Antisense Aims for a Renaissance

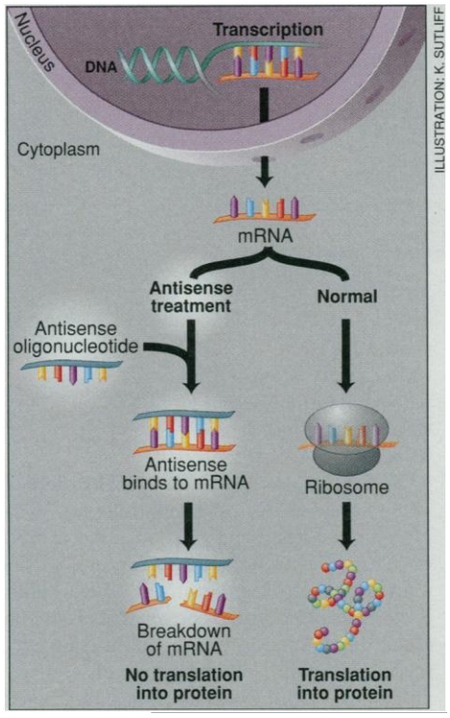
Antisense therapies, once plagued by side effects and mysterious failures, have now shed their troubled image, thanks to encouraging new clinical results and basic research on past problems

CAMBRIDGE, MASSACHUSETTS—The upscale stretch of the Charles River between Harvard University and the Massachusetts Institute of Technology could be renamed Biotech Brook. First, there is Genzyme's new $150 million facility, pointing skyward like a brick version of Chartres Cathedral. Downstream, the red-brick behemoth of Millennium Pharmaceuticals looms arrogantly over the river in a former Ford Motor Co. factory. But, for years, the next building, across the railroad tracks and down the river, was the ne'er-do-well of the neighborhood, a drab tire warehouse–turned-office-building with little architectural—or scientific—presence. Then last year, the building was renovated in a snazzy postmodern style, complete with penthouse and checkerboard-brick ornamentation.

This architectural rebirth mirrors a scientific one, for the new occupant is Hybridon Inc., a major manufacturer of antisense oligonucleotides—short, synthetic stretches of DNA and RNA designed to block the action of specific genes by binding to their RNA transcripts. Back in 1992—after studies suggested antisense might be a promising source of precisely targeted therapies for cancer and other diseases—*Science* named antisense technology one of the 10 hottest research areas of the year. But its prospects have been in doubt ever since. By 1995, researchers faced depressing evidence that their antisense molecules just didn't seem to work as intended (*Science*, 27 October 1995, p. 575). In addition, the technology was experiencing menacing glitches, including unforeseen—and sometimes lethal—side effects in animals.

Now, however, scientists are discovering that some of the problems stemmed from peculiarities of the first-generation antisense drugs, some of which bound to all sorts of molecules in addition to their targets, and second-generation antisense molecules are on the way. The result is a biotech boomlet, symbolized by Hybridon's new headquarters and the investor dollars flowing into the field; Hybridon netted $52 million in its initial public offering last year. Also, early results from new clinical trials of first-generation drugs seem promising. Just this week, at the American Society of Clinical Oncology meeting in Denver, researchers reported that antisense drugs shrank ovarian tumors in small clinical trials. Early trials of drugs against AIDS and Crohn's disease, an inflammation of the bowel, also look favorable. "It seems that many of the underlying problems that have been endemic to the field are on the way to being worked out," says analyst Michael Sheffery of the New York biotech investment firm Mehta and Isaly. Biochemist Fritz Eckstein of the Max Planck Institute for Experimental Medicine in Göttingen, Germany, agrees, noting that "antisense has come of age."

Indeed, a recent Cambridge antisense conference* turned into an "antisense lovefest," according to Mark Matteuci, director of bioorganic chemistry at Gilead Sciences of Foster City, California, one of a handful of companies heavily invested in antisense. Even so, Matteuci and some others are still cautious about the technology. Despite the new mood of optimism, a number of serious wrinkles—such as the difficulty of getting enough antisense molecules into cell nuclei, and continuing doubts about whether they exert their effects through true antisense mechanisms—have yet to be ironed out, warns Matteuci.



Antisense strategy. Antisense oligonucleotides are designed to turn off certain genes by binding to stretches of their messenger RNA.

* "Antisense 97: Targeting the Molecular Basis of Disease," 1–2 May.

Making sense of antisense

The idea behind antisense is simplicity itself. In the first step of protein synthesis, the bases on one strand of DNA's double helix are transcribed into the complementary sequence in a messenger RNA (mRNA) molecule. As Harvard biochemist Paul Zamecnik and others first showed in the 1950s, the sequences of these "sense" molecules are translated into a series of amino acids, giving rise to proteins. Then, in 1978, Zamecnik showed that an oligonucleotide tailored to complement part of a particular mRNA—an "antisense" strand—could bind to that section of the mRNA, causing a cutting enzyme to home in on the mRNA and destroy it. This prevents protein expression and turns off a gene's activity. Zamecnik quickly saw that antisense drugs might forestall a host of diseases linked to abnormal gene activity, including cancers and viral infections, and he founded Hybridon in 1989 to exploit the idea.

Researchers had some early test-tube successes, for example, showing in cell cultures that antisense molecules could reduce replication in HIV, the virus that causes AIDS. But putting the antisense strategy into practice proved difficult. The first generation of drugs—the so-called phosphorothioates, whose nucleotide backbones carry extra sulfur atoms to slow their degradation in cells—behaved unexpectedly. Some compounds were apparently unable to get into their target cells, and control oligos that didn't even complement the target mRNA seemed to work almost as well as the antisense oligos, throwing a monkey wrench into the logic of antisense drug design. Such "non–sequence-specific" effects plagued Hybridon's antisense oligo called GEM91, designed to block expression of a crucial HIV gene, and many others. The antisense technique, so precise in theory, began to look like a biological blunderbuss in practice.

There were other worrisome signs as well. Researchers found that antisense seemed to trigger extreme—and sometimes dangerous—immune responses. A group led by University of Iowa immunologist Arthur Krieg, for example, reported in 1995 that antisense oligos carrying a certain brief sequence (a cytosine nucleotide followed by a guanine) somehow pump up the body's production of immune-system warrior cells—a vexing result, for Krieg had been trying to treat diseases such as lupus that involve unbridled immune activity. In addition, several monkeys at Hybridon died

after injections of phosphorothioates.

But explanations—and solutions—have now emerged for many of these setbacks. For starters, researchers now realize that the added sulfur ions in the phosphorothioates may be responsible for some of the non–sequence-specific effects. These compounds carry a large negative electrical charge, which makes them chemically sticky, explains nucleic acid chemist Sudhir Agrawal, Hybridon's chief scientific officer. Hence, they are liable to hitch up willy-nilly not just with mRNAs, but with antibodies and intracellular messenger molecules, and so interfere with many biological processes.

As a consequence, antisense firms are developing a second generation of phosphorothioates that carry other clusters of atoms with less charge. Hybridon, for example, has found that antisense oligos bearing uncharged chemical groups have fewer unwanted side effects and survive longer inside cells, says Agrawal.

Another big stumbling block, researchers say, has been the naïve assumption that all mRNA sequences make equally attractive targets for antisense drugs. For some reason, antisense oligos bind to some sequences with much greater affinity than others, explains cancer researcher Stanley Crooke, founder, chair, and CEO of antisense leader Isis Pharmaceuticals, based in Carlsbad, California. "Randomly making antisense oligos gives random results, and when it works, it's luck," says Crooke. Cy Stein, a Columbia University pharmacologist who has questioned whether the early positive results were due to true antisense mechanisms, agrees. "You can't just pull a reagent off the shelf—you have to be prepared to screen 30 or 40 molecules to find the ones that work."

But synthesizing and testing large numbers of ultrapure oligos are expensive and time-consuming. Hybridon, which does commercial-scale manufacturing of antisense oligos, charges about $2000 per gram. Academic scientists can't afford such costs, but companies can. Isis, for example, has found success by sifting through dozens of oligos that complement slightly differing segments of the targeted mRNA, and selecting those that work. As a result, although academics are energetically applying antisense to research questions, both corporate and academic researchers agree that today many of the hottest antisense results come from companies rather than university labs. "Isis has brought sense to antisense," says Iowa's Krieg.

Hybridon researchers have also begun to make sense of their disturbing monkey fatalities. Giving monkeys a large, one-time injection of phosphorothioates triggered a systemic and lethal inflammation by activating complement, the part of the immune system responsible for organ rejection after transplants, says Agrawal. He says that giving the same drugs more slowly avoids the problem.

Even the problem of slipping antisense compounds into the cell nucleus, where they can bind to their target mRNAs, looks a bit less daunting than before. The previous round of experiments was largely done in culture, where the molecules often failed to make it past the cell membrane. So, some scientists spent years trying to craft lipid-based costumes to disguise the antisense molecules and enable them to slip through. But it appears—again for reasons not well understood—that antisense molecules do better at reaching their targets in living animals than in culture, says Crooke. Data from Hybridon and Isis show that plain, uncloaked antisense molecules injected into live animals are taken up by many different organs, although questions remain about exactly where the molecules bind once they are inside cells.

GRAHAM G. RAMSAY

New look. His company's snazzy new headquarters symbolizes a comeback for antisense, says Hybridon's Sudhir Agrawal.

Success in the clinic

While putting these old questions to rest is key to antisense's future, "Nothing is going to make a bigger difference than positive clinical data," says analyst Sheffery. In recent months, even the first generation of antisense oligos has begun to yield encouraging results. This week, Stanford University oncologist and pharmacologist Branimir Sikic reported at the oncology meeting that ISIS 3521, a 20-base oligonucleotide developed by Isis, stopped the spread of ovarian cancer in three patients out of 17 and caused few toxic side effects in Phase I clinical trials. A promising antisense oligo that blocks replication of cytomegalovirus—the virus that destroys the retinas of many AIDS patients—is already in Phase III trials at Isis. The company hopes to file a new drug application with the Food and Drug Administration by early 1998.

The largest crop of encouraging data came in late February, when Isis announced results from a Phase II trial of the antisense drug ISIS 2302 as a treatment for Crohn's disease. The inflammation response that causes Crohn's depends in part on a cell adhesion protein that helps inflammatory blood cells punch through the walls of blood vessels; the new antisense compound inhibits the synthesis of this protein. After 1 month of treatment, the disease had gone into remission in nearly half of the 15 patients treated, compared to zero out of five patients taking a placebo. That outcome "was much stronger than I expected it to be," says Sheffery.

Some optimistic researchers say that if certain lab experiments blossom into clinical applications, more successes could be on the way. At the meeting, pharmacologist Ryszard Kole of the University of North Carolina, Chapel Hill, for example, reported a potential breakthrough in the treatment of thalassemia, a hereditary form of anemia common in many parts of the world. In some individuals, this disease is caused by mutations in the gene encoding the hemoglobin subunit βglobin. These mutations cause part of an intron in the gene—a nucleotide sequence that interrupts the gene and is not intended to code for protein—to be left in the mRNA by mistake. The resultant mutant mRNA is unstable and degrades before it can be translated into protein. But Kole found that infusing mutant cells in culture with certain antisense oligos caused the intron to be snipped out, as it should be, allowing normal βglobin to be made. Reassuringly, mismatched antisense molecules injected as controls had no effect.

To the optimists, this and many other such experiments indicate that the new antisense molecules are living up to the original vision, homing in accurately on their target mRNAs. But others note that the compounds still exhibit many non–sequence-specific effects—Hybridon's GEM91, for example, not only blocks HIV replication but inhibits its binding to cells. That suggests that antisense molecules bind profligately to unintended targets and so could create a new crop of unforeseen problems. To these researchers, the atmosphere at the Cambridge conference was too utopian. "A couple of years ago, some of the more negative issues got blown out of proportion, and I think that this year things went in the opposite direction," says cell biologist Richard Wagner, director of Gilead Sciences.

Yet, even skeptics such as Wagner and Columbia's Stein—who co-organized a much more negative gathering 2 years ago—agree that antisense is moving up in the world and deserves its renewed status as an up-and-coming research technology. "It's clear that things are a lot better," says Stein. "A lot of the theoretical problems have been resolved. ... There is really a chance of seeing what people have wanted to see."

–Wade Roush