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REVIEW PAPER

miRNAs in the crosstalk between phytohormone signalling pathways

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Abstract

Phytohormones are signal molecules produced within the plant that control its growth and development through the regulation of gene expression. Interaction between different phytohormone pathways is essential in coordinating tissue outgrowth in response to environmental changes, such as the adaptation of root development to water deficit or the initiation of seed germination during imbibition. Recently, microRNAs (miRNAs) have emerged as key regulators of phytohormone response pathways *in planta* by affecting their metabolism, distribution, and perception. Here we review current knowledge on the miRNA-mediated regulations involved in phytohormone crosstalk. We focus on the miRNAs exhibiting regulatory links with more than one phytohormone pathway and discuss their possible implication in coordinating multiple phytohormone responses during specific developmental processes.

Key words: *Arabidopsis*, genetic regulations, microRNAs, phytohormones, plant development.

Introduction

Phytohormones control most, if not all, developmental stages across the plant life cycle, and often the same process involves multiple phytohormones (Fig. 1). In this review we focus on six classes of phytohormone: auxin (AUX), cytokinin (CK), abscisic acid (ABA), gibberellic acid (GA), ethylene (ET), and jasmonic acid (JA). AUX was the first class of phytohormones to be discovered and perhaps the most central in coordinating plant growth and development. Dynamic regulation of AUX distribution within the plant allows the establishment of an AUX gradient crucial in promoting lateral organ development. AUX function is tightly linked to the CK pathway and both hormones are known to control lateral organ initiation from stem cell niches, a phenomenon well described during root formation and apical dominance in plants. GA is also considered a major plant growth hormone known to stimulate stem elongation, flowering, and germination. ABA is often considered as the primary antagonist of GA based on the observation that ABA positively regulates seed dormancy and represses germination. ABA is also well known for its role during stress-induced responses, such as plant adaptation to drought. Along with ABA, JA and ET can also be classified as stress-induced hormones, involved in both biotic and abiotic stress responses. ET, which is perhaps best known for its role in promoting fruit ripening, and leaf and flower senescence, is a pleiotropic hormone like AUX, whose function is associated with a wide range of plant developmental processes.

Communication between phytohormone signalling pathways is essential to appropriately integrating and coordinating multiple developmental programmes (such as germination, organ morphogenesis, phase transition, and senescence) in response to endogenous and exogenous cues (age, photoperiod, temperature, biotic and abiotic stresses). Perhaps the best examples illustrating the complexity of hormonal crosstalk

Abbreviations: ABA, abscisic acid; AUX, auxin; CK, cytokinin; ET, ethylene; GA, gibberellic acid; GRF, growth responding factor; JA, jasmonic acid; miRNA, microRNA; tasiRNA, *trans*-acting small interfering RNA.

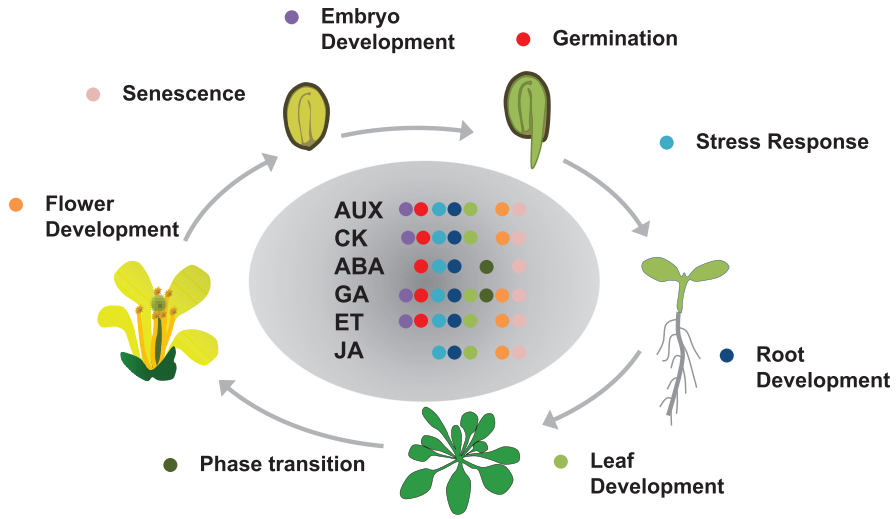

Fig. 1. Examples of plant developmental processes involving six classes of phytohormones. AUX, auxin; CK, cytokinin; ABA, abscisic acid; GA, gibberellic acid; ET, ethylene; JA, jasmonic acid.

are those involved in the control of root development, a process involving all six hormone pathways which interconnect to coordinate cell determination along the proximodistal axis of the root tip (Vanstraelen and Benkova, 2012). AUX and CK act in an antagonistic manner to coordinate the balance between cell division and differentiation. AUX sustains stem cell division in the root apical meristem while CK promotes cell differentiation towards the elongation zone by repression of polar auxin transport. This mechanism involves the regulation of *SHY2*, an AUX co-receptor that functions as negative regulator in AUX signalling (Tian, 2002; Calderon Villalobos *et al.*, 2012), whose expression is activated by the CK pathway and protein product SHY2 is degradated by the AUX signal (Dello Ioio *et al.*, 2008). GA also participates in this regulation by repressing the CK-dependent transcriptional activation of *SHY2*, therefore acting as positive regulator of cell division in the root apical meristem (Moubayidin *et al.*, 2010). In the elongation zone, GA promotes cell elongation through the controlled degradation of transcriptional repressors, DELLA proteins. The control of DELLA stability is central to the GA response and represents a major entry point for other phytohormones, such as AUX, ET, and ABA, to affect root cell elongation (Fu and Harberd, 2003; Achard *et al.*, 2006). ET is an inhibitor of root elongation, which acts, at least partially, by stimulating AUX biosynthesis and transport through the upregulation of several AUX biosynthesis genes and genes coding for AUX efflux carriers (Vanstraelen and Benkova, 2012). Additionally, several lines of evidence suggest that the control of cell expansion by the ET-AUX pathway involves CK and JA, which stimulate the ET signal through the activation of two *AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE* (*ACS*) genes (Chae *et al.*, 2003) and the derepression of the *ETHYLENE INSENSITIVE 3* (*EIN3*) gene (Zhu *et al.*, 2011), respectively. Feedback regulation also exists in which AUX controls other phytohormone biosynthesis pathways. For instance, AUX represses CK biosynthesis by promoting the expression of *CYTOKININ OXIDASE* (*CKX*) genes, and stimulates ET and GA biosynthesis by upregulating the expression of *ACS* and *GA20ox* genes, respectively (Vanstraelen and Benkova, 2012). These examples briefly illustrate the complexity of hormonal regulatory networks controlling plant growth and development. Yet we have only just started to understand the molecular bases beyond phytohormone crosstalk, and only a few mediators have been identified thus far, including microRNAs (miRNAs).

miRNAs are small RNA regulatory molecules, typically 20–21 nucleotides in length, that trigger the post-transcriptional repression of one to several target genes (Voinnet, 2009) (Fig. 2). In brief, each miRNA originates from one to several MIR gene(s), of which the transcript, named pri-miRNA, is able to form an imperfect stem-loop structure that is processed into a small double-stranded RNA molecule containing the miRNA. This RNA editing process requires the activity of several proteins, including the RNaseIII endonuclease DICER-LIKE1 (DCL1) and the double-stranded RNA-binding protein HYPONASTIC LEAVES1 (HYL1). Once exported outside the nucleus, the miRNA is subsequently incorporated into the RNA-induced silencing complex (RISC) to carry out the cleavage and/or translational repression of the targeted mRNAs.

Over the last decade, the number of annotated miRNAs has exponentially increased (Kozomara and Griffiths-Jones, 2011). In the Plant Kingdom there are over 5400 mature miRNAs known (miRBase release 19), including approximately 20 highly conserved miRNA families. Functionally, miRNAs are involved in a wide range of developmental processes, including phase transitions, organs morphogenesis, cell division, and stress responses (Voinnet, 2009), and highly conserved miRNAs often control more than one developmental process, which is a characteristic shared by phytohormones. Evidence of functional connections between miRNAs and phytohormones is shown by the phenotype of plants carrying the *hyl1* mutation which exhibit pleitropic developmental defects, reduce level of miRNAs accumulation, and show abnormal sensitivity to ABA, AUX and CK (Han *et al.*,

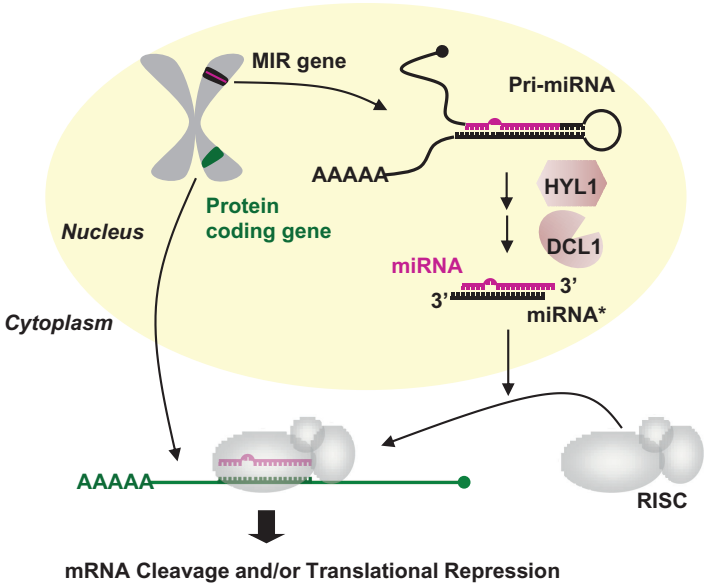

Fig. 2. A simplified model of miRNA biogenesis and function in plants. miRNAs are synthesized from a MIR gene transcript whose imperfect stem-loop secondary structure is recognized and processed by a protein complex containing HYPONASTIC LEAVES1 (HYL1) and DICER-LIKE1 (DCL1). Once exported outside the nucleus, the miRNA is incorporated into the RNA-induced silencing complex (RISC) to trigger the post-transcriptional regulation of one or several target genes through a base-pairing mechanism.

2004). Hormone and stress-response *cis* elements have been found to be enriched in many *MIR* gene promoters (Zhao and Li, 2013). Additionally, miRNAs have been shown to act non-cell-autonomously and, consequently, are able to create a gradient of regulation between adjacent tissues (Marin-Gonzalez and Suarez-Lopez, 2012). Several miRNAs have also been shown to induce the production of *trans*-acting small interfering RNAs (tasiRNAs), which are thought to act as a mobile signal (Marin *et al.*, 2010; Si-Ammour *et al.*, 2011). Because of their crucial developmental functions and their *trans*-regulation capabilities miRNAs are excellent candidates for the coordination of multiple hormonal responses.

Here, we review some of the recent knowledge on the molecular connections of miRNAs involved in multiple hormonal responses and discuss their implication as potential mediators of hormonal crosstalk in specific developmental processes.

miR396 functions in the control of cell fate determination by multiple phytohormone pathways

miR396 is a negative regulator of mitotic cell division through the downregulation of *GROWTH RESPONDING FACTOR* (*GRF*) genes in shoot meristems, leaves, and roots (Liu *et al.*, 2008; Rodriguez *et al.*, 2010; Wang *et al.*, 2011a; Hewezi *et al.*, 2012). In *Arabidopsis*, miR396 expression increases with leaf age and acts in a dose-dependent manner, in synergy with the tasiRNA pathway, to control leaf morphogenesis (Debernardi *et al.*, 2012; Mecchia *et al.*, 2013). The dynamic regulation of miR396 expression during leaf development allows for the establishment of a cell proliferation gradient that contributes to polarizing the adaxial–abaxial and proximodistal axes of the leaves (Rodriguez *et al.*, 2010; Wang *et al.*, 2011a). Additionally, using a target mimic construct (MIM396), miR396 was recently shown to participate in the control of leaf growth in response to solar radiation (Casadevall *et al.*, 2013). A homeostatic regulatory loop also exists as the over-expression of *AtGRF1* and *AtGRF3* represses the expression of *AtMIR396a* and *AtMIR396b* (Hewezi and Baum, 2012).

Despite its important developmental role, miR396 was only recently shown to be involved in the control of phytohormone-related genes. Hewezi *et al.* (2012) performed a transcriptome analysis to identify miR396-growth responding factor (GRF)-regulated genes in *Arabidopsis* using a *grf1 grf2 grf3* triple mutant and two miRNA-resistant forms of *AtGRF1* and *AtGRF3*. More than 60 genes were found to be involved in all six phytohormone signalling pathways, including many genes coding for ET- and AUX-response factors as well as genes directly involved in the biosynthesis of bioactive GA, CK, and ABA (Table 1). According to these data, miR396 appears to act as a strict repressor of the GA and CK pathways while promoting the ABA responses (Fig. 3A).

For the GA and CK pathways positive and negative regulators have been found to be antagonistically regulated by the same GRFs (Hewezi *et al.*, 2012), reinforcing the idea of upstream control by miR396. In the *grf1 grf2 grf3* triple mutant background, *GA-METHYL TRANSFERASE2* (*GAMT2*; whose ectopic expression causes a GA-deficient phenotype; Varbanova *et al.*, 2007) is upregulated, and the expression of two genes involved in GA biosynthesis, *AtGA3ox1* and an *AtGA20oxydase*, is inhibited (Table 1). The potential down-regulation of the GA pathway by miR396 correlates with the dwarfism phenotype observed in miR396-over-expressing lines (Liu *et al.*, 2009a). Interestingly, *AtGA20oxydase (AtGA20ox1)* is part of a positive regulatory loop between AUX and GA pathways, which is proposed to enhance the

Table 1. *Primary and secondary miRNA target genes involve in the regulation of a phytohormone pathway*

The primary and secondary targets refer to the genes that are directly and indirectly regulated by the miRNA, respectively. Tissue refers to the tissue (WP, whole plant; L, leaf; FL, flag leaf; S, shoot; F, flower; An, anther; St, stem; R, root; Al, aleurone; H, hypocotyls; TE, transient expression) in which the regulation of the targets has been identified [usually by 5′-rapid amplification of cDNA ends (RACE), mRNA quantification, or GUS staining]. References: 1, Si-Ammour et al., 2011; 2, Jones-Rhoades and Bartel, 2004; 3, Navarro et al., 2006; 4, Chen et al., 2012a; 5, Xia et al., 2012; 6, Bian et al., 2012; 7, Mallory et al., 2005; 8, Liu et al., 2007; 9, Gutierrez et al., 2012; 10, Wu et al., 2006; 11, Kinoshita et al., 2012; 12, Mallory et al., 2004; 13, Guo et al., 2005; 14, Xie, 2000; 15, Kim et al., 2009; 16, Koyama et al., 2010; 17, Palatnik et al., 2003; 18, Nag et al., 2009; 19, Schommer et al., 2008; 20, Rodriguez et al., 2010; 21, Ori et al., 2007; 22, Yanai et al., 2011; 23, Millar and Gubler, 2005; 24, Achard et al., 2004; 25, Reyes and Chua, 2007; 26, Alonso-Peral et al., 2010; 27, Tsuji et al., 2006; 28, Aya et al., 2009; 29, Wu and Poethig, 2006; 30, Wu et al., 2009; 31, Jung et al., 2011; 32, Yu et al., 2012; 33, Aukerman and Sakai, 2003; 34, Mathieu et al., 2009; 35, Marin et al., 2010; 36, Adenot et al., 2006; 37, Williams et al., 2005; 38, Liu et al., 2009a; 39, Wang et al., 2011a; 40, Debernardi et al., 2012; 41, Mecchia et al., 2013; 42, Hewezi et al., 2012; 43, Hewezi and Baum, 2012; 44, Zhou et al., 2013.

miR	Direct targets	Tissue	References	Secondary targets	Tissue	References
156	*AtSPL3/4/5*	S	29			
	AtSPL9/15	WP	30	*AtMIR172b*, *AtMIR156a*	WP,L	30,31
				AtGA2ox1/2, *DELLAs*	WP	32
159	*AtMYB33/65*	WP,L,F	17,23–26			
	AtMYB101	WP,TE	25	*ABA-responsive genes,*	An,Al	27
	OsGAMYB	F,An,St	27	*GA-responsive genes*	An	28
	OsGAMYBL1	F,An,St	27			
160	*AtARF10/16/17*	WP	7,8	*AtGH3.2/3.3/3.5/3.6*	WP,H	7,9
				ABA-responsive genes	WP	8
164	*AtNAC1*	WP,R,TE	12,13	*AUX-responsive genes*	R,TE	14
	AtNAC2	L	15			
167	*AtARF6/8*	F	10			
	AtIAR3	WP	11			
172	*AtAP2*	F	33			
	AtTOE1/2	F	33	*AtSPL3/4/5*, *AtMIR172b*	WP,L,S	30,31
	SMZ	L	34			
319	*AtTCP2/3/4*	L,F	16–18	*AtMIR164a*, *AtIAA3/4*, *SAUR*	WP,TE	16
	SlTCP4 (LA)	S	21	*AtLOX2/3/4*	L,S	19
	AsPCF5/6/8	R,L	44	*Ath-miR396*, *AtGRFs*	S	20
	AsTCP14	R,L	44	*SlGA2ox4*, *SlGA20ox1*	S	22
				AtGA2ox4, *AtGA20ox1*	WP	22
				AsNAC60	R,L	44
390	*AtTAS3*	WP,R	35,36	*AtARF2/3/4*	WP,F,R	35–37
393	*AtTIR1*	WP,L,F,R	1–4	*AtIAA12/17*, *AtGH3L*	WP	3
	AtAFB1	L,F	1,2	*OsIAA1*	TE	6
	AtAFB2	WP,L,F,R	1–4	*OsAUX1*	WP	5
	AtAFB3	WP,L,F	1–3			
	OsTIR1	WP,FL	5,6			
	OsAFB2	WP,FL	5,6			
	AtGRF1	WP,S,F,R	2,20,38,39,42	*AtMIR396a*, *AtMIR396b*	R	43
	AtGRF2	WP,S,F	2,20,38–40	*AtGAMT2*, *AAtGA3ox1, AtGA20ox*,	R	42
396	*AtGRF3*	WP,S,F,R	20,38–42	*AtCRF1/2*, *AtCKX4*, *AtCKI2*,		
	AtGRF4	WP,S	20,38–40	*ABI1/2*, *ABA1/4*,		
	AtGRF5	WP	39	*EIN2*, *ERF1/2/5/011*,		
	AtGRF6	WP,S	20,39	*JAZ3/7*, *JAR1*,		
	AtGRF7/8/9	WP,S,L,F	2,20,38,39	*ERFs*, *ARFs…**		

*Non-exhaustive list of AtGRF1/3-regulated genes; consult Hewezi *et al.*'s (2012) supplementary data for the complete set.

AUX signal and promote stem cell division in the root apical meristem (Vanstraelen and Benkova, 2012). The potential downregulation of GA/AUX pathways by miR396 could contribute to explain the reduction of root biomass produced by *Medicago* plants over-expressing miR396 (Bazin *et al.*, 2013). For the CK pathway, three genes coding for CK oxidases (*AtCKX3*, *4*, and *5*) that catalyse the degradation of CK are upregulated in the *grf1 grf2 grf3* triple mutant, and two *CK RESPONSE FACTOR* genes (*AtCRF1* and *2*) are repressed (Table 1). Additionally, the *CYTOKININ INDEPENDENT2* (*AtCKI2*; a histidine kinase activator of the CK response) gene is over-expressed in plants expressing a miR396-resistant

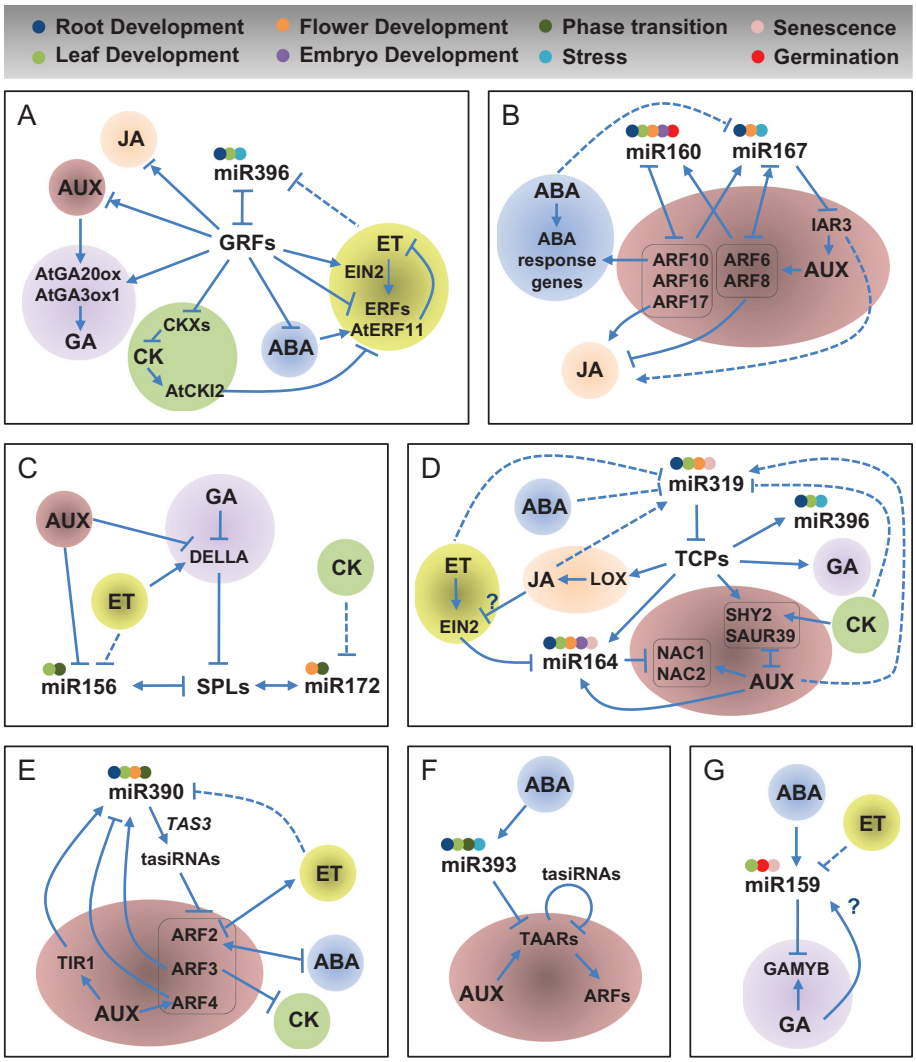

Fig. 3. Overview of the miRNA-mediated regulations involved in phytohormone crosstalk and associated developmental functions. A–G summarize the molecular connections between the phytohormone pathways and specific miRNAs with some of their associated developmental functions (upper panel): A, miR396; B, miR160 and miR167; C, miR156 and miR172; D, miR319 and miR164; E, miR390; F, miR393; G, miR159. Solid lines represent regulations observed in *Arabidopsis* and dashed lines represent regulations observed in other plant species. Arrows indicate positive regulations and blunt-ended bars indicate inhibitions. A line does not necessarily represent a unique or direct regulation. A question mark refers to unclear regulations. ERF, ETHYLENE RESPONSE FACTOR; CKX, CYTOKININ OXIDASE; GRF, GROWTH RESPONDING FACTOR; ARF, AUXIN RESPONSE FACTOR; IAR3, INDOLEACETIC ACID ALANINE-RESISTANT3; SPL, SQUAMOSA PROMOTER BINDING-LIKE; LOX, JA LIPOXYGENASE; EIN2, ETHYLENE INSENSITIVE2; TCP, TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR; NAC, NAM/ATAF1-2/CUC2 domain-containing proteins; TAAR, TIR1/AFB clade of Auxin Receptor (TAAR).

form of *AtGRF1* and *AtGRF3*. Considering the known function of AUX, CK, and GA in promoting cell division, differentiation, and elongation, respectively, miR396 could be an important player in the control of cell fate by acting as an upstream regulator of multiple hormonal signals to turn off cellular activities.

Other lines of evidence suggest a central role for miR396 in controlling the CK, ABA, and ET pathways. In plants expressing a miR396-resistant form of *AtGRF1* and *AtGRF3,* the *AtCKI2* gene, which codes for a histidine kinase activator of the CK response, is upregulated (Table 1). Interestingly, *AtCKI2* was shown to act as repressor in the signalling pathway by which ET and ABA inhibit root elongation (Iwama *et al.*, 2007). This activity correlates with the reduction of root length observed in plants over-expressing miR396 (Hewezi *et al.*, 2012; Bazin *et al.*, 2013), indicating that miR396 could control root expansion through an ABA/ET pathway. miR396-GRF regulation of the ET and ABA pathways may be conserved as it was shown to control the expression of several *ETHYLENE RESPONSE FACTOR* (*ERF*) genes and ABA-related genes. *ERFs* are transcription factor-coding genes known to function in stress-adaptation processes (Mizoi *et al.*, 2012). One of these genes, *ERF11*, is an important molecular link between ABA and ET biosynthesis (Li *et al.*, 2011) and was significantly upregulated in the *grf1 grf2 grf3* triple mutant. Also in the same mutant, two *ABA-INSENSITIVE* genes (*ABI1* and *ABI2*) were upregulated; and in plants over-expressing GRF3, two *ABA-DEFICIENT* genes (*ABA1* and *ABA4*) were downregulated (Hewezi *et al.*, 2012) (Table 1). These regulations

suggest that the miR396-GRF pathway is an important player in the CK-ABA-ET crosstalk involved in root-stress adaptation. ET is known to inhibit microbial associations in *Medicago* roots through an *ETHYLENE INSENSITIVE2*-(*EIN2*-; positive regulator of the ET response) dependent pathway (Penmetsa *et al.*, 2008). Interestingly, ET treatment of *Medicago* roots inhibits miR396 expression (Chen *et al.*, 2012b) (Table 2) and AtGRF1 promotes *EIN2* expression in *Arabidopsis* (Hewezi and Baum, 2012) (Table 1), suggesting that miR396 may be part of the regulatory pathway by which ET controls cell division and the pathogen infection response. Indeed, recent studies reported that a dynamic regulation of miR396 expression is required for the formation and maintenance of the syncytium during nematode infection in *Arabidopsis* roots (Hewezi *et al.*, 2012), and mycorrhization in *Medicago* roots (Bazin *et al.*, 2013). Additionally, upregulation of miR396 by salt stress (Liu *et al.*, 2008) and the over-expression of *AtMIR396a* and *AtMIR396b* was shown to improve drought tolerance by affecting leaf morphology (Liu *et al.*, 2009a), suggesting that miR396 may contribute to the ABA-mediated adaptation of the plant to draught conditions. In contrast, the expression level of *OsMIR396c* in rice is inhibited by salt treatment, and its over-expression by transgenic approach reduces salt stress tolerance in both rice and *Arabidopsis* (Gao *et al.*, 2010). However, comparison of mature sequences of Osa-miR396c and Ath-miR396b were found identical. This observation highlights the complexity of the stress-mediated regulation of miR396 and suggests that the precursor sequences used to over-express the miRNA (Osa-pri-miR396c and Ath-pri-miR396b) might be differentially processed in response to drought stress, thereby potentially generating additional small RNA species. Taken together, these data suggest that miR396 might be an important gateway for both ABA and ET to control cell proliferation in response to biotic and abiotic stresses.

In light of these recent data and considering the involvement of the different phytohormones in regulating specific cellular activities (such as ET, GA, and CK promoting cell division, elongation, and differentiation, respectively), it would be of interest to further characterize the role of miR396 in hormonal signals controlling cell-fate determination. Application of exogenous hormones or inhibitor of hormone biosynthesis could, for example, be use to assess the function played by the different phytohormones in the phenotype of plant with modified miR396 activity. For instance, could a GA-treatment rescue the dwarfism of plant over-expressing miR396? Or could the use of paclobutrazol restore the sensitivity of leaf cell proliferation to UV-B radiation in MIM396 plants?

Table 2. *Phytohormone treatments affecting the expression of a miRNA*

Tissue refers to the tissue (WP, whole plant; R, root; S, shoot; F, flower) in which the regulation of the miRNA has been identified. Effect refers to the up- or downregulation (represented by an arrow) observed in the corresponding study with the maximum estimated fold change indicated in parentheses (if known). Detection indicates the technique used to detect the miRNA or its precursor. References: 1, Sunkar and Zhu, 2004; 2, Liu et al., 2009b; 3, Marin et al., 2010; 4, Yoon et al., 2010; 5, Guo et al., 2005; 6, Srivastava et al., 2013; 7, Chen et al., 2012b; 8, Zuo et al., 2012; 9, Achard et al., 2004. ACC, aminocyclopropane carboxylic acid; 6-BA, 6-benzyladenine; IAA, indole-3-acetic acid; RT-qPCR, real-time reverse transcription PCR.

Hormone	Reference	Plant species	Treatment	Tissue	miRNA	Effect	Detection
ABA	1	*Arabidopsis thaliana*	ABA, 100 mM	WP	393	↑	Northern blotting
	2	*Oryza sativa*	ABA, 1 mg/l	WP	167	↓	Northern blotting
					319	↓	Northern blotting
AUX	3	*A. thaliana*	IAA, 10 mM	R	156	↓ (2)	Northern blotting
	4	*A. thaliana*	IAA, 10 mM	R	390	↑ (2)	Northern blotting
	5	*A. thaliana*	NAA, 10 mM	R	390	↑	pMIR390a-GUS
	6	*Brassica juncea*	IAA, 5 mM	S	164	↑	Northern blotting
				S,R	167	↑ (14)	pMIR390a-LUC
					319	↑ (8)	RT-qPCR of *MIR390b*
						↑ (1.5)	Northern blotting
						↑ (2)	RT-qPCR
						↑ (2)	RT-qPCR
CK	2	*O. sativa*	6-BA, 5 mg/l	WP	172	↓	Northern blotting
					319	↓	Northern blotting
ET	7	*Medicago truncatula*	ACC, 10 mM	R	159a	↓ (10)	Deep sequencing, RT-qPCR
	8	*Solanum lycopersicum*	ET, 50 µl/l	Fruit	164a/b/c	↓ (2)	Deep sequencing, RT-qPCR
					319	↓ (10)	Deep sequencing, RT-qPCR
					390	↓ (2)	Deep sequencing, RT-qPCR
					396a	↓ (2)	Deep sequencing, RT-qPCR
					156	↓	RT-PCR
GA	9	*A. thaliana* (ga1-3 mutant)	GA3, 100 mM	F	159	↑	Northern blotting
JA	6	*B. juncea*	JA, 0.1 mM	S	319	↑ (4)	RT-qPCR

miR160 and miR167 regulate the AUX signal affecting JA and ABA responses throughout plant development

miR160 and miR167 are dynamic components of the AUX response pathway by regulating the expression of several *AUXIN RESPONSE FACTOR* (*ARF*) genes, which code for transcription factors that directly regulate the expression of primary AUX-responsive genes (Fig. 3B; Table 1). In *Arabidopsis*, miR160 regulates *AtARF10*/*16*/*17* (Mallory *et al.*, 2005; Liu *et al.*, 2007) and miR167 regulates *AtARF6*/8 (Wu *et al.*, 2006). At least some of these regulations are conserved in monocotyledons, where *AtARF* orthologues have been shown to be regulated by the miR167 family in barley (Curaba *et al.*, 2012). These regulations are essential for the proper development of the plant as shown by the pleitropic developmental defects observed in plants expressing a miRNA-resistant form of some of these *ARFs*. Feedback regulation also exists by which AUX, via ARFs, controls miR160 and miR167 expression (Gutierrez *et al.*, 2009). This regulatory feedback appears to be conserved in *Brassica* plants; the level of miR167 increased in response to AUX treatment when *Brassica* plants were exposed to arsenic stress (Srivastava *et al.*, 2013) (Table 2).

miR160, miR167 and *AtARF6*/*8*/*17* are part of a regulatory network that control adventitious rooting in *Arabidopsis* (Gutierrez *et al.*, 2012). This network regulates the expression of three AUX-inducible *GRETCHEN HAGEN3* (*GH3*) genes, which, in turn, modulate JA homeostasis and control adventitious root initiation (Mallory *et al.*, 2005; Gutierrez *et al.*, 2012), suggesting a function of miR160 and miR167 in the crosstalk between AUX and JA during root formation (Fig. 3B; Table 1). miR167 also regulates lateral root development by targeting the mRNA coding for INDOLEACETIC ACID ALANINE-RESISTANT3 (IAR3), a hydrolytic enzyme that generates an active form of AUX (indole-3-acetic acid; IAA) (Kinoshita *et al.*, 2012). In tobacco, an orthologue of *AtIAR3* has been shown to act in the biosynthesis pathways of both JA and AUX (Woldemariam *et al.*, 2012), suggesting that miR167 might also control both AUX and JA biosynthesis through the downregulation of *IAR3*.

In addition to the control of root development, the miR167/AtARF6/8 regulatory pathway is essential for both male and female organ maturation (Ru *et al.*, 2006; Wu *et al.*, 2006). In developing flower buds, the level of AUX does not change, but the fine-tuned regulation of *AtARF6*/*8* gene expression is essential for the maturation of the flower and promotes JA production (Nagpal *et al.*, 2005). These results suggest that miR167 might play a critical role during the late stages of flower development by coordinating the regulation of the AUX transduction signal and the dynamic biosynthesis of JA.

The regulation of *AtARF10* by miR160 is essential for germination and post-embryonic development (Liu *et al.*, 2007). Transgenic plants expressing a miR160-resistant form of *AtARF10* exhibit an enhanced expression of several ABA-regulated genes and hypersensitivity to ABA in a dose-dependent manner, suggesting a potential path for AUX to influence the ABA response. It has been proposed that the downregulation of *AtARF10* by miR160 is required to reduce ABA sensitivity to allow radicle elongation during germination (Nonogaki, 2008). Interestingly, miR167 expression level was shown to decrease in response to ABA treatment in rice seedlings (Liu *et al.*, 2009b) (Table 2). If such regulation happens at germination, the inhibition of the ABA pathway by miR160 could also promote the expression of miR167 and, consequently, the promotion of lateral root development by AUX through the derepression of IAR3 (Kinoshita *et al.*, 2012).

Overall, the network constituted by miR160/miR167/*ARFs* represents a potent regulatory node by which AUX can influence both the ABA and JA pathways. Such regulation appears to be involved in a wide range of developmental processes throughout the plant life cycle, including post-embryonic development, root initiation, and reproductive organ maturation.

miR156 and miR172 are a potential gateway for phytohormones to control the timing of shoot development

miR156 and miR172 are part of a highly conserved regulatory module that controls the timing of vegetative-phase transition and flowering competency across all angiosperms (Huijser and Schmid, 2011). miR156 is the major miRNA coordinating vegetative-phase changes, as shown by rescue of phase defects in the *hyl1-2* mutant over-expressing miR156 (Li *et al.*, 2012). In *Arabidopsis*, over-expression of miR156 causes vegetative-phase transition delay and increases leaf initiation rate (Schwab *et al.*, 2005), whereas over-expression of miR172 promotes adult leaf traits and flowering (Jung *et al.*, 2011). The regulation of both miRNAs is tightly connected as their expression is affected by age, temperature, and light, in an opposite manner. For instance, miR156 expression gradually decreases with age in the shoot, whereas miR172 expression increases (Wu *et al.*, 2009; Jung *et al.*, 2011). At a molecular level, miR156 and miR172 act through the downregulation of genes coding for the SQUAMOSA PROMOTER BINDING-LIKE (SPL) and APPETALA2-like (AP2-like) transcription factor families, respectively, and are subjected to feedback regulation by their targets (Wu *et al.*, 2009). Additionally, several *SPL* genes targeted by miR156 are connected to miR172, such as *AtSPL9* and *10* which directly upregulate *AtMIR172b* expression and *AtSPL3*, *4*, and *5* whose expression are indirectly promoted by miR172 (Wu *et al.*, 2009; Jung *et al.*, 2011) (Table 1), therefore highlighting a molecular link between both miRNAs.

Thus, it is important to determine whether there is a connection between miR156/miR172 and phytohormone signalling pathways in the control of shoot development. Recently, this connection has been shown by Yu *et al.*, who demonstrated that GA promotes flowering in *Arabidopsis* through a miR156-dependent pathway (Yu *et al.*, 2012). GA is well known for its role in promoting floral transition through the degradation of transcription repressors, such as DELLA

proteins. The authors found that several DELLA proteins interact and repress SPL protein activities. Under long-day conditions the GA-mediated degradation of DELLA allows for the SPL-mediated activation of miR172 and floral identity genes, resulting in floral transition. Interestingly, the formation of trichomes, which are characteristic of abaxial adult leaves, is independently promoted by GA and miR156 in *Arabidopsis* (Yu *et al.*, 2010), suggesting that the GA and miR156/miR172 pathways may exert synergic control on vegetative-phase transition. The control of DELLA stability, which directly affects SPLs and miR172 activity, is not only regulated by the GA signal but also by several other phytohormones, including AUX, ET, and ABA (Vanstraelen and Benkova, 2012) (Fig. 3C). For instance, the AUX signal participates in the GA-mediated destabilization of RGA, a member of the DELLA family involved in root elongation (Fu and Harberd, 2003). Interestingly, the accumulation level of miR156 has been shown to decrease in response to AUX treatment in *Arabidopsis* roots (Marin *et al.*, 2010) (Table 2), and over-expression of miR156 in rice generates more roots with smaller size (Xie *et al.*, 2012), suggesting that the AUX signal can affect root development through the regulation of miR156-*SPLs*. ET also was shown to regulate the GA-mediated degradation of DELLA proteins (Achard *et al.*, 2003) and to slightly repress miR156 expression in tomato fruit (Zuo *et al.*, 2012) (Table 2), therefore expanding the developmental function of miR156-*SPLs*, at the interplay between ET-AUX-GA, for the regulation of both root and fruit development.

Taken together, these data suggest that the miR156/miR172 pathway may not be as 'autonomous' as once thought and that both miRNAs may integrate signals from multiple phytohormones to coordinate vegetative-phase transition and lateral organ development in response to environmental changes. For instance, considering the conserved function of miR156 in promoting aerial branching in both monocotyledon and dicotyledon plant species (Schwab *et al.*, 2005; Xie *et al.*, 2006; Chuck *et al.*, 2007), it would be interesting to investigate the potential role of miR156 in the AUX transduction signal that inhibits axillary bud outgrowth, a process known as apical dominance. Interestingly, in addition to miR156 repression by AUX, miR172 expression was shown to be inhibited by CK treatment in rice seedlings (Liu *et al.*, 2009b) (Table 2), suggesting that a correlation may exist between the antagonistic function of AUX and CK, required to coordinate the balance between cell division and differentiation, and the apparent opposite role play by miR156 and miR172 during vegetative-phase transition.

miR164 and miR319 are two players of major phytohormone crosstalk to control lateral organ development and senescence

In *Arabidopsis*, miR164 and miR319 are important players of shoot organ morphogenesis by acting through the down-regulation of genes coding for NAC (NAM, ATAF1/2, and CUC2 domain-containing proteins) and TCP (TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR) transcription factor families, respectively (Palatnik *et al.*, 2003; Mallory *et al.*, 2004; Nag *et al.*, 2009; Koyama *et al.*, 2010). miR319 is primarily known for its role as upstream regulator of leaf morphogenesis by indirectly repressing the expression of *AtMIR164a*, *SHY2* (*AtIAA3*), and two orthologues of the *SMALL AUXIN UP RNA39* (*SAUR39*) rice gene (Fig. 3D; Table 1) (Palatnik *et al.*, 2003; Koyama *et al.*, 2010). SHY2 and SAUR39 are both inhibitors of the primary AUX responses (by repressing AUX synthesis and transport), therefore indicating miR319 as a positive regulator of the AUX signal (Tian, 2002; Kant *et al.*, 2009). Additionally, miR319 was also shown to affect leaf cell differentiation by indirectly inhibiting GA biosynthesis when over-expressed in *Arabidopsis* and tomato leaves (Ori *et al.*, 2007; Yanai *et al.*, 2011), suggesting that the control of leaf organogenesis by miR319 involves the regulation of the production of at least two hormones, AUX and GA. Interestingly, AUX upregulates the production of miR319 in *Brassica* while its antagonist, CK, represses miR319 expression in rice, suggesting that miR319 function may be critical in coordinating the antagonistic function of the AUX and CK pathways (Liu *et al.*, 2009b; Srivastava *et al.*, 2013) (Fig. 3D; Table 2). In a similar manner, miR319 could also be involved in the control of the antagonistic function by the GA and ABA pathways, as its expression was shown to be inhibited by ABA treatment in rice seedlings (Liu *et al.*, 2009b) (Fig. 3D; Table 2).

In comparison to miR319, miR164 has been involved in a broader range of developmental processes, including root development (Fig. 3D). AUX induction of miR164 and the subsequent degradation of *AtNAC1* mRNAs provide a homeostatic mechanism to control lateral root formation (Xie, 2000; Guo *et al.*, 2005) (Tables 1 and 2). Interestingly, SHY2 activity is antagonistically regulated by AUX and CK to control the balance between cellular division and differentiation (Dello Ioio *et al.*, 2008). Taken together these data suggest that miR319 might acts as an upstream regulator of lateral root development by affecting the AUX/CK balance through the regulation of SHY2 and miR164 activity. Indeed, the expression of miR319 has been detected in root tissues of *Arabidopsis* and *Brassica* plants (Sobkowiak *et al.*, 2012; Srivastava *et al.*, 2013), while over-expression of miR319 in creeping bentgrass was shown to reduce the expression of both miR319 and miR164 target genes in the root and decrease root biomass production (Zhou *et al.*, 2013).

The central role of miR164/miR319 in lateral organ development extends with their implication in the phytohormone-mediated regulation of leaf senescence. During leaf aging, miR164 expression gradually decreases due to increasing repression by EIN2, resulting in progressive derepression of the NAC transcription factor *ORESARA1* (*ORE1*; also known as *AtNAC2*) and the promotion of leaf senescence (Kim *et al.*, 2009). miR319 also was shown to be a repressor of leaf senescence by indirectly repressing the expression of three LIPOXYGENASE-coding genes (*AtLOX2*, *3*, and *4*) required for the production of JA (Schommer *et al.*, 2008) (Table 1). Interestingly, it has been recently shown that reduced JA levels restore the ET sensitivity of *ein2* mutants (Kim *et al.*,

2013), suggesting that the indirect repression of miR164 by miR319 could be enhance by a JA-mediated derepression of EIN2 activity (Fig. 3D). Additionally, these data suggest that miR319 could control the regulation of senescence by JA and ET through two distinct pathways, with one pathway being miR164-/age-dependent and the other not. It is also worth noting that miR319 accumulation level was shown to be inhibited by ET in *Medicago* root and promoted by JA in *Brassica* shoot, suggesting the presence of a feedback-regulatory loop (Chen *et al.*, 2012b; Srivastava *et al.*, 2013) (Table 2).

miR319 and miR396 are the miRNAs with the most molecular connections to phytohormone signalling pathways (Figs 3 and 4). Interestingly, the over-expression of an miRNA-resistant form of *AtTCP4* results in an increase of miR396 accumulation level and a corresponding decrease in *AtGRF* gene expression (Rodriguez *et al.*, 2010), suggesting that the control of phytohormone response by miR319 may partially be relayed by miR396. Taken together, these data point to a central role for miR319 in coordinating multiple miRNA and phytohormone pathways to control lateral organ development and senescence. Although the role of miR319 in leaf morphogenesis has been clearly established further research on its function during root development needs to be investigated. Additionally, miR319 expression seems to be subject to several types of phytohormone regulation in plant such as in rice, *Medicago*, and *Brassica* (Liu *et al.*, 2009b; Chen *et al.*, 2012b; Srivastava *et al.*, 2013) (Fig. 3D; Table 2). Such regulation should be investigated to give us a better understanding of the role played by miR319 in phytohormone crosstalk.

miR390 involvement in the hormonal control of tissue outgrowth and senescence

miR390 is tightly connected to the AUX signalling pathway through the production of tasiR-ARFs from *TAS3* mRNAs, which then downregulate *AtARF2*, *3*, and *4* mRNA accumulation (Williams *et al.*, 2005) (Fig. 3E; Table 1). In turn, AUX influences the levels of miR390 by feedback regulation involving AtTIR1 as well as AtARF2, 3, and 4 (Marin *et al.*, 2010; Yoon *et al.*, 2010). AUX treatment promotes the expression of *AtMIR390a* and *AtMIR390b*, leading to an increased accumulation of miR390 in a dose-dependent manner in seedling roots. This feedback regulation is dependent on AtARF2 and/or AtARF3, which positively regulate miR390 accumulation, and on AtARF4, which represses *AtMIR390a* expression. During lateral root elongation, miR390 is specifically expressed at the root initiation site, where it restricts the spatiotemporal expression of *AtARF4* to allow the outgrowth of the emerging lateral root (Marin *et al.*, 2010; Yoon *et al.*, 2010). miR390 function also extends to the shoot, where the tasiRNA-mediated regulation of *AtARF3* and *AtARF4* is required for proper leaf morphogenesis and the timing of vegetative-phase change (Adenot *et al.*, 2006; Hunter *et al.*, 2006). Accordingly, plants expressing a tasiRNA-resistant form of *AtARF3* show precocious vegetative-phase change as well as severe leaf and flower developmental defects (Fahlgren *et al.*, 2006; Hunter *et al.*, 2006).

miR390 has no known connections with phytohormones other than AUX in *Arabidopsis*. The accumulation of miR390 is unaffected by ABA, GA, or CK treatment in *Arabidopsis* seedlings (Yoon *et al.*, 2010). In *Medicago* roots, however, miR390 is slightly downregulated by ET treatment (Chen *et al.*, 2012b). Despite this apparent lack of hormonal connections, miR390 may still participate in the regulation of multiple phytohormone responses through the downregulation of *AtARF2* and *AtARF3* (Fig. 3E). AtARF2 is a repressor of the AUX signal and is known to control shoot development by repressing cell growth and promoting senescence during the late stages of the plant life cycle (Ellis *et al.*, 2005; Okushima *et al.*, 2005; Schruff *et al.*, 2006; Lim *et al.*, 2010). Its expression is induced by ABA and contributes to the downregulation of the ABA-responsive gene *HOMEODOMAIN GENE33* (*HB33*), which is involved in seed germination and primary root growth (Wang *et al.*, 2011b). AtARF2 proteins also quickly degrade in the presence of ET (Li *et al.*, 2004), and the biosynthesis of ET was shown to be repressed in loss-of-function *arf2*, which suggests an implication of the ET pathway in the control of senescence by *AtARF2* (Ellis *et al.*, 2005; Okushima *et al.*, 2005;

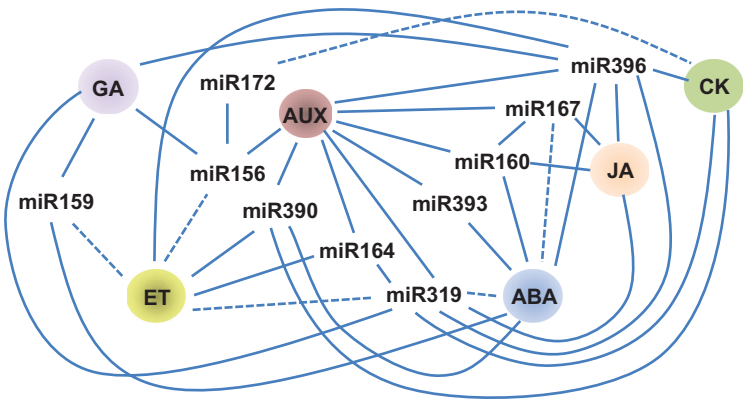

Fig. 4. The central role of miRNA-mediated regulation in phytohormone crosstalk. Solid lines represent molecular connections observed in *Arabidopsis* and dashed lines represent molecular connections observed in other plant species (*Medicago* and/or tomato).

Lim *et al.*, 2010). In addition to *AtARF2* function in multiple hormone responses, *AtARF3* has recently been shown to be involved in a communication pathway from AUX to CK by spatially restricting the biosynthesis of CK during *de novo* shoot induction (Cheng *et al.*, 2013).

Taken together, these data indicate that miR390 contributes to coordinating multiple phytohormone signals for the regulation of cell growth and senescence by controlling the level of *AtARF2* and *AtARF3* mRNAs. The miR390-tasiRNAs-ARF module might act as a sensor of *AtARF2*, *3*, and *4* mRNA levels, in which a change in the expression of one of the three *ARFs* could affect the production and recruitment of tasiRNAs and, consequently, modulate the regulation of the other *ARFs*. Considering the potential of tasiRNAs to act non-cell autonomously, such a regulatory module may serve as a communication signal to sharpen *AtARF2*, *3*, and *4* spatial expression patterns between adjacent cells and to precisely control cell growth during organogenesis in response to different hormonal signals.

miR393 is a mediator of an ABA-to-AUX signal during stress responses

The regulatory function of miR393 has been implicated in the unidirectional control of ABA to AUX signalling. ABA treatment upregulates miR393 biosynthesis (Sunkar and Zhu, 2004) and miR393 represses the perception of the AUX signal by downregulating the expression of all four members of the *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*)/*AUXIN SIGNALLING F-BOX* (*AFB*) clade of auxin receptor (*TAAR*) genes (*AtTIR1*, *AtAFB1–3*) present in *Arabidopsis* (Fig. 3F; Table 1) (Navarro *et al.*, 2006; Si-Ammour *et al.*, 2011; Chen *et al.*, 2012a). Under drought conditions, the inhibition of lateral root development has been linked to an increase of ABA levels resulting in the miR393-dependent repression of the AUX signalling pathway through the downregulation of *AtTIR1* and *AtAFB2* (Chen *et al.*, 2012a). This mechanism is conserved in rice, where the over-expression of miR393 results in altered root growth and reduced drought tolerance by suppressing the expression of *OsTIR1* and *OsAFB2* (Bian *et al.*, 2012; Xia *et al.*, 2012). The binding of AUX to its receptor promotes the degradation of AUX/IAA proteins, which inhibit ARF activities through heterodimerization, resulting in the de-repression of ARFs that triggers the transcriptional regulation of primary AUX-responsive genes. In *Arabidopsis*, miR393 can originate from two genes (*AtMIR393a* and *b*) and is mainly synthesised from *AtMIR393b* in aerial tissues (Si-Ammour *et al.*, 2011). However, a dramatic reduction of the miR393b level in the *mir393b-1* mutants shows only mild developmental defects, suggesting a sensitive regulatory mechanism in which a low concentration of miR393 could be enough to efficiently regulate its targets (Si-Ammour *et al.*, 2011). Additionally, the miR393-mediated cleavage of *TAAR* mRNAs generates tasiRNAs that might amplify the downregulation of the *TAAR* genes (Si-Ammour *et al.*, 2011). Therefore, miR393 represents a potential strong and sensitive path by which ABA can regulate the AUX signal.

Our current knowledge of miR393 function is limited by the absence of a full knockout of its expression, but over-expression experiments in rice suggest a wider function for miR393 in various development processes such as tillering and flowering competency (Bian *et al.*, 2012; Xia *et al.*, 2012). Antibacterial resistance triggered by bacterial flagellin also involves the miR393-mediated repression of the AUX signal through the downregulation of *AtTIR1*, *AtAFB2*, and *AtAFB3* (Navarro *et al.*, 2006). Considering the known function of ABA in stress responses, the miR393-AUX antibacterial resistance pathway may be triggered by an increase of ABA level upon bacterial infection. These data suggest that ABA-miR393-AUX could constitute an important regulatory pathway involved in biotic and abiotic stress responses.

miR159 is at the crosstalk of GA, ABA, and ET pathways for the control of programmed cell death

miR159 is known to post-transcriptionally regulate *GAMYB-like* genes and function in leaf, flower, and seed maturation (Cheng *et al.*, 2004; Millar and Gubler, 2005; Tsuji *et al.*, 2006; Reyes and Chua, 2007) (Fig. 3E; Table 1). The *GAMYB* gene in barley is upregulated by the GA transduction pathway in both anthers and seeds (Gubler *et al.*, 2002; Murray *et al.*, 2003). Over-expression of miR159 or disruption of the GA biosynthesis pathway both delay flowering and reduce fertility (Achard *et al.*, 2004; Cheng *et al.*, 2004). However, the question of whether GA regulates *GAMYB* genes dependently or independently of miR159 remains unclear and most likely depends on the tissues and developmental stages observed. For instance, miR159 expression has been shown to be upregulated by GA in the *Arabidopsis ga1-3* mutant (Achard *et al.*, 2004) and unaffected by GA in the rice flower, rice aleurone tissue (Tsuji *et al.*, 2006) and the *Arabidopsis* shoot apex (Alonso-Peral *et al.*, 2010). Nonetheless, miR159 interacts with the GA pathway by affecting the spatiotemporal expression of *GAMYB* genes to control tissue outgrowth. In *Arabidopsis* and rice, the proper development of the pollen cells relies on the spatial restriction of *GAMYB-like* gene expression in the anthers by miR159 (Millar and Gubler, 2005; Tsuji *et al.*, 2006) and their induction by GA to promote the programmed cell death of the tapetum (Aya *et al.*, 2009). Similarly, in *Arabidopsis* seeds, the coordinated regulation of three *GAMYB-like* genes (*AtMYB33*/*65*/*101*) by miR159 and GA is required for the vacuolation of the aleurone cells, which is a GA-mediated programmed cell death process required for germination (Alonso-Peral *et al.*, 2010).

The seed dormancy/germination process is well known to be antagonistically regulated by both GA and ABA. Reyes and Chua (2007) showed that the over-expression of miR159-resistant versions of *AtMYB33* and *AtMYB101* increases germination sensitivity to ABA, therefore indicating that miR159 acts as a negative regulator, and that AtMYB33 and AtMYB101 act as positive regulators, of the ABA response. Interestingly, these authors also show that miR159 is upregulated by ABA treatment in young seedlings, suggesting that

ABA could induce the accumulation of miR159 in some tissues where *AtMYB33/101* expression needs to be repressed but allowing *AtMYB33/101* expression and activity in other tissues where miR159 is not present. In addition to GA, ET was also documented to promote germination and act as an antagonist of ABA (Linkies and Leubner-Metzger, 2012). In *Arabidopsis*, the relationship between miR159 and the ET pathway is not known but recent evidences in *Medicago* roots and tomato fruit showed that the accumulation level of miR159 is efficiently repressed by ET treatment (Chen *et al.*, 2012b; Zuo *et al.*, 2012). The hypothesis of an antagonistic control of miR159 expression by ET and ABA may help to explain the opposite effects of these phytohormones on seed germination.

Taken together, these data suggest that miR159, which regulates the spatiotemporal expression pattern of *GAMYB* genes, constitutes a major connection among at least three hormones—namely GA, ABA, and ET—for the control of developmental processes involving programmed cell death, such as the maturation of the tapetum in the anther and the maturation of aleurone tissue in the seed. However, further investigations to clarify the interdependence of the miR159 and GA pathways, for example by investigating the expression profile of miR159 in various tissues and developmental stages of GA-deficient mutant plants, remain to be undertaken.

Concluding remarks

The coordination of plant growth and development in response to environmental changes involves complex hormonal regulatory networks. The examples from studies outlined in this review show that miRNAs are clearly involved in this crosstalk (Figs 3 and 4).

Interestingly, most miRNAs involved in hormonal crosstalk connect the AUX pathway with the ABA and/or ET pathway(s). The evolution of land plants required an adaption to environmental changes, including seasons and stress resistance, for which communication between AUX (the major hormone controlling plant organogenesis) and ABA/ET (stress-related hormones) was essential, suggesting that miRNAs were part of the evolutionary path that led to plant stress adaptation.

The control of hormonal responses by miRNAs occurs mostly downstream of the phytohormone transduction signal by regulating the expression of early phytohormone-responsive genes, therefore affecting only part of the developmental processes regulated by the hormones. Only four miRNA families (miR167, miR393, miR319, and miR396) have been found to act upstream by regulating genes involved in phytohormone biosynthesis, transport, or perception, and consequently may play a more crucial role in controlling the hormonal response. In this manner, miR393, miR319, and miR167 may be an important gateway for ABA to influence the AUX signal.

With the growing number of studies using small RNAs and mRNA degradome libraries from different plant species, the number of hormone-regulated miRNAs and miRNA targets involved in hormone signalling continues to increase. In addition to the major plant hormones described in this review, miRNA regulations can extend to other phytohormone pathways (e.g. brassinosteroids, salicylic acid, plant peptide hormones, polyamines, nitric oxide, strigolactones, and karrikins), which have only recently begun to be studied. As research progresses, the miRNA/hormone network increases in complexity, thus requiring more detailed analyses using, for example, loss of miRNA function to unravel the genetic regulations beyond hormonal crosstalk in the spatiotemporal context of a biological process.

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