Technical note 2

Genetic engineering techniques: cloning and vectors

There are several techniques of genetic engineering that are fundamental to efforts at human gene therapy. The most basic of these is cloning, or making multiple copies of a specific single gene. Once a gene has been cloned, it may be made in as many copies as desired (and thus easily studied), or moved from place to place through the use of specialized agents known as *vectors.* **There are several types of vectors; viruses** (bacteriophages, or phages), plasmids, or transposable elements.

Cloning involves several different steps. First the gene of interest must be identified; if it exists in only one copy per haploid genome (as with single gene, or Mendelian defects) then that one copy must be selected from perhaps as many as 100,000 other genes—a formidable task. As daunting as this problem is, however, there are some elegantly simple solutions.

The most favored of these is to identify the messenger RNA **used by ribosomes to assemble the protein of interest. Although mRNA is short-lived and notoriously delicate, this can often be done. From this mRNA a complementary DNA (cDNA) molecule can be synthesised, labeled with a tracer (radioactivity or a dye) and then used as a probe to identify the gene.**

If an mRNA cannot be identified, then it is possible to start with the protein product itself. This protein can be analysed and its amino-acid sequence determined. The amino-acid sequence can be used to deduce the nucleotide base sequence of the gene encoding the protein. A DNA molecule can then be synthesised and used as a probe to locate the relevant gene with its associated control sequences.

Once identified and located, special enzymes (restriction endonucleases or restriction enzymes) make it possible to isolate the entire intact gene and insert it into the appropriate vector. Plasmid (circular DNA molecules found in the cytoplasm of bacteria such as E. coli) or virus (phage) vectors make it possible to produce enormous numbers of copies of the gene of interest.

Plasmids.—In addition to the genetic information required for the existence of a simple bacterium, which is contained in its own genes, on its own chromosome, many bacteria also carry in their cytoplasm small circular molecules of DNA that replicate on their own. These are called plasmids and any number of them, from none to hundreds, can be found in individual bacteria. They are transmitted to progeny cells with the cytoplasm (hence the name) as the parent cell divides. The genetic information encoded in plasmid DNA often determines specialized characteristics of the bacteria, such as resistance to antibiotics. Their small size and simplicity have made them handy tools for the precise duplication and delivery of genetic information.

Some plasmids can be injected into the cells of higher animals where they replicate or integrate and pass from cell to cell as the cells divide. They are widely used in copying and multiplying genes because the special characteristics (e.g., antibiotic resistance) are easily engineered. These can he used to selectively promote the growth of cells that contain the plasmid, and thus also the desired genes.

Phage.—Phage (or bacteriophage) are viruses that infect bacteria, commandeer the bacterial machinery, and use it to translate the genetic information contained in the phage into phage products. Normally this leads to an infected bacterium producing phage offspring, but if the genes for building phage are replaced with a gene of interest to researchers, then the infected bacterium will produce copies of that gene instead. Phage can thus be used in much the same way that plasmids can, to make multiple copies of a given gene. The choice between using phage or plasmids as cloning vectors is based on the ease with which genes of different sizes or composition can be cloned with the different methods, and the advantages of different screening methods that can be used with the different vectors.

Transposable Elements.—Transposable elements (transposons) are relatively small molecules of DNA that can insert themselves into the genome of the host organism and move from site to site within it. Their origin is uncertain, but they seem closely related to some viruses. They have been called infectious or parasitic DNA and behave in some ways very much like infectious agents.

Genes of interest can be inserted into a transposable element, and thus be incorporated into the host genome along with the transposable element at specific sites. Although there are no transposable elements presently in use in human cells, they have been successfully used to "treat" genetic defects in fruit flies of the genus *Drosophila* (Rubin and Spradling, 1982; Spradling and Rubin, 1983). A mammalian equivalent to a transposable element would be a welcome discovery, as it could be used to control points of insertion into a human genome very precisely. Some viruses being considered as vectors for human gene therapy have similarities to transposable elements, including precise insertion sites.