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Review

The Whereabouts of microRNA Actions: Cytoplasm and Beyond

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MicroRNAs (miRNAs) are a conserved class of approximately 22 nucleotide (nt) short noncoding RNAs that normally silence gene expression via translational repression and/or degradation of targeted mRNAs in plants and animals. Identifying the whereabouts of miRNAs potentially informs miRNA functions, some of which are perhaps specialized to specific cellular compartments. In this review, the significance of miRNA localizations in the cytoplasm, including those at RNA granules and endomembranes, and the export of miRNAs to extracellular space will be discussed. How miRNA localizations and functions are regulated by protein modifications on the core miRNA-binding protein Argonaute (AGO) during normal and stress conditions will be explored, and in conclusion new AGO partners, non-AGO miRNA-binding proteins, and the emergent understanding of miRNAs found in the nucleoplasm, nucleoli, and mitochondria will be discussed.

Trends

Mature miRNAs localize in multiple subcellular locations in the cytoplasm, such as RNA granules, endomembranes, and mitochondria, and are secreted out of cells via exosomes.

Recent studies have revealed that mature miRNAs can also localize to the nucleus, where they could function in epigenetic regulation.

The distributions of canonical and non-canonical forms of miRNA-induced silencing complexes suggest that different subcellular locations are required for the processing and degradation of miRNA itself, or for silencing or activation of miRNA targets.

These subcellular distributions are differentially regulated by post-translational modifications as a function of cellular conditions, but one major question is whether such location-specific miRNAs are physiologically relevant.

miRNA: A Moving Target

In animals, most miRNA genes are transcribed by RNA polymerase II in the nucleus (reviewed in [1]). The primary miRNA transcript is processed by Drosha in the nucleus to become one or more precursor miRNAs (pre-miRNAs), which bear a hairpin structure with a 2-nt overhang at the 3′ end. The overhang structure is recognized by Exportin 5 for export to the cytoplasm, where pre-miRNAs are processed by Dicer to become an approximately 22-nt duplex. The duplex is then loaded onto one of the AGO family members (e.g., AGO1–4 in humans), which selectively retains one strand as the mature miRNA. Emergent data indicate that mature miRNAs then localize in subcellular compartments in the cytoplasm and, surprisingly, the nucleus (Figure 1, Key Figure). In this review, the potential functions of miRNAs in these compartments will be discussed.

To effect gene silencing, miRNAs require AGOs and other silencing factors to form differently sized miRNA-induced silencing complexes (miRISC), ranging from approximately 100 kDa to 3 MDa [2–4]. Depending on the degree of sequence complementarity between miRNAs and their RNA targets, as well as the protein composition of miRISCs, the outcomes of miRNA binding are different. Perfect complementarity between miRNAs and RNA targets allows AGO2 (the only AGO that has slicer activity) to cleave RNA targets. By contrast, nearly all animal miRNAs identify RNA targets with perfect complementarity at the so-called 'seed sequence' (2nd–7th nucleotide), where the recruitment of the AGO-binding protein GW182 forms a canonical miRISC to repress translation and/or accelerate deadenylation and degradation of mRNAs. Thus, when considering the whereabouts of miRNA functions, it is important to consider the localizations of miRNAs, the core miRNA-binding AGO family members, and other miRISC components.

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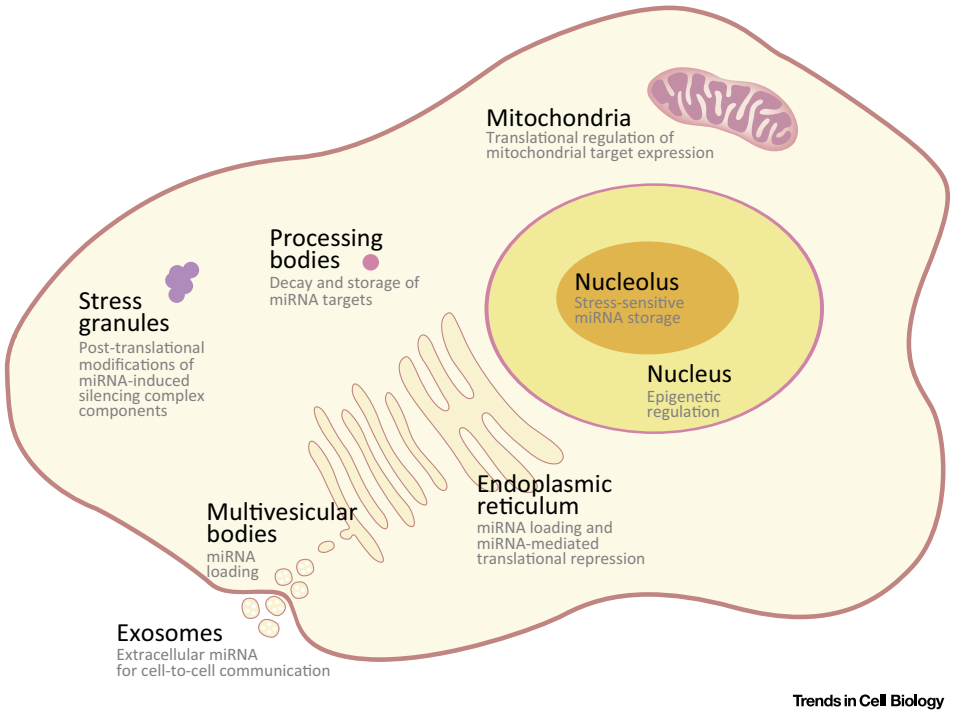
Key Figure

Figure 1. Proposed Location-Specific Functions of Mature miRNAs



The Possible Roles of P-bodies in miRNA Silencing

Given that AGO is required for functional miRISCs, its localization has been used as the proxy for identifying sites of miRNA functions ([5,6]; reviewed in [7]). Fluorescent microscopy studies indicate that mammalian AGO members are localized prominently in cytoplasmic bodies known as P-bodies (PBs) [8], which are enriched with RNA decay factors, including deadenylases. Besides AGO, PBs are also enriched with GW182, which bridges AGO proteins with deadenylases. Importantly, both miRNAs and their targets are recruited to PBs [5]. Such localizations suggest that PBs are a major site for miRNA-directed deadenylation and subsequent decay of mRNA targets. However, quantitation of the cytoplasmic localization of AGO2 indicates that, even though it has greater than 10-fold higher concentration at PBs than the neighboring cytoplasmic space, <1% of cytoplasmic AGO2 is localized in PBs [9]. Moreover, cells depleted of microscopically visible PBs have normal miRNA-mediated translational repression and degradation of mRNAs [7,10]. Although PBs could be sites for decay of miRNA targets, these data argue that the major actions of miRNAs occur elsewhere in the cytoplasm, likely as submicroscopic complexes.

PBs are also proposed to be storage sites of miRNA-targeted mRNAs destined for future translation [11]. For example, a subpopulation of CAT-1 mRNAs repressed by miR-122 is localized at PBs, but upon amino acid starvation, these mRNAs are delocalized from PBs and translation of this transcript resumes [11]. Given that such starvation-induced translation occurs even with treatment of a transcriptional inhibitor, it is likely that formerly repressed CAT-1 mRNAs are relocated to polysomes for translation. A possible source of these repressed mRNAs is from