GENETIC ENGINEERING

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Although recombinant DNA (rDNA) methodology is only a very recent addition to the experimental methods available for investigating the molecular bases of life processes, it has had a very noisy and in many ways undeserved reception by both scientists and non-scientists. On the one hand, it has been heralded as the latest scientific miracle to provide the solution to all the unresolved problems in biology, and on the other, it has been depicted as an unjustified meddling that will result in irreparable damage to existing life forms on this planet.

Furthermore, it has suffered at a rather higher than useful frequency, the calumny of mistaken identity. This has been predominantly a problem of semantics which has been compounded by a journalistic style in which accuracy has never achieved the same status as news. The technique that I wish to describe tonight, I shall refer to as recombinant DNA or as Gene Cloning. It has nothing whatever to do with "Cloning Organisms" as has been achieved with some Amphibia and with many varieties of plants. Nor has it anything to do with "test-tube babies", eugenics or the production of a "super-race". As is probably obvious, I believe that both the good news and the bad news have been overdone in the past, and in this lecture, I will try to give you what I believe is a more balanced perspective for recombinant DNA and the experiments in which it is involved.

First I will endeavour to describe to you the fundamental aspects of the technique and then comment on its relevance to current biological research.

The genetic material of all cells is composed of deoxyribose nucleic acid or DNA. As the genetic material of the cell, DNA has to have the ability both to be replicated and to act directly or indirectly as a library of information for the structure of all of the cell's many thousands of individual components. We now understand a number of the basic principles as to how both of these functions are achieved. The molecule of DNA consists of two strands. Each of these is composed of alternating residues of the sugar deoxyribose and a phosphate group. Attached to each sugar residue is one of four

nitrogenous bases: adenine, guanine, thymine and cytosine. Each strand of a single molecule of DNA may contain as many as ten million sugar-base-phosphate units joined together, but in each DNA molecule each of the two strands of the DNA double helix bears a special relationship to each other. A guanine base on one strand is always found opposite a cytosine on the other and an adenine always opposite a thymine. This so called "complementary base pairing" provides the key to DNA replication and is also the mechanism that allows recombination to occur between homologous DNA molecules. This general recombination between homologous DNA molecules is a major mechanism by means of which the exchange of genes occurs between chromosomes at meiosis and genetic exchange occurs in the more primitive bacterial cells.

About ten years ago very few scientists would have considered the possibility of recombination between anything but homologous DNA molecules. Today, however, we have a significant array of methods for joining together DNA molecules which do not share large sequences in common. In these reactions molecules are joined together by their ends. These ends referred to as "sticky ends" contain a short series of bases on one unpaired single strand which is able to pair with the complementary sequence of bases on the single strand of another "sticky end". This end to end joining reaction is the basis of recombinant DNA technique. Sticky ends are generated by a particular class of enzymes called restriction endonucleases. These enzymes recognise particular short sequences of 4 to 6 bases in the DNA and cut the DNA backbone between the sugar and the phosphate residues.

The probability with which any particular recognition sequences of 6 bases will occur randomly in the DNA is about once per 4,000 bases.

Bearing in mind that the size of an average gene is about one thousand bases, we would expect an enzyme that recognises a six base sequence to cut DNA into fragments each of which would contain about 3 to 4 genes. A second essential component of recombinant DNA work is another DNA molecule called a vector. This molecule usually has the following attributes:

1. it can replicate autonomously in the host cell,
2. it can be readily isolated from such cells and introduced back into the same cell,

and

3. it generally contains a single cut site for the restriction enzyme being used in the experiment.

In end-to-end joining, one cuts both the foreign DNA which one wishes to introduce into the cell and the vector molecule with the same restriction endonuclease, allows the fragments to pair by their sticky ends and joins the molecules together with an enzyme called ligase to produce a recombinant or chimeric molecule. This chimeric molecule can then be introduced into the host cell by a process called transformation and in the host, the molecule is replicated and passed on to progeny cells. The host cell is frequently a bacterial cell such as *E. coli* and individual cells can be recovered and grown under conditions in which a single cell will produce as many as a billion progeny cells each one of which will contain identical copies of the chimeric vector.

If, however, the foreign DNA used in this experiment is, for example, the entire genome of mouse or human cells, the number of different bacterial clones, each carrying a different fragment of human or mouse DNA, that could be generated is of the order of one half to one million. The scientist now has to find the particular bacterial clone that he is seeking and this presents considerable problems. The problem can be resolved if there is a simple method for detecting the desired clone in the presence of all the others or if instead of using the entire genome at the first stage of the experiment, the DNA that is used has been greatly enriched for the gene in question. I would like to tell you how this latter enrichment is achieved as it impinges on some of the work that I want to discuss later on.

Although all the cells of the body are believed to carry the same complement of genes, not all of these genes are active. Differentiation produces cells in which particular genes are more or less permanently switched on and others switched off. Hence while most of the genes of the cell are silent, certain genes are being actively expressed. This expression involves the formation of molecules of messenger RNA which possess a base sequence complementary to one of the DNA strands of the gene in question and from which the specific protein coded for by that gene will be synthesized. Whereas the gene for that m-RNA molecule may be present in the cell as only one of one million genes, that particular species of m-RNA may be the predominant m-RNA species in the cell. Making use of this fact, messenger RNA is isolated from cells in which the desired gene is very active. By means of a series of enzymic steps these m-RNA molecules are converted to molecules of double-stranded DNA called cDNA or copy DNA. These molecules are then provided with sticky ends and ligated into a suitable vector. This is the method that has been used in a number of cases to clone cDNA coding for the structure of important eukaryotic proteins. Examples include insulin, somatostatin and

larger molecules such as the immunoglobulins. The availability of these cDNA probes can also allow one to detect bacterial clones carrying DNA sequences homologous to the probe.

To summarise then, recombinant DNA techniques allow the addition of a small number of genes to a cell using as a carrier a vector molecule which is able to replicate in that particular cell. Although most of the work to date has used the bacterium *Escherichia coli* as the recipient cell, the method can theoretically be applied to any cell type for which appropriate vectors are available.

What have these experiments to offer either to the research scientist or to the technologist and what is there to fear from the possible use of these techniques? We have all seen the "bad news" predictions in the media from time to time and I would first like to comment on some of these concerns that have been expressed.

Much of the early concern that was expressed arose from a series of speculative hypotheses which were not at that time supported by data.

"Recombinant DNA experiments will result in the creation and dissemination of novel pathogenic microorganisms which will either induce cancer or produce some other disabling disease". The major hypothesis was that the random addition of a small number of genes to the bacterium *E. coli* could convert it into an epidemic pathogen. Without going into the evidence, I would like to reassure you that this is no longer considered a possibility. The particular strain of *E. coli* that is used does not colonise the intestinal tract and cannot be converted to a pathogen even by the addition of genes specifically associated with pathogenicity in related Gram-negative organisms. Furthermore, it has been demonstrated that a bacterium into which the entire genome of an animal virus has been cloned is by many orders of magnitude less infectious than the viral agent itself. In fact, it is currently suggested that the safest way to store highly dangerous viruses like smallpox virus is to clone subgenomic fragments of the virus into different strains of *E. coli*.

"The creation of pathogenic strains of bacteria resistant to antibiotics". Although it is possible to use the techniques of recombinant DNA to convert bacterial strains to antibiotic resistance, we have, for the most part, already achieved this on a worldwide basis by our indiscriminate and widespread application of antibiotics. It is a serious problem but it will not be affected by recombinant DNA work.

"The creation of bacterial strains that will multiply in oil tanks, petrol bowsers and so on". I mention this only because this had been featured in the popular press. A scientist in the USA has made a strain of bacteria with an enhanced ability to break down hydrocar-

bons. As it turns out he did not use recombinant DNA to make this strain but recombinant DNA methodology could achieve the same endpoint. The strain was to be tested for its ability to clean up oil slicks. It is not known at the moment whether it would have any application at all. What is known however, is that microorganisms that break down hydrocarbons occur naturally, that in order to multiply they need nutrients, air and water and would therefore only be able to exist at oil-water interfaces. It is impossible to envisage any strains of bacteria multiplying in oil or petrol. We may be in danger of being deprived of our oil supplies but not by recombinant DNA!

"The cloning of people or the construction of super-beings". These proposals have no relevance to recombinant DNA.

"The development of new germ warfare agents that would have distinct advantages over existing pathogens". At the moment, there exists an awesome array of pathogenic microorganisms and viruses that have arisen as a result of natural evolution.

Many of these would be relatively cheap to produce and the major barrier to some malevolent government using them as weapons of war would seem to be the inability to provide suitable defences against a similar attack from the other side. This situation is not altered by recombinant DNA whose further modification of existing pathogens would seem hardly worth the effort.

To look at the other side of the coin it is also true that the technique has been considerably over-sold to the public and long-term projections have been paraded as certainties of tomorrow. I will discuss a few of these.

(1) "Recombitant DNA and cancer". The technique does not offer a cure for cancer. There is no doubt that recombinant DNA techniques have already greatly facilitated our capacity to follow complex interactions occurring in the cell's nucleus. They will certainly assist our understanding of the molecular processes which occur when a cell is transformed to a tumourigenic state but beyond that it is difficult to predict.

(2) "The application of recombinant DNA technology will feed the starving millions of the world". Again, it is possible that with time recombinant DNA experiments will provide improved crops either more nutritious or resistant to particular adverse environments. However, the problem of starvation in the world is a socio-economic problem concerned with distribution of resources and will not be affected by rDNA.

(3) "Another much publicised possibility is the production of super strains of bacteria that will, under natural conditions, carry out all sorts of chemical reactions ranging from leaching mineral ores to

removing pollutants from waste waters". My own view is that this proposal needs to be treated cautiously as under natural conditions microorganisms find themselves in a highly competitive world. In my view, the strain that devotes most of its resources to carrying out the reaction of our choosing will almost certainly be a poor competitor.

(4) "The possibility of a super-race", is included again largely for those who may have been disappointed at seeing it in the "bad news" category. However, the same comments apply. It is in fact now apparent, that even the task of creating a new nitrogen-fixing plant poses so many problems that it can in no way be justified as an immediate possibility.

I am glad to say, however, that there are a number of positive comments that can be made about this technique which has already had a very significant effect on biological research and which will without doubt continue to do so. Much of the knowledge that we have on the structure and function of DNA comes from studies of the simple prokaryotic bacterial cells, and of viruses whether they infect bacteria or man. The very complex eukaryote cell has on the other hand been much more difficult to study. A number of recent developments of which the most striking is recombinant DNA have made it possible to study the structure and function of individual genes in eukaryotic cells at the molecular level. Already, experiments using recombinant DNA have shown that the gene in a eukaryotic cell is a much more complex unit than prokaryotic studies had suggested. Interspersed in the nucleotide sequence which specifies the structure of a particular protein are intervening sequences of DNA (introns) which are later spliced out of the m-RNA before the message is translated. The significance of these introns for theories of evolution and gene control is currently a matter of intensive study.

Recombinant DNA experiments have demonstrated that genes concerned with antibody production undergo a specific translocation at some stage between embryonic and adult life. No doubt much more of the complexities of eukaryotic cells will be unravelled with these techniques.

There has been considerable reporting in the newspapers recently of successful attempts to create strains of bacteria able to produce important mammalian proteins. Although a large number of problems had to be resolved, scientists have now succeeded in introducing cDNA coding for a number of important proteins into the bacterium *E. coli*. Cells of *E. coli* that can produce detectable quantities of human insulin, somatostatin and interferon have already been produced. Unfortunately, some of this information has only appeared in the popular press and not in the scientific journals but there seems lit-

tle doubt after further research to increase yields of product, that these strains will be used commercially to produce these important biological components. Some of them are extremely difficult to produce at the moment. In the pharmaceutical industry this is currently an area of considerable speculation and large sums of money are being floated overseas for ventures in this type of rDNA work.

Although I expressed reservations about the construction of "super-bugs" to let loose in the environment, I have no such reservation about the use of cDNA to design more efficient strains of microorganisms for carrying out specified chemical reactions in pure cultures. It will almost certainly be of assistance in the improvement of strains of microorganisms currently used by industry to produce a variety of products.

In another area rDNA has already demonstrated its usefulness in the production of diagnostic probes. As previously mentioned the technique allows one to prepare quantities of purified gene sequences. By the use of radioactive isotopes and complementary base pairing these nucleic acid probes can be used to detect the presence of their homologues in tissues or cells. Two investigations with applications of this method have occurred recently in Adelaide. Using a nucleic acid probe of part of the hepatitis B viral genome, Professor Marmion and collaborators are searching for the hepatitis B genome in tissues of infected people. Dr Symons is using the same technique to identify latent viruses in avocado plants. By extracting DNA from plant cells and testing its ability to form a specific hybrid with his virus DNA probe, he can very simply and reliably determine whether or not a plant is free of that particular virus. This is of particular value to fruit growers who want disease-free stock.

It has been suggested that recombinant DNA techniques will also greatly facilitate the diagnosis of certain hereditary diseases. Cells obtained by amniocentesis could be examined to detect specific changes in the DNA. Although I can see that this will be a very active area of research during the next few years, it will, I think, be some time before any routine tests will be developed. In a recent article in *Newsweek* it was suggested that recombinant DNA techniques would enable scientists to sequence the entire human genome. In a more sober text I read the other day that, at the current rate of sequencing of 1000 nucleotides a week, it would take 60,000 years to sequence the entire human genome. It is possible to study selected fragments but not the whole genome.

Gene therapy is another subject that is often raised as a possible outcome of recombinant DNA work. Certainly recombinant DNA methodology should allow the identification and purification of cer-

tain human genes. If it is possible, by adding this DNA to cells carrying a defective gene, to obtain some replacement of the defective gene by the normal one, gene therapy may be a possibility. It is obviously an area of research that will receive much attention in the next few years but also an area where many problems have to be solved.

Finally, recombinant DNA techniques do offer real possibilities for producing vaccines against certain viruses which cannot at the moment be propagated artificially. Hepatitis B virus and herpes virus are two examples. If one could clone the genes for the viral antigens into *E. coli* or other microorganisms under conditions in which those genes are expressed, it may well be possible to produce a virus-specific vaccine. The first stage of this programme, namely the cloning, has already been achieved with hepatitis B virus.

It is always difficult to predict scientific developments with any accuracy. I have endeavoured not so much to be the clairvoyant but rather to give you some perspective for the current applications of recombinant DNA in research and perhaps later in applied technology.