

Hitting the Genetic OFF Switch

A host of start-ups is speeding development of a new class of drugs that block the action of RNA **By Gary Stix**

In 1996 *Worth* magazine proclaimed that Isis Pharmaceuticals could become the next Microsoft, a prediction that turned out to be a particularly egregious example of hyperbole run amok. To be sure, Isis remains a leader in the gene-blocking technology called antisense. But the road to successful treatments for cancer and other diseases has been littered with disappointments.

During the past few years, a new gene-silencing technology has emerged that may be poised to fulfill the promise that was once trumpeted for antisense. "I've been writing in grants for 25 years that during the next five years I'm going to test this process or that process to see if I can do gene inactivation studies in mammalian cells in culture. And I did them, and they were so awkward and so complicated that you just couldn't apply them generally," says Phillip A. Sharp, director of the McGovern Institute for Brain Research at the Massachusetts Institute of Technology. "Lo and behold, all of the time right there in front of me was a process that I could have used."

Sharp, a co-winner of the 1993 Nobel Prize in Physiology or Medicine, was referring to a series of relatively recent dis-

coveries that cells have a mechanism, dubbed RNA interference (RNAi), which blocks gene expression. It prevents RNA transcripts of genes from giving rise to the proteins those genes encode. This natural method of gene silencing comes into play, for example, when viruses try to commandeer a cell's protein-making machinery to produce viral proteins.

A milestone arrived in 1998, when Andrew Z. Fire, now at the Stanford University School of Medicine, and Craig C. Mello of the University of Massachusetts Medical School identified in worms double-stranded RNAs that acted as the switch to turn off genes in RNAi. And in 2001 Thomas Tuschl, now at the Rockefeller University, found that an abbreviated version of double-stranded RNAs—short interfering RNAs (siRNAs)—could shut off genes in mammalian cells. The number of research papers on RNAi has mushroomed from a dozen-plus in 1998 to multiple hundreds last year. Even if the promise for therapeutics never materializes, it is quite likely that some of the seminal discoveries will garner Nobel Prizes. "This has touched everything we do in biological science, from plants to man," Sharp notes. [See "Censors of the Genome," by Nelson C. Lau and David P. Bartel; *SCIENTIFIC AMERICAN*, August 2003.]

The excitement about siRNAs as drugs relates to how they differ in critical ways from antisense therapeutics. At first glance, siRNAs seem very similar to antisense. An antisense drug consists of an artificially synthesized chain of nucleotides, or genetic building blocks, that binds to a messenger RNA containing a complementary sequence. This binding blocks gene expression. An siRNA also silences genes—and it even uses a complementary RNA, or antisense, strand to do so. Once inside a cell, an siRNA attaches to an aggregate of proteins called an RNA-induced silencing complex (RISC), which retains only the antisense strand. The siRNA-bearing RISC then binds to the targeted messenger RNA and degrades it or prevents it from functioning [*see box on page 100*].

Unlike the antisense drugs that have been under development for the past 15 years, siRNAs do not disrupt only a single messenger RNA. They act as catalysts, doing the same job over and over, one explanation for their apparent potency. "They are 100- to 1,000-fold more effective than antisense," says Judy Lieberman, a senior investigator at the CBR Institute for Biomedical Research in Boston and one of the first researchers to show the therapeutic potential of the technique in animals.

Already almost 100 companies are

involved in RNAi; nearly half supply the chemicals and technology needed to perform experiments, and the others are biotechnology or pharmaceutical companies doing commercial research with RNAi, according to Kewal K. Jain, chief executive officer of Jain PharmaBiotech, a Basel, Switzerland, market research firm. “All of this has happened within the last two or three years,” Jain says.

A small fraction of these companies have dedicated themselves to producing therapies using siRNAs. As soon as Tuschl’s paper documenting siRNAs in mammalian cells was published, the venture-capital community sprang into action. “It was worth it to make a bet realizing in vivo efficacy was not guaranteed,” says Christoph H. Westphal, one of the

Paul R. Schimmel, a professor of molecular biology and chemistry at the Scripps Research Institute, and the founder of several biotechnology companies before this one, insisted on the name Alnylam, an Arabic word meaning “string of pearls” that is also the designation for the middle star of Orion’s belt. Schimmel made the case, over the protests of others, that the name—pronounced “al-NIGH-lam”—was difficult to pronounce but impossible to forget. Barry Greene, the company’s chief operating officer, furnishes a simpler explanation: “The URL was open,” he joked at a recent investors’ conference.

The founders constituted an all-star scientific advisory team, and some also filled slots on the board of directors. But

scientific smarts, would determine who would thrive or falter as drug development and clinical trials got under way. “We were very focused and running very hard,” he remembers. “We recognized that if we weren’t first, others would grab it from us.”

The fledgling Alnylam even bought the German firm Ribopharma to get a hold of a key patent. The stir created by RNAi—tagged by *Science* magazine as “breakthrough of the year” in 2002—helped to bring in venture money. The total take thus far has reached about \$85 million, including a somewhat disappointing initial public offering in the spring, and provides enough to keep Alnylam going for another two years, until the first drug makes it through the preliminary safety phase of clinical trials.

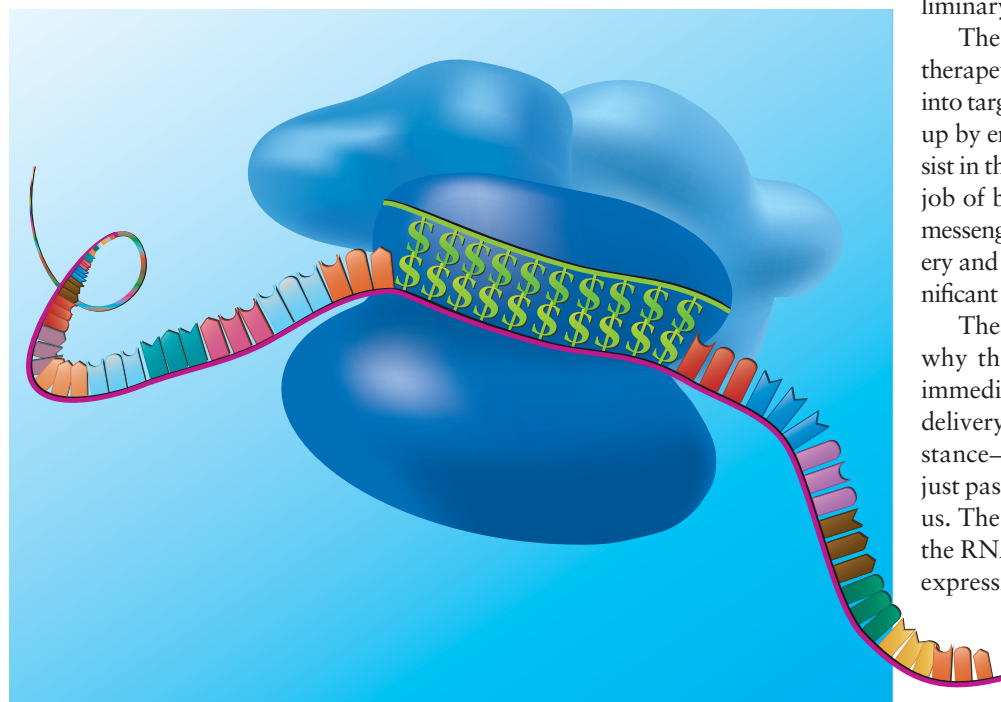
The success or failure of RNAi as a therapeutic will hinge on getting the drug into target cells without its being chopped up by enzymes. The drug must then persist in the cell long enough to carry out its job of binding to and inhibiting specific messenger RNAs. The challenge of delivery and stabilization has also posed a significant hurdle for the success of antisense.

These difficulties serve as one reason why the newly formed Alnylam team immediately discarded one approach to delivery: using a vector—a virus, for instance—to ferry a stretch of DNA, not just past the cell wall, but into the nucleus. The gene would then go on to make the RNA that would interfere with gene expression. “In my mind, nothing about

RNAi solves the problem of gene therapy,” Maraganore notes, referring to the downsides of using viruses to deliver

the drug to the right location and the unwanted side effects that they sometimes provoke. Consequently, short interfering RNAs are synthesized in the laboratory from a soup of nucleotides until they form double-stranded molecules that have 21 nucleotide pairs. Some other companies, such as Benitec in Australia, are still pursuing a gene therapy approach [see table on page 101].

Key expertise and intellectual property to accomplish this task came from an unlikely source. Alnylam had hired away



BINDING A MESSENGER RNA (long strand) to complementary RNA and an aggregate of proteins (blob) could potentially become a lucrative new approach to drug development.

founders of Alnylam in Cambridge, Mass., and a general partner with Polaris Venture Partners. Many of the early innovators in RNAi technology, including Tuschl, Sharp and David P. Bartel of M.I.T., among others, got together to form Alnylam Pharmaceuticals in 2002. Sharp, a founder of biotech giant Biogen, brought together this banner group after conversations with more established companies failed to generate sufficient interest.

when John M. Maraganore, the company’s first permanent chief executive, a transplant from Millennium Pharmaceuticals, hired the people who actually would be entrusted with the task of making siRNA drugs, he did not at first seek out postdoctoral students of these research heavyweights. “Five people were focused on intellectual property and one on science,” Maraganore says. A bulletproof patent estate, as much as

from Isis an executive, Muthiah Manoharan, to become vice president of drug discovery. Maraganore called Isis president Stanley T. Crooke last summer and reassured him that Alnylam still wished to be on good terms with the antisense manufacturer. A few months later a dialogue between the two companies resulted in an agreement in which Alnylam would pay \$5 million to license Isis's ex-

tensive patent portfolio of chemical techniques for delivering and stabilizing RNA. "We will be able to take advantage of the 10-plus years of development of chemistry used in antisense," Maraganore says. In turn, Isis invested \$10 million in Alnylam, giving it a 5 percent equity stake in the company and a stream of royalties and fees once siRNA products hit the marketplace. It will also get

the rights to make some siRNA drugs. The development trajectory for siRNA recapitulates the path that antisense has taken. The only antisense drug approved to date is Isis's Vitravene, intended to treat an eye disease once prevalent in AIDS patients. The drug is injected directly into the eye, concentrating the compound at its target while impeding it from producing adverse side effects in

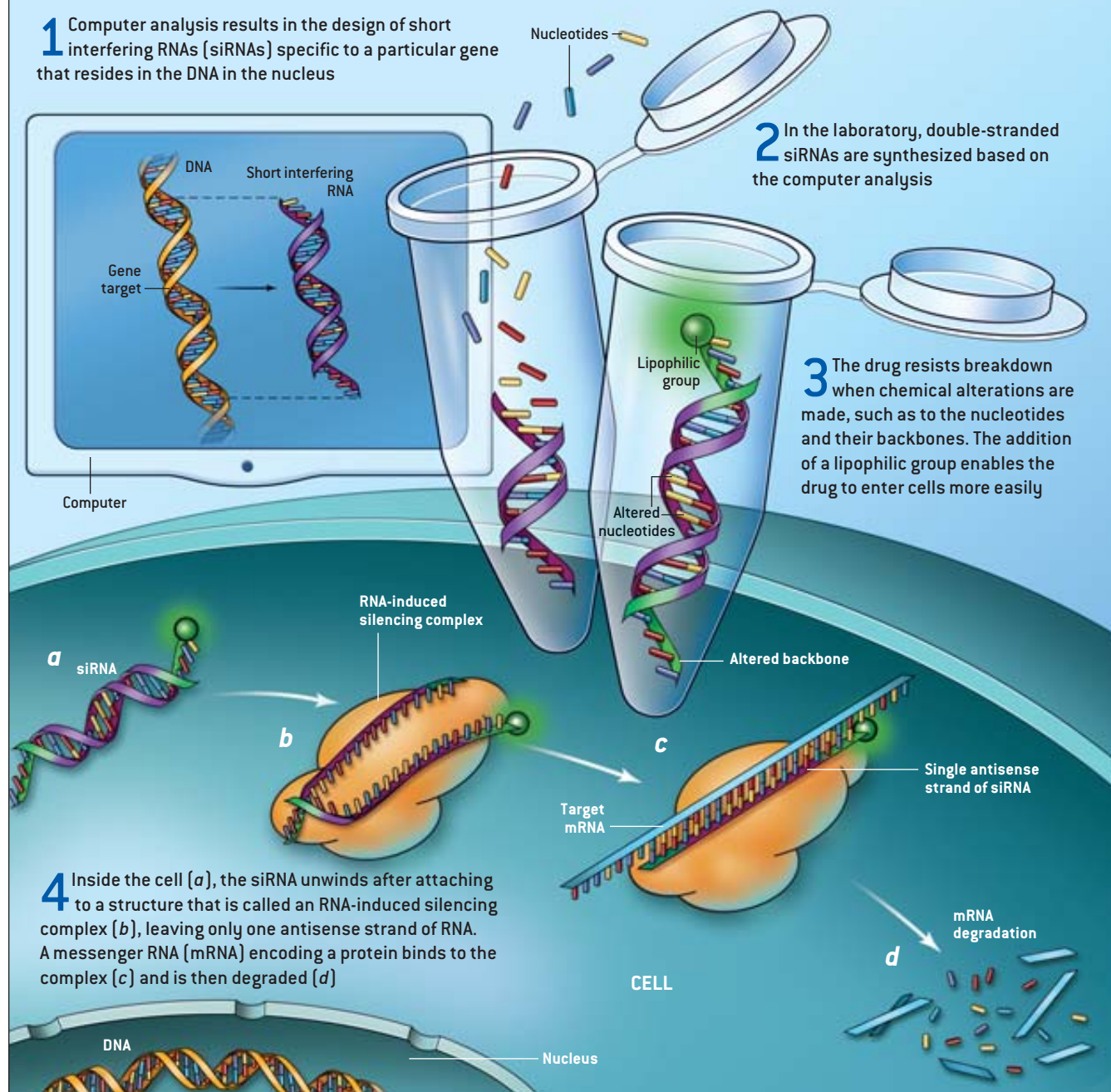
MAKING A GENETIC SILENCER

1 Computer analysis results in the design of short interfering RNAs (siRNAs) specific to a particular gene that resides in the DNA in the nucleus

2 In the laboratory, double-stranded siRNAs are synthesized based on the computer analysis

3 The drug resists breakdown when chemical alterations are made, such as to the nucleotides and their backbones. The addition of a lipophilic group enables the drug to enter cells more easily

4 Inside the cell (a), the siRNA unwinds after attaching to a structure that is called an RNA-induced silencing complex (b), leaving only one antisense strand of RNA. A messenger RNA (mRNA) encoding a protein binds to the complex (c) and is then degraded (d)



BIOTECHS DEVELOPING DRUGS BASED ON RNAi

COMPANY	THERAPIES
Acuity Pharmaceuticals Philadelphia	Age-related macular degeneration and diabetic retinopathy
Alnylam Pharmaceuticals Cambridge, Mass.	Age-related macular degeneration, Parkinson's disease and, over the longer term, cancer, metabolic and autoimmune diseases
atugen Berlin	Cancers and metabolic diseases, for systemic applications, and ocular and skin diseases, for topical delivery
Benitec Queensland, Australia	Hepatitis C and, over the longer term, cancer, autoimmune and viral diseases using a gene therapy approach
CytRx Los Angeles	Amyotrophic lateral sclerosis, cytomegalovirus, obesity and type 2 diabetes
Intradigm Rockville, Md.	Cancer
Nucleonics Horsham, Pa.	Hepatitis B and C and, over the long run, cancer, inflammatory and viral diseases using a gene therapy approach
Sirna Therapeutics Boulder, Colo.	Macular degeneration, hepatitis C and, later, cancer, metabolic, inflammatory, dermatological and central nervous system diseases

other parts of the body. But the market for the drug virtually disappeared as other treatments for AIDS became available and prevented most cases of the cytomegalovirus retinitis infection.

Short RNAs may eventually be delivered to the bloodstream to treat systemic diseases. But, reprising the antisense experience, the first petition to begin a clinical trial was filed in August by Acuity Pharmaceuticals, a Philadelphia company that will attempt to treat age-related macular degeneration by intraocular injection. Other companies, including Alnylam, will follow suit with their own drug trials for macular degeneration. One of these filings will bring Alnylam head to head with the other drug developer that stands a chance of becoming a leader in this emerging market niche.

A Boulder, Colo., company called Sirna Therapeutics was expected to submit an application to the FDA for a macular degeneration drug in early September, perhaps half a year or more before Alnylam. Unlike Alnylam, Sirna is no start-up. It is a reincarnation of another firm, Ribozyme Pharmaceuticals, which for a decade staked its fate on a different type of RNA-related drug. Ribozymes are RNAs acting as catalytic enzymes that, in principle, can cut up messenger RNA and prevent a protein from being

produced. But, as with antisense, the potency of ribozymes came into question. A drug to combat hepatitis C caused a monkey to go blind, probably because of the massive doses injected. And another drug did not seem to slow growth of tumors in patients with advanced breast cancer. At the time, Howard W. Robin, a new chief executive, who had managed development of drugs like Betaseron for multiple sclerosis at Berlex Laboratories, was faced with a decision about whether to close shop. The company had only \$2 million in cash left and risked being delisted from the Nasdaq.

The darkest days coincided with Tuschl's publication about RNAi in mammalian cells. Instead of turning out the lights, Ribozyme Pharmaceuticals became Sirna Therapeutics. Sirna rejiggered the chemical techniques it had used to deliver and stabilize ribozymes and adapted them to siRNAs. Robin claims that single doses of its siRNAs have remained active inside cells of live animals for up to 22 days. The revamping succeeded in attracting \$72 million in new investment

during an 18-month period. Besides macular degeneration, the company has programs in hepatitis, oncology and Huntington's disease, among others. "It's not often that you take the skills and intellectual property from a technology that's not working very well and transfer it to the hottest area of biology," Robin says.

Sirna has filed for 90 patents that Robin believes cover the most attractive drug prospects. Patent fights may loom as the technology gets nearer the marketplace. "If you look at our competitors, we believe almost everything they're doing violates our patents," Robin proclaims. But Maraganore begs to differ: "We have a bit of a toll road for anyone doing therapeutics." Any dispute is not likely to emerge until siRNA drugs are much closer to approval. In the meantime, investigators will be closely watching whether siRNAs produce unwanted immune responses or shut down genes they are supposed to leave intact.

For the time being, optimism about RNAi reigns. "If you do with RNAi in man what you do in cell culture, you have the most unbelievably powerful technology for making pharmaceuticals," Maraganore says. "It's the dream of medicine to do selective and efficient gene silencing," Robin asserts. But Isis's Crooke, tempered by the failure of trials for a few of his company's antisense drugs, has a slightly different perspective: "Any time you think something is magic, you're going to get in trouble. [RNAi] is a complicated system with lots of interesting nuances that mechanistically should lead to some unexpected effects as well as those you desire."

RNAi has become a preeminent research tool in a remarkably short time. But its potential as a genetically based pharmaceutical will not become clear for several years, when the first clinical trials prove whether a simple injection is capable of shutting down the effects of a disease-causing gene. SA

MORE TO EXPLORE

The RNAi Revolution. Carl D. Novina and Phillip A. Sharp in *Nature*, Vol. 430, pages 161–164; July 8, 2004.

The Silent Revolution: RNA Interference as Basic Biology, Research Tool and Therapeutic. Derek M. Dykxhoorn and Judy Lieberman in *Annual Review of Medicine*, Vol. 56 (in press).