let-7 nips translation in the bud

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The role of microRNAs (miRNAs) in the repression of translation is well known, but exactly how they achieve this, and at what stage in the translation process, has been the subject of much debate. New work shows that, in the case of *lethal-7* (*let-7*), translation is suppressed at the initiation step.

Both the 3' polyadenylated (polyA) tail and the 5'-cap structure of mRNA are crucial for translation. The polyA tail recruits polyA-binding protein (PABP), which in turn recruits initiation factors that interact with the 5' cap and bring about translation. Changes in the length of the polyA tail regulate protein expression: polyadenylation stabilizes the mRNA and promotes translation,

whereas deadenylation has the opposite effect. miRNAs repress translation by forming part of protein complexes (miRNPs) that bind to the 3' end of target mRNAs.

Yokoyama and colleagues combined cell extracts from human embryonic kidney cell lines over-expressing different components of the miRNA pathway (such as the miRNP components AGO2 and GW182) to create a cell-free system that recapitulates miRNA-mediated translational repression. To visualize *let-7* function, they created a reporter construct that encoded a luciferase mRNA containing six *let-7* target sequences. After addition of *let-7*, AGO2 and GW182 were found to associate with the reporter transcript, indicating that *let-7* recruits a miRNP containing these proteins to the mRNA.

The authors then looked at the role of the mRNA polyA tail. *let-7* reduced translation of the reporter mRNA to <30%, but had no effect when a deadenylated form was used. Translational repression was also lost using a version in which the *let-7* target sequences were mutated; so repression is sequence specific. Next, they investigated the involvement of the 5' cap. *let-7* could not repress translation of the reporter mRNA when it carried a

non-physiological 5' cap, regardless of whether the transcript was polyadenylated or not. So, both the polyA tail and the 5' cap are needed for *let-7*-mediated translational repression, consistent with *let-7* functioning at the initiation step.

But what is the mechanism by which *let-7* represses translation? Northern blots showed that reporter mRNAs were shorter following incubation with *let-7*, indicating that the polyA tails were deadenylated in a *let-7*-dependent manner; the cap structure of the mRNA had no effect on this deadenylation. Furthermore, deadenylation occurred even in the presence of a translational inhibitor, suggesting that it is the cause, not a result, of translational repression.

The authors propose that *let-7* represses translation by recruiting miRNP complexes that dedenylate the mRNA polyA tail, abolishing its crucial synergy with the 5' cap. The system used here by Yokoyama and colleagues provides a valuable platform for future studies into miRNA-mediated pathways.

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ORIGINAL RESEARCH PAPER Wakiyama, S. et al. *let-7* microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. *Genes Dev.* **21**, 1857–1862 (2007)