Gene therapy : the Wellcome Trust lecture 1993 / Marcus Pembrey.

THE WELLCOME CENTRE
FOR MEDICAL SCIENCE

Association for Science Education

The Wellcome Trust Lecture 1993

"Gene Therapy"

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Mothercare Professor of Clinical Genetics
Institute of Child Health, London

THE WELLCOME TRUST

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The Wellcome Lecture

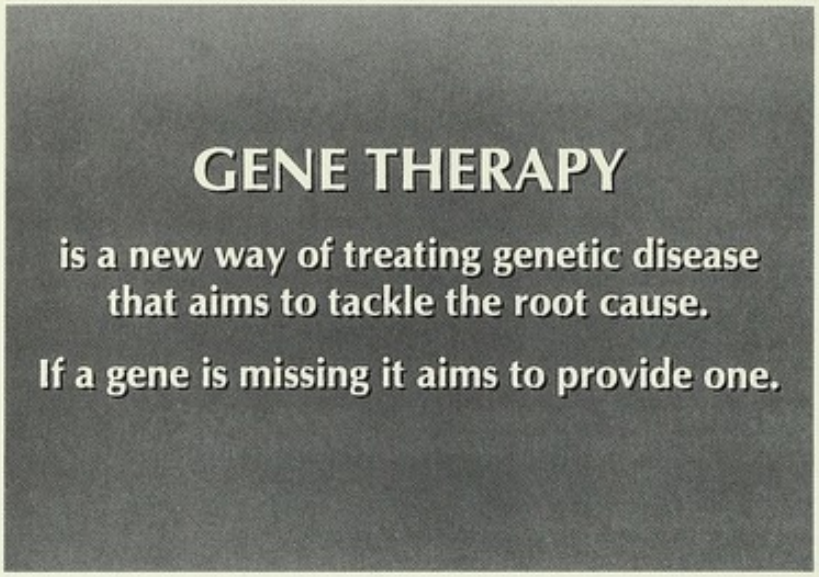
This Wellcome Lecture coincides with the launch of the Wellcome Centre for Medical Science. It is hoped that it will be the first of many in collaboration with the Association for Science Education and will contribute to the professional exchange between the Centre and those charged with teaching science in schools and colleges.

The Wellcome Lecturer

Professor Marcus Pembrey is Head of the Mothercare Unit of Clinical Genetics and Fetal Medcine, and Vice-Dean at the Institute of Child Health, the Medical School of The Hospital for Sick Children, Great Ormond Street. The Institute of Child Health has a well-established research programme aimed at providing gene therapy for inherited deficiencies of the immune system. Professor Pembrey holds many additional appointments including Consultant Advisor in Genetics to the Department of Health and Member of the Standing Committee of the British Paediatric Association on Medical Ethics. He is also Chairman of the Trustees of the Progress Educational Trust, an organisation devoted to education about human reproduction, the embryo and the causes of congenital disorders.

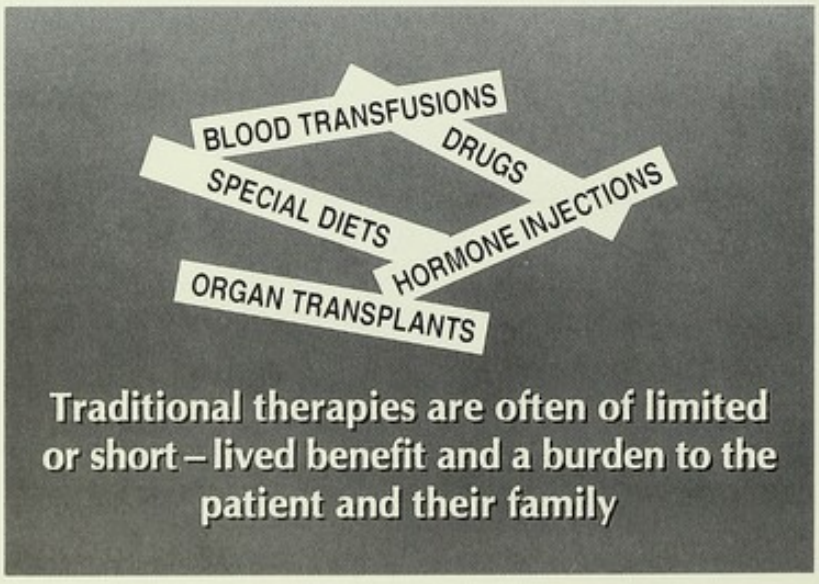
This Booklet

This booklet gives an outline of the lecture on gene therapy, with most of the illustrations used in the lecture included as diagrams. Full-sized black-and-white copies of these illustrations will be available from the Wellcome Centre for Medical Science.



The Burden of Genetic Disease

There are some 4,000 known, simply-inherited genetic disorders and, in aggregate, they are the cause of much suffering in 1–2% of the population. The commoner inherited disorders include cystic fibrosis, sickle cell disease, the thalassaemias, fragile X syndrome, Duchenne muscular dystrophy, haemophilia A, Huntington's disease, neurofibromatosis, and adult polycystic kidney disease.



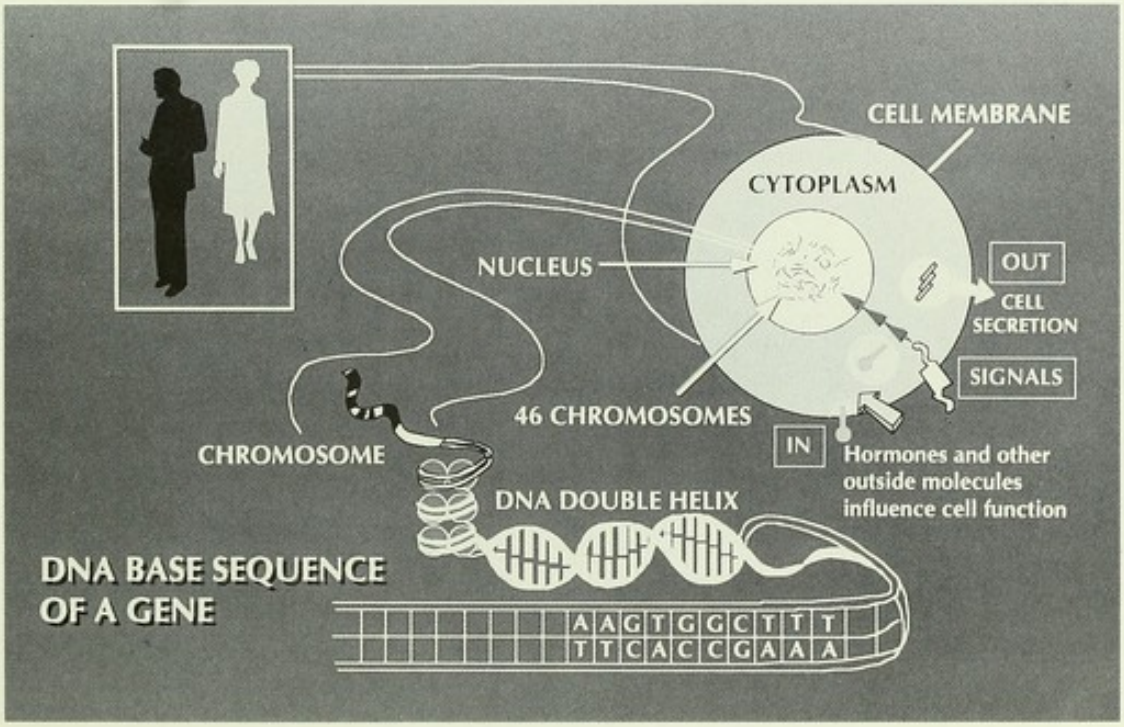
Hopes and Fears

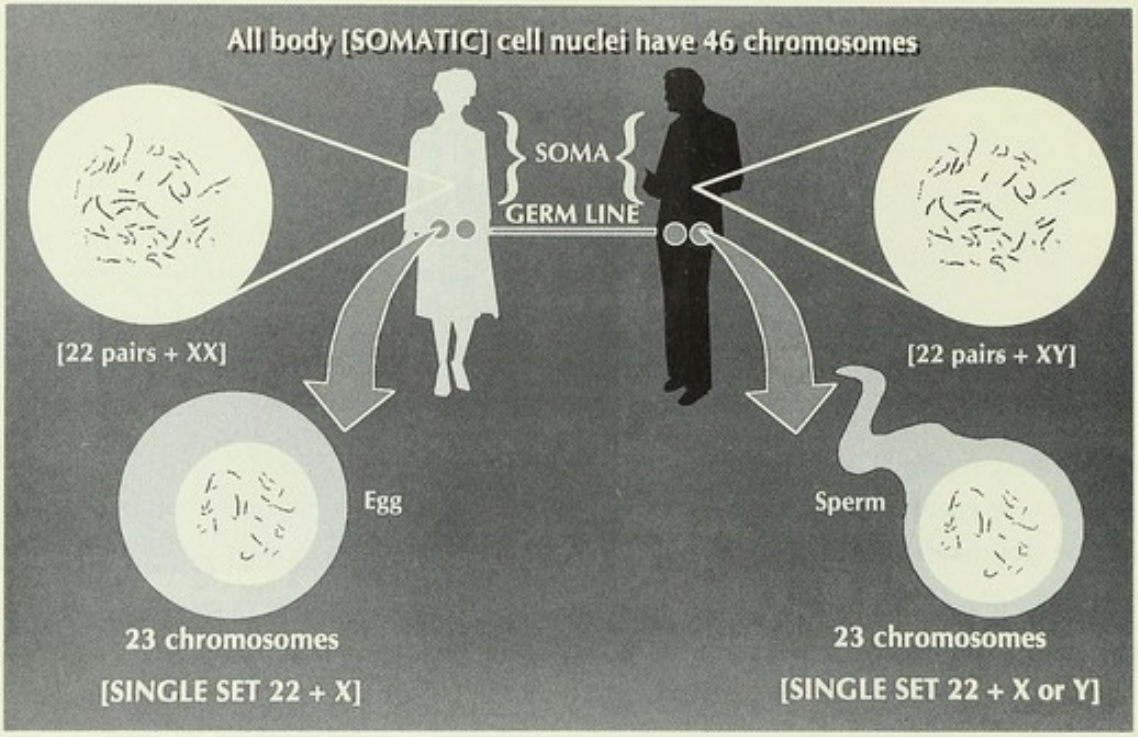
Whilst it is widely recognised that improvements in the treatment of genetic disease are desperately needed, many fear that 'tinkering' with our genes and the use of viruses as vehicles, or vectors, to deliver new genes to the body might be dangerous in some way for the population at large.

We 'fight' germs, but such language seems inappropriate for genetic disease because our genes, whether faulty or not, are an integral part of our makeup. Informed public debate needs an informed public. When they ask about the nature of genes, how they work and why they fail, and how faulty genes might be put right, the appropriate information and explanations must be available. Simple analogies can help the teacher explain molecular genetic concepts and some examples are included in this booklet.

Genes – How they work and sometimes fail

Cells play a central role in the development and functioning of the body. In the end, gene therapy is concerned with correcting the function of particular cells in the body.

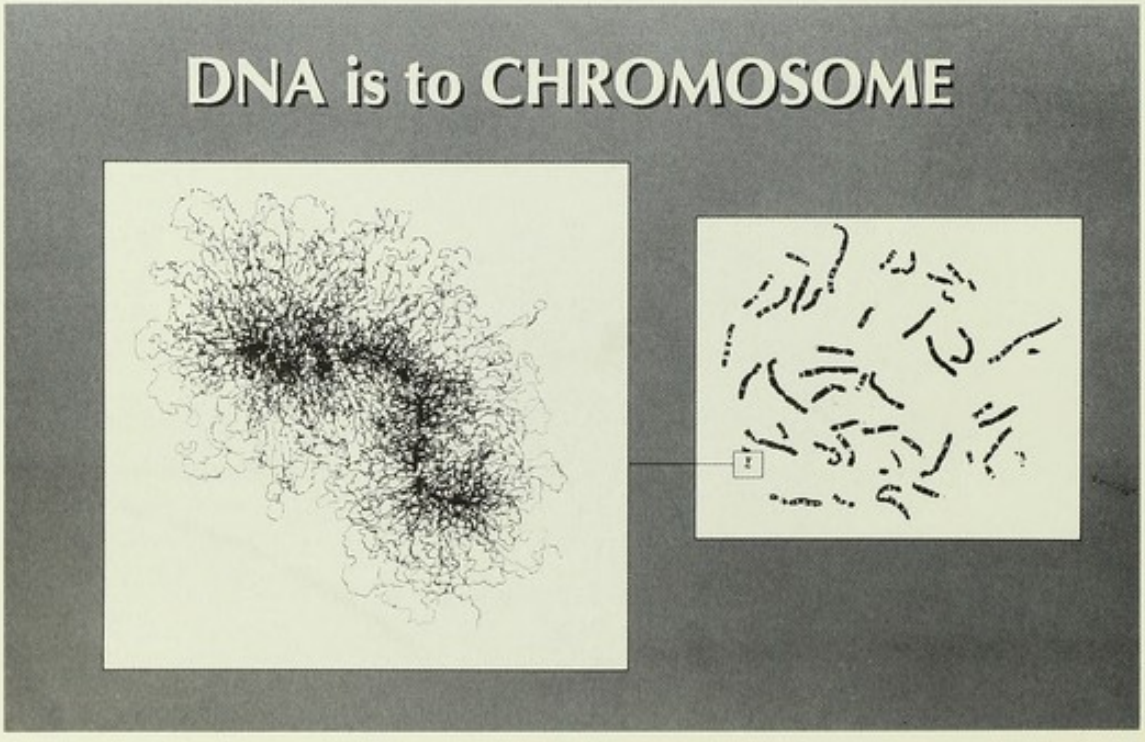


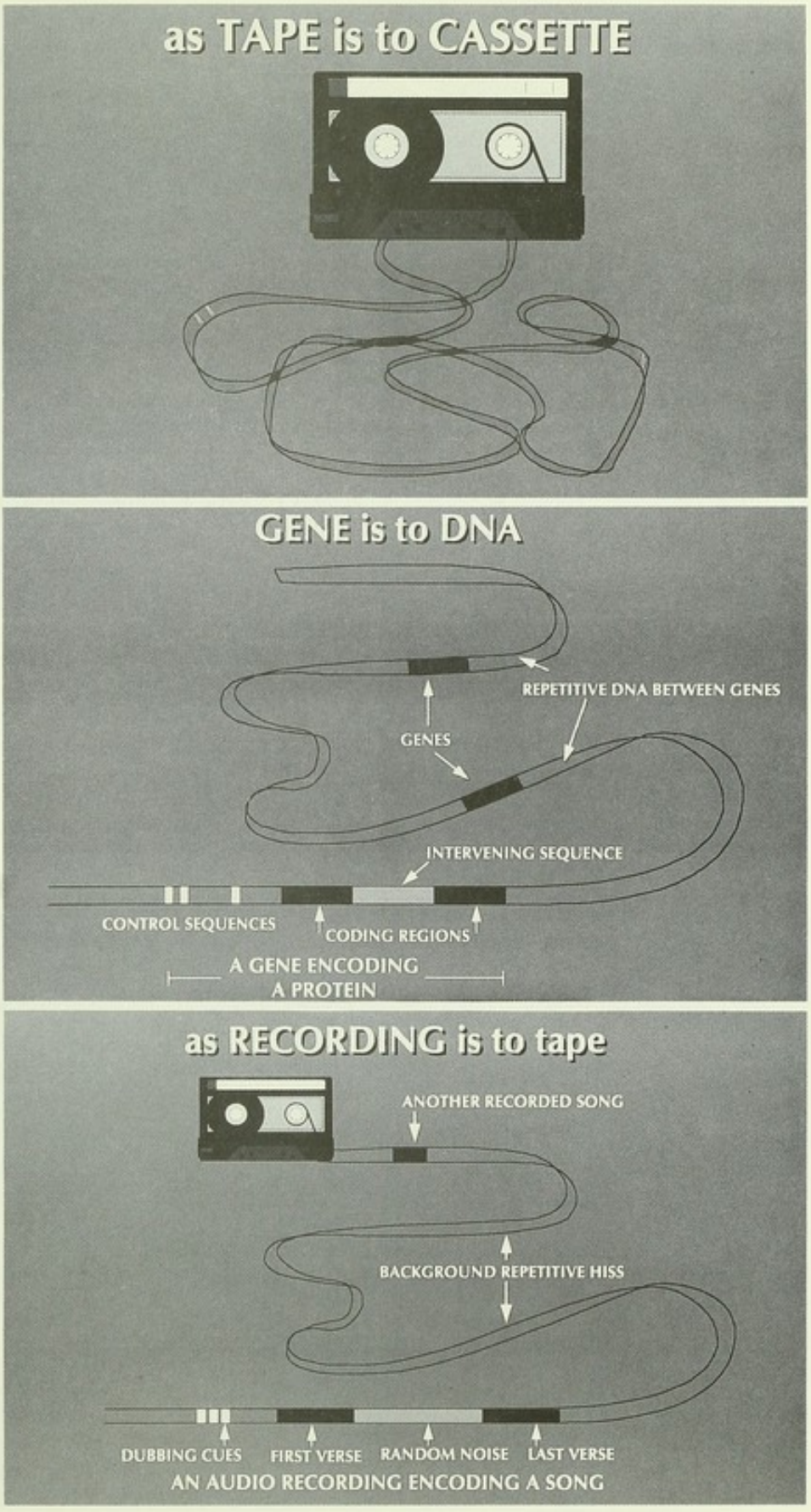


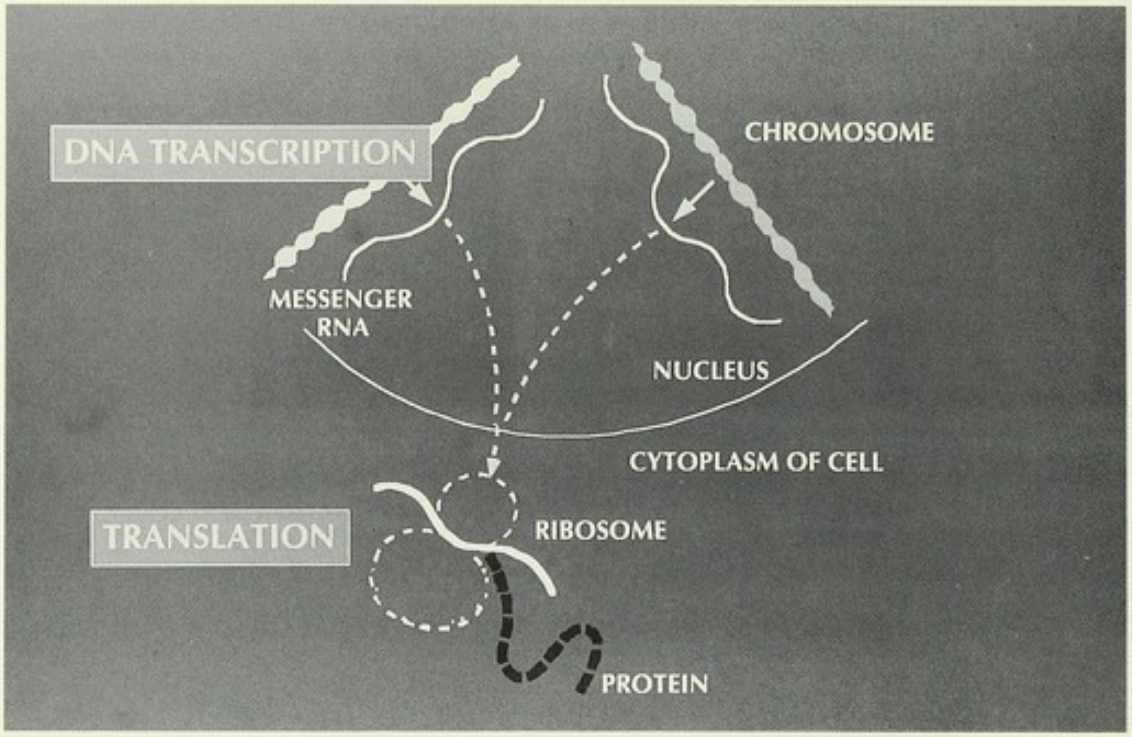
The key structures in inheritance are the chromosomes.

Genetic changes in the somatic cells (as naturally occur in most cancers) are not passed on to the next generation. It is the germline cells culminating in eggs and sperm that carry the genetic information from one generation to the next.

The ordinary audiotape cassette can be used to explain molecular genetic concepts.



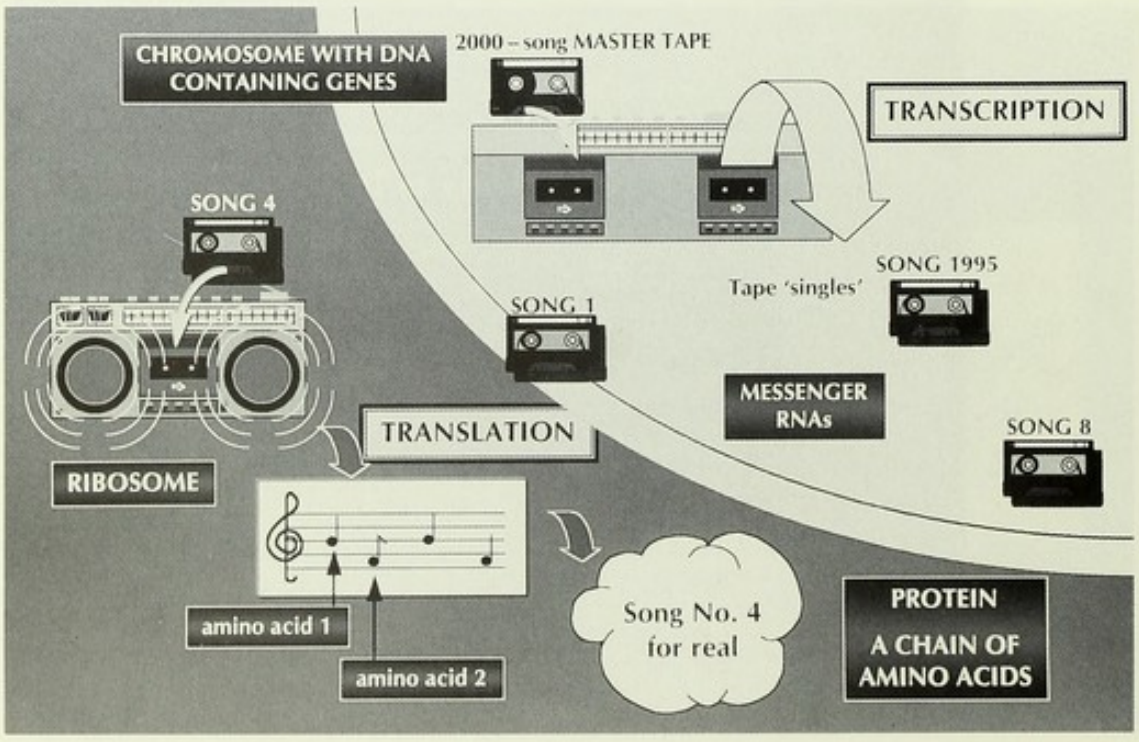

as TAPE is to CASSETTE
GENE is to DNA
REPETITIVE DNA BETWEEN GENES
GENES
INTERVENING SEQUENCE
CONTROL SEQUENCES
CODING REGIONS
A GENE ENCODING
A PROTEIN
as RECORDING is to tape
ANOTHER RECORDED SONG
BACKGROUND REPETITIVE HISS
DUBBING CUES
FIRST VERSE
RANDOM NOISE
LAST VERSE
AN AUDIO RECORDING ENCODING A SONG



A mutation is a fault in the gene (or recording) leading to a protein product (song) that is altered or absent.

The way genes work to dictate the manufacture of specific proteins can also be illustrated by the audio recording.

For a gene to work, particularly genes that are only meant to work in specific cell types (e.g. haemoglobin genes in red blood cells), their transcription has to be regulated by

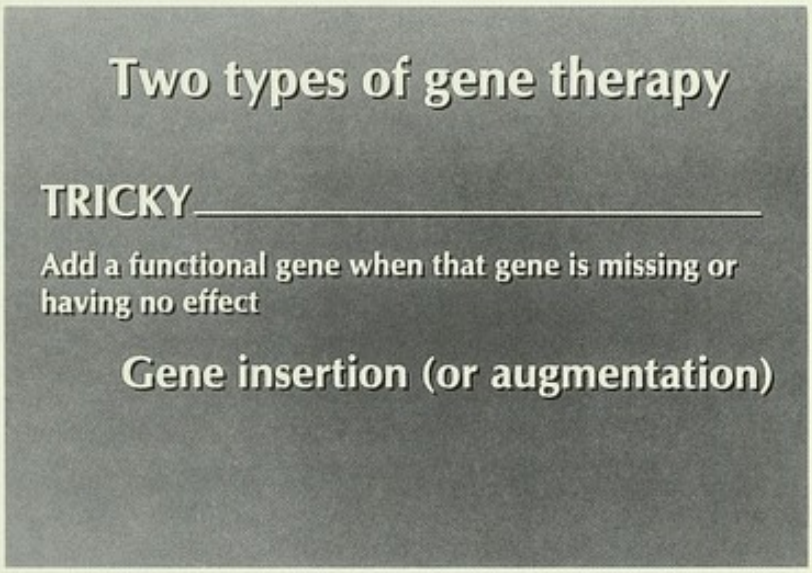


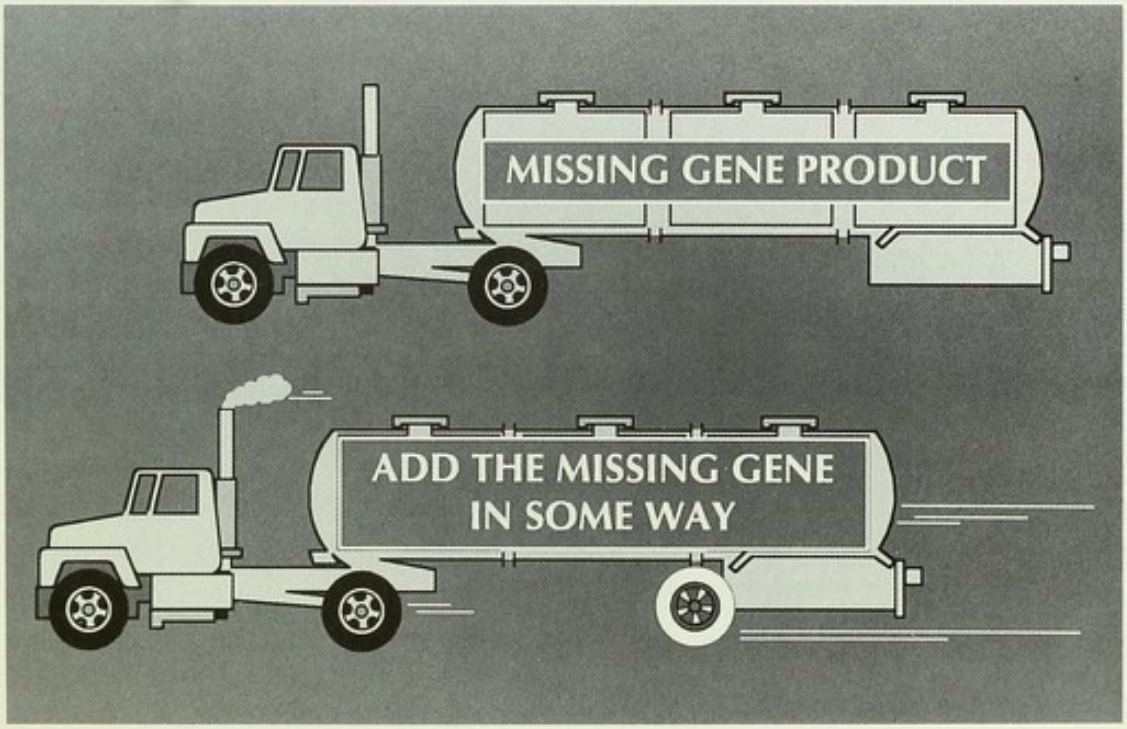
molecules that interact with DNA sequences 'upstream' of the gene, e.g. the 'Dominant Control Region'.

If gene therapy of tissue-specific genes is going to work, these regions must also be included in the new DNA added to the cells.

Gene Therapy

There are two types of gene therapy.



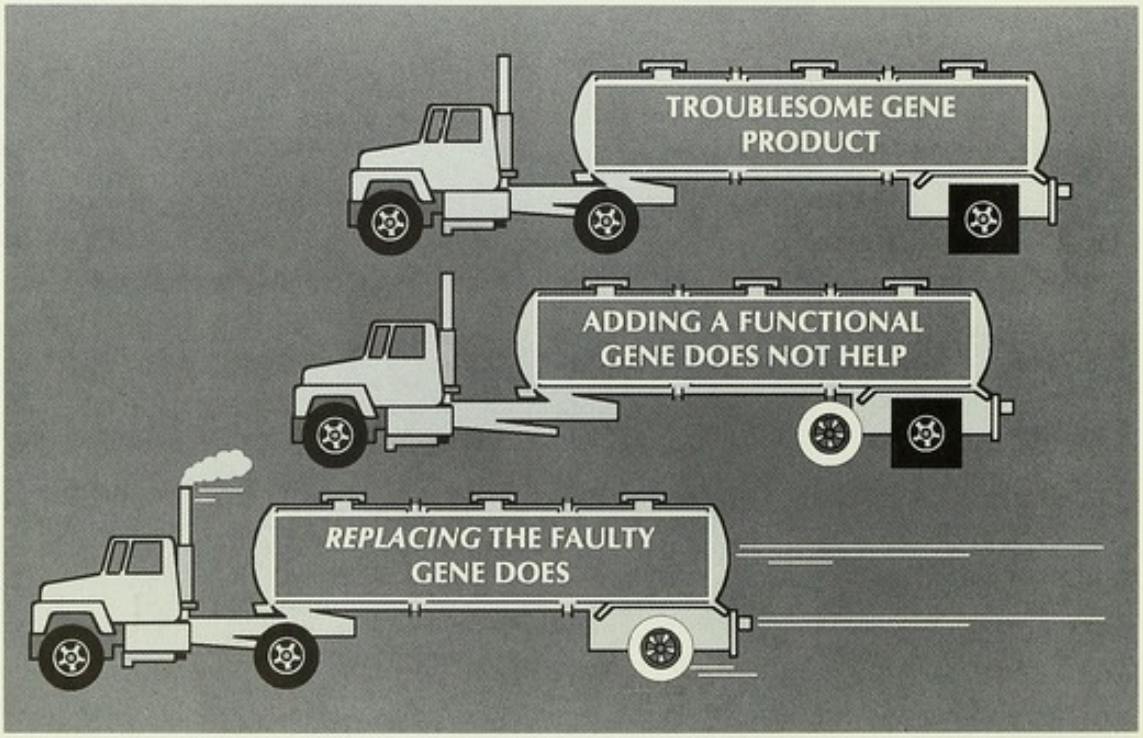


Two types of gene therapy

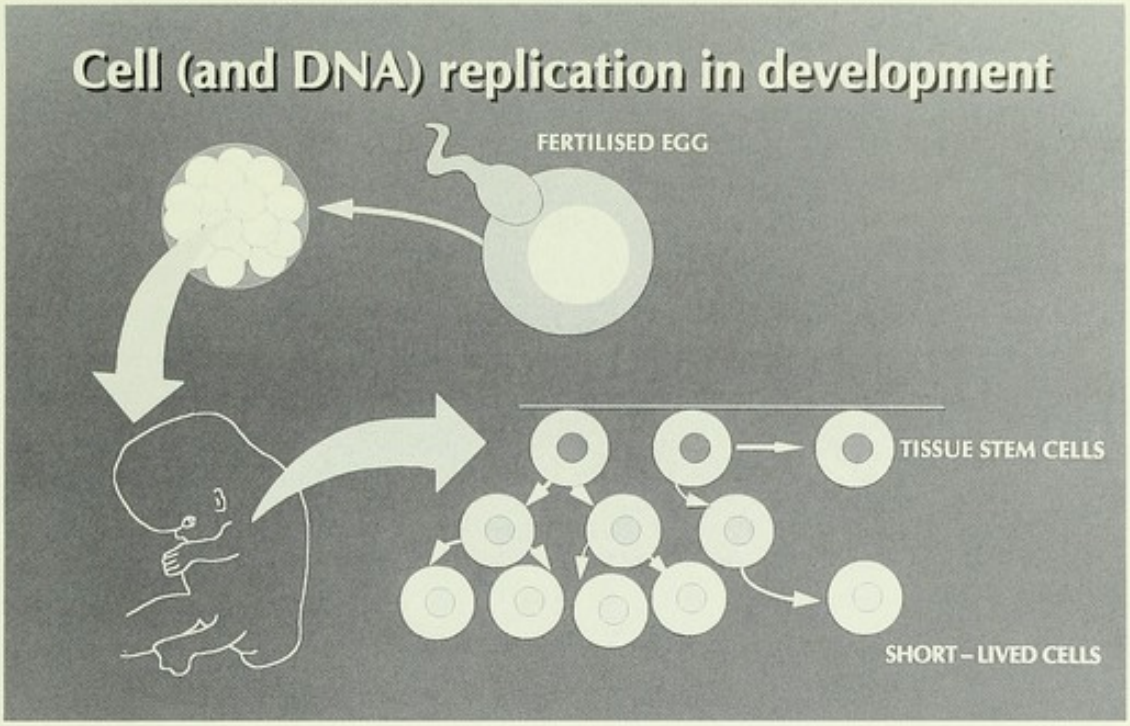
VERY TRICKY

Exchange a troublesome gene for a functional one

Gene replacement



Ideally, gene therapy requires that the new DNA not only gets into the right cells, but replicates with the cellular genes so that it is passed on to progeny cells. Otherwise the effect will 'grow out' like peroxide blonde hair.



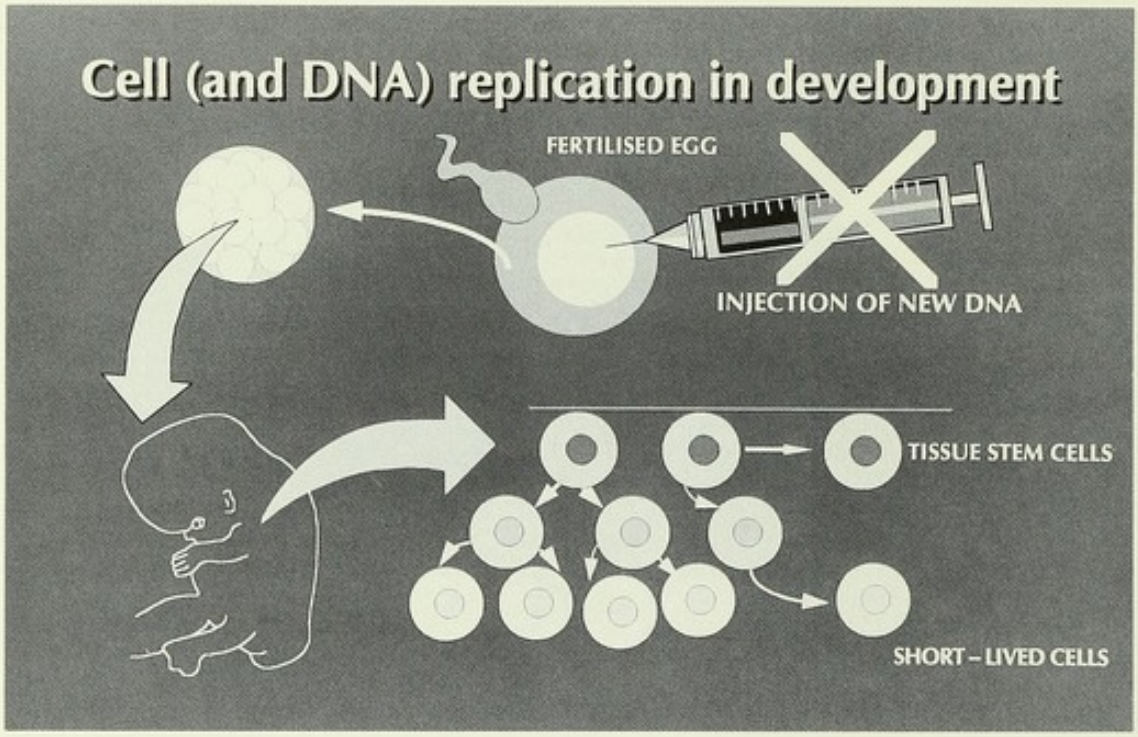
The aim is to reach the self-renewing tissue-stem cells whilst avoiding the germline cells.

Gaining entry – Tricks for getting new genes into cells and then replicating

Genetic engineers

do not usually create their own tools for manipulating DNA; they exploit naturally occurring enzymes etc. from a variety of organisms, especially bacteria.

• to cut DNA	restriction enzymes
• to join DNA	ligases
• to replicate DNA	DNA polymerase (plus nucleotides)



The simplest way to add new DNA to the cells is to copy what the sperm does and 'get in at the beginning'. Genetic modification of laboratory animals (often to create animal models of human genetic disease in order to devise new treatments) is usually done by adding the DNA at the beginning of development.

This would lead to genetic modification of the germline cells and is outlawed in humans.

Methods for gene delivery to cells may be physical or viral.

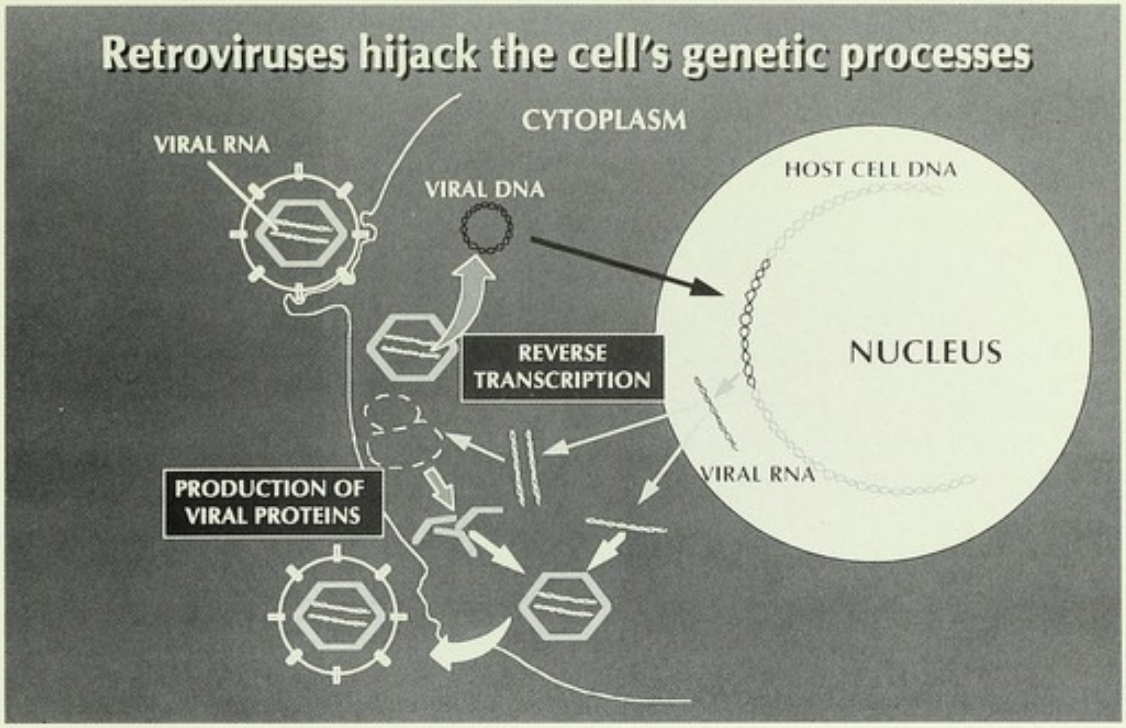
Gaining entry – Physical

- **INJECTION**
- **ZAPPING** (electroporation)

  A brief electric shock causes transient holes in the cell membrane to let the DNA in
- **MEMBRANE MERGE** (liposome – mediated – gene – transfer)

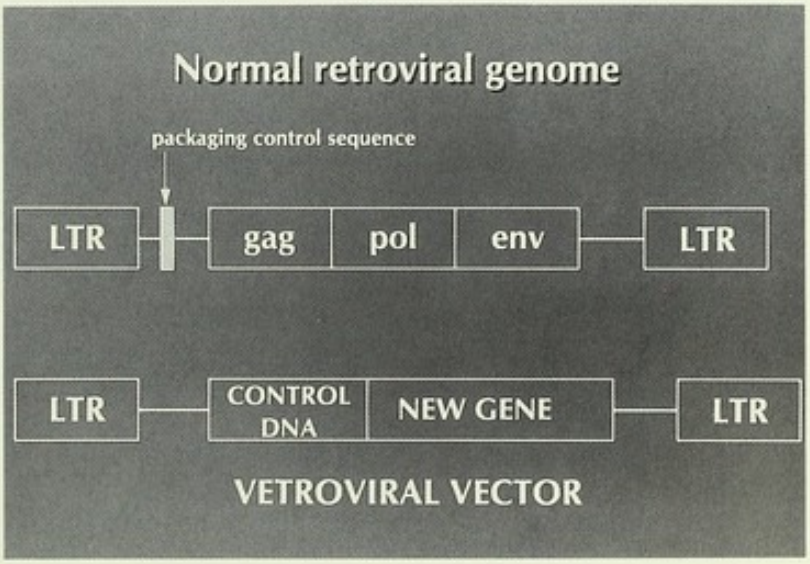
  The new DNA is placed in artificial / natural lipid – bilayer vesicles that will fuse with the cell membrane
- **THE IMPOSTER** (receptor – mediated – endocytosis)

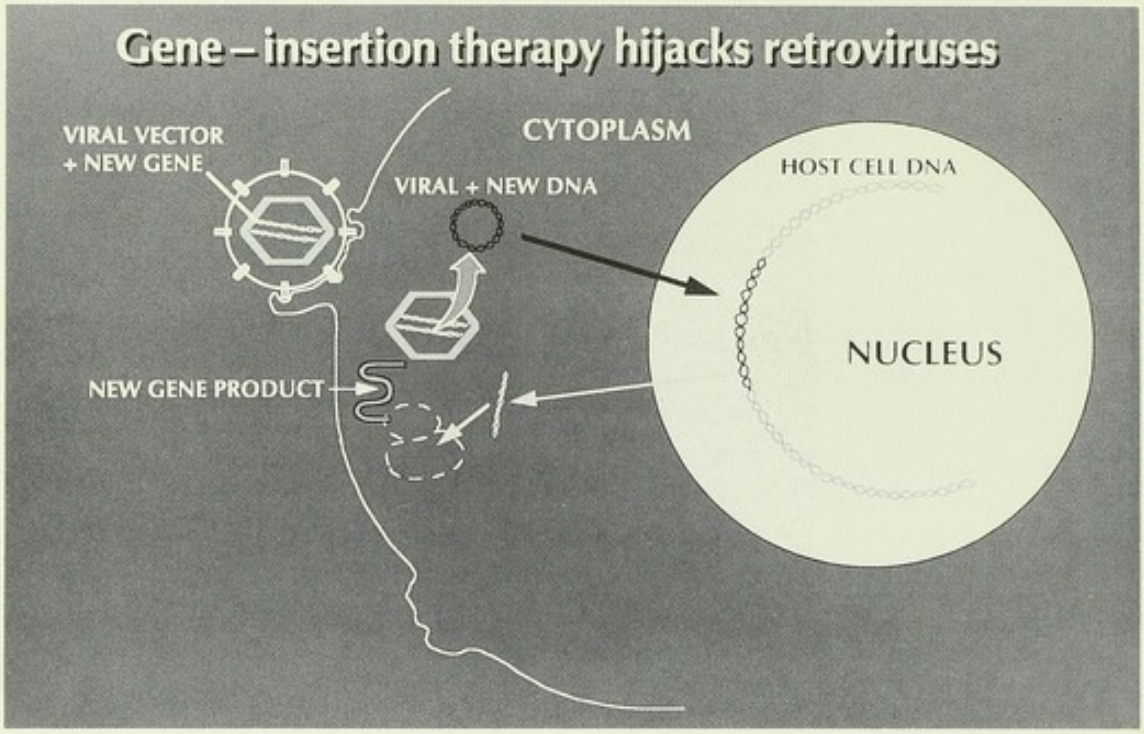
  The new DNA is attached to a protein that normally enters cells via a special receptor



Viral gene delivery methods commonly use *Retroviruses*, although other viruses (e.g. adenoviruses) are also being tried. To understand how retroviruses can be exploited one must understand the natural habits of retroviruses. The relevant bits of the retroviral life cycle are illustrated.

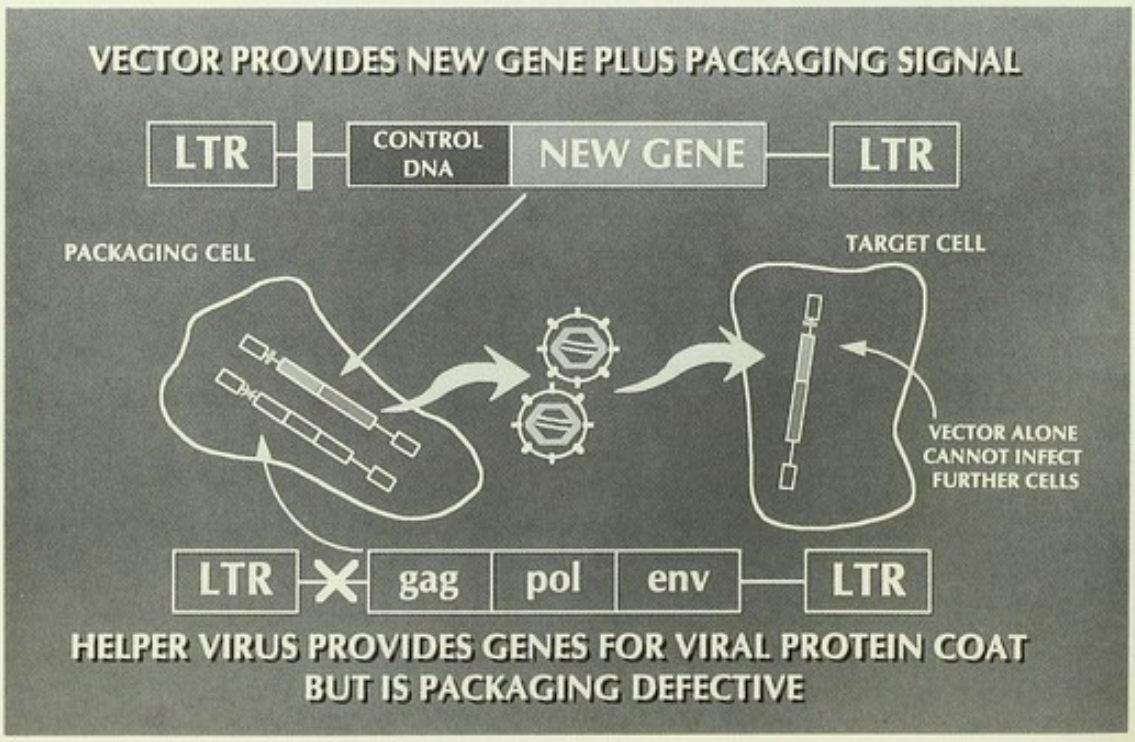
The trick is to replace the retroviral genes that allow it to make new infectious viruses, with the new gene you want to add to the patient's cells.





But how do you get one round of infection of the target cells – the cells that are going to function in the patient? It is done with the aid of packaging cells that contain a helper virus.

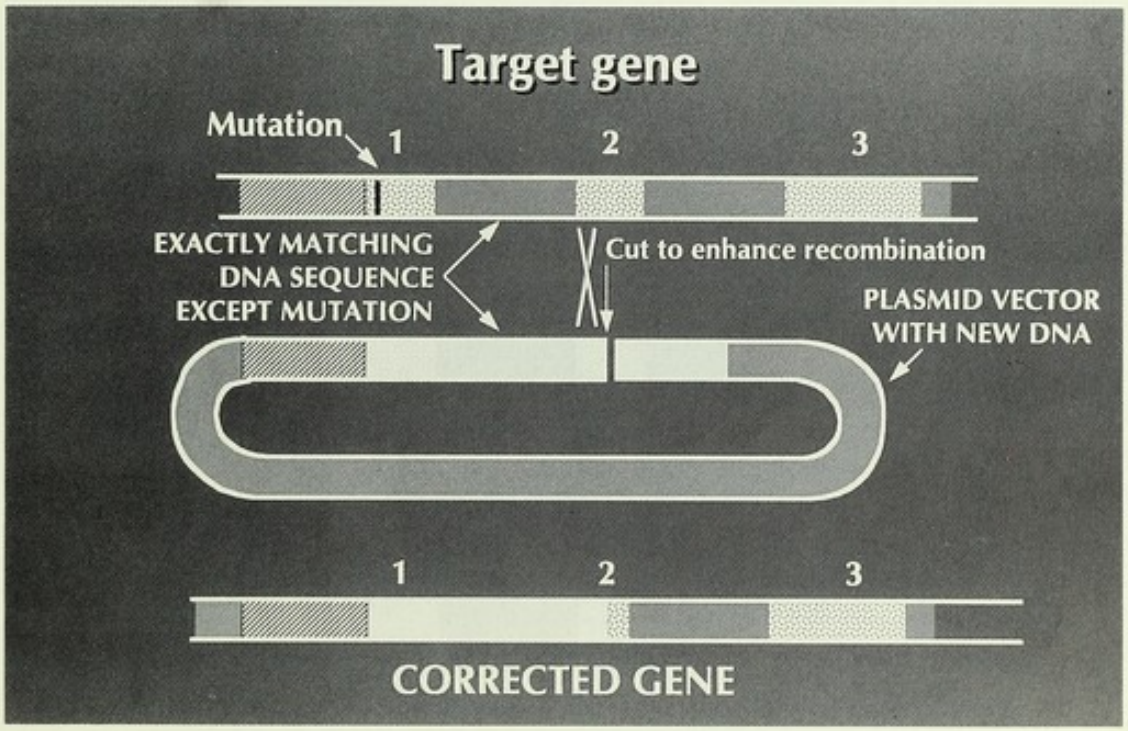
Whilst retroviral vectors are the only ones used in patients at present, they are not ideal. The ideal would be true replacement of the defective gene (targeted recombination). This is being achieved in experimental systems, usually following physical introduction of the new, 'replacement' DNA into the cells.



New gene integration

Retroviral vectors	Vectors for targeted recombination
High efficiency	Low efficiency
Random site(s)	Site – specific integration

Targeted recombination exploits the natural tendency of DNA to 'pair up' with DNA of identical sequence and exchange pieces. This is one of the processes by which genes are naturally switched between chromosomes of the pair during egg and sperm formation to create new combinations.



Some problems in gene therapy

Remove all cells, modify and return; the *ex – vitro* approach

- Extracting stem cells
- Making them divide to allow retroviral integration
- Selective proliferation of corrected cells – ablation of competing cells
- Delivery to a suitable site

Treating body cells in place; the *in – vitro* approach

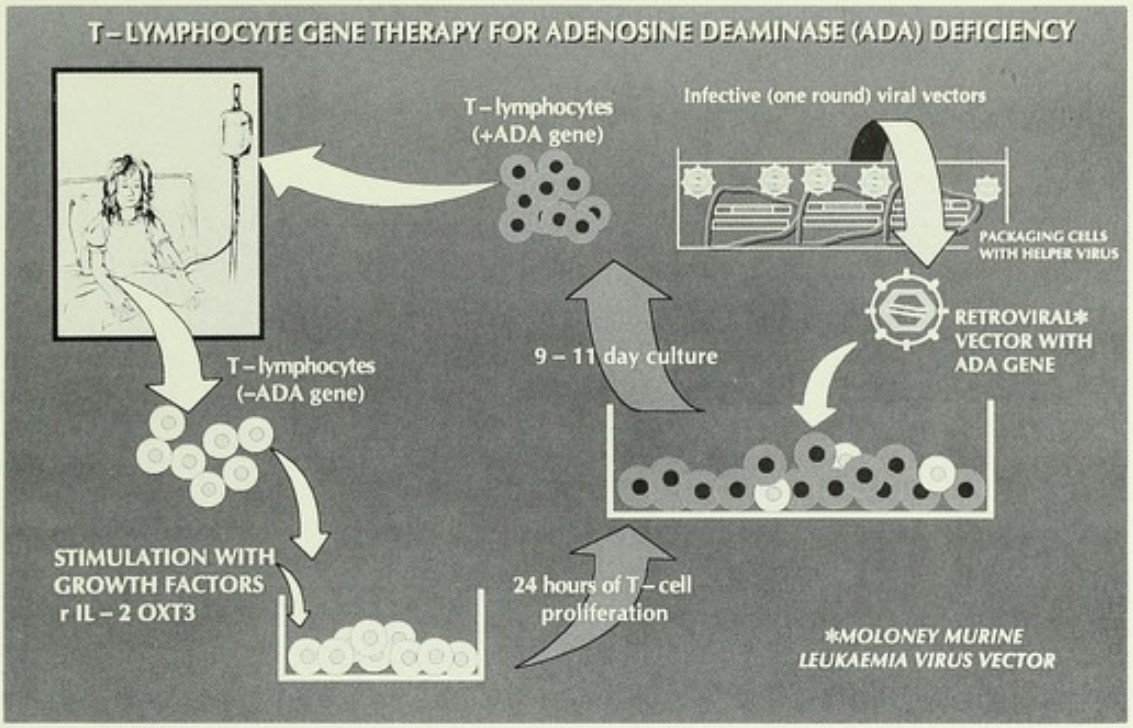
- Delivery to only the right cells in a controlled way

Getting enough corrected cells into the patient

Nowadays it has become relatively straightforward to introduce a selected gene into cells cultured in the laboratory and to get it to work well enough to detect some synthesis of the new gene's protein product in those cells. However, this achievement is a long way from the goal of adequate long-term production of the new gene product in the patient. Animal models, when available, allow techniques to be refined. Several problem areas remain.

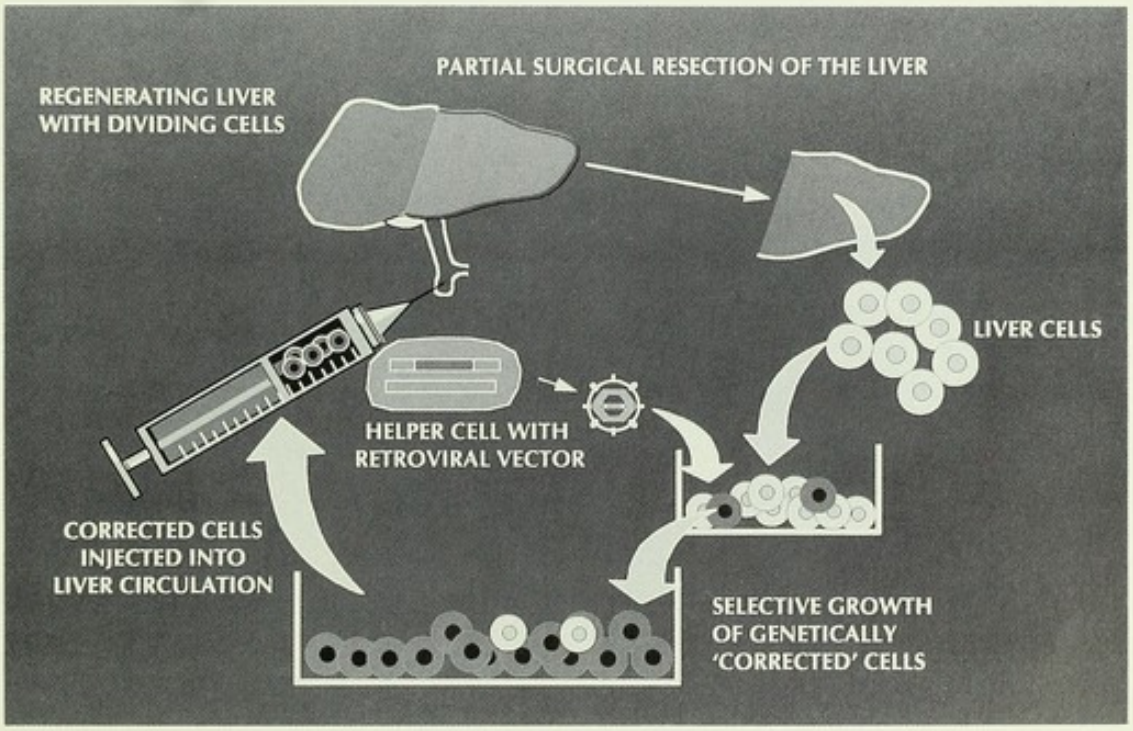
Two examples, both from the USA, illustrate some of the clinical aspects. The first is the only clinical trial of gene therapy for a genetic disease so far. The second is based on animal models, but is similar to a planned clinical trial involving gene transfer into liver cells.

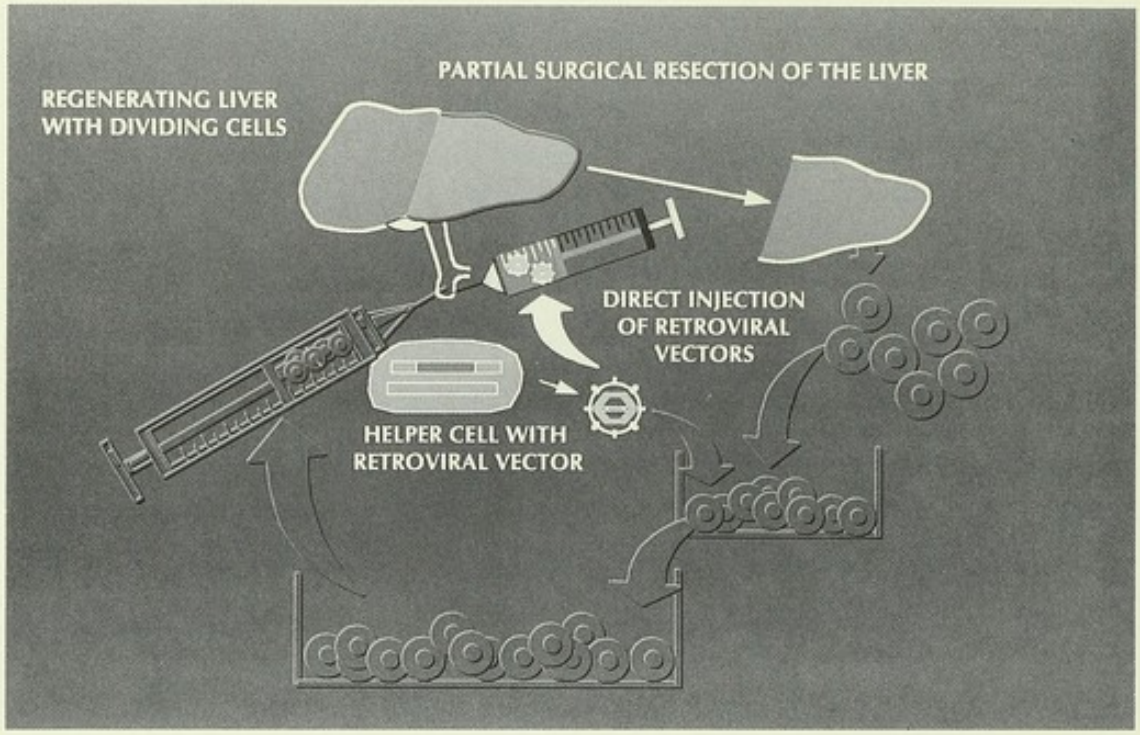
In September 1990, a four-year-old girl lacking adenosine deaminase (ADA) received a transfusion of some of her white blood cells (T-lymphocytes) into which the missing ADA gene had been inserted. ADA deficiency causes a failure of the immune system (T-cells) and, as a consequence, life threatening infections. ADA deficiency was a good first choice, because the ADA gene does not require complicated regulation and even a slight production of ADA can rescue the T-cells which are very long-lived cells. Now, this girl has a much stronger immune system, with corrected T-cells surviving six months and more after cell infusions.



Although T-cells are long-lived, the long-term aim is to insert the ADA gene into bone marrow stem cells. Purifying these and getting them to divide so that integration of the retroviral vector occurs is proving difficult.

Liver cells are somewhat easier to handle in this respect and many genetic diseases could be treated by correcting liver cell function.





Liver regenerates by cell division when part of it is removed surgically. This has allowed animal studies, in which the retroviral vector is infused directly into the blood vessel supplying the liver, with good transfer of new genes into liver cells, without having to go through the laboratory cell culture stage.

Possible Dangers

1. It might not work.
2. The correcting gene goes to the wrong type of cell or is expressed inappropriately.
3. The new gene might disrupt a normal gene when it inserts, with a risk of cancer.
4. The viral vector may be faulty, leading to it becoming 'infective' and spreading to other cells.

These potential dangers have to be assessed as experimental animal studies give way to clinical trials. The main safeguard is adequate supervision. In Britain, the Clothier Report (Report of the Committee on the Ethics of Gene Therapy, London, HMSO, 1992) has recommended the establishment of a new expert supervisory body.