INTRODUCTION

A recent review of the Biotechnology Industry listed 48 small companies (Genentech being the largest of these) and 9 Pharmaceutical majors in this field. The areas being investigated ranged from Immune Modifiers and anti-Cancer agents through Blood Proteins and Hormones to Vaccines and Anti-infectives. Indeed, a number of these products, namely human insulin, human growth hormone and interferon alpha are now being marketed while others such as tissue plasminogen activator and hepatitis B vaccine are in advanced trials.

In all cases so far the products on the market are sold as injectables. Insulin and growth hormone are replacement products and so this method of administration is not questioned at present. Interferon is being used to treat hairy-cell leukaemia and again an injection is acceptable. Indeed injections will probably be acceptable in most situations where life is threatened; a good example of this is the use of tissue plasminogen activator on patients with coronary thrombosis. But what of products that are not aimed at life threatening diseases or situations, those that may reduce pain in arthritis sufferers or reduce the incidence of fractures associated with osteoporosis. What will patient compliance be if these have to be injected frequently? Is there any alternative to injections?

The answer to the last question is yes. There are two main strategies that are being pursued. The first is to develop novel systems to deliver these new protein and peptide drugs. This concept is not really new. What is new is the type of molecule under consideration. In general these are molecules with relatively high molecular weights and probably low stability. The problem is how to get them absorbed efficiently and this is really the subject of this meeting. The second strategy is more radical and long term. Here the proteins and peptides are seen as a means to an end rather than an end in themselves. It is accepted that delivery of large proteins is difficult and that a solution may not be found. Instead the proteins and their effects are analysed in detail in the belief that an understanding of protein-protein interactions will allow the rational design of small chemical molecules that will mimic those actions of the particular protein and be orally active.
Which of these approaches is better cannot be stated at present. What is clear however is that for either to even begin, a source of protein or peptide is needed and this is where Biotechnology comes in. The Biotechnologist now has available to him a choice of systems for producing proteins. These have evolved over the last four or five years as knowledge has accumulated and some of the problems become clearer. The remainder of this paper will review these systems and discuss some of the problems and their implications. References to original papers have not been included in this discussion. If required they can be found in reviews by Harris (1983), Harris and Emtage (1986), Carter et al (1986) and Bebbington and Hentschel (1986).

THE SYSTEMS

Gene expression means taking the genetic information stored in DNA and decoding it so that a protein molecule is produced. This is done by transcribing the DNA into RNA and translating the RNA into protein. Three types of cell are currently used for protein production by expression of foreign genes. These are Escherichia coli, the yeast Saccharomyces cerevisiae and various mammalian cells. Although the general requirements for gene expression do not vary greatly among these cells, the specific mechanisms of transcription and translation are quite different. Thus there are specific transcriptional and translational barriers between the three types of cells mentioned above. The important conclusion from this is that to optimally express, for example, a gene from a human cell in E.coli, one must mimic and/or exploit the features of E.coli genes that result in high levels of both transcription and translation. Usually this is done by creating for the foreign gene a molecular biological environment as similar as possible to that of the genes from the host cell itself. From this comes the concept of an 'expression cassette' for the different cell types into which different genes may be placed. It is comprised of three main features, a region of DNA capable of binding RNA polymerases and initiating transcription (the promoter), the gene itself and a region downstream of the gene that is involved either in transcription termination (for E.coli and yeast) or mRNA maturation and polyadenylation (mammalian cells).

It is beyond the scope of this paper to describe the details of either prokaryotic and eukaryotic promoters or transcription termination. Suffice it to say that many systems are now available and that moving from E.coli through yeast to the higher eukaryotic cells results in increased complexity and choice of system. In E.coli the situation is quite simple; promoters are well characterised and function effectively in most E.coli strains. In yeast, strain differences start to become important. For example the α-factor promoter functions in α cells but not in a cells or a/α cells. In animal cells the situation is even more complex and the final choice of cell type and expression cassette will depend on whether one is trying to establish permanent cell lines, whether the DNA should be maintained episomally or integrated into the chromosome, whether a suspension cell is favoured over an anchorage dependent one and whether the protein will be correctly modified by one particular cell.

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There are two general strategies for obtaining expression of the foreign genes in E.coli. Either the protein is produced as a fusion by cloning the foreign gene downstream of and in frame with a bacterial coding sequence or as a 'native' protein by direct expression of the gene.