RIGHT, BSIP, CAVALLINI JAMES/SPL; M

RNA to the rescue?

Disease therapies based on a technique for gene silencing called RNA interference are racing towards the clinic. Erika Check investigates molecular medicine's next big thing.

Medicine's molecular revolution is overdue. By now, enthusiasts led us to believe, gene therapy and related treatments should have transformed clinical practice. Diseases, they told us, would be cured at their genetic roots, by repairing defective human DNA or by disabling the genes of infectious microbes. But it has proved frustratingly difficult to make these methods work in the clinic — if you get sick, your doctor will probably still treat you with the pills and potions of old-fashioned medicinal chemistry.

Given this chastening experience, you would expect experts to be cautious about the prospects of molecular medicine's latest hope — a gene-silencing mechanism known as RNA interference, or RNAi. But instead, researchers can barely contain their enthusiasm. "Right now, everybody's excited," says Anastasia Khvorova, director of biology with Dharmacon in Lafayette, Colorado, a company that supplies RNAi technologies to researchers and companies that develop therapeutics.

The term RNAi was coined just five years ago, in a paper documenting the phenomenon in the nematode worm *Caenorhabditis elegans*[1]. Yet doctors and biotech executives are now talking about beginning human trials within the next two or three years — an astonishing rate of progress. Part of the excitement stems from the knowledge that, unlike techniques such as gene therapy, RNAi is a natural defence mechanism that is thought to have evolved to protect organisms from viral diseases.

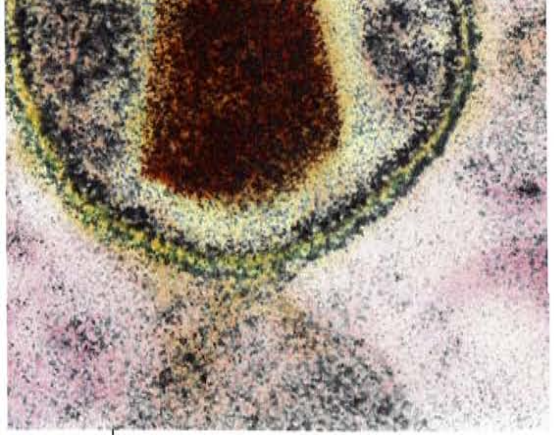
Dicey defence

Many viruses have a genetic blueprint made from RNA, rather than DNA. When they infect a cell, they make double-stranded copies of their genetic material. In response, the RNAi pathway strikes back. An enzyme known as Dicer first chops the double-stranded viral RNA into small segments of genetic code, each around 22 'letters' long. These segments, known as small interfering RNAs, or siRNAs, then separate into single strands and some bind to intact stretches of single-stranded viral RNA. Finally, proteins target this tagged viral RNA and destroy it[2]. As a result, RNAi shuts off key viral genes, potentially nipping infections in the bud.

Biologists are exploiting RNAi as an experimental tool to find out what genes do. When a gene is activated, its sequence is read to produce messenger RNA (mRNA), which contains the information necessary to manufacture a particular protein. So by using siRNAs or double-stranded RNAs that correspond to a specific mRNA sequence, researchers can trick a cell into destroying this mRNA and silencing the gene in question.

As soon as it became obvious that the phenomenon operates in mammals[3] as well as in lower organisms, clinicians pricked up their ears. In theory, RNAi could be used to treat any disease — forms of cancer, for instance — that is linked to an overactive gene or genes. But for the time being, most of the clinical interest lies in applying RNAi in its natural role: as a means of combating pathogenic viruses by disabling their RNA.

One of the obvious targets is HIV — a virus for which there is no cure and no vaccine. Last year, for instance, molecular virologist Bryan Cullen of Duke University Medical Center in Durham, North Carolina, introduced siRNAs against two HIV genes into the human immune cells that are destroyed by the virus. The siRNAs allowed these cells to resist viral replication better than those that had not been triggered to undergo RNAi



(ref. 4). Meanwhile, other researchers have shown that, in cultures of human cells, RNAi can similarly combat viruses as diverse as respiratory syncitial virus[5], and those that cause influenza[6] and polio[7].

RNAi may work like a charm in petri dishes — but what about in live animals? Mark Kay, a geneticist at Stanford University in California, addressed this question by fusing a genetic sequence from the hepatitis C virus to a gene for the enzyme luciferase, which stimulates a reaction that emits light. When Kay injected the fused gene into mouse livers, he could track its location by detecting the glow. And when the mice were treated with siRNAs targeted against the hepatitis C gene, this glow dimmed dramatically[8]. Hepatitis C doesn't make mice sick, but Kay and his colleagues have since gone on to show that RNAi can drastically reduce signs of infection by hepatitis B (ref. 9), which can damage the animals' livers.

Firm plans

Results such as these are attracting intense commercial interest. In August, Kay announced that he has licensed his work on hepatitis C to a company called Avocel in Sunnyvale, California, which aims to develop RNAi therapies against the disease. Other RNA pioneers are lining up with their own start-up biotech firms. For instance, Phillip Sharp of the Massachusetts Institute of Technology in Cambridge, who shared the Nobel Prize in Physiology or Medicine for his earlier work on RNA splicing, is one of the co-founders of Alnylam Pharmaceuticals, also based in Cambridge. US$43 million from investors. Sirna aims to develop therapies for hepatitis C and an eye condition called macular degeneration. For now, these companies are maintaining amicable relations. But the situation could get messier as RNAi moves towards the clinic, because patent offices around the world have not yet decided who owns the rights to some key RNAi-based technologies.

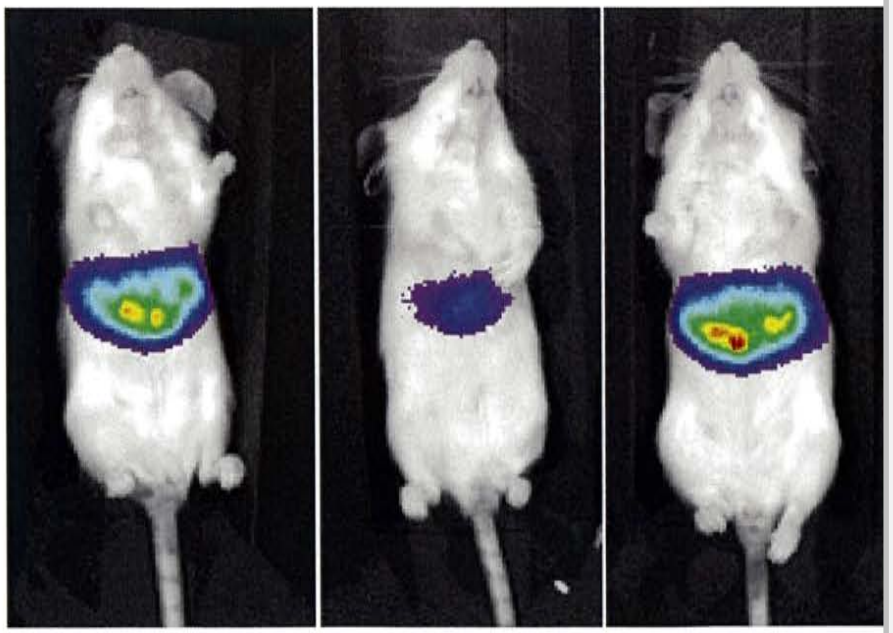
Before worrying about the ownership of key intellectual property, however, scientists must figure out how to make RNAi therapies work. They are facing some formidable technical barriers, chief among which is the problem of getting siRNAs into the right cells. This is not a trivial issue, because RNA is rapidly broken down in the bloodstream, and our cells don't readily absorb it through their membranes. And even when RNA gets into its target cell, scavenger proteins quickly chew it up. "The major hurdle right now is delivery, delivery, delivery," says Sharp.

Researchers are exploring a variety of ways to combat the problem. Some involve techniques developed to facilitate an older technology known as 'antisense'. The idea behind antisense is to muffle a cell's single-stranded mRNA — the 'sense' strand — using a piece of antisense RNA with a 'complementary' But we've looked at a lot of the delivery methods that have been used for antisense, and so far I haven't been impressed," she says.

Harmless HIV

Another option is to use a harmless virus as a vector to ferry RNAi-triggering genes into their target cells. Molecular biologist John Rossi of the Beckman Research Institute of the City of Hope Medical Center in Duarte, California, is experimenting with one such vector, based on a version of HIV from which the disease-causing genes have been stripped. Together with colleagues led by Ramesh Akkina of Colorado State University in Fort Collins, Rossi engineered this vector to contain sequences encoding siRNAs targeted against HIV genes. The researchers used their vector to infect the human stem cells that develop into immune cells. Next, they either grew the cells into mature cells in the lab, or injected them into mice from a special strain that accepts human transplants. In both cases, the mature immune cells fought off HIV when researchers tried to infect them with disease-causing HIV in culture dishes[10].

Rossi hopes that a similar technique could work in human patients with HIV. Doctors



In mice containing a glowing version of a hepatitis C gene (left), a small interfering RNA (siRNA) against the gene reduces liver fluorescence (middle), but an unrelated copy of the siRNA (right) does not.

This may be so, but there are nagging safety concerns about vectors made from viruses in the same family as HIV, which are called retroviruses. This is due to the fact that retroviruses work by forcing their way into a cell's own DNA. If the vector lands in the wrong place it can damage important genes and even cause cancer. These concerns were borne out by last year's revelation that a retroviral vector had triggered leukaemia in some children in a gene-therapy trial[11]. Because of these concerns, Rossi says that he will not use stem cells in his first clinical trial. Instead, he will initially treat mature immune cells, because these cells are less likely to grow out of control.

Safe delivery

Kay, meanwhile, is pinning his hopes for an RNAi vector on a virus known as adeno-associated virus, or AAV. He has already used AAV-based vectors in clinical trials of gene therapy against haemophilia[12]. AAV does not cause disease in people, and so far there has been no cause for any serious safety concern — even though AAV can also integrate into a cell's own DNA.

Another important question mark hanging over RNAi is its specificity. Before regulators give the go-ahead for a clinical trial, scientists need to prove that that RNAi will not shut down vital human genes as well as the target viral sequences.

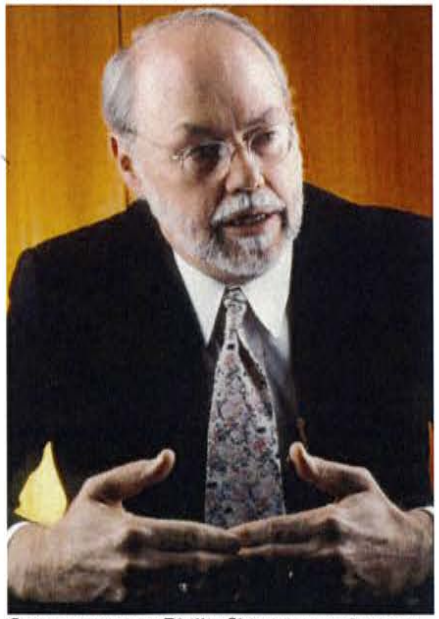
Some studies on specificity have yielded encouraging results. In May this year, for instance, researchers led by Patrick Brown of Stanford University reported on experiments in which they engineered human kidney cells to produce a fluorescent protein. They shut down the gene for this glowing protein by using RNAi, and then used DNA microarrays to monitor some 20,000 other genes — none of which seemed to be affected by the treatment[13].

But just a couple of weeks later, researchers with Rosetta Inpharmatics in Kirkland, Washington, cast a shadow over this rosy picture. The Rosetta team, led by Aimee Jackson and Steven Bartz, used a range of different siRNAs to target two genes in cultured human cells. Disturbingly, the treatment caused changes in the expression of dozens of other genes. Depending on the precise sequence of the siRNA concerned, a different range of 'off-target' genes seemed to be affected[14].

Little helpers: Mark Kay hopes to use harmless viruses to deliver RNAi therapy to patients.

Jackson and Bartz are not sure why their results were so different from those obtained by Brown's team, but one possible explanation is that they used larger doses of siRNA. The Rosetta researchers also tested for off-target effects sooner after beginning their experiment than other groups have in their studies. But whatever the explanation, the findings have shaken up the RNAi camp. "We've had some really lively discussions," says Bartz.

New data from a group at Case Western Reserve University in Cleveland, Ohio, seem to support the Rosetta findings[15]. Last week, Bryan Williams and his colleagues reported that when they introduced siRNAs into cells, certain genes that are part of the interferon



Going to market: Phillip Sharp is one of several RNAi researchers to form start-up biotech firms.

tem works. For instance, we still don't know for sure how many proteins work together to shut down a target mRNA. It's also unclear why some siRNAs are incredibly effective, whereas others, targeted at a different region of the same gene, don't work as well. Given these unknowns, some researchers urge caution before rushing into clinical trials. "Before you know what you could perturb, you have to know what's there," says Tom Tuschl, a biochemist and RNAi pioneer at Rockefeller University in New York.

Even some of the scientists working in the commercial sector, where excitement about the clinical prospects of RNAi is most intense, agree that a great deal of groundwork remains to be done. "For real clinical development, this has to be done right," says Khvorova. "Investing a little more time on the basic steps will pay back in years of time saved later on."

But despite all of these caveats, most researchers working in this fast-moving field have high hopes that RNAi will deliver on its therapeutic promise. "This is the honeymoon period; things are looking great," says Kay. "We will encounter technological issues along the way, but our goal is to solve these problems and get it to work." ■

Erika Check is *Nature*'s Washington biomedical correspondent.

1. Fire, A. *et al. Nature* **391**, 806–811 (1998).
2. Hannon, G. J. *Nature* **418**, 244–251 (2002).
3. Elbashir, S. M. *et al. Nature* **411**, 494–498 (2001).
4. Coburn, G. A. & Cullen, B. R. *J. Virol.* **76**, 9225–9231 (2002).
5. Bitko, V. & Barik, S. *BMC Microbiol.* **1**, 34 (2001).
6. Ge, Q. *et al. Proc. Natl Acad. Sci. USA* **100**, 2718–2723 (2003).
7. Gitlin, L., Karelsky, S. & Andino, R. *Nature* **418**, 430–434 (2002).
8. McCaffrey, A. P. *et al. Nature* **418**, 38–39 (2002).
9. McCaffrey, A. P. *et al. Nature Biotechnol.* **21**, 639–644 (2003).
10. Banerjea, A. *et al. Mol. Ther.* **8**, 62–71 (2003).
11. Check, E. *Nature* **420**, 116–118 (2002).
12. Manno, C. S. *et al. Blood* **101**, 2963–2972 (2003).
13. Chi, J.-T. *et al. Proc. Natl Acad. Sci. USA* **100**, 6343–6346 (2003).
14. Jackson, A. L. *et al. Nature Biotechnol.* **21**, 635–637 (2003).
15. Sledz, C. A., Holko, M., de Veer, M. J., Silverman, R. H. & Williams, B. R. G. *Nature Cell Biol.* doi:10.1038/ncb1038 (2003).

Dharmacon
www.dharmacon.com
Avocel
www.avocel.com
Alnylam Pharmaceuticals
www.alnylam.com
Sirna Therapeutics
www.sirna.com
Nature RNA Insight
www.nature.com/nature/insights/6894.html