Gene technology

Key definitions

Gene technology – this term really covers techniques such as genetic engineering, the creation of genomic libraries of DNA and DNA fingerprinting.

• *Genetic engineering* – the transfer of a gene from one organism (the donor) to another (the recipient) e.g. the genes coding for human insulin, growth hormone or the blood clotting factor, Factor VIII may be removed from human cells and transferred to bacteria.

• *Promoter* – a length of DNA (usually about 40 bases long) situated next to genes and which identify the point at which transcription should begin.

• *Marker* – a gene which is deliberately transferred along with the required gene during the process of genetic engineering. It is easily recognised and used to identify those cells to which the gene has been successfully transferred.

• *Genetic fingerprinting* – the analysis of DNA in order to identify the individual from which the DNA was taken to establish the genetic relatedness of individuals. It is now commonly used in forensic science (for example to identify someone from a blood sample) and to determine whether individuals of endangered species in captivity have been bred or captured from the wild.

• *DNA sequencing* - the determination of the precise sequence of nucleotides in a sample of DNA or even a whole genome e.g. the Human Genome Project.

The benefits and hazards of Gene technology

Benefits

Through gene technology, it is now possible to produce:

• genetically modified organisms for a specific purpose. Previously, such genetic change would have to be brought about by selective breeding which requires organisms to be of the same species (able to breed successfully together), takes many generations and involves transfer of whole genomes, complete with undesirable background genes. Gene technology is much faster and involves transferring one or few genes, which may come from completely unrelated organisms, even from different kingdoms.

• specific products, such as human insulin and human growth hormone,

thereby reducing the dependence on products from other, less reliable sources, such as pig or cow insulin.

• reduce use of agrochemicals such as herbicides and pesticides since crops can be made resistant to particular herbicides, or can be made to contain toxins that kill insects

• clean up specific pollutants and waste materials – bioremediation

• potential for use of gene technology to treat genetic diseases such as cystic fibrosis (see below) and SCID (Severe Combined Immune Deficiency) as well as in cancer treatment.

Hazards

Genes inserted into bacteria could be transferred into other bacterial species, potentially including antibiotic resistance genes and those for other materials, which could result in antibiotic resistance in pathogens, or in bacteria that can produce toxic materials or break down useful materials. Regulation is designed to minimise the risks of escape of such genes. There is little evidence that such genes have escaped into wild bacterial populations. Crop plants have, by their nature, to be released into the environment to grow, and many millions of hectares of genetically engineered crops, both experimental and commercial, are planted across the globe. So far, fears that they might turn out to be 'super-weeds', resistant to herbicides and spreading uncontrollably, or that their genes might transfer into other closely related wild species, forming a different kind of 'super-weed', or that they might reduce biodiversity by genetic contamination of wild relatives seem to have proved unfounded. A paper was published in Nature in 2001 showing that Mexican wild maize populations were contaminated with genes from genetically manipulated maize, but the methods used were flawed and subsequent studies have not confirmed this contamination, suggesting that the wild maize is not genetically contaminated. There is some evidence that Bt toxin, geneticially engineered into plants such as cotton and maize, whilst very effective in killing the target species, may kill other, desirable, insects such as bees and butterflies, and may also cause natural selection of Bt toxin resistant insects. Future events may show that such environmental risks are greater than they look at present. Food that is derived from genetically engineered organisms may prove to be unexpectedly toxic or to trigger allergic reactions when consumed. There is little reliable evidence that this has been so, but the risk remains. Food containing the expressed products of antibiotic resistance marker genes could be consumed at the same time as treatment with the antibiotic was occurring, which would potentially reduce

the effectiveness of the treatment. No examples of this are known.

The social and ethical implications of gene technology

The social impact of gene technology is to do with its potential and actual impact of human society and individuals. In terms of social impact, gene technology could:

- enhance crop yields and permit crops to grow outside their usual location or season so that people have more food
- enhance the nutritional content of crops so that people are better fed
- permit better targeted clean-up of wastes and pollutants
- lead to production of more effective and cheaper medicines and treatments through genetic manipulation of microorganisms and agricultural organisms to make medicines and genetic manipulation of human cells and individuals (gene therapy)
- produce super-weeds or otherwise interfere with ecosystems in unexpected ways, reducing crop yields so that people have less food
- increase costs of seed and prevent seed from being retained for sowing next year (by inclusion of genes to kill any seed produced this way) reducing food production
- reduce crop biodiversity by out-competing natural crops so that people are less well fed
- damage useful materials such as oil or plastic in unexpected ways
- cause antibiotics to become less useful and cause allergic reactions or disease in other unexpected ways

The ethical impact is about the application of moral frameworks concerning the principles of conduct governing individuals and groups, including what might be thought to be right or wrong, good or bad. So in the context of gene technology, it is to do with issues of whether is right or wrong to conduct research and develop technologies, whether it is good or bad. Judgements may be that

- It is good to conduct such research to develop technologies that might improve nutrition, the environment or health
- It is good to use the results of such research to produce food, to

enhance the environment or improve health

- It is wrong to continue such research when the potential impact of the technology is unknown and many aspects of it remain to be understood.
- is wrong to use the results of such research even when the organisms are kept in carefully regulated environments such as sterile fermenters as the risks of the organisms or the genes they contain escaping are too great and unknown
- It is wrong to use the results of such research when this involves release of gene technology into the environment as once it is released it cannot be taken back – the genes are self-perpetuating, and the risks that they might cause in future are unknown

The social and ethical implications of gene technology are complex and relatively unfamiliar to people who are not scientists, including those involved in the media and in government. This complexity and unfamiliarity is the cause of considerable concern and debate. In considering the implications of gene technology the best approach is to avoid the general (e.g. avoid 'it is bad to play God') and stick to the specific and balanced (e.g. it is possible to increase food crop yields with gene technology so more people can be fed, but there is enough food already if it is properly distributed, so people should not be forced to eat products with unknown risks).

The use of electrophoresis in genetic fingerprinting and DNA sequencing

Electrophoresis

Electrophoresis is a method of separating substances and analyzing molecular structure based on the rate of movement of each component in a liquid medium while under the influence of an electric field. In genetic fingerprinting and DNA sequencing, the components being separated are fragments of DNA.

this case, the type of electrophoresis used is gel electrophoresis – the gel appears solid but is actually a colloid in which there are spaces between the molecules through which other molecules can move. Electrodes are placed at either end of the gel, as a result of which the DNA molecules move under the influence of an electric current. Usually the DNA is fragmented (cut across) into a series of fragments using a restriction enzyme or mixture of restriction enzymes. These enzymes cut the DNA at specific restriction sites (see above), but these sites are randomly distributed along the length of the DNA so the

fragments are of varied lengths.

The direction of movement depends on the fact that DNA molecules and fragments of DNA are negatively charged and thus move towards the positive electrode (anode). The distance moved in a given time will depend on the mass of the molecule of fragment. The smaller fragments move further in a given time, and the larger fragments of DNA move less far. Taking humans as an example, almost everyone has 46 chromosomes: 23 pairs if you are female and 22 pairs plus two odd ones if you are male. The longest of these kinds of chromosomes has been numbered as chromosome 1 and the smallest as 22, the sex chromosomes being out of sequence and called X and Y. The base sequence of every chromosome 1 in every human being is similar, but not identical due to the existence of mutations and therefore of different alleles of genes. What this means is that when the DNA is fragmented with a restriction enzyme, the fragments are similar but not exactly the same in DNA from different people. The DNA is transparent and invisible, so the fragments must be treated to make them visible. There are two key ways of doing this:

- One is based on staining all of the DNA fragments, for example using ethidium bromide (toxic, fluoresces in short wave UV radiation), methylene blue (fades quickly and stains gel as well as DNA)and nile blue A (does not stain gel and visible in ordinary light).
- The other is based on creating a gene probe that is complementary:
- either to a commonly repeated bit of DNA that will therefore be present on many of the fragments,
- or to a base sequence that is specific to a particular gene or allele of a gene which will therefore be present on no more than one of thefragments. The gene probe is a single stranded piece of DNA with a base sequence complementary to the DNA that you wish to identify.

In order to make it possible to locate which fragment or fragments the gene probe has attached itself to, the gene probe must be labelled. The most common forms of labelling are:

- to make the probe radioactive and to detect it by its ability to expose the photographic film used to make X-ray photographs
- to stain the probe with a fluorescent stain such as vital red, that will fluoresce with bright visible light when placed in ultraviolet light, making the location of the probe and therefore of the fragment or fragments visible.

Genetic fingerprinting

Once the DNA fragments have been separated by gel electrophoresis they can be compared with other samples of DNA, thereby allowing determination of the source of the DNA (as in forensic investigations) or whether the samples are derived from related individuals, as shown below: Image

DNA sequencing

The most publicised example of DNA sequencing is the Human Genome Project. Electrophoresis is used to separate fragments of DNA to enable determination of the order of bases within genes and chromosomes. The fragments vary in length by one base at a time and the last base on each can be identified. Because the fragments are different lengths, they can be separated by electrophoresis as shown below: image

The causes and symptoms of Cystic Fibrosis

Cystic Fibrosis (CF) is a genetic condition in humans. It is inherited and although it reduces considerably the life expectancy of people with the condition, improved treatments have been helping such people to live longer so that the average life-span is now about 35 years. There are estimated to be around 50,000 people with CF worldwide.

Causes

Cystic fibrosis is caused by several different alleles of a key gene coding for a transmembrane protein that transports chloride ions through cell surface membranes (cystic fibrosis transmembrane regulator, CFTR). Its inheritance is autosomal (i.e. it is NOT sex-linked) and recessive. The gene is located on chromosome 7. CF alleles originate by mutation of the CFTR protein, but can then be inherited through many generations.

As CF alleles are recessive, individuals with a single copy of such an allele are heterozygous and do not have the condition. There are about 10 million such carriers worldwide.

To have CF, it is necessary to be homozygous for CF alleles, most often by inheriting one CF allele from each parent.

Effects of CF

Reduced chloride transport through cell membranes leads to production of thick, sticky mucus that particularly affects the lungs, pancreas and reproductive organs.

- The mucus remains in the lungs rather than being swept out by the tracheal cilia, leading to wheezing and repeated infections. The mucus may be removed by physiotherapy
- The mucus may block the pancreatic duct, preventing amylase and protease enzymes from reaching the small intestine, compromising digestion and nutrition, and also causing a build-up of protease in the pancreas, damaging the pancreatic tissue including the cells that produce insulin, increasing the chance of diabetes.
- The mucus may block the sperm ducts, causing male infertility and may slow the progress of eggs and sperm through the oviducts, reducing female fertility.

Progress towards treating Cystic Fibrosis with gene technology

Current treatments for CF deal with the symptoms rather than the causes, for example physiotherapy to remove mucus from lungs, antibiotics to combat recurrent lung infections and enzyme supplements to enhance digestion. These have been very successful in improving people's quality of life and lifespan, but research continues to try and develop techniques for adding functional copies of the CFTR gene to the cells of people with CF.

Since it is a recessive condition, such gene therapy does not need to remove or replace the existing genes in the person's cells – adding a working copy of the gene to a cell and having it expressed would be sufficient to permit that cell to transport chloride ions normally. Since it is the mucus in the lungs that generally limits lifespan in people with CF, it is these cells that have been the focus of effort. It is thought that if even a proportion of lung cells could be given a working copy of the gene, this would thin the mucus sufficiently to allow the cilia to operate normally.

The approach that has been trialled with another recessive genetic condition, SCID, is to remove cells from the body, add working copies of the gene and put the cells back. The working copies of the gene integrate themselves into random positions in the genome of the treated cells. The blood cells involved in this case only live for a few weeks so it has to be frequently repeated. Of 14 boys in one French trial, 3 have developed cancer, probably because the gene has been inserted into a critical portion of one of the cells at some point. Clearly this approach cannot be used with CF because the lung surface cells cannot be extracted from the body.

For CF, a vector must be used to deliver the DNA containing the functional CFTR gene into the lung cells.

- *Viral delivery systems* – some viruses such as Adenoviruses can be used asthe vector. Normally, viruses which infect lung cells are used – their virulence (ability to cause disease) is removed and they are genetically engineered to carry the functional human CFTR gene. Early trials have involved either injection with the genetically engineered viruses or inhale them from an aerosol directly into the lungs. The intention is that the lung surface cells are infected with the virus, which releases the genetic material into the cells where it is expressed.

- *Non-viral delivery systems* – other systems are also being developed and have been trialled for safety but have not been used therapeutically e.g.

1. Creation of a lipid sphere or liposome, containing the DNA. An aerosol is sprayed into the lungs where the liposome will be able to pass through the target cell membrane and carry the DNA into the cell.

2. DNA can be compressed into a very small volume which may directly enter cells.

Whether the DNA is introduced into the cells by viruses or some other system, the intention is that the gene will be incorporated into the cell's genome and will start to be expressed, to produce CFTR protein to carry chloride ions through its membrane.

There is not yet a successful example of treatment of CF by gene therapy. This is because:

- current viral vectors have been found to stimulate allergic or other immune responses

- current liposome vectors have proved inefficient at delivering genes into cells

- the effect of the therapy on chloride ion transport has, so far, lasted only a few days

Research continues to solve these problems to develop a workable treatment for lung symptoms. Further into the future, similar approaches may be possible for pancreatic symptoms. A cure would require every one of the 50 x 1013 cells in the body to be altered, which is not currently thought to be technically possible and would raise significant further ethical issues. To enable people with CF to have children would require germ-line gene therapy where changes are made to human gamete cells that are inherited by the next

generation. This would also raise very significant further ethical issues and does not appear to be realistic at present.

Genetic screening and counseling

There are now many conditions known to be caused by varied alleles of varied genes and which can therefore be inherited. The pattern of inheritance varies, according to whether the allele is dominant, recessive or sex-linked.

Individuals may be tested for the presence of such alleles – such tests may be requested because there is a history of a particular condition in the family of that person or because the person belongs to an ethnic group which has a high percentage of individuals with a particular allele, such as the alleles that cause Tay Sachs in people who are Ashkenazi Jews.

Genetic screening: The testing of samples of DNA from a group of people to identify the presence or absence of particular alleles and thus the risk of having or passing on particular genetic conditions. Such screening may be:

Carrier screening- all the individuals in a family may be screened if one family member develops a particular condition that may be genetic. potential parents may be screened where there is the possibility that one or both of them might carry a recessive allele for some particular condition e.g. cystic fibrosis

- *Prenatal screening* – this is used to determine aspects of the genetic makeup of an unborn child. Such testing can detect a number of genetic conditions:
- *Chromosomal abnormalities,* such as Downs Syndrome (of particular importance if the mother is over 34), trisomy 13 and trisomy 18.
- *Single gene disorders*, such as haemophilia, sickle cell anaemia and cystic fibrosis
- *Neural tube defects*, such as spina bifida and anencephaly Pre-natal screening may be carried out in different ways and at different stages of the pregnancy :
- *Chorionic villus sampling* – where the early placental tissue is sampled, usually done at 10 – 12 weeks of the pregnancy
- *Amniocentesis* – where fetal cells in amniotic fluid are sampled, usually done at 13 – 18 weeks of the pregnancy
- *Intra-uterine blood test* – where fetal blood is sampled, usually done at 16 – 18 weeks of the pregnancy

- *Newborn screening* – in some countries, all newborn babies are screened for genetic conditions such as phenylketonuria (pku) by a simple blood test. This test enables the affected individual to be put onto a protective diet low in the amino acid phenylalanine, for the rest of their life, to protect them from the damaging symptoms of the condition.

Once the results of a genetic test are known, it will be necessary for those involved to receive Genetic Counselling. This will involve an explanation of the results and the implications in terms of probabilities, dangers, diagnosis, and treatment.

- For the individual – depending on the nature of any detected allele (dominant or recessive), it will be necessary to explain the possible future consequences in terms of the health of the individual and whether this is likely to have repercussions on their education or employment. In some cases, it might affect their prospects of obtaining insurance.
- For couples who want to have children – again, depending on the nature of the inheritance, it will need to be explained what the probabilities are of any children inheriting the defective allele – and the chances of any child actually having the disease i.e. it showing in their phenotype. All of this will depend on whether the allele is dominant, recessive or sex-linked.

In addition to the practical considerations of genetic screening and counselling, there are also some ethical considerations :

- Who decides who should be screened or tested?
- Which specific disorders should be screened?
- Who should be providing the screening?
- Should we screen or test for disorders for which there is no known treatment or cure?
- What psychological impact might the results have on the individuals involved?
- Should the results be confidential?
- If not, who should be able to have access to the information?

- Should the results be made available to potential employers, insurers etc.?

Questions and answers

Outline the need for energy in living organisms using named examples. [9]

- ATP as universal energy currency ;
- light energy needed for photosynthesis ;
- ATP used conversion of GP to TP ;
- ATP used to regenerate RuBP ;
- (energy needed for) anabolic reactions ;
- protein synthesis / starch formation / triglyceride formation ;
- activation energy ;
- (activate) glucose in glycolysis ;
- active transport ;
- example ; e.g. sodium / potassium pump
- movement / locomotion ;
- example ; e.g. muscle contraction / cilia beating
- endocytosis / exocytosis / pinocytosis / bulk transport ;
- temperature regulation ;

Explain the advantages of treating diabetics with human insulin produced by genetic engineering. [6]

— constant/reliable supply all year round/unlimited supply;

— less risk of contamination/infection;

— identical to insulin produced in the body;

— less/no risk of allergic reaction;

— does not stimulate the immune system;

— fewer side effects;

— can be produced without the killing of animals/ethical reason;

— cheaper/easier to extract and purify;

— more available/large amount;

— more rapid response;

Describe the use of recombinant DNA technology in the synthesis of human insulin by bacteria [9]

— mRNA coding for insulin/isolate gene for human insulin;

— from beta cells of islets of Langerhans/pancreas;

— reference to reverse transcriptase;

— to cDNA;

— reference PCR/DNA polymerase/double strand;

— reference sticky ends/AW;

— use of vector/virus/plasmid;

— reference endonuclease/restriction enzymes;

— to cut plasmid;

— reference DNA ligase to join DNA;

— inserted into suitable host cell/E.coli/bacteria;

— reference method of insertion;

— identification of modified bacteria;

— reference growth/culture of engineered bacteria in fermenters;

Explain why mammalian cells are used as host cells in recombinant DNA technology during production of human factor VIII

— human genes contain exons and introns

— mammalian cells have enzymes to remove introns/ enzymes for methylation and splicing of mRNA;

— mammalian cells have enzymes / Golgi apparatus for post –translational modification;

— presence of human regulator genes

State any biological methods other than the use of plasmids; of introducing genes into host cells.

— Liposome transfer

— Electroporation

— Microinjection/ micropipette;

— a DNA gun fires tungsten or gold particles coated with DNA

— ballistic impregnation

— use of vector