Biotechnology and general practice. 1. Recombinant DNA, monoclonal antibodies and genetic probes

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Introduction

FOR most of us biotechnology is an obscure area of biology with apparently little relevance to general practice. Genetic engineering and gene splicing appear to be esoteric techniques which we can safely leave to the experts. The aim of these two articles is to undermine this complacency: biotechnology is going to have far-reaching implications for general practice and some of the results of research are with us already. This article introduces some basic molecular biology, describes some of the practical problems of recombinant DNA techniques and monoclonal antibodies, and explains the principles underlying genetic probes. Wider issues that are implicit in these advances will only be briefly touched on as they are explored in greater detail in the second article.

What is DNA?

Deoxyribonucleic acid (DNA) is the substance from which genes are made. Two features of its structure are important. First, there are two chains of phosphates and sugars that coil round each other to form a double helix. Secondly, packed between these outer chains are chemical bases, which can be of four types: adenine (A), cytosine (C), thymine (T) and guanine (G). The bonds along the chains are strong and require special enzymes to break them. The bonds joining pairs of bases together are weak and allow DNA to split into two separate chains that can form templates for new double helices.

The bases, taken in groups of three, form a coded message. Each triplet, or codon, codes for either a particular amino acid or an instruction such as 'start' or 'stop'. The sequence of bases CGT for instance codes for the amino acid arginine. Thus what is often referred to as 'genetic information' consists of sequences of bases within DNA which, when read in triplets, code for a particular chain of amino acids. The resultant polypeptide may be further processed within the cell to form specific proteins, hormones or enzymes.

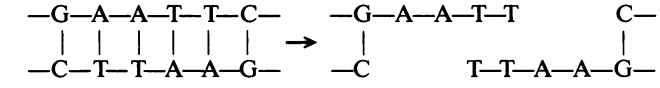
Recombinant DNA

Techniques for creating hybrid or recombinant DNA molecules form the basis of much of genetic engineering. In essence these are simple: a desired gene — that is, a particular sequence of bases — is isolated from a mass of fragmented cellular DNA and is inserted ('recombined') into another easily handled microorganism, usually a bacterium or yeast. Once in its new host the gene can then be expressed — that is, read by the protein synthesis machinery of the bacteria — and the desired gene product extracted from the bacterial culture. Ideally, the host organism will secrete into the surrounding medium the molecule it has been reprogrammed to make.

Putting it as simply as this conceals a multitude of technical complexities but captures the important features of the technology.

Restriction enzymes

One of the major breakthroughs in genetic engineering was the discovery of a way to cut the DNA molecule at specific locations. This is possible using naturally occurring 'restriction enzymes' which only act at a particular sequence of bases on the DNA molecule and then cut the molecule in two at that 'restriction site'. For example, when the *Eco RI* restriction enzyme finds the sequence of bases GAATTC it divides the backbone of both chains of DNA as shown below:



There are now over 300 restriction enzymes known, each of which will split DNA at a certain point. This wide choice ensures that a suitable restriction site can usually be found which lies near the beginning or end of any desired sequence of bases of DNA.

Digesting DNA (which is easily obtained from leucocytes) with a restriction enzyme yields a mass of fragments because the enzymes will chop up the DNA at every restriction site along the length of all the DNA molecules. Fortunately, it is fairly easy to separate out the particular fragment carrying the gene of interest using electrophoresis. Each piece of DNA is a unique length and travels a particular distance in a given time. The various bands on an electrophoresis gel thus represent fragments of different sizes. The particular band that corresponds to the gene of interest can be identified by a probe of DNA that is complementary to a short part of the known DNA sequence of the gene. This probe, which is labelled by the incorporation of radioactive atoms ^{3}H or ^{14}C, will only bind to the gene sequence of interest. Its radioactive label allows direct identification of the fragment.

Insertion into the host

After the gene has been isolated it is inserted into a suitable microorganism. For bacterial hosts this is usually achieved using plasmids, which are naturally occurring loops of DNA that bacteria exchange between themselves. By splitting the plasmid ring with a restriction enzyme and then rejoining it to include the foreign DNA, the gene is parcelled up in a form acceptable to the bacteria. The bacteria are plated out in a culture medium that selects for the tiny minority of cells which have taken up and expressed the inserted gene. Large quantities of these recombinant bacteria can then be grown as a clone. Large scale production can still be difficult since the bacteria tend to loose their plasmids and extracting the gene product from the culture may not be easy.[1]

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Table 1. Some physiological substances that have been cloned.

Polypeptide	Number of amino acids	Possible use
Insulin[a]	51	Treating diabetes
Growth hormone[a]	191	Treating small stature, wound healing, treating senile osteoporosis
Interferon[a]	Variable	Treating viral infections and cancer
Calcitonin[a] and calcitonin gene related product	32	Treating Paget's disease, vasodilation
MSH (melanocyte-stimulating hormone)	13	Improving memory
Cholecystokinin	33	Suppressing appetite
Endorphins	31	Treating pain, altering mood
Factor VIII	2332	Treating haemophilia

[a] Already produced in commercial quantities. Adapted from: Office of Technology Assessment USA 1982.

Implications for general practice

The most immediate result of recombinant technology is the ability to clone and produce many physiologically active polypeptides (Table 1). Some of the smaller molecules — those of about 30 amino acids or less — have in the past been available through chemical synthesis and others, such as insulin, have been extracted from animals, but many are now being produced for the first time.

Although some of these substances might at first seem obscure they are of major practical importance to medicine. Insulin, factor VIII and perhaps interferon have direct therapeutic uses and many of the others are leading to significant advances in our understanding of disease. In the somewhat longer term such new knowledge will generate novel therapeutic agents.

A major barrier to be overcome in designing these molecules is to make them orally active. All the direct products of gene transcription are proteins and hence are susceptible to the processes of digestion. The secondary process of designing molecules which mimic endogenous peptides but which are protected from breakdown in the gut has already been achieved for at least one of the substances in Table 1.[2] This is equivalent to devising an orally active insulin (something which a number of pharmaceutical companies are also working on).

Endorphins provide a good example of the benefits, and problems, we can expect from these advances. These opiate-like substances occur in brain tissue and have revolutionized thinking about the perception of pain and the placebo effect. Genetic engineering techniques have made the analysis and synthesis of these products much quicker than they would otherwise have been and work is in progress to develop drugs that will exploit and perhaps amplify the analgesic and mood altering characteristics of endorphins. Although there are obvious benefits from this there is also the risk of introducing new drugs of addiction. It has also been suggested that endorphins are the biochemical mediators of ecstatic religious and sexual experiences. Not only does this underline the potential for abuse it is also one example of how easily this new understanding can devalue the symbolic and emotional dimensions of human experiences to the level of biochemical events.

New vaccines

The essential requirement for an effective vaccine is a good supply of pure antigen that, when introduced into the host, will induce the production of antibodies that are lethal to the invading organism. Viruses consist of genetic material surrounded by a coat of protein. The protein coat alone can be immunogenic and is non-infective. Since these surface antigens are coded by sequences carried in the pathogen's genetic material, theoretically, they can be isolated and cloned. Thus, recombinant technology provides the potential for an efficient method of obtaining large amounts of antigen that are totally uncontaminated by the pathogenic organism. This has already been achieved for hepatitis B and has led to a genetically engineered vaccine that will soon replace the expensive agent painstakingly recovered from individuals affected by hepatitis B. Genetic engineering is being used to provide pure antigens that may prove effective as vaccines for a number of diseases, such as:

Hepatitis B
Malaria
Acquired immune deficiency syndrome
Herpes simplex
Rabies
Trypanosomiasis
Gonorrhoea
Dental caries

Antigenic sequences have already been cloned for the first four of the above, although this does not necessarily mean that they can induce effective immunity.

If vaccines for these diseases can be produced this would be a milestone in medicine with enormous implications. The vaccines would be particularly useful in the Third World, where, for the first time, immunization might be available for some protozoal diseases. More prosaically, there will also be the attendant practical questions. Which groups require immunization? Who is best placed to carry out such a programme? Who will pay? As with other immunization programmes there are good arguments for many of these new vaccines to be given in a primary care setting. Yet to be successful such programmes will demand time and commitment, teamwork and a high degree of organization. Can general practice deliver these?

Monoclonal antibodies

Antibodies are made by plasma cells. Each of the numerous antibodies produced by the body is made by a single cell line of plasma cells dedicated to making that antibody and no other. If plasma cells could be grown in culture as a pure clone then this would yield a single, or monoclonal, antibody. Unfortunately, plasma cells cannot be grown *in vitro*. However, by combining them with other types of cell to form what is known as a 'hybridoma' it is possible to induce them to replicate and preserve their ability to make their own particular antibody. The process is technically very complicated and it is usually far from easy to isolate and propagate desired hybridomas. Nevertheless, a rapidly increasing range of ultra-pure monoclonal antibodies is now available.

Applications of monoclonal antibodies

Antibodies have a number of useful properties quite apart from their role in fighting disease. First, they provide a powerful tool for investigating physiological and pathological processes. Since any given antibody recognizes only one particular antigen among millions a pure supply of this antibody allows minuscule quantities of antigen to be detected. Mice, for example, can be made to produce antibodies to human proteins and in this way monoclonal antibodies to many normally occurring cellular materials have been made. In addition to extending our knowledge of physiology this technique has many direct

diagnostic applications. For example, it is likely that over-the-counter urine-testing kits will soon be available that will predict and detect when ovulation has occurred. More impressive still is the intense search to identify an antigen which is characteristic of carcinoma cells. A monoclonal antibody to such an antigen could lead to a generalized screening test for some, or possibly all carcinomas. Such a test has already been advertised in the medical press, although it is not clear how effective it actually is.

A second proposed use for monoclonal antibodies is as highly discriminating messengers taking drugs to specific sites. For example, it is hoped that by linking cytotoxic drugs to tumour-derived antibodies it might be possible to deliver the drugs very accurately to the site of the tumour and avoid many of the systemic side-effects. In practice this has, as yet, proved hard to achieve.

Recombinant techniques are at the heart of biotechnology. They allow us to cut up DNA and splice sequences into unrelated organisms. However, DNA consists of three billion bases and it is estimated that only 10% of the molecule is required to code for all known human proteins. The purpose, if any, of the rest of the DNA molecule is a matter of much debate. In order to be able to use recombinant techniques we must be able to identify the relevant sequence of bases. Gene probes provide a way of mapping chromosomes in this way and of finding out which genes lie where.

Genetic probes

Genetic probes are another of the new tools of biotechnology. They have immense clinical potential and during the next decade they are likely to lead to a revolution in the understanding and therapy of many diseases. By allowing a genetic probe to bind with naturally occurring DNA the position and sequence of different genes can be identified. The use of such probes may allow pathology to be directly related to disordered sequences of bases within DNA. New forms of treatment are likely to follow these insights.

Direct probes

The concept underlying genetic probes is simple: a probe is a short strand of single chain radioactively labelled DNA. Two single strands of DNA that have a complementary sequence of bases will bind together. By binding with their complementary sequences in natural DNA probes can be used to recognize specific sequences of bases on the molecule, that is, identify the position of different genes.

Studies on sickle cell anaemia provide a good example of how the pathology of a condition can be traced back to disordered DNA. By 1949 classical genetics was able to demonstrate that this condition is inherited in an autosomal recessive fashion. In the 1950s the pathological basis of sickling was found to be due to the substitition of a single amino acid on the beta protein of haemoglobin. This change of one amino acid is caused by the alteration of a single codon, or triplet of bases, within DNA. This knowledge has allowed the construction of a probe that will match the DNA that codes for this protein. By changing the relevant triplet of bases this probe can be made so that it will only bind to DNA that codes for the sickling trait, that is, it can directly recognize the difference of one codon among the one billion that go to make up our total complement of DNA.

This direct identification of abnormal genes can be applied only to those cases where details of the genetic fault are known. However, gene probes can also be used when the gene sequence is not known.

Indirect probes

If DNA is incubated with a restriction enzyme that cuts the DNA at a certain point, the resultant array of fragments of DNA of differing lengths can be separated out by electrophoresis. Since restriction sites can arise or be deleted by natural mutations the pattern of such fragments (technically known as restriction fragment length polymorphisms — RFLPs) varies between individuals; for close relatives the pattern will be very similar while non-relatives may have quite dissimilar patterns.

Consider a gene defect that confers a serious disease but about which nothing else is known. By looking at the way particular restriction sites are inherited among relatives at risk of the disease and by combining this with knowledge of where a particular probe binds, it has been possible to locate the approximate site of a gene.

Once a restriction site has been linked to a particular condition it is then possible to use the inheritance of the restriction site as a substitute for the gene itself. By combining data from several generations on how the linked restriction site is inherited it is often possible to state the probability of any one individual carrying the gene in its mutant form. This complicated process has already been carried out for several diseases including Huntington's chorea and Duchenne muscular dystrophy.

One feature of this gene mapping should be emphasized: because the gene is being detected indirectly it is possible only to give a probability of whether it has been inherited. The more family members that have been tested the easier it is to give an accurate probability of inheritance. Thus the clarification of the genetic status of one individual can necessitate tests on many of the relatives. For this reason it is likely that the clinical use of indirect probes will be confined to serious inherited diseases.

Genetic analysis, both direct and indirect, can be used to identify people who are unaffected heterozygotes, that is, carriers of abnormal genes who do not themselves suffer the related condition. This enables parents to be counselled about the possibilities of genetic defects being passed on and expressed in their offspring.[1,3,4]

Antenatal diagnosis and chorionic biopsy

A major use of genetic probes is in prenatal diagnosis. DNA analyses can be done using material from a very small number of fetal cells. Recently an alternative to amniocentesis has been developed called chorionic villi (or villus or villous) sampling. Samples of the chorion are biopsied via the cervix so yielding cells which are genetically identical to fetal cells and from which the micrograms of DNA required for genetic probe studies can be extracted. The advantage of chorionic sampling is that it can be done as early as the seventh week of pregnancy, although the high spontaneous miscarriage rate at this stage of preganancy makes it difficult as yet to tell how much risk of abortion attaches to the procedure itself.[5]

Implications

Genetic probes offer the possibility of exploring the human DNA molecule and so identifying the structure and function of each gene. Mapping all three billion base pairs is clearly an immense task, but clinically useful information arises quite early in this process and is already changing clinical practice.

Conditions that result from a single gene defect such as the haemoglobinopathies and haemophilias have been the easiest to study so far. From the point of view of general practice such conditions may seem rare. However, since it will now be possible to locate the carriers of recessive genes, the number of people in any practice who will ultimately be affected by such techniques may be large. For example, if a probe for identifying the gene

for cystic fibrosis were to become available then screening for the carrier state would be possible. Since this recessively inherited gene is carried by one in 22 people in this country an average practice includes about 100 carriers. We must ask ourselves whether identifying and advising people who carry recessive genes should become part of general practice.

Antenatal screening

The advances in antenatal diagnosis also have practical implications for family practice. General practitioners' knowledge of patients' kinship patterns and clinical problems places them in a unique position to discover which couples might benefit from antenatal diagnosis. Family doctors are often consulted early in a pregnancy and so will bear the responsibility for the referral of any women at high risk of carrying a genetically abnormal fetus. Genetic diagnosis early in pregnancy will therefore become more important. Chorionic sampling is possible from around seven weeks of gestation but this still leaves little time to perform the test, obtain the results and arrange a first trimester termination if required.

General practitioners will also be concerned with the consequences of hospital screening programmes and will wish to support women through this experience. As general practitioners we have much to learn in this field before we can begin to offer what is needed and what will increasingly be asked of us.

Screening in adult life

As more is known about the structure and control of human DNA, the genetic component of many medical conditions will be progressively clarified. It is possible that probes identifying a genetic disposition to diseases such as diabetes will be produced. Researchers are still a long way from achieving this since for most major diseases a complex of several genes seems to be involved. If efforts in this direction are successful, however, screening for such diseases could mean testing for the presence of those genes which are known in later life to produce a high risk of the disease. Such screening could theoretically take place from the point of conception onwards.

Screening for genetic predispositions, both in the fetus and the adult raises a number of interesting and difficult questions. Being able to predict an individual's risk of developing rheumatoid arthritis in 10 or 20 years time is a feat of great technical virtuosity but of what practical use would it be? Who would want this information? Employers and insurers perhaps — but their interest will not always be benign. Would it ever be ethical to apply such screening tests antenatally with a view to terminating the pregnancy if the test were positive? How much fear and anxiety would such knowledge of personal genetic predispositions create?

Gene therapy

Our ability to manipulate genetic material has reached the stage where it may soon be possible to correct genetic defects directly at the level of the DNA molecule. Such 'gene therapy' would mean taking cells from an affected person, isolating and removing the defective gene sequence and inserting the correct sequence. If these cells can then be reintroduced to the patient so that they both multiply and express the corrected gene, then the disease may be ameliorated or even cured. An unsuccessful attempt to do this for thalassaemia has already been made. Serious discussions are going on at present in the USA concerning plans to repeat similar experiments in children who are terminally ill with several very rare genetic diseases.

DNA probes offer a quantum leap in our knowledge of our own genes. They open up the interior world of our chromosomes to understanding, screening and perhaps manipulation. The first clinical fruits of this process are already with us and the coming decade promises many practical applications of direct consequence to general practitioners. Some of the wider problems and dilemmas created by these powerful new tools will be discussed in the second article.

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Further reading

For a fuller explanation of the molecular biology underlying recombinant DNA techniques see: Wolpert L. DNA in medicine: DNA and its message. *Lancet* 1984; **2:** 853-856. For a clear exposition of both the technology and wider issues aimed at the non-expert audience see: Yoxen E. *The gene business.* London: Pan Books, 1983.

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Stress and general practice

For many physicians being a general practitioner is a stressful occupation. General practitioners often feel subjected to many demands and expectations, and the way he or she reacts to the demands made is important. Some doctors are stimulated to perform well in caring for their patients; for others the consequences are more likely to be negative, especially if the load and tension become too high. It seems likely that there is a relationship between a doctor's experience of work as a general practitioner and the quality of patient care.

The emotional reactions of 57 general practitioners to three aspects of work were assessed using questionnaires and the quality of patient care by means of observations of general practice consultations, assessment of audiotaped consulting hour contacts and an analysis of the referral and prescription figures. A distinction was made between the degree of positive and the degree of negative feelings general practitioners have about their work. Many positive feelings (satisfaction; feeling at ease) correlated with more openness to patients, more attention to psychosocial aspects of the complaints but also with a higher rate of referral to medical specialists. On the other hand, many negative feelings (frustration, tension, lack time) correlated with a high prescription rate and with giving little explanation to patients. To some extent the way the work is experienced by general practitioners correlated with the quality of care for the patients, but what constitutes cause and effect requires further study. A reflection of a doctor's own feelings about work should become part of training, continuing education and medical audit programmes.

Sources: Grol R, Mokkink H, Smits A, van Eijk J, *et al.* Work satisfaction of general practitioners and the quality of patient care. *Family Practice* 1985; **2:** 128-135.