Bio Factsheet

Number 13

Genetic Engineering

The sequence of nucleotide bases on a DNA molecule makes up the genetic code. Each triplet of three bases codes for a particular amino acid. Using this code, a cell can produce any one of the many proteins that it requires. One of the many remarkable features of the genetic code is that it is universal; the three bases CAC, for example, will code for the amino acid valine whether it is in a bacterial cell or a cell from a human liver. A gene taken from one organism should then be able to produce exactly the same product if it is introduced into another organism. This is the basis of genetic engineering, the production of new characteristics by the insertion of a gene from one organism into another.

The basic principles

Genetic engineering is now used very widely for a variety of purposes ranging from manufacturing individual gene products such as human insulin to improving the yield of crop plants. A number of different techniques are used but they usually involve the following stages:

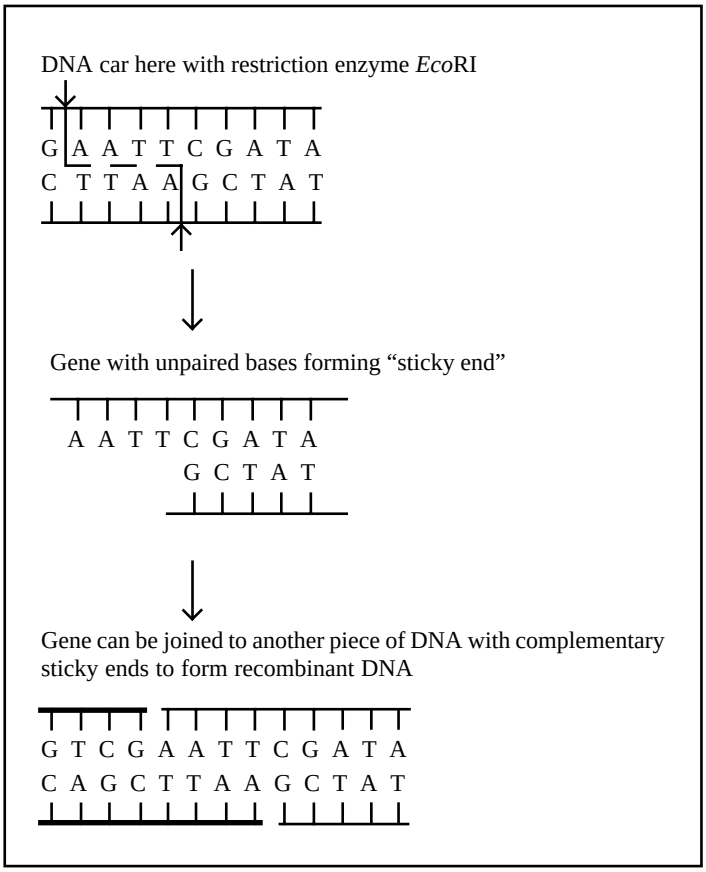
- The isolation of the gene required to produce the product,
- Insertion of this foreign gene into the DNA of a host cell by using a suitable DNA carrier called a vector,
- Checking to find the host cells which contain the new gene,
- Multiplying or cloning the organism containing the new gene to produce large numbers of genetically identical cells or organisms for commercial use

The tool kit

In order to isolate individual genes and stick or splice them into other pieces of DNA, we need a suitable tool kit. This involves three different types of enzyme:

- **Restriction endonucleases** or restriction enzymes
  In the early 1970s, researchers discovered restriction endonucleases in bacterial cells. These enzymes are now known to be part of the natural defence system of bacteria against bacterial viruses. They cut the virus DNA into small fragments and stop the infection process. There are many different types of restriction endonuclease, each one cutting the DNA at a specific nucleotide sequence. Some cut the DNA straight across. Others produce a staggered cut like that shown in Figure 1. Each cut piece of DNA will have a sequence of unpaired bases called "sticky ends". These are very important as they can join with complementary sticky ends on other pieces of DNA.
- **Ligase**
  An enzyme which is able to join two pieces of DNA. In genetic engineering, ligase enzymes are used to join DNA from two different sources to make recombinant DNA.
- **Reverse transcriptase**
  Transcription is the production of mRNA from DNA. Reverse transcriptase is therefore an enzyme which catalyses the opposite reaction. It enables a single chain of DNA to be made from the corresponding mRNA molecule. This single chain of DNA, called complementary DNA or cDNA, can then be used to make a double chain. From the mRNA in the cytoplasm of a cell we can produce a copy of the gene from which it was transcribed.

Fig 1. The production of sticky ends and recombinant DNA



Isolating the gene

The starting point for genetic engineering involves isolating the required gene. This may be done in a number of different ways:

- The DNA of an organism may be cut up using restriction enzymes. The resulting fragments will be of different size and therefore have different electrical charges. This means that they can be separated from each other by means of a technique called **electrophoresis**. Some of the fragments will contain the gene we want. Obviously this technique is much easier in organisms such as viruses which only contain a small amount of DNA.

Exam Hint *- You must be able to define: recombinant DNA, restriction endonuclease, vector, DNA ligase, plasmid, reverse transcriptase.*

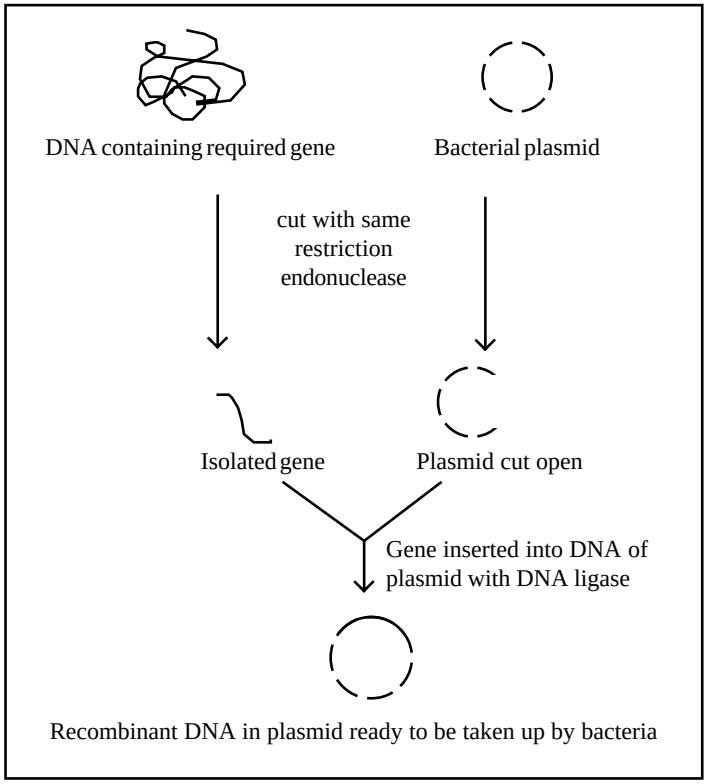
- Insulin is a protein. It is a hormone produced in large quantities in the β-cells of the Islets of Langerhans in the pancreas. Obviously, much of the mRNA produced by these cells should code for insulin. If we isolate mRNA from appropriate cells and incubate it with reverse transcriptase, we can produce a copy of the insulin gene.

- Since we know the sequence of bases which codes for each amino acid, we can work backwards to produce an artificial gene. What we need to do is to analyse the sequence of amino acids which make up the protein in which we are interested. We can then use a so-called 'gene machine' to assemble an artificial gene which will carry the correct code.

Producing recombinant DNA

The next problem that researchers face is to insert the required gene into the DNA of the host cell. Unfortunately, this cannot usually be done directly, so use has to be made of a vector. This is often a **plasmid** - a small, circular piece of DNA found in bacterial cells. Firstly, plasmids are isolated. They are then cut open with restriction enzymes and the new gene inserted with the aid of ligases. We now have recombinant DNA; DNA from different organisms joined in a single molecule. The plasmid is then put into the host organism. This process is summarised in Figure 2.

Figure 2. The production of recombinant bacteria



Checking the host cells

It is by no means certain that treating the plasmids in this way will ensure that they all take up the new gene. In practice, very few of them do and we are left with a mixture of plasmids, most of which do not contain this gene. There is another problem as well. If we incubate plasmids and bacterial cells, only some of the cells will take up the plasmids. We need a way to identify the few bacteria which contain the recombinant DNA.

Plasmids carry genetic information of their own and the ones used in genetic engineering usually contain DNA which codes for resistance to particular antibiotics. We know that the plasmid we are interested in, the one containing the new gene, is resistant to certain antibiotics. By growing the bacteria on a medium containing these antibiotics, we can identify the ones we want.

Cloning the gene

Every time a bacterial cell divides, two genetically identical individuals are produced. If the bacterial cell contains the new gene, this will also be copied and will appear in each of the daughter cells. The same is true of cells from other organisms, providing that they are dividing by mitosis. Bacteria are widely used in genetic engineering processes and have transformed many biotechnological processes. One advantage is that micro-organisms can be produced rapidly which will have a much higher yield. This is a much faster process than if we had to wait for chance mutations to arise.

Sometimes the organism that produces the desired product is slow growing, so genetic engineers insert an appropriate gene into the DNA of a faster growing organism such as the bacterium, *Escherichia coli*. It has also become possible to separate growth and production stages of culture. The bacterial cells grow rapidly and transform all the raw materials in the culture medium into molecules required for growth. Only when a certain cell density is reached are the genes responsible for making the required product switched on. In this way, much larger amounts of product can be produced much more rapidly.

A problem with large industrial-scale fermentations is that respiring micro-organisms produce a lot of heat. Cooling a fermenter is expensive so genetic engineers are experimenting with the use of thermophilic bacteria; bacteria which normally live in places such as hot springs where the temperature is high.

The uses of genetic engineering

1. Making products from genetically modified organisms

- **Human insulin**
  It has been estimated that there are approximately 60 million people in the world who have diabetes and who require daily injections of insulin. At one time this insulin was obtained from the pancreases of pigs and cattle. This only produced limited supplies and, in addition, insulin from these sources could produce an allergic reaction since it was a foreign protein. Genetically-engineered human insulin is cheaper to produce and less likely to produce allergic reactions.

- **Bovine somatotrophin (BST)**
  This is a hormone produced in very small quantities in the pituitary gland of a cow. It stimulates growth of young animals. If given to older cows, it stimulates milk production by increasing the cow's appetite and by diverting more of the food intake towards milk production. Several countries have used genetically-engineered BST to increase milk production.

- **Interferons**
  Interferons are naturally occurring chemicals produced in very small quantities when the cells of a mammal are attacked by viruses. Interferons can be used to treat certain types of cancer. Only with the development of genetic engineering techniques, however, has it become possible for enough interferon to be produced for medical use.

2. Improving the yields of crop plants

For centuries the only method available for improving the yield of crop plants was selective breeding. This, however, is slow and unpredictable. The advent of genetic engineering means that it is now possible to select desirable genes from a whole range of organisms and insert these into plants with a suitable vector. The bacterium *Agrobacterium tumefaciens* is commonly used. It contains a large plasmid which readily becomes incorporated in the DNA of the host plant cell. This plasmid can be used to introduce new genes.

Examples of improving plant yield in this way include:

- The transfer of disease resistance genes into crops. The crop will then be resistant to important diseases such as potato blight and tobacco mosaic disease.
- Producing crops which are resistant to the herbicide glyophosphate so that when the crop is sprayed with this herbicide, only the weeds are killed.
- The insertion of nitrogen-fixing genes into non nitrogen-fixing plants improving both yield and soil fertility and reducing the need for fertilisers. This has not yet been achieved commercially.
- The soil bacterium *Bacillus thuringiensis* produces a protein called Bt which is a natural insecticide. The gene coding for this protein can be inserted into crop plants making them toxic to insects.

3. Gene therapy

Eventually it may become possible to insert genes into human DNA. This would allow us to treat a number of inherited diseases. Trials are already taking place into the possibility of treating cystic fibrosis in this way. This is the commonest inherited disease in the white population of the United Kingdom. It is caused by a faulty gene which prevents the efficient transport of chloride ions across cell membranes. One of the results of this is that the lungs fill with a thick, sticky mucus which, apart from producing breathing difficulties, makes the person concerned very prone to bacterial infections. By using viruses or small lipid particles as vectors, it is hoped to be able to insert a functional copy of the gene into the lung cells so that the necessary chloride-transporting proteins are produced.

The social and ethical implications of genetic engineering

Like all new applications of biology, genetic engineering is a field which there are many social and ethical issues. You should be aware of the main arguments for and against the use of genetic engineering. The important thing from an examination point of view is to avoid such general statements as "Who are we to play God?" and produce a biological argument supported with valid examples. Some of the important issues are given below:

There may be dangers arising from the accidental release of genetically engineered organisms into the environment. The bacterium which is used in many genetic engineering experiments is *E.coli* which lives naturally in the human intestine. If a genetically engineered strain of *E.coli,* carrying a cancer-causing gene or a gene for antibiotic-resistance managed to invade the human body, the consequences could be disastrous. In addition, different species of bacteria are able to exchange genetic material with each other. A genetically engineered strain of *E.coli* might escape and transfer genes to another species of bacteria. This might mean that bacteria which are present easily controlled by antibiotics might become resistant and thus much more serious.

Genes for herbicide resistance are inserted into crop plants. If the particular herbicide is then sprayed on to a crop, any weeds present will die; the crop plants will not be affected. There is concern that this resistance gene may be able to spread from the crop plants to closely related species of weeds. These weeds will then be extremely difficult to control. By inserting genes for herbicide resistance into crop plants, use of herbicide is encouraged. We still do not know enough about the long-term biological effects of these chemicals on the soil and the organisms found in it or on human health. Recently, genetically engineered soyabeans have been introduced which are herbicide resistant. There are considerable concerns that their widespread planting will lead to even greater contamination of the environment with herbicides.

Most research has gone towards the development of crops which are dependent on high fertiliser input as well as extensive use of herbicides. The companies undertaking this genetic research often produce these chemicals as well. This may be the reason why so little research has been carried out into developing strains which require less fertiliser. The disadvantages of the present situation are clear. Dependence on chemicals which require large amounts of energy to produce cannot continue indefinitely. Most of our present fuel supplies are finite resources.

Practice Questions

Read the following passage.

Volunteers are being recruited to eat raw potatoes in the first human trials of a vaccine grown in genetically engineered potato plants. Researchers hope that people who eat the potatoes will be protected from common gut infections.

The team first tried out the technique in tobacco plants. They took a strain of *E.coli* bacteria that causes food poisoning and identified the toxin as a protein molecule. The toxin binds to receptor molecules on the cell surface membrane of its victim's gut cells. They then used a modified bacterium called *Agrobacterium tumefaciens.* Under normal circumstances these bacteria transfer pieces of DNA known as plasmids into plant cells causing the plant to manufacture the nutrients the bacteria need. In the modified bacteria, however, the gene for producing the *E.coli* toxin had been inserted into the plasmid bacteria.

(a) Use information in the passage to help explain what is meant by:
(i) recombinant DNA;
(ii) a vector (4 marks)

(b) The gene for the toxin may be isolated and put into a plasmid from Agrobacterium tumefaciens. Describe the part played by enzymes in these processes. (4 marks)

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Answers

(a) (i) Recombinant DNA is DNA from different sources which join; eg. the binding protein gene inserted into the plant DNA;

(ii) A vector is something which can be used to insert DNA; *A. tumefasciens/plasmid*;

(b) Restriction endonuclaese/enzymes;
isolating gene;
cut at specific base sequences;
also cut plasmid DNA;
Ligase;
joins pieces of DNA together

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