RNA INTEREFERENCE

MicroRNA: the perfect host

By creating an expression cassette that embeds the sequence of short hairpin (sh)RNA into the larger fold of a ubiquitous microRNA, scientists can achieve highly efficient target gene knockdown.

'Let a cell teach you how to do RNA interference most effectively', could have been the motto behind collaborative efforts by the groups of Greg Hannon and Scott Lowe at Cold Spring Harbor Laboratory and Stephen Elledge at Harvard University to generate a second generation of shRNA libraries. Their goal was to develop an effective screening tool to identify genes essential for the regulation of tumor growth; a previous shRNA expression library had given good results, but it suffered from sub-optimal efficiency of knockdown.

The scientists adopted a strategy, first introduced by Brian Cullen at Duke University, which takes advantage of the cell's endogenous RNA interference (RNAi) machinery to process and cleave an shRNA embedded in a larger microRNA fold, resulting in more stable expression of small interfering RNAs (**Fig. 1**). Hannon and Elledge created large libraries of microRNA-based shRNA vectors targeting many of the human and mouse genes, and observed more efficient knockdown than with the previous shRNA library (Silva *et al.*, 2005).

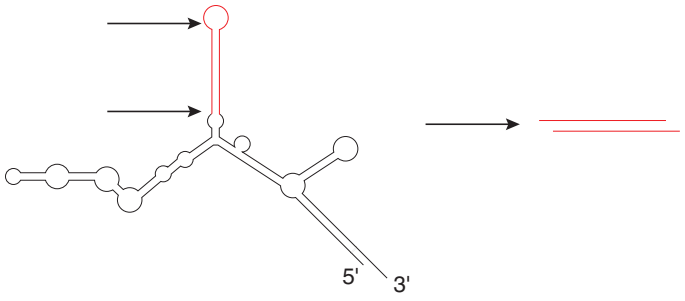

Figure 1 | Cells process a hairpin RNA (red) embedded in a microRNA fold like endogenous microRNAs. The RNAi machinery of the cell cleaves the microRNA (at the sites indicated with arrows) leading to a robust expression of small interfering RNAs.

An added bonus of the new library is the ease of cloning that allows quick insertion of any shRNA into the microRNA fold, together with a promoter of choice. Although the Hannon-Elledge library is driven by an RNA polymerase III promoter, a promoter the cell uses for small noncoding RNAs, microRNAs—like mRNAs—are transcribed from RNA polymerase II promoters. These promoters yield abundant and stable transcripts

CELL BIOLOGY

TEACHING CELL BIOLOGISTS HOW TO COUNT

To truly understand the biochemistry of cellular systems, the concentrations of the endogenous protein players must be known, and these can be obtained by careful quantification of fluorescent fusion proteins.

As tools and techniques to study biochemical processes and structures *in vivo* progress, researchers gain an increasingly detailed appreciation of the inner workings of the cell. A crucial element in understanding the biochemistry of cellular systems is knowing the concentrations of the participating molecules, but surprisingly, this information is rarely determined. "Everything in biology is just complicated chemistry and physics, so all the reactions that take place in biology depend on the concentrations of the reactants," remarks Thomas Pollard of Yale University. "But I think that not enough cell biologists are interested in quantitative aspects in their work."

Pollard and postdoc Jian-Qiu Wu hope that this sentiment will change, and offer a new method to calculate the global and local concentrations of endogenous proteins in live cells. Although immunoblotting has been used to determine the copy number of proteins in cells, local concentrations cannot be determined this way. Fluorescence is a good indicator of local presence, but few researchers have bothered to calibrate the fluorescence of tagged proteins in cells. "Pick up any biology journal, and you'll find it is full of beautiful images of fluorescent fusion proteins, but few investigators ever get any quantitative information from these pictures!" says Pollard.

By working with fission yeast, Wu and Pollard took advantage of homologous recombination to replace the endogenous protein with a fluorescent fusion protein, expressed from the native promoter. Being specifically interested in elucidating the mechanism of cytokinesis, they integrated yellow fluorescent protein (YFP) into the genome at either the amino or carboxy terminus of 27 different cytokinesis proteins. They rigorously tested the cells to ensure that the YFP fusions did not substantially change the expression or function of the endogenous proteins. Using confocal microscopy and quantitative immunoblotting, they calibrated the intensity of fluorescence per YPF molecule, which they used to literally count the number of fluorescent fusion proteins in a live cell. They determined concentrations of proteins involved in

and, most importantly, can be made inducible.

Intrigued by the possibility of an inducible shRNA library, the groups of Elledge and Lowe, working independently, cloned some microRNA-shRNA constructs under the control of a tetracycline-regulated RNA polymerase II promoter and showed tight control of gene knockdown in cultured cells even when only a single copy of the shRNA vector was integrated into the host genome (Stegmaier *et al.*, 2005; Dickins *et al.*, 2005). In addition, Ross Dickins in the Lowe lab demonstrated that the growth of tumors formed by cells infected with an oncogene and an inducible shRNA vector targeting p53, depended on the induction of the shRNA. Shutting the shRNA off caused re-expression of p53 and shrinkage of the tumors. Dickins sees an important application for such a system in the identification of therapeutic targets in cancer. He says: "Experiments like ours can provide a rational basis for targeting particular components of pathways because the short hairpin RNA can mimic a targeted therapeutic." If the knockdown of a gene causes a tumor to shrink, a drug targeting this gene will most likely have the same effect.

Learning from a cell's own RNAi machinery how to best regulate gene expression may well turn out to be a powerful tool to better understand and fight tumor cells.

Nicole Rusk

RESEARCH PAPERS

Dickins, R.A. *et al*. Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. *Nat. Genet.* **37**, 1289–1295 (2005).

Silva, J.M. *et al.* Second-generation shRNA libraries covering the mouse and human genomes. *Nat. Genet.* **37**, 1281–1288 (2005).

Stegmeier, F. *et al.* A lentiviral microRNA-based system for single-copy polymerase II–regulated RNA interference in mammalian cells. *Proc. Natl. Acad. Sci. USA* **102**, 13212–13217 (2005).

cytokinesis ranging from 600 copies per cell (formin Cdc12p) to 1.43 million copies per cell (actin).

Even in a genetically 'friendly' organism like yeast, it is not possible to tag every protein coded by the genome. Wu and Pollard ran into this problem with actin, but worked out a suitable solution: a fluorescently tagged fusion protein can be expressed from a plasmid at a low level such that it is still possible to visualize the localization of the protein without considerably increasing its total endogenous concentration. This strategy could potentially also be used when working with organisms incapable of homologous recombination.

Whereas traditionally biochemical processes have been studied in isolation *in vitro*, the ability to obtain quantitative data from living cells should provide biologists with a much more accurate picture of cellular systems. Pollard summarizes: "Biology, at the end of the day, is going to be all about putting together quantitative information to understand how whole huge systems such as cytokinesis work."

Allison Doerr

RESEARCH PAPERS

Wu, J.Q. & Pollard, T.D. Counting cytokinesis proteins globally and locally in fission yeast. *Science* **310**, 310–314 (2005).

NEWS IN BRIEF

IMAGING AND VISUALIZATION

Signal amplification in molecular imaging by pretargeting a multivalent, bispecific antibody

Radiolabeled antibodies are a useful tool for *in vivo* imaging of tumors. Sharkey *et al.* show that by injecting mice first with bispecific antibodies that target a particular tumor antigen, then with radioisotope conjugated to a hapten recognized by the same antibodies, one can target and image tumors more rapidly and with greater specificity than with directly radiolabeled antibodies.

Sharkey, R.M. *et al. Nat. Med.* **11**, 1250–1255 (2005).

STEM CELLS

Isolating gene-corrected stem cells without drug selection

Positive-negative drug selection is the preferred strategy for isolating gene-targeted stem cells. This method, however, can take too long for the maintenance of some stem cell types. Using fluorescent protein genes instead of drug resistance markers for their targeting construct, Hatada *et al*. demonstrate a more rapid, fluorescence-activated cell sorting (FACS)-based positive-negative strategy to isolate targeted cells.

Hatada, S. *et al*. *Proc. Natl. Acad. Sci. USA* **102**, 16357–16361 (2005).

MOLECULAR LIBRARIES

Cell-specific targeting of nanoparticles by multivalent attachment of small molecules

Designing molecules that specifically target only certain cell types is a delicate craft; Weissleder *et al.* present a high-throughput, nanoparticle-based assay for characterizing the interactions of a wide variety of functional groups with cells of different types or activation states. The resulting data can be used to dramatically improve the design of highly specialized, targeted compounds.

Weissleder, R. *et al. Nat. Biotechnol.* **23**, 1418–1423 (2005).

PROTEOMICS

Metalloproteomics: high-throughput structural and functional annotation of proteins in structural genomics

An estimated 15–25% of the proteins in the protein data bank are coordinated with at least one metal atom. Chance *et al.*, working in conjunction with the New York Structural GenomiX Research Consortium, have developed a high-throughput, X-ray absorption spectroscopy–based approach for identifying and characterizing such metalloproteins with ~95% accuracy.

Shi, W. *et al. Structure* (*Camb.*) **13**, 1473–1486 (2005).

CELL BIOLOGY

Marker-specific sorting of rare cells using dielectrophoresis

Fluorescence-activated cell sorting (FACS) is widely recognized as a useful tool for sorting even very rare populations of cells from a heterogeneous mixture. The throughput of FACS, however, is relatively low, and so Hu *et al.* demonstrate 'DACS', a higher-throughput alternative wherein cells tagged with antibody-conjugated polymeric beads are sorted by dielectrophoresis.

Hu, X. *et al. Proc. Natl. Acad. Sci. USA* **102**, 15757–15761 (2005).