phophorothioates were used to inhibit selectively the expression of either p65 or p50 whilst the adhesion properties of various cell types were monitored. Inhibition of p65 resulted in the complete removal of the cells ability to adhere to extracellular matrices such as fibronectin and laminin. Similar results were obtained with cells expressing antisense p65 RNA constructs confirming that phosphothioates, which are known to bind extensively to membranes[5], were not inhibiting cell adhesion by simply masking cell surface adhesion sites. Furthermore, both sense and antisense oligonucleotides targetted to p50 had no effect on cell adhesion in most cell types studied. This suggests that p65 is the major player in regulating cell adhesion, with little or no involvement of p50. Narayan also illustrated the potential for antisense therapy in adhesion-dependent processes (such as inflammation and cell invasion and metastases in cancer). Antisense inhibition of p65 in a whole animal model actually imparted anti-tumourigenic properties, but the details of this mechanism are not entirely clear.

Other groups demonstrated the potential of antisense therapy in whole animals. Miller and T'so (John Hopkins Medical School) successfully treated herpes simplex infections of the ears of mice with psoralen-conjugated methylphophonate oligonucleotides, applied topically, followed by irradiation. Psoralen is a pheto-reactive cross-linking agent which enables the oligonucleotide to attach covalently to the target mRNA upon irradiation with long wavelength UV light.

Despite the excitement over antisense therapy in whole animal studies (see Table 1), there is a fundamental problem that needs addressing. Efficient incorporation of oligonucleotides into cells is hampered by their large molecular weight, often polar nature and sensitivity to nuclease digestion. Different oligonucleotides enter cells by different mechanisms: most are thought to enter cells by endocytosis-polyanionic phosphodiesters and phosphorothioates by receptor mediated endocytosis and apolar methylphosphonates by adsorptive or fluid phase endocytosis. Escape from the vescicular compartments associated with endocytosis is then a prerequisite if the oligonucleotides are to interact with cytosolic and nuclear bound targets[6].

Chris Mirabelli and colleagues (ISIS Pharmaceuticals, California) followed the cellular destiny of fluorescently labelled phospho-rothioates in a number of different cell lines. They discoverd that the cell type appeared to influence the fate of the oligonucleotides. Whereas fibroblasts, endothelial cells and hepatocytes exhibited largely vesicular distributions (characteristic of endocytic entry) with little or no nuclear uptake, keratinocytes displayed a cytosolic and nuclear distribution of the oligonucleotides. It is not yet clear whether this respresents non-endocytotic uptake in keratinocytes or merely suggests that endosomal escape is relatively rapid in these cells. It also appears that cell types are sensitive to the particular oligonucleotide chemistry with, for example, those conjugated to cholesterol having a general cytosolic and nuclear distribution (A.Kreig, University of Iowa).

Given these problems, it is not surprising to learn that many groups are attempting to improve the endosome to cytosol transfer of nucleic acids. A particularly elegant approach, reported by E. Wagner (Institute of Molecular Pathology, Vienna, Austria) aims to take advantage of the natural ability of the influenza virus to rapidly exit the acidic endosome by transiently destabilizing the membrane following low pH induced conformational changes to the viral haemagluttinin subunit, HA2. By synthesizing a 20mer N-terminal fragment of HA2 (or its dimer), which can transiently disrupt the endosome membrane, and coupling this to polylysine-transferin-DNA complexes, significantly improved gene transfer was observed in some cell lines. If problems regarding the efficient delivery of nulceic acids to the correct cells can be overcome *in vivo*, then both antisense and gene therapy may yet end up making sense. □

Correction

In the June issue of *Nature Genetics*, the addresses for the authors of the News & Views article "Trinucleotide repeat instability: when and where?" were omitted inadvertently. The addresses should have read as follows: David L. Nelson, Institute for Molecular Genetics and Human Genome Center, Baylor College of Medicine, Houston, Texas 77030, USA and Stephen T. Warren, Department of Biochemistry and Howard Hughes Medical Institute, Emory University School of Medicine, Atlanta, Georgia 30322, USA.