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MicroRNAs Visit the ER

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Little is known about where microRNAs (miRNAs) regulate their targets within the cell. In this issue, Li et al. identify a new player in the plant miRNA pathway that implicates the endoplasmic reticulum in miRNA-mediated gene silencing.

MicroRNAs (miRNAs) are short regulatory RNAs that bind Argonaute proteins to affect gene expression through base-pairing with target messenger RNAs (mRNAs). Twenty years have passed since the discovery of miRNAs, and yet the mode in which they guide gene silencing is still a matter of contention. In animals, miRNAs were first shown to regulate their targets by inhibiting translation, whereas in plants, miRNAs were originally shown to direct target cleavage, resulting in mRNA decay (Llave et al., 2002; Wightman et al., 1993) (Figure 1A). However, mounting evidence suggests that the mechanism of miRNA-mediated gene repression in both plants and animals is more complicated than can be explained by a single model (Bazzini et al., 2012; Brodersen and Voinnet, 2009; Djuranovic et al., 2012). A new study by Xuemei Chen and colleagues (Li et al., 2013) provides further support for a role of miRNAs in translational inhibition in plants and moreover reveals that this process occurs in the endoplasmic reticulum. The subcellular localization of miRNAs is critical to facilitate proper protein and RNA interactions and may ultimately determine how miRNAs regulate their targets.

miRNA maturation involves cleavage from a longer precursor molecule in the nucleus followed by transport to the cytoplasm for additional processing and loading into an effector complex containing an Argonaute protein. How are miRNA-Argonaute complexes sorted within the cytoplasm, and where do they encounter their targets? An early study showed that, in rat cells, Argonaute2 (Ago2) associates with the endoplasmic reticulum (ER) (Cikaluk et al., 1999). However, subsequent analysis of miRNA pathway components suggested that cytoplasmic foci called P bodies are the primary sites of miRNA activity (Eulalio et al., 2009).

In a genetic screen designed to identify mutants that activate a transgene silenced by DNA methylation in *Arabidopsis*, Li et al. (2013) identify a mutant with pleiotropic developmental defects that are reminiscent of miRNA-defective plants, which fail to downregulate particular developmental control transcription factors. Although the mutant does not activate the transgene and is therefore unlikely to have defects in DNA methylation, the authors focus on it because of its phenotypic similarity to miRNA pathway mutants. They map the causal mutation to *ALTERED MERISTEM PROGRAM1* (*AMP1*), which encodes a highly conserved glutamine carboxypeptidase containing a transmembrane domain, a protease domain, a peptidase domain, and a transferrin receptor domain. The pleiotropic defects in *amp1* mutants, including abnormal tissue patterning, stunted growth, and reduced fertility, are exacerbated when combined with a mutation in the closely related gene *LAMP1*, indicating that the two genes share a common function.

To investigate whether or not *AMP1* is indeed involved in the miRNA pathway as its phenotype would suggest, Li et al. (2013) first examine the levels of several miRNAs using northern blot assays to determine whether *AMP1* is required for miRNA formation or stability. None of the miRNAs tested are substantially affected by the *amp1* mutation. Alternatively, *AMP1* could function downstream of miRNA formation in the repression of target mRNAs. To address this possibility, the authors first ask what happens to miRNA-target mRNA levels in *amp1* mutants using quantitative RT-PCR. Similar to miRNAs, miRNA-target mRNA levels are unchanged. Next, the authors examine the levels of proteins produced from several miRNA target genes by western blot analysis. Relative to wild-type plants, *amp1* mutants accumulate elevated levels of protein from each of the miRNA targets tested. Given that mRNA levels are unchanged, this result suggests that the *amp1* mutation affects translational inhibition of miRNA targets without disrupting mRNA decay, thereby decoupling these two silencing mechanisms (Figure 1B).

ARGONAUTE1 (AGO1) is the key protein component of the plant miRNA effector complex. Does AMP1 interact with AGO1? Li et al. (2013) find that AMP1 and AGO1 coimmunoprecipitate; however, it is possible that the interaction is an artifact caused by their proximity to one another within the cell. Using fluorescently tagged transgenes, the authors discover that AMP1 and AGO1 both localize to the ER, although AMP1 is diffuse, whereas AGO1 forms punctate foci on the ER meshwork. In cell extracts, both AMP1 and AGO1 are enriched in the crude membrane fraction relative to the soluble fraction, suggesting that they are both membrane bound. Under high salt or high pH, AGO1 is driven into the soluble nonmembrane fraction, suggesting that it is a peripheral membrane protein, whereas AMP1 remains in the membrane fraction under these conditions, indicating that it is an integral membrane protein.

The rough ER is coated with membrane-bound ribosomes that form polysomes along mRNAs during protein synthesis. Membrane-bound polysomes can be fractionated from total polysomes

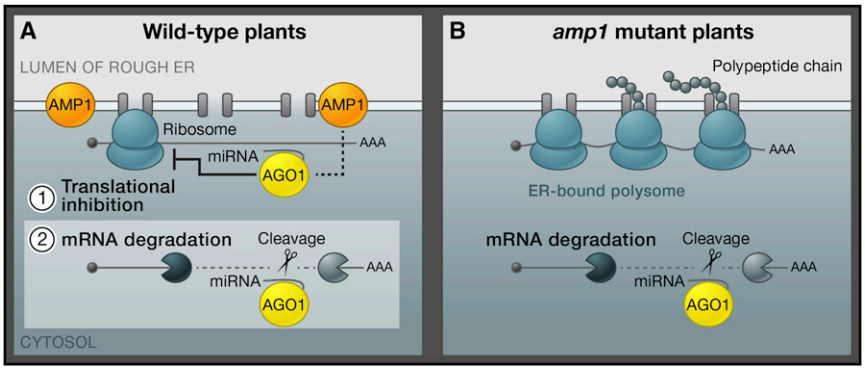

Figure 1. Mechanisms of miRNA-Mediated Gene Silencing

(A) In wild-type plants, miRNAs function in two distinct modes: (1) translational inhibition and (2) mRNA degradation initiated by endonucleolytic cleavage by AGO1. Translational inhibition likely occurs on mRNAs bound to the rough endoplasmic reticulum, although the location where mRNA cleavage occurs is still unknown. Following cleavage, the resulting mRNA fragments are degraded by nucleases. AMP1 is an integral membrane protein that interacts with AGO1 to facilitate translational inhibition.

(B) In *amp1* mutant plants, mRNA cleavage and decay occur normally, whereas translational inhibition is impaired.

in a sucrose density gradient. Using this approach, Li et al. (2013) show that miRNA target mRNAs are unchanged in total polysomes in *amp1 lamp1* mutants relative to wild-type plants using quantitative RT-PCR. In contrast, the levels of several miRNA-target mRNAs are elevated in membrane-bound polysomes in *amp1 lamp1* mutants, suggesting that, in wild-type plants, mRNAs bound to ER-associated ribosomes are subjected to translational inhibition involving AMP1.

The specific role of AMP1 in translational inhibition is unclear. *AMP1* is not required for AGO1 localization to membrane-bound polysomes, suggesting that it is not involved in tethering AGO1 to the ER. One possibility is that AMP1 facilitates interaction between AGO1 and mRNAs bound to ribosomes localized to the ER. The slicer activity of AGO1, which cleaves target mRNAs at the central point of complementarity to the miRNA, is essential for plant development, indicating that translational inhibition involving AMP1 is not sufficient for miRNA-mediated gene silencing (Carbonell et al., 2012). Further characterization of AMP1 may shed additional light on the mechanism of translational repression involving miRNAs and its role in plant development.

Given that animal miRNAs also direct translational inhibition, that *AMP1* is conserved in animals, and that Ago2 associates with the ER in rat cells, the mechanism and subcellular location of miRNA-mediated translational repression are likely similar between plants and animals. In fact, human Ago2, as well as several proteins required to process or load small RNAs into Ago2, also associate with the rough ER (Stalder et al., 2013).

Do miRNAs direct mRNA decay on the ER as well, or are translational inhibition and mRNA decay physically separated from one another within the cell? One possibility is that translational repression on the ER is transient and ultimately leads to mRNA decay in separate compartments such as P bodies. It will be important to decipher the specific contributions and roles of mRNA decay and translational repression in miRNA-mediated gene silencing.

REFERENCES

Bazzini, A.A., Lee, M.T., and Giraldez, A.J. (2012). Science *336*, 233–237.

Brodersen, P., and Voinnet, O. (2009). Nat. Rev. Mol. Cell Biol. *10*, 141–148.

Carbonell, A., Fahlgren, N., Garcia-Ruiz, H., Gilbert, K.B., Montgomery, T.A., Nguyen, T., Cuperus, J.T., and Carrington, J.C. (2012). Plant Cell *24*, 3613–3629.

Cikaluk, D.E., Tahbaz, N., Hendricks, L.C., DiMattia, G.E., Hansen, D., Pilgrim, D., and Hobman, T.C. (1999). Mol. Biol. Cell *10*, 3357–3372.

Djuranovic, S., Nahvi, A., and Green, R. (2012). Science *336*, 237–240.

Eulalio, A., Tritschler, F., and Izaurralde, E. (2009). RNA *15*, 1433–1442.

Li, S., Liu, L., Zhuang, X., Yu, Y., Liu, X., Cui, X., Ji, L., Pan, Z., Cao, X., Mo, B., et al. (2013). Cell *153*, this issue, 562–574.

Llave, C., Xie, Z., Kasschau, K.D., and Carrington, J.C. (2002). Science *297*, 2053–2056.

Stalder, L., Heusermann, W., Sokol, L., Trojer, D., Wirz, J., Hean, J., Fritzsche, A., Aeschimann, F., Pfanzagl, V., Basselet, P., et al. (2013). EMBO J. Published online March 19, 2013. http://dx.doi.org/10.1038/emboj.2013.52.

Wightman, B., Ha, I., and Ruvkun, G. (1993). Cell *75*, 855–862.