Techniques of gene therapy

Gene therapy involves isolating a gene, putting it into cells where it will be used, and ensuring that the inserted gene functions in the new cells in a way that does not harm the patient.

Genes are copied and passed on by DNA replication

Genetic information is transmitted from one cell to its progeny by duplication, or replication, of its DNA. When a cell divides, it copies its DNA and distributes a copy to each of two offspring cells. A new therapeutic gene introduced into a cell in the laboratory can thus be reproduced through the process of cell division when the cell is placed into a patient and proliferates.

Many breakthroughs in molecular genetics have come from discoveries about how DNA replicates, how it can be specifically cut and reassembled, and how to re-introduce the altered DNA back into cells in such a way that its expression, or translation into protein, can be controlled (Judson, 1980). Many of the techniques for splicing and controlling the expression of genes were first discovered between 1970 and 1974, using some of the same techniques that led to the development of recombinant DNA (Watson, 1984).

Isolation and cloning of the normal gene

The usual first step in approaching gene therapy is identification of the abnormal gene. (This step can be skipped when the corresponding normal genes are already available, as was the case for sickle cell disease.) Once the abnormal gene has been found, then copies of the corresponding normal gene must be isolated and copied. There are several ways to identify abnormal genes. These involve analysis of patterns of inheritance of a disease, study of the metabolism of patients who have the disease, and analysis of the genes of those who have the disease, Identification of the gene that causes a particular disease requires hundreds of experiments, luck, and extensive resort to recombinant DNA technology.

Once the gene that causes a disease has been identified, the corresponding normal gene must be isolated, unless it is already available because it has been studied for some other purpose. Using an abnormal gene to find its normal counterpart is usually done by exploiting the extensive similarity between the sequences of the normal and defective genes; they rarely differ greatly in overall sequence (although the *functional* results are quite different, or there would be no disease).

After the normal gene has been identified and isolated, then it must be copied. The process of making multiple copies of a single gene is called cloning.[7] Cloning involves combining the gene of interest with DNA sequences that allow it to be copied in lower organisms—usually bacteria or yeasts. The DNA containing the gene of interest is then inserted into bacteria or yeast (or, more recently, into some types of mammalian cells growing in culture). The DNA is copied as the cells proliferate. The numerous copies of DNA are then purified from other cell components, and the gene of interest can be cut away from unwanted DNA sequences, One now has millions or billions of copies of a single gene.

These copies are then combined with DNA that is suitable for insertion into human cells.

Insertion into human cells

The DNA that contains the normal gene can be administered to human cells in several ways: using viruses, physically injecting it, treating the DNA chemically so that cells take it up, treating the cells so that they are induced to take in the DNA, or by fusing the cells with membranes that

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[7]For details of cloning, see the Technical Notes. (honing a gene should not be confused with cloning an organism, The term "cloning" refers to reproduction without mating: in the case of a gene or DNA sequence, this merely means making copies of the relevant stretch of DNA. Cloning a whole organism, in contrast, involves copying *all* of a cell's DNA so that a completely new organism that shares all its genes with the original is produced. The techniques for cloning genes are completely' different frum those for cloning organisms. Cloning an individual human would not help in the prevention of genetic disease, and is not d irectly related to the questions raised by human gene therapy

contain the DNA. In the distant future, designed viruses or genetic elements may be used to transfer genes to specifically targeted human cells. At present, however, more primitive methods are used.

VIRUSES

Viruses are small packages of genetic information in the form of DNA or RNA that enter cells and either insert their information into that of the infected cell or duplicate themselves using the cell's biochemical machinery. Viruses are usually covered with a coat of protein or membrane, but their most distinguishing characteristic is the genetic information that they contain. Some viruses promise to be practical for gene transfer because they are relatively simple and controllable, and contain sequences that permit insertion of genes into the host's DNA. Modified viruses are the most likely candidates for gene therapy in the long run, because they are highly efficient, can affect many cells, and are relatively easy to manipulate in the laboratory (Rawls, 1984).

Several scientists are developing viruses that would not injure cells, would not propagate uncontrollably, and would enter only target cells (Anderson, 1984). Such viruses have been successfully used to insert new genes into blood-forming cells of mice with relatively high efficiency (A. D. Miller, 1984; Williams, 1984). At some point, scientists may be able to design a virus that could be used for cloning as well as delivery, saving yet more steps.

MICROINJECTION

Microinjection of DNA involves putting the DNA one wants to insert into a solution that can be pushed directly into individual cells through extremely small needles made of glass. The technique is highly reliable, in that a high proportion of cells that receive genes express them (Capecchi, 1981), but limited by the number of cells that can be directly injected. Investigators can inject hundreds or thousands of cells, at most, for a given experiment, compared to billions that can be treated using viruses or chemical treatments. Microinjection has been the method of choice for experiments involving gene transfer in mice, because of its reliability, but its applicability to humans is questionable because it is not *com-pletely* reliable, and often results in cell death (an

alternative that is ethically unacceptable for human experiments) (Anderson, 1984).

CHEMICAL AND PHYSICAL METHODS

Some early experiments in gene transfer employed mixing DNA with chemicals and subsequently applying the DNA to a large number of cells. Most cells would pick up the DNA, and some would insert it into their own DNA, and, in some cases, express it. The usual chemical treatment employed calcium phosphate with relatively large amounts of the desired DNA. The most common physical method involved "electroporation") in which electrical treatment of the cells induced uptake of DNA and other constituents from the fluids bathing the cells.

Chemical and physical treatments have the advantage of not requiring a vector to cause insertion, but have two major disadvantages. First, the DNA is only stably incorporated into a small proportion of cells, usually only one in ten thousand to one in a million. (This small proportion nevertheless usually represents hundreds or thousands of times more cells than could be directly injected.) This feature requires that cells that take up and incorporate the desired DNA must somehow be separated from cells that do not, and there must be a very large number of cells to treat in the first place. Second, the DNA usually inserts at random into the cell's genome, and often in multiple copies. DATA insertion following chemical and physical insertion methods is thus relatively uncontrolled and unpredictable (Anderson, 1984).

MEMBRANE FUSION

The final way to get DNA into cells involves putting it inside of membranes that can then be fused with the outer membrane of target cells, allowing the contents to spill into the cells. The membrane sacs, called liposomes, can be made of artificially constructed lipid mixtures or derived from specially treated cells such as red blood cells or bacteria. The advantage of cell fusion is that it is relatively simple, and large numbers of cells can be treated. It is, like chemical treatment, unreliable and nonspecific at delivery. The technique might prove useful in the future, however, if membranes are constructed that target specific cells with highly reliable delivery.