Not lost in translation: stepwise regulation of microRNA targets

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MicroRNAs (miRNAs) are endogenous, ~22-nucleotide-long, noncoding RNAs that play critical roles in physiology and disease via mechanisms that remain obscure. Although numerous studies implicate miRNAs in repression of translation, more recent reports suggest that the major role of miRNAs is in reduction of target mRNA stability. Because mRNA translation and stability are intimately connected, it has been a challenge to establish whether miRNAs induce translational repression, mRNA decay, or both. If miRNAs reduce both mRNA translation and stability, the timing and contribution of each process to overall repression is unclear. Indeed, it has been debated whether mRNA decay is a cause or consequence of miRNA-mediated translational repression. On the other hand, if these events are mutually exclusive, what determines which mechanism is used? In a recent issue of *Science*, Bazzini *et al* (2012) use genome-wide ribosome footprinting and RNA sequencing (RNA-Seq) to demonstrate that in developing zebrafish embryos, miR-430 naturally represses translation initiation of target mRNAs, followed by their deadenylation and decay.

The earliest studies in *Caenorhabditis elegans* indicated that miRNAs repress translation after initiation (Olsen and Ambros, 1999). However, subsequent cell-free and cell-based reporter systems in worms, flies, and mammals demonstrated repression of translation at initiation (Pillai *et al*, 2005; Wang *et al*, 2006; Mathonnet *et al*, 2007; Thermann and Hentze, 2007; Wakiyama *et al*, 2007) and mRNA deadenylation and decay (Behm-Ansmant *et al*, 2006; Wu *et al*, 2006; Wakiyama *et al*, 2007). All of these studies examined the effects of individual miRNAs on individual target mRNAs and thus, by definition, did not capture the global effects of miRNAs on repression of mRNA translation and stability.

To assess the global effects of an miRNA on its target mRNAs in mammals, microarrays and RNA-Seq have been used to measure mRNA levels, stable isotope labelling with amino acids in cell culture (SILAC) has been used to measure translation rates (Baek *et al*, 2008; Selbach *et al*, 2008), and ribosome footprinting has been used to measure ribosome occupancy (Guo *et al*, 2010). These reports concluded that mRNA decay predominantly contributes to overall repression of target mRNAs, with only a minor contribution from

repression of translation (initiation). However, these studies used artificially-expressed miRNAs or miRNA knockouts and assessed translation and mRNA levels after 12–48 h, at which point only the most downstream effects of miRNA function may have been observed. Though these global approaches indicated major effects on target mRNA levels, they did not rule out important and likely earlier effects on translation.

Like Guo *et al* (2010), Bazzini *et al* (2012) combined ribosome footprinting and RNA-Seq to study the global effects of an miRNA on its target mRNAs. They examined miR-430, a predominant miRNA naturally induced in zebrafish embryos at the onset of zygotic transcription, which has been previously shown to promote deadenylation and degradation of maternal transcripts at 5 and 9 hours post fertilization (hpf) (Giraldez *et al*, 2006). In this most recent study, Bazzini *et al* (2012) assessed ribosome occupancy and mRNA levels of miR-430-targeted mRNAs at earlier time points, 4 and 6 hpf (corresponding to 2 and 4 h after miR-430 induction). At 4 hpf, ribosome density along miR-430-targeted mRNAs uniformly decreased without a corresponding decrease in the

Figure 1 Stepwise model of miRNA repressive events. MiRNAs first block translation initiation (1) and then promote deadenylation (2). As a consequence, target mRNAs are degraded in processing bodies (3).

mRNA levels, indicating repression of translation initiation. At 6 hpf the levels and/or poly(A) tail lengths of the majority (70%) of mRNAs translationally repressed at 4 hpf were reduced, indicating that mRNA decay followed translational repression (Figure 1).

At 4 hpf, individual miR-430-targeted mRNAs were slightly deadenylated. It therefore remained possible that partial deadenylation triggers translational repression or that deadenylation and translational repression occur simultaneously. To exclude these possibilities, Bazzini *et al* (2012) artificially blocked deadenylation of a miR-430-targeted reporter at 4 hpf and showed that translational repression can occur in the absence of deadenylation. Still, it remains unclear whether endogenous miR-430-targeted mRNAs that are naturally polyadenylated are translationally repressed prior to initiation of deadenylation. Indeed, initial shortening of the poly(A) tail at 4 hpf may cause translational repression by reducing the occupancy of the poly(A) binding protein (PABP) that promotes translation initiation.

Although Bazzini *et al* (2012) demonstrate that repression of translation initiation can precede mRNA decay of the majority of endogenous miR-430-targeted mRNAs, there was variation in the modes and timing of repression events. For 30% of mRNAs translationally repressed at 4 hpf, mRNA decay was not observed at 6 hpf, indicating either that these targets are subject to pure translational repression or that the onset of mRNA decay is delayed beyond 6 hpf. For 47% of mRNAs reduced at 6 hpf, translational repression was not detected at 4 hpf, indicating either that translational repression occurred sometime between 4 or 6 hpf or that these targets are subject to pure mRNA decay. For 12% of mRNAs reduced at 6 hpf, translational repression was not

detected at 4 hpf but these mRNAs were undergoing both translational repression and decay at 6 hpf. Importantly, no miR-430-targeted mRNAs were subject to decay prior to the onset of translational repression.

Bazzini *et al* (2012) provide several insights into the order of events of endogenous miRNA-mediated repression. However, this study also raises new questions. Although the majority of translationally-repressed mRNAs are later decayed, the mechanisms mediating these processes are not known. Bazzini *et al* (2012) determine time points that can be used for isolation of the factors mediating translational repression and mRNA decay. Additionally, some miR-430-targeted mRNAs were purely translationally repressed while others were purely decayed at the examined time points. These outlier mRNAs should be examined more carefully to identify protein or mRNA determinants of the different outcomes or altered timing of miRNA-mediated repression. Ribosome footprinting combined with RNA-Seq at early time points should be applied in other natural non-steady-state conditions to define the contribution of each of these modes of repression in development, differentiation, or stress responses in other organisms.

Note added in proof

While this feature was in press, Rachel Green and colleagues reported that miRNA-mediated translational repression precedes deadenylation and decay of natural and artificial reporter mRNAs in *Drosophila* (*Science* (2012) **336**(6078): 237).

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Baek D, Villen J, Shin C, Camargo FD, Gygi S.P, Bartel DP (2008) The impact of microRNAs on protein output. *Nature* **455**: 64–71
- Bazzini AA, Lee MT, Giraldez AJ (2012) Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. *Science* **336**: 233–237
- Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E (2006) mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev* **20**: 1885–1898
- Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF (2006) Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* **312**: 75–79
- Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* **466**: 835–840
- Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L, Murata T, Biffo S, Merrick WC, Darzynkiewicz E, Pillai RS, Filipowicz W, Duchaine TF, Sonenberg N (2007) MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. *Science* **317**: 1764–1767
- Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. *Dev Biol* **216**: 671–680
- 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
- Selbach M, Schwanhaussner B, Thierfelder N, Fang Z, Khanin R, Rajewsky N (2008) Widespread changes in protein synthesis induced by microRNAs. *Nature* **455**: 58–63
- Thermann R, Hentze MW (2007) *Drosophila* miR2 induces pseudopolysomes and inhibits translation initiation. *Nature* **447**: 875–878
- Wakiyama M, Takimoto K, Ohara O, Yokoyama S (2007) Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. *Genes Dev* **21**: 1857–1862
- Wang B, Love TM, Call ME, Doench JG, Novina CD (2006) Recapitulation of short RNA-directed translational gene silencing in vitro. *Mol Cell* **22**: 553–560
- Wu L, Fan J, Belasco JG (2006) MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci USA* **103**: 4034–4039