Emerging Connections between Small RNAs and Phytohormones

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Small RNAs (sRNAs), mainly including microRNAs (miRNAs) and small interfering RNAs (siRNAs), are ubiquitous in eukaryotes. sRNAs mostly negatively regulate gene expression via (post-)transcriptional gene silencing through DNA methylation, mRNA cleavage, or translation inhibition. The mechanisms of sRNA biogenesis and function in diverse biological processes, as well as the interactions between sRNAs and environmental factors, like (a)biotic stress, have been deeply explored. Phytohormones are central in the plant's response to stress, and multiple recent studies highlight an emerging role for sRNAs in the direct response to or the regulation of plant hormonal pathways. In this review, we discuss recent progress on the unraveling of cross-regulation between sRNAs and nine plant hormones.

Highlights

- An increasing number of studies identified a large variety of sRNAs responding to diverse phytohormones, and in-depth validation revealed molecular mechanisms underneath this.

- Conversely, multiple sRNAs and central proteins in sRNA pathways can regulate biosynthesis or signaling of nine phytohormones.

- Some sRNA modules interconnect with more than one hormonal pathway, thereby providing new bridges in plant hormonal cross-talk.

- In response to environmental stimuli, phytohormones enable plant adaptation and part of this reaction could be attributed to sRNAs and their targets

Small RNAs and Hormones: two Mutually Influenced Systems for Plant Growth and Stress Response

Phytohormones are important signaling molecules involved in almost all biological processes of the plant's life cycle [1-5]. To date, auxin, ethylene (Et), gibberellic acid (GA), cytokinin (Ck), abscisic acid (ABA), brassinosteroids (BR), jasmonic acid (JA), salicylic acid (SA) and strigolactones (SL) have been identified as the main plant hormones. They are synthesized via different routes and are perceived by receptor proteins, which subsequently initiate intracellular signal transduction [6]. Ultimately, the transduction reaches transcription factors (TFs) that control the downstream hormonal response. Hormones cooperate to modulate diverse processes including vascular root patterning, cell elongation, abiotic stress response, or biotic stress defense [7-12].

Other endogenous molecules also participate to all these biological processes: the sRNAs, 18-25nt in length RNAs mainly consisting of siRNAs and miRNAs (Box 1) [13]. sRNAs constitute important regulators of plant development under favorable conditions, for example, for the establisment of leaf patterning and leaf growth [14-17]. Moreover, they also participate to environmental stress responses. For example, upon virus infection, siRNAs trigger the cleavage of viral RNAs to protect the plant, while miRNAs participate by silencing the negative regulators of the plant's immune system [18,19]. sRNAs are pivotal in abiotic stress response as well. Numerous sRNAs are produced upon abiotic stress exposure and in turn regulate the expression of genes involved in stress defense [20,21].

Even though the metabolic and transduction pathways of hormones and sRNAs are very different, they participate in common biological processes and multiple recent studies highlighted interplay between hormones and sRNAs. Their connections enable plants to rapidly and efficiently adapt to environmental stresses by opting for sRNAs as intermediates to control hormone levels or, conversely, by using hormones to modulate the levels of specific sRNAs. Aiming at overviewing these new connections, we discuss the involvement of sRNAs in the regulation of biosynthesis and signaling of the nine major phytohormones, grouped based on their biological function (Table 1).

sRNAs Form Novel Hubs in Growth-Promoting Hormonal Networks

Gibberellins

GAs are crucial for developmental processes like seed germination, stem elongation and flower initiation. They are synthesized through the activity of GA-OXIDASES (GA20OX and GA3OX) and perceived by the receptor that promotes degradation of DELLA proteins, key repressors of the GA response [22]. Treating plants with GA not only affects the level of protein-coding transcripts, but also triggers the production of more than hundred miRNAs in plants [23,24] (Table 1). How this GA-mediated control of sRNA levels occurs, as well as whether sRNAs can act on GA biosynthesis and signaling, has attracted researchers' attention.

Because they are central factors in GA response, DELLAs were proposed to mediate the regulation of several sRNAs via interaction with protein partners. A first example is the os-miR396 of rice (*Oryza sativa*) that is directly induced by INDETERMINATE DOMAIN2 (IDD2). IDD2 interacts directly with the rice DELLA protein SLENDER RICE1, and the induction of os-miR396 is disabled upon GA treatment, when DELLAs are absent, strongly suggesting that DELLAs are necessary for the regulation of this sRNA (Figure 1). The induction of os-miR396 further reduces the expression of its targets, the *GROWTH-REGULATING FACTORs* (*GRFs*) transcription factors involved in growth promotion. Consistently, os-miR396 overexpression results in dwarfism that resembles GA-deficient plants [25]. Secondly, DELLAs can also function through the degradation of PIF4 that regulates miRNA levels via binding to the *MIR* promoter or via destabilizing the miRNA-processing complex [26,27]. As such, the *MIR172a* overaccumulates in the *pif4* arabidopsis mutant (*Arabidopsis thaliana*), thus, PIF4 is a negative regulator of ath-miR172 [27] (Figure 1). In contrast, its homolog *MIR172b* is

repressed by the DELLA protein, pointing towards a regulatory mechanism different from *MIR172a*. This inhibition of *MIR172b*, which causes flowering delay, might be indirect via the regulation of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) (Figure 1). DELLAs directly bind and thereby inhibit the activity of SPLs, transcription factors that positively regulate ath-miR172 and that are, in turn, targeted by ath-miR156 [28]. Finally, a third possible DELLA-regulated sRNA is ath-miR171, a sRNA targeting the transcription factor SCARECROW-LIKE27 (SCL27), which elevates ath-miR171 levels via a negative feedback loop [29] (Figure 1). ath-miR171 abundance is decreased in *pif4* mutants [27], and an independent study shows that DELLA interferes with the activity of SCL27 in chlorophyll biosynthesis. Thus, by regulating SCL27 activity, DELLA might negatively affect the ath-miR171 [29].

In turn, GA-biosynthesis genes can be positively affected by sRNAs indirectly, and sl-miR171 is one of them. Similarly to the module in arabidopsis, an SCL6-ortholog in tomato (*Solanum lycopersicum*), *SlGRAS24*, is targeted by sl-miR171 (Figure 1). *SlGRAS24* expression is quickly elevated by GA, leading to a reduction in the expression of *SlGA20OX1* and *SlGA3OX1*, which results in a GA-deficiency phenotype [30]. In contrast, one sRNA is reported to negatively regulate GA synthesis. The wheat (*Triticum*)-specific tri-miR9678 triggers a delay in seed germination by decreasing the expression of GA biosynthesis genes [31].

Finally, GA signaling can be regulated by a negative feedback loop involving one sRNA in particular: ath-miR159, whose expression is induced by GA in arabidopsis [32]. In turn, ath-miR159 targets *GAMYB* or *GAMYB-like* transcription factors involved in GA signaling, affecting also their downstream targets. For example, *LEAFY*, a potential target of MYB33 that is induced by GA, is negatively regulated by ath-miR159 (Figure 1). LEAFY stimulates transition to flowering and, consequently, overexpression of ath-miR159 in the Landsberg arabidopsis accession delays flowering in short-day conditions [33]. In rice, os-miR159 can also affect GA biosynthesis. Although os-miR159 level is not altered by GA treatment, os-miR159 has a positive effect on GA biosynthesis by cleaving its target *OsGAMYBL2*, a negative regulator of GA biosynthesis [34]. Taken together, the miR159-GAMYB(L)s module seems to constitute a key modulator of GA response, also affecting GA biosynthesis in some species.

In conclusion, the phytohormone GA can alter the level of multiple sRNAs. This might occur in part via DELLA proteins and their interactors, such as IDD2, PIF4 or SCL. To explore the extent of DELLA-mediated sRNA regulation, a comparison of the miRNA content of DELLA gain-of-function plants with that of *della* mutants could be helpful. In turn, sRNAs are able to directly regulate GA biosynthesis and signaling via miR156-SPL, miR171-SCL and miR159-GAMYB(L)s modules, respectively.

Brassinosteroids

BRs are steroid hormones mainly involved in plant growth, vascular differentiation and stomatal development [35]. In arabidopsis, miRNAs from 48 known families and 23 unknown miRNAs are differentially expressed upon BR treatment [36] (Table 1). While the role of these BR-induced sRNAs has not been studied in arabidopsis yet, recent research performed in rice has shown that, conversely, sRNAs influence BR synthesis and signaling.

sRNAs can directly target transcripts of BR biosynthesis and signaling genes for cleavage. As such, OsDCL3a (Box 1) produces 24-nt siRNAs from transposable elements, resulting in the downregulation of the BR-biosynthesis gene *OsBR6ox*, and reduced BR levels [37] (Figure 1). Moreover, the os-miR1848 silences *OsCYP51G3* transcripts, encoding a cytochrome P enzyme that mediates BR biosynthesis. Consequently, overexpression of this miRNA causes BR deficiency under salt stress condition [38]. Also in rice, a line overexpressing os-miR397 shows higher grain yield and hypersensitivity to BR. This might be attributed to the cleavage of the target gene *OsLAC*, encoding a laccase-like protein involved in BR-related gene expression [39]. In contrast, os-miR444 induces BR-biosynthetic genes by silencing their transcriptional repressor *OsMADS57* and, thereby, promotes BR-mediated inhibition of root elongation [40] (Figure 1).

Remarkably, two sRNAs bridge BR with GA, contributing to the control of rice architecture and grain yield. Upon BR treatment, the level of os-miR159 rapidly decreases, leading to accumulation of OsGAMYBL2, which stabilizes the ortholog of the BRASSINOSTEROID-INSENSITIVE2 kinase. Interestinlgy, this also leads to decreased expression of the positive BR response regulator *BRASSINOSTEROID UPREGULATED1* and to inhibition of GA biosynthesis [34] (Figure 1). Moreover, GA biosynthesis can also be inhibited by BR via another sRNA-mediated pathway. The

BR-responsive TF BRASSINAZOLE-RESISTANT (OsBZR1) directly promotes the expression of *OsMIR396d*, which results in silencing of *OsGRF6* and reduced expression of GA-biosynthesis genes *OsGA20OX* and *OsGA3OX* [41] (Figure 1).

Altogether, evidence obtained from studies in rice suggest that BR biosynthesis and signaling can be controlled via siRNA- and miRNA-mediated mechanisms. However, the link between sRNAs and BR, as well as the involvement of sRNAs in GA-BR crosstalk in other species still need further investigation and genetic validation to understand the involvement of the different modules in these interactions.

Auxin

Auxin (INDOLE-3-ACETIC ACID, IAA) is another pivotal hormone that contributes mainly to root patterning and leaf morphology. It can be synthesized via flavin-containing monooxygenases (YUCCA, YUC) and transported via auxin influx and efflux carriers. Auxin signaling is then initiated by the co-receptors TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), which interacts with the Aux/IAA transcriptional repressors to promote their degradation, releasing AUXIN RESPONSE FACTORs (ARFs) [42]. Interestingly, the miRNA biogenesis mutant *hyl1* (Box 1) exhibits reduced sensitivity to exogenous auxin and the *ago1* mutant shows less IAA accumulation in roots [43,44]. These findings indicate that sRNAs and regulation of auxin are tightly linked and raise the questions of whether sRNAs affect auxin biosynthesis or transduction, which sRNAs are involved, and how plants integrate these two signals to precisely orchestrate plant morphology (Figure 1).

While the lower IAA level in *ago1* mutants suggest that sRNAs are positively involved in auxin biosynthesis [43], few sRNAs are robust candidates for this regulation. One notable exception is ath-miR10515, which stimulates IAA production by downregulating its target gene *SUPERROOT1* (*SUR1*) that encodes an enzyme antagonizing IAA production (Figure 1). Consequently, IAA-responsive genes are highly expressed in the ath-miR10515 overexpression line and reduced in ath-miR10515 mutant [45]. On the contrary, several miRNAs decrease auxin levels. As such, *miR165/166* silences *REVOLUTA* (*REV*) transcripts, which encode a direct positive regulator of *YUC5* [46] (Figure 1). Similarly, in rice, attenuating *OsMIR396d* or *OsMIR1432* levels causes an increase of auxin-biosynthesis gene expression or of

auxin transport, resulting in plants performing better in terms of grain filling and yield [41,47]. Finally, *IAA-Ala RESISTANT3* (*IAR3*), encoding an enzyme that releases bioactive auxin, is targetted by the ath-miR167, a miRNA that mainly targets ARFs [48] (Figure 1). As *IAR3* is induced by high osmotic stress and contributes to drought tolerance, whereas ath-miR167 is downregulated under stress, this module could participate in fine-tuning auxin levels under water-limiting conditions. Besides these miRNAs, also siRNAs can negatively affect auxin biosynthesis. *YUC2* expression is controlled by thermo-regulated heterochromatic siRNAs (hc-siRNAs) (Figure 1). With increasing temperature, the level of hc-siRNAs able to bind to the *YUC2* promoter is reduced, leading to the elevation of *YUC2* expression. This hc-siRNAs–YUC2 pattern is consistent with previous observations showing that auxin accumulates upon higher temperature [49].

The reduced root elongation in *hyl1* in response to IAA treatment indicates that sRNAs could also be involved in auxin response and, indeed, several sRNAs directly target almost all auxin signaling members, from the receptor to the transcriptional regulators. For example, ath-miR393 and secondary ath-siRNAs promote *TIR1/AFB* transcript degradation [50], while the Aux/IAA IAA28 transcripts are recognized by ath-miR847 [51] (Figure 1). However, these miRNAs are not affected in the *hyl1* mutant, so they cannot explain *hyl1*'s auxin-hyposensitive phenotype. More downstream, multiple ARFs are targeted by sRNAs forming a crucial network in plant development [52-55]. A well-documented example is the ath-miR165/166-ARF3/4 module in the establishment of adaxial-abaxial polarity of leaves. The ath-miR165/166 targets *PHABULOSA* (*PHB*), a direct activator of ARF5, which in turn triggers the expression of *MIR390* [56,57] (Figure 1). Increased miR390 levels directly cause accumulation of tasi-RNAs of ARF3/4 (tasi-ARF3/4) [58]. The opposite movement of both sRNAs, ath-miR165/166 and ath-tasiARF3/4, along the adaxial/abaxial axis of the leaf, alters the distribution of ARF3/4 and PHB/REV, creating robust developmental boundaries for maintaining a flat leaf architecture [59].

Overall, these examples demonstrate a large involvement of sRNAs at multiple levels of auxin biosynthesis and signaling (Table 1), explaining why compromising sRNA biogenesis can alter auxin sensitivity. However, the available literature currently does not explain why plants lacking miRNAs (*hyl1*) are auxin-hyposensitive, as the overall effects of sRNAs on the auxin pathways are negative. Another exciting question is how

sRNA spatial dynamics might help to establish the specific auxin distribution, a point that has greatly contributed to our understanding of leaf polarity, but that has not been explored in roots yet. Finally, with the exception of the ARF5-*miR390* module, our knowledge on if and how auxin or ARFs control the expression of sRNAs is still rather limited.

Cytokinin

CKs were discovered because of their pivotal role in cell division. CKs are synthesized via the adenylate-isopentenyltransferase (IPT), activated by the enzyme LONELY GUY (LOG), and perceived by receptors. This results in the activation of the transcriptional regulators, B-Type ARABIDOPSIS RESPONSE REGULATORS (ARRs), thereby triggering the induction of CK-responsive genes [60]. In addition to the reduced sensitivity to auxin treatment, *hyl1* exhibits hyposensitivity to CK, raising the question of how sRNAs contribute to the control of CK response [44].

sRNAs seem to affect CK biosynthesis and, to a lesser extent, CK signaling. For example, st-miR156 increases CK levels by indirectly inducing *LOG1* in potato (*Solanum tuberosum*), which results in more pronounced CK-induced branching [61] (Figure 1). Oppositely, ath-miR159 and ath-miR319 suppress the expression of *SHOOTMERISTEMLESS* and *BREVIPEDICELLUS*, which enhances *IPT* expression and promotes CK biosynthesis in the shoot apical meristem [62,63]. Additionally, in tomato, the sl-miR208 directly silences *IPT2*, causing a reduction in CK level and an early leaf senescence phenotype [64]. Besides miRNAs, also siRNAs contribute to the regulation of CK biosynthesis. In petunia (*Petunia hybrida*), anti-sense transcription of the *Sho* (*PhIPT*) locus generates natural cis-antisense siRNAs (nat-siRNAs). They target *Sho* sense transcripts, encoding an enzyme responsible for CK biosynthesis [65] (Figure 1). At CK transduction level, the ath-miR156-target SPL9 inhibits B-type ARRs, hence, the ath-miR156-SPL9 module regulates CK-related shoot regenerative capacity [66] (Figure 1).

Notably, sRNAs also affect CK biosynthesis and transduction via auxin. This is particularly interesting as sRNA biogenesis mutants are hyposensitive to both hormones, suggesting that sRNAs are involved in maintaining the CK/auxin balance. For example, the above-described ath-miR165 target, PHB, not only promotes auxin response but also CK accumulation via activation of *IPT7*. In turn, ARR1 prevents *PHB*

and *MIR165a* expression for the inhibition of root growth [67] (Figure 1). Another example is the ath-miR160-target ARF10 that promotes callus formation through direct repression of a negative regulator of CK response [68]. Supporting this, in soybean (*Glycine max*), gm-miR160-overexpression inhibits CK-related nodule development [69]. These examples suggest that miRNAs participate in the CK/auxin crosstalk.

Conversely, very few studies explored how CK affects sRNAs. In the context of nodule initiation, CKs trigger the induction of *NODULATION SIGNALING PATHWAY2* (NSP2) genes, that are responsible for nodule formation. In parallel, however, CKs also stimulate the medicago (*Medicago truncatula*) miR171h, which is capable of NSP2 silencing. Therefore, this miR171h-NSP2 module forms a balance mechanism in the control of nodule initiation [70,71].

In conclusion, several studies pointed out a direct effect of sRNAs on CK at the level of CK biosynthesis in multiple species (Table 1). In most known cases, this occurs through sRNA-mediated control of *IPT* genes, encoding rate-limiting enzymes in CK biosynthesis. sRNAs also have a role in modulating the auxin/CK balance in different tissues, with the ath-miR165 emerging as an additional player in the auxin/CK homeostasis in root growth regulation. Whether other sRNAs affect this balance in other tissues or organs forms an exciting question for future research. Answering this might contribute to a better understanding of why sRNA mutants have altered sensitivity to both hormones.

Strigolactones

Unlike other phytohormones, strigolactones (SLs), involved in shoot branching, were discovered relatively recently. In rice, SLs are perceived by the α/β-hydrolase DWARF14 (D14), which stimulates the degradation of D53, causing the induction of SL-responsive genes [72]. Although not many connections between SLs and sRNAs have been elucidated yet, the expression of key proteins in SL signaling is partly controlled by sRNAs (Table 1).

In rice, the SL suppressor D53 interacts with the os-miR156-target SPL14 and suppresses its transcriptional activity, while SPL14 positively regulates *D53* (Figure 1). Consequently, os-miR156-overexpressing plants have more branches and are SL insensitive [73,74]. Furthermore, the arabidopsis D53-orthologs, *SMXL4/5*, act

as templates for RDR6-DCL2-dependent siRNAs biogenesis (Box 1). Interestingly, DCL4, the homolog of DCL2, plays a negative role in the generation of these *SMXL4/5*-derived ath-siRNAs (Figure 1). Hence, these siRNAs accumulate in the *dcl4* mutant, leading to the silencing of *SMXL4/5* and the phenocopy of the *smxl4smxl5* double mutant [75].

Although our current knowledge about how sRNAs and strigolactones interplay is still very limited, the miR156-SPL module sheds light on a new level of post-transcriptional regulation of SLs. When further uncovering the SL pathways, it will be interesting to consider the potential regulatory role of these miRNAs and siRNAs, as they can affect key transcripts, such as those of *SMXL4/5*.

sRNAs Coordinate Salicylic and Jasmonic Acid in Response to Biotic Stress

When plants are infected by pathogens, the PATTERN RECOGNITION RECEPTORS detect damage-associated molecular patterns and stimulate plant immunity [76]. In this context, SA, JA, and sRNAs are induced to counteract the pathogen invasion [8,9,19]. Interestingly, *hyl1* mutants show over-activated JA signaling, and *HYL1*-overexpressing plants are more susceptible to *Botrytis cinerea* infection [77]. Moreover, knocking-out RNA Pol V subunits (Box 1) leads to reduced induction of JA-resposive genes and more pronounced induction of SA-responsive genes upon infection with the bacterial pathogen *Plectospharella cucumerina* [78,79]. These observations raise the question of whether JA, SA and sRNAs are connected in the defense reponse towards pathogens, besides their well-known role in silencing of viral RNA (Box 2).

Upon pathogen infection, SA, a phenolic compound crucial for plant pathogen defense, accumulates and is perceived by the receptor NONEXPRESSER OF PR-GENES (NPR), activating transcription factors called TGACGTCA CIS-ELEMENT-BINDING PROTEIN (TGA) and WRKY DNA-BINDING PROTEIN (WRKY) [80]. Interestingly, the promoters of *AGO*, *DCL* and *RDR* genes contain predicted binding sites for TGAs and WRKYs. Accordingly, SA application triggers changes in *AGO1* and *DCL2/3/4* transcript levels, suggesting SA can affect sRNAs in a wider context than upon virus invasion, although the biological consequences of these expression changes are unclear [81]. Conversely, increasing evidence suggests that SA levels and signaling can be affected by endogenous sRNAs and that pathogens can misuse this system. In tomato, sl-miR396a, which targets *GRF1* transcripts to reduce *TGA1/2* and

PATHOGEN-RELATED1 (*PR*) transcript levels, is repressed upon fungal infection (Figure 2). Consequently, sl-miR396-overexpressing tomato plants are more susceptible to *Phytophthora infestans* and *Botrytis cinerea* infection, even though they show a higher SA concentration and *NPR* expression [82].

Also related to biotic stress, JA is responsible for the wounding response and antagonizing insect attacks. JA biosynthesis occurs by the conversion of unsaturated fatty acids into JA via diverse enzymes, such as lipoxygenase (LOX). Triggered by JA, the CORONATINE INSENSITIVE1-JASMONATE-ZIM-DOMAIN PROTEIN (COI1-JAZ) co-receptor complex is activated and elicits degradation of the suppressor JAZ, allowing transcription factors (i.e. MYC2) to promote JA-responsive genes [83]. Main regulators of sRNAs function, like AGO1 and HSP70/90, are emerging as positive regulators of the JA signaling pathway; AGO1 incorporates sRNAs derived from JA-responsive genes, like JAZ, MYC2 or LOX2 and promotes these JA-responsive genes' expression in response to JA application [84] (Figure 2). Moreover, HSP70/90 can stabilize COI1 to stimulate the JA response [85]. In turn, two JA-induced sRNAs, miR319 and ath-miR156, provide feedback regulation in the JA pathway. First, miR319 targets *TCP4* in arabidopsis and tomato, and *TCP21* in rice, thereby inhibiting *LOX* in response to biotic stress, which alters the sensitivity to diverse pathogens [86-88]. More downstream, ath-miR156 targets the gene encoding SPL9, which physically interacts with JAZ3 and promotes JAZ3 stability, resulting in attenuated insect resistance [89] (Figure 2).

Because these two hormones participate in the plant's immunity, SA and JA are influenced by RNA silencing suppressor (RSS) encoded by viruses for antagonizing virus-activated siRNA pathways (Box 2). For example, *Cauliflower mosaic virus* (*CaMV*) P6 protein suppresses SA accumulation by activation of TARGET OF RAPAMYCIN, which downregulates the expression of *NPR1* and *WRKY45* [90,91] (Figure 2). An alternative strategy is used by the *Turnip mosaic virus*, where RSS Hc-Pro physically interacts with a homolog of SA–BINDING PROTEIN and represses the SA-mediated immune response [92]. On the contrary, *Potato virus A* Hc-Pro and *Geminiviridae* AC2, another RSS, promote expression of JA biosynthesis- and JA-related genes, like *LOX* and *VSP1* [93,94], while several other RSSs were reported to hinder the JA response [95,96].

In conclusion, it is clear that several recent evidences suggest a role for sRNAs, both from endogenous and viral origin, in the fine-tuning of JA and SA levels and response during biotic stress defense (Table 1). However, except for Pol V subunits, no other endogenous sRNA regulators are known to affect both JA as well as SA, but this would be an interesting question for future studies. On the other hand, only few studies reported the effect of SA/JA on sRNA levels, which raises the question of whether these hormones mainly act downstream of sRNAs. This is most likely not the case, as recent sRNAseq data in arabidopsis revealed that 87 ath-miRNAs and 4 ath-tasiRNAs show differential expression upon JA treatment [97]. Further characterizing the functional importance of these sRNAs in plant immunity is an exciting area for future research, both from an academic and a more applied point of view, as engineering these sRNAs could contribute to increased resistance to plant pathogens.

sRNAs and Abiotic Stress-Related Hormones Abscisic Acid and Ethylene

When plants encounter abiotic stress such as drought, ABA is synthesized to facilitate plant adaptation. Activated by ABA, the kinase SNF1-RELATED PROTEIN KINASE2 (SnRK2) phosphorylates the ABA-RESPONSIVE ELEMENTS-BINDING FACTORs (AREB/ABFs) TFs to promote transcription of ABA-responsive genes [98]. Similarly, osmotic stress also promotes the production of ethylene, which activates or stabilizes ETHYLENE INSENSITIVE (EIN) proteins of the ethylene signaling pathway. Two downstream TFs, EIN3 and EIN3-LIKE1 (EIL1) further induce numerous ETHYLENE RESPONSIVE FACTORs (ERFs) [5], which promote stress-responsive genes. Although the roles of ABA and ethylene in abiotic stress response are extensively studied, sheding light on the ABA/Et-controlled gene expression changes, it is unclear how and why ABA/Et also alter the levels of sRNAs in multiple species [99-107]. Excitingly, overexpression of some of these ABA-responsive sRNAs, like ath-miR168/393/394, renders arabidopsis more resistant to drought or salinity, suggesting that miRNAs are involved in the ABA-mediated drought response [108-110].

More than the other hormones discussed so far, ABA was reported to promote miRNA biogenesis through different pathways in arabidopsis (Figure 3). First, ABF transcription factors directly bind to the promoter of *MIR168A* for the induction of ath-miR168, the main miRNA targetting *AGO1* transcript for degradation. Conversely, mutating AGO1 or overexpressing ath-miR168 results in ABA hypersensitivity and enhanced drought tolerance, suggesting that the miR168-AGO1 module is involved in

the ABA-dependent drought resistance [110]. Secondly, ABA stabilizes the CBP20/80 complex required for the stability of pre-miRNA transcripts (Box 1) [111]. This is, for example, the case for ath-miR159, which targets *MYB33/101*, encoding proteins required for ABA-mediated inhibition of seed germination (Figure 3). Accordingly, *cbp80* is hypersensitive to ABA, salt and osmotic stress during seed germination, which could in part be attributed to this ath-miR159-MYB33/101 module [112]. Moreover, SE and HYL1 are phosphorylated by SnRK2, which promotes HYL1 abundance. Although the SE protein level is not altered, it was suggested that this phosphorylation might affect SE in its interaction with other factors [113]. Among the ABA-responsive miRNAs, ath-miR842/846 forms another noteworthy example. Both miRNAs arise from the same functional isoform, with and without intron, respectively. ABA accumulation causes alternative splicing, resulting in accumulation of yet another isoform, thereby reducing ath-miR842/846 [114]. However, the biological impact of this splicing-mediated sRNA control, is still unclear.

Besides ABA, ethylene also acts on CBP20 by promoting its phosphorylation, possibly re-inforcing its activity, resulting in upregulation of ath-miR319 and downregulation of its target *MYB33,* but not *TCP2/4* in root (Figure 3). The *cbp20* mutant is less sensitive to ethylene, while the *MIR319b* overexpression line shows hypersensitivity to this hormone [115]. In turn, ethylene biosynthesis and salt tolerance genes are altered in pv-miR319-overexpressing switchgrass (*Panicum virgatum*) upon ACC treatment, enabling enhanced salt-tolerance[116]. While no research reported direct induction of *MIR* transcription by ERFs, in petunia (*Petunia hybrida*), PhERF2 promotes *RDR2/6*, *DCL2* and *AGO1* expression to regulate siRNAs biogenesis and induce RNA silencing in response to viral infection [117] (Figure 3). Finally, ethylene and siRNAs are directly connected by *EIN5*, member of ethylene signaling pathway encoding a 5'-3' exoribonuclease necessary to enable aberrant transcript degradation. In *ein5* mutants, aberrant transcripts accumulate and generate siRNAs which induce post-transcriptional gene silencing (PTGS) [118,119].

ABA/Et biosynthesis or response are also regulated by miRNAs (Table 1). For example, in the case of the gh-miR157–GhSPL10 module, overexpression of *GhSPL10* increases ethylene contents, promotes *ERF1/2* expression, and stimulates callus iniation in cotton (*Gossypium hirsutum*) [120]. Moreover, ethylene-mediated leaf senescence was reported to occur via EIN3-triggered repression of ath-miR164, which

targets *ORESARA/NAC2*, encoding a TF crucial for leaf senescence induction [121] (Figure 3). Finally, the kinase CONSTITUTIVE TRIPLE RESPONS4 (SlCTR4) of tomato can be silenced by sl-miR1917. As SlCTR4 acts as a negative regulator of ethylene signaling, this silencing stimulates early fruit ripening [122]. On the ABA side, ABA induces ath-miR399f that targets *ABF3*, itself encoding a positive regulator of the ABA response, thereby creating a feedback loop [123] (Figure 3). Consistently, ath-miR399f overexpressing plants show a reduced sensitivity to ABA but decreased survival rate upon severe drought. Finally, ath-miR165/166 represses ABA response by targeting the TF *ABA INSENSITIVE4* (*ABI4*) and also indirectly represses *β-1,3-GLUCANASE1* (*BG1*), an enzyme mediating ABA production. Therefore, miR165/166-defective mutant exhibits ABA- and drought hypersensitivity [124].

Overall, ABA/Et-regulated common phenotypic traits such as seed germination or leaf senescence can, at least in part, be attributed to sRNAs that affect the ABA/Et level and response [111,121]. Conversely, ABA/Et pathways also control sRNA levels by regulating core sRNAs biogenesis proteins, particularly CBP20, whose function is promoted by these hormones. This protein appears to have an important role in the ABA/Et response, but the precise mechanisms by which ABA/Et affect its phosphorylation and stability, are still unknown. Besides unraveling these molecular regulations, it would be interesting to investigate whether ABA/Et act on the same or distinct CBP20 phosphorylation site, by regulating a common or a specific kinase, respectively.

sRNAs Act as Crosstalk-Mediating Agents during Hormonal Communication

Mutants in core sRNA regulators display hyper- or hyposensitivity to a range of hormones, as discussed earlier for the *hyl1* mutant [21,37,44,75,77] (Box 1, Figure 4A). In addition, several miRNAs can be regulated by genes from more than one hormonal pathway (Figure 4B), and the same holds true for general sRNA-regulatory proteins like CPB20 that is regulated by the two abiotic stress hormones [111,112,115]. This suggests that sRNAs can act as hubs in hormonal networks, and two sRNAs in particular illustrate this.

First, miR159 levels are altered by BR, GA and ABA in different species [33,34,125]. In turn, miR159 is involved in the control of not less than 4 hormonal pathways: it promotes GA and BR biosynthesis, but, on the other hand, inhibits CK biosynthesis, and interferes with ABA in inhibition of seed germination. In the GA, BR and ABA

pathways, miR159 does so by targetting a TF of the MYB- or MYB-LIKE family [34,63,112,125,126]. Supporting a crucial role for miR159 in hormonal connections, supression of *miR159* in arabidopsis and rice causes pleiotropic effects on plant growth that may be attributed to alteration of hormone levels [127,128].

Another clear example of miRNA contributing to the hormonal crosstalk is the miR156, the major orchestrator in age phase-transition [129]. miR156 inhibits GA or SL signal transduction by declining *SPL* expression. This miR156 thereby participates in, respectively, flowering time, branching, and JA-dependent insect defense [28,74,120]. Therefore, the miR156–SPL module may act as a hub for hormones in the regulation of diverse biological processes. More interestingly, these two crosstalking sRNAs also interact with each other, as deficiency in miR159 elevates miR156 levels, which leads to a delay of vegetative development [130]. Whether the miR159–miR156 balance is responsible for vegetative phase transition and its precise role in this process, is still elusive.

Concluding Remarks and Future Perspectives

In the emerging sRNA-hormone network, it is clear that different steps of hormone biosynthesis can be affected by endogenous sRNAs, which is especially the case for GA, auxin, CK, and JA. By contrast, at the signal transduction level, the role of sRNAs seems to be more complex since either the sRNA regulators interplay with hormone responses or sRNA target genes belong to or participate in hormonal signaling. Conversely, hormones shape plant phenotypic plasticity under optimal and stress conditions and part of this regulation is achieved via sRNAs, as illustrated in the GA, Et, and ABA response. High-throughput sRNA-sequencing in different species in response to hormone treatment demonstrated that large sets of sRNAs are altered (Table 1). Hormones influence sRNAs biogenesis members and hormone-responsive factors regulate miRNA precursors. On the other hand, RSS proteins are also suggested to interfere with hormones, and thus offer another crucial link between sRNAs and hormones.

Although substantial advances have been achieved to understand the crosstalk between sRNAs and hormones, more research is required to elucidate several remaining questions (see Outstanding Questions). For example, some miRNAs and their targets, such as miR156-SPL and miR159-GAMYB, are evolutionarily conserved among species. Remarkably, the hormonal effect on these conserved

modules can be different between species, as illustrated by the promotion of miR159 in arabidopsis but not in rice. Therefore, it would be exciting to unravel the evolutionary basis underneath the hormonal, or even environmental, responses of miRNAs. On the other hand, newly identifed miRNAs like os-miR444 and ath-miR842 were currently only described in one species. It would be interesting to identify the orthologous miRNAs based on these novel miRNAs' features. Additionally, small non-coding transfer-RNA derived fragments (tRFs) are emerging actors in the sRNA-hormone network: some are known to be stress- or ABA-responsive [131] or to modify auxin-regulatory enzymes [132], but what is the exact relationship between hormones and tRFs? Even regarding the above-described miRNAs and siRNAs, their precise mode-of-action in hormonal regulation is not completely clear. Finally, hormones can undergo long-distance transfer. Interestingly, sRNAs are able to transfer between cells, organisms, and species, for which they could be considered as "RNA hormones" [133,134]. Whether hormones and sRNAs would meet and regulate mutually in these crossroads forms another fascinating path for future research.

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Outstanding Questions

- What are the more precise molecular mechanisms underlying hormone-mediated regulation of sRNA?
- How do SLs and recently identified tRFs integrate in the sRNAs–hormone network?
- Regarding the mobility of sRNAs and hormones in plants, are hormone-related proteins participating in the regulation of sRNA trafficking, thereby indirectly influencing gene silencing?

FIGURES

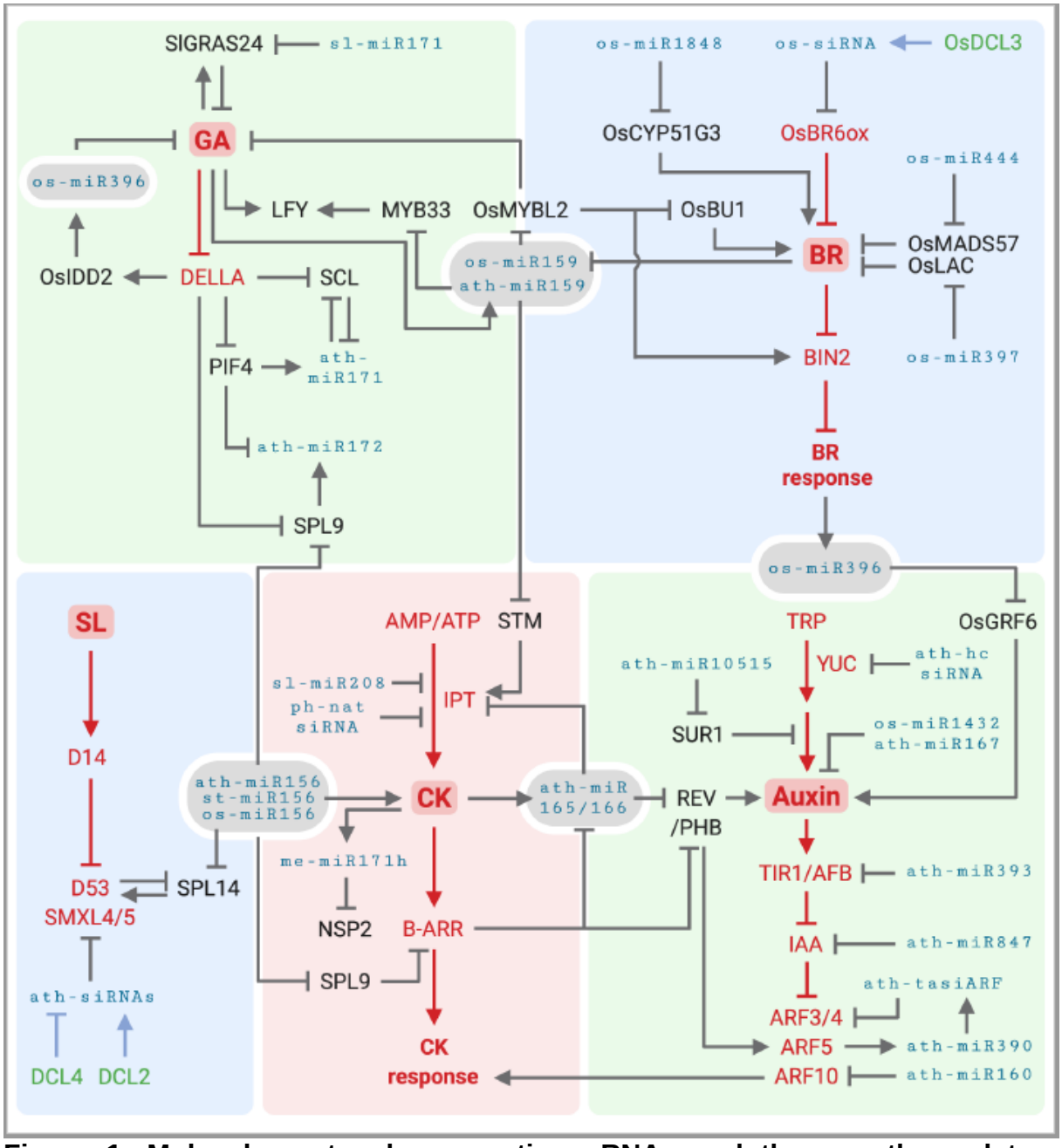

Figure 1. Molecular network connecting sRNAs and the growth-regulatory hormones. During plant development, gibberellic acid (GA), auxin, cytokinins (CK), brassinosteroids (BR) and strigolactones (SL) control multiple aspects of plant growth, particularly cell division and differentiation. Hundreds of sRNAs are responsive to these hormones (Table 1), and multiple miRNAs (blue) participate in the fine-tuning of hormone biosynthesis or signaling (red). Because some miRNAs are responsive to a subset of hormones and in turn regulate other hormones, they could form new connections in hormonal networks (grey modules). Abbreviations: GROWTH-REGULATING FACTOR (GRF), SCARECROW-LIKE27 (SCL27), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), INDETERMINATE DOMAIN2 (IDD2), PHYTOCHROME INTERACTING FACTOR 4 (PIF4), LEAFY (LFY), DICER-LIKE (DCL), BRASSINOSTEROID-INSENSITIVE2 (BIN2), LACCASE (LAC), BRASSINOSTEROID UPREGULATED1, BRASSINOSTEROID-6-OXIDASE (BR6ox),

AUXIN RESPONSE FACTOR (ARF), YUCCA (YUC), SUPERROOT1 (SUR1), TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), INDOLE-3-ACETIC ACID INDUCIBLE (IAA), ARABIDOPSIS RESPONSE REGULATORS (ARRs), PHABULOSA/REVOLUTA (PHB/REV), SHOOTMERISTEMLESS (STM), NODULATION SIGNALING PATHWAY2 (NSP2), DWARF14 (D14), SUPPRESSOR OF MAX2 1-LIKE (SMXL), ISOPENTENYLTRANSFERASE (IPT), Tryptophan (Trp), Adenosine monophosphate/Adenosine triphosphate (AMP/ATP), *Oryza sativa* (os), *Solanum lycopersicum* (sl), *Medicago truncatula* (me), *Arabidopsis thaliana* (ath) *Solanum tuberosum* (st).

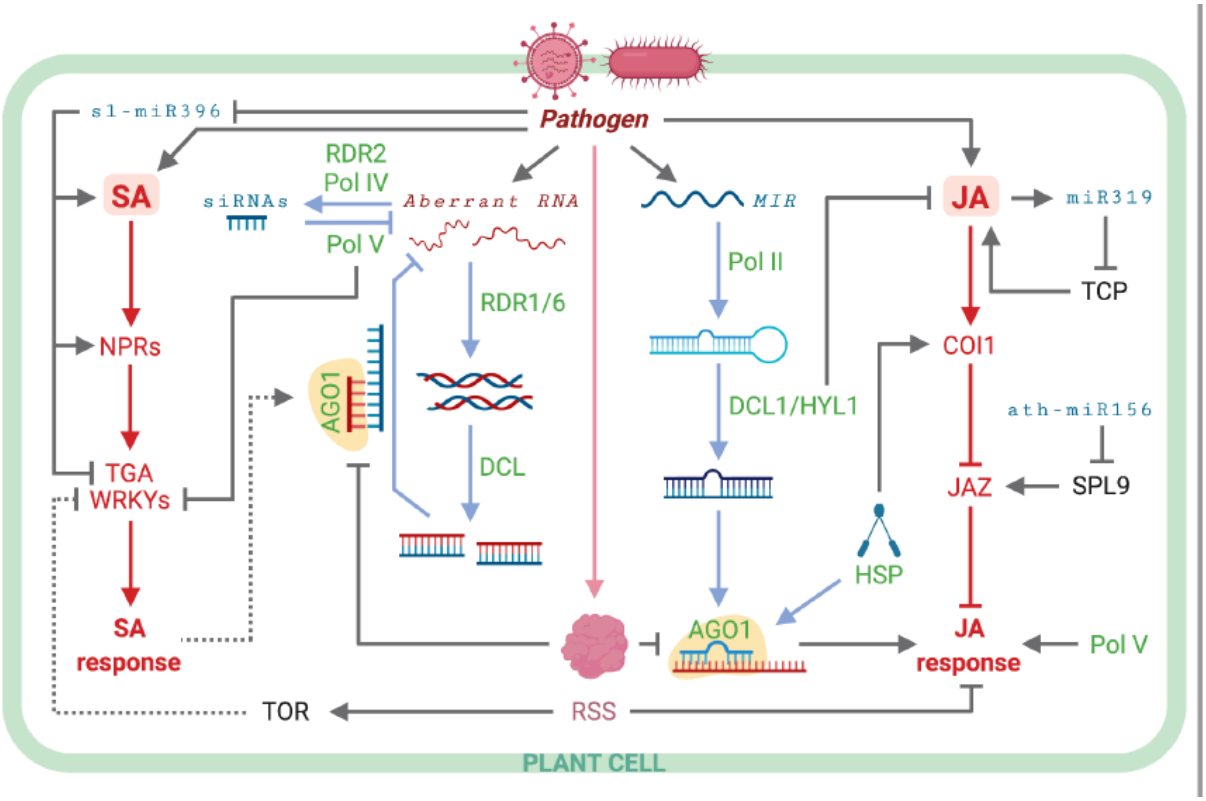

Figure 2. Regulation of the biotic stress-induced hormones salicylic acid (SA) and jasmonate (JA) by sRNAs. Upon viral or bacterial infection, plants stimulate biosynthesis of SA or JA depending on the pathogen. Key proteins of sRNA biogenesis or function (green) can regulate SA- or JA-biosynthesis genes and downstream transcription factors (red), a mechanism that is hijacked by pathogens. Additionally, virusses produce RNA silencing suppressor (RSS) proteins that repress the plant's ARGONAUTE 1 (AGO1) proteins, central players in post-transcriptional regulation of defense genes. Finally, sRNA species (blue) act directly on SA- or JA-biosynthesis or signaling genes, by either promoting or inhibiting it. Abbreviations: RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), NONEXPRESSER OF PR-GENES (NPR), TGACGTCA CIS-ELEMENT-BINDING PROTEIN (TGA), DICER-LIKE (DCL), HYPONASTIC LEAVES1 (HYL1), CORONATINE INSENSITIVE1 (COI1), JASMONATE-ZIM-DOMAIN PROTEIN (JAZ), HEAT SHOCK PROTEIN (HSP), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), *Solanum lycopersicum* (sl), *Arabidopsis thaliana* (ath).

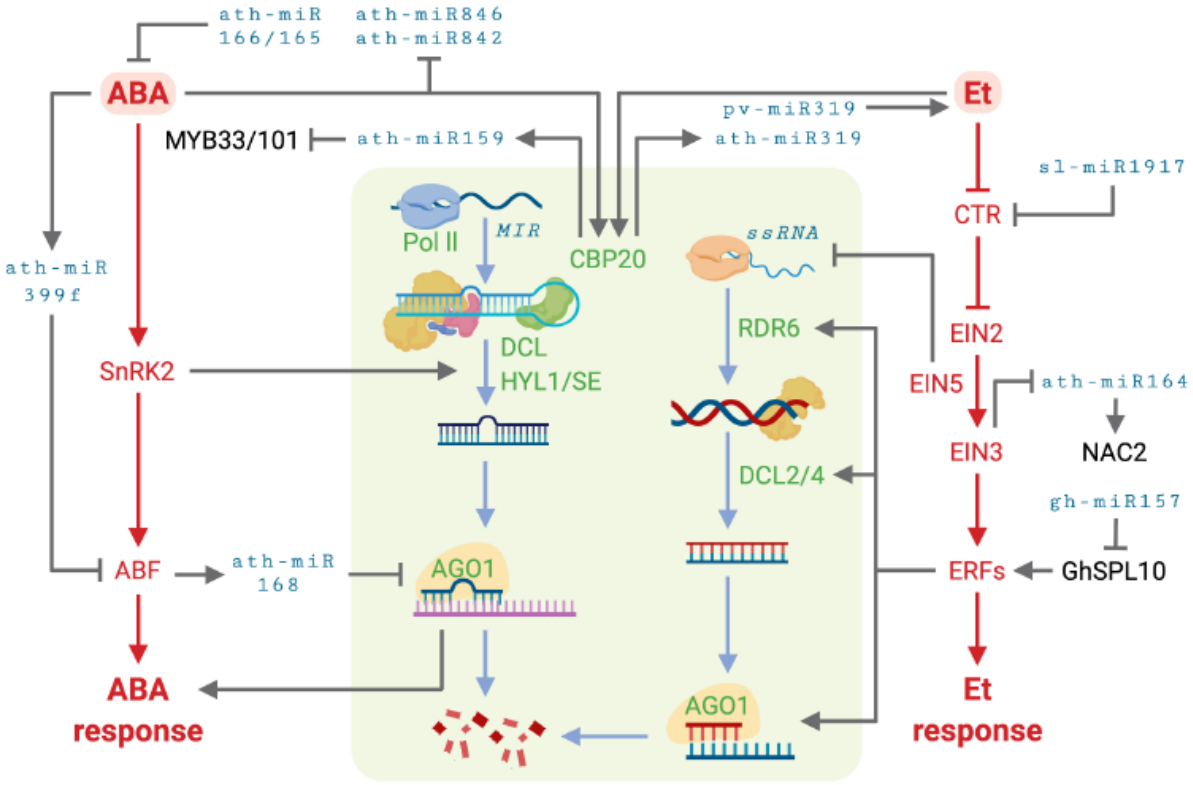

Figure 3. Interconnection of abiotic stress-responsive hormones ABA and ethylene with miRNAs. When plants experience abiotic stress, abscisic acid (ABA) and ethylene (Et) are rapidly synthesized. The biosynthesis of these hormones (red) is regulated by miRNAs (blue), themselves affected by the hormone in a feedback mechanism. Other hormone-induced miRNAs can fine-tune the downstream signaling pathway of ABA and Et (red), respectively. Finally, key effectors in sRNA biogenesis or function (green) can be controlled by ABA, Et, or their respective downstream transcription factor. Abbreviations: SNF1-RELATED PROTEIN KINASE2 (SnRK2), ETHYLENE INSENSITIVE (EIN), ETHYLENE RESPONSIVE FACTOR (ERF), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), ABRE BINDING FACTOR (ABF), DICER-LIKE (DCL), HYPONASTIC LEAVES1 (HYL1), ARGONAUTE1 (AGO1), RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), CAPING BINDING PROTEIN (CBP), *Gossypium hirsutum* (gh), *Panicum virgatum* (pv), *Arabidopsis thaliana* (ath).

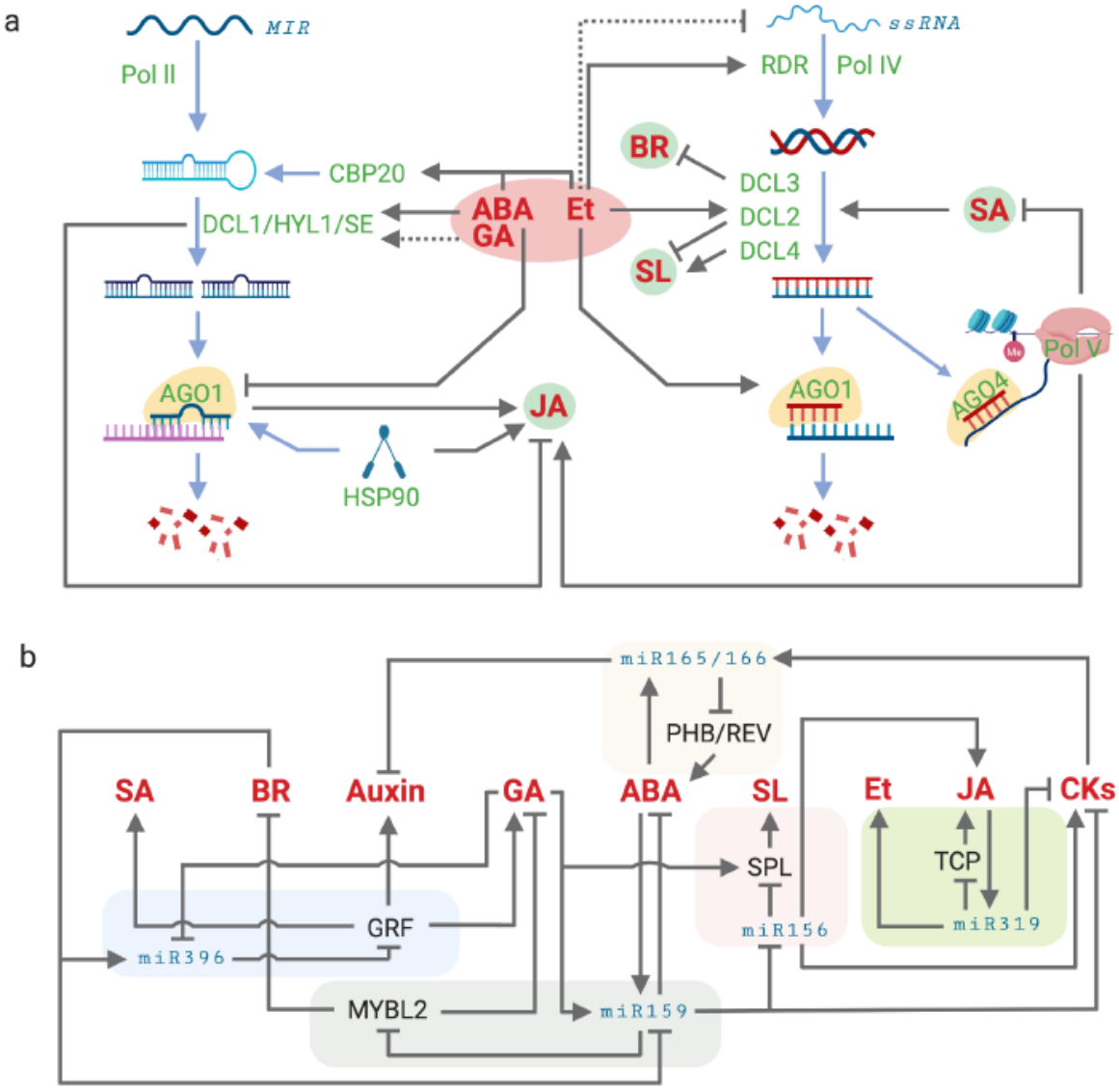

Figure 4. sRNA-modules as novel hubs in hormonal crosstalk. (A) Central proteins in sRNA biogenesis or function (green) can be induced by multiple hormones, including abscisic acid (ABA), ethylene (Et), and salicylic acid (SA). In turn, they can affect strigolactones (SL), brassinosteroids (BR) or jasmonate (JA). Their regulation by hormones and their capacity to, in turn, fine-tune other hormonal responses, places them as new possible hubs in plant hormonal crosstalks. (B) Multiple miRNA species can form new bridges in hormonal crosstalk. miR156, miR159, miR165/166, miR319, and miR396 are regulated by ABA, BR, JA, or gibberellic acid (GA) and in turn participate in the control of SA, auxin, cytokinin (CK) or SL, thereby providing miRNA-regulated connections between hormones. Abbreviations: PHABULOSA/REVOLUTA (PHB/REV), GROWTH-REGULATING FACTOR (GRF), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), DICER-LIKE (DCL), HYPONASTIC LEAVES1 (HYL1), ARGONAUTE1 (AGO1), RNA-DEPENDENT RNA POLYMERASE (RDR), RNA polymerase (Pol), CAPING BINDING PROTEIN (CBP), SERRATE (SE), HEAT SHOCK PROTEIN (HSP).

TABLES

Table 1. Overview of the important sRNA modules in hormonal responses[a]

Hormones	Genome-wide analysis	Involvement	Key miRNAs	Target	Biological process	Species	Refs.
Gibberellin (GA)	miRNAs: 79 down, 58 up (grape)	GA biosynthesis	miR396	GRF6	Plant growth	rice	[23,28-30,33,34,41]
			miR159	GAMYBL2	Development		
		GA signaling	miR159	MYB33	Flowering	tomato, arabidopsis	
			miR156	SPL	Flowering	arabidopsis	
			miR171	SCL27	Chlorophyll biosynthesis		
				GRAS24	Fruit set	tomato	
Brassinosteroids (BR)	miRNAs: 256 (arabidopsis)	BR biosynthesis	miR397	LAC	Grain filling, panicle branching	rice	[34,36-41]
			miR444	MADS57	Root growth		
			miR1848	CYP51G3	Salt response		
			BR6ox-siRNA	BR6ox	Plant height, lamina bending		
		BR signaling	miR159	GAMYBL2	Development	rice	
			miR396*	GRF6	Plant growth		
Auxin	miRNAs: 30 (cotton)	Auxin biosynthesis	miR10515	SUR1	Hypocotyl growth	arabidopsis	[41,47-51,56-59,135]
			miR165/166	REV/PHB	Leaf polarity, shade-avoidance		
			miR167	IAR3	Root architecture		
			YUC2-siRNA	YUC2	Leaf morphology		
			miR396	GRF6	Grain filling	rice	
			miR1432	ACOT	Grain filling		
		Auxin signaling	miR393	TIR1/AFB	Development	arabidopsis	
			miR847	IAA28	Lateral root initiation		
			tasi-ARF3/4	ARF3/4	Leaf polarity		
			miR390*	TAS3	Primary root meristem		
Cytokinin (CK)	miRNAs: 70 (balfour spruce)	CK biosynthesis	miR156	SPL	Tuber yield (potato), shoot regeneration (arabidopsis)	potato, arabidopsis	[61,63-65,67,68,71,136]
			miR159	MYB	Development	arabidopsis	
			miR319	TCP			
			Sho-siRNA	Sho		petunia	
			miR165/166	REV/PHB	Root patterning and growth	arabidopsis	
			miR160	ARF10	Callus formation	arabidopsis	
			miR208	IPT4	Leaf senescence	tomato	
		CK signaling	miR171h	NSP2	Nodule initiation	medicago	
Strigolactones (SL)		SL signaling	miR156	SPL14	Shoot branching	rice	[74,75]
			SMXL4/5-siRNA	SMXL4/5	Anthocyanin production	arabidopsis	
Salicylic acid (SA)		SA signaling	miR396	GRF1	Pathogen immunity response	tomato	[82]
Jasmonate (JA)	sRNAs: 57 up, 24 down (arabidopsis)	JA signaling	miR319	TCP	Pathogen immunity response	arabidopsis, tomato, rice	[87-89,97,137]
	miRNAs: 189 up, 182 down (wheat)		miR156	SPL9	Insect resistance	arabidopsis	
Abscisic acid (ABA)	miRNAs: 14 up, 16 down (poplar)	ABA signaling	miR399f	ABF3	Drought tolerance	arabidopsis	[104-107,110,111,114,123,124,138]
	miRNAs: 107 up, 28 down (rice)		miR165/166	ABI4	Drought tolerance		
	miRNAs: 26 (strawberry)		miR159	MYB33/101	Seed germination		

	miRNAs: 63 up,73 down (tomato)		miR168*	AGO1	Drought tolerance		
	miRNAs: 4 up, 29 down (knotweed)	Unknown	miR842/846	AT5G28520	Unknown		
Ethylene (Et)	sRNAs: 21(tomato)	Et signaling	miR319	MYB33	Root growth	arabidopsis	[99-102,115,116,120-122]
	miRNAs: 93 up.69 down (grape)			PCF5	Salt tolerance	switchgrass	
	miRNAs: 12 up.10 down (banana)		miR164	NAC2	Leaf senescence	arabidopsis	
	miRNAs: 8 (medicago)		miR1917	CTR4	Fruit ripen	tomato	
			miR157	SPL10	Callus proliferation	cotton	

[a] Abbreviations: GROWTH-REGULATING FACTOR (GRF), SCARECROW-LIKE27 (SCL27), SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), SUPPRESSOR OF MAX2 1-LIKE (SMXL), PHABULOSA/REVOLUTA (PHB/REV), LACCASE (LAC), BRASSINOSTEROID UPREGULATED1, BRASSINOSTEROID-6-OXIDASE (BR6ox), AUXIN RESPONSE FACTOR (ARF), YUCCA (YUC), SUPERROOT1 (SUR1), TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), INDOLE-3-ACETIC ACID INDUCIBLE (IAA), SHOOTMERISTEMLESS (STM), NODULATION SIGNALING PATHWAY2 (NSP2), ABRE BINDING FACTOR (ABF), ARGONAUTE1 (AGO1), PALLIATIVE CARE FORMULARY (PCF), ABA INSENSITIVE4 (ABI4), CONSTITUTIVE TRIPLE RESPONSE (CTR). * indicates a direct regulatory connection between the hormone-responsive transcription factors and the *MIR* gene.

Box 1. Current model for sRNA (miRNAs, siRNAs, and tRFs) biogenesis and function in plants

In plants, miRNAs originate from *MIR* genes, which are transcribed by RNA polymerase II (Pol II) into primary miRNAs (pri-miRNAs) containing a hairpin-like structure (Figure 4A). Associated with miRNA-processing-complexes, including DICER LIKE1 (DCL1), HYPONASTIC LEAVES1 (HYL1), and SERRATE (SE), the pri-miRNAs are processed into mature miRNA duplexes [21], which are then loaded into ARGONAUTE1 (AGO1) proteins in the nucleus to form AGO:miRNA complexes with the help of HEAT SHOCK PROTEIN (HSP70/HSP90) chaperones. Via the CRM1/EXPORTIN1 (EXPO1) pathway, the AGO:miRNA complex is exported to the cytosol, where it forms part of the RNA-INDUCED SILENCING COMPLEX (RISC) [139].

Unlike miRNAs, siRNAs arise from the splicing of long double-stranded RNAs (dsRNAs) by DCL2-4 proteins into 21-24nt siRNAs, which later assemble into the RISC with AGO1 in the cytosol. These dsRNAs are produced by RNA-DEPENDENT RNA POLYMERASEs (RDRs) and SUPPRESSOR OF GENE SILENCING3 (SGS3) from aberrant transcripts or exogenous sources, including viral genomes or synthetic constructs, or by RNA POLYMERASE IV (Pol IV) and RDR2 based on single-stranded RNAs (ssRNAs) [13].

Guided by 21-22nt miRNAs or 21-24nt siRNAs, the RISC binds to complementary mRNAs and cleaves them into 5’ and 3’ end fragments, which are degraded by exonucleases [13]. Alternatively, some fragments are subjected to the RDR6-mediated secondary, phased siRNA (phasiRNA) biogenesis pathway [21]. For example, tasi-RNAs, one type of phasiRNAs in plants, are derived from the miRNA-cleaved fragments of *TAS* transcripts [13]. Besides mRNA cleavage, miRNAs can also block ribosome-mediated translation initiation and elongation through binding to the UTR or ORF of target genes in the endoplasmic reticulum [21]. Similarly, in addition to target degradation, some 21-22nt siRNAs and most 24nt siRNAs, like hc-siRNAs, recruit methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) to transposable elements (TEs), transgenes or viral DNA for RdDM and transcriptional gene silencing (TGS) with the help of RNA POLYMERASE V (Pol V) [140].

Similar in size as miRNAs and siRNAs, tRFs constitute a novel class of sRNAs cleaved from mature tRNAs or as byproducts of pre-tRNA processing. Based on the cleavage

location, tRFs are categorized as tRF-5a and tRF-3a [131]. Currently, the biogenesis of tRNAs in plants is unclear. Recent research showed that the level of tRFs is strongly reduced in *rns1* mutants, which are RNase T2 knockouts, but not *dcl1* mutants, implying that RNases T2 are mainly required for tRF synthesis [141,142]. Although the function of tRFs is not widely studied yet, the interaction between tRFs and AGOs suggests that they may trigger RNA silencing similarly to miRNAs and siRNAs [143].

Box 2. siRNAs in response to pathogen attack

An important component in antiviral immunity is RNA silencing: the single/double-stranded viral RNAs are processed by the plants into 21-24nt virus-derived siRNAs (vsiRNAs) and also activate endogenous virus-activated siRNAs (vasiRNAs) that are synthesized via the RDR6-mediated secondary siRNA biogenesis pathway (Box 1, Figure 2) [118,144] [19]. Subsequently, vsiRNAs are incorporated in AGOs to target complementary viral RNA for degradation and vasiRNAs trigger host genes decay [145]. In the case of DNA viruses, vsiRNAs target viral DNA for RdDM [146]. In response, the viral genome encodes RSS proteins for RNA silencing suppression, such as P1/helper-component proteinase (P1/Hc-Pro) from *Potyvirus genus*, P0 from *Turnip yellows virus*, and P6 proteins from *Cauliflower mosaic virus (CaMV)*. These proteins intend to compromise RNA silencing by inhibiting key components of the miRNA biogenesis pathway or attenuating AGO1/4 binding ability [147,148].

GLOSSARY

AGO: ARGONAUTE family contains ten members (AGO1-10) in arabidopsis. Generally, AGO1/5/10 bind siRNAs or miRNAs and cleave mRNAs, while AGO 4/6/9 act as effectors for siRNAs-directed DNA methylation and thus induce transcriptional gene silencing.

IPT: adenosine phosphate-isopentenyltransferase, which catalyzes the rate-limiting step in cytokinin biosynthesis.

PIF4: basic helix–loop–helix transcription factor that negatively regulates photomorphogenesis and is degraded by red-light activated phyB-Pfr via physical interaction.

PRRs: PATTERN RECOGNITION RECEPTORS are receptors recognizing pathogen-associated molecular patterns (PAMPs) from pathogens or damage-associated molecular patterns (DAMPs) from plants, rapidly triggering immunity response.

PTGS: POST-TRANSCRIPTIONAL GENE SILENCING; carried out by siRNAs or miRNAs, which incorporate in AGO1 that further cleaves complementary mRNAs for RNA decay.

PHB/REV: PHABULOSA/REVOLUTA, two TFs belonging to the high auxin levels activating class III homeodomain leucine zipper (HD-ZIP III) transcription factors. They are directly targeted by miR165/166 and participate in modulation of tissue patterning.

RDR: RNA-DEPENDENT RNA POLYMERASES; the RDR family in arabidopsis consists of six proteins. Among them, RDR1/2/6 are involved in the synthesis of dsRNA molecules which are later processed to siRNAs, like vsiRNAs, hcsiRNAs, and nat-siRNAs.

RdDM: RNA-directed DNA methylation; an epigenetic process which is executed by 21-24nt siRNAs. These siRNAs loaded in AGO4-, AGO6-, or AGO9-RISC target to Pol V-dependent RNAs and then recruit DNA methyltransferases to methylate target genomic loci, resulting in transcriptional gene silencing.

RNA polymerase IV and V: both are evolved from RNA polymerase II and possess 12 subunits, which are involved in RdDM; RNA polymerase IV aids RDR2 to generate 24nt siRNAs, while RNA polymerase V transcribes genomic loci and recruits complementary siRNAs:AGO complex for cytosine methylation.

RISC: RNA-INDUCED SILENCING COMPLEX; a multiprotein complex comprising core unit AGO protein and other unclear members; the complex integrates with siRNAs or miRNAs to target mRNAs and then activates RNase for cleavage.

SCL: scarecrow-like protein, containing a conserved GRAS domain at the C-terminus. Gibberellin signaling repressors RGA and GAI belong to this family, and three SCL mRNAs (SCL6-II, SCL6-III, and SCL6-IV) are targets of miR171.

SGS3: SUPPRESSOR OF GENE SILENCING3; required for PTGS and belonging to an unknown protein family, which stabilizes cleavage fragments of the primary ta-siRNA transcripts and associate with RDR for the production of siRNAs.

sRNAs: a type of ~18 to 25 nucleotides (nt) in length non-coding RNA which appears ubiquitously in eukaryotes. They silence gene expression at transcriptional level by DNA methylation or at post-transcriptional level by cleaving mRNA transcript or by mediating translation inhibition.