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Interferon Synthesis by Micro-organisms

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Introduction

Interferon, from its discovery in 1957, has always held out a promise of important clinical utility, first as an antiviral agent and more recently in the treatment of cancer. Proper evaluation of this therapeutic potential and even study of the underlying biological mechanisms involved were hampered by the minute amounts of interferon produced by mammalian cells and by the difficulties encountered in attempts to purify it. The development of recombinant DNA technology during the 1970s opened new avenues to the characterization and production of scarcely available proteins. Interferon inevitably became a major target for this new technology in both academic and commercial laboratories. The result has been a breathtakingly rapid series of advances, both in the definition and availability of interferons and in the elaboration of techniques for gene manipulation.

After a brief description of the interferon system, this review will survey the methods employed to clone genes for the interferons and to synthesize the interferon proteins in micro-organisms. A final section presents techniques which have been devised to design and produce non-natural interferon analogues for structure–activity studies. It is hoped that these may lead to new products with improved therapeutic properties.

The interferons

Interferons are defined as proteins able to inhibit the replication of a variety of RNA and DNA viruses through cellular metabolic processes involving the synthesis of new RNA and protein (Committee on Interferon Nomenclature, 1980). They are produced in cells of a wide variety of species in response to viral infection, a range of chemical inducers and immune stimulation. Three main classes of interferons have been distinguished to date and are currently named

Abbreviations: bp, base pairs; HuIFN, human interferon; IFN, interferon; PGK, phosphoglycerate kinase; SD sequence, Shine–Dalgarno sequence; ds, double-stranded RNA; cDNA, complementary DNA.

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Table I. Interferon classification

Class	Old nomenclature	Subtypes	Inducers
IFN-α	Leukocyte (Lc) Lymphoblastoid Type I pH 2 stable	At least 13	Viruses Synthetic dsRNA Some chemicals
IFN-β	Fibroblast (F) Fibroblastoid Type I pH 2 stable	1, probably more	Viruses Synthetic dsRNA Some chemicals
IFN-γ	Immune Type II pH 2 labile	1 only	Antigens Mitogens

alpha (IFN-α), beta (IFN-β) and gamma (IFN-γ) on the basis of antigenic types. Essentially the same three classes were defined previously on the basis of their most common cells of origin (*Table 1*). The three classes also differ from each other physically and chemically and are induced by different agents. In addition to their antiviral activity, all three classes of interferon also exhibit potent inhibition of cell proliferation and modulation of various facets of the immune system (Toy, 1983). IFN-γ is reported to potentiate both the antiviral and anticellular effects of IFN-α and of IFN-β.

Human IFN-α is now known to be a family of homologous single-chain proteins which differ from each other by no more than about 25% of their 165 or 166 amino-acid residues. This knowledge derives in part from direct protein-sequencing studies (Allen and Fantes, 1980; Allen, 1982), but primarily from translation of the nucleotide sequences of genes identified by probing with cloned IFN-α cDNA (for a review *see* Weissmann *et al.*, 1982). At least 13 distinct functional IFN-α genes are known, together with several variants of these believed to be alleles. Many have been expressed in *E. coli* to produce biologically active proteins. In addition, at least six distinct IFN-α pseudogenes have been identified which would not normally be translated into interferon proteins.

The amino-acid sequence of IFN-α_1, the first human IFN-α species to be defined (Mantei *et al.*, 1980), is shown in *Figure 1*. Four of the five cysteine residues in IFN-α_1 are conserved across all the human α-IFNs and these are believed to be linked by the disulphide bridge between Cys^{1}–Cys^{99} and Cys^{29}–Cys^{139} (Wetzel, 1981).

So far, work in several laboratories has led to only one human IFN-β gene being clearly identified and expressed (Taniguchi *et al.*, 1979, 1980a; Derynck *et al.*, 1980b; Houghton *et al.*, 1981a). This gene encodes a protein, IFN-β_1, again consisting of 166 amino-acid residues and with 29% homology with IFN-α_1 (Taniguchi *et al.*, 1980b). The α-IFNs and IFN-β, all share the unusual feature that their genomic DNA sequences do not contain introns.

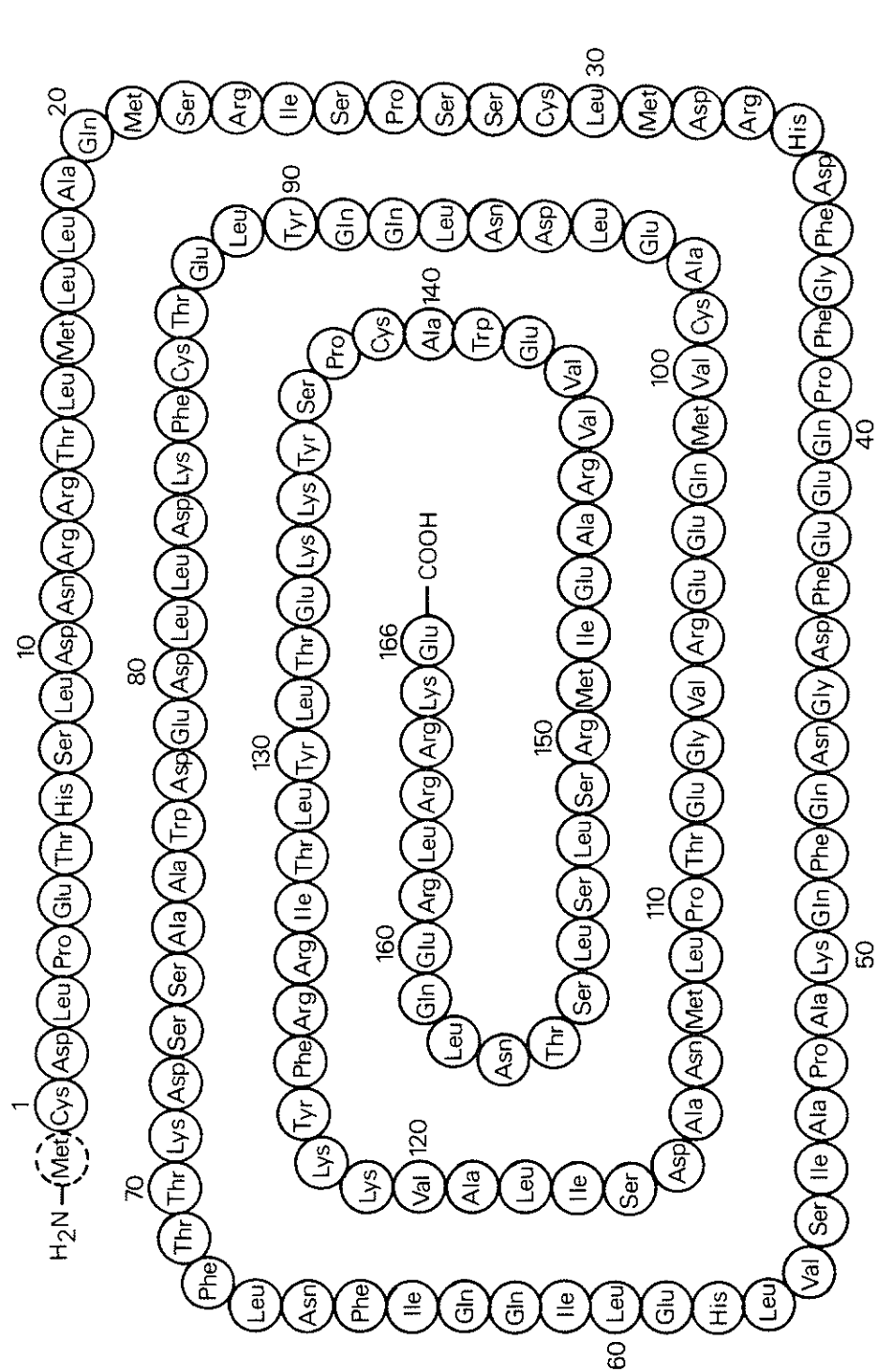

Figure 1. Amino-acid sequence of mature IFN-α_1. The additional methionine residue at the *N*-terminus may or may not be present in bacterially derived protein.

There are reports of several other human IFN-β mRNA species (Sagar *et al.*, 1982, Sehgal, 1982) but these remain to be characterized properly.

Only one human IFN-γ gene has been detected to date and this codes for a 146 amino-acid residue protein with no significant homology to either the α-IFNs or IFN-β_1 (Devos *et al.*, 1982; Gray *et al.*, 1982). The genomic DNA for IFN-γ does include introns (Gray and Goeddel, 1982). This distinctiveness of IFN-γ at the gene and protein structural level is also reflected at the functional level: whereas α-IFNs and IFN-β_1 appear to bind to the same cellular receptors, IFN-γ has a different receptor (Branca and Baglioni, 1981). Furthermore, exposure of cells to IFN-γ appears to induce an extra set of intracellular proteins in addition to a common set also induced by α-IFNs and IFN-β_1 (Weil *et al.*, 1983).

Cloning and expression

GENERAL CONSIDERATIONS

For any bacterial cell to produce a human protein, some means must be found of transforming the cell with DNA (a gene) encoding the foreign protein. Fortunately, DNA encoding the human protein can be incorporated into plasmids or bacteriophages which have suitable properties to enable them to be used as vectors or cloning vehicles. Plasmids and bacteriophages are DNA molecules which can be isolated easily in an intact form and are able to replicate autonomously in *Escherichia coli*. The range of techniques involved in the process generally referred to as gene cloning has been comprehensively reviewed (Old and Primrose, 1981). In essence four steps are involved: generation of a DNA fragment encoding the foreign gene; incorporation of the fragment into the vector to produce a recombinant; introduction of the recombinant vector into the host bacterial cell in which it can replicate; selection of a clone of cells that has acquired the foreign gene. In addition, for production of the foreign protein in the bacterial cell it is important that the gene be placed in the vector in such a way that the protein will be expressed. This is usually achieved by further genetic manipulation or gene editing of the DNA sequences adjacent to the foreign gene and is discussed in detail later in the review.

SOURCES OF DNA FOR CLONING OF IFN GENES

The interferons are a group of proteins, the genes of which have been cloned and expressed in a variety of organisms. Examples of the majority of 'systems' for the production of foreign proteins in bacteria have been used on this group of molecules. The sources of DNA encoding interferons are widespread. Initially, as with the majority of cloned genes, the most commonly used procedure for preparing DNA for IFN-cloning experiments involved the enzymatic synthesis of complementary DNA (cDNA) to the messenger RNA (mRNA) population isolated from a cell line, tissue or organ. The preparation and screening of cDNA clone banks has been reviewed (Williams, 1981). Briefly, the mRNAs isolated from eukaryotic cells have poly (A) tracts at their

3′ ends which serve as template for the oligonucleotide, oligo (dT), which can be used to prime synthesis by the enzyme reverse transcriptase (*Figure 2*). This furnishes DNA strands complementary to the complete mRNA templates. The RNA strand is removed, usually by alkali hydrolysis and the double-stranded DNA molecules produced (again enzymatically with DNA polymerase) for ligation or annealing to a plasmid vector and used to transform bacterial cells. This procedure has been used to prepare cDNA libraries encoding human interferon genes from human fibroblasts (Taniguchi *et al.*, 1979; Derynck *et al.*, 1980a; Goeddel *et al.*, 1980a), human leukocytes (Nagata *et al.*, 1980), the human myeloblastoid line KG-1 (Goeddel *et al.*, 1980b), Namalwa cells (Skup *et al.*, 1981; Dworkin-Rastl, Dworkin and Swetly, 1982) peripheral blood lymphocytes (Gray *et al.*, 1982) and human splenocytes (Devos *et al.*, 1982). For the first cloning experiments with interferon genes (Taniguchi *et al.*, 1979; Derynck *et al.*, 1980a; Goeddel *et al.*, 1980a; Nagata *et al.*, 1980) this was a formidable task. Not only was the structure of the protein unknown (information which usually aids the identification of cloned sequences) but the mRNA levels for interferon are extremely low.

Priming with oligo (dT) produces copies of all mRNA molecules. In the absence of a more specific primer it is usually necessary to fractionate mRNA in order to enrich the desired mRNA before priming with oligo (dT) and reverse transcriptase. It is, however, important to assess fractionation by an *in vitro* translation procedure. The *Xenopus* oocyte system (Gurdon *et al.*, 1971) has been used extensively to translate IFN mRNA and the protein then detected by its antiviral activity (Stewart, 1979). The process of priming cDNA synthesis by reverse transcriptase can be refined once some of the DNA or the amino-acid sequence of the gene/protein is known. A cDNA produced by specific priming with a synthetic oligonucleotide, complementary to a specific site in the gene, can be used to screen the libraries produced by oligo (dT) priming. The fact that interferon is an inducible protein has proved useful in the isolation of cDNA clones. For example, if mRNA is produced from cells both induced and uninduced for interferon production, only the induced system should have the mRNA and cDNA for interferon. By hybridizing the cDNA from the induced system to the mRNA from uninduced cells the unbound cDNA is enriched for IFN coding sequences (Taniguchi *et al.*, 1979). Similarly, by using oligonucleotide primers to produce separate cDNA screening mixtures, from both induced and uninduced mRNA, clones hybridizing only with the cDNA from the induced cells are likely to contain the IFN genes (Goeddel *et al.*, 1980b). Once cloned, the cDNA sequences can be used as a hybridization probe to obtain DNA from genomic sources. This led to the identification of the presence of several other genes coding for α-IFNs (Nagata, Mantei and Weissmann, 1980).

A completely different approach, once the complete sequence of the gene or protein is known, is to synthesize the gene chemically. The first example of the application of this procedure to the interferons described the total chemical synthesis of a human IFN-α_1 gene (Edge *et al.*, 1981). This synthesis involved the assembly of 67 oligonucleotide fragments to give a double-stranded DNA molecule, each strand of which contained 514 base pairs (bp) (*Figure 3*). Prior

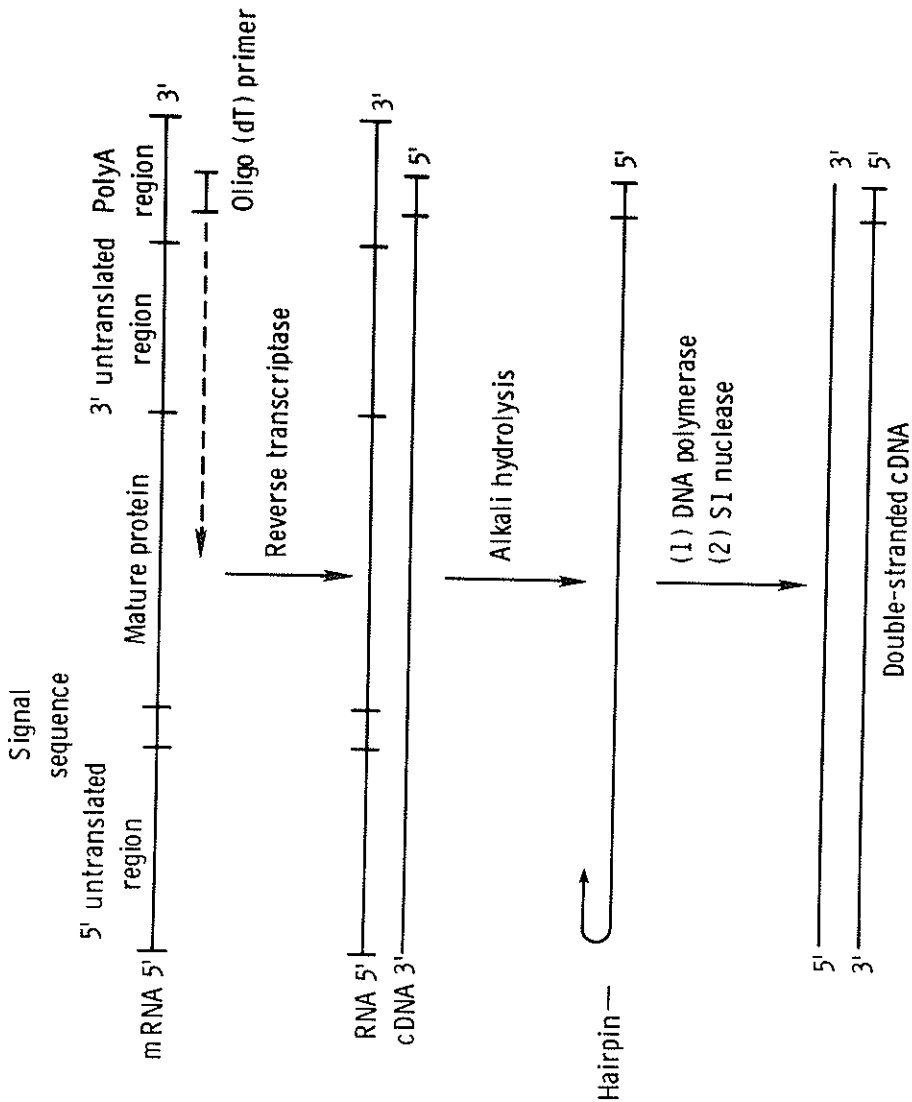

Figure 2. The preparation of double-stranded cDNA from mRNA.

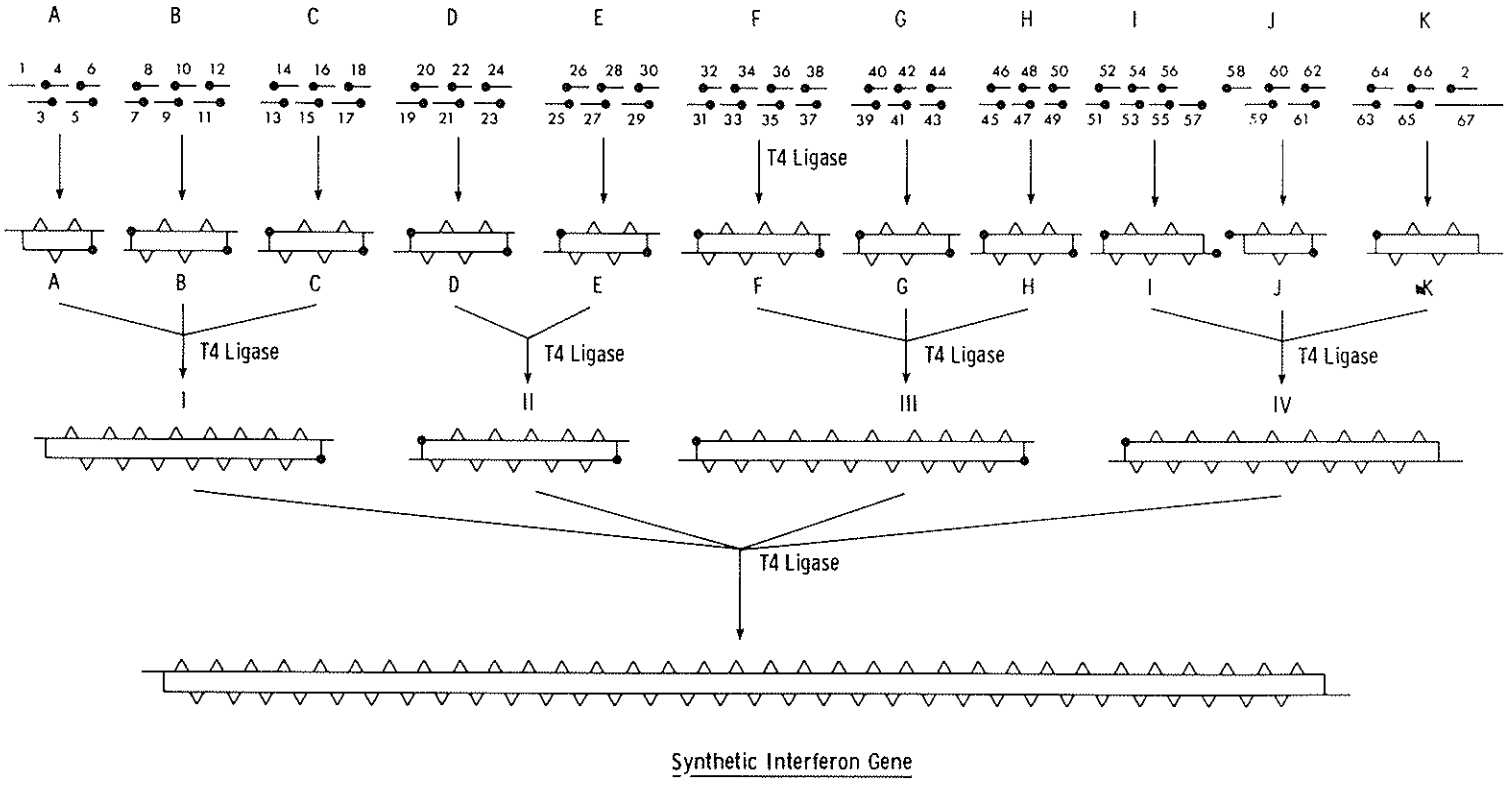

Figure 3. Ligation of chemically synthesized oligonucleotides to produce the IFN-α_1 gene. Aliquots of the chemically synthesized oligonucleotides 2–66 were phosphorylated with T4-induced polynucleotide kinase and [γ-^{32}P] ATP. The terminal phosphate group is represented by the dot. Eleven separate T4 ligase-catalysed joining reactions were performed to give fragments A to K. In groups A and K oligonucleotides 1 and 67, respectively, were not phosphorylated. Fragments I–IV were constructed from fragments A–K. The final ligation of fragments I–IV gave the complete gene.

to this work, the largest gene to have been chemically synthesized in a reasonable time scale was only just over 100 bp. Genes for IFN-γ (Tanaka *et al.*, 1983), IFN-α_2 (Edge *et al.*, 1983) and several IFN analogues (Alton *et al.*, 1983b, *see* pages 244–245) have since been reported. Synthetic genes may have certain advantages over the natural gene sequence for good expression in micro-organisms. Each organism has a different pattern of preferred codon usage. In *E. coli*, for example, the genes for abundantly expressed proteins contain a very low percentage of some codons (Gouy and Gautier, 1982) which are likely to be present in genomic or cDNA sequences for human proteins. A synthetic gene can therefore incorporate those codons thought to be preferred for expression in the host organism. Additionally, the synthetic design is likely to eliminate sequences which might give rise to secondary structures in the DNA or mRNA (to aid assembly of the oligonucleotide fragments). This might also affect the efficiency of gene expression.

FACTORS AFFECTING EXPRESSION OF CLONED IFN GENES

The synthesis of a human protein in bacteria depends on two basic steps: transcription of the gene to produce an mRNA and translation of the mRNA to produce the protein. Transcription of a cloned gene requires a promoter sequence which enables RNA polymerase to bind and initiate transcription at a site adjacent to the coding region of the gene. In order to control the transcriptional process, regulatory proteins interact with an operator region, which can be part of, or adjacent to, the promoter. This control can be exploited by inducing transcription at a specific period in the growth of the bacterial culture. For efficient translation of mRNA, a region known as the ribosome-binding site, located near the beginning of the coding sequence, is required to interact with the ribosomal RNA. The region of the DNA (3–9 bases) within the ribosome-binding site which interacts with the 3′ end of 16S ribosomal RNA is known as the Shine–Dalgarno (SD) sequence and generally precedes the translation start or initiation codon (normally AUG coding for methionine) by 3–12 bases (Steiz, 1979). A general scheme for this overall process is shown in *Figure 4*. A more detailed account of the expression of eukaryotic genes in *E. coli* can be found elsewhere (Harris, 1983).

Additional stages may be required to convert the primary translation product into functional protein. For the interferons this would include the removal of a signal sequence and glycosylation in the case of IFN-β and IFN-γ. Although IFN-β and IFN-γ are believed to be glycosylated, when produced in bacteria without their carbohydrate chains they seem to possess full biological activity. Natural IFNs are synthesized initially with a hydrophobic *N*-terminal signal sequence which is removed during processing for secretion. It is possible that the natural signal sequence functions to allow secretion to the periplasmic space in *E. coli*, but it has been observed that for IFN-β_1 the presence of a signal sequence can lead to increased instability and degradation of the pre-protein (Taniguchi *et al.*, 1980b).

Intramolecular disulphide bridges exist in IFN-α and IFN-β (Shepard *et al.*, 1981; Wetzel, 1981) and one of these is important for biological activity. In contrast, IFN-γ does not appear to form such a disulphide bridge.

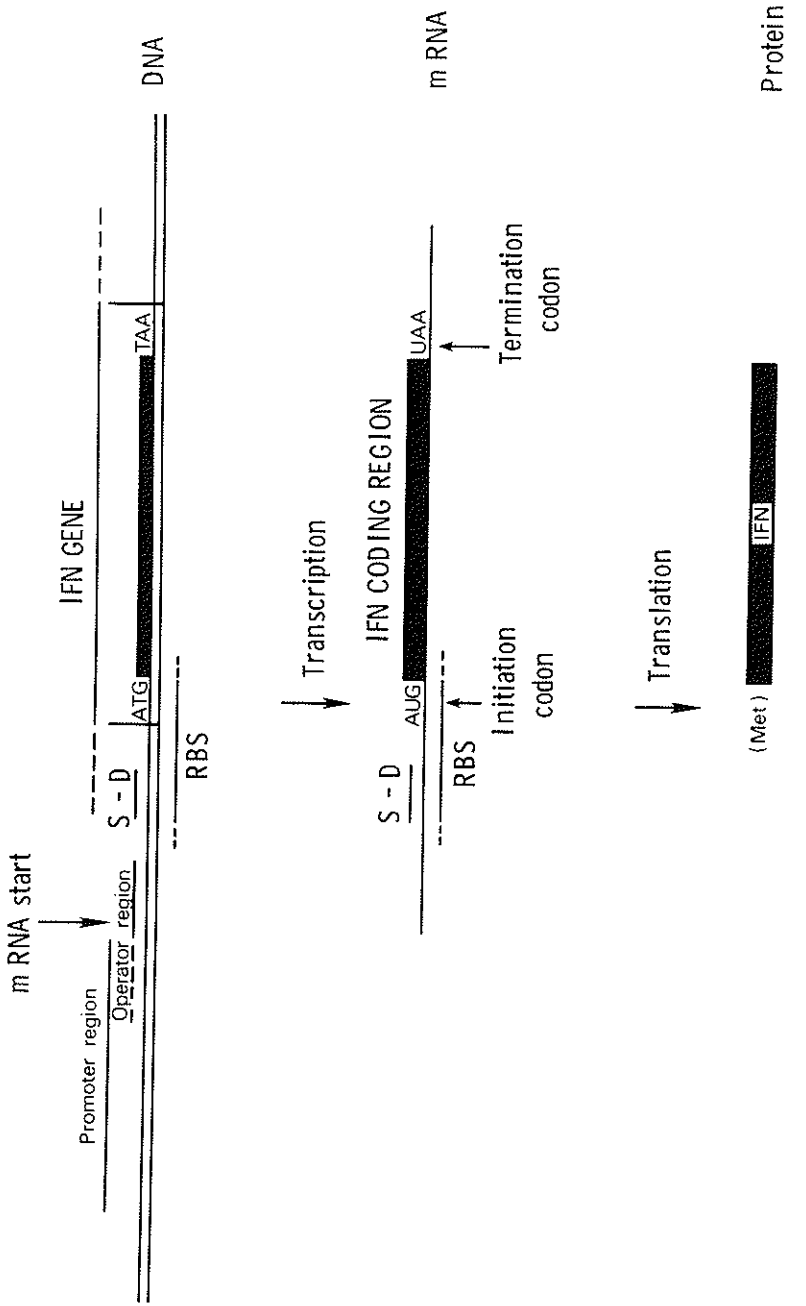

Figure 4. Gene transcription and translation. A general outline of the principal steps and structural features involved. RBS, ribosome-binding site; S–D, Shine–Dalgarno sequence.

Systems that have been used for the expression of IFN genes are presented in the appropriate sections of this review and are summarized in *Tables 2–7*. It has been possible to take the basic elements of the expression system and to vary these to maximize the production of interferon in *E. coli*. As stated above, the natural interferon-coding sequence produces pre-IFN, mature peptide preceded by the signal sequence. Thus, in order to express mature IFN the interferon gene must be engineered or edited to remove the region coding for the signal peptide in such a way that the resulting gene can be joined to the promoter system. In carrying out this manipulation, an initiation codon (ATG in the DNA molecule) must be provided at the start of the coding sequence of the mature protein. This has often been achieved by the use of synthetic oligonucleotides. It is possible to prime DNA synthesis from the codon for the first amino acid of the mature protein and then to join this DNA, after addition of linkers which include the initiation codon and a restriction enzyme site, to a plasmid vector for direct expression of the mature protein in bacteria. Alternatively, it may be possible to cut the gene near to the 5′ end of the coding sequence for the mature gene with a restriction enzyme. The region coding for the signal sequence and part of the mature protein can then be removed and replaced with a synthetic fragment to rebuild a gene coding for mature protein together with an initiation codon (*see* Edge and Markham, 1982 for a review). The translational product of these constructions is therefore a modified interferon, Met-IFN, except for IFN-β_1 where the mature peptide does have methionine at its *N*-terminus. Ironically, it appears that *E. coli* removes the *N*-terminal methionine on IFN-β_1 (cited in Stebbing *et al.*, 1982) whereas it is partially retained for the α-IFNs (Wetzel *et al.*, 1981).

It is often more convenient to join the interferon gene to a promoter system in such a way that an extended protein is formed containing extra amino acids at the *N*-terminus. These 'extra' amino acids may be part of a natural *E. coli* gene product (producing a hybrid protein), part of the signal sequence of the interferon or a combination of both. Such molecules have biological activity similar to the mature protein and have been useful for providing material for biological studies even though in some cases these extended proteins can be relatively unstable in the bacterial environment.

Synthetic genes can take into account the need to place the gene under appropriate transcriptional/translational control and so generally produce Met-IFN as the protein. A further advantage of a synthetic gene is that codons compatible with the host organism can be used. The nucleotide sequence may also be altered to avoid formation of secondary structure, particularly inverted repeats, giving rise to stem-loop structures, which may affect gene expression. A gene for bovine growth hormone, for example, is very inefficiently expressed in *E. coli* due to the formation of secondary structure (De Boer, Seeburg and Heyneker, 1983). When this region, near the *N*-terminus, was replaced with a synthetic fragment to eliminate the stem-loop, good expression was obtained.

Another phenomenon affecting the yield of foreign proteins in bacteria is protein degradation. It is known that *E. coli* can rapidly degrade nonsense proteins and in certain circumstances can degrade its own non-essential proteins. It is clear, therefore, that host strains with mutations in the

degradative systems can affect levels of expression and/or processing of recombinant proteins (*see* for example Chang, Kung and Pestka, 1983).

A comparison of the various systems used to express IFN in *E. coli* and other bacteria is difficult. Expression levels are usually assessed on the level of activity in an antiviral assay (Finter, 1969). The standard errors in such assays are notoriously large and the titres obtained vary widely depending on the cell type or virus used (Weck *et al.*, 1981a). Without knowing the specific activity of the particular molecule being expressed in the assay system used, a direct correlation is impossible. For example, the polypeptide referred to as IFN-α_1 or IFN-αD has a specific activity on human cells of only $3{\cdot}2 \times 10^6$ units per mg of protein as against $2–4 \times 10^8$ units per mg for other IFN-α species. On bovine cells it has a specific activity similar to that of other interferons at $2{\cdot}2 \times 10^8$ units per mg of protein (Weck *et al.*, 1982). A further source of confusion is the variety of ways in which different authors report expression levels. In summarizing the systems used (*Tables 2–7*), we have presented expression levels as shown in the original reference.

Human IFN from *E. coli*

HUMAN IFN-β EXPRESSION (*TABLE 2*)

The first reported cloning of an interferon gene (Taniguchi *et al.*, 1979) made use of the mRNA-to-cDNA approach described above. The mRNA was isolated from human foreskin fibroblast strain DIP 2 which had been induced for interferon production with poly (I):poly (C) and cyclohexylamine. The mRNA was further purified by sucrose gradient centrifugation and fractions enriched for IFN-producing species were used to produce cDNA. This cDNA was inserted into plasmid pBR322 and used to transform *E. coli*. A clone containing the interferon gene was identified by hybridization to a cDNA highly enriched for IFN-containing sequences by removing those cDNAs hybridizing to total mRNA from mock-induced cells. The hybrid plasmid (TpIF319) from this clone was shown to contain the sequence for HuIFN-β_1 by an *in vitro* hybridization translation assay. In a second paper by these workers (Taniguchi, Fujii-Kuriyama and Muramatsu, 1980) this hybrid plasmid was used as a probe and another recombinant plasmid, TpIF319–13, the cDNA insert of which consists of about 800 bp, was described. The nucleotide sequence analysis of about 100 bases, corresponding to the *N*-terminal region of the gene, confirmed that it coded for HuIFN-β_1 of which the first 13 amino acids had been sequenced (Knight *et al.*, 1980). The full sequence of the gene and hence of the derived protein was established independently by two other groups (Derynck *et al.*, 1980a; Houghton *et al.*, 1980) and published separately by the Japanese workers (Taniguchi *et al.*, 1980a).

Expression of HuIFN-β_1 has been achieved by several groups. The first full-length IFN-β_1 gene to be cloned (Taniguchi *et al.*, 1979) was placed under the control of a *lac* promoter in two separate plasmids in such a way that either pre-HuIFN-β_1 or mature IFN-β_1 was produced (Taniguchi *et al.*, 1980b). The

lac promoter is controlled by a repressor protein and can be conveniently induced by adding the compound isopropylthiogalactoside. Mature HuIFN-β_1 with a MW of about 20 000 and pre-HuIFN-β_1 with a MW of about 23 000 were produced at the level of 5000–10 000 molecules per cell when assayed by incorporation of radioactivity during a 5 min pulse label experiment in minicells. Bacterial extracts of the HuIFN-β_1 showed activity in an antiviral assay which corresponded to only 50 molecules per cell; extracts containing pre-HuIFN-β_1 were devoid of antiviral activity.

Other workers inserted the HuIFN-β_1 gene in a thermo-inducible expression plasmid under control of the phage lambda P_L promoter (Derynck *et al.*, 1980b). One of the constructs should produce a hybrid protein consisting of 82 amino acids of the β-lactamase protein, one amino acid coded for at a fused *Hind* II site and the complete polypeptide of pre-HuIFN-β_1. A second construct should give a protein derived from 90 amino acids of the *N*-terminus of MS2 polymerase, 27 amino acids coded for by sequences between the *Bgl* II site and the initiation codon of the HuIFN-β_1 gene, followed by the complete pre-HuIFN-β_1 gene. To estimate the MW of the interferon produced, bacterial extracts were fractionated by polyacrylamide gel electrophoresis under denaturing conditions and antiviral activity determined on eluates from sucessive gel slices. In addition to the expected hybrid proteins (or a slightly processed form), a second peak of activity with approximate MW 15 000–18 000 was produced, presumably as a result of some post-translational cleavage. The level of HuIFN-β_1 production was very low at 100 units per 5×10^8 cells/ml (equivalent to about 10 molecules/cell). The interferon produced in these systems had antiviral activity together with biological and immunological characteristics closely resembling authentic HuIFN-β. Thus, it appears that glycosylation of the interferon is not necessary for its biological and immunological properties and that additional amino acids can be accommodated at the *N*-terminus without gross effects on its activity. In a later construct, the replicase gene of the RNA phage MS2 was placed downstream from the P_L promoter and the mature HuIFN-β_1 gene coupled to the MS2 AUG initiation codon (Remaut, Stanssens and Fiers, 1983). The activity of the promoter could again be regulated by temperature. Induced cells produced 4×10^9 antiviral units/litre of culture, equivalent to 4% of the total cellular protein.

Workers at Genentech (Goeddel *et al.*, 1980a) produced a cDNA library using mRNA from human fibroblasts induced with poly (I):poly (C). Synthetic oligonucleotides based on the known *N*-terminal amino-acid sequence of IFN-β_1 (Knight *et al.*, 1980), which hybridize specifically to IFN mRNA, were used to provide a cDNA probe. This cDNA probe identified a single clone containing the IFN-β_1 gene. The size of the cDNA insert was 550 bp, which was probably too short to contain the entire coding region for a protein of the size of HuIFN-β_1. A ^{32}P-labelled DNA probe, produced from this fragment by random priming with calf thymus DNA, was used to identify a full-length gene from a newly constructed cDNA library. Oligonucleotide primers were again used in a series of elegant constructions to place the gene encoding mature HuIFN-β_1 under control of either a *lac* UV5 or a *trp* promoter system. The *lac* UV5-directed synthesis produced $9{\cdot}0 \times 10^6$ units of antiviral activity per litre of

Table 2. Expression of human interferon-β genes in *E. coli*

Interferon type	Source of DNA	Molecule expressed	Promotor or expression system	Level of expression	Reference
IFN-β	cDNA	Pre IFN-β Mature IFN-β	lac lac	50 mol/cell 5000–10 000 mol/cell	Taniguchi *et al.*, 1980b
IFN-β	cDNA	Mature IFN-β	lac trp 3×trp	$9{\cdot}0 \times 10^6$ units/ℓ $1{\cdot}8 \times 10^7$ units/ℓ $8{\cdot}1 \times 10^7$ units/ℓ	Goeddel *et al.*, 1980a
IFN-β	cDNA	β-lac or MS-2 polymerase-IFN-β or mature IFN-β	λ P_L	10 mol/cell	Derynck *et al.*, 1980b
IFN-β	cDNA	Mature IFN-β	λ P_L	4×10^9 units/ℓ	Remaut, Stanssens and Fiers, 1983
IFN-β	cDNA	Mature IFN-β	trp	10^7 units/ℓ	Houghton *et al.*, 1981a
IFN-β	cDNA	Mature IFN-β	trp	2×10^4 mol/cell	Shepard, Yelverton and Goeddel, 1982
IFN-β	Genomic	Pre IFN-β	rec A	2×10^5 units/ℓ	Feinstein *et al.*, 1983
IFN-β	cDNA	β-gal-IFN-β	lac	10^6 units/ℓ	Siggens *et al.*, 1983

culture which is equivalent to 2250 molecules per cell. The *trp* promoter construct doubled the production to 4500 molecules per cell. Moreover, when three *trp* promoters were placed in tandem and used to express the interferon, 20 200 molecules per cell were produced. A *trp* promoter system has been used by a group at G.D. Searle and Co. Ltd (Houghton *et al.*, 1981a) to express mature HuIFN-β_1 with a yield of about 10^7 antiviral units per litre of culture.

The *trp* promoter system is kept fully repressed by a protein from the *trp* repressor gene, but can be derepressed by reducing the tryptophan content of the medium or by adding the compound 3-indolylacrylic acid. For proteins other than interferon, constructions with the *trp* promoter have been used to achieve very high levels of expression, exceeding 100 000 molecules per cell. The relatively low levels of expression for IFN-β_1 cannot be explained but are probably caused by more than one factor. By optimizing the structure of the ribosome-binding site, a considerable variation in IFN-β_1 production can be achieved (Shepard, Yelverton and Goeddel, 1982). The result suggests that the efficiency of translation initiation may be one of the limiting steps in the production of protein from cloned genes in *E. coli*.

A DNA fragment, containing the human genomic IFN-β_1 gene on a 1840 bp *Eco* RI fragment, has been isolated (Mory *et al.*, 1981) and since it contains no introns (Houghton *et al.*, 1981b) was used to express IFN-β_1 in *E. coli*. The DNA was cloned in a λ Charon 4A vector and the promoter which directs transcription of the IFN-β_1 gene in the phage could be the P_L promoter or a promoter on the human DNA itself. Interferon antiviral activity of up to 7×10^6 units per litre was recovered from phage lysates after chromatography on Cibacron Blue–Sepharose.

Recently, the *rec* A promoter, thought to be one of the stronger *E. coli* promoters, has been used to express a HuIFN-β_1 gene from a human genomic library (Feinstein *et al.*, 1983). The *rec* A gene is normally repressed in *E. coli* by the product of the *lex* A gene, but can be induced by treatment with nalidixic acid. Two types of plasmid were constructed. One type contains the IFN gene at some distance from the *rec* A promoter — remote promoter fusion. The other type of plasmid contains the *rec* A promoter, ribosome-binding site and the first three codons fused to the bulk of the mature IFN-β_1 gene. With either type of construction, only 10^5 units per litre of culture were obtained on induction with optimal amounts of nalidixic acid. A fivefold increase was observed if the host strain was changed to P678–54, a minicell producer. The protein product in the remote fusion construct has not been accurately defined, but is thought to result from re-initiation at the natural IFN start codon to produce pre-IFN-β_1. The gene fusion construction substitutes the codons for the tripeptide sequence –Ala–Ile–Gln– for the serine codon at the second position of the mature IFN-β_1 gene. This interferon is still antivirally active but requires thirty times more anti-β polyclonal antibody per antiviral unit for neutralization. The authors suggest that the specific activity of this fusion product may be much lower than that of authentic IFN-β, leading to underestimation of the true amount of the fusion product present. A further consequence of this hypothesis is the implication that the region near the *N*-terminus of the IFN-β_1 molecule is important for its interaction with target cells.

An *N*-terminally modified IFN-β_1 molecule has been produced in which the codons for the first seven amino acids in an IFN-β_1 cDNA are replaced by those for 11 amino acids from β-galactosidase, EC 3.2.1.23 (Siggens *et al.*, 1983). The hybrid molecule was produced at 10^6 units per litre in *E. coli* under control of the *lac* Z gene in the single-stranded DNA phage M13 mp7. A second construction removed a tripeptide sequence –Pro–Asp–Pro– from within the *N*-terminal β-galactosidase region of the fused polypeptide and produced a sequence in which the seven amino acids of authentic IFN-β_1 were replaced by only eight amino acids of β-galactosidase. The yield (expressed as antiviral titre) was not increased. The authors conclude that the *N*-terminal amino-acid sequence can be modified fairly extensively without loss of biological activity, although the specific activities of the products were not determined. The proteins had the predicted molecular weights, but were produced at only 1% of that found for an M13mp7 construct containing the IFN-α_2 gene (Slocombe *et al.*, 1982).

To date, all the work with IFN-β has used genes which coded for the same IFN-β_1 molecule. There have been reports that a second mRNA, coding for fibroblast IFN, is produced by human fibroblasts (Sehgal and Sagar, 1980; Weissenbach *et al.*, 1980). More recently, human DNA from Namalwa lymphoblastoid cells has been examined and the presence of a novel functional IFN-β gene was strongly indicated (Sehgal *et al.*, 1983).

HUMAN IFN-α EXPRESSION (*TABLE 3*)

Very soon after the report of the cloning of a HuIFN-β gene (Taniguchi *et al.*, 1979) a report of the cloning and expression of a HuIFN-α gene appeared (Nagata *et al.*, 1980). Double-stranded cDNA prepared from the 12S fraction of mRNA from IFN-producing human leukocytes was cloned in *E. coli* using the pBR322 vector. The cDNA was inserted in the β-lactamase gene at the *Pst*I site with the expectation that it could be expressed as a fusion protein or perhaps as an independent polypeptide. A cDNA clone containing an IFN-α gene was identified by an mRNA hybridization translation assay in which DNA from successively smaller pools of clones was screened. The *Pst*I insert contained only 320 bp, about one-third of the expected length of a complete IFN cDNA. Screening of the colonies with the ^{32}P-labelled 320 bp *Pst*I fragment as a probe revealed a clone with a 900 bp *Pst*I insert. The cDNA hybridized selectively to IFN mRNA and was able to direct the synthesis, in *E. coli*, of a polypeptide with the biological activity of IFN. Plasmids were also constructed to allow synthesis of the IFN molecule fused to an earlier part of β-lactamase. Biologically active material was produced by hybrids with the IFN gene in the three different reading frames. There was no detectable difference in the size of the biologically active peptide, apparently the precursor form, made by the three clones, indicating that initiation must have occurred within the interferon gene fragment. Up to 10 000 units of antiviral activity per gram of cells was obtained from some of the clones.

The gene for IFN-α_1 was later expressed from a *lac* UV5 promoter (Stewart *et al.*, 1980). Although SDS-polyacrylamide gel electrophoretic data appear to show that the biologically active product expressed by this construction does

not carry a substantial number of supernumerary amino-acid residues, a later report (Sarkar, 1982) suggests that six additional amino acids may be present at the *N*-terminus. In yet another construction (Streuli *et al.*, 1981) the IFN-α_1 gene was joined to the trimmed 3′ end of a *lac* UV5 promoter fragment extending to the beginning of the β-galactosidase gene. The best producer clone had an initiator triplet followed by codons for the first six amino acids of β-galactosidase, a proline codon generated by the fusion, 13 codons of the signal sequence and the mature IFN-α_1 sequence. This should generate IFN-α_1 with 21 extra amino acids at the *N*-terminus.

A gene coding for HuIFN-α_1 has been chemically synthesized (Edge *et al.*, 1981) with appropriate restriction sites for placing it under the transcriptional and translational control of suitable promoters. The synthetic gene was expressed (De Maeyer *et al.*, 1982) from a wild-type *lac* and the mutant *lac* UV5 promoter which normally allows higher expression levels. The gene was indeed controlled by the *lac* promoter and the protein was expressed from the *lac* UV5 promoter at $1{\cdot}9 \times 10^5$ to $1{\cdot}3 \times 10^6$ antiviral units per 0·15 ml of bacterial extract when assayed on bovine MDBK cells. (In this report antiviral units were laboratory units and were not converted against the international standard). Other properties of the protein expressed in *E. coli* were also characteristic of IFN-α_1, including inactivation by antisera directed against leukocyte or Namalwa cell interferon, species cross reactivity, stimulation of (2′–5′) oligoadenylate synthetase activity and acid stability. It appears, however, that the *lac* UV5 construction used in this instance may not initiate translation at the expected position but at a position further along the gene (P.A. Meacock and D. Secher, unpublished data) resulting in formation of a slightly truncated protein with many of the properties of IFN-α_1. Indeed, IFN-α_1 lacking the first five or ten amino acids at the *N*-terminus has been reported to have antiviral activity (Weissmann, 1981).

The synthetic IFN-α_1 gene reported above has also been expressed from a synthetic *trp* promoter system which also reconstructs the natural ribosome-binding site/initiator codon of the *trp* LD 102 mutant *trp* species (Windass *et al.*, 1982). Based on specific activity described in the literature (Weck *et al.*, 1982) expression levels were either 23 000 (MDBK cells) or 36 000 (WISH cells) molecules of interferon per cell. (This difference probably reflects the inherent variability of antiviral assays). The product of *trp* promoter expression was purified on a monoclonal antibody column. Protein sequencing confirmed that the 10 *N*-terminal amino-acid residues were in agreement with the expected sequence of full-length IFN-α_1 (D. Secher, unpublished data).

The human myeloblastoid line KG-1 has been used as a source of mRNA enriched for IFN coding sequences (Goeddel *et al.*, 1980b). After formation of double-stranded cDNA and cloning in *E. coli*, a gene coding for a complete IFN-α gene (later designated IFN-αA) was isolated. The gene was inserted in a plasmid containing part of the *trp* operon to produce a fusion product made up from amino acids of the leader peptide and the pre-IFN-αA. The product was biologically active with a yield of 480 000 antiviral units per litre of culture. Synthetic oligonucleotides were used to link three fragments of this gene to a *trp* promoter system to achieve direct expression of mature IFN-αA. A yield of

Table 3. Expression of human interferon α genes in *E. coli*

Interferon type	Source of DNA	Molecule expressed	Promoter or expression system	Level of expression	Reference
IFN-α_1	cDNA	(Pre-) IFN-α_1	β-lactamase	2×10^4 units/ℓ	Nagata *et al.*, 1980
IFN-αA	cDNA	(Pre-) IFN-αA	trp	$4{\cdot}8 \times 10^5$ units/ℓ	Goeddel *et al.*, 1980b
		Met-IFN-αA	trp	$2{\cdot}5 \times 10^3$ units/ℓ	
IFN-α_1	cDNA	(Pre-) IFN-α_1	lac-UV5	no details	Stewart *et al.*, 1980
IFN-α_2	cDNA	(Pre-) IFN-α_2	β-lactamase	5000 units/g cells	Streuli, Nagata and Weissmann, 1980
IFN-α_1	cDNA	β-gal-IFN-α_1	lac-UV5	no details	Streuli *et al.*, 1981
IFN-α_2	cDNA	β-gal-IFN-α_2	lac-UV5	no details	Streuli *et al.*, 1981
IFN-αB	cDNA	Met-IFN-αB	trp	$8{\cdot}0 \times 10^7$ units/ℓ	Yelverton *et al.*, 1981
IFN-αD	cDNA	Met-IFN-αD	—	$2{\cdot}0 \times 10^4$ mol/cell	Cited in Hitzeman *et al.*, 1981
IFN-α_1	Synthetic	IFN-α_1*	lac UV5	$1{\cdot}3 \times 10^6$ units/0·15 ml extract	De Maeyer *et al.*, 1982
IFN-α_1	Synthetic	Met-IFN-α_1	trp	$1{\cdot}7 \times 10^6$ units/10^{10} cells	Windass *et al.*, 1982
IFN-αA	cDNA	Met-IFN-αA	trp	1×10^5 mol/cell	Shepard, Yelverton and Goeddel, 1982
α-IFNs	Genomic	No details	trp	$4\text{–}6 \times 10^6$ mol/cell	Innis *et al.*, 1982
IFN-α_2	cDNA	β-gal-IFN-α_2	lac	$1{\cdot}5 \times 10^8$ units/ℓ	Slocombe *et al.*, 1982
IF-αF	cDNA	Met-IFN-αF	lacUV5	10^5 units/ℓ	Ovchinnikov *et al.*, 1982
			2 × lac UV5	10^6 units/ℓ	
			trp	$10^7\text{–}10^8$ units/ℓ	
IFN-α_2	Synthetic	Met-IFN-α_2	trp	$1{\cdot}8 \times 10^7$ units/ℓ	Edge *et al.*, 1983
IFN-αA	cDNA	Pre-IFN-αA	trp	5×10^5 units/ℓ	Dworkin-Rastl, Swetly and Dworkin, 1983
		Met-IFN-αA	trp	2×10^7 units/ℓ	
IFN-αC	cDNA	Met-IFN-αC	trp	$8{\cdot}2 \times 10^5$ units/ℓ	
IFN-αC	Genomic	(Pre)-IFN-αC	rec A	5×10^4 units/ℓ	Feinstein *et al.*, 1983
IFN-αI	Genomic	Met-IFN-αI	trp	$6{\cdot}3 \times 10^6$ units/ℓ	Franke *et al.*, 1982

* Probably lacking some *N*-terminal amino-acid residues (*see* text)

$2{\cdot}5 \times 10^8$ antiviral units per litre of culture was obtained. This was estimated as equivalent to 600 μg of IFN per litre of culture. Subsequent determination of the specific activity of IFN-αA indicates that this really corresponds to a yield of about 1 mg per litre of culture. Attempts at optimization of IFN-αA production in *E. coli* have been reported (Shepard, Yelverton and Goeddel, 1982) by altering the distance between the Shine–Dalgarno region and the initiation codon. The optimum distance was found to be nine bases with production of mature IFN-αA at 10^5 molecules per cell.

Essentially the same protein (IFN-α_2; one amino-acid residue changed) was encoded by the second IFN gene isolated by Weissmann and co-workers (Streuli, Nagata and Weissmann, 1980) and this has been expressed in a variety of systems. Initially, a construction identical to that used to express IFN-α_1 as a β-galactosidase fusion product (Streuli *et al.*, 1981) was used. Protein from minicells was labelled with ^{35}S-methionine and fractionated by polyacrylamide gel electrophoresis. When compared with IFN-α_1 production in an identical system it appears that IFN-α_2 is produced at only 10–20% of the IFN-α_1 yield. A later report (Weissmann *et al.*, 1982) described how the signal sequence of IFN-α_2 was replaced by a DNA fragment containing the *lac* promoter and extending to the initiator codon (ATG). Although no expression figures were reported for this construction it was stated that a similar construction involving the β-lactamase promoter proved even more effective and was adapted for large-scale production of IFN-α_2. Yields of about 2 mg per litre of culture were attained on a 30 000 ℓ scale. Both IFN-αA and IFN-α_2 interferon have been purified, crystallized and are in phase I and phase II trials in man.

A cloned IFN-α_2 gene has been placed into the *lac* Z gene of bacteriophage M13mp7 (Slocombe *et al.*, 1982). Two recombinant phages containing the IFN-α_2 sequence in the correct orientation for expression from the *lac* promoter were characterized in detail. DNA sequence analysis showed that the inserted IFN-α_2 gene was in phase with the initiation codon of the *lac* Z gene. The polypeptide product has an additional 19 amino acids at the amino terminus of the mature IFN-α_2. The first 11 amino acids are derived from the amino-terminus of β-galactosidase and nine amino acids are part of the signal sequence of pre-IFN-α_2. Infection of *E. coli* with these phages, followed by induction of the *lac* promoter with isopropylthiogalactoside, gave average yields of $1{\cdot}5 \times 10^8$ units per litre of the modified IFN-α_2.

A totally synthetic IFN-α_2 gene has been produced and cloned in *E. coli* (Edge *et al.*, 1983). When the *lac* UV5 promoter was used for expression of the gene, the level of interferon was only just detectable by an immunoradiometric assay. This poor expression level is probably a consequence of secondary structure in the mRNA molecule which makes the ribosome-binding site and initiation codon inaccessible. In contrast, when expressed from a synthetic *trp* promoter, a protein was produced with the same electrophoretic mobility as a sample of authentic IFN-α_2. This product showed biological effects similar to those of Namalwa interferon and cross-reacted with the monoclonal antibody NK2 (Secher and Burke, 1980). Production of IFN-α_2 in this system was $1{\cdot}8 \times 10^7$ antiviral units per litre of culture but like many of the systems described in this review, has not been optimized.

Following the cloning and expression of these first IFN-α genes, other cDNAs for α-IFNs were identified (Streuli, Nagata and Weissmann, 1980; Goeddel *et al.*, 1981) together with related and additional chromosomal genes (Nagata, Mantei and Weissmann, 1980; Lawn *et al.*, 1981). There are at least six pseudogenes that are probably transcribed but their coding sequences contain mutations which preclude the expression of a full-length interferon-like protein, e.g. IFN-αE, -α_9, -β_{10}, -α_{11} and -α_{12} (Weissmann *et al.*, 1982). Other workers have produced cDNA clones of IFN genes from sources such as human leukocytes (Maeda *et al.*, 1980) and Namalwa cells (Skup *et al.*, 1981).

Expression of some of these sequences has been reported: IFN-αA is described above (Goeddel *et al.*, 1980b), IFN-αB from a *trp* promoter (Yelverton *et al.*, 1981), IFN-αD from a *trp* promoter (cited in Hitzeman *et al.*, 1981) and IFN-αF from *lac* and *trp* promoters (Ovchinnikov *et al.*, 1982). Leukocyte IFN gene fragments, IFN-αA and IFN-αC, isolated from a cDNA library from human lymphoblastoid cells, were expressed using a *trp* operon derived from *Serratia marcescens* and a synthetic ribosome-binding site (Dworkin-Rastl, Swetly and Dworkin, 1983). The resulting recombinant plasmid directed the synthesis of up to 5×10^5 units of A-type preinterferon, 2×10^7 units of mature IFN-αA and 8×10^5 units of mature IFN-αC per litre of culture.

The genomic sequence for IFN-αI has been edited for expression from a *trp* promoter system (Franke *et al.*, 1982) and a genomic fragment encoding IFN-αC has been expressed from a *rec* A promoter (Feinstein *et al.*, 1983). Three IFN-α genes which were isolated from a human gene library have been expressed from a *trp* promoter and leader peptide ribosome-binding site (Innis *et al.*, 1982). Although no firm details are given, the authors report expression levels of 4–6 $\times 10^6$ molecules per cell.

HUMAN IFN-γ EXPRESSION (*TABLE 4*)

Of all the IFN species, the one related to the immune system (IFN-γ) is perhaps the most interesting. The anti-proliferative effect of IFN-γ on transformed cells has been reported to be 10–100-fold greater than that of IFN-α or -β (Blalock *et al.*, 1980; Rubin and Gupta, 1980) suggesting a potential use in the treatment of cancers. Before 1982, very little was known about the sequence and structure of this protein. The first cloning of the IFN-γ gene was described early in 1982 (Gray *et al.*, 1982). A cDNA library was prepared from gel-fractionated IFN-γ mRNA from human peripheral blood lymphocytes (PBLs) stimulated to produce IFN-γ. Bacterial clones from the cDNA library were screened with a ^{32}P-labelled cDNA probe prepared either from induced or from unstimulated PBLs. Only those clones hybridizing to the probe from the induced PBLs were selected for further study. Subsequent rescreening of the cDNA library with a DNA fragment, isolated from a selected clone, identified clone 69 containing a cDNA sequence of around 1250 bp. The DNA sequence coded for a polypeptide of 166 amino acids, 20 of which could constitute a signal peptide. The procedure used to express the cDNA insert in *E. coli* was similar to that used previously for IFN-β_1 and α-IFNs. A restriction site located at codon 4 of the presumed mature coding sequence was used to remove the signal peptide

Table 4. Expression of human interferon-γ genes in *E. coli*

Interferon type	Source of DNA	Molecule expressed	Promoter or expression system	Level of expression	Reference
IFN-γ	cDNA	Met IFN-γ	trp	250 units/ml extract	Gray *et al.*, 1982
IFN-γ	Synthetic	Met IFN-γ	lac UV5	850 units/ml lysate	Tanaka *et al.*, 1983

Table 5. Expression of human interferons in bacteria other than *E. coli*

Interferon type	Organism	Source of DNA	Molecule expressed	Promoter or expression system	Level of expression	Reference
IFN-α_2	*B. subtilis*	cDNA	α-Amylase-IFN-α_2	α-amylase	Secreted—2×10^5 units/ml original culture Cellular—6×10^3 units/ml original culture	Palva *et al.*, 1982
IFN-α_1	*M. methylotrophus*	Synthetic	IFN-α_1	lac	$3{\cdot}8 \times 10^4$ units/0·15 ml extract	De Maeyer *et al.*, 1982

coding region. Two synthetic oligonucleotides were used to restore the codons for amino acids 1–4, incorporating an ATG initiation codon and creating a restriction site cohesive terminus for joining to a *trp* promoter. Production of IFN-γ was low, only about 250 units per ml of extract, but the product had properties characteristic of IFN-γ. A ^{32}P-labelled IFN-γ cDNA probe was used to screen human genomic DNA. The results indicated that human DNA contains only a single gene cross-hybridizing with the IFN-γ cDNA sequence. The gene also gives rise to a single class of mRNA (Derynck *et al.*, 1982). The genomic DNA contains three introns (Gray and Goeddel, 1982; Taya *et al.*, 1982), unlike IFN-β_1 or the α-IFNs, and cannot therefore be expressed directly in bacteria. Several publications describe the production of IFN-γ, from a cDNA sequence in yeast cells—*see* pages 236–237). Fiers and co-workers have also described the production of IFN-γ, again from a cDNA sequence, in higher eukaryotic cells such as AP8 monkey cells (Devos *et al.*, 1982) and Chinese hamster ovary (CHO) cells (Scahill *et al.*, 1983). The IFN-γ from monkey cells was secreted into the culture medium and was not distinguishable from HuIFN-γ by serological criteria or by target-cell specificity. The protein was also secreted from CHO cell lines, reaching 50 000 units per ml of culture medium. The CHO-produced HuIFN-γ migrates as two bands corresponding to MW of 25 000 and 21 000 on an SDS polyacrylamide gel.

A chemically synthesized gene for IFN-γ has been expressed in *E. coli* (Tanaka *et al.*, 1983) from a *lac* UV5 promoter. Antiviral activity was present in cell lysate and could be neutralized by addition of anti-IFN-γ serum, but not by anti-IFN-α or anti-IFN-β sera. The MW of the synthetic gene product was shown to be 17 000 by SDS polyacrylamide gel electrophoresis of ^{35}S-Met labelled proteins synthesized in minicells. On the other hand, when extracts of *E. coli* strain WA802 were fractionated by gel filtration chromatography, antiviral activity was found to elute as a single peak estimated at MW 32 000. The results suggest that a dimer form is produced in *E. coli* although it is also possible that the protein is associated with some *E. coli* component.

Human IFNs from other bacteria (*Table 5*)

The majority of cloned human genes have been expressed in *E. coli* essentially because so much is known about the genetics and expression systems in this organism. Whereas interferon synthesized in *E. coli* is retained within the cell, for production purposes it might prove advantageous if the protein is secreted into the medium. The organism *Bacillus subtilis* can secrete protein into the medium and is being intensively studied and developed as a host system for production of recombinant proteins (*see* Chapter 5 of this volume). The α-amylase gene from *Bacillus amyloliquifaciens* has been cloned in *B. subtilis* (Palva, 1982) and a series of secretion vectors consisting of promoter, ribosome-binding site and signal sequence region of the pre-α-amylase gene have been constructed (Palva *et al.*, 1982). Sequences encoding mature IFN-α_2 have been joined to these α-amylase derived secretion vectors (Palva *et al.*, 1983). The hybrid preproteins were cleaved precisely following the last amino acid of the α-amylase signal sequence. Two constructions described produced

IFN-α_2 protein preceded by either one or six amino acids; both products were secreted into the medium at 0·5 – 1 mg/litre. The amino-terminal sequences of the products were confirmed by automated Edman degradation. It seems likely that constructions in which the signal peptide is fused precisely to the IFN-α_2 polypeptide will be cleaved to release mature IFN-α_2 (Palva, 1983). This approach may therefore be superior to expression in *E. coli* systems where the eukaryotic protein is normally produced as the *N*-terminal methionine derivative. Since the foreign protein is not accumulated within the cell, where it may impair cell metabolism and growth, higher yields may also be obtained. Strains of *B. subtilis* with low exoprotease activity are likely to be required to prevent degradation of proteins in the medium.

The organism *Methylophilus methylotrophus* has many attractive features as a host. It is able to grow efficiently on the cheap substrates methanol and ammonia (*see* Chapter 10 of this volume). Additionally, the toxicology of the organism has been extensively studied and being an obligate methylotroph, it is unlikely to infect man. A DNA fragment containing the synthetic IFN-α_1 gene (Edge *et al.*, 1981) linked to a *lac* promoter was transplanted to a broad host range vector plasmid that is capable of stable maintenance in both *E. coli* and *M. methylotrophus* (De Maeyer *et al.*, 1982). IFN activity was detected in extracts of both organisms containing the recombinant hybrid plasmid and the level of expression was found to be equivalent in these two hosts.

Human IFNs from yeast (*Table 6*)

The preceding sections have reviewed the strategies used to express IFNs in prokaryotic systems and in a few cases, for the sake of completeness, in higher eukaryotic cells. Expression of foreign genes in the lower eukaryote *Saccharomyces cerevisiae* (yeast) has received much attention. Yeast has several advantages over bacteria as a recombinant DNA host: yeast glycosylates proteins (cf. IFN-β and IFN-γ) and might excise introns (such as those present in the chromosomal gene of IFN-γ). Yeast can also secrete protein and should have the advantage over bacteria in that it may process the eukaryotic signal sequence more readily.

The gene for IFN-αD has been linked to the yeast alcohol dehydrogenase I gene in a plasmid capable of autonomous replication and selection in both yeast and *E. coli*. Yeast cells transformed with these plasmids synthesize up to 10^6 molecules of biologically active IFN-αD per cell (Hitzeman *et al.*, 1981). Since a signal sequence was not used in this case, the protein was not secreted from the yeast cell. The *TRP* 1 gene of yeast codes for *N*(5′-phosphoribosyl)-anthranilate isomerase and has been used to direct the synthesis of IFN-α_2 in yeast (Dobson *et al.*, 1983). Yields of $1{\cdot}7 \times 10^3$ molecules per cell were obtained. Very high levels of a phosphoglycerate kinase gene (PGK) – IFN-α_2 fusion peptide have been produced in yeast (Tuite *et al.*, 1982). The system can produce as much as 15 mg of IFN per litre of batch culture. The PGK promoter system has been modified to allow expression of foreign genes with translation initiation from their own initiator codon (Hitzeman *et al.*, 1983). Plasmids were constructed to direct synthesis of the human proteins IFN-α_1, IFN-α_2 and IFN-γ

in yeast. The mature IFN-α_1 and IFN-α_2 genes were expressed in the yeast at 1% of the total cellular protein, based on IFN activity bioassays. No secretion of these proteins was observed, nor with a mature IFN-γ gene construct. Secretion of IFN did occur when a signal sequence was present and varied with the nature of the gene, with a maximum of 21% for IFN-γ. Secretion of IFN-α_1 and IFN-α_2 from similar constructs was between 3% and 8% and a large proportion of the protein in the growth medium has the same amino terminus as the natural mature IFN. Some processing to give proteins with additional amino acids at the *N*-termini was also observed. Several plasmids which direct the expression of HuIFN-γ in yeast under transcriptional control of the PGK promoter have been constructed (Derynck, Singh and Goeddel, 1983). The level of IFN-γ production reached $2{\cdot}5 \times 10^7$ units per litre of culture.

A more detailed account of the vector systems used for expression of IFN in yeast has been presented recently (Kingsman and Kingsman, 1983).

Non-human IFNs (*Table 7*)

The interferons are generally regarded as having species specificity in their biological activity. Whereas HuIFN-α and -β generally exhibit some antiviral action on cell lines of other species (Stewart, 1979), HuIFN-γ has strict species specificity. Consequently, animal models may not be at all suitable for the study of the potential biological and toxicological properties of human interferons or their analogues. A source, therefore, of animal interferons and their evaluation in that species may eventually aid the evaluation of the clinical potential of HuIFNs.

The mouse has an IFN-α gene family comprising not less than four, and probably more than 10 members (Shaw *et al.*, 1983). A chromosomal IFN-α gene and its cloned cDNA sequence were expressed in monkey cells under control of an SV40 promoter and in *E. coli* under control of the β-lactamase promoter. In all cases, biologically active interferon was produced. The chromosomal gene product, MuIFN-α_1 had no detectable activity on human cells, whereas the cDNA derived product, MuIFN-α_2 was 20% as active on human as on mouse cells. A murine IFN-β has been cloned and expressed from an SV40 promoter in COS-7 monkey cells (Higashi *et al.*, 1983). The MuIFN-β gene was isolated from a cDNA library derived from mouse L cells infected with Newcastle disease virus. The COS-7 cells directed the synthesis and secretion of a protein product indistinguishable from authentic mouse IFN-β. In contrast to the human genome, there may be more than one gene for mouse IFN-β (Skup *et al.*, 1982).

Recently, a gene for mouse IFN-γ has been prepared from a recombinant murine-λ phage library (Gray and Goeddel, 1983). The MuIFN-γ gene was ligated into an SV40-based expression vector and after some modification to the vector, the plasmid was used to transfect COS-1 cells. Media from the transfected COS-1 cells contained 6000 units per ml of IFN activity. The transfected COS-1 cells and mitogen-induced murine spleen cells were used to prepare cDNA for MuIFN-γ. The cDNAs from both cell lines coded for identical proteins. This MuIFN-γ cDNA was tailored for expression in *E. coli*

Table 6. Expression of human interferons in yeast

Interferon type	Source of DNA	Molecule expressed	Promoter or expression system	Level of expression	Reference
IFN-αD	cDNA	Mature IFN-αD	ADH	10^6 mol/cell	Hitzeman *et al.*, 1981
IFN-α_2	cDNA	TRP-IFN-α_2	TRP1	$1{\cdot}3 \times 10^3$ mol/cell	Dobson *et al.*, 1983
IFN-α_2	cDNA	PGK-IFN-α_2	PGK	$1{\cdot}2 \times 10^7$ mol/cell	Tuite *et al.*, 1982
IFN-γ	cDNA	mature IFN-γ	PGK	$16–25 \times 10^6$ units/ℓ	Derynck, Singh and Goeddel, 1983
IFN-α_1	cDNA	PreD-IFN-α_1	PGK	4*	Hitzeman *et al.*, 1983
		Mature IFN-α_1	PGK	0	
		PreD/A-IFN-α_2	PGK	8	
		Mature IFN-α_2	PGK	0	
		Preγ-IFN-γ	PGK	21	
		Mature IFN-γ	PGK	0	

* Percentage of IFN secreted into the medium

Table 7. Expression of non-human interferons in micro-organisms

Interferon type	Organism	Molecule expressed	Promoter or expression system	Reference
Mouse IFN-α	*E. coli*	MuIFN-α_1, -α_2	β-lactamase	Shaw *et al.*, 1983
	COS-7 (Monkey cells)	MuIFN-α_1, -α_2	SV-40	
Mouse IFN-β	COS-7 cells	MuIFN-β	SV-40	Higashi *et al.*, 1983
Mouse IFN-γ	COS-1 cells	MuIFN-γ	SV-40	Gray and Goeddel, 1983

and placed under control of a *trp* promoter. *E. coli* containing the MuIFN-γ expression plasmid produced about 5×10^4 units per ml of bacterial culture in a murine antiviral assay.

A patent describing several bovine interferons has appeared (Capon and Goeddel, 1983). The genes for these bovine interferons, both BovIFN-αs and BovIFN-βs (Leung, Capon and Goeddel, 1983) have been expressed from a *trp* promoter in *E. coli*. These interferons may find application as antiviral and antitumour agents in this animal species.

Interferon analogues

Heterogeneity in IFN-α preparations from human buffycoat leukocytes and lymphoblastoid cells had been recognized for several years prior to the first successful gene-cloning experiments (*see* Stewart, 1979 for a review). This had commonly been ascribed to probable differences in the length or degree of glycosylation of a single interferon protein. Indeed, one purification scheme for lymphoblastoid interferon incorporated a glycosidase treatment step because this seemed to reduce the number of components with interferon activity (Zoon *et al.*, 1979). It has since emerged that none of the native IFN-α species examined to date is in fact glycosylated (Allen and Fantes, 1980; Rubinstein *et al.*, 1981). The demonstration (Streuli, Nagata and Weissmann, 1980; Goeddel *et al.*, 1981) of a whole family of genes for human α-interferons suggested that the multiple components might correspond to the different protein products of the various genes.

Thus, unlike the accepted situation with the majority of hormonal and regulatory proteins and peptides, there is not just one parent human IFN-α protein. This immediately raised the possibility, so far unsubstantiated, that the different IFN-α species might have different targets within the same organism, or that they might show different relative potencies among the biological properties and side-effects ascribed to the interferon cocktails from normal cell extracts. Earlier sections of this review have described how recombinant DNA technology has made it possible to clone and express the individual IFN genes and to isolate each interferon species for detailed examination. In addition, techniques have been developed to create new genes which synthesize novel non-natural interferons. This section examines the early results available from such experiments which it is hoped could lead eventually to the design of interferon analogues with optimized biological and clinical profiles.

HYBRIDS FROM DIFFERENT SPECIES OF IFN-α

The gene and protein sequences of the different IFN-α species show 70–90% sequence homology. As a result, the recombinant DNA molecules give similar restriction patterns and, by exploiting common restriction endonuclease sites on different genes, several laboratories have constructed, by *in vitro* recombination, hybrid interferon genes containing portions from each parent molecule.

In the first published report (Streuli *et al.*, 1981) starting plasmids for IFN-α_1

and IFN-α_2 were used which contained codons for the first six amino acids of β-galactosidase, a proline codon generated by the fusion, and 13 codons of the IFN signal sequence between the initiation triplet and the start of the IFN gene sequence. These plasmids synthesized fused IFN proteins extended at the *N*-terminus by some 21 amino-acid residues. Hybrids between the IFN-α_1 and IFN-α_2 genes were constructed using as crossover points the *Pvu* II site at codon 92 and the *Bgl* II site at codon 63 of the IFN genes. In *E. coli* under *lac* promoter control these produced four hybrid IFNs (*Table 8*, 1–4), each carryng the same *N*-terminal extension as the parent species.

Essentially the same hybrids were also constructed from plasmids coding for mature IFN-αD and IFN-αA (these differ from IFN-α_1 and IFN-α_2 respectively at just one amino-acid position). This time the hybrid protein products produced in *E. coli* under *trp* promoter control carried no *N*-terminal extensions (*Table 8*) (Weck *et al.*, 1981b). Both groups examined the relative antiviral activities of crude extracts of the parent and hybrid interferons on a range of human and other mammalian cell lines. Detailed discussion of structure activity results is not appropriate to this review, but briefly, the hybrid interferons did indeed exhibit different profiles of activity from either parent molecule. The pattern of antiviral activity in the cell lines tested seemed to depend primarily on the amino and carboxy termini and not on the sequence of amino acids (62–92) between the two restriction sites. The *C*-terminal part of IFN-α_1 was important for high specific activity on mouse cells and the *N*-terminal part of IFN-α_2 conveyed high activity on human cells.

The same hybrids from the same IFN-αD and IFN-αA expression plasmids were also reported by yet a third group, but in this case each of the hybrid interferons was purified using a monoclonal antibody to human leukocyte interferon before biological evaluation (Rehberg *et al.*, 1982). A new hybrid was also constructed by digesting the *Pvu* II hybrid gene for IFN-α_1 (1–92)/α_2 (92–165) with *Bgl* II (EC 3.1.23.10) and replacing the fragment coding for the *N*-terminal region with the corresponding fragment from the gene for IFN-α_2. The protein synthesized (*Table 8*, 5) differs from IFN-α_2 at just three amino-acid positions in the region (62–92): the serine at position 69, the threonine of position 80 and the tyrosine at position 86 were replaced by threonine, aspartic acid and cysteine respectively. After purification this analogue showed a significant increase in antiviral activity over IFN-α_2 on feline lung cells and mouse L-cells and a decrease in activity on human cells.

Using the highly purified parent and hybrid IFNs, the ratios of specific molecular antiproliferative/antiviral activity on human lymphoblastoid cell lines were shown to vary over a 12-fold range. Such differences are consistent with these two distinct biological activities being mediated by different mechanisms, and provide the first evidence that the two effects may be separable. Detailed biological comparisons of all five hybrids are now appearing from several laboratories (Lee *et al.*, 1982; Weck *et al.*, 1982; De La Maza, Peterson and Czarniecki, 1983; Ortaldo *et al.*, 1983).

Another series of IFN-α hybrids explored the effects of sequence changes in just the *C*-terminal 15 amino acids of the molecules (Franke *et al.*, 1982). The plasmids directing the synthesis of mature IFN-αA and IFN-αD and a new

Table 8. Hybrid IFN-α analogues

	Target protein*	Amino-acid residues† Total	Changes from α_2	Changes from α_1	Original designation	Reference
1.	(21)-IFN-α_1(1–92)/α_2(92–165)	(21)+166	16	13	fIFNα-1(P)α-2	Streuli *et al.*, 1981
	IFN-α_1(1–92)/α_2(92–165)	166	16	13	LeIF-DA(Pvu)	Weck *et al.*, 1981b
	IFN-α_1(1–92)/α_2(92–165)	166	16	13	IFLrD/A(Pvu)	Rehberg *et al.*, 1982
2.	(21)-IFN-α_1(1–62)/α_2(62–165)	(21)+166	13	16	fIFNα-1(B)α-2	Streuli *et al.*, 1981
	IFN-α_1(1–62)/α_2(62–165)	166	13	16	LeIF-DA(Bgl)	Weck *et al.*, 1981b
	IFN-α_1(1–62)/α_2(62–165)	166	13	16	IFLrD/A(Bgl)	Rehberg *et al.*, 1982
3.	(21)-IFN-α_2(1–91)/α_1(93–166)	(21)+165	13	16	fIFNα-2(P)α-1	Streuli *et al.*, 1981
	IFN-α_2(1–91)/α_1(93–166)	165	13	16	LeIF-AD(Pvu)	Weck *et al.*, 1981b
	IFN-α_2(1–91)/α_1(93–166)	165	13	16	IFLrA/D(Pvu)	Rehberg *et al.*, 1982
4.	(21)-IFN-α_2(1–61)/α_1(63–166)	(21)+165	16	13	fIFNα-2(B)α-1	Streuli *et al.*, 1981
	IFN-α_2(1–61)/α_1(63–166)	165	16	13	LeIF-AD(Bgl)	Weck *et al.*, 1981b
	IFN-α_2(1–61)/α_1(63–166)	165	16	13	IFLrA/D(Bgl)	Rehberg *et al.*, 1982
5.	IFN-α_2(1–61)/α_1(63–92)/α_2(92–165)	165	3	26	IFLrA/D/A(Bgl-Pvu)	Rehberg *et al.*, 1982
or	[Thr^{69}, Asp^{80}, Cys^{86}]IFN-α_2					
6.	IFN-α_2(1–149)/αI(151–166)	165	6	—	IFN-αAI	Franke *et al.*, 1982
7.	IFN-αI(1–150)/α_2(150–165)	166	26	—	IFN-αIA	Franke *et al.*, 1982
8.	IFN-α_2(1–149)/α_1(151–166)	165	3	26	IFN-αAD	Franke *et al.*, 1982
9.	IFN-α_1(1–150)/α_2(150–165)	166	26	3	IFN-αDA	Franke *et al.*, 1982

* The possible presence of an *N*-terminal methionine residue derived from the translation initiation codon is ignored. Target proteins are indicated as derived from IFN-α_1 and IFN-α_2, the single amino-acid changes in IFN-αD and IFN-αA being ignored for simplicity.

† Mature IFN-α_2 (IFN-αA) and IFN-α_1 (IFN-αD) contain 165 and 166 amino acids respectively; an aspartic-acid residue at position 44 in all other IFN-α sequences so far determined is deleted in IFN-α_2 (IFN-αA).

expression plasmid containing the gene for IFN-αI (isolated from a human genomic library) have common *Bgl* II and/or *Sau* 3A restriction endonuclease sites at amino-acid positions (150–151). Gene fragments were prepared from each plasmid by sequential restriction-enzyme digestion including cuts at this common site, and recombined to yield four new hybrid plasmids which were expressed using an identical *trp* promoter expression system (*Table 8*, 6–9). A *C*-terminal truncated protein, IFN-αA (1–154), lacking the last eleven amino-acid residues of IFN-αA was also synthesized by deleting from the IFN-αA plasmid a *Hinc* II restriction fragment containing the last 34 coding nucleotides. A TAG stop codon was created by inserting, by blunt end ligation, a restriction fragment from a plasmid expressing IFN-β_1 (Goeddel *et al.*, 1980a).

Antiviral activity measurements on crude *E. coli* extracts, and after expression of fragments of plasmid DNA in a coupled transcription – translation system, again showed significant differences between the hybrids and the parent IFNs. However, although these sequence changes in the *C*-terminal region do affect the relative levels of antiviral activity, none of the amino-acid residues in this region can be essential for activity as the truncated IFN-αA (1–154) still showed up to 30% activity. This conclusion was confirmed by limited thermolysin digestion of purified IFN-αA to give IFN-αA (1–152) (with an additional peptide bond cleaved at position 109) which retained almost all the antiviral activity of the intact protein (Wetzel *et al.*, 1982) and was able to compete with radio-labelled IFN-α_2 for binding to bovine kidney cells (Arnheiter *et al.*, 1983). Also, the 10 carboxy-terminal amino-acid residues are absent in two natural leukocyte IFN proteins which have been isolated and sequenced (Evinger, Rubinstein and Pestka, 1981; Levy *et al.*, 1981).

Alternative confirmation of the non-essential nature of the carboxy-terminal region was provided by the construction of a plasmid which synthesizes an interferon in which the eleven carboxy-terminal amino-acid residues of IFN-αA were removed as described above for IFN-αA (1–154) and replaced by a sequence of nine residues encoded by the β-lactamase gene of pBR322. This chimeric protein showed some 20% of the antiviral and antiproliferative activity of IFN-αA and was probably more susceptible to proteolytic degradation in *E. coli* (Chang, Kung and Pestka, 1983).

All the hybrids described above involve *in vitro* recombination techniques and are limited by the number of common restriction sites for which isolated endonucleases are available. The recombinant machinery of *E. coli* cells *in vivo* has also been exploited to produce hybrids of IFN-α_1 and IFN-α_2 genes with junctions at points not easily accessible by the *in vitro* methodology (Weber and Weissmann, 1983). Restriction fragments from plasmids containing each of the two parent genes were linked to give a linear DNA structure with one parent IFN gene at each end joined by the rest of the vector sequences. This linear structure was transfected into the *E. coli* host where it circularized by recombination at a number of different points within the two different IFN genes to give replicating plasmids expressing various IFN hybrids. It was predicted that the positions of the junctions between the two parent genes may be confined to predetermined regions. The proteins synthesized by these hybrid genes have not yet been characterized.

HYBRIDS FROM IFN-α AND IFN-β

The sequence homologies between the different IFN-α species and IFN-β_1 are much less than those between any two IFN-α species. Thus, IFN-α_1 and IFN-β_1 show only 45% homology at the nucleotide and 29% at the amino-acid level (Taniguchi *et al.*, 1980c). Common restriction sites do occur nevertheless in both genes and a *Hinf* I site at around amino acid 70 of both proteins has been exploited to construct plasmids synthesizing two hybrid proteins: IFN-α_1 (1–72)/β_1(74–166) and IFN-β_1(1–72)/α_1 (72–166) (Mark and Creasey, 1983). Extracts containing each of these inter-class hybrid interferons are claimed to show good growth-inhibitory activity, but substantially less natural killer-cell activation activity than the parent IFNs and a total absence of detectable antiviral activity. The profile of growth-inhibitory activity seemed to be determined by the *N*-terminal part of the protein. Both IFN-α_1 and IFN-α_1 (1–72)/β_1(74–166) inhibited the growth of Daudi cells but did not inhibit HS294T clone 6 cells, whereas IFN-β_1 and IFN-β_1(1–72)/α_1(72–166) both inhibited the growth of both cell lines. These analogues are the first known examples of modifications to the sequences of native interferons which lead to a massive loss of just part of the range of biological activities associated with the parent molecules.

A further hybrid, IFN-α61A(1–40)/β_1(43–166) was obtained using a *Dde* I restriction enzyme site in the genes at around amino-acid position 40 of both proteins. (IFN-α61A is the full-length protein probably corresponding to the IFN-αG for which a partial gene sequence was described earlier) (Goeddel *et al.*, 1981). This hybrid was devoid of growth-inhibitory activity, did not activate NK-cells and showed no detectable antiviral activity on human GM2504 cells. It did show some antiviral activity on bovine cells.

ANALOGUES BY SITE-SPECIFIC MUTAGENESIS

Hybrid interferon genes generate novel permutations of the various amino-acid residues which occur naturally at any given position in the native protein chains. Other techniques are required to make changes at amino-acid positions which are conserved between proteins or to substitute completely different amino acids at the variable positions. In site-specific mutagenesis, a synthetic oligonucleotide complementary to part of a particular gene sequence but containing one or more mismatched base pair(s) is used as a primer in DNA repair reactions. This can produce mutant genes in which a single base has been changed and hence proteins in which a specific amino-acid residue has been altered in a predetermined fashion.

The technique has been applied very successfully to human IFN-β_1 to change the cysteine residue at position 17 to serine (Mark, Lin and Lu, 1984). Human IFN-β_1 contains three cysteine residues, two of which are involved in an intra-molecular disulphide bridge, the integrity of which is important for retention of biological activity. The third cysteine residue at position 17 is free and can lead to covalent dimer formation with a consequent loss of potency. By specifically switching this cysteine residue to serine the possibility of dimer formation was eliminated. The [Ser^{17}]IFN-β_1 produced had the same specific

activity as native IFN-β but was stable on storage for at least 3 months, and is now in clinical trials. Recombinant IFN-β_1 produced previously had lost biological activity too rapidly to be useful in the clinic.

Another analogue of IFN-β_1 in which the cysteine residue at position 141, normally involved in a disulphide bridge, was changed to tyrosine (Shepard *et al.*, 1981) may have been the result of inadvertent site mutagenesis. During attempts to produce recombinant plasmids coding for human fibroblast IFN a partial-length cDNA was isolated containing about two-thirds of the coding region of IFN-β_1 but with a G→A transition at position 485 of the published sequence which converts a cysteine codon to a tyrosine codon. A hybrid gene between this partial cDNA and the *N*-terminal region of the authentic IFN-β_1 cDNA was then constructed in order to express the protein analogue. Among other possibilities, the origin of the G→A transition might have been a mutagenic error during reverse transcription and this variant might not occur naturally. The [Tyr^{141}]IFN-β_1 analogue product showed no detectable antiviral activity.

ANALOGUES FROM CHEMICALLY SYNTHESIZED GENES

Clearly the most versatile approach to analogue design is to employ genes obtained by total chemical synthesis. In principle any genetically coded amino-acid residue can be substituted at any desired position throughout the entire length of the polypeptide chain. This potential was recognized in the very first report demonstrating the feasibility of total synthesis of a gene for human IFN-α_1 (Edge *et al.*, 1981). Since then, plasmids containing totally synthetic genes have been constructed to express IFN-α_2 (Edge *et al.*, 1983) and IFN-γ (Tanaka *et al.*, 1983) and reports are just beginning to appear on the synthesis and expression of analogue genes. The synthetic methodology has reached a state of development where, even relying on total synthesis for each analogue gene, by using common intermediate double-stranded segments for those regions of genes not being changed we are able to prepare and clone genes for up to two different interferon analogues a week (M.D. Edge and co-workers, unpublished data).

By introducing restriction endonuclease cleavage sites at the ends of large intermediate synthetic segments these can be inserted into a selected vector, cloned and amplified in a micro-organism and then re-isolated for further assembly, so economizing on the required scale of the chemical synthesis. In the same manner, the final totally assembled gene sequences can include two or more unique endonuclease cleavage sites at points along the sequence. This permits excision and replacement of segments, so further facilitating the generation of analogues (Alton *et al.*, 1983a). This modular approach has been used to produce genes for IFN-αF and for three 'consensus' interferons based on comparisons of the structures of all the different IFN-α genes defined to date. The consensus interferons expressed from the genes showed relative differences in antiviral activity and one of them showed a specific antiviral activity on human cells about 20 times higher than those of known natural IFN-α species (Alton *et al.*, 1983b). Genes for IFN-γ and for some 13 analogues

of the IFN-γ sequence were also described by the same workers. Although detailed biological data are not yet available, it seems that changing a single tryptophan residue at position 39 or a single methionine residue at position 48 leads to a tenfold decrease in antiviral activity whereas removal of the three *N*-terminal residues only slightly decreases antiviral activity.

ANALOGUES CONTAINING NON-CODED STRUCTURAL FEATURES

The direct products from transcription–translation of recombinant genes in bacteria are polypeptide chains containing the α-L-amino-acids programmed by the genetic code. To extend structure–activity studies into analogues incorporating non-coded amino acids or other structural features, either post-translational protein modification (*see* Means and Feeney, 1971 for a review) or protein semi-synthesis (*see* Offord, 1980 for a review) techniques would need to be applied to the bacterial-derived proteins. Some results are available of the effects on biological activity of enzymatic or chemical modification of purified IFN-α_2 (Wetzel *et al.*, 1982) but no semi-synthesis studies with IFN-related proteins have yet appeared.

In the past, protein semi-synthesis has been limited by two intrinsic factors: an adequate supply of the parent protein of interest, and the location of useful specific cleavage sites within the polypeptide chain(s) of the parent protein. Recombinant DNA technology can obviously resolve the first problem in many cases, but it should also be able to alleviate the second. Using chemically synthesized genes, analogues of the parent protein can be prepared rather than the parent protein itself. These can incorporate specific enzymatic or chemical cleavage sites at just the desired positions along a polypeptide chain. If such cleavage sites already exist in the parent protein but at undesired positions, they may be eliminated by appropriate substitution with amino acids compatible with retention of biological activity. In this manner, the starting protein sequence can be tailored or engineered to facilitate subsequent, semi-synthetic manipulation.

Conclusion

The biological and clinical interest in the interferons provided an enormous stimulus to the exploitation and development of recombinant DNA technology. Not only have cDNA sequences for a whole range of human and animal interferons been successfully cloned and expressed in a variety of micro-organisms and higher eukaryotic cells in the space of just four years, but also new techniques have been devised for the generation of structural variants of the natural interferon proteins. The success of these techniques has demonstrated the feasibility of actually being able to produce specifically designed proteins and has re-kindled interest in what is fashionably titled 'protein engineering'. Without doubt, such techniques will be applied over the next few years to other proteins of medicinal and industrial interest.

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