RNA SILENCING SPEAKS FOR ITSELF

Technology for RNA silencing has matured rapidly. Dr Thomas Tuschl, associate professor at Rockefeller University, tells *World Pharmaceutical Frontiers* that its potential is becoming clearer and predicts investment in its future.

The application of RNA interference (RNAi) to adult mammalian cells was discovered only three years ago, but it has caught the attention of researchers worldwide. RNAi relies on the introduction of double-stranded RNA (dsRNA) into a cell, so that it degrades messenger RNA (mRNA) containing an identical or complementary sequence and the targeted gene is switched off.

Small interfering RNA (siRNA) is also important in this process, being an RNA sequence of 21 to 23 nucleotides that mediates catalysis of mRNA. The understanding of siRNA's action and the technique of gene silencing has rapidly matured to become one of the most exciting issues in molecular biology.

Exciting potential

Although big pharmaceutical companies remain conservative, none are ignoring this new technique. The reason for this excitement lies in the significance of the potential uses of the technology. Researchers are looking to use RNA silencing as a direct therapeutic treatment. Objectives closer at hand are the use of the technique to help pharmaceutical and biotech companies identify new targets for drug development as well as validating existing targets. Part of the reason for the rapid growth in the popularity of RNAi is that the benefits of gene silencing have been proven using other techniques. New developments in RNAi have simply given the pharmaceutical and biotech industries a cheaper, more accessible tool to achieve the desired results.

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The discovery of siRNA has given researchers a better way to perform gene suppression. Traditionally, loss-of-function experiments have been conducted using knockout technologies, such as antisense or ribozyme.

These methods, however, proved in many cases to be unreliable or inefficient, rendering high-throughput screening impossible or impractical. With the advent of siRNA, molecular biologists now have a much more efficient way to engage in gene suppression.

'Oligonucleotides and antisense are not widely available,' says Dr Thomas Tuschl, associate professor and head of the laboratory for RNA molecular biology at Rockefeller University. 'RNA silencing is a platform that lets you do whatever you want and is available to everyone. Previous techniques for expressing or over-expressing genes might take months or years, but now you can take part of a sequence and rapidly make siRNA.'

Speed is one advantage of the new technology, but flexibility and predictability are equally important. 'What you have now is a reproducible way of knocking down target genes,' says Tuschl. 'You can find many molecules that are capable of knocking down a single gene, so you have a pool of siRNA to target any given gene.' Add to this versatility the fact that using siRNA for gene knockdown is cheaper than the techniques that have been deployed in the past, and you have the fact that using siRNA for gene knockdown is cheaper than the techniques that have been deployed in the past, and you have a solution that meets the commercial needs of pharmaceutical companies, which find themselves under growing pressure to cut costs and improve efficiency. It is hardly surprising that the industry has rallied around this innovative technology so swiftly. 'Using siRNA is easy and is moderate in cost compared with antisense,' notes Tuschl. 'As soon as it came on the market people knew it was the right tool and it has become the industry standard very quickly.'

Unleashing the potential

However, some potential applications of RNA silencing are some way off. Principally, the use of the technique as a direct therapeutic treatment is far in the future compared with other applications.

RNA silencing is recognised as a mechanism for cellular protection and cleansing. It has the capacity to defend the genome against molecular parasites, such as viruses and transposons. It can also be effective in removing plentiful but non-functional mRNAs. One of the working groups in the laboratories of Rockefeller University is looking into the molecular mechanism of RNAi and its application for analysis of gene function and as a therapeutic.

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So far, the group's efforts have identified that delivery of siRNA duplexes into cultured mammalian cells is followed by degradation of targeted mRNAs, producing knockdown cells with characteristic knockdown phenotypes. Targeting of essential genes is seen to cause growth arrest or trigger apoptosis. Thus, siRNA-based knockdown technology is suitable for large-scale analysis of mammalian gene function. The group is now developing protocols for genome-wide analysis of human gene function and for therapeutic applications.

'It must be remembered, however, that it is only two or three years since its initial discovery,' says Tuschl, 'and though it has come far, it is not yet in clinical trials. At the end of 2004 there should be phase 1 trials on animals underway. It is very early days for this technique.'

One of the key issues that must be resolved before the use of siRNA as a direct therapy can be fully developed is delivery. Problems with delivery are limiting the extent to which the efficacy of the technique can be assessed. Also, much more research needs to be conducted into the safety and sustainability of the technology for direct use in humans.

'Delivery needs to be worked out,' explains Tuschl. 'We need to look at localised delivery or find a way to develop systemic delivery. We can build on the literature from antisense use, where long, negatively charged molecules are used. These are cleared through the kidney or liver if they are made complex with liposomes, for instance. We can compare RNA silencing with antisense and then systematically analyse its performance.'

As understanding of how RNAi could be used as a therapy steadily develops, other uses of the technique are finding traction more quickly. The potential of the technology for drug development companies already appears to be huge as more firms start to use it to identify new drug targets and to validate existing targets.

There are already drugs in phase I or phase III trials that have been evaluated using RNA silencing techniques. Some of these provoke an immune response to fight cancer, for example, while others target the liver and could potentially be used to treat metabolic disorders.

'It is a little bit early, but the proof of concept literature is certainly exciting to the industry,' says Tuschl. 'The big pharmaceutical companies are looking to RNA silencing for target identification and validation mostly. With new technology you are always very conservative, so people will start with a conservative attitude to target choice, but we may see developments in treatments for hepatitis B or C, hypercholesterolemia and type 2 diabetes.'

The choice of such targets is hardly surprising given that they relate to conditions into which drug developments companies have invested and that they represent potentially sizeable markets for final products. There is little doubt, however, that the range of potential targets will grow as assessments of the efficacy and safety of RNAi technologies is supported by a growing body of research.

Taking RNAi into the future

Rockefeller University is one institution leading the way in RNAi research. Numerous laboratory facilities are focused on the biochemical potential of RNA silencing technology. A considerable amount of research has been done on the natural process of RNA silencing to allow better design and understanding of siRNAs and competing mechanisms.

The results of such ongoing research are likely to push RNAi much further as a therapeutic treatment and as a tool for more detailed diagnosis of important medical conditions. 'Cancers are diverse in their genetic origin and siRNA technology could yield new diagnostic tools in this area,' says Tuschl. 'We will be able to look at genetic predisposition and inherited conditions. Treatments may take many years to develop, but we will get there in incremental steps. We need to find a lead candidate for delivery and then look at optimisation. We need to arrive at a standardised formulation for drug treatments.'

In the recent past, RNA did not seem like a sustainable market, but now it is a growth area. It is a real, natural biological mechanism with huge potential, so there is now less scepticism about its future. Just as the last few years have seen a radical re-evaluation of RNA silencing technology, it can be expected that the next two or three years will see a similar revolution in its application and development. **END**

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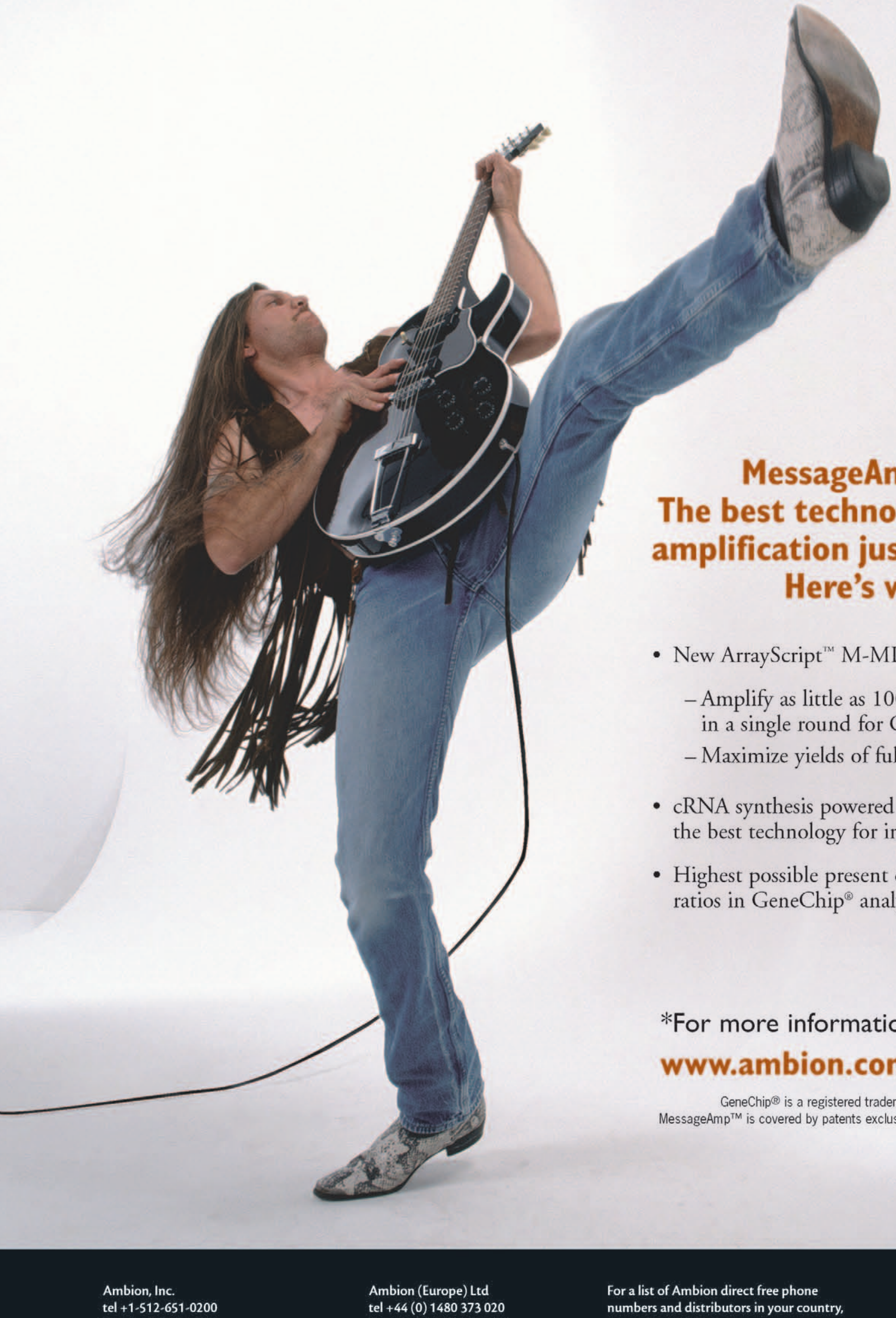

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