

Running Interference

The revolution in RNA interference has galvanized basic research. Now, some biopharmas are pushing the technology from the laboratory to the clinic.

BY NANCY WEIL

"I have frequently said that RNA interference, as far as I'm concerned, is the most important discovery of the past decade," says MIT's Phillip A. Sharp. As co-founder of Biogen and Alnylam Pharmaceuticals, and Nobel laureate for his discovery of gene splicing, Sharp knows of what he speaks.

His small office at the MIT Center for Cancer Research is crammed with stacks, albeit neat ones, of journals and papers. Since his September announcement to step down as director of the McGovern Institute for Brain Research at MIT, Sharp can once again devote his considerable energies to lab research — and evangelizing RNA interference (RNAi).

"RNAi may be the most important discovery for a number of decades," Sharp continues. "It is among the discoveries that shift the way we understand the paradigms of biology as well as a new approach to therapeutics. It's very rare that you have an advance in the field that really changes the way we understand all biological systems, and as well suggests new means of therapeutic intervention."

Even if RNAi never leads to a new class of drugs targeting myriad diseases, as many believe it will, it remains a fascinating and important biomedical breakthrough. Its rapid acceptance as an indispensable laboratory tool is helping scientists dissect gene function and unlock cellular processes.



Phillip A. Sharp, MIT professor and Nobel laureate, says there is a "platform of science for us to move forward in drug discovery."

While Sharp's views are widely shared by scientists, venture capitalists, and company executives, they recognize the challenges that must be overcome for RNAi therapeutics to become a reality.

RNAi is a natural process that evolved eons ago to help organisms ward off viruses — which makes its recent discovery all the more exciting and its applications all the more promising. From yeast to mammals, RNAi is a complex cellular reaction that not only fends off foreign invaders but also regulates aspects of cell development and death.

But while armies of scientists dedicate themselves to understanding how RNAi works at the molecular level, a handful of biotechs intend to apply RNAi to the creation of therapeutics for a host of diseases, including macular degeneration, hepatitis, Parkinson's disease, Huntington's disease, Lou Gehrig's disease, cancer, and HIV.

Mystery Solved

For several years scientists puzzled over the RNAi phenomenon they observed in various plants, fungi, and other organisms (see "Silence is Golden", July 2003 Bio-IT World, page 26). More than a decade ago, plant biologists trying to increase gene activity in petunias found they were inadvertently producing white or variegated flowers instead of dark purple. They dubbed the phenomenon "co-suppression" because the transgene and endogenous gene were mutated.

The breakthrough came in 1998, in studies on the nematode *Caenorhabditis elegans*. Andrew Fire, now at Stanford University School of Medicine, and Craig Mello, at University of Massachusetts Medical School, were exploring "why some things were working much better than they should," Fire recalls. Su Guo, then a student at Cornell University, had found that preparations of antisense or sense RNA could inhibit gene expression in *C. elegans*. Even more surprising, Mello's lab found that this "interference" spread into the germ line even if the injection missed the germ line.

Fire and Mello subsequently found that double-stranded RNAs (dsRNAs) caused the gene silencing, and published a seminal paper on RNAi in *Nature* in 1998. A few years later, Rockefeller University's Thomas Tuschl determined that small interfering RNAs (siRNAs) could do the same thing in mammalian cells.



RNA RICHES: Alnylam's John Maraganore sees no end of potential drug targets and potential applications

Recent studies have demonstrated that RNAi and microRNAs control the expression of hundreds of known genes — perhaps as much as 10 percent of the genome. "Some of those genes appear to be critical for development, some seem to be important for cell death control, and some for growth, so it's touching every aspect of biology," Sharp says. Scientists didn't take long to realize that this major new tool in genomics research also had profound implications for drug discovery and development. "Not only can one contemplate using RNAi to cure a disease, we can use it now to understand how a disease works," Fire says.

"There's a lot that's happened in a very short time, and there's certainly a lot more that's going to happen," Fire says, noting that RNAi is used as a tool in hundreds of studies a week. "That base of users will encourage the use of RNAi as a tool, and that will benefit the clinical work."

RNA Eye

This summer, two companies — Acuity Pharmaceuticals, a small Philadelphia-based startup, and Sirna Therapeutics in Boulder, Colo. — filed investigational new drug (IND) applications with the FDA to begin human RNAi trials for treatment of the "wet" form of age-related macular degeneration (AMD), the leading cause of age-related irreversible vision loss in developing countries.

The procedure targets the vascular endothelial growth factor (VEGF) pathway, by suppressing the growth of new blood vessels and causing existing vessels to atrophy. In wet AMD, an abnormal number of blood vessels form behind the retina and leak, destroying cells in the central part of the retina (see "Wet Age-Related Macular Degeneration RNAi Therapy"). "RNAi offers the ideal pharmacological mechanism to inhibit the protein that is causing the vision loss and wet AMD," says Sam Reich, Acuity's co-founder and senior director of R&D. "The eye is an ideal organ to move forward with revolutionary technologies because it is easily accessed."

Acuity recently signed a manufacturing agreement with Avecia Biotechnology to receive supplies of Avecia's siRNA drug, Cand5, which is in Phase I trials with AMD patients. These are the first human clinical trials for pharmaceutical siRNA.

At Sirna, pre-clinical work went so well that the company beat its planned IND filing date by about three months, says Nassim Usman, chief operating officer and senior vice president. The Sirna-027 Phase I trial, targeting the VEGF receptor, began in November. Both trials for AMD will involve the injection of RNAi-based therapeutics directly into the eyes of patients at their doctor's offices, with close monitoring to determine efficacy and toxicity.

Alnylam, which says it is the first company formed specifically to develop RNAi therapies, is on target to apply for regulatory approval for clinical trials in macular degeneration in 2005, as well as for pre-clinical testing by the end of this year on therapies for Parkinson's disease. President and CEO John Maraganore says the Acuity and Sirna applications were taken in stride. "We're actually encouraged to see the field advance," he says, adding that clinical trials by any company will mean that the benefits of chemically modified, stable siRNAs can be studied and established, which has to happen for the market to progress.

While the AMD trials involve direct application of RNAi-based drugs to the sites where they are needed, systemic delivery of RNAi may follow if direct delivery to target tissues is a success. This could open the way for treatment of viral diseases such as HIV or influenza, which could be an important RNAi target given the expectation of another global pandemic and shortage of drugs to treat the flu. "That's where I think the biggest challenges are," says Cold Spring Harbor Laboratory's Gregory Hannon of systemic delivery.

Solving Stability

But before systemic delivery of RNAi drugs becomes feasible, researchers must clear other hurdles. Stability is another problem. RNA molecules are large and don't pass readily through cell membranes. They are also subject to degradation, excretion, and are fragile to work with in the laboratory. Nevertheless, researchers are confident that both delivery and stability issues will be licked, and that synthetically produced RNAi will behave the same way as the naturally occurring process.

"We're well on the way to solving these challenges for different cell types, though it's different for each cell," says Sirna CEO Howard Robin. "Once we understand how to make these stable to deliver them in a more broad sense, if you know the sequence of the RNA you have a drug," he says. "That's not around the corner, but that's where we're headed."

Although some are skeptical, Robin believes it is "very reasonable" to think that if delivery and stability are solved, companies could be on the way to robust pipelines in the next five years. Depending on the disease and how patients respond, regular injections would likely be required for some duration, perhaps permanently. "We're not going to take a pill of RNAi," Sharp says.

In the chemical approach taken by Sirna, Alnylam, and Acuity, dosages can be altered to diminish negative side effects, or the therapy can be terminated if necessary. Researchers are reasonably confident there won't be long-lasting side effects, but that is not yet proven. Commercial RNAi ventures are also pondering what Alnylam's Maraganore terms "an embarrassment of riches." There are so many potential drug targets and so many potential

applications for RNAi-based therapies that it is difficult to stay focused on specific diseases. This is particularly true with areas of unmet medical need, which those in the RNAi industry widely agree should be the overarching initial emphasis for such a novel approach.

"How do you bring your research and everything you do to focus on a valuable target that is going to work clinically?" Sharp asks. "That's the real challenge."

The strongest immediate focus among most RNAi therapeutic companies is on diseases for which there currently are few or no effective treatments. When Fire was at Johns Hopkins University, he found, in conversations with those working with HIV-positive patients, that they "felt they could treat someone with HIV, and they didn't feel that there was ... an impetus to find revolutionary new treatments that have substantial risks associated with them."

Property Rights

Today, many scientists feel compelled to help develop therapies for diseases such as AMD and Parkinson's, which, in the absence of a cure, leave patients willing to try novel therapies that might have greater risks. There is also a sense that the FDA is more likely to approve novel therapies for untreatable diseases, even allowing for the greater risks involved. Big Pharma is also pursuing RNAi research and therapies, while leaving much of the heavy lifting to biotechs. Eli Lilly has a deal with Sirna to work on cancer therapeutics, and Merck has a pact with Alnylam.

The chances of Alnylam and Sirna surviving among others enjoying long-lasting success depends largely on intellectual property rights. Alnylam protected its IP position by loading its employee roll with leaders in the field. The company was founded by Sharp and Tuschl, who teamed with RNA experts Paul Schimmel, David Bartel, and Phillip Zamore.

Alnylam further has a key deal with antisense leader Isis Pharmaceuticals to license all of Isis' IP estate and important chemistry work, which is a cornerstone of RNAi research, in return for Isis investing in Alnylam. When it hasn't been able to license or develop the IP it needs, Alnylam has bought it — acquiring Ribopharma of Germany to obtain an important patent. Although some competitors grumble about Alnylam's aggressive approach to IP, Maraganore says, "we don't want to use our IP to block development of these important new drugs," and emphasizes that the company is amenable to licensing its IP.

Both Sirna and Alnylam develop synthetic dsRNAs, but not all companies take that approach. Australian biotech Benitec Ltd. introduces constructs that lead to creation of "hairpin" RNAs that fold up to make a double-stranded molecule. The idea is to induce the body to produce the RNAi mechanism, without the need for ongoing drugs.

Benitec is working with the City of Hope, in Duarte, Calif., to study that method in HIV patients suffering from a specific type of lymphoma. To qualify for the clinical trials, patients cannot have developed full-blown AIDS. However, they represent "an extreme case" in that they will have qualified for bone marrow transplants to treat lymphoma, says John Rossi, chair of molecular biology with the Beckman Research Institute at the City of Hope. Their own stem cells will be genetically modified to make them HIV resistant, and they will be placed back into their bodies with the hope that the modified stem cells will re-populate.

"Theoretically, this is a great way of doing it," Rossi says. "The virus would have a very difficult time establishing resistance." HIV-based vectors will be used to deliver the modified genes. "It's an interesting turn of events that we could take the type of virus that is making patients sick and potentially make them well," he says.

Even here, however, patent challenges are looming. Pennsylvania's Nucleonics recently challenged the validity of a key Benitec patent, charging that prior art abolishes the novelty of the patented technology. Benitec has aggressively protected its IP, helped by the experience of CEO John McKinley, who was a U.K. lawyer specializing in patent law before he jumped into biotech.

Beyond the companies developing RNAi-based therapeutics, the booming market also has led to the emergence of vendors for laboratory hardware and software. For instance, Ambion, in Austin, Texas, specializes in products for stabilizing, synthesizing, handling, isolating, storing, detecting, and measuring RNA.

Qiagen, with corporate offices in the Netherlands and Germany; Promega in Madison, Wis.; and Dharmacon in Lafayette, Colo., are among established life science companies that expanded their portfolios to include RNAi as that market became hot. OligoEngine, in Seattle, was formed in 2001 to offer siRNA sequence-design software, while Open Biosystems, founded in 2002 in Huntsville, Ala., has added to the resources it offers short hairpin RNA (shRNA) expression vectors developed at Hannon's Cold Spring Harbor lab.

RNAi research is moving ahead so quickly that Hannon admits it is difficult to keep up. "You always wonder what you're going to read in the journals," he says. RNAi is, for once, a technology that actually warrants the hype and attention it has received. "There are a lot of great biological discoveries, and this is certainly a fun one to have been involved in," Fire says. "It's been an exciting ride."

Sharpest Knife in the Drawer

Phil Sharp shared the 1993 Nobel Prize in physiology or medicine with Richard J. Roberts for RNA splicing, a seminal discovery that not only transformed scientists' understanding of gene regulation but also helped give rise to biotechnology. Twenty years ago, Sharp co-founded Biogen (now Biogen Idec). Convinced of the therapeutic potential of RNA interference (RNAi), in 2002 he helped launch Alnylam Pharmaceuticals. From his office at the MIT Center for Cancer Research, Sharp spoke to Nancy Weil.

BITW: Which therapeutic areas do you think have the most promise for RNAi-based treatments?

PS: The most obvious ones are probably viral infections of the liver: hepatitis B and C. Clinical trials are going to begin on macular degeneration. I have a colleague at MIT who has shown that you can inhibit the replication of flu in mice using this technology. That's a very nice piece of work. And if you think of what you can deliver to the liver, there are metabolic diseases as well that you could potentially have an impact on.

There's the CNS (central nervous system). The big issue in any drug, but particularly in CNS, is specificity. This technology lends itself to answering that problem because it's driven by sequence specificity, so I think you could probably anticipate treatment of some CNS situations ... and, hopefully, maybe glioblastoma-type tumors. If we can deliver this to cells circulating in the bloodstream, then I think there are a whole host of leukemias and other diseases — blood cancers — that we know a lot about molecularly (that could be treated with RNAi). We know what genes we would like to inhibit, and that should become possible.

What about the issues of stability and delivery?

That's where the cutting-edge biotech research is. I'd be pretty confident, given what we now know, that if we can deliver these to all the cells of the liver or all the cells of any organ that we could have a significant therapeutic impact.

The other thing is these are all going to be deliverable by injection. We're not going to take a pill of RNAi — we're going to take an injection or an infusion into the bloodstream or into the muscle. Therefore, we need longevity of action that is appropriate for that kind of delivery. So I'd love to get two weeks to a month of circulating a drug to inhibit these processes, or once the drug is in the cell, it persists that long and changes the character of the cell ... Chemical modifications to stabilize the RNAs so they won't be degraded by blood nucleases or modified by intracellular activities, as well as to promote their uptake, are things that are just cutting-edge research to take this to therapeutics.

Alnylam is a serious company this way, and I think Sirna is also working in this area. Some of the early work was developed by Isis in antisense, which had much the same problems of delivery and stability [as RNAi]. Alnylam and Isis have a [licensing] partnership, so all of that chemistry work is available to Alnylam.

Do you anticipate that some other mechanisms for silencing genes will be discovered?

Yes. We have mechanisms that silence at the level of protein synthesis, RNA stability, and transcription. That's all three of the major steps. I suspect there will be insights into how those processes occur that will give us indications as to how to take our chemistry, or select sequences, or introduce these things so that they're more effective.

How will you figure out how the RNAi mechanism actually works?

We know much more than we did three or four months ago. We now know that the Argonaut 2 protein is likely the major nuclease. We've got an atomic structure of it, even. We know several other proteins are probably in part of the complex. We know it's catalytic, and we know how it recognizes, at least in a descriptive way, this small siRNA and picks one strand versus another. All that sort of hurtled forward, and it's provided a platform of science for us to move forward in drug discovery.

Horizons

RNA INTERFERENCE

Silence Is Golden

BY MALORYE BRANCA

Bio-IT World

It's so quick, easy, powerful, and cost-effective, RNA interference (RNAi) almost sounds like the too-good-to-be-true subject of a late-night television infomercial.

Certainly, in labs desperately hunting for better ways to study gene function, these tiny, exquisitely specific gene-silencing molecules seem nothing short of miraculous. RNAi promises to transform investigations of gene function in organisms from microbes to mammals.



The color purple: The phenomenon of "co-suppression" was first described in petunias by Richard Jorgensen and Carolyn Napoli

RNAi rapture has also consumed the biotech and venture capital community like wildfire, inspiring resurgence in a previously struggling sector of the biotech industry that sees unlimited potential for RNAi in the clinic. A recent article in *Fortune* heralded "Biotech's Billion-Dollar Breakthrough." In 2002, Alnylam Pharmaceuticals, founded by a bevy of RNAi pioneers including Nobel laureate and Biogen founder Phillip Sharp, raised \$17 million in early financing. And earlier this year, just a week after changing its name from Ribozyme Pharmaceuticals, reborn Sirna Therapeutics netted a cool \$48 million in venture capital. But now the tough questions loom large: Will RNAi prove to be the missing link needed for genomic drug discovery? And must RNAi-based drugs endure the same type of roller-coaster ride that earlier technologies, such as monoclonal antibodies and RNA antisense, endured?

Or will past experiences in gene therapy and antisense make for an easier transition?

The first hints that there was a natural way to suppress genes emerged from the fertile field of plant genomics. Richard Jorgensen, currently at the University of Arizona, stumbled upon this realization in the late 1980s, when he tried adding more of a "purple" gene to deepen some petunias' purple hue. The flowers turned white instead.

The underlying phenomenon — RNAi — was described in 1998 by Andy Fire and Craig Mello at the Carnegie Institute in Washington, D.C. The researchers showed that small interfering double-stranded RNA (siRNA) fragments could specifically target and eliminate natural messenger RNA molecules.

Early RNAi work focused on plants and lower animals such as worms. Then, two years ago, a paper in *Nature* revealed that RNAi also worked in mammalian cells (Elbashir, S.M. et al. 411: 494-498; 2001). The precise role of RNAi in nature is still vague, but thousands of scientists

are applying the tool to examine the effects of specifically shutting off genes. These experiments can take as little as a week — lightning speed compared to the months required to knock out a single gene in mice.

But Not Quite Magic

Despite its promising start, RNAi still has some rough edges. At Gene Expression Systems' recent RNAi 2003 meeting in Waltham, Mass., Joanne Kamens from Abbott Bioresearch Center described her struggles to use the technique in macrophages. These immune cells are notoriously resistant to the introduction of foreign genetic material. "Everyone agreed, surprisingly quickly, that we should do this, but then it wasn't as easy as we hoped," Kamens said.

Working in collaboration with Sequitur, the Abbott group was eventually able to shut down certain genes, but curiously the proteins they were trying to eliminate were still being expressed at high levels in the cell. "One possibility is that there are redundant mechanisms, and that shutting down one gene actually leads to increased protein production by other means," Kamens said.

Producing effective siRNAs — the actual gene-silencing molecules — can also be difficult. Companies including Dharmacon and Cenix BioScience are trying to work out the rules for what makes a good siRNA.

"Not all genes silence equally," said Stephen Scaringe, chief scientific officer at Dharmacon, who also spoke at the Waltham meeting. Certain sequences are just more difficult to shut down by RNAi. "You can change the siRNA by one base and suddenly gain or lose functionality," he said. Meanwhile, some siRNAs can interact with parts of the cellular machinery beyond their intended targets.

Single nucleotide polymorphisms (SNPs) and alternative splicing can also cause problems.

People who use poorly designed silencing molecules have a hard time getting good results. "Adding more bad siRNA does not make it work better," Scaringe said. Scientists are therefore relying on bioinformatics to help design the molecules and categorize them: Some siRNAs work, but poorly, while others are "superfunctional." Dharmacon's scientists have also found that using a specific set, or pool, of many siRNAs works best.

More drug companies are catching on to the "intelligent design" approach to siRNAs: Last month, Dharmacon announced a deal with Exelixis to produce a library of siRNA reagents targeted against 600 top drug candidates, including kinases. The library will be used for "high-throughput functional genomic studies designed to characterize drug targets and pathways in mammalian model systems," according to a joint announcement.

Pitfalls remain, but many companies are streamlining the technique, quickly making it easier.

But Can You Make Drugs with It?

RNAi is not *just* a spectacular lab tool — it offers a tantalizing mechanism for producing actual drugs as well. No sooner was RNAi's utility in mammals demonstrated than investors started pouring money into RNAi-based companies (see "RNAi-Fever Heats Up Novel Drug Category Funding." Oct. 2002 *Bio-IT World*, page 11). A string of recent successes in using RNAi to attack viruses such as HIV and hepatitis B and C in animal models has kept investors salivating.

One of the more glamorous startups in the field is Alnylam, with its advisory board of RNAi luminaries including David Bartel, Thomas Tuschl, Phillip Zamore, and Greg Hannon. The company is working on RNAi-based drugs for cancer, infectious diseases, and inflammation.

German biotech Cenix BioScience raised \$4.9 million last year and also plans to make drugs. Cenix is working with Ambion to develop validated synthetic siRNAs for every gene in the human genome, according to David Brown, senior R&D scientist at Ambion.

Even more dramatic is the number of established companies suddenly embracing RNAi. Groups that have worked through some of the devilish details surrounding the use of other

RNA-targeting technologies, such as antisense and ribozymes, have jumped on the RNAi bandwagon. Colorado-based Ribozyme Pharmaceuticals had nearly dissolved because its ribozyme drugs weren't passing clinical milestones. But applying its RNA insights to RNAi, capped off with a new name — Sirna Therapeutics — resurrected investor interest.

To produce RNAi-based therapeutics, companies need to develop both a molecule to specifically silence genes and a way to deliver that molecule inside the targeted cell. Several firms have one of those ingredients and are looking for the other.

In the siRNA Space

RNAi was *Science* magazine's "breakthrough of the year" in 2002. Based on a recent survey, BioInformatics LLC predicts a surge of RNAi-related studies, driving market growth. Here are some of the companies riding the RNAi boom.

Amaya Biosystems	Invitrogen
Ambion	InvivoGen
Applied Biosystems	Mirus
BD Biosciences	New England Biolabs
Benitec	OligoEngine
Clontech	Proligo
ChemGenes	Qiagen
Compugen	Promega
Dharmacon	Qbiogene
Gene Expression Systems	Roche Applied Science
Gene Tools	Sequitur
Imgenex	Upstate Group
	U.S. Genomics

Source: Bio • ITWorld

Intradigm and Sequitur, for example, have just signed what will undoubtedly be one of many deals involving cross-licensing of RNAi technologies. Intradigm now has access to Sequitur's Stealth RNAi in certain disease areas, while Sequitur can use Intradigm's Targeted Synthetic Gene Vector technology.

Sequitur is an experienced service provider that uses antisense and RNAi to help drug companies confirm that they are hitting good molecular targets in the body with their new drugs. Stealth RNAi is a modified form of siRNA that is "more effective, more stable in serum, and less likely to generate an interferon [immune] response," according to Sequitur President Tod Woolf.

Intradigm, meanwhile, has several staffers from former Novartis subsidiary Genetic Therapy, where they were also tackling the daunting problem of introducing genetic material into human cells. Their solution for delivering siRNA is both technological and practical: Besides using a special chemical formulation that protects the drug as it is entering the body, they target contained areas, such as the eye, the joint space, and tumors.

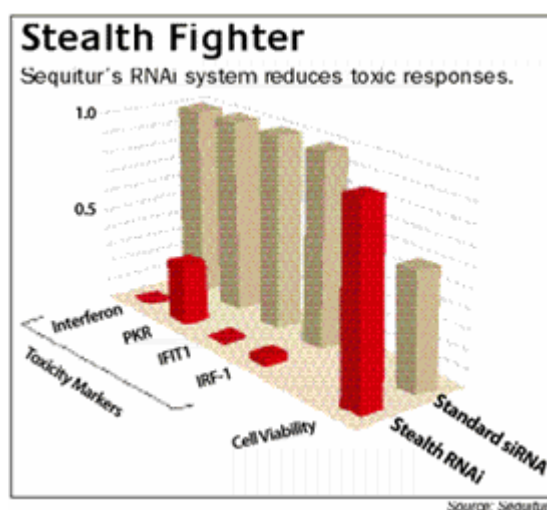
Both companies want to make their own drugs as well as work with collaborators. "Our philosophy is to find groups to work with, rather than to fight," says Martin Woodle, Intradigm's founder and chief scientific officer.

Litigating RNAi

But not everyone is going to work things out through deals. "If RNAi turns out to be as valuable as people anticipate, there will inevitably be litigation," says Irene Abrams, technology licensing officer at MIT, which is part owner of one of the key siRNA patents. The number of RNAi-related patents has, naturally, proliferated as the field heated up. Many of the major patents are pending, and many may overlap.

Because of the economic climate, the need for quick results, and the uncertainty over intellectual property, several deals are being negotiated. "With patents, you can never proceed with certainty until they are issued," Woodle says. Still, some bitter contests are likely. With so many players and so much at stake, "It's hard for people to compromise or fairly determine the value of their own intellectual property," Abrams says.

Even if the thorny patent issues are resolved, companies still have to get drugs into the clinic and then to the market. "Anybody who has experienced any kind of drug discovery and development realizes that there will be surprises ahead [for RNAi]," cautions Frank Bennett, vice president at Isis Pharmaceuticals.

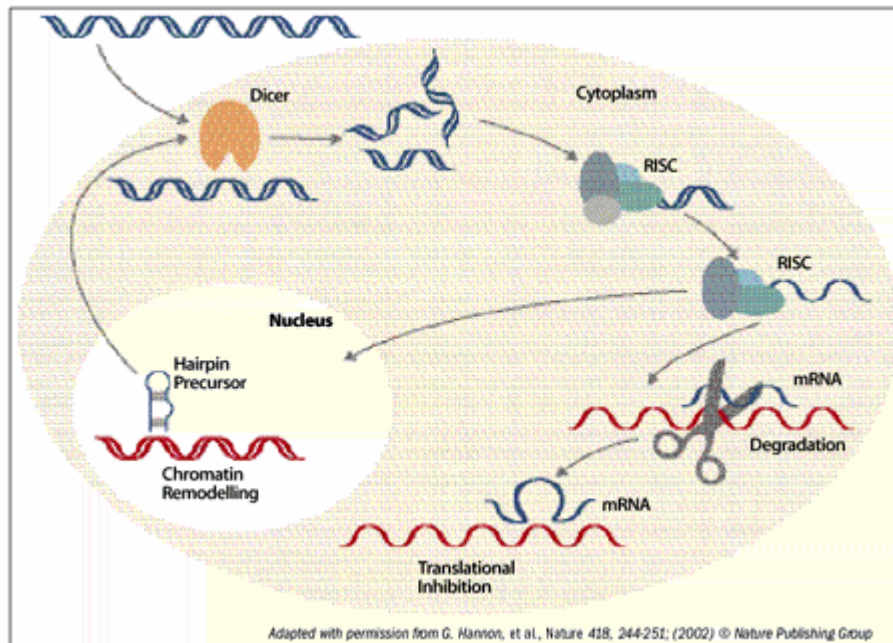


Woolf believes the key to keeping the field hot long enough for bona fide drugs to get made is keeping the science high-quality. During the peak of antisense fever, he says, "Every type of chemistry tried was claimed to work in animals." But many of those results turned out to be spurious. Such "bad" science can proliferate when many inexperienced investigators are trying a new technique. Some companies also pushed hard too early with antisense- or ribozyme-based products that really weren't ready for the clinic. The result was a backlash, and only now is antisense technology getting a fresh chance in clinical trials.

Things could go differently with RNAi. Sequitur's Woolf points out: "The academic researchers who discovered [RNAi] are extremely high-grade scientists, and the work that has followed has been carefully done too."

And a decade of experience with related RNA technologies will prove invaluable as well. "I think antisense has helped RNAi a lot, in showing what the rate-limiting steps will be and how to solve some of the pharmacokinetic issues," Bennett says.

Although Isis is studying RNAi, Bennett is bullish on antisense. And with about a dozen antisense-based drugs still working their way through clinical trials, the older technology could yet eclipse any possible wave of RNAi-based drugs. Both techniques face the same major hurdle — delivery into the appropriate cells. But once that is solved, it would appear that RNAi will have finally arrived.



RNAi roundabout: Double-stranded RNA molecules are processed into small interfering RNAs (siRNAs) by the enzyme Dicer. These siRNAs pass to the RISC (RNA-induced silencing complex), where they become unwound and activated. Gene silencing probably occurs via several mechanisms, including RNA degradation, translational inhibition, and chromatin remodeling.



Snapshot of Drug-Discovery Companies Working with RNAi

Name: Alnylam Pharmaceuticals

Founded: 2002

Founders include: Phillip Sharp, Paul Schimmel, David Bartel, Thomas Tuschl, Phillip Zamore, Christoph Westphal

Financial note: \$17 million private equity financing.

URL: www.alnylam.com

Name: Benitec

Founded: 1998

Financial note: Market capitalization ~ \$25 million.

Note: "First company to demonstrate RNA interference in human cells..."

URL: www.benitec.com.au

Name: Cenix BioScience

Founded: 1999

Founders: Christophe J. Echeverri, Pierre Gönczy, Anthony A. Hyman

Financial note: €11 million raised to date

URL: www.cenix-bioscience.com

Name: Intradigm

Founded: 2002

Founders/Advisors include: Martin C. Woodle, Patrick C. Lu, Paul Tolstoshev, Richard Heller, Richard Gilbert

Financial note: \$1.35 million in series-A financing

URL: <http://www.intradigm.com>

Name: Ribopharma (merged with Alnylam in July; will maintain operating unit in Kulmbach, Germany)

Founded: 2000

Founders: Roland Kreutzer, Stefan Limmer

Financial note: EUR 2 million from Abingworth in 2003

URL: www.ribopharma.de

Name: Sequitur

Founded: 1996

Founders/Advisors: Tod Woolf, Craig Mello, Richard Wagner

Financial note: less than \$20,000 invested initially by founder Tod Woolf

URL: www.sequiturinc.com

Name: Sirna Therapeutics (formerly Ribozyme Pharmaceuticals)

Founded: 1992

Founders: "No longer relevant," according to Marvin Tancer, CFO and vice president of operations

Financial note: \$29 million initial financing; \$48 million private stock placement in 2003; \$145.7 million total private stock placement

URL: www.sirna.com