EXHIBIT T

Applications of Genetic Engineering to the Pharmaceutical Industry

MICHAEL KONRAD

Cetus Corporation
600 Bancroft Way
Berkeley, California 94710

INTRODUCTION

One of the major tasks of genetic engineers is to take a gene from one organism and transfer it to another. The motive for gene transfer is usually that the host cell is difficult, expensive, or impossible to grow. It may also be that the level of expression (rate of synthesis per cell or per unit time) is unpractically low, or that a rather exotic induction procedure is required to cause the cell to make the protein. In the case of a protein with pharmaceutical use, the donor cell is most likely to be human. The choice of the cell species in which this gene is best transferred and expressed may require knowledge of the structure, and in some cases the function, of the particular protein.

INSULIN

An extreme example, in terms of biological difference between donor and recipient cell type, is the production of human insulin in the bactrium *E. coli*. The donor cell is one of the major cell classes present in the pancreas, the Islets of Langerhans. To my knowledge, no one has proposed culturing these cells as a source of insulin, although a varient cell type, from insulinoma tumors, continues to make insulin even though transformed, and is relatively easy to grow in tissue culture.

The first step in the tranfer of genetic information is to obtain a copy of the coding region of the gene that can be read by the bacterial cell. Although the genetic code is universal (almost) the majority of eukaryotic genes, including insulin,[1] contain nucleotide segments called introns, imbedded in the sequence that codes for the amino acid sequence of the protein. These segments are removed from the messenger RNA by an enzymatic processing system that is missing in bacteria. One must then isolate messenger RNA from the insulinoma and copy this enzymatically *in vitro* to produce a cDNA fragment.

The second problem is that the promoter that caused the insulin gene to be transcribed in the human cell doesn't work in bacterium. In a sense, this is no problem if we have a cDNA gene, since the promoter sequence is not represented in the RNA anyway. The solution is then to splice a bacterial promoter in front of the insulin gene. If the protein is going to be toxic to the bacterial cell, and it may be for direct or indirect reasons, it is important to use a promoter that can be turned off. In any case, it is best to use a promoter that can be controlled, because until you have the gene expressed in the cell you will not know if it's toxic or not. If it is toxic, it will prevent you from isolating the desired bacterial clone and you may never guess the reason.

The next problem is that the gene codes not for insulin, but for preproinsulin (see FIGURE 1). Let's take this problem in two steps, first assuming we had proinsulin. We

can now take advantage of
given to this hormone sec
number of protein chemist
available as almost a bypro
pancreas tissue, and bioche
for the enzymatic cleavage
peptide.[2] After this, separa

The next problem is
polypeptide region that cau
which it is removed. In the

FIGURE 1. The conversion
proinsulin to insulin in the pan
The precursor to insulin is the
polypeptide chain at the top
figure. The NH_2-terminal
segment is removed during
cretion, yielding proinsulin,
contains three intramolecular
nde bonds as illustrated in the
structure. An enzyme system
specificity similar to trypsin
the peptide at both ends of
region to produce insulin (the
disulfide bond remains, but
shown in this diagram).

fact that most is transporte
awaits subsequent release
of most proteins that are
usually be active outside
(if not they wouldn't be
must have been secreted by

One solution is to
peptide. It is then likely
that removes N-formylmet

A second solution

can now take advantage of the fact that for several decades, in addition to the attention given to this hormone because of its widespread clinical importance, a significant number of protein chemists have taken the insulin system as a model. Proinsulin is available as almost a byproduct of the purification of insulin from bovine and porcine pancreas tissue, and biochemists have been able to develop procedures and conditions for the enzymatic cleavage of the protease-sensitive regions at both ends of the C peptide.[1] After this, separation of insulin from C peptide is straightforward.

The next problem is how to remove the "pre" or leader sequence. It is this polypeptide region that causes insulin to be secreted through the cell membrane, after which it is removed.[4] In the case of insulin, the secretion process is complicated by the fact that most is transported to localized storage areas where as a complex with zinc, it awaits subsequent release. The presence of a leader sequence is a quite general feature of most proteins that are of pharmaceutical interest. This is because such proteins will usually be active outside the cell or at the cell surface or be able to be taken up by cells (if not they wouldn't be effective after being administered extracellularly). Thus they must have been secreted by cells and have had a leader sequence.

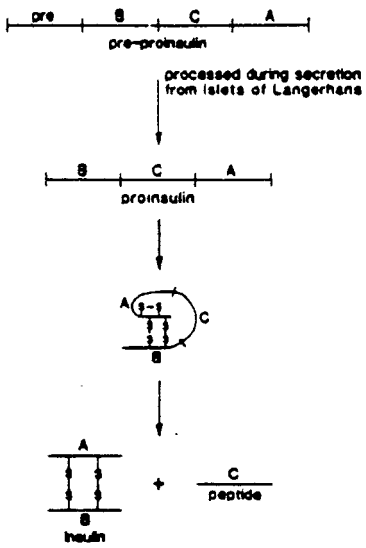

FIGURE 1. The conversion of pre-proinsulin to insulin in the pancreas. The precursor to insulin is the single polypeptide chain at the top of the figure. The NH_2-terminal "pre" segment is removed during secretion, yielding proinsulin, which contains three intramolecular disulfide bonds as illustrated in the third structure. An enzyme system with specificity similar to trypsin cuts the peptide at both ends of the C region to produce insulin (the third disulfide bond remains, but is not shown in this diagram).

One solution is to construct the codon for methionine just before the desired peptide. It is then likely, but not assured, that the normal bacterial processing system that removes N-formyl-methionine from bacterial proteins will remove it also.

A second solution is to add the proinsulin sequence to a bacterial protein with an

amino acid that can be selectively cleaved. This was the approach used by the Genentech-Lilly group when in taking advantage of the absence of methionine in proinsulin, they constructed a chimera of the NH_2-terminal end of tryptophan synthetase, joined to proinsulin at a methionine residue. Treatment of this protein with cyanogen bromide, which cleaves the polypeptide chain after methionine residues, gives proinsulin.

A third approach is to utilize the secretion system of the bacterium. In the case of gram-negative organisms like *E. coli*, secretion is into the periplasmic space, which is defined as the compartment whose contents are released by osmotic shock. There is a convenient DNAase reaction site in the middle of the gene for beta lactamase, into which the cDNA insulin gene has been inserted by our colleagues at the University of Chicago, Prof. Donald Steiner and Dr. Shu Chan (see FIG. 2). However, this plasmid did not produce insulin presumably because the insulin gene was out of phase with the beta-lactamase gene. In such a situation, the ribosomes reading the chimeric mRNA will produce nonsense protein as they move into the insulin gene, and will be released when they hit one of the three termination condons.

Drs. Chander Bahl and David Mark at Cetus Corporation, utilized two convenient restriction sites in the beta-lactamase sequence upstream or 5' to the insulin gene to remove a DNA fragment, and an exonuclease acting at each of the ends to enlarge the deletion even further. The two ends were then joined back to regenerate a circular plasmid and the DNA was used to transform bacteria. We now had a heterogenous collection of plasmids, some of which must be expressing at least a chimeric peptide containing the insulin sequence. Thousands of bacterial colonies were then screened by Dr. Tom White, using antibodies raised to insulin,[3] and a group producing insulin antigen were identified. Analysis of the size of the deletion in the positive clones allowed us to pick two that were producing a peptide close to the size of proinsulin. Small cultures were then grown in medium containing high specific activity ^{35}S, which would label only the 6 cysteine residues in insulin, and the small fraction of the bacterial protein that had been secreted into the periplasmic space was released by osmotic shock and shipped to our colleagues in Chicago. This material was purified by precipitation with antibody to insulin, and subjected to Edmond degradation, a process that strips amino acids sequentially from the NH_2-terminal end of the peptide. Two peaks of activity were seen at the 7th and 19th cycle corresponding to the two cysteine residues in the B chain of insulin.[4]

After determination of the DNA sequence of the plasmid, it was evident that what

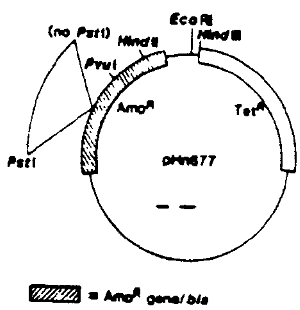

FIGURE 2. Insertion of the insulin cDNA copy into the pBR322 plasmid. The plasmid was cut at the single *Pst*I DNAase restriction site which is in the middle of the gene conferring resistance to ampicillin (the gene codes for the enzyme beta-lactamase). Poly dG segments were added to both 3' ends of the cut plasmid, and poly dC segments were added to the corresponding ends of the insulin cDNA copy. Complementary base pair hydrogen bonding between the insulin and plasmid ends generated the plasmid pHn67.[7] The 3' *Pst*I site was regenerated as expected, while the 5' one was not, presumably due to contaminating nuclease activity.

KONRAD: APPLICA

FIGURE 3. An ... construction of a plasm... high levels of human ... plasmid, pJW 3000 ... has been made in the ... the insulin segment. The ... coding for the first ... the Amp leader seque... fused to the last coding ... amino acids of the insu... chimeric peptide is ... by the bacterial cell to p... ... A small DNA ... ... the lac UV prom... tryptophan promoter ... ... has been ... EcoRI site 21 base ... ... the insulin gene

we had done was ... to the last 13 amino ... that was still corre... produce proinsulin ... using the gene for ...

The signal to sta... synthesis, the ... lactamase, and the re... containing the corres... interferons in *E. col*... expression. Unfortun... NH_2-terminal end of ... tactics one can use in ... Emerick to produce a ...

The plasmid was ... insulin sequence (Fi... exonuclease action. T... the Bam H1 site, and ... closed by blunt-end li... about one chance in ... specific site will be re... site as close as possib... This construction wa... DNA polymerase pro... these conditions, the ... chain until the first ... incorporates dCTP ... indefinitely, but and ... generated a series of ... ATG starting the ins... the *trp* promoter and ... increase over the orig...

AM-ITC 014468

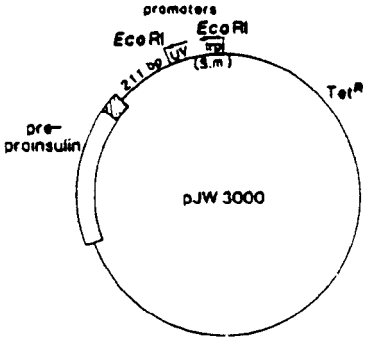

FIGURE 3. An intermediate in the construction of a plasmid expressing high levels of human insulin. In this plasmid, pJW3000, a large deletion has been made in the Amp gene 5' to the insulin segment. The 36 base pairs coding for the first 12 amino acids of the Amp leader sequence have been fused to the last coding for the last 13 amino acids of the insulin gene. This chimeric peptide is cleaved correctly by the bacterial cell to produce proinsulin. A small DNA fragment containing the lac UV promoter and the tryptophan promoter (from *S. marcescens*) has been inserted at the *Eco*R1 site 211 base pairs upstream from the insulin gene.

we had done with the first 12 amino acids of the beta-lactamase leader sequence to the last 13 amino acids of the insulin leader sequence, providing a chimeric leader that was still correctly recognized by the bacterial secretion-protease system to produce proinsulin.[7] Results similar to these have been reported by Talmadge *et al.* using the gene for rat preproinsulin.[8]

The signal to start RNA synthesis, or transcription, and the signal to start protein synthesis, the ribosomal binding site (RBS), were still those belonging to beta lactamase, and the resulting level of expression was low. We had used a small fragment containing the corresponding sites for the tryptophan synthetase operon to express interferons in *E. coli* and as I will show later, had obtained quite high levels of expression. Unfortunately, there were no convenient restriction sites close to the NH_2-terminal end of our insulin construction to insert this fragment. There are several tactics one can use in a case like this, and I'll briefly describe the one used by Dr. Anne Emerick to produce a clone with a higher level of expression.

The plasmid was opened at the *Eco*R1 site about 200 base pairs upstream of the insulin sequence (FIG. 3), and DNA was removed from both ends by random exonuclease action. The deletion in the distal direction was repaired by first cutting at the Bam H1 site, and then adding back the intact fragment. Finally, the plasmid was closed by blunt-end ligation. Most of the time the *Eco*R1 site will be lost, but there is about one chance in four that the nucleotide on the insulin end will be a C, and the specific site will be regenerated. Such a construction was isolated that contained the site as close as possible to the start of the coding sequence, only 14 nucleotides away. This construction was then opened, and the insulin-proximal end digested with the DNA polymerase produced by the bacterial virus T4 in the presence of dCTP. Under these conditions, the exonuclease activity of this enzyme allows it to chew back the 3' chain until the first C is removed. When that happens, the polymerase activity incorporates dCTP in the chain to regenerate the damage. This cycle then goes on indefinitely, but under our conditions designed to remove only a few nucleotides, generated a series of plasmids with the RBS being various distances upstream of the ATG starting the insulin sequence. The best clone from this series when placed after the *trp* promoter and RBS produced 0.16 percent of its protein as insulin, a 16-fold increase over the original clone, pJW 2172.

INTERFERON

Interferons are a group of proteins that protect cells against viral infections. They are typically produced by cells that have been infected and are then secreted by these cells. They then bind to cell membrane receptors on neighboring cells and trigger the synthesis of several special enzymes that inhibit viral expression and replication. Thus while the original cell may not escape the virus, it has altruistically protected its neighbors. Interferons also have growth-inhibitory activity, and there are many who believe that they may be involved in normal growth regulation and are one of the organism's defenses against cancer cells.[1] Indeed, Cetus Corporation and its partner in this venture, Shell Oil Company, plan to start clinical trials with cancer patients quite soon.

Interferons can be divided into three groups. The alphas number at least 12, and typically show 70% amino acid homology. Only one member of the beta and gamma class have been isolated, and they are both glycosylated, whereas the alphas appear not to be. Beta is clearly related to the alphas, with about 30% amino acid homology. The genes for beta and the alpha interferons do not contain introns. Gamma has no obvious sequence homology to the other interferons, and there are several introns in its genomic sequence.[4]

The function of the sugar residues on beta and gamma interferons (IFNs) are not understood. Some experiments with beta interferon suggest that the terminal carbohydrate residues may be sialic acid, which when enzymatically removed expose galactose. The galactose residue may be recognized by specific receptors in the liver, and the protein is removed from circulation.[5] Thus one function of the sugar may be to provide a mechanism for removing IFN from the body after a definite time. However, the residence time in the serum of the alpha IFNs, which are thought not to

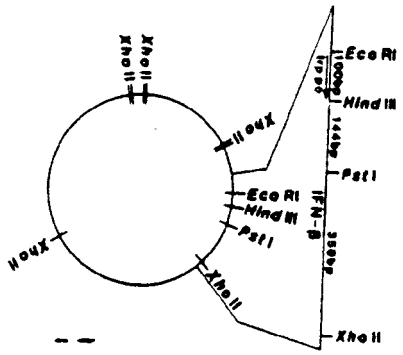

FIGURE 4. The gene coding for the human beta interferon gene inserted into the plasmid pBR322. The DNA fragment diagramed linearly at the top of the figure has been inserted in place of the *EcoR*I to *Xho*II segment seen at the top of the circular pBR322 plasmid map. The inserted fragment contains the promoter and ribosomal binding site of the tryptophan synthetase operon on a 100 base pair long *EcoR*I to *Hind*III fragment at the top left of the figure. This segment promotes transcription and translation of the gene coding for interferon extending to the *Xho*II site.

FIGURE 5. Induction of
described in FIGURE 4 we
tryptophan (14C]leucine wa
nduced culture. Both cultu
ncorporation. The lysates w
by a sodium dodecylsulfate
nduced culture (open circles
the gel are compared to the p
the figure (weights in kiloc
interferon

be glycosylated, is rather-
We have obtained prelim
thus not glycosylated,
Hopefully one of the con
possible that will more
IFNs are certainly not u
only serum albumin is n

The construction of
Cetus Corporation heade
Figure 4. The cDNA fr
tryptophan promoter fr
plasmid. The portion of t

CPM

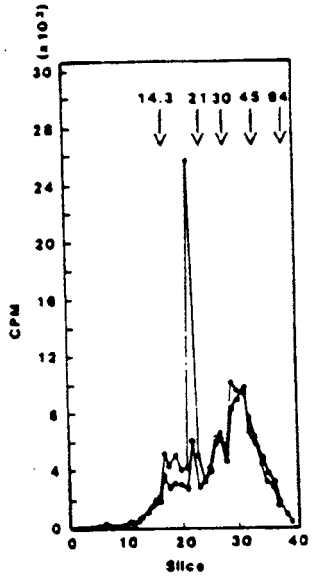

FIGURE 5. Induction of *E. coli* to produce beta interferon. Bacteria containing the plasmid described in FIGURE 4 were grown in the presence (repressed) and absence (induced) of tryptophan [^{14}C]leucine was added to the repressed culture and [^{3}H]leucine was added to the induced culture. Both cultures were chilled and the cells lysed after one minute of amino acid incorporation. The lysates were mixed and the proteins separated according to molecular weight by a sodium dodecylsulfate gel electrophoresis system. The distribution of both ^{3}H from the induced culture (open circles) and ^{14}C from the repressed culture (closed circles) of 2 mm slices of the gel are compared to the positions of molecular weight standard proteins indicated at the top of the figure (weights in kilodaltons). The peak at an apparent molecular weight of 19 kd is interferon.

be glycosylated, is rather modest, thus there must be other mechanisms for clearance. We have obtained preliminary pharmacokinetic data on beta produced in bacteria, and thus not glycosylated, and it certainly does not stay in the circulation indefinitely. Hopefully one of the contributions of genetic engineering will be to make experiments possible that will more completely elucidate the role of the carbohydrate residues. IFNs are certainly not unique in being glycosylated. Of the major proteins in the blood, only serum albumin is not glycosylated.

The construction of a plasmid expressing beta IFN was accomplished by a group at Cetus Corporation headed by Dr. David Mark. The structure of the plasmid is seen in FIGURE 4. The cDNA fragment containing the coding sequence was placed behind the tryptophan promoter mentioned previously in connection with the expression of insulin. The portion of the total cell protein synthesis that has been converted to

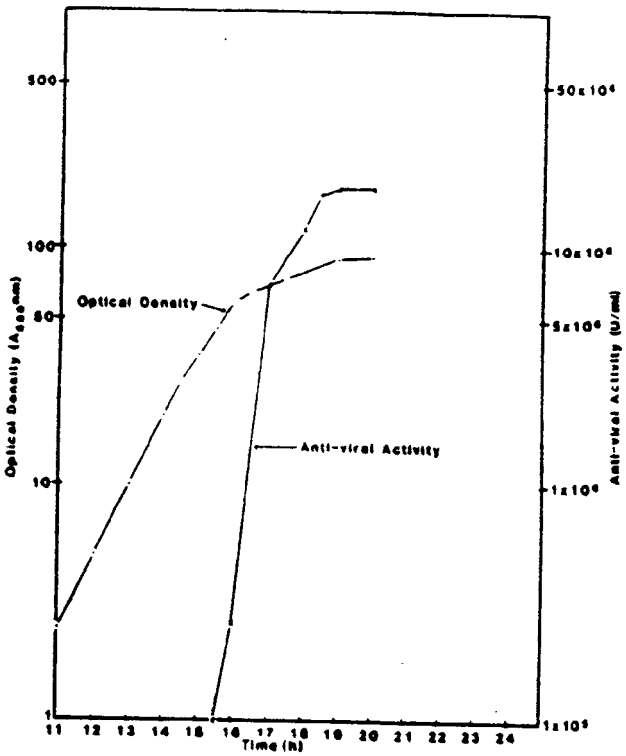

FIGURE 6. Production of human *d*. interferon by *E. coli* K-12 in a fermenter. The optical density of the culture is indicated on the left. At a value of about 60 and a run time of 16 hours, induction of interferon starts. The induction can be followed by the rapid appearance of antiviral activity in the cells as plotted on the right axis. Antiviral activity is assayed on human fibroblast cells challenged with VSV and is relative to an NIH standard interferon preparation.

making beta IFN is considerable, and is illustrated in FIGURE 5. A small culture of cells was suspended in tryptophan-free medium and the proteins made under these conditions were radioactively labeled for 1 minute with [^{3}H]leucine. A similar culture of the same cells, but growing in the presence of free tryptophan, was labeled with [^{14}C]leucine. The two cultures were chilled, mixed, sonicated, and then analyzed by SDS PAGE, which separates proteins by molcular weight. The distribution of both isotopes along the gel is seen in FIGURE 5. The very noticeable peak of ^{3}H at an apparent molecular weight of 18,000 daltons is beta IFN, and represents about 15% of

the total instantaneous rate of protein synthesis. In FIGURE 6 we see the turbidity and total IFN concentration in a 10-liter fermentation run producing IFN to be used in the development of the purification process, and for biological characterization. In FIGURE 7 we see stained protein in an SDS PAGE analysis of cells from such a fermentation run. The band corresponding to beta can be clearly seen even in the crude extract, although it is not so conspicuous as seen in the isotopically labeled protein where only the proteins made after induction are visible.

A number of constructions producing beta interferon in *Bacillus subtilis* have been made by a group headed by Dr. Shing Chang. That the beta expressed in bacteria is not glycosylated can be seen by a technique developed to determine the molecular weight distribution of nanogram amounts of proteins present in mixtures. The proteins are first resolved by SDS PAGE, and transferred to activated paper where they become covalently bound. After extensive washing, the paper is incubated in a solution of antibody, in our case to beta IFN, and then with radioactive protein A, which selectively binds to antibody. The protein distribution is then determined by autoradiography. Bands corresponding to the beta produced in *E. coli* and *B. subtilis* that are about 20% lower in molecular weight than the band of beta induced in human fibroblast cells in tissue culture by poly I:C, which mimics viral infection.

In order to produce beta interferon that is glycosylated, the plasma seen in FIGURE 8 was constructed and characterized by Drs. Michael Innis and Frank McCormick at Cetus Corporation and a colleague at Stanford University, Prof. Gordon Ringold. The plasmid contains a segment of pBR322, which was used in the bacterial systems

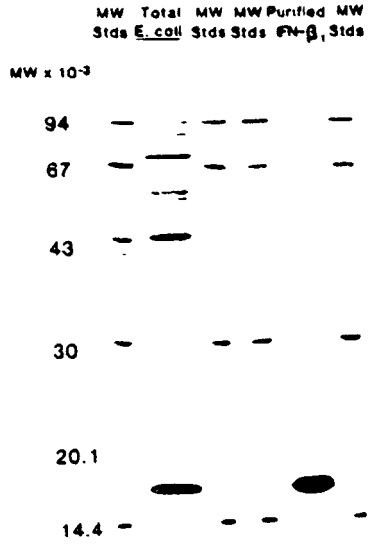

FIGURE 7. Purification of human β interferon produced in *E. coli*. The gel electrophoresis system described in FIGURE 5 was used to determine protein distribution in a whole cell lysate at the end of a fermentation, and a purified preparation of interferon. The proteins were visualized by staining, which is approximately proportional in intensity to the concentration of proteins in the bands. The positions of six proteins of known molecular weights are seen in lanes 1, 3, 4, and 6. The original bacterial lysate is seen in lane 2, while the purified material is seen in lane 5.

described above. In that segment is the origin of replication, which allows growth of the entire plasmid in *E. coli* in which it was constructed, and the ampicillin resistance gene, which allows easy selection of bacterial cell transformed by the plasmid. In another segment is the origin of replication from the animal virus SV40, and promoter from the mouse mammary tumor virus that allows transcription and expression of a gene coding for the enzyme dihydrofolate reductase. This gene allows selection in the animal cell host, if that host is dhfr – Finally, it contains the gene for IFN-b, with its own promoter.[11] The host animal cell chosen is one of the oldest cell lines, derived from fibroblast Chinese hamster ovary cells (CHO) by Puck several decades ago. It grows rapidly, and through continuous passage has become transformed so that it no longer requires a solid support, but can be cultured in a spinner flask. Fortunately for us this cell line has lost the ability to produce its own IFN, and is not sensitive to human *β*-IFN. Finally, Drs. Chasen and Urlaub at Columbia University had isolated a dhfr – derivative of this line.[12]

After transformation and selection, cells were obtained in which the plasmid had been integrated into the chromosome producing a stable cell line. Some of these cells secreted IFN-*β*, into the tissue culture medium at the level of several hundred U/ml, but when subjected to induction by poly I C produced 100,000 U/ml. This IFN was analyzed by the SDS electrophoresis technique discussed previously, and the material binding to beta interferon antibody had a molecular weight indistinguishable from the native material produced by fibroblasts, that is, it is glycosylated.

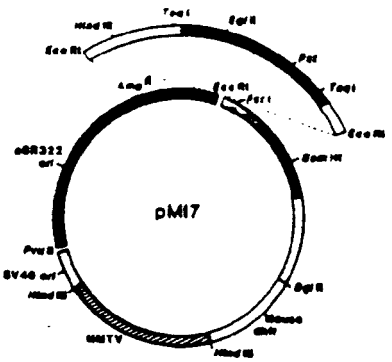

FIGURE 8. A plasmid that produces human beta interferon in hamster cells. Starting from the top of the circle and going counter-clockwise the plasmid pM17 contains: **Amp R:** The gene for ampicillin resistance allowing the selection of bacteria containing the plasmid. The fragments making up pM17 were assembled in a bacterial host. **pBR322 ori:** The origin of replication needed for DNA replication in bacteria. **SV40 ori:** A DNA origin of replication from the SV40 animal virus that enables replication in the hamster cell. **MMTV:** A promoter from the mouse mammary tumor virus, needed for transcription of the next gene. **Mouse dhfr:** The gene from the mouse that codes for the essential enzyme dihydrofolate reductase. The hamster cells into which this plasmid was introduced could not make this enzyme, thus cells containing the plasmid had a selective advantage. **Human beta interferon:** The structure of the genomic fragment that contains the gene and its promoter is diagramed on the upper semicircle.

Choice of the dhfr
selective pressure can b
produced by one gene
increasing concentratio
concentration is picked
derivatives that have in
the gene are able to repl
nearby often are also c
stages of this process,
methotrexate and that

While we are encou
the ultimate, final syst
induction protocol (or
obtain IFN-*d* from hun
treating the cells with p
synthesis, but allows th
with actinomycin D, w
but blocking induction
as preventing the indu
synthesis of beta, that
has never been directly
higher levels of IFN th
no time trying to impr
protein and RNA syr
temperature-sensitive
happening, there migh
likely that the easiest a
with one that is strong
active development at

However, even in it
magnitude higher than
pattern of sugar resic
fibroblasts, although it
investigations of just wh
IFN.

A frequent motive
protein from a cell tha
inexpensive to handle.
gene coding for a prote
While bacterial cells
produce proteins wh
while there are exar
interest in expression
knowledge of the fu
successes and problen
been presented.

Choice of the dhfr gene as a selection marker was motivated by the fact that selective pressure can be exerted on the cell to increase the amount of dhfr above that produced by one gene copy. This is accomplished by growing the cells in ever-increasing concentrations of a competitive inhibitor of the enzyme methotrexate. A concentration is picked where growth is inhibited but not stopped. Spontaneous derivatives that have increased enzyme levels due to the creation of multiple copies of the gene are able to replicate more rapidly and outgrow the other cells. Genes that are nearby often are also carried along piggyback by this process.[1] We are in the early stages of this process, but have obtained derivatives that have greater resistance to methotrexate and that also produce correspondingly higher levels of β-IFN.

While we are encouraged and excited by this work, it seems unlikely that it will be the ultimate, final system used for the production of IFN-β. For one thing, the induction protocol (or superinduction as it is usually called), the same one used to obtain IFN-β from human fibroblasts, is rather involved and not cheap. It consists of treating the cells with poly I:C in the presence of cycloheximide, which inhibits protein synthesis, but allows the build-up of IFN-β mRNA. The drug is removed but replaced with actinomycin D, which now allows transcription of the RNA producing IFN-β_1, but blocking induction of synthesis of new mRNA. This protocol has been rationalized as preventing the induction of a protein by IFN-β, which would then inhibit the synthesis of beta, that is, circumventing a natural negative feedback loop. This model has never been directly verified, and is doubted by some, but in fact it does produce higher levels of IFN than poly I:C alone. On the optimistic side however, we have spent no time trying to improve this induction scheme. If it is really necessary to block protein and RNA synthesis, there are probably easier ways to do it, reversible temperature-sensitive mutations, for example. If we know more about what was really happening, there might be easy ways to obtain the same result. However, it seems likely that the easiest and most general approach will be to replace the beta promoter with one that is stronger, and perhaps easily controllable. Such systems are under active development at Cetus Corporation.

However, even in its present form, this cell line produces levels that are an order of magnitude higher than that produced from regular fibroblasts. It is unlikely that the pattern of sugar residues will be exactly the same as that produced by human fibroblasts, although it may be quite close. It will enable us to proceed more rapidly in investigations of just what the sugar means to the biochemical properties of this kind of IFN.

SU

A frequent motive for genetic engi
protein from a cell that is difficult or ic
inexpensive to handle. In the pharmac
gene coding for a protein secreted by hu
While bacterial cells are an inexpensiv
produce proteins which are glycosylat
while there are examples of successfu
interest in expression in animal cells. Tl
knowledge of the function, or even t
successes and problems in production o
been presented.

REFERENCES

1. BELL, G. I., R. L. PICTET, W. J. RUTTER, B. CORDELL, E. TISCHER & H. M. GOODMAN 1980. Nature (London) 284: 26–32.
2. STEINER, D. F. & J. L. CLARK 1968 Proc. Natl. Acad. Sci. USA 60: 622–629.
3. KEMMLER, W., J. D. PETERSON & D. F. STEINER 1971 J. Biol. Chem. 246: 6786–6791
4. MILSTEIN, C., C. G. BROWNLEE, T. M. HARRISON & M. B. MATHEWS. 1972. Nature (London) New Biol. 239: 117–120.
5. BROOME, S. & W. GILBERT 1978 Proc. Natl. Acad. Sci. USA 75: 2746–2749
6. CHAN, S. J., J. WEISS, M. KONRAD, T. WHITE, C. BAHL, S-D. YU, D. MARK & D. F. STEINER. 1981. Proc. Natl. Acad. Sci. USA 78: 5401–5405.
7. TALMADGE, K., J. KAUFMAN & W. GILBERT 1980 Proc. Natl. Acad. Sci. USA 77: 3988–3992.
8. LENGYEL, P. 1982. Ann. Rev. Biochem. 51: 251–282.
9. CRAY, P. W. & D. V. GOEDDEL 1982 Nature (London) 298: 859–863
10. BOCCI, V., A. PACINI, G. P. PESSINA, V. BARIGLI & M. RUSSI. 1977 J. Gen. Virol. 35: 525–534
11. OHNO, S. & T. TANIGUCHI 1981 Proc. Natl. Acad. Sci. USA 78: 5305–5309
12. URLAUB, G. & L. A. CHASIN 1980 Proc. Natl. Acad. Sci. USA 77: 4216–4220.
13. RINGOLD, G., B. DIECKMANN & F. LEE. 1981 J. Mol. Appl. Genet. 1: 165–175

Using Genetical Vacc

Di

460 Po
South San

All vertebrates possess an immune such as viruses or other diseas recognizing foreign invaders and One response involves the produc enabling the host to inactivate it. can be quite severe before the h immune response is significantly e organism or by the use of v organisms. Vaccines stimulate invader's surface. Because of the used to help prevent or fight infec

The large-scale production of tricky problem, dependent in par Edward Jenner found, for examp would protect them for life from recently led to the worldwide era from yellow fever virus, rubella v been highly successful. For many suitable vaccine product exists, hepatitis, the vaccine products ar are expensive.

When it became possible to s recognized that vaccines might be more effective, or for diseases whe for the antigenic proteins could techniques, introduced into rela proteins. The protein antigens is vaccines, or possibly the whole org

Over the last five years, a nur the use of *E. coli* for the product

This paper reviews research done by Dennis Kleid, Daniel Yansura Maureen Hoatlin, Neal Clayton Ste John Ogez and at the United State Center (PIADC), Greenport, New Y D. McKercher, Donald O. Morgan Shuot, JoAnn Henry, Edward Whit