Box 1 RNA interference: a primer

RNA interference silences a target gene through the specific destruction of that gene's messenger RNA, the intermediary molecule between DNA and protein. Double-stranded RNA (dsRNA) is central to the technique: when dsRNA with identical sequences to a specific mRNA is introduced into cells, the mRNA is recognized and degraded by a multiprotein body called the RNA-induced silencing complex. Destruction of the target mRNA leads to a drop in the levels of its encoded protein, and thus to inhibition of the target gene.

In worms and flies, dsRNAs of hundreds of nucleotides can be used to target a gene. However, in mammalian cells long dsRNAs induce a potent anti-viral response, shutting down the synthesis of all proteins. So more sophisticated strategies are required, and small interfering RNAs (siRNAs) are used instead. These siRNAs are about 21 nucleotides long, and are efficiently used by the RNA-induced silencing complex but are too short to activate a full-blown anti-viral dsRNA response.

siRNAs can either be made *in vitro* and subsequently introduced into cells, or they can be made directly in cells through the expression of short hairpin RNAs (shRNAs). shRNAs fold back on themselves, creating a region of dsRNA and a loop. This hairpin is processed enzymatically to remove the loop and generate a mature siRNA. Expression of shRNAs can be used to induce RNAi in transgenic mice as well as in cell lines, so the technique can be applied to investigate gene function in whole animals. **A.F.**

genes can be targeted using their clones. This library of easily transferable shRNAs is a beautifully designed resource, and should permit an impressive range of analyses in diverse cell types.

To increase the speed of RNAi screening, both groups[4,5] borrow a sequence identifier (bar-code) system, developed in studies on yeast, for the quantitative analysis of pools of genes[8]. Each shRNA construct has a unique bar-code — Berns *et al.* use the shRNA sequence itself, whereas Paddison *et al.* have an independent bar-code, which they report as being of far greater effectiveness. The abundance of each shRNA construct in a pool of constructs can be assessed by monitoring the relative levels of each bar-code using a microarray. Thus any screen for genes that confer a growth advantage (or defect) can be carried out by the simultaneous screening of large pools of shRNA-expressing vectors, greatly increasing the throughput. Bar-coding is still in its infancy but has great potential for analysing RNAi selection screens.

There are still some uncertainties surrounding mammalian cell RNAi, especially regarding both specificity and efficiency of targeting. According to one report[9], a sequence identity of as few as 11–12 nucleotides between an interfering RNA and a messenger RNA may be sufficient for interference to occur. If it is, cross-reactivity is a substantial problem: far from targeting one gene, many expressed shRNAs may target several genes simultaneously. Similar analyses[10] came to the opposite conclusion, however, so it remains to be seen whether this is a general problem. Even if cross-reactivity does occur, there are straightforward controls for specificity: most simply, if two independent shRNAs targeting the same gene give similar effects, it is probably safe to conclude that this is specific to the targeted gene, and not due to some 'off-target' cross-reaction. This is precisely the approach adopted by Berns *et al.* and the presence in each of the libraries reported here of multiple shRNAs against each gene should make these internal controls relatively easy.

As regards RNAi targeting efficiency, it is clear that — as in worms or flies — different genes in mammalian cells are turned off with differing efficiencies. For example, Paddison *et al.* screened their library to identify components of the proteasome, a cellular machinery that degrades many unwanted proteins and that is implicated in certain diseases. Although genes encoding some subunits (those for the 19S base, for example) were apparently easily identified, others (such as those of the 19S lid or 20S core) were harder to hit. Like any screening tool, RNAi is unlikely ever to be perfect. As the rules for predicting effective shRNAs continue to improve, however, the false-negative rate will drop, and the libraries will improve.

Despite these notes of caution, we will no doubt see an explosion in RNAi screening of mammalian cells over the coming months. As with any genetic screen, the power of each RNAi screen depends on the appropriate choice of functional read-out, and that will require development of a variety of cell-based assays (such as the assay for proteasomal function reported by Paddison *et al.*). As no single laboratory can specialize in every aspect of gene function, the general availability of these shRNA libraries as communal resources is a major step forward, harnessing the screening expertise of the entire mammalian-cell research community. Pulling together the data from these varied RNAi screens in a common, central database will take our understanding of mammalian gene function a further giant stride forward. ■

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100 YEARS AGO

It is not surprising to find that at last a 'motor' pocket book has appeared; in fact, it is a wonder such a work has not appeared sooner… Our author has a breezy style of expression which adds largely to the pleasure of reading the book. Take, for instance, his treatment of that all-important worry of the motorist, the 'police'. Mr O'Gorman says, "to pass unchallenged at a speed in excess of the legal limit — a thing which is daily accomplished by carts, hansoms, and even by the London omnibuses on almost every run when the gradients favour them… remember that by sitting upright with a calm face (on a quiet car) you produce no impression of speed except on turning a corner. If you turn a corner without being able to see down the road you are entering at over 20 miles per hour you deserve to be punished. If, however, you stoop forward… jamb your hat over your eyes, screw up your face, stare intently and anxiously, do a great deal of steering with visible swinging of your body, blow your horn in such a manner as to say 'Get out of my way' frequently, instead of pressing it slowly and peaceably, you will invariably be arrested."

From *Nature* 24 March 1904.

50 YEARS AGO

Another statement claimed by Prof. Dingle to be fallacious is connected with an underlying assumption in experimental science; this assumption is that the repetition of an experiment will reproduce the original results. But experimental science is not based on an assumption; "it is an adventure in which you accept whatever you find, and although you may be guided in a particular case by an expectation, the experiment may reveal something totally different". An instance of this is found in the case of Schwabe, who counted sunspots with the object of finding an intra-Mercurial planet, and instead of doing so he found the eleven-year solar period... it would be futile to believe that the achievements of experimental science would necessarily lose all significance if it were discovered that some assumption proved baseless. In the realm of psychology it is accepted that no experiment when repeated produces the original result, and even in physics it has been held for a long time that no experiment is repeatable, the entropy of the universe never being twice the same.

From *Nature* 27 March 1954.