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Argonaute10 as a miRNA Locker

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The stability and translation efficiency of many messenger RNAs is regulated by microRNAs (miRNAs), which exert their effects through associated Argonaute proteins. In this issue, Zhu, Zhang, and colleagues reveal that plants also exploit miRNA binding by Argonautes as a sequestering mechanism that prevents miRNAs from fulfilling their normal roles.

One of the most important levels of post-transcriptional regulation in eukaryotes relies on small RNAs. Among them, one particular group, the 21 or 22 nucleotide long microRNAs (miRNAs), are critical for many aspects of plant and animal development through their effects on mRNA stability and translation efficiency. Two main protein families required for their biogenesis and action are Dicer proteins, which generate small RNAs, and Argonaute proteins, which use these small RNAs to find their targets. In this issue of *Cell*, Zhu, Zhang, and colleagues (Zhu et al., 2011) report that Argonaute proteins of the plant *Arabidopsis thaliana* are not only required for miRNAs silencing their targets but can also prevent miRNAs from doing so.

In plants and animals, small RNAs are typically generated from longer precursors. The four Dicer-like (DCL) proteins in plants have unique substrate specificities or produce differently sized small RNAs, with a complex hierarchical relationship between them. The result of DCL action is the formation of short RNA duplexes with 2 nucleotide long 3′ overhangs (Vazquez, 2006). In the case of miRNAs, one strand stably associates with an Argonaute (AGO) protein, which is then guided by the miRNA to its target (Mallory and Vaucheret, 2010).

Several modes of negative regulation of miRNA production and function have been identified in plants (Figure 1). The first mechanism is interference with the activity of HYL1, a DCL1 accessory protein, by poisoning it with an RNA derived from a SINE element (Pouch-Pélissier et al., 2008). Alternatively, viral suppressor proteins that lack any catalytic activity may compete with AGO proteins for miRNA binding (Kasschau et al., 2003). Other viral proteins interact directly with AGO proteins and thereby either destabilize them or suppress their activity (Mallory and Vaucheret, 2010). Finally, even if a properly functioning miRNA-AGO complex is available, decoys known as target mimics can sequester the active AGO-miRNA complex and prevent it from reaching its normal original target (Franco-Zorrilla et al., 2007).

Zhu and colleagues (2011) now describe yet another level of negative regulation of miRNA activity: competition of AGO10 with AGO1 for a specific miRNA. Plant Argonaute proteins fall into four distinct clades with different properties (Czech and Hannon, 2011; Mallory and Vaucheret, 2010). AGO1, the namesake of the family, binds predominantly 21 nucleotide miRNAs (and to a lesser extent 22 nucleotide miRNAs) with a 5′ uridine (U). Target mRNAs with different degrees of sequence complementarity to the miRNAs are negatively regulated by miRNA-loaded AGO1, either through slicing or translational inhibition. Because AGO1 is required to execute the function of many miRNAs, its inactivation severely impairs normal plant development.

Other AGOs prefer different spectra of small RNAs and have different biological roles. AGO7 associates largely with a single miRNA, miR390. This miRNA guides AGO7 to noncoding TAS RNAs, which are then processed into *trans*-acting small interfering RNAs (ta-siRNAs). AGO2 (and probably the very similar AGO3) has a preference for 21 nucleotide ta-siRNAs with a 5′ adenine (A), whereas AGO5 binds small RNAs of different sizes that have a 5′ cytosine (C) and originate from intergenic regions. Finally, it is thought that 24 nucleotide siRNAs with a 5′ A guide AGO4/6/9 to noncoding RNAs that mediate DNA methylation and chromatin modifications.

The closest *Arabidopsis thaliana* homolog of the *AGO1* gene is *AGO10*, also known as *ZWILLE* or *PINHEAD*. Some of the *ago10* mutant phenotypes are superficially similar to those of *ago1* mutants, and double-mutant analyses had suggested that *AGO1* and *AGO10* have overlapping functions (Lynn et al., 1999). Furthermore, as a potential explanation for functional differences between AGO1 and AGO10 proteins, it had been proposed that AGO10 specializes in translational repression (Brodersen et al., 2008), rather than slicing.

Zhu and colleagues (2011) set out to clarify AGO10 function by applying the same straightforward approach that had been used for several other AGOs, sequencing of the small RNA population bound in vivo by AGO10 (Mallory and Vaucheret, 2010). Much to their surprise, they found that AGO10 behaves very differently from AGO1. It has a narrow preference spectrum and primarily associates with small RNAs from the miR165/166 family. Testing a large set of mutant variants, several structural features in the miRNA/miRNA* duplex were identified as essential for the specific interaction with AGO10. These are highly conserved in the miR165/166 family, which is found in all vascular plants, but they are absent from other miRNAs. This observation is in agreement with earlier findings from flies

and nematodes that sorting of small RNAs into AGOs depends on the structure of the double-stranded RNA duplex that is excised from the larger precursor (Czech and Hannon, 2011). How AGO10 recognizes the relevant structural features so specifically will be an obvious direction for future research.

The second surprising discovery by Zhu and colleagues (2011) was that slicing ability is apparently dispensable for miR165/166-dependent AGO10 activity in plants. This, in turn, led the authors to hypothesize that AGO10's main function is to attenuate miR165/166 activity by preventing it from associating with the catalytically active AGO1 (Figure 1). In support of this idea, expressing in plants a version of miR166 that can be recruited by the more promiscuous AGO1, but not by AGO10, indeed mimics *ago10* mutant phenotypes.

Zhu and colleagues (2011) also make several intriguing yet unexplained observations that have the potential to teach us more about AGO10. For example, AGO10 seems to decrease miR165/166 levels in plants, whereas conversely more AGO10 accumulates when there is an excess of miR166. AGO1 is known to be feedback regulated by miR168 via the regular miRNA slicing pathway (Mallory and Vaucheret, 2010), but the mode of negative feedback control of AGO10 by miR165/166 must be distinct because miR165/166 targets a family of genes that is unrelated to *AGO*s. Finally, it is noteworthy that *AGO10* is largely dispensable in the *Arabidopsis thaliana* reference strain Columbia and that strong phenotypes are only observed in the Landsberg *erecta* background. Because no additional *AGO10*-like genes are present in the fully sequenced Columbia genome, it is unlikely that this is a case of conventional genetic redundancy. As behooves an exciting discovery, the work of Zhu and colleagues (2011) raises as many new questions as it answers old puzzles.

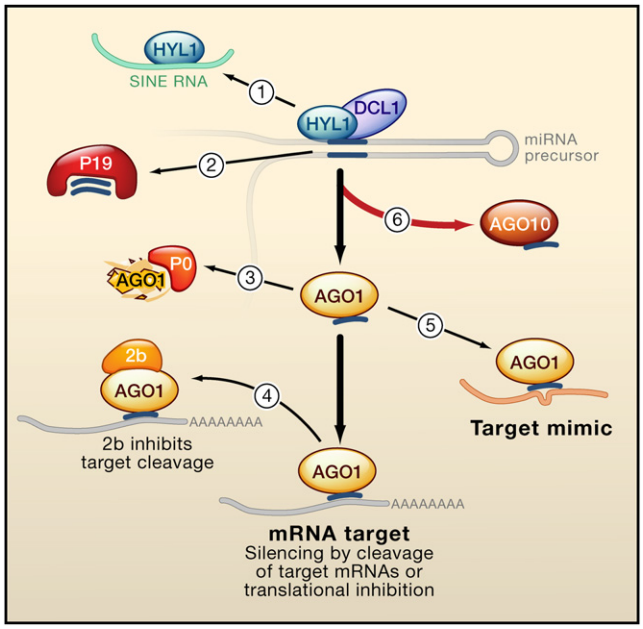

Figure 1. Suppression of miRNA Activity in Plants
(1) Sequestration of HYL1 protein, which normally acts together with DCL1 in miRNA processing. (2) Sequestration of miRNA/miRNA* duplex by viral P19 protein. (3) Degradation of AGO1 by viral P0 protein. (4) Inhibition of AGO1 activity by viral 2b protein. (5) Sequestration of miRNA-loaded AGO1 by endogenous target mimics. (6) Sequestration of miRNA by AGO10.

REFERENCES

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L., and Voinnet, O. (2008). Science *320*, 1185–1190.

Czech, B., and Hannon, G.J. (2011). Nat. Rev. Genet. *12*, 19–31.

Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., Leyva, A., Weigel, D., García, J.A., and Paz-Ares, J. (2007). Nat. Genet. *39*, 1033–1037.

Kasschau, K.D., Xie, Z., Allen, E., Llave, C., Chapman, E.J., Krizan, K.A., and Carrington, J.C. (2003). Dev. Cell *4*, 205–217.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M.K. (1999). Development *126*, 469–481.

Mallory, A., and Vaucheret, H. (2010). Plant Cell *22*, 3879–3889.

Pouch-Pélissier, M.N., Pélissier, T., Elmayan, T., Vaucheret, H., Boko, D., Jantsch, M.F., and Deragon, J.M. (2008). PLoS Genet. *4*, e1000096.

Vazquez, F. (2006). Trends Plant Sci. *11*, 460–468.

Zhu, H., Hu, F., Wang, R., Zhou, X., Sze, S.-H., Liou, L.W., Barefoot, A., Dickman, M., and Zhang, X. (2011). Cell *145*, this issue, 242–256.