Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

# Evolution, functions, and mysteries of plant ARGONAUTE proteins

Han Zhang[1], Rui Xia[2], Blake C Meyers[2] and Virginia Walbot[1]

ARGONAUTE (AGO) proteins bind small RNAs (sRNAs) to form RNA-induced silencing complexes for transcriptional and post-transcriptional gene silencing. Genomes of primitive plants encode only a few AGO proteins. The *Arabidopsis thaliana* genome encodes ten AGO proteins, designated AGO1 to AGO10. Most early studies focused on these ten proteins and their interacting sRNAs. AGOs in other flowering plant species have duplicated and diverged from this set, presumably corresponding to new, diverged or specific functions. Among these, the grass-specific AGO18 family has been discovered and implicated as playing important roles during plant reproduction and viral defense. This review covers our current knowledge about functions and features of AGO proteins in both eudicots and monocots and compares their similarities and differences. On the basis of these features, we propose a new nomenclature for some plant AGOs.

**Addresses**
[1] Department of Biology, Stanford University, Stanford, CA 94305, USA
[2] Department of Plant and Soil Sciences and Delaware Biotechnology Institute, University of Delaware, Newark, DE 19716, USA

Corresponding author: Zhang, Han (zhanghan@stanford.edu)

**Current Opinion in Plant Biology** 2015, **27**:84–90

This review comes from a themed issue on **Cell signalling and gene regulation**

Edited by **Xiaofeng Cao** and **Blake C Meyers**

For a complete overview see the Issue and the Editorial

Available online 17th July 2015

http://dx.doi.org/10.1016/j.pbi.2015.06.011



## Introduction

In eukaryotes, sRNA-mediated gene silencing is crucial for developmental regulation, responses to environmental stimuli, and epigenetic control of transposable elements (TEs) [1,2]. All types of described sRNAs are known to associate with AGO proteins to form RNA-induced silencing complexes (RISCs). Each RISC is guided by the bound sRNAs to achieve specific interactions with target transcripts based on sequence complementarity. These interactions can result in mRNA cleavage, translational repression, or chromatin modification [3,4]. On the basis of their functional domains, eukaryotic AGO proteins can be divided into two major groups: the AGO and the PIWI subfamilies. PIWIs and their interacting sRNAs (piRNAs) are both found predominantly in animal germ lines [5]. Plant genomes encode multiple AGO proteins, all in the AGO subfamily [3,4].

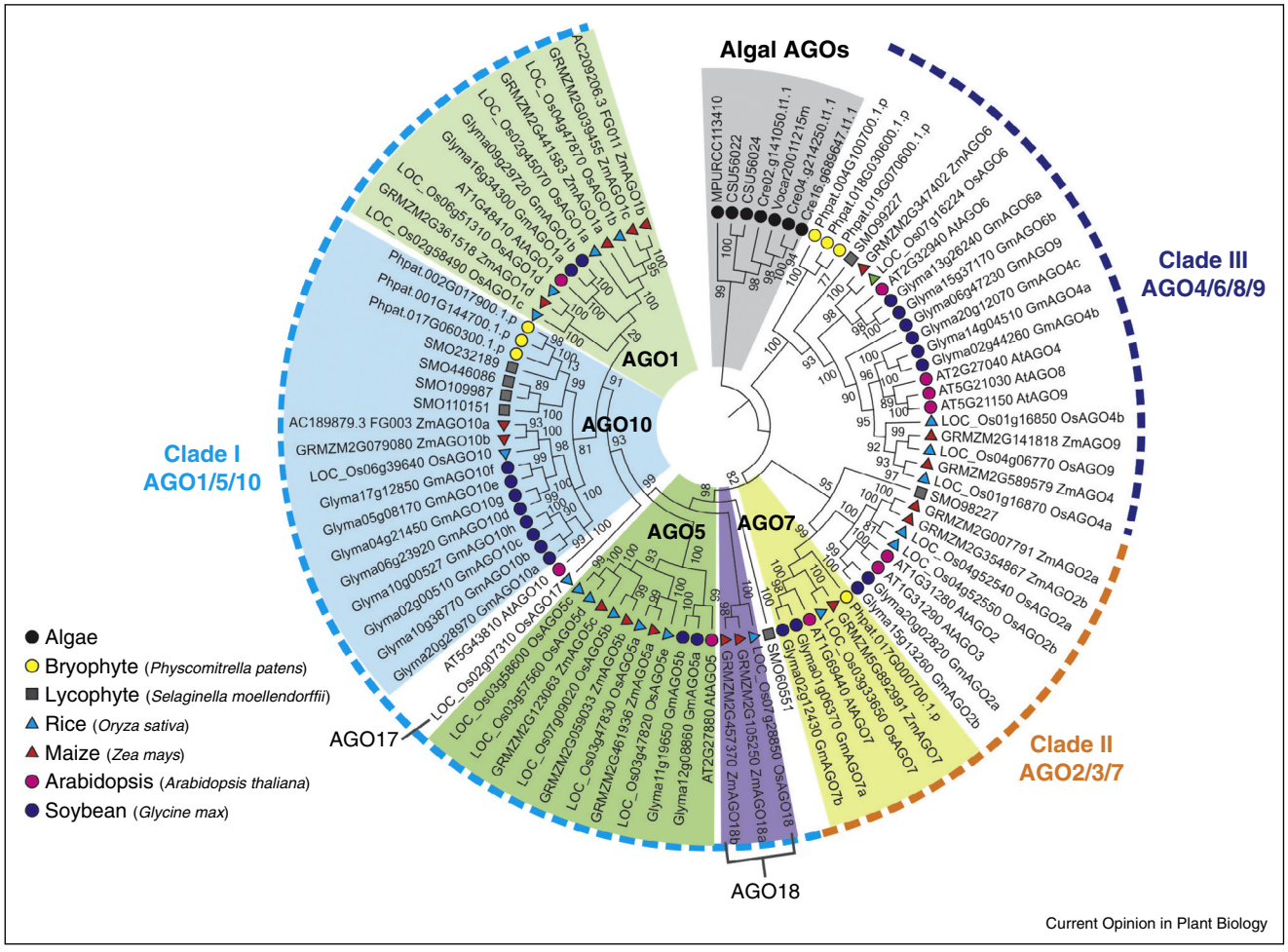
Taking advantage of sequenced plant genomes, we examined the AGO phylogenetic relationships using four selected angiosperms (two eudicots and two grasses) and utilized several basal non-flowering plant taxa to track the expansion of AGOs over a long period of plant evolution (Figure 1). The Arabidopsis genome encodes ten AGO proteins [3], but this number is increased in other flowering plants: 22 AGO proteins in soybean (*Glycine max*, a paleopolyploid) [6], 19 in rice (*Oryza sativa*) [7], and 17 in maize (*Zea mays*) [8,9] (Figure 1). All flowering plant AGOs group into three major clades: AGO1/5/10, AGO2/3/7, and AGO4/6/8/9 [3]. For simplicity, we designated these as clades I, II, and III (Figure 1). The grasses exhibit an expanded first clade and equivalent or similar size second and third clades. Additionally, grasses have an extra subclade — AGO18, a deep branch in the AGO1/5/10 clade (Figure 1). Unless specified, the term 'AGO' in this review is referring to a class of AGOs in all flowering plants, while particular AGOs in a species are named with two letters representing the abbreviated species names, for example, AtAGO for an Arabidopsis AGO protein.

## Features and functions of plant AGO proteins

The AGO family expanded during plant evolution with numerous duplications and losses [10]. In ancient unicellular or multicellular green algae (*Micromonas pusilla*, *Volvox carteri*, *Coccomyxa subellipsoidea*, *Chlamydomonas reinhardtii*), few (≤3) AGO genes are present [11]. The family expanded to seven members in mosses (*Physcomitrella patens*) and lycophytes (*Selaginella moellendorffii*), and further to ten or more members in flowering plants (Figure 1). The expansion of the plant AGO family suggests a functional diversification of AGO proteins presumably reflecting expanding sRNA-directed regulatory pathways.

Our understanding of AGO functions and their small RNA binding preferences is informed by sequencing of sRNAs associated with AGO proteins and by functional analysis of *ago* mutants in Arabidopsis and other plants. In Table 1, well-characterized plant AGO proteins are listed with their interacting sRNAs, binding preferences, and demonstrated functions. Because of the rapid rate of whole genome sequencing, AGO protein identification via genome annotation has outpaced functional characterization, the latter

**Figure 1**




Phylogenetic tree of AGO proteins in plants. Representative plant species were selected for the illustration of AGO protein evolution in plants. Full length protein sequences were aligned using Clustal Omega (http://www.clustal.org/omega/) and the tree was constructed using FastTree with default settings. AGOs from different lineages or species were coded with various shapes of distinct colors. Abbreviations for AGOs from basal non-flowering plants were as follows, SMO: *Selaginella moellendorffii*; Phpat: *Physcomitrella patens*; Cre: *Chlamydomonas reinhardtii*; Voca: *Volvox carteri*; CSU: *Coccomyxa subellipsoidea*; MPURCC: *Micromonas pusilla* CCMP1545.

typically done via mutant analysis [6–9]. Because of the errors in genome annotation and the naming history, AGO nomenclature in flowering plants is somewhat inconsistent and confusing. We propose a phylogeny-based nomenclature and list names assigned in previous literature, as a guide to published analyses (Table 2).

### Clade I — AGO1/5/10

*AtAGO1* is the founding member of the *AGO* gene family, so named because Arabidopsis *ago1* mutants resemble the tentacles of an *Argonauta* squid [12]. AGO1 preferentially associates with sRNAs with a 5′ uridine; it plays an essential role in miRNA-mediated regulation of development and stress responses [12–14,15••]. Although only one *AGO1* exists in *A. thaliana*, four *AGO1* homologs have been identified and characterized in rice [15••]. Deep sequencing of sRNAs bound in OsAGO1a, OsAGO1b, and OsAGO1c complexes in seedlings showed that most miRNAs are evenly distributed in the three AGO1 complexes tested, but a subset of miRNAs are specifically incorporated into or excluded from one of these three AGO1s [15••]. These observations suggest that there are both redundant and specialized functions among the rice *AGO1* genes. The binding specificity of OsAGO1d has not yet been analyzed.

*AtAGO10* is closest to *AtAGO1*, and it regulates shoot apical meristems by sequestering miR165/166 [16–18]. The miR165/166 family is produced from nine Arabidopsis loci and targets class III homeodomain-leucine zipper (HD-ZIP III) transcription factors. AtAGO10 recognizes distinct structural features in miR165/166 duplexes that

**Table 1**

**Characterized AGO proteins in plants**[a]

| Clade | AGO name | Function | 5′-Nucleotide preference | sRNA bound | Reference |
|---|---|---|---|---|---|
| Clade I: AGO1/5/10 | AtAGO1 | Plant development regulation and stress responses | U | 21-nt miRNAs | [12,14] |
| | OsAGO1a | miRNA-directed target gene regulation | U | 21-nt miRNAs | [15••] |
| | OsAGO1b | miRNA-directed target gene regulation | U | 21-nt miRNAs | [15••] |
| | OsAGO1c | miRNA-directed target gene regulation | U | 21-nt miRNAs | [15••] |
| | AtAGO5 | Initiation of megagametogenesis | C | siRNAs | [14,21] |
| | OsAGO5c | Regulation of cell division of premeiotic germ cells | C | 21-nt siRNAs | [22,23••] |
| | AtAGO10 | Regulation of shoot apical meristems | n/d | miR165/166 | [16–18] |
| | OsAGO18 | Broad-spectrum virus resistance | n/d | n/d | [28••] |
| | ZmAGO18b | Tapetum and germ cell development | n/d | n/d | [9,25••] |
| Clade II: AGO2/3/7 | AtAGO2 | Antibacterial immunity, viral defense, and DSB-induced sRNAs activity | A | 21-nt miRNAs, vsiRNAs, diRNAs | [30–33] |
| | AtAGO3 | n/d | n/d | n/d | |
| | AtAGO7 | *TAS3*-derived tasiRNA biogenesis | n/d | miR390 | [34] |
| | OsAGO7 | *TAS3*-derived tasiRNA biogenesis | n/d | miR390 | [35,38] |
| | ZmAGO7 | *TAS3*-derived tasiRNA biogenesis | n/d | miR390 | [36] |
| Clade III: AGO4/6/8/9 | AtAGO4 | RdDM pathway | A | 24-nt siRNAs | [39,40,42] |
| | AtAGO6 | Methylation of tasiRNA-generating loci and transcriptionally active TEs | A | 24-nt siRNAs and 21-22-nt endo-siRNAs | [45••] |
| | AtAGO8 | Proposed to be a pseudogene | n/d | n/d | |
| | AtAGO9 | Germ cell fate repression in the somatic companion cells surrounding MMC | A | 24-siRNAs | [46] |
| | ZmAGO9 | Somatic cell fate repression in the germ cells | n/d | n/d | [47] |

n/d, not determined.
[a] Due to space limitations, only the main function and major associated sRNA type is listed.

are absent from other miRNAs and it has a higher affinity for miR166 than does AtAGO1. As a result, AtAGO10 specifically sequesters miR165/166, preventing their loading into AtAGO1, and thereby upregulating *HD-ZIP III* transcripts [16–18]. One and two AGO10 homologs were identified in rice and maize, respectively [7,8], while eight were found in soybean (Figure 1) [6]. The expansion of the GmAGO10 family probably co-evolved with the expansion of the miR165/166 family, as 21 copies of miR165/166 are annotated in the soybean genome [19,20].

*AtAGO5* is specifically expressed in the somatic cells surrounding megaspore mother cells and in megaspores [21]. While Arabidopsis *ago5* null mutants lack obvious defects, a semi-dominant allele is impaired in initiation of megagametogenesis [21]. The grass *AGO5* family has expanded, with five homologs in rice and three in maize (Figure 1). Among these, only *MEL1* (*MEIOSIS ARRESTED AT LEPTOTENE1*, *OsAGO5c*) has been well studied. Rice *mel1/ago5c* mutants display abnormal periclinal cell division in the somatic tissues of anther lobes and later exhibit defects in pollen mother cells (PMCs) [22]. It is still unclear whether MEL1 affects rice sporogenesis directly by acting in PMCs and meiocytes, or whether the earlier somatic defects indirectly cause meiotic arrest. Recently it was shown that MEL1 binds to 21-nt phased small RNAs (phasiRNAs), a highly abundant sRNA group in grass anthers [23••,24,25••]. Like AtAGO5, MEL1 binds preferentially to sRNAs with a 5′ cytosine [23••]. Interestingly, this preference was not observed in the total 21-nt phasiRNA population of rice or maize [24,25••,26•,27•], suggesting that MEL1 may not be the only AGO protein that binds 21-nt phasiRNAs.

The *AGO18* subclade has been found only in grass genomes. In rice, AGO18 protein is not detectable in normal seedlings but is induced by viral infection [28••]. Overexpression of *OsAGO18* confers broad-spectrum virus resistance in rice. A Tos17 retrotransposon insertional mutant of *ago18* is more sensitive to viral infection, although no other plant phenotypes were observed [28••]. Interestingly, viral siRNAs are highly enriched in OsAGO1 complexes but only account for a small percentage of sRNAs bound to OsAGO18 [28••]. Therefore, OsAGO18 regulates or indirectly contributes to antiviral defense response by a strategy other than directly binding viral siRNAs.

In maize, two copies of *AGO18* exist, one of which (*ZmAGO18b*) is enriched in the tapetum and germinal cells during male meiosis [9]. This pattern of expression mimics the accumulation of the 24-nt phasiRNAs that are derived from unique intergenic regions and are processed by DICER-LIKE 5 (DCL5)/DCL3b (originally named

**Table 2**

**Proposed nomenclature of AGO proteins in the four selected flowering plants**[a]

| Gene identifier (eudicots) | Proposed name | Previous name | Gene identifier (monocots) | Proposed name | Previous name |
|---|---|---|---|---|---|
| AT1G48410 | AtAGO1[b] | | LOC_Os02g45070 | OsAGO1a[b] | |
| AT1G31280 | AtAGO2[b] | | LOC_Os04g47870 | OsAGO1b[b] | |
| AT1G31290 | AtAGO3[b] | | LOC_Os02g58490 | OsAGO1c[b] | |
| AT2G27040 | AtAGO4[b] | | LOC_Os06g51310 | OsAGO1d[b] | |
| AT2G27880 | AtAGO5[b] | | LOC_Os04g52540 | OsAGO2a | OsAGO2 [7] |
| AT2G32940 | AtAGO6[b] | | LOC_Os04g52550 | OsAGO2b | OsAGO3 [7] |
| AT1G69440 | AtAGO7[b] | | LOC_Os01g16870 | OsAGO4a[b] | |
| AT5G21030 | AtAGO8[b] | | LOC_Os01g16850 | OsAGO4b | OsAGO15 [7] |
| AT5G21150 | AtAGO9[b] | | LOC_Os03g47830 | OsAGO5a | OsAGO11 [7] |
| AT5G43810 | AtAGO10[b] | | LOC_Os07g09020 | OsAGO5b | OsAGO14 [7] |
| Glyma16g34300 | GmAGO1a[b] | | LOC_Os03g58600 | OsAGO5c | OsMEL1 [7] |
| Glyma09g29720 | GmAGO1b[b] | | LOC_Os03g57560 | OsAGO5d | OsAGO13 [7] |
| Glyma20g02820 | GmAGO2a | GmAGO3a [6] | LOC_Os03g47820 | OsAGO5e | OsAGO12 [7] |
| Glyma15g13260 | GmAGO2b | GmAGO3b [6] | LOC_Os07g16224 | OsAGO6 | OsAGO16 [7] |
| Glyma14g04510 | GmAGO4a[b] | | LOC_Os03g33650 | OsAGO7 | SHL4 [7] |
| Glyma02g44260 | GmAGO4b[b] | | LOC_Os04g06770 | OsAGO9 | OsAGO4b [7] |
| Glyma20g12070 | GmAGO4c[b] | | LOC_Os06g39640 | OsAGO10 | OsPNH1 [7] |
| Glyma12g08860 | GmAGO5a[b] | | LOC_Os02g07310 | OsAGO17[b] | |
| Glyma11g19650 | GmAGO5b[b] | | LOC_Os07g28850 | OsAGO18[b] | |
| Glyma13g26240 | GmAGO6a[b] | | GRMZM2G441583 | ZmAGO1a[b] | |
| Glyma15g37170 | GmAGO6b[b] | | AC209206.3_FG011 | ZmAGO1b | ZmAGO1c [9] |
| Glyma01g06370 | GmAGO7a[b] | | GRMZM2G039455 | ZmAGO1c | ZmAGO1b [9] |
| Glyma02g12430 | GmAGO7b[b] | | GRMZM2G361518 | ZmAGO1d | ZmAGO1f [9] |
| Glyma06g47230 | GmAGO9[b] | | GRMZM2G007791 | ZmAGO2a | ZmAGO2 [8] |
| Glyma20g28970 | GmAGO10a[b] | | GRMZM2G354867 | ZmAGO2b | ZmAGO7 [8] |
| Glyma10g38770 | GmAGO10b[b] | | GRMZM2G589579 | ZmAGO4[b] | |
| Glyma02g00510 | GmAGO10c[b] | | GRMZM2G461936 | ZmAGO5a | ZmAGO5d [9] |
| Glyma06g23920 | GmAGO10d[b] | | GRMZM2G059033 | ZmAGO5b | ZmAGO5a [9] |
| Glyma05g08170 | GmAGO10e[b] | | GRMZM2G123063 | ZmAGO5c | ZmAGO5b [9] |
| Glyma17g12850 | GmAGO10f[b] | | GRMZM2G347402 | ZmAGO6 | ZmAGO5c [9] |
| Glyma04g21450 | GmAGO10g[b] | | GRMZM5G892991 | ZmAGO7[b] | |
| Glyma10g00527 | GmAGO10h[c] | | GRMZM2G141818 | ZmAGO9 | AGO104 [45] |
| | | | | | ZmAGO4d [8] |
| | | | AC189879.3_FG003 | ZmAGO10a[b] | |
| | | | GRMZM2G079080 | ZmAGO10b[b] | |
| | | | GRMZM2G105250 | ZmAGO18a[b] | |
| | | | GRMZM2G457370 | ZmAGO18b | ZmAGO18c [8] |

[a] Our review of the literature may not be complete.
[b] Indicates previous and proposed names are identical; numbers indicate the reference, where previous names are used.
[c] Newly identified in this work.

DCL3b, renamed as DCL5 thereafter) [25••,26•]. DCL5, AGO18, as well as the 24-nt phasiRNAs are all absent from eudicot genomes analyzed to date [25••,29]. On the basis of the co-expression of *ZmAGO18* and 24-nt phasiRNAs, and the coincident novelty of their appearance, ZmAGO18b is hypothesized to bind the 24-nt phasiRNAs [25••]. How and by virtue of what selective pressures this apparently novel sRNA pathway evolved in grasses is still a mystery.

### Clade II — AGO2/3/7

In Arabidopsis, AGO2 primarily associates with miRNAs with a bias for a 5′ adenosine [14,30] and functions in antibacterial immunity [31]. Besides miRNAs, it also binds virus-derived small interfering RNAs (vsiRNAs) [32] and sRNAs induced by double-strand DNA breaks (diRNAs) [33]. *AtAGO3* is very similar to *AtAGO2*, and they are 3 kb apart in a direct tandem repeat [3]. Neither rice nor maize encodes an *AGO3* ortholog, but both have a pair of similar *AGO2* genes, designated *AGO2a* and *AGO2b* [7,9].

The function of the third member of this clade is better known and highly conserved: AGO7 predominantly binds miR390 and triggers the generation of *TAS3*-derived *trans*-acting siRNAs (tasiRNAs). *TAS3*-tasiRNAs target several *AUXIN RESPONSE FACTORS* and regulate dorsiventral leaf patterning and lateral organ development in flowering plants [34–38].

### Clade III — AGO4/6/8/9

Arabidopsis AGO4 primarily binds 24-nt, repeat and heterochromatin-associated siRNAs and functions in RNA-directed DNA methylation (RdDM) [39–40]. Transcripts from RNA POLYMERASE IV (Pol IV) are copied into double-stranded RNAs (dsRNAs) by

RNA-DEPENDENT RNA POLYMERASE 2 (RDR2). The dsRNAs are processed by DCL3 into 24-nt siRNAs and incorporated into AtAGO4. These AtAGO4-bound siRNAs guide the targeting of nascent scaffold transcripts from RNA POLYMERASE V by sequence complementarity and recruit DNA methyltransferase activity to mediate *de novo* methylation [41].

Besides the RdDM pathway directed by 24-nt siRNAs, a recent report found that Arabidopsis 21-nt tasiRNAs can also direct RdDM [42]. Instead of being processed by DCL4 and loaded into AGO1 for target mRNA cleavage in *trans*, these tasiRNAs are processed by DCL1 and loaded into AtAGO4 or AtAGO6; these RISCs direct methylation of *TAS* loci [42]. Pol II transcripts from TEs can be converted to dsRNAs by RDR6 to produce 21–22 -nt endogenous siRNAs (endo-siRNAs), which can be loaded into AtAGO1 for post-transcriptional gene silencing [43,44] or loaded into AtAGO6 to promote methylation of transcriptionally active TEs [45••]. DNA methylation directed by both tasiRNAs and endo-siRNAs is independent of Pol IV, DCL3 and RDR2, but does depend on RDR6 [41].

In Arabidopsis, AGO9 controls female gamete formation by repressing the specification of germ cell fate in the somatic companion cells [46]. *ago9* mutants of *A. thaliana* contain multiple megaspore mother cells. AtAGO9 preferentially interacts with 24-nt sRNAs derived from TEs and is necessary to silence TEs in female gametes [46]. *AtAGO8* and *AtAGO9* are very similar in sequence [3], but *AtAGO8* transcripts are barely detectable in all tested tissues; *AtAGO8* is proposed to be a pseudogene [4].

*ZmAGO9* (initially named *ZmAGO104* in the literature, Table 2) is also specifically expressed in the somatic cells surrounding the precursors of the gametic cells [47]. But unlike AtAGO9 which represses germ cell fate in the somatic cells, ZmAGO9 represses somatic cell fate in the germ cells. The *ago104* mutant exhibits unreduced (diploid) gametes, abnormal chromatin condensation during meiosis, and hypomethylated centromeric and knob-repeat DNA at non-CG sites [47]. In rice, an AGO member (previously OsAGO4b) shows a close homology to ZmAGO9 (Figure 1), we therefore suggest renaming it as OsAGO9.

## Why so many AGOs?

In ancient alga, all AGOs belong to a single group, which diversified into distinct clades in bryophytes (Figure 1). The presence of these AGO clades in primitive plants suggests that sRNA-guided processes were already well-established long before the seed plants. Ferns (present ~200 mya prior to flowering plants) are a major missing link in terms of genome sequences, required for connecting the lycophytes to the more recent gymnosperms and angiosperms. Within the angiosperms, the major groups of AGO proteins have all been retained; certain subgroups have expanded differently in monocots and eudicots (Figure 1).

Genome duplication is one explanation for the proliferation of the AGOs. For example, soybean is a paleopolyploid [48] and its genome encodes two copies of AGO1, AGO2, AGO4/9, AGO5, AGO6, and AGO7 (Figure 1). Gene duplication events and promoter subfunctionalization could generate cell type specificity or the ability to respond to specific conditions. *AtAGO2* and *AtAGO3*, *AtAGO8* and *AtAGO9*, *OsAGO2a* and *OsAGO2b*, *OsAGO5a* and *OsAGO5e*, as well as *OsAGO4a* and *OsAGO4b* have all been suggested to arise from gene duplication [3,7]. These now-diverged genes have non-redundant expression profiles [4,7] and hence may be amenable to analysis by genetic disruption in single mutants. Functional specialization of AGOs could also occur to bind a certain type of sRNAs for a specific function, as exemplified by the specialization of AGO10-miR165/166 and of AGO7-miR390 [2].

Although *AGO* genes have been extensively studied in Arabidopsis, only a few members of the AGO family have been analyzed in depth in the monocots and other eudicots (Table 1). In those species, many fundamental questions regarding plant AGOs remain to be answered. Do they function exactly the same as or similarly to their homologs in Arabidopsis? If multiple homologs exist, do they have redundant or specific roles? Why are some genes, such as *OsAGO17*, found only in rice and are missing in other species tested? From where did the grass-specific AGO18 originate? Biochemical studies are also a high priority for future research: are all sRNAs bound to an AGO? Can RISC functions be redirected by exchange of sRNAs?

In summary, the diversification of sRNA-mediated regulation in plants is a fascinating story. This regulation is mediated by AGO proteins bound to guide sRNAs. In this review we have attempted to summarize what is currently known about the phylogeny of AGO proteins in flowering plants, the diverse roles and preferences of the individual AGO types, and the directions for future research.

## Acknowledgements

This work was supported by grants from the National Science Foundation including award #1339229 from the Plant Genome Research Program and IOS award #1257869.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Axtell MJ: **Classification and comparison of small RNAs from plants**. *Annu Rev Plant Biol* 2013, **64**:137-159.

2. Bologna NG, Voinnet O: **The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis**. *Annu Rev Plant Biol* 2014, **65**:473-503.

3. Vaucheret H: **Plant ARGONAUTES**. *Trends Plant Sci* 2008, **13**:350-358.

4. Mallory A, Vaucheret H: **Form, function, and regulation of ARGONAUTE proteins**. *Plant Cell* 2010, **22**:3879-3889.

5. Ross RJ, Weiner MM, Lin H: **PIWI proteins and PIWI-interacting RNAs in the soma**. *Nature* 2014, **505**:353-359.

6. Liu X, Lu T, Dou Y, Yu B, Zhang C: **Identification of RNA silencing components in soybean and sorghum**. *BMC Bioinform* 2014, **15**:4.

7. Kapoor M, Arora R, Lama T, Nijhawan A, Khurana JP, Tyagi AK, Kapoor S: **Genome-wide identification, organization and phylogenetic analysis of Dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice**. *BMC Genomics* 2008, **9**:451.

8. Qian Y, Cheng Y, Cheng X, Jiang H, Zhu S, Cheng B: **Identification and characterization of Dicer-like, Argonaute and RNA-dependent RNA polymerase gene families in maize**. *Plant Cell Rep* 2011, **30**:1347-1363.

9. Zhai L, Sun W, Zhang K, Jia H, Liu L, Liu Z, Teng F, Zhang Z: **Identification and characterization of Argonaute gene family and meiosis-enriched Argonaute during sporogenesis in maize**. *J Integr Plant Biol* 2014, **56**:1042-1052.

10. Singh RK, Gase K, Baldwin IT, Pandey SP: **Molecular evolution and diversification of the Argonaute family of proteins in plants**. *BMC Plant Biol* 2015, **15**:23.

11. Kim EJ, Ma X, Cerutti H: **Gene silencing in microalgae: mechanisms and biological roles**. *Bioresour Technol* 2015, **184**:23-32.

12. Bohmert K, Camus I, Bellini C, Bouchez D, Caboche M, Benning C: **AGO1 defines a novel locus of Arabidopsis controlling leaf development**. *EMBO J* 1998, **17**:170-180.

13. Rogers K, Chen X: **Biogenesis, turnover, and mode of action of plant microRNAs**. *Plant Cell* 2013, **25**:2383-2399.

14. Mi S, Cai T, Hu Y, Chen Y, Hodges E, Ni F, Wu L, Li S, Zhou H, Long C *et al.*: **Sorting of small RNAs into Arabidopsis argonaute complexes is directed by the 5′ terminal nucleotide**. *Cell* 2008, **133**:116-127.

15. •• Wu L, Zhang Q, Zhou H, Ni F, Wu X, Qi Y: **Rice MicroRNA effector complexes and targets**. *Plant Cell* 2009, **21**:3421-3435.
This is the first paper that compares functions and sRNA binding preferences of an Arabidopsis AGO to its homologs in rice. Using antibodies specific for each AGO1 proteins, the authors showed that, like AtAGO1, OsAGO1a, OsAGO1b and OsAGO1c each has a strong preference for sRNAs with a 5′ uridine. The authors also demonstrated that these three proteins have both redundant and specialized sRNA bindings.

16. Zhu H, Hu F, Wang R, Zhou X, Sze SH, Liou LW, Barefoot A, Dickman M, Zhang X: **Arabidopsis Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development**. *Cell* 2011, **145**:242-256.

17. Zhang Z, Zhang X: **Argonautes compete for miR165/166 to regulate shoot apical meristem development**. *Curr Opin Plant Biol* 2012, **15**:652-658.

18. Zhou Y, Honda M, Zhu H, Zhang Z, Guo X, Li T, Li Z, Peng X, Nakajima K, Duan L *et al.*: **Spatiotemporal sequestration of miR165/166 by Arabidopsis argonaute10 promotes shoot apical meristem maintenance**. *Cell Rep* 2015, **10**:1819-1827.

19. Xu F, Liu Q, Chen L, Kuang J, Walk T, Wang J, Liao H: **Genome-wide identification of soybean microRNAs and their targets reveals their organ-specificity and responses to phosphate starvation**. *BMC Genomics* 2013, **14**:66.

20. Zhang B, Pan X, Stellwag EJ: **Identification of soybean microRNAs and their targets**. *Planta* 2008, **229**:161-182.

21. Tucker MR, Okada T, Hu Y, Scholefield A, Taylor JM, Koltunow AM: **Somatic small RNA pathways promote the mitotic events of megagametogenesis during female reproductive development in Arabidopsis**. *Development* 2012, **139**:1399-1404.

22. Nonomura K, Morohoshi A, Nakano M, Eiguchi M, Miyao A, Hirochika H, Kurata N: **A germ cell specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice**. *Plant Cell* 2007, **19**:2583-2594.

23. •• Komiya R, Ohyanagi H, Niihama M, Watanabe T, Nakano M, Kurata N, Nonomura K: **Rice germline-specific Argonaute MEL1 protein binds to phasiRNAs generated from more than 700 lincRNAs**. *Plant J* 2014, **78**:385-397.
This article characterizes sRNAs bound to MEL1/AGO5c in rice reproductive tissues. The authors showed that MEL1/AGO5c specifically binds 21-nt phasiRNAs and like AtAGO5, MEL has a preference for sRNAs with a 5′ cytosine. In a previous publication, it was shown that *mel1* mutants display abnormal somatic cell divisions and meiotic arrest. Therefore, it is inferred that 21-nt phasiRNAs are important for male reproduction.

24. Johnson C, Kasprzewska A, Tennessen K, Fernandes J, Nan GL, Walbot V, Sundaresan V, Vance V, Bowman LH: **Clusters and superclusters of phased small RNAs in the developing inflorescence of rice**. *Genome Res* 2009, **19**:1429-1440.

25. •• Zhai J, Zhang H, Arikit S, Huang K, Nan GL, Walbot V, Meyers BC: **Spatiotemporally dynamic, cell-type-dependent premeiotic and meiotic phasiRNAs in maize anthers**. *Proc Natl Acad Sci U S A* 2015, **112**:3146-3151.
This pioneer study provides the first comprehensive analysis of phasiRNA dynamics during anther development in maize, and demonstrates the cell-type dependence of the two phasiRNA size classes: epidermis is required for 21-nt phasiRNAs biogenesis and tapetum is important for 24-nt phasiRNA production.

26. • Song X, Li P, Zhai J, Zhou M, Ma L, Liu B, Jeong DH, Nakano M, Cao S, Liu C *et al.*: **Roles of DCL4 and DCL3b in rice phased small RNA biogenesis**. *Plant J* 2012, **69**:462-474.
These two articles characterized the genetic pathway responsible for phasiRNA biogenesis: RDR6 is required to convert *PHAS* transcripts into dsRNAs before processing by DCL4 and DCL5 (originally named DCL3b) to produce 21-nt and 24-nt phasiRNAs, respectively.

27. • Song X, Wang D, Ma L, Chen Z, Li P, Cui X, Liu C, Cao S, Chu C, Tao Y *et al.*: **Rice RNA-dependent RNA polymerase 6 acts in small RNA biogenesis and spikelet development**. *Plant J* 2012, **71**:378-389.
See annotation to Ref. [27•].

28. •• Wu J, Yang Z, Wang Y, Zheng L, Ye R, Ji Y, Zhao S, Ji S, Liu R, Xu L *et al.*: **Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA**. *Elife* 2015:4.
This is the first paper showing the importance and functions of AGO18. The authors showed that AGO18 protein is not detectable in rice seedlings but is highly induced by viral infection. Interestingly, AGO18 mostly binds to non-viral siRNAs, indicating that it plays an indirect role during virus defense.

29. Arikit S, Zhai J, Meyers BC: **Biogenesis and function of rice small RNAs from non-coding RNA precursors**. *Curr Opin Plant Biol* 2013, **16**:170-179.

30. Takeda A, Iwasaki S, Watanabe T, Utsumi M, Watanabe Y: **The mechanism selecting the guide strand from small RNA duplexes is different among argonaute proteins**. *Plant Cell Physiol* 2008, **49**:493-500.

31. Zhang X, Zhao H, Gao S, Wang WC, Katiyar-Agarwal S, Huang HD, Raikhel N, Jin H: **Arabidopsis Argonaute 2 regulates innate immunity via miRNA393(*)-mediated silencing of a Golgi-localized SNARE gene, MEMB12**. *Mol Cell* 2011, **42**:356-366.

32. Pumplin N, Voinnet O: **RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence**. *Nat Rev Microbiol* 2013, **11**:745-760.

33. Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, White CI, Rendtlew Danielsen JM, Yang YG, Qi Y: **A role for small RNAs in DNA double-strand break repair**. *Cell* 2012, **149**:101-112.

34. Montgomery TA, Howell MD, Cuperus JT, Li D, Hansen JE, Alexander AL, Chapman EJ, Fahlgren N, Allen E, Carrington JC: **Specificity of ARGONAUTE7-miR390 interaction and dual**

**functionality in TAS3 trans-acting siRNA formation**. *Cell* 2008, **133**:128-141.

35. Nagasaki H, Itoh J, Hayashi K, Hibara K, Satoh-Nagasawa N, Nosaka M, Mukouhata M, Ashikari M, Kitano H, Matsuoka M *et al.*: **The small interfering RNA production pathway is required for shoot meristem initiation in rice**. *Proc Natl Acad Sci U S A* 2007, **104**:14867-14871.

36. Douglas RN, Wiley D, Sarkar A, Springer N, Timmermans MC, Scanlon MJ: ***ragged seedling2* encodes an ARGONAUTE7-like protein required for mediolateral expansion, but not dorsiventrality, of maize leaves**. *Plant Cell* 2010, **22**:1441-1451.

37. Zhou C, Han L, Fu C, Wen J, Cheng X, Nakashima J, Ma J, Tang Y, Tan Y, Tadege M *et al.*: **The trans-acting short interfering RNA3 pathway and no apical meristem antagonistically regulate leaf margin development and lateral organ separation, as revealed by analysis of an *argonaute7/lobed leaflet1* mutant in *Medicago truncatula***. *Plant Cell* 2013, **25**:4845-4862.

38. Shi Z, Wang J, Wan X, Shen G, Wang X, Zhang J: **Over-expression of rice OsAGO7 gene induces upward curling of the leaf blade that enhanced erect-leaf habit**. *Planta* 2007, **226**:99-108.

39. Havecker ER, Wallbridge LM, Hardcastle TJ, Bush MS, Kelly KA, Dunn RM, Schwach F, Doonan JH, Baulcombe DC: **The Arabidopsis RNA-directed DNA methylation argonautes functionally diverge based on their expression and interaction with target loci**. *Plant Cell* 2010, **22**:321-334.

40. Wu L, Zhou H, Zhang Q, Zhang J, Ni F, Liu C, Qi Y: **DNA methylation mediated by a microRNA pathway**. *Mol Cell* 2010, **38**:465-475.

41. Matzke MA, Mosher RA: **RNA-directed DNA methylation: an epigenetic pathway of increasing complexity**. *Nat Rev Genet* 2014, **15**:394-408.

42. Wu L, Mao L, Qi Y: **Roles of dicer-like and argonaute proteins in TAS-derived small interfering RNA-triggered DNA methylation**. *Plant Physiol* 2012, **160**:990-999.

43. McCue AD, Nuthikattu S, Reeder SH, Slotkin RK: **Gene expression and stress response mediated by the epigenetic regulation of a transposable element small RNA**. *PLoS Genet* 2012, **8**:e1002474.

44. Pontier D, Picart C, Roudier F, Garcia D, Lahmy S, Azevedo J, Alart E, Laudie M, Karlowski WM, Cooke R *et al.*: **NERD, a plant-specific GW protein, defines an additional RNAi-dependent chromatin-based pathway in Arabidopsis**. *Mol Cell* 2012, **48**:121-132.

45. •• McCue AD, Panda K, Nuthikattu S, Choudury SG, Thomas EN, Slotkin RK: **ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation**. *EMBO J* 2015, **34**:20-35.

This paper demonstrates that AGO6 is the effector protein that incorporates siRNA products from transcriptionally active TE to direct DNA methylation in a RDR6-dependent RdDM pathway.

46. Olmedo-Monfil V, Duran-Figueroa N, Arteaga-Vazquez M, Demesa-Arevalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP: **Control of female gamete formation by a small RNA pathway in Arabidopsis**. *Nature* 2010, **464**:628-632.

47. Singh M, Goel S, Meeley RB, Dantec C, Parrinello H, Michaud C, Leblanc O, Grimanelli D: **Production of viable gametes without meiosis in maize deficient for an ARGONAUTE protein**. *Plant Cell* 2011, **23**:443-458.

48. Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J *et al.*: **Genome sequence of the palaeopolyploid soybean**. *Nature* 2010, **463**:178-183.