Making Sense of Antisense

Novel methods of blocking gene expression are already proving themselves in plant development and may also lead to new therapies for human disease

OUT IN THE SAN JOAQUIN VALLEY OF CALIFORNIA grows a field of tomato plants that look like ordinary tomato plants. But these tomatoes are special—one of the first fruits of a new technology that may revolutionize not just commercial plant development but human medicine as well.

The new technology uses novel RNAs, called antisense RNAs, to block the activity of specific genes. At first, researchers were mainly interested in antisense RNA as a tool for probing gene function. In the late 1970s, when the technology was first developed, molecular biologists didn't have a good way of mutating genes in the cells of higher organisms so that they could see what happens when the gene activity is lost. Antisense technology, in effect, provided a way of doing that.

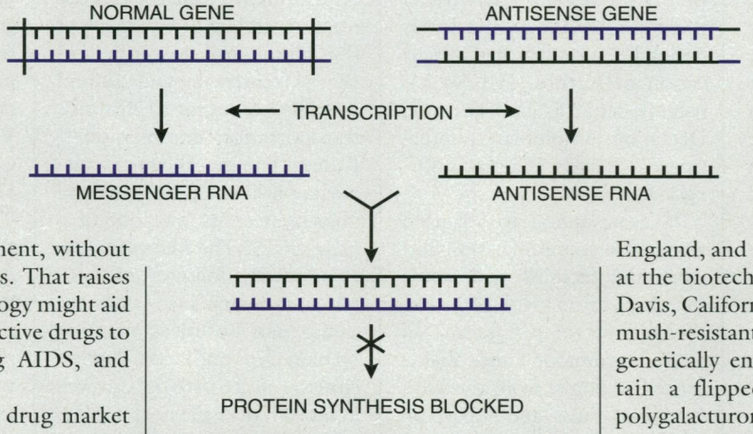
But the biotechnology industry soon recognized the immense practical potential of a technique that could be used to knock out the activity of "bad" genes. To make the tomato plants, for example, plant scientists used antisense RNAs to shut off the expression of the gene encoding an enzyme that makes tomatoes mushy, thereby yielding a product that may travel better and last longer on grocery shelves. Indeed, as pioneer antisense researcher Jonathan Izant of Yale University, points out: "This should be the first example of a commercial use of antisense that will have an impact on large numbers of people."

But there's more: Recent work by various labs suggests that it may be possible to design antisense compounds that inhibit the activity of viral genes or of the oncogenes thought to contribute to cancer development, without affecting normal cellular genes. That raises the possibility that the technology might aid in producing better, more selective drugs to treat viral diseases, including AIDS, and cancer.

With a potentially lucrative drug market as a stimulus, it's no wonder then that new biotech companies are springing up to try to exploit antisense technology. The new companies include Gilead Sciences of Foster City, California; Genta, Inc., of San Diego; Hybridon, Inc., of Worcester, Massachusetts; and Isis Pharmaceuticals of Carlsbad, California, among others. In addition, several established pharmaceutical companies, including Glaxo, Inc., in Research Triangle Park, North Carolina, and Sterling Drugs in New York City, have begun antisense research programs.

Even as attempts to apply antisense technology spread, however, there are indications that the approach may not be as straightforward as it may have seemed at first—and this may complicate plans to apply antisense technology eventually to human disease therapy.

During gene expression, the information encoded in a gene is first transcribed into a messenger RNA molecule that in turn directs protein synthesis. The original idea behind antisense technology was to create a piece of RNA with a base sequence complementary to that of a particular messenger RNA. This antisense RNA would be able to bind to the messenger, preventing it from making its protein. Researchers knew that this happens in the natural world: Bacteria and the viruses that infect bacteria sometimes turn off gene expression by making just such antisense messages. So if bacteria and viruses were clever enough to turn this trick, researchers asked, might not it work in the cells of higher organisms, too?



Showstopper. *One way that antisense RNA prevents gene expression is by binding to messenger RNA and blocking protein synthesis.*

Early experiments showed that higher organisms were also susceptible to antisense strategies—although researchers have to manipulate the target cells to provide them with the necessary antisense RNA molecules. One way of doing this was developed in 1984 by Izant, who was then working as a postdoc with Harold Weintraub at the Fred Hutchinson Cancer Center in Seattle.

Genes are, of course, composed of two strands of DNA, only one of which is normally transcribed into messenger RNA. But if the protein-coding portion of the gene was flipped over, Izant and Weintraub reasoned, the gene's regulatory sequences would cause the other—or "wrong"—strand to be transcribed, allowing the cell to produce its own antisense RNA. The Seattle workers went on to show that the synthesis of the enzyme thymidine kinase could be blocked in mouse cells if a flipped version of the thymidine kinase gene was introduced into the cells along with the normal gene. The work suggested that the RNA produced by the flipped gene bound and inactivated the RNA produced by the normal gene, just as the researchers proposed.

Not long afterwards, Douglas Melton of Harvard University devised a second way of using antisense methodology to block gene activity. He showed that synthesis of specific proteins could be prevented in frog eggs simply by injecting them with synthetic antisense RNAs.

Those early experiments provided the basis for the two general antisense strategies used today. Donald Grierson's group at the University of Nottingham, England, and a second group of researchers at the biotechnology firm Calgene, Inc. of Davis, California, independently created the mush-resistant tomatoes, for example, by genetically engineering the plants to contain a flipped version of the gene for polygalacturonase, an enzyme that breaks down plant cell walls. As a result, production of the enzyme was reduced by 99%. Otherwise, the tomato plants, which are now in the third generation, appear normal.

"We were delighted to have tampered selectively with one gene with no nasty side-effects," Grierson says.

Transfer of antisense genes has also been used to produce virus-resistant plants and animals. In the August issue of the *Proceedings of the National Academy of Science, USA*, Anthony Day of the Imperial College of Science, Technology, and Medicine in London and his colleagues describe experiments in which they put an antisense gene construct that blocks the replication of tomato golden mosaic virus into tobacco plants. The resulting transgenic plants were much more resistant to infection by the virus than were controls.

And in the May *Proceedings*, Thomas Wagner, Lei Han, and Jeung Yun of Ohio University in Athens describe a similar experiment in mice. These researchers genetically engineered mice with an antisense construct of a gene needed to make infectious particles of a leukemia-causing virus. When these mice were injected with the virus shortly after birth, none developed symptoms of leukemia, although 31% of the control animals did. "We were surprised at the results," Wagner says. "It (the antisense construct) absolutely turned off viral packaging."

The Wagner group's results have caused molecular geneticist John Rossi of the City of Hope Medical Center in Duarte, California, to declare that "antisense is going to be a powerful antiretroviral tool." Potential targets for antisense therapy include AIDS, which is caused by a retrovirus. It might be possible, Wagner suggests, to genetically engineer lymphocytes, one of the major cell types infected by the AIDS virus, with antisense constructs that prevent the virus from replicating.

Alternatively, it may be possible to develop an approach more like Melton's, which uses an external source of synthetic antisense compounds, to treat human diseases. A number of companies, including Gilead and Hybridon, are trying to develop such antisense compounds for treating AIDS and herpes virus infections. And on p. 562, Bruno Calabretta and his colleagues, who have just moved from Temple University Medical School to Jefferson Medical College in Philadelphia report that antisense compounds can target and stop the replication of human cancer cells growing in culture.

The cells used by the researchers carry a particular chromosomal abnormality, named the "Philadelphia chromosome translocation" after the city where it was discovered, that is relatively common in human leukemias. The translocation causes an oncogene, designated ABL, to be moved from its normal location on chromosome 9 to chromosome 22, resulting in the formation of a hybrid gene. The activity of ABL is increased in the cells with the Philadelphia chromosome, presumably as a result of the translocation, and cancer biologists assume that the activity contributes to the increased growth of the leukemia cells.

DNA Plant Technology, Inc.

Moneymakers. *Genetic engineering turned pink petals white in these aptly named Moneymaker chrysanthemums.*

What Calabretta and his colleagues have now done is make a short, single-stranded antisense DNA, just 18 nucleotides long, that specifically recognizes the junction of the ABL hybrid. The antisense construct, the researchers found, stops the growth of the cancer cells, but not that of the normal cells from which the cancer cells were derived. "These findings demonstrate the feasibility," says Calabretta, "of using antisense compounds for gene-targeted, selective killing of neoplastic cells."

And the approach need not be limited to leukemias. Many of the common solid tumors, such as lung and colon cancer, also carry oncogenes that have been activated by gene mutations. Antisense compounds could be designed for those mutations, too.

But as Rossi acknowledges, a number of "ifs, ands, and buts" need to be worked out before antisense technology can be applied to human disease therapy. For one thing, antisense compounds can be unstable, breaking down before they reach their targets. For another, cell culture experiments have shown that it's sometimes hard to get the compounds into cells at the right time to block messenger RNA activities. Because of these problems, it may be necessary to use large quantities of the agents, raising questions about their potential toxicity in the body. Such drugs may also be very expensive.

What's needed to design better antisense drugs, Rossi says, is a clearer understanding of how they work. Although conventional wisdom has it that they bind to, and inactivate, messenger RNAs, either by inhibiting their translation or causing their degradation, there are reasons to think that this may not be the whole story. So far, for example, the telltale sense-antisense RNA hybrids have been tough to find.

And last year, a research team led by Richard Jorgensen, who was then with DNA Plant Technology's Oakland lab and is now at the University of California, Davis, came up with some unexpected findings that show that the antisense picture is more complicated than expected. While working on a program aimed at genetically engineering plants for novel colors, Jorgensen put an extra gene for an enzyme needed to make the purple pigment anthocyanin into petunia plants. He thought this would give the flowers a deeper color. The actual result? The blossoms turned white. In other words, the sense gene apparently behaved like an antisense construct.

Nor are the petunias an isolated example. Other researchers, including Joseph Mol of the Free University in Amsterdam, Holland, Bill Hiatt at Calgene, and Grierson, have repeated Jorgensen's experiment with additional genes, including the polygalacturonase gene, and have obtained comparable results. That's all right for the plant industry, since biotech companies are already using this "sense" technology to develop flowers, including petunias and chrysanthemums, with attractive new color patterns.

But it adds confusion to efforts to understand how antisense technology works. One possibility is that antisense mechanisms in plants may be different from those in animals. Or antisense may work differently, depending on the developmental stage of the organism in which it's applied. Some researchers have also suggested that what really determines what happens is the location where a transferred gene ends up in the recipient genome, not whether it's sense or antisense. Examples of such "position effects" on gene expression have been documented in other experiments. Other researchers say that the RNAs transcribed from antisense genes may have unusual three-dimensional structures that disrupt transcription of the corresponding sense gene. "There are all these possibilities," says Jorgensen. "At the very least, it is dangerous to assume that antisense constructs work only by producing antisense RNAs that form hybrids with messenger RNAs." And only when researchers understand antisense mechanisms will they feel comfortable applying a technology that has worked so well on tomatoes to human beings. ■ ANNE SIMON MOFFAT