Basic Molecular and Cell Biology

Production and use of therapeutic agents

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All proteins—whether they are hormones such as insulin, growth hormone, or calcitonin, enzymes such as the clot forming and clot lysis components of blood, or antibodies—have a structure which is determined by the gene that specifies them. Genes are composed of the chemical deoxyribonucleic acid (DNA). Proteins are linear molecules composed of large numbers of 20 amino acids; their structure is determined by the order in which the amino acids are arranged. This order is in turn determined by the order of the components of DNA, which is also a linear molecule.

Each cell has its component of DNA molecules, but not all of them are expressed—that is, used to give rise to the proteins they specify. The choice of genes which are expressed depends on the stage in the life cycle of the cell or on what kind of cell it is. Fibrinogen, for example, is made in the liver, haemoglobin in erythrocyte precursors, and myosin in muscles. The genes for these proteins are not expressed in cells which do not make them. This means that within each cell is a set of control mechanisms which determine which of its genes are expressed and in what order. Thus simply introducing a gene into a foreign cell does not guarantee that the protein product of that gene will be made by the cell. The correct control mechanisms are also needed.

The expression of recombinant DNA in a cell was first achieved with the bacterium *Escherichia coli*, an organism whose genetics have been so well studied that it was easier to predict the requirements for expression. Since then expression systems have been devised for many types of cell, including yeast and animal cells growing in culture. All of them allow for the controlled production of the protein in fermentation systems similar to those used for producing antibodies. The cell system which a researcher chooses nowadays to express a protein depends on the nature of that protein and the use to which it will be put. For example, if a large quantity of the protein is required he would probably choose a micro-organism as the means of production. If the protein is large and complex he would be more likely to choose an animal cell in culture.

One special technique for producing potential therapeutic agents is the monoclonal antibody technique, also referred to as the hybridoma technique. The end result of this approach is the production of a protein, the desired antibody, in a tissue culture cell; operationally, therefore, it is similar to the systems described above. The method of deriving the cell line is different, however, as it depends on the fusion of two different cell types to introduce the gene into the production cell rather than introducing it as a piece of purified DNA. The earlier methods of making antibodies—that is, injection of a suitable animal with the antigen—resulted in serum which contained a mixture of antibodies with a wide variety of properties. The hybridoma technique allows the selection of a cell

making only one of the whole repertoire of antibodies which an intact animal would make when exposed to the antigen.

The processes of fermentation and protein purification which are used in the production of proteins are well understood so that, although there have been some new problems and some special features to consider, the production of high quality therapeutic proteins is becoming increasingly easy.

Therapeutic use of recombinant protein

What are these proteins used for? What advantages do they bring, and what are their problems and limitations? The most important reasons for preparing therapeutic proteins in this way are that they cannot be obtained by more traditional means such as extraction from natural sources or, if they can, that the product is in some way unsatisfactory.

HUMAN INSULIN

The first product of any consequence to be made by recombinant DNA techniques was human insulin. Diabetics have been treated with insulin extracted from animal pancreases (mainly pig and beef) for some time. Natural human insulin has not been available because, in the face of the satisfactory performance with the animal products, it was not considered worth while to set up the complex procedures for collecting human pancreases from cadavers, even if the supply would have been adequate. Now, however, a product identical to the natural human product is available through fermentation in *E. coli*. Although it may be some time before we know whether this product results in any improvements in treatment compared with the animal products, it is certainly equally effective and safe.

GROWTH HORMONE

Unlike insulin, growth hormone is species specific. Patients who suffer from hypopituitarism and need replacement treatment must therefore be given the human hormone. Until recently this came from human pituitaries obtained from cadavers. Such treatment was considered satisfactory until suspicions were aroused that a few patients had been infected through the treatment with a slow acting virus which gave rise to the Creutzfeldt-Jakob syndrome. The natural product has now been largely removed from the market. Fortunately, replacements from a number of sources, made by means of recombinant DNA, were almost ready for introduction, and they have been developed rapidly.

This then is an example of the use of a recombinant product to substitute for a natural product which proved unsatisfactory because of viral contamination. A similar problem has arisen with the contamination of factor VIII preparations, used for treating haemophilia, with the virus which causes the acquired immune

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deficiency syndrome (AIDS). In this case, however, the recombinant product is not yet available, apparently because of the great complexity of the protein. Alternative means have been used to make the factor VIII preparations safe.

HEPATITIS VACCINE

Hepatitis B is caused by a small DNA virus and has hitherto been impossible to control by a vaccine because the virus could not be grown in any of the normal laboratory systems used for preparing viruses—that is, tissue culture cells or embryonated eggs. Until recently the most promising vaccine has been a killed virus preparation made from the plasma of infected individuals. This has obvious drawbacks. The collection process is laborious, and there is no possibility of developing safer attenuated forms of the virus as has been done, for example, for polio. Ultimately, the more successful the vaccine the more difficult it would be to prepare because the number of infected individuals would decrease. It has to be admitted, however, that this limitation is a long way off.

Again, the solution to this problem has been to clone the appropriate gene from the virus into a micro-organism and to express it to obtain viral antigen. One of the more promising preparations has come from a yeast clone of this gene. This preparation is safe both for the patient and for the people responsible for its manufacture because there is no virus present. The yeast cell contains the information for only one gene from the virus. Thus there is no way that a complete virus or any other infectious agent could arise in the vaccine.

This technology therefore represents a promising way of making vaccines for agents which are difficult or dangerous to grow in culture or where the vaccine itself may be a danger to the patient, who is not usually ill at the time of treatment. It will undoubtedly be central to the production of a vaccine for AIDS. The techniques are also being used to dissect the molecular biological intricacies of the agents causing malaria, schistosomiasis, and trypanosomiasis, with the eventual aim of vaccine control.

ANTICANCER MONOCLONAL ANTIBODIES

The immune system has long been implicated in the body's defence against cancer, a defence which obviously goes awry when the disease becomes established. This thought has led to the proposition that stimulating or substituting for the presumed defects of the endogenous system may be a route to controlling or curing the disease.

One of the routes of attack has been to try to obtain tumour specific antibodies and to attach chemotherapeutic or other cell killing agents to them. If successful this would result in the targeting of the killing agent on the tumour, greatly reducing the toxicity in the rest of the body. Promising results were obtained some time ago in animal models with polyclonal antibodies, but for a variety of reasons these could not be reproduced in man. One of the reasons may lie in the fact that a polyclonal serum contains many different antibodies and, indeed, many non-antibody proteins. If an antibody to a particular tumour cell determinant was required, its concentration in the mixture would be so low that it would be ineffective. Monoclonal antibodies clearly represent a way of overcoming this problem. High concentrations of a single antibody which is specific to a selected determinant on the target cell can be obtained.

Much work has been done to discover appropriate antibodies, and many promising candidates have been found. One important principle has been agreed on as a result of this work—that is, that it is unlikely that a truly tumour specific antigen exists. "New" antigens which appear on tumours have been found on other cells at

some time in the lifetime of the individual, either during development or on stem cells in the tissue in which the tumour originates. This means that if antibodies can be used as cytotoxic agents care will have to be taken over dosage and distribution among tissues and cells.

One further feature of antibodies which needs to be taken into account is that they are not designed to be used as antitumour agents. There are therefore a number of features of the antibody molecule which could mitigate against its usefulness. The methodology of recombinant DNA now provides the opportunity of modifying the structure of antibodies in a controlled way to overcome this problem. The genes for a candidate antibody may be cloned, then modified to alter or delete unwanted portions, and finally reinserted into a suitable cell for production.

It is too early to say how successful this approach will be in a wide variety of tumours. The early results are promising, however, and it seems likely that modified antibodies will be among the collection of drugs that will be used to control tumour growth before long.

LIMITATIONS OF THE TECHNOLOGY

A number of other important products under development are emerging from the use of this technology in health care. Examples are tissue plasminogen activator for use in myocardial infarction, erythropoietin for use in various forms of anaemia, and lymphokines and growth factors, which may find a variety of uses.

The major limitation of this approach is that all the therapeutic agents are proteins. They cannot be taken by mouth because they would not survive exposure to the enzymes in the gut and would not be absorbed from it. They must therefore be given by injection, which limits their use mainly to patients in hospital.

Treatment of the diseases under consideration by the first wave of products coming from the technology will not be hindered by this problem as the patients are already being treated in hospital. There are many other conditions, however, where frequent attendance at hospital and treatment by injection is undesirable or impossible. These include the various forms of arthritis, hypertension, and osteoporosis. This does not mean that molecular biology has nothing to contribute to the discovery of treatments for these diseases.

New approaches for the future

The development of modern drugs in the past 30 years or so has depended on our understanding of the basic biology of the disease and the quality or relevance of the model systems used to test potential drugs. It has become apparent during this period that active compounds achieve their effects by combining with macromolecules in the organism, termed receptors, and consequently modifying or mimicking the effect of a natural molecule *in vivo*.

Many drugs have been discovered and developed without knowledge of the nature or function of the receptor concerned. It is clear, however, that a better understanding of the nature of the receptor and its natural activation will help the design of active drugs. This is where molecular biology will have a major impact on drug design. The genes for many receptors and their activators are now being cloned. In many cases this will lead to the design of model systems using the receptor, which would not be possible in the natural state. In the longer term the structure of both natural molecules can be elucidated by x ray crystallography or by other techniques so that the detail of their interactions may be examined by computer graphic modelling. This will allow a much more precise design of potentially active compounds.