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Review

The Functions of MicroRNAs: mRNA Decay and Translational Repression

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MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs, which regulate complementary mRNAs by inducing translational repression and mRNA decay. Although this dual repression system seems to operate in both animals and plants, genetic and biochemical studies suggest that the mechanism underlying the miRNA-mediated silencing is different in the two kingdoms. Here, we review the recent progress in our understanding of how miRNAs mediate translational repression and mRNA decay, and discuss the contributions of the two silencing modes to the overall silencing effect in both kingdoms.

Trends

Animal miRNAs promote mRNA decay by recruiting deadenylases and decapping factors onto the target mRNAs through GW182/TNRC6.

Plant miRNAs do not promote deadenylation but cleave nearly perfectly complementary targets. The 3′ end of the 5′ fragment is uridylated, and both the 5′ and 3′ fragments are decayed by the 5′-to-3′ exoribonuclease.

Animal miRNAs repress translation initiation by promoting dissociation of eIF4F through GW182-mediated displacement of PABP, recruitment of translational inhibitors via GW182, and displacement of the ATP-dependent RNA helicase eIF4A from the translation initiation complex eIF4F.

Plant miRNAs repress translation via various organelle-bound factors. Although the mechanism is unclear, *in vitro* studies suggest that AGO1–RISC can block translation initiation and ribosome movement.

miRNAs Mediate Two Modes of Silencing

miRNAs are endogenous, small noncoding RNAs approximately 20–22 nucleotides (nt) long that regulate gene expression by binding to their complementary target mRNAs. To date, over 2000 miRNAs have been identified in the human genome, while the model plant *Arabidopsis thaliana* has ~300 miRNAs [1]. They control a broad array of biological processes, including development, differentiation, proliferation, and stress responses [2–6].

miRNAs cannot work alone. To silence target mRNAs, they need to form a ribonucleoprotein complex, called RNA-induced silencing complex (RISC) [7,8]. The minimal RISC is composed of a small RNA and Argonaute protein (Ago) [9]. In mammals, all four Agos (Ago1–4) function in the miRNA pathway, while in *Drosophila* one of the two Agos (Ago1) is functionally specialized for miRNAs [10]. Via RISC, miRNAs mediate two modes of gene silencing: mRNA decay and translational repression [8]. mRNA decay can be induced by endonucleolytic cleavage by RISC. Indeed, Argonaute proteins have a domain homologous to RNase H, and when the small RNA is perfectly or nearly perfectly complementary to the target mRNA, RISC cleaves the target mRNA at the position facing nucleotides 10 and 11 of the small RNA [8]. The cleavage mode of RISC action is commonly seen in plants, in which most of miRNAs are nearly complementary to a single or a few target mRNAs [11]. In contrast to this, animal miRNAs recognize their target mRNAs through partial base pairing, especially within the ‘seed’ sequence at nucleotides 2–7 or 2–8 of the miRNA [8,12]. Such partial complementarity prevents the cleavage activity of RISC, but animal RISC can still silence target genes by recruiting additional effector proteins, which induce translational repression and/or mRNA decay in a manner independent of endonucleolytic cleavage.

In recent years, genetic, biochemical, and structural analyses have provided a detailed picture of the mechanism of miRNA-mediated mRNA decay. By contrast, it still remains unclear how animal miRNAs repress translation of target mRNAs, even though considerable progress was made in the past several years. Similar to animals, miRNAs also induce translational repression in plants, but the underlying mechanism has been poorly understood. In this review, we focus on the effector step of the miRNA-mediated gene silencing, that is, how miRNAs silence their target genes, in animals and plants. Specifically, we review recent progress on the molecular mechanisms of miRNA-mediated

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mRNA decay and translational repression in animals and plants. Moreover, we discuss the relative contribution of mRNA decay and translational repression in miRNA-mediated gene silencing in both kingdoms.

Mechanism of miRNA-Mediated mRNA Decay in Animals

Although animal miRNAs were initially thought to repress translation of target mRNAs with little or no decrease in mRNA abundance [13,14], later studies revealed that miRNAs can also promote mRNA destabilization by recruiting deadenylases onto target mRNAs through GW182 protein (TNRC6A–C in mammals and GW182 or Gawky in *Drosophila*) [15–27]. GW182 protein plays key roles in the animal miRNA pathway through interaction with Ago. Biochemical and structural studies revealed that the tryptophan residues in the N-terminal glycine–tryptophan (GW) repeat domain of GW182 protein are recognized by the tandem tryptophan-binding pockets in the PIWI domain of Ago [20,28–36]. GW182 protein serves as a hub protein, and recruits several factors to the target mRNAs including poly(A)-binding protein (PABP) and two deadenylase complexes, CCR4–NOT and PAN2–PAN3 complexes (Box 1) [21–23,37–46].

Knockdown of CCR4–NOT components or overexpression of dominant negative form of CAF1, one of the two deadenylases in the CCR4–NOT complex, severely impeded miRNA-mediated deadenylation and mRNA decay [20,22,24–26,47]. By contrast, depletion of PAN3 or over-expression of catalytically inactive form of PAN2 had only a modest effect on poly(A) shortening [22,24,26]. These results indicate that CCR4–NOT complex, rather than PAN2–PAN3 complex, is the major trigger of miRNA-mediated deadenylation and mRNA decay (Figure 1) [20,22,24–26,47].

miRNAs can promote mRNA decay not just by recruiting deadenylases on the target mRNAs but also by increasing the accessibility of the poly(A) tail to deadenylases (Figure 1). Indeed, recent studies demonstrated that miRNA targeting or GW182 tethering promoted dissociation of PABP before deadenylation starts [48]. Furthermore, even when deadenylation was blocked by an internalized poly(A) tail, PABP was released from target mRNAs by the recruitment of the CCR4–NOT deadenylase complex via GW182 [49]. As such, dissociation of PABP as well as dead-enylation via GW182 can be a cause of translational repression and subsequent mRNA decay (see later).

After deadenylation, target mRNAs undergo degradation in the 5′-to-3′ mRNA decay pathway [20,27,50–52]. Importantly, RISCs can directly promote decapping and the subsequent mRNA

Box 1. Interaction between GW182 Protein and Two Deadenylase Complexes

GW182 protein interacts with the two deadenylase complexes, CCR4–NOT and PAN2–PAN3, through its tryptophan (W) motifs located in the C-terminal silencing domain (the W motifs in the N-terminal domain of GW182 in *Drosophila* can recruit deadenylase complexes) [21–23,43,45,46]. Recent structural studies have provided important molecular insights into these interactions. The rod-shaped CN9BD (CAF40/CNOT9-binding domain) of CNOT1 (NOT1 ortholog in vertebrates) interacts with the ARM repeat domain of the CNOT9 (NOT9/CAF40/RQCD1/RCD1). This CNOT1–CNOT9 complex binds to TNRC6 through interaction between W motifs in TNRC6 and the two hydrophobic tryptophan-binding pockets located in the ARM repeat domain of the CNOT9 [45,46]. In addition to the CNOT9-mediated interaction, CNOT1 can interact with TNRC6 via the C-terminus region and the tristetraprolin-binding site of CNOT1 [45,46].

The crystal structure of PAN3 forms intertwined and asymmetric homodimers [43,140–142]. The crystal structure of the PAN2–PAN3 complex reveals that the catalytic subunit PAN2 and PAN3 interact with 1:2 stoichiometry [43,140–142]. The PAN3 dimerization interface harbors a W-binding pocket, which is required for the interaction with TNRC6 [43].

Interestingly, *Caenorhabditis elegans* GW182 homolog protein, AIN-1, has less than 12% sequence identity to *Drosophila* GW182 and human TNRC6, but still can interact with both *C. elegans* and *Drosophila* PAN3 and NOT1 [42]. Given that the AIN-1 mutant, in which all tryptophan residues were substituted with alanine residues, did not interact with NOT1 or PAN3, the W-motif-mediated recruitment of deadenylase complexes may be conserved among animal GW182 proteins. For more details on the structural bases for the interactions between GW182 and its interactors, see Jonas and Izaurralde [143].

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