Scanning for a unified model for translational repression by microRNAs

Tamiko Nishimura^{1,2} & Marc R Fabian^{1,2}

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. GW182 also interacts with the poly(A) binding protein (PABP) and facilitates mRNA deadenylation by recruiting the PAN2–PAN3 and CCR4–NOT deadenylase complexes. While the mechanism by which miRNAs promote mRNA decay is relatively well understood, it is still unclear how miRNAs inhibit translation.

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; eIF4AI, 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 (García-Vázquez *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 eIF4AI (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

¹ Department of Oncology, McGill University, Montreal, QC, Canada. E-mail: marc.fabian@mcgill.ca

² Segal Cancer Centre, Jewish General Hospital, Lady Davis Institute for Medical Research, Montréal, QC, Canada
DOI 10.15252/embj.201694324 | Published online 20 April 2016

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).

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, Kuzuoğlu-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

- Elfakess R, Sinvani H, Haimov O, Svitkin Y, Sonenberg N, Dikstein R (2011) Unique translation initiation of mRNAs-containing TISU element. *Nucleic Acids Res* 39: 7598–7609
- Fukao A, Mishima Y, Takizawa N, Oka S, Imataka H, Pelletier J, Sonenberg N, Thoma C, Fujiwara T (2014) MicroRNAs trigger dissociation of eIF4AI and eIF4AII from target mRNAs in humans. *Mol Cell* 56: 79–89
- Fukaya T, Tomari Y (2012) MicroRNAs mediate gene silencing via multiple different pathways in *Drosophila*. *Mol Cell* 48: 825–836
- Fukaya T, Iwakawa HO, Tomari Y (2014) MicroRNAs block assembly of eIF4F translation initiation complex in *Drosophila*. *Mol Cell* 56: 67–78
- Galicía-Vázquez G, Chu J, Pelletier J (2015) eIF4AII is dispensable for miRNA-mediated gene silencing. *RNA* 21: 1826–1833
- Gingras AC, Raught B, Sonenberg N (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* 68: 913–963
- Humphreys DT, Westman BJ, Martin DI, Preiss T (2005) MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci USA* 102: 16961–16966
- Jonas S, Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* 16: 421–433
- Kuzuoğlu-Öztürk D, Bhandari D, Huntzinger E, Fauser M, Helms S, Izaurralde E (2016) miRISC and the CCR4-NOT complex silence mRNA targets independently of 43S ribosomal scanning. *EMBO J* 35: 1186–1203
- Meijer HA, Kong YW, Lu WT, Wilczynska A, Spriggs RV, Robinson SW, Godfrey JD, Willis AE, Bushell M (2013) Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation. *Science* 340: 82–85
- Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W (2005) Inhibition of translational initiation by Let-7 MicroRNA in human cells. *Science* 309: 1573–1576
- Ricci EP, Limousin T, Soto-Rifo R, Rubilar PS, Decimo D, Ohlmann T (2013) miRNA repression of translation *in vitro* takes place during 43S ribosomal scanning. *Nucleic Acids Res* 41: 586–598