Scanning for a unified model for translational repression by microRNAs

Tamiko Nishimura¹,² & Marc R Fabian¹,²

MicroRNAs (miRNAs) silence target mRNAs by inhibiting translation and subsequently initiating mRNA decay. The mechanism by which miRNAs silence translation is still poorly understood, with a number of competing models proposed. In this issue of The EMBO Journal, Kuzuoğlu-Öztürk et al (2016) investigated miRNA silencing in human and insect cells. Their data support a model whereby miRNAs inhibit translation initiation. However, in contrast to several recent reports, their data suggest that translational inhibition is independent of 43S ribosomal subunit scanning, eIF4A translation factor activity, and 5’UTR secondary structure.

See also: D Kuzuoğlu-Öztürk et al (June 2016)

MicroRNAs (miRNAs) are a class of short (~21 nt) RNAs that post-transcriptionally repress gene expression by hybridizing to target mRNAs (Jonas & Izaurralde, 2015). In general, miRNAs inhibit protein synthesis by first repressing mRNA translation and subsequently initiating mRNA deadenylation, decapping, and decay. miRNAs recruit the miRNA-induced silencing complex (miRISC), a ribonucleoprotein complex composed of a miRNA-loaded Argonaute (AGO) protein, and the AGO-interacting protein GW182, and the CCR4–NOT complex. The authors show that the W-binding pockets in AGO proteins, which bind GW182, are critical for it to silence miRNA targets in human cells. However, they also found this to be the case in Dm S2 cells, where AGO1 has been reported to silence a target mRNA in the absence of GW182 protein (Fukaya & Tomari, 2012). It is possible, as Kuzuoğlu-Öztürk et al (2016) propose, that the Dm AGO1 utilizes its W-binding pockets to interact with another protein in the absence of GW182 in Dm that also mediates gene silencing. However, the identity of this protein and the biological significance of its interaction with AGO1 remain to be determined.

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Several early studies pointed to miRNAs inhibiting cap-dependent translation at the initiation step via an unknown mechanism (Humphreys et al, 2005; Pillai et al, 2005). Translation initiation depends on a number of factors, including the eIF4F complex, which is composed of three subunits: eIF4E, which binds to the mRNA 5’-cap structure; eIF4A1, an RNA helicase; and eIF4G, a scaffold that binds both eIF4E and eIF4A and helps recruit the small ribosomal subunit 43S pre-initiation complex (PIC) (Gingras et al, 1999). eIF4A activity is essential for unwinding highly structured 5’UTRs to allow for 43S PIC scanning.

A number of mechanisms have recently been proposed to explain how miRNAs suppress translation (reviewed in Jonas & Izaurralde, 2015). Several groups have demonstrated that the CCR4–NOT complex can repress translation independent of its deadenylase activity. In addition, miRNA translational repression requires the DEAD-box helicase DDx6, which binds the CCR4–NOT complex. miRNA translational repression has also been proposed to specifically utilize the eIF4AI paralog, eIF4AII, which was reported to interact with the CCR4–NOT complex (Meijer et al, 2013). However, this model has been questioned based on the observation that (i) eIF4AII is vertebrate-specific and (ii) eIF4AII knockout cells display no defects in miRNA silencing (Galicia-Vazquez et al, 2015). Other groups have reported that miRNAs inhibit 43S ribosomal scanning, with miRNA reporters harboring unstructured 5’UTRs being refractory to miRNA silencing (Ricci et al, 2013). Several laboratories have also reported that translational repression somehow intersects with eIF4A1 (Fukao et al, 2014; Fukaya et al, 2014) by showing that eIF4A depletion in Drosophila melanogaster (Dm) S2 cells impairs miRNA-mediated translational repression. Furthermore, experiments carried out in Dm and human cell-free extracts showed that eIF4A is displaced from miRNA-targeted reporters. Finally, it has also been reported that Dm AGO1 can facilitate eIF4A dissociation and repress translation in GW182-depleted S2 cells (Fukaya & Tomari, 2012).

In this issue of The EMBO Journal, Kuzuoğlu-Öztürk et al (2016) investigate miRNA-mediated translational repression in human and Dm cells to determine whether parallel and potentially species-specific mechanisms exist. They use a multitude of reporter RNAs that cannot be deadenylated in order to investigate “pure” translational repression mediated by miRNAs, AGOs, GW182, and the CCR4–NOT complex. The authors show that the W-binding pockets in AGO proteins, which bind GW182, are critical for it to silence miRNA targets in human cells. However, they also found this to be the case in Dm S2 cells, where AGO1 has been reported to silence a target mRNA in the absence of GW182 protein (Fukaya & Tomari, 2012). It is possible, as Kuzuoğlu-Öztürk et al (2016) propose, that the Dm AGO1 utilizes its W-binding pockets to interact with another protein in the absence of GW182 in Dm that also mediates gene silencing. However, the identity of this protein and the biological significance of its interaction with AGO1 remain to be determined.

Kuzuoğlu-Öztürk and colleagues provide evidence that miRNAs, AGO, GW182, and the CCR4–NOT complex engender a common
mechanism to degrade target mRNAs (i.e. deadenylation followed by decapping). The authors also tested whether mRNAs that are translated via 43S scanning-independent mechanisms are refractory to miRNA- or CCR4–NOT-mediated translational silencing, as suggested by several previous studies. To this end, they utilized luciferase reporters containing short (< 10 nt) 5’UTRs, including the translation initiator of short 5’UTR (TISU), which direct efficient cap-dependent translation via a scanning-independent mechanism (Elfakess et al., 2011). Both the miRISC and the CCR4–NOT complex were able to efficiently suppress the translation of these reporters, suggesting that miRNAs silence translation initiation at a step other than 43S ribosomal subunit scanning. In addition, varying the 5’UTR secondary structure had no noticeable impact on translational repression, again demonstrating that miRNA silencing does not seem to require eIF4A helicase activity.

So how do miRNAs inhibit translation? In keeping with previous studies, Kuzuoglu-Öztürk et al (2016) show that the DDx6 is critical for translational repression. They also demonstrate that DDx6 represses translation initiation in a 43S scanning-independent manner, and must interact with CNOT1 in order to do so. DDx6 also binds a number of decapping and decay factors (e.g. PatL1, EDC3, LSm14, and 4E-T) via a FDF motif-binding surface. Cells complemented with a DDx6 mutant that cannot bind to these factors were also defective in miRNA silencing. How DDx6 brings about translational repression and through which DDX-interacting protein(s) remains to be established.

In summary, these data support a model whereby the translational repression by the miRISC and CCR4–NOT is DDx6-dependent but occurs independently of 43S scanning and eIF4AI activity. However, the authors also point out that their data do not discount a role for eIF4AI in miRNA silencing. Indeed, eIF4A has been reported to dissociate from miRNA targets in vitro, and knocking down eIF4A in Dm S2 cells significantly impairs miRNA translational repression (Fukao et al., 2014; Fukaya et al., 2014). Moreover, the addition of the eIF4A-binding protein HuD impairs miRNA-mediated translational silencing in vitro, whereas a HuD mutant that cannot bind eIF4A does not (Fukao et al., 2014). It is important to note that while eIF4A helicase activity is not required for scanning-independent translation mediated by TISU, eIF4A is still recruited in the context of eIF4F. One possible explanation that could tie these data together is that the miRNA machinery may interact with eIF4A, directly or indirectly, in order to repress eIF4F-mediated translation. Thus, the miRISC could silence a mRNA whose translation is less dependent on eIF4A activity (i.e. scanning-independent), as long as eIF4A is present and accessible.

References

Figure 1. A unifying model for miRNA-mediated translational repression
miRNAs inhibit translation initiation via DDx6 but in a manner that is independent of 43S scanning and eIF4AI activity. One possibility is that DDx6-interacting proteins associate with eIF4A to inhibit 43S recruitment (depicted as question marks).