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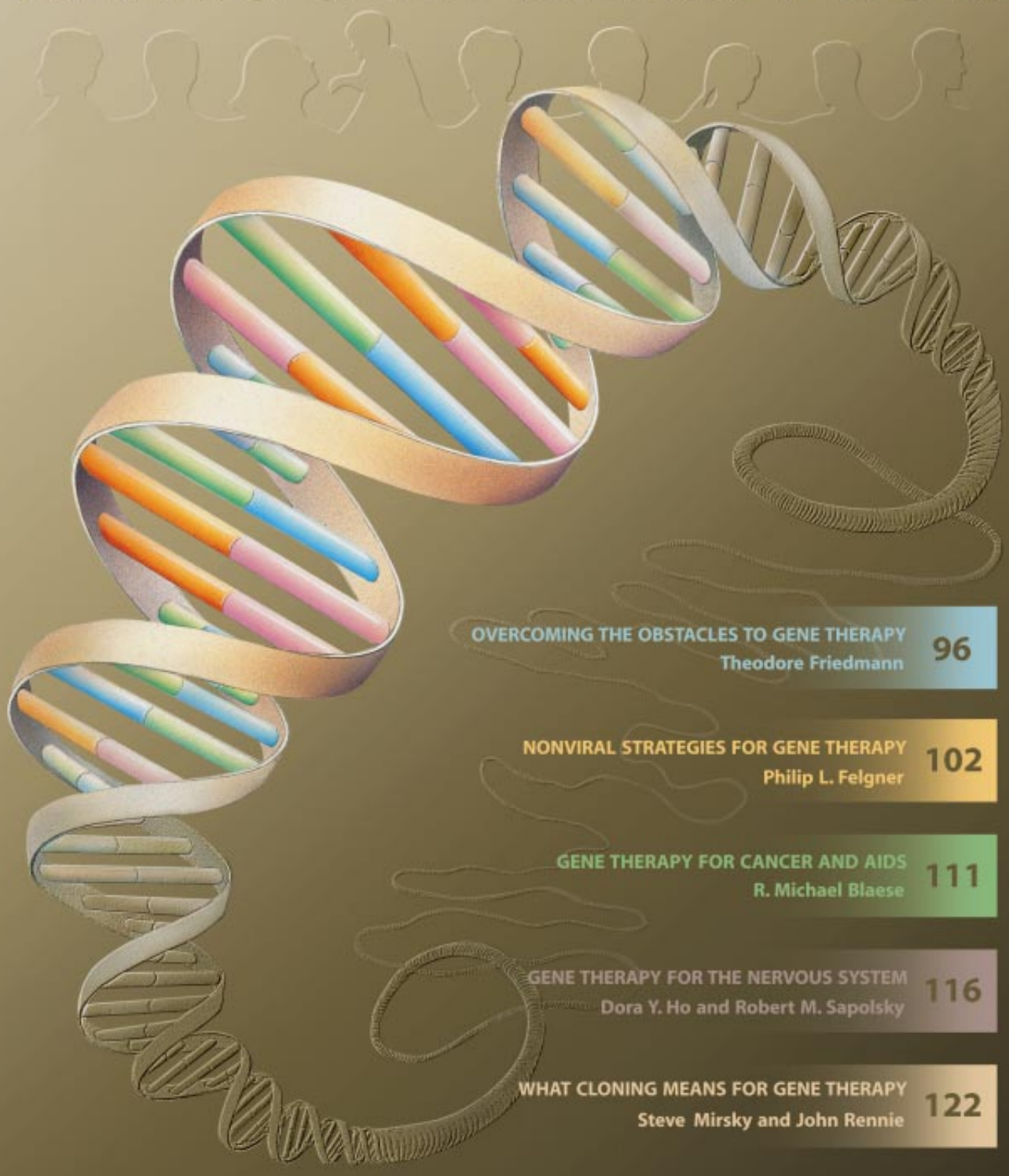
— SPECIAL REPORT —

MAKING GENE THERAPY WORK



TOMO NARASHIMA AND LAURIE GRACE

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Overcoming the Obstacles

Treating disease by providing needed genes remains a compelling idea, but clinical and basic researchers still have much to do before gene therapy can live up to its promise

by Theodore Friedmann

In the late 19th century, when the pioneering architect Daniel H. Burnham was planning some of the first modern skyscrapers, his associates were skeptical about erecting buildings that soared into the clouds. Burnham reportedly warned the skeptics against making "little plans," having "no magic to stir men's blood." He urged them to reach beyond traditional architectural boundaries, to think once inconceivable thoughts and to perform previously unimagined deeds—the hallmarks of revolutions.

Revolutionary changes have also occurred in medicine over the past few centuries. Witness the new understandings and practices that issued from the introduction of microscopy, anesthesia, vaccination, antibiotics and transplantation. Medicine is now preparing to undergo another epochal shift: to an era in which genes will be delivered routinely to cure or alleviate an array of inherited and acquired diseases.

Preparing for a radical change, yes, but not yet in the midst of it. By emphasizing hopes and downplaying uncertainties, some overzealous researchers, representatives of industry and members of the lay and scientific media have implied that gene therapy is already advanced enough for widespread application. It is not.

Arguably, the conceptual part of the gene therapy revolution has indeed occurred. Whenever a new gene is discovered, researchers and nonscientists immediately ask whether it can be used to treat some disorder, even when more traditional approaches might be applied. But the technical part of the revolution—the ability to correct disease—is another story. Investigators have accomplished the requisite first steps: they have shown that transferred genes can be induced to function in the human body, at times for several years. So far, however, no approach has definitively improved the health of a single one of the more than 2,000 patients who have enrolled in gene therapy trials worldwide.

This lack of a convincing therapeutic benefit is sobering. Yet it would be a mistake to doubt gene therapy's powerful future. Remember, the field is young; in the U.S., trials in patients have been carried out for fewer than 10 years. A more realistic interpretation of the unspectacular clinical results thus far is that they reflect researchers' imperfect initial gropings toward a difficult new technology and that the obstacles are more formidable than many of us had expected.

A central challenge, as a federally commissioned critique of the gene-therapy research effort noted in 1995, is perfecting methods for delivering therapeutic genes to cells. Often genes introduced into patients do not reach enough of the appropriate cells or, for reasons that are not always clear, function poorly or shut off after a time. Under those conditions, a gene that could potentially be helpful would have little chance of affecting a disease process.

In this article I will outline some of the most pressing technological stumbling blocks to successful gene transfer and the strategies being considered to cope with those difficulties. I will deal only with therapy affecting somatic cells, the kinds that are neither sperm nor egg. To date, research aimed at human gene therapy has avoided manipulations that would deliberately affect descendants of the treated individuals, perhaps in unintended ways. The need for enlightened public debate over the merits and risks of germ-line therapy has, however, been made more urgent by the recent cloning of an adult sheep [see "What Cloning Means for Gene Therapy," by Steve Mirsky and John Rennie, on page 122].

How Genes and Gene Therapy Work

Anyone who wants to understand the obstacles to gene therapy should first know a bit about what genes do and about how attempts at gene therapy are currently carried out. An individual gene in the human cell is a stretch of DNA that, in most cases, acts as a blueprint for making a specific protein; it spells out the sequence of amino acids composing that protein. All cells in a body carry the same genes in the chromosomes of the nucleus. But neurons, say, behave unlike liver cells because different cells use, or express, distinct subsets of genes and hence make separate sets of proteins (the main functionaries of cells). Put more precisely, each cell copies only selected genes into individual molecules of messenger RNA, which then serve as the templates from which proteins are constructed.

If a particular gene is mutated, its protein product may not be made at all or may work poorly or even too aggressively. In any case, the flaw may disturb vital functions of cells and tissues that use the normal gene product and can thereby cause symptoms of disease.

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to Gene Therapy

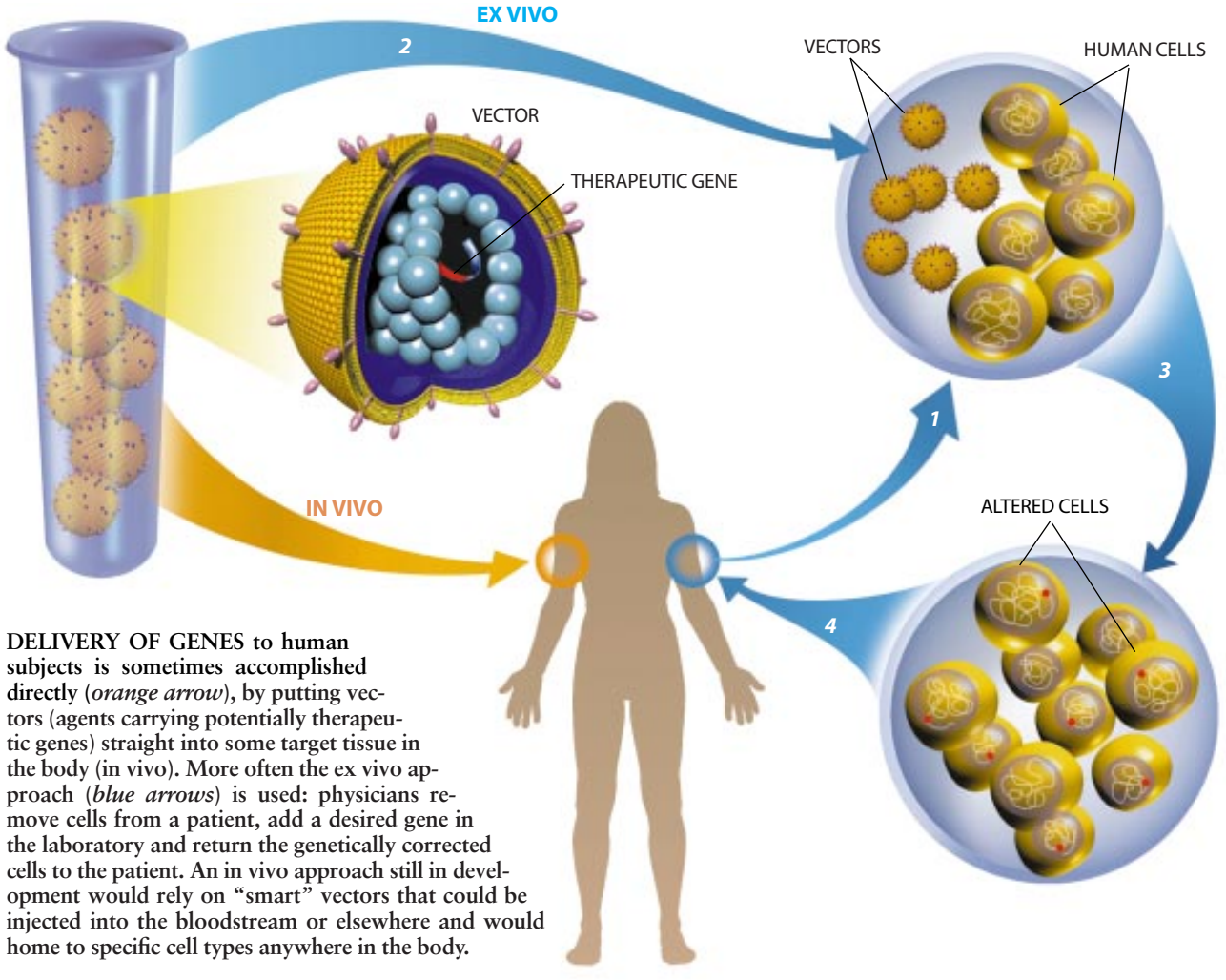
Historically, physicians have treated disorders stemming from inherited genetic mutations not by altering genes but by intervening in the biological events resulting from a mutation. For example, dietary restriction has long been prescribed for phenylketonuria, in which loss of a gene leads to the toxic buildup of the metabolic products of the amino acid phenylalanine. Unfortunately, nongenetic manipulations are usually only partly effective against inherited ills.

In the early 1970s this fact—combined with growing understanding of how genes function and with discovery of the genes underlying many inherited ills—led to the suggestion that better results might be achieved by attacking inborn diseases at their source. Among the genetic diseases that have been studied are cystic fibrosis (which mainly affects the lungs), muscular dystrophy, adenosine deaminase deficiency (which severely impairs immunity), and familial hypercholesterolemia (which leads to the early onset of severe atherosclerosis).

Surprisingly, as time went on, it became clear that even acquired maladies often have a genetic component that can theoretically be a target of a genetic correction strategy. Indeed, quite unexpectedly, more than half of all clinical trials for gene therapy these days aim at cancer, which in most cases is not inherited but results from genetic damage accumulated after birth [see "Gene Therapy for Cancer," by R. Michael Blaese, on page 111]. A number of trials also focus on AIDS, which is caused by the human immunodeficiency virus (HIV).

In principle, a normal gene can be delivered so that it physically takes the place of a flawed version on a chromosome. In practice, such targeted insertion of a gene into a chromosome is not yet achievable in people; fortunately, it often is not required. Most attempts at gene therapy simply add a useful gene into a selected cell type to compensate for a missing or ineffective version or to instill some entirely new property. Many proposed anticancer gene therapies under study take this last tack: they aim to induce cancer cells to make substances that will kill those cells directly, elicit a potent attack by the immune system or eliminate the blood supply that tumors require for growth.

Some gene therapy groups are also devising strategies to compensate for genetic mutations that result in destructive proteins. In one approach, called antisense therapy, short stretches of syn-



DELIVERY OF GENES to human subjects is sometimes accomplished directly (*orange arrow*), by putting vectors (agents carrying potentially therapeutic genes) straight into some target tissue in the body (in vivo). More often the ex vivo approach (*blue arrows*) is used: physicians remove cells from a patient, add a desired gene in the laboratory and return the genetically corrected cells to the patient. An in vivo approach still in development would rely on "smart" vectors that could be injected into the bloodstream or elsewhere and would home to specific cell types anywhere in the body.

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thetic DNA act on messenger RNA transcripts of mutant genes, preventing the transcripts from being translated into abnormal proteins. Related tactics deploy small RNA molecules called ribozymes to degrade messenger RNA copied from aberrant genes. A rather different plan provides a gene for a protein, called an intracellular antibody, that can block the activity of the mutant protein itself. Some therapeutic strategies rely on the design of hybrids of DNA and RNA that might direct the repair of mutant genes.

Genes are currently provided to patients in two basic ways. In both cases, the genes are usually first put into transporters, or vectors, able to deposit foreign genes into cells. In the more common method, scientists remove cells from a selected tissue in a patient, expose them to gene-transfer vectors in the laboratory (ex vivo) and then return the genetically corrected cells to the individual. Other times researchers introduce the vectors directly into the body (in vivo), generally into the tissue to be treated. Our ultimate goal, of course, is to deliver vectors into the bloodstream or other sites and to have them act like homing pigeons, finding their own way to the desired cells—say, to organs that are hard to reach or to hidden cancer deposits. No such targeted carriers are yet ready for testing in patients, but work toward that end is advancing quickly.

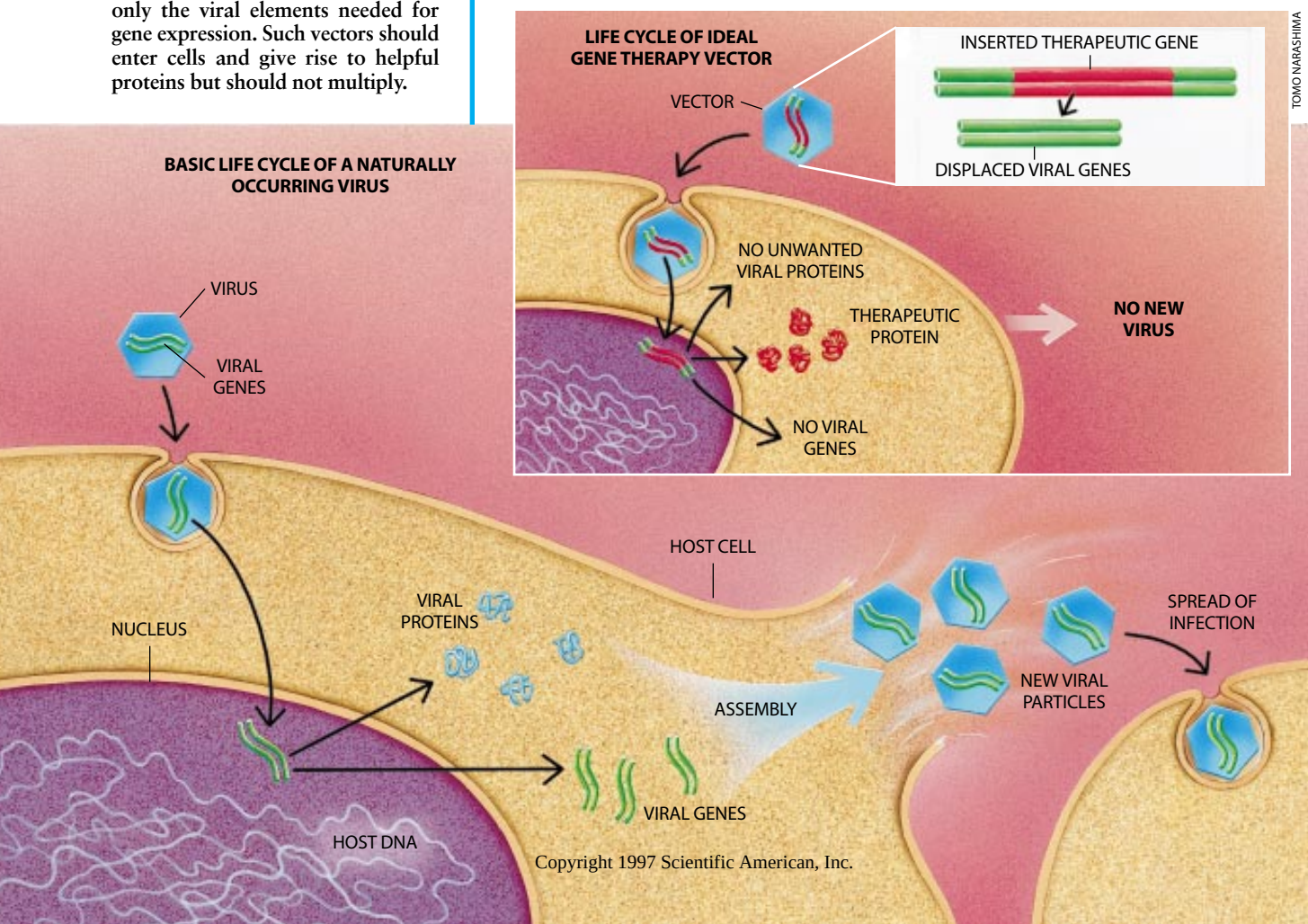
In the body, certain genes will be helpful only if their expression is regulated tightly: in other words, they must give rise to just the right amount of protein at the right times. Biologists have yet to achieve such precise control over foreign genes put into the body. For many gene therapy applications, however, exquisite regulation will not be essential. Nor will it always be necessary to put genes into the cells that are in need of fixing. Sometimes more accessible cell types (say, muscle or skin) might be turned into protein factories; these factories would release proteins needed by nearby cells or might secrete proteins into the bloodstream for transport to distant sites.

Retrovirus Vectors: Flaws and Fixes

The key to success for any gene therapy strategy is having a vector able to serve as a safe and efficient gene delivery vehicle. From the start, viruses—which are little more than self-replicating genes wrapped in protein coats—have drawn the most attention as potential vectors. They are attractive because evolution has designed them specifically to enter cells and express their genes there. Further, scientists can substitute one or more potentially therapeutic genes for genes involved in viral replication and virulence. In theory, then, an altered, tamed virus should transfer helpful genes to cells but should not multiply or produce disease.

The viruses that have been examined most extensively are retroviruses, which splice copies of their genes permanently into the chromosomes of the cells they invade. Such an integrated gene is cop-

NATURALLY OCCURRING virus (*bottom panel*) releases its genetic material into cells. Whether or not the genes become integrated into the DNA of the infected cell, they soon direct the synthesis of new viral particles that can injure the cell and infect others. To convert a wild-type virus into a safe gene therapy vector, scientists replace viral genes with ones specifying therapeutic proteins (*top panel*), while ideally leaving only the viral elements needed for gene expression. Such vectors should enter cells and give rise to helpful proteins but should not multiply.



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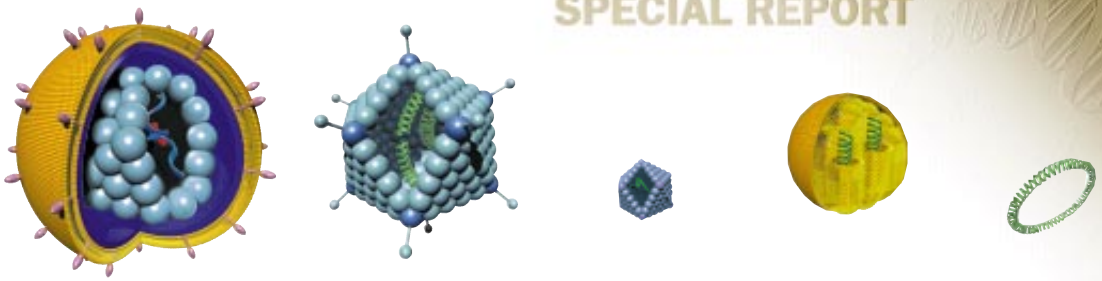

SLIM FILMS

	Retroviruses	Adenoviruses	Adeno-Associated Viruses	Liposomes	"Naked" DNA
Some Potential Advantages	Integrate genes into host chromosomes, offering chance for long-term stability	Most do not cause serious disease; large capacity for foreign genes	Integrate genes into host chromosomes; cause no known human diseases	Have no viral genes, so do not cause disease	Same as for liposomes; expected to be useful for vaccination
Some Drawbacks of Existing Vectors	Genes integrate randomly, so might disrupt host genes; many infect only dividing cells	Genes may function transiently, owing to lack of integration or to attack by the immune system	Small capacity for foreign genes	Less efficient than viruses at transferring genes to cells	Inefficient at gene transfer; unstable in most tissues of the body

VECTORS UNDER STUDY as gene delivery vehicles include viral and nonviral carriers, only some of which are listed. Each vector type has its own set of advantages and disadvantages, and all are being modified rapidly to improve their effectiveness in patients.

ied and passed to all future generations of those cells. In contrast, many other kinds of viruses do not integrate their genetic material into a host's chromosomes. Their genes generally function in the body more transiently—in part because the genes do not replicate when recipient cells divide.

One group of ideal target cells for retrovirus vectors consists of so-called stem cells, which persist indefinitely and also produce more specialized descendant cells. Blood-forming stem cells, for example, give rise to every other type of blood cell (red cells, white cells of the immune system, and so on) and reconstitute the blood as needed; they also make more copies of themselves. At the moment, however, it is extremely difficult to identify human stem cells and modify them in safe, predictable ways.

Despite the appeal of retroviruses, which were first introduced as vectors in the early 1980s, they pose several challenges. They are promiscuous, depositing their genes into the chromosomes of a variety of cell types. Such lack of fine specificity for host cells can militate against direct delivery of the vectors into the body; uptake by cells that were not intended to receive the foreign gene could reduce transfer to the targeted population and might have unwanted physiological effects. Conversely, the retroviruses now receiving the most study fail to transfer genes to cell types that cannot divide or that do so only rarely (such as mature neurons and skeletal muscle cells). Current retrovirus vectors reach chromosomes only when the membrane surrounding the nucleus of the host cell dissolves, an event that occurs solely during cell division.

Also problematic is the fact that retroviruses splice their DNA into host chromosomes randomly, instead of into predictable sites. Depending on where inserted genes land, they might disrupt an essential gene or alter genes in ways that favor cancer development. Tumors would probably result only rarely, but even the remote chance of increasing cancer risk must be taken seriously.

Researchers have made good progress recently in confronting the shortcomings of retroviruses as gene delivery vehicles. For instance, to increase specificity and thus enable retrovirus vectors to direct themselves to particular cells in the body, researchers are altering the viral envelope (the outermost surface). Like other viruses, retroviruses deposit their genetic cargo into a cell only if proteins projecting from their surface find specific mates, or receptors, on the cell. Binding of the viral proteins to the cellular receptors enables a retrovirus to fuse its envelope with the cell membrane and to release viral genes and proteins into the cell's interior. To make retroviruses more selective about the cells they invade, investigators are learning how to replace or modify natural envelope proteins or to add new proteins or parts of proteins to existing envelopes.

In an experiment showing that the replacement strategy is feasible, Jiing-Kuan Yee of the University of California at San Diego, with my laboratory at that university, substituted the envelope protein of the mouse leukemia virus with that of the human vesicular stomatitis virus. (The mouse virus, which causes no known disease in people, is the retrovirus that has been evaluated most extensively as a gene therapy vector.) The altered mouse retrovirus then infected cells bearing receptors for the human vesicular stomatitis virus instead of cells with receptors for the mouse virus.

Work on modifying existing envelope proteins is also proceeding well. Yuet Wai Kan and his colleagues at the University of California at San Francisco have recently linked a protein hormone to the envelope protein of the mouse leukemia virus. This hormone enabled the virus to infect human cells that displayed the receptor for that hormone.

Prospects for generating retrovirus vectors able to insert therapeutic genes into the chromosomes of nondividing cells are looking up as well. Inder M. Verma, Didier Trono and their colleagues at the Salk Institute for Biological Studies in San Diego have capitalized on the ability of HIV, a retrovirus, to deposit its genes into the nucleus of nondividing brain cells without waiting for the nuclear wrapping to dissolve during cell division.

The team removed genes that would allow HIV to reproduce and substituted a gene coding for a protein that was easy to trace. This vector then brought the traceable gene into nonreplicating cells, not only when the vector was mixed with cells in culture but also when it was injected directly into the brains of rats. HIV itself might one day prove to be a

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useful vector if worry that the disabled vectors might somehow become pathogenic can be allayed. Another tactic would transfer certain of HIV's useful genes—particularly those coding for the proteins that transport genes to the nucleus—into retroviruses that do not cause human disease.

Finally, efforts are under way to ensure that retrovirus vectors will place genes less randomly into human chromosomes. Workers toiling in this taxing realm have recently been assisted by new understanding of how genes integrate into predictable sites in the DNA of other organisms, such as yeast.

Pros and Cons of Other Virus Vectors

Vectors derived from viruses other than retroviruses present their own sets of advantages and disadvantages. Those based on the ubiquitous human adenoviruses have gained the most popularity as alternatives to retroviruses in part because they are quite safe; the naturally occurring forms typically cause nothing more serious than chest colds in otherwise healthy people. Moreover, they infect human cells readily and, initially at least, tend to yield high levels of the therapeutic protein.

Adenovirus vectors dispatch genes to the nucleus but apparently do not insert them into chromosomes. This feature avoids the possibility of disturbing vital cellular genes or abetting cancer formation, but, regrettably for some applications, the genes are often effective only temporarily. Because the DNA eventually disappears, treatments for chronic conditions, such as cystic fibrosis, would have to be repeated periodically (every so many months or years). In some situations, though—say, when a protein is needed only temporarily to induce an immune response to cancer or to a pathogen—short-term expression of a foreign gene may be preferable. Another drawback, shared with retroviruses, is lack of specificity for target cells. As is true for retroviruses, however, scientists are rapidly devising ways to target adenovirus vectors to tissues of the researchers' choosing.

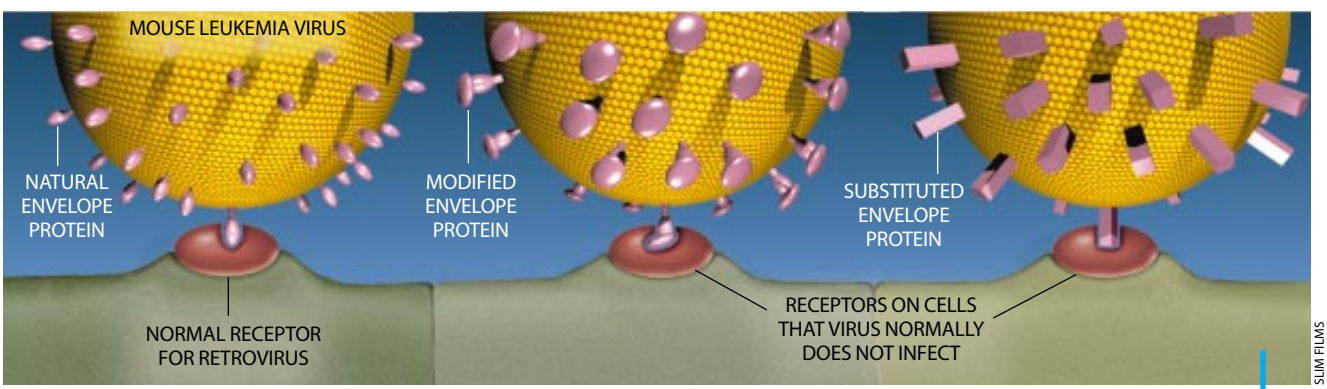
At the moment the more serious stumbling block to use of adenovirus vectors in patients is the body's strong immune response against them. During an initial round of treatment, such vectors might infect the appropriate cells and generate high amounts of the desired proteins. But soon host defenses come into play, killing the altered cells and inactivating their new genes. Further, once the immune system is alerted to the viruses, it eliminates them quickly if they are delivered a second time. Such responses probably have contributed to a shutdown of gene expression in a number of adenovirus gene-transfer studies in patients. Advancing understanding of the shortcomings of adenoviruses is now leading to a new generation of vectors that should reduce defensive interference. These enhancements have been achieved in part by removing or mutating the adenovirus genes most responsible for eliciting immune attacks.

Several other viruses are being explored as vectors as well—among them, adeno-associated viruses, herpesviruses, alphaviruses and poxviruses. None is perfected yet, but each is likely to have its own therapeutic niche. For example, adeno-associated viruses appeal because they cause no known diseases in people. What is more, in their natural form, they integrate their genes into human chromosomes. They are likely to be useful for some applications that now depend on retroviruses, but they are smaller and so may not be able to accommodate large genes. Herpesviruses, in contrast, do not integrate their genes into the DNA of their hosts. But they are attracted to neurons, some of which retain the viruses in a more or less innocuous state for the lifetime of the affected person. Herpesviruses therefore have potential as vectors for therapy aimed at neurological disorders [see "Gene Therapy for the Nervous System," by Dora Y. Ho and Robert M. Sapolsky, on page 116].

Perfecting Nonviral Delivery Systems

As a group, vectors produced from viruses continue to show great promise, although researchers must always work to ensure that the viruses will not change in ways that will enable them to cause disease. This consideration and others have encouraged development of various nonviral methods for therapeutic gene transfer. In common with viruses, these synthetic agents generally consist of DNA combined with molecules that can condense the DNA, deliver it to cells and protect it from degradation inside cells. And, like virus vectors, they will almost certainly be used in medical practice eventually but are still in need of refinement. The genes transferred by nonviral vectors become integrated into the chromosomes of recipient cells in the laboratory but have done so only rarely after delivery into the body. Whether lack of integration will be an advantage or disadvantage depends, as I have noted, on the particular goal of therapy.

Liposomes, which are small fatty (lip-



VIRUSES MUST BIND to particular surface molecules, or receptors, to gain entry into cells. The naturally occurring mouse leukemia virus normally binds through its envelope protein to a receptor found on many cell types (*left*). By altering the envelope protein to include new components (*center*) or replacing it with other proteins (*right*), scientists have directed the virus to cells it would otherwise ignore. Similar tactics can target other vectors to selected cell types.

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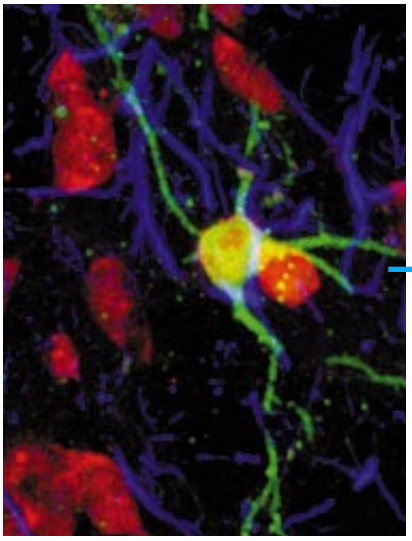

U. BLÖMER, F. H. GAGE AND INDER M. VERMA

HUMAN BRAIN CELL took up an HIV-based vector containing a gene for a traceable protein (*shown in yellow*). This success implies that disarmed forms of HIV, a retrovirus, can potentially be used to deliver therapeutic genes into neurons, which, being unable to divide, are resistant to traditional retrovirus vectors.

id) spheres, have been studied almost as long as retrovirus vectors. These synthetic bubbles can be designed to harbor a plasmid—a stable loop of DNA derived from bacterial viruses known as phages—in which original genes have been replaced by those intended to be therapeutic. Gene transfer by liposomes (or "lipoplexes," as current versions are increasingly called) is much less efficient than virus-mediated transfer but has advanced enough for these vectors to enter clinical trials for such diseases as cancer and cystic fibrosis. Meanwhile alterations in the chemical composition of liposomes are addressing the efficiency problem and are beginning to produce vectors that mimic viruses in their targetability and prowess at gene transfer [see "Nonviral Strategies for Gene Therapy," by Philip L. Felgner, on page 102].

Newer kinds of vectors sheathe DNA in nonlipid coats. These coats include amino acid polymers and other substances intended to target therapeutic genes to the proper cells in the body and to protect the genes from being broken down by cellular enzymes. These complexes—studied intensively by Max Birnstiel and Matt Cotten of the Institute of Molecular Pathology in Vienna and by David T. Curiel of the University of Alabama at Birmingham—have performed well in cell culture experiments. They are now being further modified and are undergoing testing in animal studies and in patients.

Some scientists are also exploring injecting so-called naked DNA—without a lipid wrapping—into patients. Initial results suggest that the naked-DNA strategy has exciting potential for immunization against infectious diseases, and even against certain kinds of cancer.

Alternatives to plasmids are being pursued as well. Notably, workers are learning to construct miniature chromosomes, or artificial human chromosomes, into which therapeutic genes can be spliced. These constructs will contain the minimum amount of genetic material needed to avoid degradation in the nucleus and loss during cell division. They will also incorporate elements that enable the artificial chromosomes to copy themselves accurately (and only once) each time a cell divides, just as ordinary chromosomes do.

Looking Ahead

In the future, as now, investigators will choose one or another gene delivery method on the basis of their therapeutic goal. If a patient inherited a genetic defect and needs a continuing supply of the normal gene product throughout life, a vector that can integrate the therapeutic gene into the patient's chromosomes, where it will stay in perpetuity, might be best. Then a retrovirus or adeno-associated virus may be selected. If only short-term activity of a gene is needed, such as to arouse the immune system against cancer cells or an infectious agent, nonintegrating delivery vehicles, such as adenovirus vectors, liposomes or even naked DNA may be more suitable.

But the tools that finally come into common use almost certainly will not be the prototypes being tested today. And because no single technique will be perfect for every disorder, there will be many choices. The ideal gene transfer systems of the future will combine the best features of different vectors. Each system will be tailored to the specific tissue or cell type requiring modification, to the needed duration of gene action and to the desired physiological effect of the gene product. Scientists will also want to develop ways to alter the level of gene expression at will and to shut off or completely remove introduced genes if therapy goes awry.

Even when these gene delivery vectors are perfected, the challenges will not end. For instance, cells often modify foreign genes in ways that ultimately cause the genes to stop working. This activity is being addressed vigorously but is not yet solved. In addition, we still have few clues as to how the defensive systems of patients will respond when they encounter a seemingly foreign protein from a therapeutic gene. To prevent an inactivating immune reaction, physicians might have to treat some patients with antirejection drugs or try to induce immune tolerance to the encoded protein by carrying out gene therapy very early in a patient's life (before the immune system is fully competent).

Although I have dwelled on certain technical challenges to gene therapy, I am nonetheless highly optimistic that it will soon begin to prove helpful for some diseases. Our tools are improving rapidly, and some of the burgeoning clinical trials clearly are on the verge of demonstrating real merit in ameliorating disease, even with today's imperfect techniques. Notably, it seems likely that gene-based immunotherapies for some malignancies, such as neuroblastoma and melanoma, will be shown convincingly in the next few years to slow the development of further disease and to force existing tumors to regress; they should then become helpful additions to existing therapies. But I must emphasize that it is only through insistence on rigorous science, carefully designed clinical studies and less exaggerated reporting of results that researchers can ensure the timely, ethical and effective flowering of this exciting new field of medicine. SA

The Author

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