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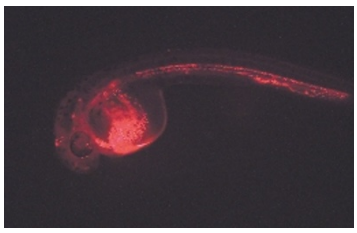
Targeting zebrafish



When MOre is less. One of the phenocopies (informally known as 'the vampire') obtained by Nasevicius and Ekker[11] is deficient in uroporphyrinogen decarboxylase and hence a model of hepatoerythropoietic porphyria. The decarboxylase removes carboxy groups from the porphyrin ring and permits its binding of iron; deficiency results in an accumulation of photosensitive carboxylated porphyrins. These fluoresce and break down when exposed to light.

Ablation of gene expression is central to the dissection of genetic mechanism in model organisms. Until recently, however, facile effective strategies by which to selectively repress the expression of specific genes have been limited to a small group of organisms. Progress on several fronts therefore gives cause for optimism. Robust demonstrations of gene targeting in *Drosophila melanogaster* and sheep have been described within the last few months (see page 159 for a review[1] of these developments). And RNA interference (RNAi), a method whereby injection of double-stranded RNA into cells results in the degradation of existing cognate mRNA and repression of further synthesis, is receiving increased attention[2]. Whereas its success in *Caenorhabditis elegans* and *Drosophila* has been recognized for over two years, it has only recently been extended to targeting gene expression in the mouse oocyte and early embryo[3,4]. The zebrafish is a model organism that is in need of an effective means to carry out 'reverse genetics' experiments—and is therefore a natural focus for new strategies that target gene expression.

Some studies indicate that RNAi can ablate gene expression in the zebrafish[5,6], although a recent set of 'blind' experiments[7] indicates that the frequency of nonspecific effects consequent to RNAi application renders the method impracticable for investigating early zebrafish development. The current level of insight into the way in which RNAi works is limited, albeit evolving, and so it is difficult to modify the method so as to render it more effective—although some success has been achieved in obtaining heritable effects in *C. elegans*[8] and *Drosophila*[9].

Non-specific effects are also endemic to more traditional antisense strategies. These typically involve the injection of an antisense oligonucleotide, designed to block translation of mRNA by binding to it and recruiting RNase H. This is achieved by chemically modifying the oligonucleotide. For example, oligonucleotides in which a sulphur atom is substituted for (a non-bridging) oxygen atom on the phosphate intersubunit linkages, resulting in phosphorothioated DNA, effects the recruitment (upon hybridization with target) of RNase H. If all goes according to plan, degradation of the targeted mRNA ensues. One source of non-specific effect is the promiscuity of RNase H; it seems to require but a small degree of hybridization (about five or six bases) for recruitment. Non-specific effects may also be generated through the interaction of oligonucleotide with other molecules, such as heparin-binding proteins and cell surface proteins[10]. There are also issues of toxicity (these are especially relevant to fish and *Xenopus laevis* embryos). The elegant simplicity of

the antisense approach appeals in a similar manner to other forms of 'gene therapy'. Likewise, it has run up against substantial and unforeseen obstacles that are common to the issue of "contextual dependency," as observed by C.A. Stein[10].

Data presented by Aidas Nasevicius and Stephen Ekker (Univ. Minnesota) on page 216 of this issue[11] provide support for a comparatively new antisense strategy, using 'Morpholino' oligonucleotides (MO). These have been developed primarily by James Summerton (now of Gene Tools) and have the advantages of being inert and, it would seem, having the ability to invade RNA secondary-structure[12]. Because of the way in which they are synthesized, which involves the piecing together of converted ribosides, they are relatively inexpensive. They are designed to bind between the 5′ cap of an mRNA and its start codon, thereby inhibiting translation by blocking the path of the initiation complex before it assembles into a full ribosome at the start codon.

On injecting zebrafish embryo yolks with MOs, Nasevicius and Ekker obtained 8 zebrafish embryos (see inset on preceding page) that phenocopy mutants generated by chemical mutagenesis. With one exception, full penetrance of the phenotype exceeded 90% of animals injected. Targeting a globally triggered promotor hooked up to an open reading frame encoding green fluorescent protein indicates that access to cells in all tissues is good. So will the MO work for zebrafish where other agents have failed? The current study is promising, as is another that achieves knockdown of β-catenin expression in *X. laevis*[13]. Clearly, additional studies are warranted.

It should be noted that the current study indicates two predictable weakness of the strategy: not all genes will be equally good targets and, as with other methods, non-specific effects are possible. Nasevicius and Ekker found that only half of the embryos injected with a construct designed to block expression of *one-eyed-pinhead* (*oep*) showed loss of function, which the authors hypothesize is due to polymorphism. Attempts to generate two phenocopies (*oep* and *bozozok*) resulted in the generation of non-specific effects when MOs were used in moderate quantities. Ekker reckons that the most significant limitation of the technique will prove to be mis-targeting, although he points out that targeting the same gene using different MOs (in different embryos) should help to sort the 'pure' effects from those generated by inadvertent targeting. It is possible that the MO may be trumped by genes that transcribe mRNA in response to low protein levels encoded by that mRNA. Moreover, it seems unlikely that the method is suited to investigating the function of genes expressed in embryos older than two days, owing to the effect of cell proliferation on the cellular concentration of MO. It is therefore likely to complement another strategy for obtaining zebrafish mutants, one that permits the selection of fish with point mutations in genes of interest by breeding mutagenized 'parental' fish with those containing identified deletions[14]. The latter method, however, depends on a comprehensive series of deletion mutants that cover the genome in a robust manner, the generation of which is currently underway.

Whereas the suppression of gene expression by MO during early stages of development is a new approach to exploring gene function in the early embryo, demonstration of MO efficacy cannot be said to be conceptually novel. In this regard, one might query the publication of its use in zebrafish in *Nature Genetics*. As is the case with the success of an antisense construct, so is the case for scientific publications: they are considered in context. Technologies that seem likely to have significant impact and are convincingly demonstrated to be effective are worthy of broad communication. Without method, there would be no results.

1. Capecchi, M. *Nature Genet.* **26**, 159–161 (2000).
2. Gura, T. *Nature* **404**, 804–808 (2000).
3. Wiany, F. & Zernicka-Goetz, M. *Nature Cell Biol.* **2**, 70–75 (2000).
4. Zernicka-Goetz, M. *Nature* **405**, 733 (2000).
5. Wargelius, A., Ellingsen, S. & Fjose, A. *Biochem. Biophys. Res. Commun.* **263**, 156–161 (1999).
6. Li, Y.X., Farrell, M.J., Liu, R., Mohanty, N. & Kirby, M.L. *Dev. Biol.* **217**, 394–405 (2000).
7. Oates, A., Bruce, A.E.E. & Ho, R.K. *Dev. Biol.* **224**, 20–28 (2000).
8. Tavernarakis, N., Wang, S.L., Dorovkov, M., Ryazanov, A. & Driscoll, M. *Nature Genet.* **24**, 180–183 (2000).
9. Kennerdell, J.R. & Carthew, R.W. *Nature Biotechnol.* **18**, 896–898 (2000).
10. Stein, C.A. *Nature Biotechnol.* **17**, 209 (1999).
11. Nasevicius, A. & Ekker, S. *Nature Genet.* **26**, 215–220 (2000).
12. Summerton, J. *Biochim. Biophys. Acta* **1489**, 141–158 (1999).
13. Heasman, J., Kofron, M. & Wylie, C. *Dev. Biol.* **222**, 124–134 (2000).
14. Appel, B. *et al. Curr. Biol.* **9**, 247–256 (1999).