DNA makes protein makes money

from J. S. Emtage

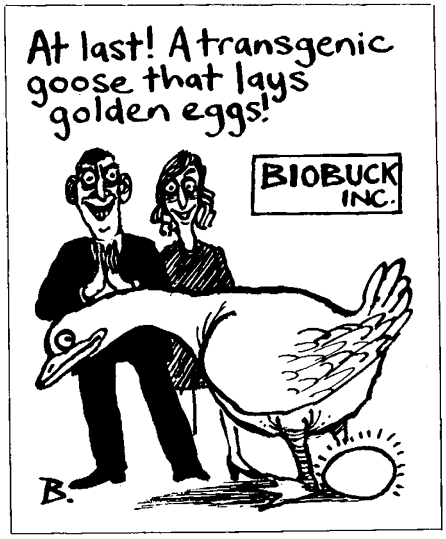
The problems involved in producing unlimited quantities of hormones and similarly useful proteins by recombinant DNA techniques are fast being solved. With their solution comes the chance for those involved to reap almost equally unlimited financial rewards.

It is just twelve years since Stan Cohen, Herb Boyer and co-workers published the results of their work on transformation of *Escherichia coli* by R-factor (plasmid) DNA and on the construction of biologically functional bacterial plasmids *in vitro*. This work provided the cornerstone of recombinant DNA technology — the ability to cut and join DNA molecules very specifically and to introduce these molecules into *E. coli* in such a way that individual molecules, or clones, are selected. Of course, as with all major advances, other discoveries were soon made that expanded the power of the technology. In 1975 Ed Southern described a method for detecting specific sequences among DNA fragments separated by gel electrophoresis, the so-called Southern blot. We now have 'Northern' and 'Western' blot techniques for RNA and protein molecules respectively. Subsequently, in 1977, Maxam and Gilbert in Harvard and Sanger and colleagues in Cambridge independently developed methods for sequencing DNA. It is this combination of methods that has revolutionized molecular biology in the last decade. One practical result of this revolution is the renaissance of biotechnology with its potential applications in medicine, agriculture and industrial microbiology which a host of small companies hope to exploit.

It was the prospect of being able to produce unlimited quantities of proteins like insulin, growth hormones and the interferons that provided the driving force for the formation of these companies. At that time it was tacitly assumed that expression of heterologous genes in prokaryotic hosts like *E. coli* and the purification of the resulting protein would be simple. This has turned out not to be so and we now know that *E. coli* is not the perfect host for producing human or animal proteins. Many of them are found, after synthesis, in an insoluble, denatured form within the bacterium and tedious procedures involving chemicals like urea and guanidinium hydrochloride are required to render them soluble and active. *E. coli* has other problems too — frequently the initiation methionine is not removed from the protein and thus human growth hormone (hGH) for example is produced as MethGH. Moreover, *E. coli* does not add the carbohydrate side chains found on some eukaryotic proteins and, in situations where glycosylation is necessary for biological activity, this will clearly be a major problem. Last but not least the complete removal of endotoxins from the product must be achieved during purification.

Because of these difficulties major efforts have been made to develop expression systems for yeast and mammalian cells. Yeast because of the existing knowledge of how to grow yeast on a large scale and because of its potential for secreting proteins and peptides into the medium and lack of toxins. Mammalian cells have been used because they will glycosylate proteins as well as secrete them. In both cases, secretion should result in a product with the correct N-terminal amino acid.

In the longer term the general opinion is that, for production, none of the above systems will predominate. Rather it will be 'horses for courses' and much will depend on the final use of the protein, how much is required, the productivity of the different systems and the final cost. For some medical products where the volume required is low and a premium price can be charged, an animal cell system might be suitable. For other products like animal growth hormones where the predicted requirement is greater than 100 tonnes per year and the cost per dose must be low to make economic sense, then a route using a fermentable microorganism seems the only option at present.



As well as the progress discussed above on gene expression, all of which stems from an understanding at the molecular level of the interactions taking place during transcription and translation, recombinant DNA technology has had a fundamental effect on our ability to access genes for proteins whose messenger RNAs are present in cells at low abundance. There are three methods that are commonly used at present. Genomic libraries made in phage vectors or complementary DNA libraries made in plasmids or phage vectors are screened using either a mixture of short oligonucleotides or a single long oligonucleotide as a probe. Obviously this approach is dependent on knowledge of some sequence of the protein of interest. In the second method cDNA libraries are made in an expression vector, such as the phage λgtll, so that a fusion protein is produced consisting of the majority of β-galactosidase with the information encoded by the cDNA insert as a C-terminal fragment. Recombinant phage are then screened for the presence of antigenic fragments using an antibody to the protein.

The final method, unlike those above, does not require any detailed knowledge of the protein itself; all that is needed is a biological assay for the activity of the protein. In this system, libraries are made in *E. coli* in 'shuttle' vectors which are capable of high efficiency, transient gene expression in animal cells. Libraries are split into pools, DNA isolated from these pools and transfected into the animal cells. After incubation for 2–3 days medium from these cells is removed for bioassay. Once an active pool has been identified the specific clone in it is purified by sib selection. One or other of these procedures has been used in recent months in the cloning of the cDNA genes for protein such as tumour necrosis factor (TNF), colony stimulating factor (GM–CSF), human transforming factor-β as well as a number of receptor molecules.

Through genes and gene probes, recombinant DNA technology is also impacting on the diagnosis of (but not yet the treatment of) inherited diseases such as the haemoglobinopathies, haemophilia, phenylketonuria and Down's syndrome. The aim at present is to offer antenatal diagnosis and the possibility of a termination to pregnant women identified as at risk. This is done by isolating fetal cells by amniocentesis or from chorionic villi and culturing these to provide sufficient DNA for analysis. Gene probes are then used in Southern blots to show the presence or absence of the particular gene. This is convenient for gene deletions but what about point mutations? Until recently this has been a problem. However, work with

short oligonucleotide probes (19-mers) has shown that conditions can be defined where they will hybridize to normal but not to mutant alleles. Thus, provided the nucleotide sequence around the mutant site is known, then diagnosis using this approach should be possible.

Once a gene, or a gene family, has been cloned the present technology allows us to produce novel proteins either by 'shuffling' domains or by the more specific techniques of *in vitro* mutagenesis. Domain shuffling has been used mainly for the α-interferon family and really involves switching restriction fragments between two related genes. In this way a hybrid A–D α-interferon has been produced which shows different specificities from either parent interferon. *In vitro* mutagenesis however has far more potential and, if we can ever predict accurately what effect a particular amino acid change will have on the structure and function of a protein, the technique will have very wide application. It may, for example, be possible to increase the temperature stability of enzymes, to alter their *p*H profile or their substrate specifity. The most significant work done in this area is that of Fersht, Winter and colleagues on tyrosyl-tRNA synthetase. They have shown that single amino-acid substitutions can have dramatic effects on the catalytic parameters of the enzyme. This is certainly a good start along the road attempting to lay the ground rules for future protein engineering.

As well as being able to modify proteins by manipulating their genes it is also possible to modify pathways and so produce novel chemicals. An elegant example of this is the production of a hybrid antibiotic in *Streptomyces* by the transfer of biosynthetic genes between streptomycetes. Although the particular antibiotic produced in this experiment has no commercial value, just the demonstration of its production must be heartening to those interested in 'discovering' new antibiotics.

The most recent and interesting application of recombinant DNA technology is in the production of transgenic animals by injecting foreign DNA into the pronuclei or nuclei of eggs from superovulated animals. This produces offspring with the inserted DNA in terminally differentiated cell types. So far transgenic mice, rabbits, sheep and pigs have been produced, in most cases containing a fusion between the mouse metallothionein promoter/regulator region and either the rat or human growth hormone structural gene. Now that the technique has been established, however, it offers the possibility of investigating the mechanism of tissue-specific gene expression and maybe eventually of producing useful proteins in the milk of cows or in the eggs of chickens.

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Big business for suppliers

The increasing number of laboratories engaged in recombinant DNA research is supporting a thriving industry.

• Instrument Research has introduced a high voltage pulse generator, the Model 958A, designed specifically for cell fusion systems. The instrument delivers a positive pulse adjustable from 0 to over 3.5kV, with pulse currents to 2A and peak powers over 7kW. This large voltage range permits adequate fusion gradients for large cells, and offers the further possibility of using series resistance in the cell circuit to approach a constant current characteristic. A digital pulse control module — 921A — permits direct setting of pulse width from 1μs to 99μs in 1μs steps, control of pulse period from 0.1 to 9.9s in 0.1s steps, and burst control of counted pulses from 1 through 99. An analog driver, Model 904A, offers continuous pulse control from less than 0.5μs up to seconds in 11 ranges, and also features triggered delay, burst, and single-shot modes. For maximum flexibility, the user can order both the analog and digital front ends, selecting either control via a rear panel connector. Applications include monoclonal antibody production (see M. Lo *et al. Nature* **310,** 792; 1984).
Reader Service No. 100.

• Cambridge Biotechnology Laboratories, a division of Uniscience Limited, has added a further four restriction enzymes to the company's range. The new enzymes are *Ban*I, *Bcl*I, *Gsp*AII and *Sca*I. *Gsp*AII is exclusive to CBL and recognizes the sequence TGCGCA.
Reader Service No. 101.

• Transcriptaid RT from P & S Biochemicals of Liverpool is a new multicomponent system for cDNA generation. By removing the need for the S_1 nuclease digestion step of ordinary procedures, Transcriptaid RT makes cDNA synthesis simpler and complete cDNA libraries can be constructed even from low abundance mRNA. In the new method, after the primary DNA strand has been synthesized with reverse transcriptase, the original mRNA strand is partially digested with RNase H. The remaining fragments then act as primers for synthesis of the secondary DNA strand using DNA polymerase 1. The reaction sequence is then completed by the removal of the non-transcribed 3′ overhang with T4 DNA polymerase. A Transcriptaid RT kit contains five special reagents plus all the necessary buffers and nucleotides and control mRNA. The five reagents are used sequentially in a simple, step-by-step protocol. Each kit has enough material for ten complete reactions starting with 5 μg of mRNA.
Reader Service No. 102.

• A new range of *in vitro* RNA transcription products is now available from Pharmacia's Molecular Biology Division. MultiProbe pSPT 18 and pSPT 19 are two new SP6/T7 dual promoter plasmids containing the multiple cloning site from pUC 18 and 19 respectively, and together allow both strands of an inserted DNA to be transcribed. New FPLC*pure* T7 RNA Polymerase has exceptionally high functional purity, and has provided transcripts of up to 4.1 kilobases. Also now available are new RNaStop= RNAguard ribonuclease inhibitor, a RNase/DNase-free bovine serum albumin and the ^{7}mGpppN nucleotide caps necessary for translation of RNA transcripts.
Reader Service No. 103.

• The lambda GT 11 sequencing primer is available as a kit from Clontech. A synthetic 15-mer adjacent to the cloning *Eco*RI site in lambda 11 is used for rapid DNA sequencing using the dideoxy method. This enables researchers to sequence clones identified by immunoscreening and other methods and eliminates the necessity to subclone in M13. The protocol is as simple as using M13 and yields sequence lengths comparable to M13. M13 primers are also available from Clontech.
Reader Service No. 104.

• Bethesda Research Laboratories' biotinylated lambda DNA/*Hin*dIII fragments provide a convenient method for estimating the size of genomic DNA directly on nitrocellulose in Southern blot analysis. Following electrophoresis and transfer to a nitrocellulose filter, the fragments are visualized within 3h using a non-radioactive streptavidin/biotin detection method, such as BRL's DNA detection system. The size of the genomic DNA can be estimated by following three simple steps: (1) the biotinylated fragments and the biotin-labelled sample DNA are electrophoresed side-by-side on an agarose gel; (2) they are then transferred together onto a sheet of nitrocellulose by Southern blotting; and (3) the sample and the markers are visualized colorimetrically.
Reader Service No. 105.

• Beta-lactamase screening filters are now available from Bio-Rad for the rapid detection of Amp^s recombinant clones. The filters provide a simple, rapid and reliable chromogenic assay for recombinant plasmids resulting from insertion of DNA into the ampicillin resistance gene of cloning vectors. After transfer to the sterile filters, recombinant (Amp^s) col-

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