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Plant ARGONAUTES: Features, Functions and Unknowns

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Running head: Plant ARGONAUTE proteins

Abstract

ARGONAUTES (AGOs) are the effector proteins in eukaryotic small RNA(sRNA)-based gene silencing pathways controlling gene expression and transposon activity. In plants, AGOs regulate key biological processes such as development, response to stress, genome structure and integrity, and pathogen defense. Canonical functions of plant AGO-sRNA complexes include the endonucleolytic cleavage or translational inhibition of target RNAs, and the methylation of target DNAs. Here, I provide a brief update on the major features, molecular functions and biological roles of plant AGOs. A special focus is given to the more recent discoveries related to emerging molecular or biological functions of plant AGOs, as well as to the major unknowns in the plant AGO field.

Key words: ARGONAUTE, small RNA, RNA silencing, microRNA, Arabidopsis

1 Introduction

In eukaryotes, ARGONAUTES (AGOs) are the effector proteins functioning in small RNA (sRNA)–guided gene silencing pathways regulating gene expression and transposon activity [1]. AGO–sRNA complexes target and silence complementary DNA or RNA through posttranscriptional gene silencing (PTGS) or transcriptional gene silencing (TGS), respectively. Silencing of target transcripts occurs through either direct endonucleolytic cleavage (slicing) or through other cleavage–independent mechanisms such as target destabilization or translational repression [2].

AGOs have an ancient origin, as they are present in bacteria, archae and eukaryotes [3]. In plants, the AGO family includes a variable number of members depending on the plant species [4], with flowering plants encoding more AGOs. For example, *Arabidopsis thaliana* (Arabidopsis) and rice have 10 and 19 AGO members [5,6] respectively, while the algae *Chlamydomonas reinhardtii* and the moss *Physcomitrella patens* have three and six [7-9] respectively. The expansion of the plant AGO family suggests a functional diversification of AGO proteins most likely during the specialization and evolution of endogenous sRNA–based RNA silencing pathways [10,11]. Phylogenetically, flowering plant AGOs can be grouped in three major clades: AGO1/5/10, AGO2/3/7 and AGO4/6/8/9. In addition, grasses present an expanded AGO1/5/10 clade including AGO18 [10].

Crystallographic studies on eukaryotic AGOs have determined that AGOs present four functional domains: a variable N–domain, and conserved PAZ, MID and PIWI domains [12]. The MID and PAZ domains bind the 5' monophosphorylated nucleotide and the 3' nucleotide of the sRNA, respectively. The PIWI domain is the ribonucleolytic domain, with four metal–coordinating residues required for slicer activity [13,14]. Plant AGOs associate with sRNA based on the identity of the 5'

nucleotide of the sRNA and/or other sequence and structural features of the sRNA duplex and the AGO PIWI domain [15-19]. Plant AGO–sRNA complexes can function through different modes to silence complementary DNA or RNA and exert their biological role.

I present next an updated overview on the known and emerging molecular and biological roles of plant AGOs. I also highlight the main unknowns in the plant AGO field.

2 Modes of Action of Plant AGOs

The main modes of action of plant AGOs are summarized in Figure 1 and described next.

2.1 Endonucleolytic Cleavage

The PIWI domain of AGOs uses intrinsic RNase H–like activity to cleave target RNA [20], and contains a metal–coordinating Asp–Glu–Asp–His/Asp catalytic tetrad [14,21]. Slicing activity has been experimentally confirmed for Arabidopsis AGO1 [22,23], AGO2 [24], AGO4 [25], AGO7 [15] and AGO10 [18,26].

Since the initial observation that plant microRNAs (miRNAs) targeted and cleaved highly sequence complementary target RNAs [27], it was assumed that slicing was the predominant mode of action in miRNA–mediated PTGS in plants [28]. Indeed, the high degree of complementarity is a requirement for effective target slicing by plant AGOs [29]. However, later examples describing slicing–independent translational repression of certain miRNA targets (see below) have questioned this assumption. Still, growing evidence suggest that target RNA regulation by slicing is widespread in plants. First, sequencing of Arabidopsis mRNA degradome has revealed that most

miRNA targets undergo slicing [30,31]. Second, the slicing activity of Arabidopsis AGO1 (the primary miRNA–associating AGO), AGO2 and AGO7 is critical for plant development, antiviral activity and juvenile to adult phase transition, respectively [24], while AGO4 and AGO10 exert their primary functions in a slicer–independent mode [18,25]. And third, recent transcriptome profiling of *ago1* null and slicer–deficient Arabidopsis mutants confirmed that AGO1 slicer activity is necessary for the repression of the majority of miRNA targets [32].

Slicing activity of plant AGOs is also required for triggering the amplification of phased secondary small interfering RNAs (phasiRNAs) from certain target transcripts. For instance, *trans*–acting small interfering RNAs (tasiRNA), a class of secondary small interfering RNAs (siRNAs) that forms through a refined mechanism, derive from four families of *TAS* transcripts that are initially cleaved by AGO1–miR173, AGO1–miR828 or AGO7–miR390 complexes to produce *TAS1/TAS2*, *TAS4* and *TAS3*–derived tasiRNAs respectively [15,24,33–37]. Interestingly, a recent comparative analysis of tasiRNA generation in wild–type, *ago1* null and *ago1* slicer deficient Arabidopsis showed that slicing by AGO1 is required for the definition of the phase but not for the generation of *TAS*–derived tasiRNAs [38].

The subcellular location of miRNA–mediated target cleavage has been largely unknown. However, the observation that a reduced level of isoprenoids, which are essential for membrane sterols, blocks miRNA–mediated cleavage of several target transcripts suggests that membrane association of AGO1 is important for target cleavage [39]. In a recent work, Arabidopsis miRNAs were shown to associate with membrane–bound polysomes as opposed to polysomes in general, and this association was required for miRNA–triggered phasiRNA production [40]. Because slicing is

required for phasiRNA production, it appears that, at least, part of the AGO-mediated target cleavage activity occurs in membrane-bound polysomes.

2.2 Translational Repression

Translational repression by miRNAs is common in animals where miRNA-target RNA interactions require limited sequence complementarity [41]. In plants, several evidences suggest that AGO-miRNA complexes can also translationally repress their targets RNAs with almost perfect complementarity [39,42-48]. Several Arabidopsis mutants impaired in miRNA-mediated gene repression at protein but not at mRNA levels have been described [39,43,49]. In particular, AGO1-miRNA-mediated translation repression in Arabidopsis occurs in the endoplasmic reticulum and requires the integral membrane protein ALTERED MERISTEM PROGRAM 1 to exclude target mRNAs from membrane-bound polysomes [43]. AGO10, another member of the AGO1 clade, also appears to translationally repress several Arabidopsis miRNA target genes, including AGO1 [50]. Very recently, it has been shown that AGO7-miR390 binding to a non-cleavable miR390 target site included in *TAS3a* non-coding transcripts cause ribosome stacking and subsequent inhibition of translation elongation [51]. However, the global contribution to plant miRNA-mediated translation repression of direct blocking of ribosome movement through the binding of AGO-miRNA complexes appears to be limited [51].

2.3 Target mRNA Decay?

Target mRNA decay is a common outcome of AGO recruitment in animals, where the complementarity between an amiRNA and its target mRNA is generally limited to the 5' region of the miRNA and slicing is not common [52]. AGO-miRNA complexes

destabilize target mRNAs in a process requiring both deadenylation and decapping [53,54]. Recruitment of the two major deadenylases CCR4–NOT and PARN requires the adaptor protein GW182/TNRC6 that binds to hydrophobic pockets in AGOs [55,56]. Because no homolog of GW182/TNRC6 is present in plant genomes, it is unlikely that such mechanism exists in plants. Three recent studies support this statement: i) transcriptome analyses performed on either stable or conditional slicer–deficient AGO1 mutants did not show any substantial differences in gene expression between the two classes [32], ii) efficient translational repression by either wild–type or slicer–deficient AGO1 in lysates of tobacco protoplasts is not accompanied by any kind of reporter–mRNA degradation other than slicing [42], and iii) no deadenylation has been reported in sRNA–mediated translational repression in *Chlamydomonas reinhardtii* [57]. However, the possibility that a subset of plant miRNA targets could be regulated by AGO–mediated mRNA decay cannot be completely ruled out.

2.4 RNA–Dependent DNA Methylation

DNA methylation regulates gene expression, blocks transposon movement and consequently maintains genome integrity. In plants, canonical DNA methylation is primarily mediated by AGO4–siRNA complexes functioning in RNA–dependent DNA methylation (RdDM) pathways [58]. These pathways are initiated by the synthesis of double–stranded RNA (dsRNA) by the concerted action of RNA POLYMERASE IV (Pol IV) and RNA–DEPENDENT RNA POLYMERASE 2 (RDR2) [59–63]. DsRNA processing in the nucleus by DICER–LIKE 3 (DCL3) leads to the production of 24–nt siRNAs [59] that are exported to the cytoplasm where they are incorporated into AGO4. AGO4–siRNA complexes localize to the nucleus where they are recruited to target loci via base–pairing with nascent Pol V transcripts and/or

through their interaction with the glycine–tryptophane/thryptophane–glycine (GW/WG) AGO hook motifs present in in both Pol V [64-67] and its associated factor SUPPRESSOR OF TY INSERTION 5 (SPT5) [68,69]. Finally, AGO4–siRNA complexes recruit DOMAIN REARRANGED METHYLTRANSFERASE 2 (DRM2) protein that methylates target DNA [70,71]. Very recently, AGO4 interaction with DNA has been observed at RdDM targets. It appears that Pol V–dependent transcripts or their transcription are needed to lock Pol V into a stable DNA–bound configuration that allows AGO4 recruitment via Pol V and SPT5 AGO hook motifs [72].

AGO6 is also associated with RdDM in Arabidopsis, and thought initially to have partially redundant functions with AGO4 [73]. Later studies assigned more specific features and functions for AGO6, such as its preferential association for a unique set of heterochromatic sRNAs [74], or its dominant expression in shoot and root apical meristems and not in mature leaves [75]. More recently, it has been proposed that AGO6 may indeed work sequentially with AGO4 in the methylation of most target loci [76]. In addition, AGO6 also associates with RDR6–dependent 21–22 nt sRNAs to direct the methylation of transcriptionally active transposons in Arabidopsis [77].

Unexpectedly, a combination of genetic, biochemical and bioinformatic genome–wide analyses has recently showed that Arabidopsis AGO3, thought to function in PTGS as the other members of the AGO2/AGO7 clade, binds 24 nt sRNAs and can partially complement AGO4 function. The authors speculate with a role of AGO3 in RdDM in Arabidopsis [78], possibly under salinity stress when its expression is highly induced.

2.5 Emerging AGO Functions

Besides the well-characterized roles of plant AGOs in sRNA-mediated PTGS and TGS, new molecular functions have been described in the last years. First, Arabidopsis AGO10 and rice AGO18 sequester miR165/166 and miR168 from AGO1 to regulate shoot apical meristem (SAM) development [18] and antiviral defense [79], respectively. Rice AGO18 additionally sequesters miR528 from AGO1 upon viral infection to inhibit L-ascorbate oxidase (AO) mRNA cleavage by AGO1-miR528 complexes, thereby increasing AO-mediated accumulation of reactive oxygen species and enhancing antiviral defense [80]. Second, Arabidopsis AGO2 and AGO9 participate in the repair of double-strand break sites [81,82]. Third, AGO4 participates in an alternative siRNA biogenesis pathway by binding precursor transcripts that are subsequently subjected to 3'-5' exonucleolytic trimming for maturation and sidRNA (siRNA independent of DCLs) production [83]. AGO4-sidRNA complexes target Pol V transcripts to mediate DRM2-dependent DNA methylation. And fourth, a novel role for Arabidopsis AGO1 in the cotranscriptional regulation of *MIRNA* gene expression under salt stress conditions has been recently reported [84]. It seems that miRNA-loaded AGO1 interacts with chromatin at *MIR161* and *MIR173* loci, causing the disassembly of the transcriptional complex and the release of short and unpolyadenylated transcripts [84].

3 Biological Roles of Plant AGO Proteins

Plant AGOs have functionally diversified during evolution due to the expansion of the AGO family because of numerous duplications and losses [10,85,86]. The main biological roles of plant AGOs are listed in Table 1 and described next [*see* [4] for a recent review].

3.1 Plant AGOs and Development

The importance of AGOs in plant development became obvious after the characterization of the first *Arabidopsis ago1* mutants. These mutants –named “Argonaute” because of the resemblance of their leaf defects with the tentacles of a small squid of the *Argonauta* genus– presented important pleiotropic developmental defects such as dwarfing and sterility [87]. Later, developmental screens in *Arabidopsis* identified a series of hypomorphic *ago1* alleles with reduced developmental defects. The characterization of such mutants highlighted AGO1 role in leaf polarity and lateral organ development [88-90]. The organ polarity defects exhibited by *ago1* mutants suggested that AGO1 plays a role in the miRNA pathway, as these defects were similar to those of *phabulosa* (*phb*) and *phavoluta* (*phv*) miRNA gain-of-function mutants [91]. *Ago1* mutants have also been characterized in rice, and show obvious pleiotropic developmental defects such as severe dwarfism, tortuous shoots, narrow and rolled leaves and low seed-setting rates [92].

Other *Arabidopsis ago* mutants such as *ago7* or *ago10* present limited developmental defects, and others like *ago2*, *ago3*, *ago4*, *ago5* *ago6* and *ago9* have no obvious growth-related phenotypes [93]. AGO7 was identified in a screen for mutants displaying accelerated juvenile to adult phase change [94]. AGO7 associates exclusively with miR390 to target *TAS3* transcripts and initiate *TAS3*-based tasiRNA biogenesis leading to the targeting of several *AUXIN RESPONSE FACTOR* genes involved in the regulation of developmental timing and lateral organ development in *Arabidopsis* [15,35,95-98]. The observation that AGO7 also participates in *TAS3*-dependent tasiRNA biogenesis in moss [97] and in monocot species such as rice [99] and maize [100] indicates that AGO7 function in tasiRNA biogenesis is deeply conserved in plants.

Arabidopsis AGO10 mutants (previously known as *phn* from “pinhead” and *zll* from “zwille”) exhibit abnormal SAM development [101,102]. Despite that early analyses of Arabidopsis *ago1ago10* double mutants revealed functional redundancies between the two AGOs in some aspects of development [101], later observations have assigned specific roles for AGO10. Contrary to AGO1 which is expressed ubiquitously, AGO10 is predominantly expressed in the provascular, adaxial leaf primordia and the meristem [101,102]. AGO10 expression pattern is consistent with its roles in the maintenance of SAM development and leaf development in Arabidopsis [101,102] and rice [103]. More recent observations indicate that AGO10 sequesters miR165/miR166 from AGO1 to regulate SAM development [18], and associates with miR172 to favour floral determinacy [26].

3.2 Plant AGOs and Pathogen Defense

Plant AGOs play a key role in antiviral defense [for a recent review see [104]]. In antiviral silencing, highly structured or dsRNAs of viral origin are processed by plant DCLs into 21–24 nt virus-derived siRNAs (vsiRNAs). VsiRNAs associate with specific AGOs to target and repress cognate viral RNA through endonucleolytic cleavage or translational repression, or cognate viral DNA through hypermethylation or by regulating host gene expression to enhance antiviral defense [105]. Plant AGOs with roles in antiviral silencing include Arabidopsis AGO1, AGO2, AGO4, AGO5, AGO7 and AGO10, *N. benthamiana* AGO1 and AGO2, and rice AGO1 and AGO18. Plant AGOs can also bind sRNAs derived from viroids to attenuate viroid accumulation *in vivo* [106]. Interestingly, a recent report suggests that Arabidopsis AGO4 has direct antiviral activity against *Plantago asiatica mosaic virus* independent of its RdDM function [107].

In addition to their well-known role in antiviral defense, several Arabidopsis AGOs have antibacterial activity. In particular, AGO2 binds miR393b* to translationally repress the Golgi-localized *MEMB12* gene, resulting in the exocytosis of the pathogenesis-related protein PR1 with high antibacterial activity [108]. AGO4 is required for Arabidopsis resistance to *Pseudomonas syringae*, in a mode independent of other components of the RdDM pathway [109].

3.3 Plant AGO Functions In Meiosis And Gametogenesis

Plant AGOs have a key role during sexual reproduction, with specific AGOs being preferentially expressed in reproductive tissues and enriched in germline cells [110]. For instance, Arabidopsis AGO5 is expressed in the somatic cells around megaspore mother cells and in the megaspores, and *ago5* mutants are impaired in megagametogenesis initiation [111]. In rice, mutations in *MEIOSIS ARRESTED AT LEPTOTENE 1 (MEL1)* –one of the five AGO5 homologs in rice– induces precocious meiotic arrest and male sterility, with abnormal tapetums and aberrant pollen mother cells [112]. In maize, AGO9 is expressed in ovule somatic cells surrounding female meiocytes and contributes to non-CG DNA methylation in heterochromatin, and chromosome segregation is arrested during meiosis in *ago9* mutants [113]. Both Arabidopsis and maize AGO9 act in somatic cells to regulate cell fate specification in a non-cell autonomous manner. However, Arabidopsis AGO9 represses germ cell fate in somatic cells [114] while maize AGO9 inhibits somatic cell fate in germ cells [113].

4 Major Unknowns in the Plant AGO field

4.1 AGO Protein Interactors

In principle, plant AGOs likely need co-factors to exert their functions. However, in contrast to the situation observed in other organisms, a limited number of proteins interacting with plant AGOs has been described to date. Known plant AGO interactors are i) CYCLOPHILIN 40 (CYP40), HEAT SHOCK PROTEIN 90 (HSP90) and TRANSPORTIN 1 (TRN1) which interact with AGO1 and facilitate miRNA loading [115-118], and ii) the PolIV NRPE subunit [66], the transcription elongation factor SPT5 [68,69], and the putative oxidoreductase WGRP1 [119] which interact with AGO4 via their GW/WG AGO hook motifs. Systematic genome-wide scans for AGO protein interactors through more refined co-immunoprecipitation coupled with mass spectrometry analyses should identify a larger number of AGO partners, especially in response to abiotic or biotic stresses.

4.2 AGO Target RNAs

A fundamental requisite to understand AGO function is the identification of the whole spectrum of cellular target RNAs regulated by plant AGOs. Contrary to animal miRNAs, the majority of plant miRNAs regulate highly sequence complementary mRNAs [120]. This strict complementary feature of functionally relevant miRNA-target interactions made early bioinformatics studies highly successful in predicting miRNA targets in plants [28]. Molecular validation of numerous plant miRNA targets has relied on the amplification by 5' Rapid Amplification of cDNA Ends (RACE) of 3' cleavage products from cell extracts [121]. Because the isolation of loss-of-function miRNA mutants is difficult due mainly to the genetic redundancy in most miRNA families, the biological significance of individual miRNA-target interactions has been explored by other genetic approaches. These include the overexpression of miRNAs, miRNA-resistance targets or target mimics [122]. The first genome-

wide assessment of the repertoire of miRNA target RNAs regulated by cleavage corresponds to degradome sequencing analyses [30]. It appears that many conserved canonical targets have consistently strong degradome signatures, suggesting that this approach may be more likely to detect functionally relevant targets. Unfortunately, weak signatures are also recovered from several conserved canonical targets, and new potential targets do not follow the canonical parameters of base pairing. Therefore, the functional significance of degradome signatures is still not always clear [122]. All these approaches are useful to confirm or discover miRNA targets but do not reveal which specific AGO member mediates their regulation.

Genome-wide analysis of AGO-bound target RNAs have been reported in animals by applying a step of *in vivo* crosslinking (generally using ultraviolet light) in intact cells of tissues before immunoprecipitating the AGO of interest and analysing by high-throughput sequencing the co-immunoprecipitated AGO-bound RNAs [123]. Such AGO crosslinking immunoprecipitation followed by sequencing (CLIP-Seq) approaches have not been reported in plants. This could be due because, in contrast with animals where the majority of miRNA targets are not sliced, AGO-sRNA-target RNA interactions are ephemeral for the majority of plant target RNAs that might be immediately sliced upon AGO-miRNA recognition. Indeed, recent AGO RNA immunoprecipitation followed by high-throughput sequencing (RIP-Seq) analysis of AGO1-bound RNAs in Arabidopsis revealed that target RNAs are more efficiently co-immunoprecipitated with slicer-deficient AGO1 forms [24]. This suggests that AGO1 ternary complexes including miRNAs and target RNAs are more stable when AGO1 is catalytically inactive. By comparing the pool of target RNAs recovered from immunoprecipitates containing catalytically active or inactive AGO1 forms it is possible to identify the repertoire of AGO1 target RNAs regulated by slicing and

those regulated in a slicing-independent mode. Moreover, the application of this methodology to the different Arabidopsis AGOs could reveal the specific pool of target RNAs regulated by each specific AGO in different stress conditions or cell types. Understanding AGO-sRNA-target RNA dynamics is crucial to better understand sRNA-mediated gene silencing in plants.

4.3 AGO Transcriptional Regulators

While some plant AGOs such as Arabidopsis AGO1 and AGO4 are ubiquitously expressed, others have a more restricted expression. This is the case of Arabidopsis AGO9 and AGO10, which are expressed in female gamete and their accessory cells [114], or in provascular, adaxial leaf primordia and the meristem [101,102], respectively. Moreover, several AGOs are induced upon abiotic or biotic stress. For instance, rice AGO18 accumulation is induced upon viral infection [79], while AGO2 and AGO3 accumulation is induced by gamma-irradiation and bacterial infection [108], and salt stress [78], respectively. The differential spatiotemporal expression of the distinct AGO members as well as the induction of certain AGOs upon stress suggests that AGO transcription may be regulated. However, transcriptional regulators of plant AGOs are largely unknown. Only recently, it was shown that Arabidopsis AGO10 expression is activated by at least one homeodomain-leucine zipper (HD-ZIP) transcription factor [124], and inhibited by the LBD12-1 transcription factor that directly binds to AGO10 promoter [125].

5 Conclusions and Future Challenges

Intensive research over the past two decades has elucidated the main functions of plant AGOs. However, future research should identify new functions for plant AGOs,

as occurred for AGOs from other organisms. Emerging functions of non-plant AGOs include nonsense-mediated mRNA decay regulation in humans [126], alternative splicing in humans [127,128] and *Drosophila* [129], sRNA-independent association with full-length introns (called “agotrons”) to control gene expression in humans and probably in other mammals [130], nucleosome occupancy at human transcription start sites [131] and quality control of human proteins entering the secretory pathway [132]. Remarkably, DNA-guided genome editing has been recently reported in human cells using *Natronobacterium gregoryi* AGO [133], although failure to replicate these results by other groups [134] has questioned the general applicability of this approach.

Several outstanding questions remain to be answered in the plant AGO field. At the molecular level, more structural work is needed to better understand the formation of AGO ternary complexes. In particular, how AGOs scan and find target transcripts? Or, how ternary complexes dissociate? Indeed, to date no crystal structure for a complete plant AGO has been solved. Also, besides AGO4 binding to sidRNA precursors, can other AGOs regulate target RNAs in a sRNA guide-independent mode? Regarding sRNA-mediated translational repression of target RNAs, what is the degree of miRNA-target RNA complementarity necessary to support the translational inhibition activity of plant miRNAs? Can AGOs other than AGO1 or AGO10 be programmed to function in a translational repression mode? And for those target RNAs regulated by slicing and translational repression [39,46,135], what mechanism(s) underlie the choice between these two modes of action? At a cellular level, how AGO ternary complexes are programmed in different cell-types and tissues? Cell-type specific profiling of AGO-small RNA-target RNA dynamics in different cell-types and tissues should shed light on the role of the different AGO modules in the large regulatory networks established during development and stress

response. Because of the broad interest of these fundamental questions, I anticipate that at least some of them will be answered soon.

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Table 1 Biological roles of plant ARGONAUTES

Function	AGO involved ^a	References
Antibacterial immunity	AtAGO2 AtAGO4	[108] [109]
Antiviral defense	AtAGO1 AtAGO2 AtAGO4 AtAGO5 AtAGO7 AtAGO10 NbAGO1 NbAGO2 OsAGO1a/b OsAGO18	[6,136-139] [17,24,136,138,140-143] [107,142,144-147] [136,148] [136,137] [136] [149] [150-152] [79] [79]
Cell specification Gamete Somatic	AtAGO9 ZmAGO9	[114] [113]
Chromosome segregation	ZmAGO9	[113]
Development	AtAGO1 OsAGO1a/b/c SiAGO1b	[22-24,87,153] [92] [154]
DNA methylation	AtAGO3 AtAGO4 AtAGO6 OsAGO4a OsAGO4b	[78] [25,64,65,72,155,156] [73,74,76,77,156,157] [158] [158]
DNA repair	AtAGO2 AtAGO9	[81] [82]
Germ cell development	ZmAGO18b	[159]
Leaf development	AtAGO1 AtAGO7 OsAGO10 ZmAGO7	[87,88] [95,160] [103] [100]
Meiosis	AtAGO4 OsAGO5c	[161] [112]
Megagametogenesis	AtAGO5	[111]
Phase transition	AtAGO7	[15,24,94,95]
SAM development	AtAGO10 OsAGO7	[18,26,101,102,162,163] [99]
SAM maintenance	OsAGO10	[103]
Small RNA biogenesis miRNA siRNA tasiRNA	AtAGO1 AtAGO4 AtAGO1 AtAGO7 OsAGO7 ZmAGO7	[84] [83] [34] [15] [99] [100]
Stress response	AtAGO1 SiAGO1b	[164,165] [154]
Tapetum development	ZmAGO18b	[159]

^a At, *Arabidopsis thaliana*; Nb, *Nicotiana benthamiana*; Os, *Oryza sativa*; Si, *Setaria italica*, Zm, *Zea mays*

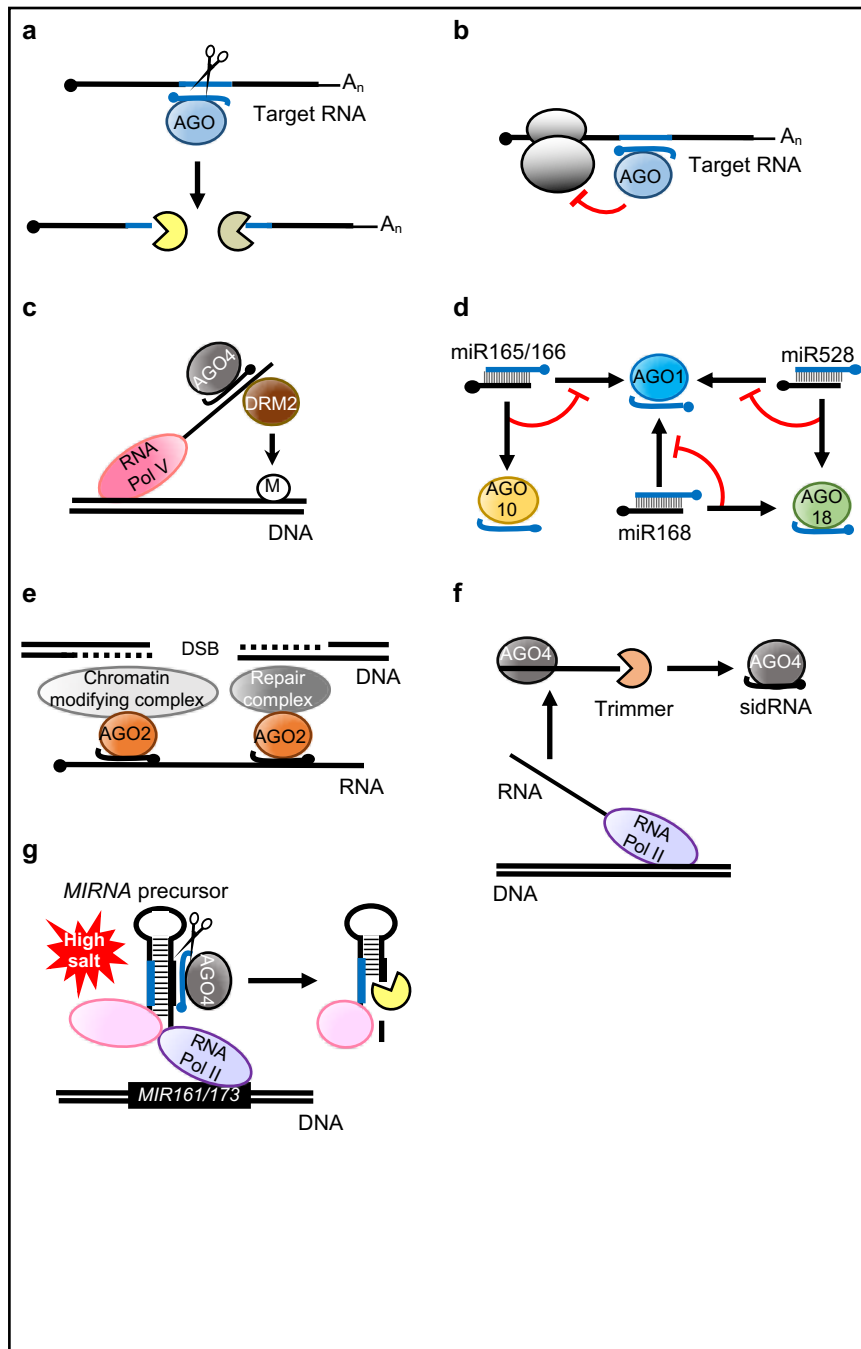


Fig.1 Modes of action of plant AGOs. **(a)** Endonucleolytic cleavage. Several AGOs bind sRNAs and slice highly sequence complementary target RNAs. Cleavage products are degraded by components of endogenous degradation pathways. **(b)** Translational repression. Plant AGOs such as Arabidopsis AGO1 and AGO10 associate with miRNAs and target highly complementary RNAs to inhibit their translation. **(c)** Canonical RdDM pathway. AGO4–siRNA complexes bind to nascent

Pol V transcripts. DRM2 is recruited to mediate DNA methylation. **(d)** MiRNA sequestration. Arabidopsis AGO10 sequesters miR165/166 from AGO1, while rice AGO18 sequesters miR168 and miR528 from AGO1. **(e)** Double-stranded break repair (DSB). Arabidopsis AGO2 binds to DSB-induced siRNAs (diRNAs) to mediate DSB repair. **(f)** DCL-independent siRNA biogenesis. AGO4 binds to nascent Pol II RNAs which are trimmed by a 3'–5' exonuclease to produce sidRNAs. **(g)** Cotranscriptional regulation of *MIRNA* gene expression. Upon salinity stress AGO4 directs the slicing of nascent *MIR161* or *MIR173* precursors to cotranscriptionally regulate miRNA production.