Trends in Plant Science

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Spotlight

Lost in translation? microRNAs at the rough ER

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MicroRNAs (miRNAs) function to post-transcriptionally regulate target RNAs, including long non-coding RNAs and mRNAs. A recent study demonstrates that *Arabidopsis* miRNAs are enriched at the rough endoplasmic reticulum (ER). This enrichment is a surprise, given that most known miRNA targets are not expected to be translated at the rough ER.

Like many other eukaryotes, plants use endogenously produced small RNAs to regulate gene expression. These small RNAs form the specificity determinants of RNA-induced silencing complexes (RISC), with Argonaute (AGO) proteins at their cores. RISC selects target RNAs based on target-small RNA complementarity. Three of the major types of plant endogenous small RNAs are microRNAs (miRNAs), Pol IV-dependent siRNAs (p4-siRNAs), and phased siRNAs (phasiRNAs) [1]. miRNAs repress gene activity at the post-transcriptional level by dampening protein output from mRNAs, and sometimes can also initiate phasiRNA production from both mRNAs and long non-coding RNAs (lncRNAs). P4-siRNAs repress gene activity at the transcriptional level by recruiting chromatin-modifying enzymes to sites transcribed by RNA polymerase V. PhasiRNAs depend upon an initial targeting event by a miRNA or phasiRNA on a primary transcript. The targeted transcript is then used as the template for phasiRNA production. PhasiRNAs can act both like miRNAs (post-transcriptional repression of mRNAs) and like P4-siRNAs (transcriptional repression in the nucleus).

Because they interact with spliced, polyadenylated mRNAs and lncRNAs, it has long been assumed that miRNAs function in the general cytoplasm, but whether miRNAs have a distinct locale within the general cytoplasm has not been clear. Li *et al.* [2] studied the sub-cellular localization of miRNAs and phasiRNAs using density gradient fractionation of lysates from *Arabidopsis thaliana* seedlings. miRNAs were enriched in microsomal fractions (crude membrane vesicles, many of which are derived from the endoplasmic reticulum), membrane-bound polysome fractions (MBP; polysomes are mRNAs complexed with multiple attached ribosomes), and in immunoprecipitations targeting an endoplasmic reticulum (ER)-localized marker (Figure 1). P4-siRNAs were, by contrast, depleted from these three fractions. There are several AGO proteins, each with specialized functions. AGO1, a major miRNA and phasiRNA-specific AGO protein, was also found in microsomes and MBPs and was required for the observed microsomal enrichment of miRNAs. Altogether these data argue that AGO1-miRNA complexes are enriched in a membrane-rich, polysome-containing area of the cell; most likely the rough ER.

Enrichment of miRNAs on the rough ER is surprising. Rough ER is peppered with membrane-associated ribosomes, which are the sites of translation for proteins that are targeted for the secretory pathway, organellar delivery, or which contain one or more membrane-spanning domains [3]. However, the proteins encoded by many well-characterized and highly conserved miRNA targets have none of these features; these include targets encoding several DNA-binding transcription factor families, F-box proteins, and various enzymes [4]. In addition, other well-known targets of miRNAs, such as the precursors for phasiRNAs, do not encode long proteins at all. So why would AGO1 and miRNAs be enriched at a location where many of their targets should not be present? A surprising answer provided by Li *et al.* [2] is that maybe the target mRNAs indeed are at the rough ER. RNA-seq from fractionated MBPs demonstrated that many mRNAs encoding non-transmembrane proteins are present at approximately equal levels in MBP vs free polysomes. This includes many predicted miRNA targets, a few of which are even enriched on MBPs relative to free polysomes. This observation challenges the basic understanding of the rough ER's function by suggesting that MBPs are not limited to membrane proteins and golgi cargo.

A final key finding of Li *et al.* [2] is that several phasiRNA precursor RNAs are in fact bound by ribosomes, based on ribosomal footprint sequencing from purified MBPs (Figure 1). Several well-studied *A. thaliana* phasiRNA precursor RNAs lack long open-reading frames, so their engagement by ribosomes was not expected. Ribosome occupancy on these nominally non-coding RNAs peaks immediately upstream of the 5′-most miRNA complementary sites, strongly suggesting a mechanistic link between ribosome occupancy and the initiation of phasiRNA biogenesis. This finding confirms and extends the results of Hou *et al.* [5], who found the same pattern of ribosome occupancy upstream of the 5′-most miR390 complementary site in the conserved phasiRNA-generating *TAS3*. The AGO specific for miR390, AGO7, is also localized in membrane-containing cytoplasmic foci [6]. Based on these observations, and the enrichment of known phasiRNA-triggering miRNAs at MBPs, the conclusion is that phasiRNA biogenesis occurs on MBPs, presumably at the rough ER, and is connected to engagement by the ribosome.

Like any seminal study, Li *et al.*'s [2] results raise more questions than they answer. Perhaps the first order of business should be directly visualizing the sub-cellular localization of miRNAs and AGO1 with high-resolution methods under native conditions. Direct visualization of

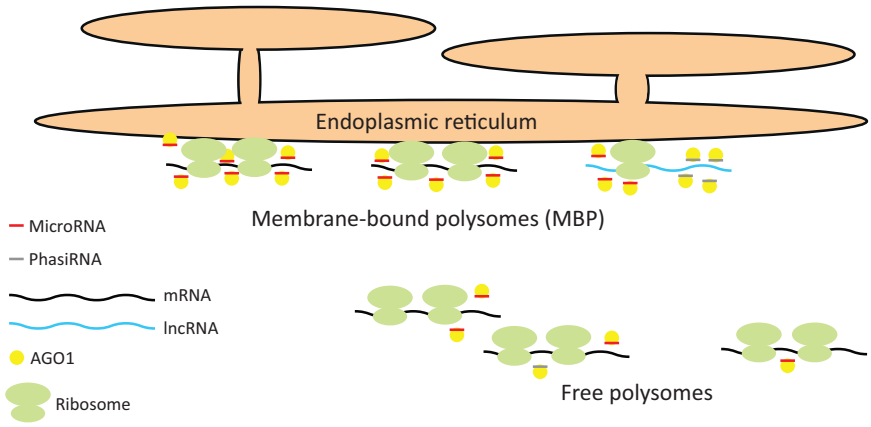

Figure 1. *Arabidopsis* miRNAs are Enriched at the Rough Endoplasmic Reticulum. Schematic summarizing Li *et al.*'s [2] observation of AGO1 and microRNA enrichment (yellow circles with red lines) at membrane-bound polysomes relative to free polysomes. Certain long non-coding RNAs (blue) that give rise to AGO1 associated phased siRNAs (yellow circles with gray lines) were also enriched on membrane-bound polysomes. Abbreviations: AGO1, ARGONAUTE1; ER, endoplasmic reticulum; lncRNA, long non-coding RNAs; phasiRNAs, phased siRNAs.

miRNAs and/or AGO1 on the rough ER would strengthen the conclusion of rough ER localization, which at present is based mostly on fractionation of cellular lysates using density-gradient centrifugation. In previously published work, Li *et al.* [7] showed that YFP-tagged AGO1 localized to punctate foci that overlapped ER in transiently transformed *Nicotiana benthamiana* leaves. However, it remains possible that this localization was influenced by the non-physiological conditions associated with transient expression and/or the large protein fusion. In the fractionation experiments reported in the current work [2], native AGO1 appears present at microsomes and MBPs, but is not particularly enriched in those fractions compared to control integral and luminal ER proteins.

Even more important is to discover the biological significance of why AGO1 and associated miRNAs are enriched at MBPs. Sub-cellular enrichment of small RNAs and their guide proteins is not unique to plants: for instance the *Drosophila* Piwi proteins AGO3 and Aubergine are found in the 'nuage', a perinuclear compartment of nusrse cell in the *Drosophila* ovary [8]. Association of plant AGO1 with general polysomes has been known for some time [9], and is not surprising given that miRNA-AGO1 complexes by nature need to 'scan' the mRNA population to identify targets; polysomes are where the mRNAs are, and so that is where we would expect to find scanning AGO1-miRNAs. In addition, plant miRNAs can cause AGO1-dependent translational inhibition of target mRNAs [10], which logically implies close proximity of AGO1-miRNA complexes to ribosomes. The question is, why the enrichment specifically on membrane-bound polysomes versus total polysomes? Earlier work from the same group might offer a clue; AMP1, an integral ER membrane protein, binds to AGO1 and is required for miRNA-mediated translational repression [7]. It is possible therefore that the enrichment of miRNAs at the rough ER reflects a stable tethering between AGO1, miRNAs, targets, and AMP1 required for repression. The difficulty with this otherwise attractive hypothesis is that wild-type AGO1 does not co-immunoprecipitate with known target mRNAs to any appreciable degree; only when catalytic residues from AGO1's endonuclease domain are mutated can a stable AGO1-target mRNA association be observed *in vivo* [11]. An alternative idea to ternary AGO1-miRNA-target complexes is that the rough ER-enrichment of miRNAs represents a 'scanning' pool of AGO1-miRNA complexes. It is unknown what fraction of AGO1-miRNA complexes are actively engaged with true targets versus scanning the transcriptome at any given time, but it is possible a large fraction are in the scanning mode. Perhaps the rough ER is a location that is particularly well-suited for target searches by AGO-miRNA complexes.

Li *et al.*'s findings place miRNAs where they were not expected to be, based on their known targets and the conventional understanding of rough ER function. Understanding the biological function for the unexpected location of plant miRNAs should be a productive and illuminating area of future research.

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References

1. Axtell, M.J. (2013) Classification and comparison of small RNAs from plants. *Annu. Rev. Plant. Biol.* 64, 137–159
2. Li, S. *et al.* (2016) Biogenesis of phased siRNAs on membrane-bound polysomes in Arabidopsis. *Elife* 5, e22750
3. Alberts, B. *et al*. The Endoplasmic Reticulum. In *Molecular Biology of the Cell* (4 edn) Garland.
4. Jones-Rhoades, M.W. (2006) MicroRNAS and their regulatory roles in plants. *Annu. Rev. Plant. Biol.* 57, 19–53
5. Hou, C.-Y. *et al.* (2016) Global Analysis of Truncated RNA Ends Reveals New Insights into Ribosome Stalling in Plants. *Plant Cell* 28, 2398–2416
6. Jouannet, V. *et al.* (2012) Cytoplasmic Arabidopsis AGO7 accumulates in membrane-associated siRNA bodies and is required for ta-siRNA biogenesis. *EMBO J.* 31, 1704–1713
7. Li, S. *et al.* (2013) MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. *Cell* 153, 562–574
8. Webster, A. *et al.* (2015) Aub and Ago3 Are Recruited to Nuage through Two Mechanisms to Form a Ping-Pong Complex Assembled by Krimper. *Mol. Cell.* 59, 564–575
9. Lanet, E. *et al.* (2009) Biochemical evidence for translational repression by Arabidopsis microRNAs. *Plant Cell* 21, 1762–1768
10. Chen, X. (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* 303, 2022–2025
11. Carbonell, A. *et al.* (2012) Functional Analysis of Three Arabidopsis ARGONAUTES Using Slicer-Defective Mutants. *Plant Cell* 24, 3613–3629