RNA INTERFERENCE

# microRNAs—subtler than you think

**Two research groups apply quantitative proteomics to study the effects of microRNAs on cellular proteins.**

MicroRNAs (miRNAs) regulate gene expression post-transcriptionally in many organisms. miRNAs are small RNAs that base-pair with mRNA transcripts and, either by destabilization of the message or by translational repression, downregulate the expression of the corresponding gene. The ubiquity of miRNAs and their role in important biological processes is undeniable. But with some exceptions, the question remains: what are the biological targets of these key regulators, and what are their effects on a global scale?

Large-scale approaches to tackle this question have mainly focused on examining the effects of a given miRNA on global mRNA levels, defining which messages go up and which go down, when an miRNA of interest is removed or overexpressed. The global effects on proteins, however, have remained unknown. Now, two independent research groups applied quantitative proteomics to examine what happens to cellular proteins when the amount of an miRNA is altered (Baek *et al.*, 2008; Selbach *et al.*, 2008).

At the Max Delbrück Centre in Berlin, Nikolaus Rajewsky and colleagues teamed up with the lab of Matthias Selbach. Meanwhile, David Bartel and colleagues at the Whitehead Institute collaborated with Steven Gygi at Harvard University. Both groups adapted the stable isotope labeling with amino acids in cell culture (SILAC) method to compare two samples that differ with respect to the presence or absence of a single miRNA (**Fig. 1**).

Bartel and colleagues used the standard SILAC approach, in which the proteins in one sample are labeled with heavy isotopes and the other sample remains unlabeled ('light'), and then both samples are analyzed by mass spectrometry. Heavy-isotope labeling allows the peptides from each sample to be distinguished, and the ratio between peak intensities can be used to determine the relative protein levels between the samples. Applying this approach, Bartel and colleagues examined the effect, on thousands of proteins, of overexpressing miR-124, miR-1 or miR-181 in HeLa cells and, more importantly, of removing miR-223, in neutrophils derived from a miR-223 knockout mouse.

In contrast, instead of labeling cells with heavy isotopes for several days such that almost 100% of proteins incorporate the label, Rajewsky and colleagues used an adapted SILAC procedure. The researchers pulse-labeled the two samples for 24 hours either with medium-heavy or heavy isotopes, and then used the fact that pre-existing proteins in the sample remain 'light' (unlabeled) to look at differences between the samples specifically in new protein synthesis. Using this pulsed-SILAC approach, they examined the effect of overexpressing five different miRNAs (miR-1, miR-30, miR-155, miR-16 and let-7b) in HeLa cells, and of knocking down let-7b with antisense oligonucleotides containing locked nucleic acids, in these cells.

The methodological differences in these two studies notwithstanding, the overall conclusions drawn are similar and are not necessarily what was expected. "Since we started these experiments," says Rajewsky, "I could not wait for the day when we would see the results. That gives some idea of the level of uncertainty about what we would see." What both groups report is that the global effects of miRNAs on proteins are quite subtle. The changes did not often exceed four fold and were typically much smaller than that, though there are some notable exceptions. Rajewsky and colleagues report somewhat larger-fold changes, which may be due to their use of the pulsed-SILAC approach. Many of the sequence rules that were thought important for miRNA-mRNA pairing, previously derived by comparative sequence analysis and by looking at global changes in mRNA, still apply at the protein level.

Notably, the miRNA 'seed' sequence and the complementary target sequence in the mRNA, which are likely to be important in miRNA-mRNA interactions, are often highly conserved between species. As Bartel puts it: "When you put the comparative sequence analysis, which tells us how many of these sites are under selective pressure to be maintained, together with the proteomics, which tells us how small an effect these sites are imparting, one of the big results here I think is that the precise amount of so many cellular proteins is important in evolution."

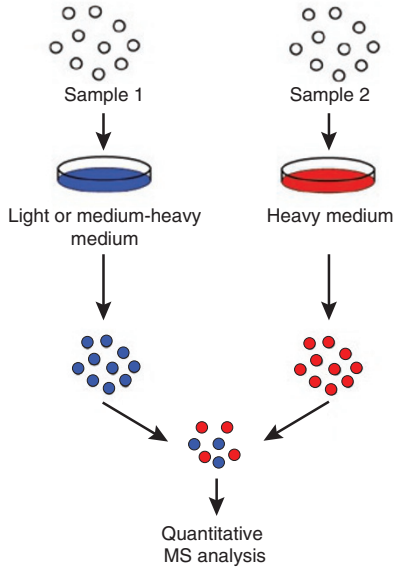


**Figure 1** | Schematic depicting the SILAC method. Samples are cultured in media containing amino acids with different isotope labels, pooled and analyzed by mass spectometry (MS). For the pulsed-SILAC adaptation, the final pooled mixture contains three classes of cells (not shown). Figure modified from the *Nature* articles.

Undoubtedly, quantitative proteomics will continue to be applied in many different contexts to further study how miRNAs keep proteins at their optimal levels in the cell.

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**RESEARCH PAPERS**

Baek, D. *et al.* The impact of microRNAs on protein output. *Nature*, published online 30 July 2008.

Selbach, M. *et al.* Widespread changes in protein synthesis induced by microRNAs. *Nature*, published online 30 July 2008.