RNA INTERFERENCE

When microRNAs activate translation

Contrary to their traditional role, microRNAs (miRNAs) contribute to an increase in translation during cell quiescence. This function may be exploited for microRNA-mediated regulation of protein expression.

Under certain stress conditions, as well as during development, cells exit the cell cycle and enter a stage of quiescence. Joan Steitz and Shobha Vasudevan from Yale University are interested in the regulators of this process and have now discovered an unexpected player: miRNAs that, in a reversal of the role traditionally ascribed to them, increase the translation of their targets.

For cells growing in culture, quiescence —the reversible exiting of the cell cycle—is brought about by several stimuli: for example, contact inhibition, loss of adhesion or, as in the case of the work by the Steitz group, serum starvation. Whereas different stimuli induce expression of different genes, there is an underlying gene expression pattern that is common to all quiescent cells that ensures that the condition is reversible and suppresses terminal differentiation. Understanding the regulation of gene expression during quiescence is important to pinpoint the differences between a quiescent and a differentiating cell.

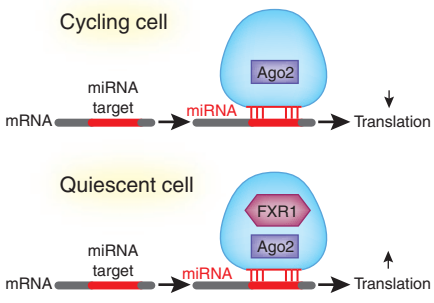

Figure 1 | In a cycling cell, a microRNA paired with its target sequence on the mRNA leads to downregulation of translation, but in a quiescent cell the same pairing results in increased protein translation.

Steitz and Vasudevan have previously identified two key components for translational upregulation during serum starvation: the proteins argonaute 2 (Ago 2) and fragile-X-metal retardation related protein 1 (FXR1). Both need to be associated with (A+U)-rich elements in the 3′ untranslated region (UTR) of the TNF alpha mRNA. Now the researchers introduce a miRNA as the mediator of this interaction.

After a computational search for miRNAs that bind to the 3′ UTR of TNF alpha, the authors experimentally confirmed one candidate, miRNA 369-3, and showed that direct base pairing between the miRNA and its target is required for translational upregulation after serum starvation.

The intriguing question then was whether this is only true for the miRNA targeting TNF alpha mRNA, or whether miRNAs in general upregulate translation during quiescence.

Vasudevan decided to test more miRNAs. She chose let7-a and miRcxcr4; both have

GENE REGULATION

GATHERING A BOUQUET OF miRNA TARGETS

Pulling down microRNA-induced silencing complexes (miRISCs) allows researchers to collect microRNAs and their mRNA targets *in vivo*.

Tiny and elusive, microRNAs (miRNAs) went unnoticed for years. Only a serendipitous mutation in a well-characterized model organism brought them to light. But because genetic tools are slow to identify miRNAs and the mRNAs they target, many researchers use bioinformatics to predict miRNA pathways. The success rate of these *in silico* searches, however, has not been satisfactory. Now, with his colleagues at University of Colorado at Boulder, Min Han reports an efficient and robust biochemical screen for miRNAs and their targeted mRNAs, allowing unprecedented *in vivo* characterization of these elusive species.

RNA-induced silencing complexes (RISCs) form around miRNA precursors to process the miRNA and disrupt the expression of targeted mRNAs. Members of the GW182 protein family are essential components of the miRISC, but mutations in *ain-1*, a *Caenorhabditis elegans* gene encoding a GW182 protein, had mild miRNA-deficient phenotypes.

Suspecting redundancy, Han and colleagues sought and identified a homolog of *ain-1*, which they called *ain-2*. They found that worms with mutations in both genes had an miRNA pathway block. AIN-1 and AIN-2, however, were not needed for miRNA production, but instead for miRNA function. The researchers proposed that AIN-1– and AIN-2–associated miRISCs acted at an 'effector stage' of the pathway and might contain mature miRNAs and their targeted mRNAs.

In coimmunoprecipitation assays for either AIN-1 or AIN-2, Han and colleagues characterized other components of these effector miRISCs, hoping to capture active complexes from living tissue. They first sequenced the collected small RNAs and found an impressive 106 of the 130 previously known miRNAs between the AIN-1– and AIN-2–derived samples. Additionally, the small RNA samples had 9 new putative miRNAs that had been missed in earlier screens, perhaps because of low expression and sequence conservation. This catalog of miRNAs suggested that this approach was effective and comprehensive.

Then, co-first authors Liang Zhang and Lei Ding isolated mRNA from these samples. In preliminary experiments with quantitative reverse transcription–PCR, they found that "many known target mRNAs appeared to be stably associated with these complexes," says Zhang. Encouraged, the researchers ran the AIN-1– and AIN-2–containing miRISCs on microarrays. They detected almost 90% of the 12 previously established targeted mRNAs in the samples, reporting a sensitivity not yet seen in computational predictions of miRNA targets or from pulldowns with other components of miRISCs.

In total, the researchers identified almost 3,600 transcripts associated with AIN-1 or AIN-2. Han and colleagues could follow mRNAs that are known to be developmentally regulated, detecting

been well studied by many groups and are known to downregulate their targets. Placing the target sites for these miRNAs in the 3′ UTR of a reporter gene, the scientists showed the expected downregulation in proliferating cells but upregulation of the reporter during serum starvation. This increase in translation depended on base-pairing between miRNAs and their targets as well as on the presence of Ago2 and FXR1 (**Fig. 1**).

This surprising role of miRNAs during quiescence could be exploited to specifically upregulate protein expression during this process. By engineering a mRNA with well-defined target sites for a miRNA, the protein will be overexpressed only as the cells exit the cell cycle, and its contribution to establishing and maintaining quiescence can be studied.

For the time being, Vasudevan is interested in the mechanism behind this process. She says, "I want to know how a microRNA can be told to switch to activate rather than repress," and she plans to look at two aspects. The first is whether the base-pairing requirements of the miRNA with its target are different during activation and repression; the second is a thorough identification of the protein components of the RNA-associated complex and their modifications.

So far Steitz and Vasudevan found a new role for miRNAs in one defined biological condition; it is conceivable that down the line the components that trigger this role reversal in miRNA function may be harnessed to fine-tune miRNA activity so that miRNAs can be made into research tools that either activate or repress protein expression.

Nicole Rusk

RESEARCH PAPERS
Vasudevan, S. *et al.* Switching from repression to activation: microRNAs can up-regulate translation. *Science* **318**, 1931–1934 (2007).

these transcripts only when they are regulated by miRNAs, demonstrating these data reflected the contents of active miRISCs. The investigators also found that 28% of AIN-1– or AIN-2–associated transcripts had been predicted to be miRNA targets by bioinformatic analysis, a significantly higher proportion than the 13% predicted of the entire transcriptome. This observation suggests that these AIN-1– or AIN-2–derived samples were enriched for transcripts that had already been predicated to be miRNA targets based on characteristics other than association with effector miRISCs.

Han hopes that this list of putative miRNA targets will become a valuable training set for algorithms, improving their predictive power. He says that "if we have enough data from many complexes under different physiological conditions, we may be able to generate a miRNA-miRNA target interaction map through correlation analysis," matching miRNAs to targeted mRNA and deducing miRNA networks.

Han says that the biochemical screen is a "very effective way to identify miRNA targets," and that this approach could be used in other systems as well. GW182 proteins have divergent sequences, but their conserved function suggests that the protein family may be a foothold into the effector miRISC, giving researchers a new vantage point on the world of miRNAs.

Katherine Stevens

RESEARCH PAPERS
Zhang, L. *et al.* Systematic identification of *C. elegans* miRISC proteins, miRNAs, and mRNA targets by their interactions with GW182 proteins AIN-1 and AIN-2. *Mol. Cell* **28**, 598–613 (2007).

NEWS IN BRIEF

MICROARRAYS

Isostable DNA

The greater stability of G·C DNA base pairs compared to that of A·T base pairs can introduce bias into DNA applications such as the use of microarrays. Ahlborn *et al.* now make isostable DNA by chemically modifying nucleic acids, introducing caps, locks and intercalators on target all-(A+T) strands to stabilize interactions and *N*4-ethylcytosine residues on target all-(G+C) strands to tune down the stability. The use of these 'decorated' nucleic acids is compatible with existing microarray platforms.

Ahlborn, C. *et al. J. Am. Chem. Soc.* **129**, 15218–15232 (2007).

CELL BIOLOGY

Separating cells by cell-cycle phase

Methods for separating cells according to cell-cycle phase are important for many biotechnology applications, but gentle ways to do so without introducing cell stress are lacking. Kim *et al.* describe a dielectrophoresis-based microfluidic device that gently separates mammalian cells by taking advantage of the relationship between cell-cycle phase and the cell volume—G2/M phase cells are much larger than G1/S phase cells.

Kim, U. *et al. Proc. Natl. Acad. Sci. USA* **104**, 20708–20712 (2007).

GENOMICS

Assembling bacterial genomes

High-throughput sequencing technologies produce an unprecedented amount of data—in the form of short reads. To assemble a whole genome from sequence fragments of no more than 300 bp in length is a computational challenge. Chaisson and Pevzner now present a new Eulerian assembler that generates long contigs from 454 sequence reads that can then be assembled into the full genome with the help of low-coverage Sanger reads.

Chaisson, M.J. & Pevzner, P.A. *Genome Res.*, published online December 14, 2007.

BIOPHYSICS

Dynamic protein interactions in live cells

Understanding signaling pathways requires analysis of their dynamic and interacting protein components. Fluorescence fluctuation spectroscopy methods can be used for such studies, but have not been extensively applied to live cells. In recent work, Slaughter *et al.* used such fluctuation techniques, along with imaging, to study the diffusion dynamics, stoichiometries and interactions between components of the MAP kinase cascade in living yeast.

Slaughter, B.D. *et al. Proc. Nat. Acad. Sci.* **104**, 20320–20325 (2007).

MICROSCOPY

Super-resolution in 3D

The diffraction limit of light microscopy has recently been broken with several new techniques. However, super-resolution in all three dimensions has remained challenging. Now, using optical astigmatism in combination with their two-dimensional STORM method, Huang *et al.* achieved three-dimensional super-resolution imaging, with 20–30 nm lateral and 50–60 nm axial resolution.

Huang, B. *et al. Science*, published online 3 January 2008.