MICRORNA

Lost in translation

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level by base pairing to partially complementary sequences in the 3′ untranslated regions (3′ UTRs) in target mRNAs. Exactly how miRNAs repress translation is unclear, although it is thought that they exert their effects through a variety of mechanisms. Two groups, reporting in *Nature Structural & Molecular Biology*, now demonstrate that miRNA-targeted mRNAs are present in translating polysomes even when protein production is inhibited.

Nilsen and colleagues analysed the subcellular distribution of three abundant miRNAs — *miR-21*, *miR-16* and *let-7a* — in exponentially growing HeLa cells. They found that the vast majority of all three miRNAs sedimented with the actively translating polysomal fractions on a sucrose gradient. Interestingly, transfection of cells with an oligonucleotide that is complementary to *let-7a* led to a shift in the sedimentation of *let-7a*. Also, in cytoplasmic extracts treated with micrococcal nuclease, which cleaves exposed regions of mRNA, *miR-21* was much more resistant to digestion than actin mRNA, and its sedimentation profile differed from that of ribosomes or ribosome-protected actin mRNA fragments. Together, these results indicate that miRNAs associate with polysomes by interacting with target mRNAs.

When the authors treated cells with puromycin, an agent that terminates polypeptide extension, the fraction of polysomes was reduced, and the sedimentation of both mRNAs and miRNAs shifted in parallel to lighter, non-translating ribosomal fractions. The Nilsen group also showed that a known miRNA target, *KRAS* mRNA, sedimented with polysomes and shifted to lighter fractions in the sucrose gradient following treatment with puromycin. These findings imply that miRNAs are associated with actively translating mRNAs, which includes target mRNAs.

The second group, led by Richter, transfected HeLa cells with a reporter mRNA construct that was linked to the *lin-41* 3′ UTR, which contains two *let-7a* miRNA target sites. The presence of *let-7a* miRNA caused a ~3.5-fold reduction in reporter mRNA expression. The majority of the reporter mRNA sedimented in the polysome-containing fraction on a sucrose gradient, and treatment with puromycin resulted in a shift in the sedimentation to the lighter ribosomal fractions. Together, these results indicate that the reporter mRNA associates with actively translating ribosomes.

The authors constructed a *lin-41*-containing reporter mRNA with an iron-response element in the 5′ UTR, which causes translation inhibition in the absence of iron. Indeed, in the presence of iron, the majority of reporter mRNA sedimented with polysomes, whereas in the absence of iron, *in vivo* ribosome 'run-off' occurred as reporter mRNAs sedimented with non-translating ribonucleoparticles.

So, if target mRNAs are seemingly translated normally by actively translating polysomes, why is almost no protein produced? The Richter group provided an initial clue when they tried to immunoprecipitate ribosomes associated with a reporter mRNA that contained several Myc tags at the N terminus of the encoded protein and either contained or lacked the *lin-41* 3′ UTR. Ribosomes associated with repressed mRNAs, but not with normally translated mRNAs, did not immunoprecipitate with an anti-Myc antibody. This indicates that the nascent polypeptide chain that is derived from the target mRNA under *let-7a* control is either destroyed immediately, masked by associated factors that mark the protein for destruction, or is in some other way lost in translation. Elucidating the mechanism of nascent-polypeptide destruction will undoubtedly be the next goal.

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ORIGINAL RESEARCH PAPERS Maroney, P. A. *et al.* Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nature Struct. Mol. Biol.* 26 Nov 2006 (doi:10.1038/nsmb1174) | Nottrott, S. *et al.* Human *let-7a* miRNA blocks protein production on actively translating polyribosomes. *Nature Struct. Mol. Biol.* 26 Nov 2006 (doi:10.1038/nsmb1173)
FURTHER READING Eulalio, A. *et al.* P-bodies: at the crossroads of post-transcriptional pathways. *Nature Rev. Mol. Cell Biol.* **8**, 9–22 (2007) | Rana, T. M. Illuminating the silence: understanding the structure and function of small RNAs. *Nature Rev. Mol. Cell Biol.* **8**, 23–36 (2007)
WEB SITES
Timothy W. Nilsen's laboratory:
http://www.rnaresearch.org/nilsen.htm
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