, the common critical regulatory factors in multiple plant hormone signaling, such as
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PKL and TPL, could be key factors in revealing the new paradigms of plant hormone crosstalks. The studies of conventional plant hormone signaling pathways are well advanced in Arabidopsis. This advance should aid in our future studies aimed at understanding how these conventional hormone signaling pathways are integrated into the new layers of hormone regulations at various epigenetic levels.

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