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ARGONAUTE 1: a node coordinating plant disease resistance with growth and development

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Abstract

Argonaute (AGO) proteins are highly conserved and widely distributed across various organisms. They mainly associate with small RNAs (sRNAs) and act as integral players of the RNA-induced silencing complex in the RNA interference (RNAi) pathway, regulating gene expression at transcriptional and post-transcriptional levels, thereby mounting diverse fine-tuning functions in a variety of biological processes. Since the discovery and functional characterization of the first AGO in Arabidopsis, our understanding of the functions of AGO proteins has grown rapidly throughout the plant kingdom. AGO1 attracts investigators' attention because it forms an autoregulatory loop with miR168 and associates with other endogenous sRNAs and cross-kingdom exogenous sRNAs to relay all-round functions. AGO1 associates with endogenous sRNAs that form a complicated regulatory network via targeting a large body of downstream genes involved in growth, development, and stress-induced responses. Host AGO1 may also be exploited by cross-kingdom exogenous sRNAs generated by parasitic organisms to facilitate their colonization via suppressing host defense genes. Moreover, many pathogenic microbes directly target host AGO1 to facilitate their infection via suppression of the host RNAi pathway. Thus, we focus on plant AGO1 and provide an overview of our current understanding of the roles of AGO1 in the coordination of plant disease resistance with growth and development. We also discuss the perspectives in the dissection of the AGO1-mediated signal transduction pathway.

Keywords AGO1, Plant-microbe interactions, Small RNA, Disease resistance, Gene silencing, RNA interference

Background

Argonaute (AGO) proteins are the core components of the RNA-induced gene silencing complex (RISC) in the RNA interference (RNAi) pathway, which regulates gene expression at the transcriptional and post-transcriptional level (Fang and Qi 2016; Feng et al. 2021). AGO proteins were named after AGO1 in Arabidopsis in which its loss of function affects general plant architecture and results in tubular-shaped leaves that make the plants look like a small squid, thus named ARGONAUTE (Bohmert et al. 1998). AGO proteins are highly conserved and presented in archaea, bacteria, and all eukaryotes (Meister 2013), except for *Saccharomyces cerevisiae*, which has lost the RNAi machinery (Drinnenberg et al. 2009). The AGO family has expanded during plant evolution, and different plant species contain a variable number of AGO family members. For example, the green algae (*Chlamydomonas reinhardtii*) has three AGOs, moss (*Physcomitrella patens*) has six, Arabidopsis has 10, and rice (*Oryza sativa*) has 19 (Fang and Qi 2016).

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AGO proteins are composed of four distinct domains, including N-terminal, PAZ (Piwi Argonaute Zwille), MID (middle), and PIWI (P-element-induced wimpy testis) domains. The N-terminal domain is variable and essential for driving duplex sRNAs unwinding and loading a guide strand to assemble mature RISCs (Kwak and Tomari 2012), whereas C-terminal PAZ, MID, and PIWI domains are conserved (Tolia and Joshua-Tor 2007; Hutvagner and Simard 2008). These domains collaboratively use sRNAs to bind and mediate the cleavage of complementary RNA targets (Jin et al. 2021). The PAZ domain contains a binding pocket and is responsible for binding the 3′-end of sRNAs (Yan et al. 2003; Lingel et al. 2004; Ma et al. 2004; Tian et al. 2011). The MID domain affords an insertion site for the 5′-end of sRNAs (Boland et al. 2011; Frank et al. 2012). The PIWI domain has functions similar to an RNase H and contains a conserved active site and strongly implicates AGOs in sRNA-directed mRNA cleavage or slicer activity (Liu et al. 2004; Song et al. 2004; Jin et al. 2021).

Different AGO proteins bind to different-sized or competitively bind to sRNAs to perform diverse functions (Martin-Merchan et al. 2023), although their typical functions are in cleavage and translational inhibition of target mRNAs to repress the expression of targeted genes in the cytoplasm (Ma and Zhang 2018; Feng et al. 2021). First, some AGOs participate in RNA-directed DNA methylation (RdDM) by binding to 24-nt siRNAs. In Arabidopsis, three closely related AGOs, namely AGO4, AGO6, and AGO9, are implicated in RdDM. They all bind to 24-nt sRNAs with a 5′ terminal adenosine but have different preferences for sRNA from various heterochromatin-associated loci (Havecker et al. 2010). In rice, OsAGO4 associates with 24-nt long miRNAs (lmiRNAs) according to hierarchical rules and then directs DNA methylation at the loci where the lmiRNAs are produced from or in *trans* at the target genes (Wu et al. 2010). Second, some AGOs are involved in sRNA biogenesis. For example, AGO1-miRNAs are associated with membrane-bound polysomes (MBPs) to generate phased siRNA (phasiRNA) from phased precursor RNAs (Li et al. 2016), whereas AGO4 is involved in the production of siRNAs independent of DCLs (sidRNAs) that are then associated with AGO4 and direct DNA methylation in Arabidopsis (Ye et al. 2016). Third, some AGOs are engaged in DNA double-strand break (DSB) repair. In Arabidopsis, AGO1 and AGO2 recruit 21-nt DSB-induced small RNAs (diRNAs), which are produced from the sequences in the DSB flanking regions. Then diRNAs-guided AGOs mediate the recognition of DNA damage to facilitate repair (Wei et al. 2012; Schalk et al. 2017). Fourth, certain AGOs function as decoys of other AGOs to competitively bind to sRNAs. For example, AGO10 in Arabidopsis competes with AGO1 for binding miR166/miR165 to prevent their incorporation into AGO1 and subsequent repression of the expression of class III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factor genes, thus regulating the development of shoot apical meristem (SAM) (Zhu et al. 2011). Similarly, OsAGO18 in rice competes with OsAGO1 for binding miR168 to alleviate the repression of OsAGO1 and mounts antiviral RNAi signaling transduction pathway, leading to broad-spectrum viral disease resistance (Wu et al. 2015). Lastly, some AGOs associate with chromatin to function as either a transactivator or a transcriptional repressor in the nucleus. For example, the nuclear-localized AGO1 binds to chromatin via its association with sRNA and SWI/SNF (switch/sucrose non-fermentable) complexes to promote the transcription of stimuli-responsive genes (Liu et al. 2018). AGO1 interacts with chromatin at the *MIR173* and *MIR161* loci and causes the transcriptional complex disassembly, thus negatively regulating the expression of *MIR161* and *MIR173* in the nucleus under salt stress conditions, whereas mature miR161 and miR173 are stabilized via their loading into AGO1 (Dolata et al. 2016). Obviously, AGO1 plays crucial roles in the mentioned diverse functions with the exception of DNA methylation. Thus, this review focuses on the functions of AGO1 in RNAi signaling pathway regulating plant growth and development, and the interactions of plants with fungal, bacterial, and viral pathogens.

AGO1 plays crucial roles in plant growth and development

AGO1 becomes attractive in the plant science community because of RNAi-mediated epigenetics. In Arabidopsis, a mutant exhibits severe defects in development with an unusual appearance resembling a small squid; thus, the mutant was named *argonaute 1* (Bohmert et al. 1998). Subsequently, AGO1 is found to determine plant stature, leaf shape, flower phenotypes, and sterility (Fagard et al. 2000; Morel et al. 2002; Vaucheret et al. 2004). Its loss-of-function results in pleiotropic phenotypes, such as rarely developed axillary meristems, lacking a leaf blade of the rosette leaves, filamentous structure along the stem, and abnormal inflorescence carrying infertile flowers with the filamentous structure (Bohmert et al. 1998). Moreover, when sRNAs were reported to play crucial roles in development around 2000, *ago1* mutants were found to be defective in the production of miRNAs in contrast to the increased expressions of their target genes (Vaucheret et al. 2004). Arabidopsis *ago1* mutant also shows defects in adventitious rooting, presumably due to the light hypersensitivity of *ago1* mutant that is deregulated in auxin homeostasis, showing down-regulation of

several auxin-inducible *GH3* genes but up-regulation of a repressor of auxin-inducible gene *ARF17* (Sorin et al. 2005). Consistently, *ago1* seedlings develop an adventitious rooting phenotype upon the dark and auxin treatment. Intriguingly, the expression of *AGO1* was found to be regulated by one of the miRNAs, namely miR168. Transgenic plants expressing a miR168-resistant *AGO1* mRNA, which loses complementarity with miR168, show over-accumulation of *AGO1* mRNA and developmental defects such as a shorter stature, asymmetric rosette leaves, and twisted leaves (Vaucheret et al. 2004). Further investigation revealed that *AGO1* and miR168 are mutually regulated with each other, thus forming an autoregulatory loop. While miR168 suppresses *AGO1* expression at the post-transcriptional level, AGO1 is required to stabilize mature miR168 (Vaucheret et al. 2006). Any perturbation on the miR168-*AGO1* module disturbs the fine-tuning functions of the miRNA pathway, leading to the observed pleiotropic morphology phenotypes in *ago1* mutants and the transgenic lines expressing miR168-resistant *AGO1*.

Moreover, AGO1 cooperates with the other AGOs to relay the miRNA pathway in plant growth and development. In screening genes responsible for the enhanced mutant of flower development, AGO10 is required for floral determinacy in partnership with AGO1 for competing binding to a subset of miRNAs, particularly miR165/166 (Ji et al. 2011). Mechanistically, AGO10 has a higher affinity than AGO1 for binding to miR165/166, which target HD-ZIP III transcription factors. Mutation in *AGO10* causes miR165/166 to be loaded into AGO1 and suppress the expression of *HD-ZIP* IIIs, leading to a defective SAM and a pinhead phenotype (Ji et al. 2011; Zhu et al. 2011).

Consistent with the observations in Arabidopsis, AGO1 plays a crucial role in the growth and development of rice (Table 1). Four genes encoding OsAGO1 in rice, namely OsAGO1a, OsAGO1b, OsAGO1c, and OsAGO1d, are partially functionally redundant. Suppressed expression of *OsAGO1*s by RNAi results in plant lethality in severe lines and pleiotropic morphological phenotypes in the less severe lines (Wu et al. 2009). Consistently, individually knocking down each *OsAGO1* gene results in much weaker or no defects in morphological phenotypes, which may depend on the rice accessions used or tested conditions. For example, specific suppression of *OsAGO1a* by RNAi results in weak adaxial leaf rolling in Nipponbare, but no obvious phenotypes in Zhonghua 11 (Li et al. 2013, 2019b). Knocking down of *OsAGO1b* in Zhonghua 11 reduces pollen fertility and seed setting rates, but no altered phenotypes were observed in knocking down of the other *OsAGO1*s (Li et al. 2019b). Nevertheless, overexpression of *OsAGO1b* in Zhonghua 11 results in adaxial leaf rolling and a variety of abnormal phenotypes, including shortened plant height and reduced tiller numbers, but no obvious change was observed in overexpression of the other *OsAGO1*s (Li et al. 2019b). Although *OsAGO1d* is transcriptionally expressed in different rice organs, its protein specifically accumulates in the anther wall cells where it associates with miR2275 and miR2118 to mediate phasiRNAs biogenesis and regulates pollen development (Shi et al. 2022; Si et al. 2023). Consistent with the organ-specific accumulation of OsAGO1d, its loss-of-function mutation in Nipponbare results in the reduction of 21- & 24- nt phasiRNAs and male sterility at lower temperatures (Shi et al. 2022; Si et al. 2023). Moreover, altering the expression of all four *OsAGO1* genes can be achieved via over-expression of miR168 (OX168) and/or expressing a target mimic of miR168 (MIM168), both of which lead to dynamic alteration of a subset of miRNAs, such as miR156, miR159, miR160, miR164, miR166, miR167, miR171, miR172, and miR535, which are involved in regulating important agronomic traits such as plant height, tillering, flowering time, grain size, and grain yield (Wang et al. 2021a). Therefore, the full functional atlas of each OsAGO1 can be unveiled by using different rice accessions combined with measuring different traits at different growth conditions.

The crucial roles of AGO1 in growth and development are also confirmed in other plant species (Fig. 1; Table 1), such as wheat (*Triticum aestivum*) (Liu et al. 2011), *Nicotiana benthamiana* (Jones et al. 2006; Ludman and Fátyol 2021), white spruce (*Picea glauca*) (Tahir et al. 2006), foxtail millet (*Setaria italica*) (Liu et al. 2016), and tomato (*Solanum lycopersicum*) (Hendelman et al. 2013). The highly functional conservation of AGO1 in plant growth and development is attributable to its role as a core component of RISC in the miRNA signal pathway. Even mild alteration of the miR168-AGO1 module in some crops can improve economically important traits, such as grain yield in rice and fruit harvest in tomato (Xian et al. 2014; Wang et al. 2021a).

AGO1 is required for plant disease resistance to viruses

The roles of *AGO1* in plant disease resistance have been unveiled through analyses of the responses of *ago1* mutants to viruses (Fig. 1; Table 1). In Arabidopsis, because *ago1* null mutants are sterile, several hypomorphic *ago1* mutants were obtained that are fertile but still defective in post-transcriptional gene silencing. These hypomorphic *ago1* mutants are super-susceptible to the cucumber mosaic virus (CMV) (Morel et al. 2002). AGO1 was found to act as the most efficient player in the clearance of viral RNAs from a suppressor-defective

Table 1 AGO1 plays diverse roles in plant growth, development, and immunity

Plants	AGO name	Locus ID	Function in plant immunity	Function in plant growth and development	References
Arabidopsis	AGO1	AT1G48410	(1) AGO1 positively regulates plant disease resistance to suppressor-defective TCV, BMV, CMV, and *S. sclerotiorum* (2) AGO1 negatively regulates plant disease resistance to BaMV, *V. dahlia*, *V. longisporum*, *B. cinerea*, and *H. arabidopsidis*	AGO1 functions in the determination of plant stature, leaf shape, flower phenotypes, sterility, adventitious rooting, and SAM development in Arabidopsis	Bohmert et al. (1998), Fagard et al. (2000), Morel et al. (2002), Vaucheret et al. (2004), Sorin et al. (2005), Qu et al. (2008), Ellendorff et al. (2009), Ji et al. (2011), Zhu et al. (2011), Dzianott et al. (2012), Weiberg et al. (2013), Shen et al. (2014), Cao et al. (2016), Alazem et al. (2017), and Dunker et al. (2020)
Rice (*Oryza sativa*)	OsAGO1	OsAGO1a: LOC_Os02g45070; OsAGO1b: LOC_Os04g47870; OsAGO1c: LOC_Os02g58490; OsAGO1d: LOC_Os06g51310	OsAGO1 positively regulates plant disease resistance to RDV, RSV, and *M. oryzae*	OsAGO1 plays a crucial role in rice growth and development	Wu et al. (2009, 2015), Li et al. (2013, 2019b), Wang et al. (2021a), and Shi et al. (2022)
Wheat (*Triticum aestivum*)	TaAGO1 TaAGO1b	tplb0006m04; JQ805149	n/d	TaAGO1 is required for the regulation of growth rate, growth features, and adventitious roots	Liu et al. (2011), and Meng et al. (2013)
Nicotiana benthamiana	NbAGO1	NbAGO1-1: DQ321488; NbAGO1-2: DQ321489; NbAGO1a: MT701525; NbAGO1b: MT701526.	(1) Loss-of-function of NbAGO1 leads to compromised temperature-dependent symptom recovery of the plants infected with ToRSV (2) Repressed expression of *NbAGO1-1* results in rapidly necrotic symptoms of a *Tombusvirus* infection. (3) Both NbAGO1a and NbAGO1b contribute to antiviral defense against tombusvirus	(1) Suppressed expression of *NbAGO1* results in developmental defects (2) *Nb*AGO1b but not *Nb*AGO1a is required for normal development	Jones et al. (2006), Ghoshal and Sanfaçon (2014), Gursinsky et al. (2015), Odokonyero et al. (2017), Paudel et al. (2018) Ludman and Fátyol (2021)
Tomato (*Solanum lycopersicum*)	SlAGO1-1; SlAGO1-2	J×9,945,381; J×9,945,382	SlAGO1 is sensitive to BWYV P0-mediated destabilization	Reduced expression of *SlAGO1* results in pleiotropic morphological defects along with restricted growth at post-germination	Hendelman et al. (2013)
White spruce (*Picea glauca* (Moench) Voss)	PgAGO	DQ068741	n/d	PgAGO is required for embryo development and is specialized for the proper shoot and root apical meristem differentiation	Tahir et al. (2006)
Foxtail millet (*Setaria italica*)	SiAGO1b	Seita.7G201100	n/d	SiAGO1b is required for the regulation of growth and development in foxtail millet	Liu et al. (2016)
Malus hupehensis	MhAGO1	MDP0000161046	MhAGO1 negatively regulates plant disease resistance to *B. dothidea*	n/d	Yu et al. (2017)

n/d: not determined

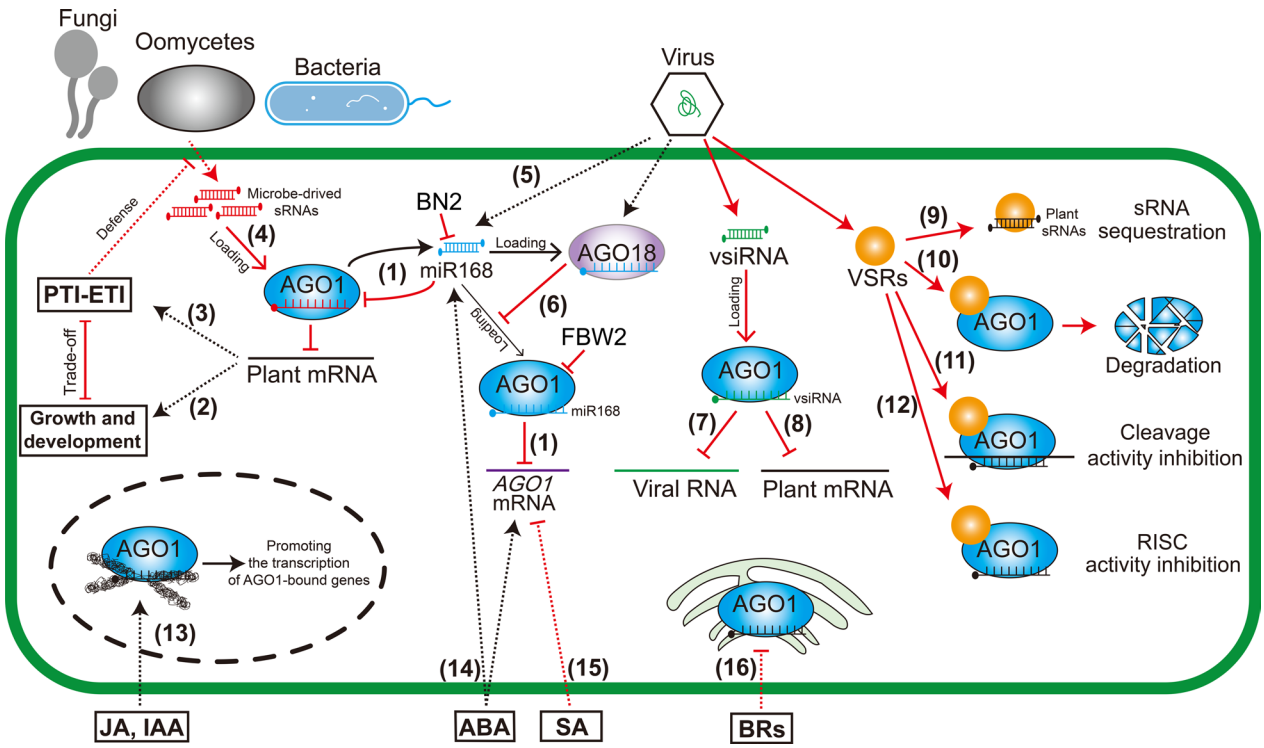

Fig. 1 AGO1 mediates all-round functions with endogenous and exogenous sRNAs. (1) Plant miR168 and *AGO1* forming an autoregulatory loop in which AGO1 stabilizes miR168, whereas miR168 suppresses *AGO1* expression. (2–3) AGO1-sRNA suppresses plant mRNAs that may play crucial roles in plant growth and development (2) and be involved in plant PTI and ETI against pathogens (3), such immunities have trade-offs with growth and development. (4) Pathogenic fungi and oomycetes may generate sRNAs that can be loaded into plant AGO1 to hijack the host RNAi pathway to facilitate their infection. (5) Viral infections may induce the expression of miR168 to suppress *AGO1* expression, thereby facilitating infection. (6) Rice AGO18 competes with AGO1 for binding miR168 to alleviate its repression on AGO1 and mounts antiviral immunity. (7–8) AGO1 may bind to vsiRNAs to mediate defense against viruses (7) or to silence plant endogenous genes for facilitating infection (8). (9–12) Virus-encoded suppressors of RNAi (VSRs) may promote viral infection via sequestering the host's sRNAs (9), or bind to AGO1 and mediate AGO1 degradation (10), or inhibit AGO1 cleavage activity (11), or block RISC activity (12). (13) JA and IAA can promote AGO1 binding to chromatin to enhance the expression of stimuli-responsive genes. (14–15) ABA induces the expression of *AGO1* and the abundance of mature miR168 (14), whereas SA inhibits the expression of *AGO1* (15). (16) BRs inhibit the distribution of AGO1 at the ER, thereby inhibiting miRNA-mediated transcriptional repression

turnip crinkle virus (TCV) among the components in the RNAi pathway required for antiviral defense, including Dicer-Like 1 (DCL1), DCL2, DCL3, DCL4, AGO2, AGO7, and RDR6 (Qu et al. 2008). Consistently, *ago1* mutants become susceptible to suppressor-defective TCV (Qu et al. 2008) and super-susceptible to brome mosaic virus (BMV) (Dzianott et al. 2012). However, *ago1* mutant exhibits reduced bamboo mosaic virus (BaMV) titers because loss-of-function of AGO1 results in significantly increased expression of *AGO2*, *AGO3*, and *AGO4*, which positively regulate plant disease resistance to BaMV (Alazem et al. 2017).

The requirement of AGO1 in antiviral defense is also conserved and confirmed in other plant species (Table 1). For instance, in rice, *OsAGO1* RNAi lines exhibit super-susceptible to rice dwarf virus (RDV) and rice stripe virus (RSV) (Wu et al. 2015). In *N. benthamiana*, defective expression of *NbAGO1* results in compromised temperature-dependent symptom recovery of the plants infected with tomato ringspot virus (ToRSV) (Ghoshal and Sanfaçon 2014; Paudel et al. 2018). Silencing of *NbAGO1-1* via VIGS results in rapid necrotic symptoms of a tombusvirus infection (Gursinsky et al. 2015). In addition, loss-of-function of *AGO1a* mutants show typical severe symptoms of TCV infection in *N. benthamiana* (Ludman and Fátyol 2021).

In general, RNAi is a crucial antiviral mechanism in plants (Fig. 1). AGO proteins, including AGO1, play an essential role in binding to virus-derived siRNAs (vsiRNAs), forming RISC to directly suppress viral RNA or DNA, leading to viral disease resistance. In plant-viral pathogen interactions, multiple layers of regulation contribute to antiviral immunity. Once the AGO1-mediated RNAi pathway is overcome by viral suppressors of RNA silencing (VSR) that target AGO1, a second layer involving AGO2 is activated to constrain virus accumulation

because *AGO2* abundance is manipulated by AGO1-loaded miR403 (Harvey et al. 2011). RNAi-mediated antiviral immunity is also enhanced via the up-regulation of AGO1 by other AGO family members. In rice, for example, the expression of *OsAGO1* and *OsAGO18* is induced upon RSV infection, as OsAGO18 has a higher binding affinity for miR168 than OsAGO1, enhanced expression of OsAGO18 results in enhanced expression of OsAGO1 and its mediated antiviral resistance (Wu et al. 2015). In rice, OsAGO2 epigenetically regulates the expression of *OsHXK1* (*HEXOKINASE 1*) to modulate reactive oxygen species (ROS) accumulation in response to the infection of rice black-streaked dwarf virus (Wang et al. 2021c).

AGO1 plays diverse roles in plant disease resistance to fungal and bacterial pathogens

Similar to the requirement of AGO1 in plant antiviral defense, *AGO1* is also essential for plant disease resistance to several fungal and bacterial pathogens (Fig. 1; Table 1). This is reasonable because defects in *AGO1* lead to a reduction of pattern-triggered immune (PTI) responses in Arabidopsis. *AGO1* loss-of-function mutants show significantly reduced callose deposition and compromised expression of PAMP-responsive genes, implying that AGO1 plays a crucial role in PTI signaling (Li et al. 2010). Arabidopsis *ago1* mutants, namely *ago1-27* and *ago1-33*, generate more severe necrotic disease symptoms upon inoculation of the fungal pathogen *Sclerotinia sclerotiorum*, indicating that *AGO1* is required in Arabidopsis resistance to *S. sclerotiorum* (Cao et al. 2016). Accordingly, transgenic Arabidopsis over-expressing *AGO1* exhibits enhanced disease resistance correlated with the abundance of *AGO1* transcripts. These observations are consistent with the up-regulated expression of defense-related genes and the marker genes involved in salicylic acid (SA) and jasmonic acid (JA)/ethylene pathways, indicating that *AGO1* acts in plant resistance to *S. sclerotiorum* via SA and JA pathways (Cao et al. 2016). In rice, down-regulation of *OsAGO1* leads to enhanced susceptibility to *Magnorpothe oryzae*; up-regulation of *AGO1* expression by suppressing miR168 leads to enhanced disease resistance, suggesting that AGO1 positively regulates rice immunity against *M. oryzae* (Wang et al. 2021a). Moreover, AGO1 and various stress-related miRNAs contribute to disease resistance by promoting callose deposition and up-regulating defense-related genes (Li et al. 2010). Another example is miR393, which is loaded into AGO1 and regulates PTI by targeting auxin receptors, thereby suppressing the auxin signaling pathway to restrict the growth of *P. syringae* DC3000 (Navarro et al. 2006), indicating that *AGO1* is necessary for plant defense against *P. syringae* DC3000.

However, AGO1 has been reported to negatively regulate plant disease resistance to *Verticillium dahlia*, *Verticillium longisporum*, *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Hyaloperonospora arabidopsidis* (Table 1) (Ellendorff et al. 2009; Weiberg et al. 2013; Shen et al. 2014; Yu et al. 2017; Dunker et al. 2020). Arabidopsis *ago1* mutants, including *ago1-25* and *ago1-27*, exhibit reduced disease symptoms and decreased fungal biomass growth in comparison with wild-type upon the inoculation of *V. dahlia* (Ellendorff et al. 2009). Additionally, *ago1-27* exhibits remarkably less severe disease symptoms than the wild-type upon infection with *B. cinerea*, a fungal pathogen that delivers sRNAs into host plants and hijacks host RNAi pathways by associating with AGO1 to promote infection (Weiberg et al. 2013). Arabidopsis mutants *ago1-t*, *ago1-25*, and *ago1-27* all showed resistance to *V. longisporum* with remarkably less severe disease symptoms and compromised *V. longisporum* development compared to wild-type (Shen et al. 2014). Moreover, Arabidopsis *ago1-27* mutant exhibits enhanced disease resistance against *H. arabidopsidis* with a remarkable change in disease phenotype and reduced accumulation of pathogen biomass (Dunker et al. 2020). Consistent with the above observations, the elevation of AGO1 via knockout of miR168 results in super-susceptible to *V. longisporum* with significantly enhanced pathogen development. Therefore, A*GO1* negatively acts in Arabidopsis disease resistance to *V. longisporum*. Similarly, *MhAGO1* plays a negative role in *Malus hupehensis* resistance to *B. dothidea*, as silencing of *MhAGO1* results in enhanced defense responses and delayed development of disease symptoms (Yu et al. 2017).

Therefore, the involvement of AGO1 is quite complicated in plant disease resistance to fungal and bacterial pathogens. Whether AGO1 is required or acts negatively in plant disease resistance depends on the pathogens and their host plants involved. For instance, AGO1 loaded with a specific sRNA may have the same or opposite functions in different plant species. Whereas miR160a positively regulates plant immunity in both Arabidopsis and rice (Li et al. 2010; Feng et al. 2022), miR398b negatively regulates immunity in Arabidopsis (Li et al. 2010) but positively regulates immunity in rice (Li et al. 2019a). This phenomenon could be due to the differences in the functional roles of downstream genes targeted by the same miRNA and the plant species involved. Additionally, AGO1 loaded with a particular sRNA may target genes with opposite functions. For example, among the four rice *SOD*s family genes targeted by miR398b, mutants of the three genes are more resistant to rice blast, whereas the mutant of the other gene is more susceptible (Li et al. 2019a). Hence, the function of target

genes also seems crucial for the role of AGO1-sRNA complexes in plant immunity.

Pathogenic microbes target AGO1 to subvert plant disease resistance

During the co-evolutionary arms race between plants and pathogenic microbes, plants are endowed with AGO1 to buffer growth with plant immunity against pathogenic microbes via its association with sRNAs (Carbonell and Carrington 2015). As a counter strategy, pathogenic microbes have evolved suppressors of RNA silencing to nullify or hijack host AGO1 to facilitate infection (Fig. 1; Table 2).

VSRs from viruses disrupt multifarious steps of the silencing pathways. Some VSRs sequester sRNAs to prevent them from being loaded into the RISC (Vargason et al. 2003; Ye et al. 2003), whereas others manipulate the host AGO1 or endogenous miR168 abundance to facilitate infection, either via interference with AGO1 cleavage activity, promoting AGO1 degradation, inhibition of RISC activity, and/or decrease the accumulation of *AGO1* mRNA. First, virus-encoded proteins may inhibit AGO1 cleavage activity. For example, CMV-encoded 2b protein (CMV 2b) interacts with AGO1 via one surface of the PAZ domain and the partial PIWI domain of AGO1 to interfere with the miRNA pathway, leading to developmental defects in CMV-infected plants that partially phenocopy *ago1* mutants in Arabidopsis (Zhang et al. 2006). Mechanistically, AGO1-associated CMV 2b specifically inhibits AGO1 cleavage activity, leading to a reduction of the production of 21-, 22-, and 24-nucleotide classes of viral small interfering RNAs (Zhang et al. 2006; Diaz-Pendon et al. 2007). Although CMV 2b also interacts with both short and long dsRNA, direct CMV 2b-AGO1 interaction is sufficient to block the AGO1 cleavage function independent of the dsRNA-binding function of CMV 2b (Feng et al. 2013). Moreover, CMV-encoded 1a protein, a component of the viral replicase complex, limits the proportion of CMV 2b molecules that bind to AGO1, thereby regulating CMV 2b and AGO1 interaction while maintaining the silencing suppressor activity of CMV 2b (Watt et al. 2020). Second, virus-encoded protein may mediate AGO1 destabilization. For example, polerovirus-encoded P0 protein targets AGO1 for degradation (Baumberger et al. 2007; Bortolamiol et al. 2008). Polerovirus P0 protein associates with AGO1 at the PAZ domain and the adjacent upstream sequences, resulting in AGO1 degradation to convert the RNA silencing-mediated antiviral immunity (Baumberger et al. 2007; Bortolamiol et al. 2008; Derrien et al. 2018). P0 protein belongs to the F box proteins, which are the components of E3 ubiquitin ligase complexes. Thus, P0 may exploit host ubiquitination machinery to degrade AGO1. Further studies have showed that P0 triggers AGO1 degradation by the autophagy pathway because AGO1 degradation is blocked by inhibiting the autophagy pathway (Derrien et al. 2012). Mechanistically, the P0 protein interacts with AGO1 at the endoplasmic reticulum (ER), forming ER-associated bodies delivered to the vacuole for degradation (Michaeli et al. 2019). In tomato, constitutive expression of P0 results in reduced SlAGO1 protein abundance and increased mRNA accumulation of miRNA target genes. Accompanying this, P0-expressed transgenic plants exhibit pleiotropic morphological defects (Hendelman et al. 2013). Besides, potato virus X P25 has been found to mediate AGO1 degradation via the proteasome pathway (Chiu et al. 2010). Third, virus-encoded proteins may block si/miRNA-programmed RISC activity. For example, TCV-encoded P38 contains two glycine/tryptophane (GW) motifs that make P38 mimic host-encoded GW-containing proteins required for RISC assembly. P38 directly binds to AGO1, resulting in the quenching of AGO1 from RISC assembly and the reduction of RISC activity (Azevedo et al. 2010). Another similar silencing suppressor is the P1 protein from sweet potato mild mottle virus (SPMMV), which contains three WG/GW motifs at its N-terminal. P1 targets the RISC complex and inhibits si/miRNA-programmed RISC activity through direct interaction with AGO1. Site-directed mutagenesis in these three WG/GW motifs of P1 completely abolishes its ability to bind and suppress the AGO1 function (Giner et al. 2010). Further studies have shown that the P1 protein contains a zinc finger domain that contributes to the silencing suppressor activity of P1 via preventing target RNA from binding to AGO1 (Kenesi et al. 2017). Besides, ToRSV coat protein (CP) contains a WG motif that makes CP interact with and destabilize AGO1 (Karran and Sanfacon 2014). Therefore, these viral-encoded proteins with WG/GW motifs function as an AGO1-hook protein and compete for AGO1 binding with the host GW/WG-containing proteins, thus blocking RISC assembly and activity. Fourth, virus-encoded proteins may modulate the endogenous miR168 abundance to impact the translational capacity of *AGO1* mRNA. The abundance of miR168 is ubiquitously up-regulated in plants upon infection of viruses, including CymRSV, crTMV, PVX, TEV, TCV, RMV, and SHMV (Várallyay et al. 2010). Viral P19 RNA-silencing suppressor is responsible for the up-regulation of miR168. However, the abundance of *AGO1* mRNA is also up-regulated in viral-infected plants, but the amount of AGO1 protein remains unchanged or down-regulated, implying translational inhibition on *AGO1* mRNA by miR168. This conclusion was confirmed through the analyses of the cleaved *AGO1* mRNA and the mutants at the miR168 target site in *AGO1* during viral infection (Várallyay et al. 2010). Thus, viruses can inhibit

Table 2 AGO1 protein is targeted by microbes facilitating their colonization

Microbes	Host (s)	Target	Function	References
Cucumber mosaic virus (CMV)	Arabidopsis	AGO1	CMV 1a protein interacts with the 2b protein and regulates the interaction of 2b protein and AGO1. The 2b protein blocks AGO1 cleavage activity to attenuate RNA silencing and counter host defense	Zhang et al. (2006), Ruiz-Ferrer and Voinnet (2007), Feng et al. (2013), and Watt et al. (2020)
Poleroviruses	Arabidopsis	AGO1	Polerovirus-encoded F box protein (P0) targets AGO1 and mediates its degradation in planta	Baumberger et al. (2007), Bortolamiol et al. (2008), and Derrien et al. (2012)
	Tomato (*Solanum lycopersicum*)	SlAGO1	P0 mediates SlAGO1 destabilization in tomato	Hendelman et al. (2013)
Turnip crinkle virus (TCV)	Arabidopsis	AGO1	A virus-encoded GW-containing protein (P38) binds directly and specifically to Arabidopsis AGO1 and inhibits its function	Azevedo et al. (2010)
Sweet potato mild mottle virus (SPMMV)	Arabidopsis	AGO1	SPMMV P1 targets AGO1 and inhibits RISC activity	Giner et al. (2010)
Turnip crinkle virus (TCV), ribbgrass mosaic virus (RMV), and cymbidium ringspot virus (CymRSV)	Arabidopsis and *Nicotiana benthamiana*	AGO1	Plant viruses mediate miR168 induction followed by the inhibition of the translational capacity of *AGO1* mRNA to alleviate its antiviral function	Várallyay et al. (2010)
Tomato ringspot virus (ToRSV)	*N. benthamiana*	NbAGO1	Tomato ringspot virus coat protein (CP) binds to AGO1 and destabilizes AGO1	Karran and Sanfacon (2014)
Turnip yellows virus (TuYV)	Arabidopsis	AGO1	TuYV P0 associates with AGO1 and mediates the delivery of AGO1 from ER to vacuole followed by the clearance of membrane-bound AGO1 via an ER-derived autophagy degradation pathway	Michaeli et al. (2019)
Botrytis cinerea	Arabidopsis and tomato	AGO1	Bc-sRNAs hijack the host RNA interference (RNAi) pathways by binding to AGO1 and selectively silencing the immunity genes of the host	Weiberg et al. (2013)
Hyaloperonospora arabidopsidis	Arabidopsis	AGO1	Oomycete sRNAs associate with host AGO1 to target and silence host defense genes for virulence	Dunker et al. (2020)

the accumulation of AGO1 by modulating the abundance of miR168 to relieve the AGO1-mediated antiviral function.

Fungal- or oomycete-generated sRNAs can be loaded into host AGO1 to hijack host RNAi pathways to facilitate infection. Unlike viral-derived sRNAs, which are processed by host RNase III and Dicer and guide AGO proteins as part of antiviral RISC (Azevedo et al. 2010), fungi and oomycetes process sRNA using their native sRNA production pathway. These kinds of sRNA can silence the genes involved in immunity in Arabidopsis and tomato (Weiberg et al. 2013). It has been shown that Bc-sRNAs bind to host AGO1 and hijack the RNAi machinery to selectively silence host genes involved in immunity. Loss-of-function mutants of host AGO1 exhibit reduced susceptibility to *B. cinerea*, whereas *dcl1 dcl2* double mutant of *B. cinerea*, which can no longer generate Bc-sRNAs, has reduced pathogenicity on tomato and Arabidopsis (Weiberg et al. 2013). Another microbial pathogen, *Hyaloperonospora arabidopsidis*, which belongs to the kingdom of oomycetes, also hijacks host AGO1 for virulence (Dunker et al. 2020). Similar to *B. cinerea*, *H. arabidopsidis* sRNAs (*Hpa*sRNAs) associate with host AGO1 and selectively silence host genes involved in the immunity in the infected cells of the host. Expression of a short-tandem-target-mimic (STTM) RNA in host blocks *Hpa*sRNAs activity and results in reduced virulence of *H. arabidopsidis* (Dunker et al. 2020). Thus, fungal and oomycete pathogens generate virulent sRNAs and deliver them into the host to suppress the host immunity to achieve colonization.

Overall, the host AGO1 protein can be nullified and/or hijacked by pathogenic microbes for degradation and/or cleavage activity inhibition, resulting in suppression or interference with the host siRNA pathways.

AGO1 associates with multiple phytohormone pathways

RNA silencing pathways modulate responses to particular stresses and are partially tuned by various hormones, such as abscisic acid (ABA), SA, JA, and brassinosteroids (BRs). These plant hormones are involved in the regulation of specific components of the RNA silencing pathways, including AGO1 (Fig. 1) (Yoon et al. 2009; Li et al. 2012; Sun et al. 2016; Alazem et al. 2017; Wang et al. 2021b).

AGO1 and plant hormone homeostasis are mutually regulated with each other. Plant hormones affect AGO1 expression and/or subcellular localization, and AGO1 is required for some plant hormone-mediated immunity. ABA affects AGO1 and *MIR168a* homeostasis in Arabidopsis. Loss-of-function of *AGO1* mutant and overexpression of *MIR168a* transgenic lines exhibit enhanced sensitivity to ABA. The transcription activity of *AGO1* is increased upon ABA treatment according to the promoter activity analysis; meanwhile, mature miR168 and its precursor are also induced under ABA treatment, implying that maintaining the stable *AGO1* mRNA abundance needs elevated *MIR168a* abundance during the stress response (Li et al. 2012). Moreover, BaMV-induced expression of *AGO1* is ABA-dependent because the expression of *AGO1* is remarkably lower in mutants defective in the ABA signaling pathway than in the wild-type upon BaMV-infection (Alazem et al. 2017). The SA signaling pathway negatively regulates the expression of *AGO1*, and SA treatment decreases *AGO1* expression (Alazem et al. 2019). Conversely, the expression of *AGO1* is positively regulated by ABA and ABA treatment enhances *AGO1* expression, especially in the SA mutants, indicating that SA and ABA exhibit mutual antagonism in the expression of *AGO1* (Alazem et al. 2019). Besides, BRs regulate the subcellular distribution of AGO1 (Wang et al. 2021b). The ER-localized AGO1 is significantly increased in the BR-deficient mutants but decreased under BR treatments. Consequently, BR-deficient mutants exhibit reduced protein abundance rather than transcript abundance of the miRNA target genes, whereas BR treatment increased protein abundance of the miRNA target genes (Wang et al. 2021b). On the other hand, AGO1 is required for long-lasting memory of JA-dependent immunity. JA treatment induces long-term susceptibility to both hemibiotrophic and necrotrophic pathogens and long-term resistance to herbivory (Wilkinson et al. 2023). JA treatment specifically enriches hypomethylated *ATREP2* transposon elements (TEs), which are regulated by the RdDM and ROS1 and produce 21 nt sRNAs binding to the nuclear-localized AGO1. Thus, AGO1, along with these sRNAs from *ATREP2* TEs, *trans*-regulates JA-dependent long-lasting memory of plant immunity (Wilkinson et al. 2023). Finally, various hormones, including JA, benzothiadiazole (BTH), and indoleacetic acid (IAA), trigger AGO1 to bind to certain chromatic regions, leading to enhanced expression of stimulus-responsive genes (Liu et al. 2018). Thus, AGO1 plays roles in many hormone-signaling pathways, and such roles help us to understand its all-round functions in growth, development, and interactions with pathogenic microbes.

Conclusions and perspectives in the AGO1-mediated pathway

Although our current knowledge of AGO1 is quite fragmental, it is pretty clear that AGO1 performs an all-round function via associating with a large number of sRNAs that could be endogenous or exogenous (Fig. 1). Its association with endogenous sRNAs leads

to regulation of the transcription of stimuli-responsive genes and the suppression of a large number of genes that are involved in growth, development, and stress-induced responses (Liu et al. 2018). Its association with pathogenic microbe-generated sRNAs results in the successful pathogenesis of the pathogens (Weiberg et al. 2013; Dunker et al. 2020). Meanwhile, AGO1 plays a paramount role in plant resistance to viral diseases, and thus it is also a preferential target of VSRs in a number of viruses (Michaeli et al. 2019). With the increasing reports of players in AGO1-mediated functions, AGO1 appears fascinating and attractive in coordinating plant growth, development, and disease resistance.

However, it is largely lagged in our understanding of the regulation of AGO1 expression and homeostasis. In addition to miR168, which regulates AGO1 expression at the post-transcriptional level, AGO1 homeostasis is controlled by an F-BOX WITH WD-40 2 (FBW2) protein that specifically targets empty AGO1 for degradation to avoid loading of illegitimate sRNAs and off-target cleavage in Arabidopsis (Hacquard et al. 2022). In turn, the expression of *FBW2* is regulated by the histone methyltransferase CURLY LEAF (CLF), an effector of the Polycomb Repressor Complex 2 (Ré et al. 2020). *AGO1* mRNA abundance is indirectly regulated by Bifunctional nuclease-2 (BN2), which functions as a ribonuclease to degrade miR168 (Wang et al. 2021c). Therefore, several issues need to be focused on to investigate the regulatory mechanism of AGO1 expression and homeostasis. First, to fully understand the crucial regulatory role of AGO1 in plant immunity, it is necessary to clarify the regulatory relationship between AGO1 and immune receptors. Several papers reported that the expression of AGO1 is up-regulated upon the infection of pathogens (Du et al. 2011; Wu et al. 2015; Alazem et al. 2017; Yu et al. 2017). Obviously, AGO1 expression responds to biotic stress, which might correlate with the activation of immune receptors. In turn, the activation of immune receptors might lead to activating certain transcription factors that would further promote the transcription of *AGO1*. Alternatively, because the abundance of *AGO1* transcripts is also regulated by miR168 at the post-transcriptional level, the activation of immune receptors could suppress certain other transcription factors that determine the transcription of *MIR168*, leading to the release of *AGO1* from suppression by miR168. Under this hypothetical scenario, the AGO1-mediated regulatory pathway could extend to the transcription factors that regulate the expression of *AGO1* either directly at the transcriptional level or via miR168 at the post-transcriptional level. Second, it is worthwhile to make out whether there are any effectors from pathogens other than viruses to target AGO1 in a way similar to that of VSRs. It might be practically doable if AGO1 is used as bait to screen interactors from pathogens' proteins. Finally, it is well-known that many proteins require modification to perform their function, such as phosphorylation, ubiquitination, etc. It is also worthwhile to investigate post-translational modifications that AGO1 is subject to, such as the one exploited by FBW2 to control AGO1 homeostasis in Arabidopsis. Overall, unveiling the regulations and functions of AGO1 will enable us to understand better its roles in regulating plant immunity, growth, and development, which will bring the gospel to the molecular breeding of crops.

Abbreviations

ABA	Abscisic acid
AGO	Argonaute
BaMV	Bamboo mosaic virus
BMV	Brome mosaic virus
BRs	Brassinosteroids
BTH	Benzothiadiazole
CLF	CURLY LEAF
CMV	Cucumber mosaic virus
CMV 2b	CMV-encoded 2b protein
CP	Coat protein
DCL1	Dicer-Like 1
diRNA	DSB-induced small RNAs
DSBs	DNA double-strand breaks
ER	Endoplasmic reticulum
ETI	Effector-triggered immunity
FBW2	F-BOX WITH WD-40 2
GW	Glycine/tryptophane
HD-ZIP III	HOMEODOMAIN-LEUCINE ZIPPER
IAA	Indoleacetic acid
JA	Jasmonic acid
lmiRNA	Long miRNA
MBPs	Membrane-bound polysomes
MIM168	Target mimic of miR168
OX168	Over-expression of miR168
PAMP	Pathogen-associated molecular pattern
PAZ	Piwi Argonaute Zwille
phasiRNA	Phased siRNA
PIWI	P-element-induced wimpy testis
PTI	pathogen-associated molecular pattern (PAMP)-triggered immunity
RdDM	RNA-directed DNA methylation
RDV	Rice dwarf virus
RISC	RNA-induced silencing complex
RNAi	RNA interference
RSV	Rice stripe virus
SA	Salicylic acid
SAM	Shoot apical meristem
sidRNA	siRNAs independent of DCLs
SPMMV	Sweet potato mild mottle virus
sRNA	Small RNA
STTM	Short-tandem-target-mimic
SWI/SNF	Switch/sucrose non-fermentable
TCV	Turnip crinkle virus
ToRSV	Tomato ringspot virus
VIGS	Virus-induced gene silencing
vsiRNAs	Virus-derived siRNAs
VSR	Viral suppressors of RNA silencing

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Author contributions
WW and ZZ wrote the manuscript. SY drew the figure. XY (Xiao-Xiao Yin) prepared the tables. XY (Xiu-Lian Yan) and BH were in charge of the literature collection. JF, YL, ZZ, and WW revised the manuscript. All authors read and approved the final manuscript.

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