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Expression of human fibroblast interferon gene in *Escherichia coli*

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The human fibroblast interferon gene was inserted in a thermoinducible expression plasmid under control of the phage lambda P_L promotor. The primary translation products predicted on the basis of the plasmid constructions were hybrid proteins starting with β-lactamase or phage MS2 polymerase information followed by the total preinterferon. On induction, antiviral activity, whose physico-chemical, immunological and biological characteristics closely corresponded to those of authentic human fibroblast interferon, was synthesized. Processing to a size compatible with mature but unglycosylated authentic product was observed.

ON exposure to viruses or other specific inducers most vertibrate cells secrete protein(s) with broad antiviral action known as interferons. Human interferons are being intensively studied for their antiviral[1], anticellular[2] and immunomodulating[3] activities. Clinical trials have been carried out mainly with leukocyte interferon but also with fibroblast interferon, and some promising results have been obtained with both types in the treatment of viral diseases and cancer[1,4–9]. Tests with fibroblast interferon have been severely restricted by its very limited availability.

In a previous report[10], we described the construction and characterization of chimaeric plasmids containing human fibroblast interferon (HF–IF) cDNA. Two other groups have constructed plasmids containing either human leukocyte[11] or human fibroblast[12] interferon cDNA and in the former case, interferon-related polypeptides, as judged by biological and immunological criteria, were detected in *E. coli* strains harbouring the chimaeric plasmids. We have now inserted the HF–IF coding sequence derived from our original clones into appropriate sites on specifically constructed expression vehicles which contain the strong leftward promoter (P_L) of bacteriophage λ. The functioning of the promoter could be controlled by using host strains which synthesize a temperature-sensitive repressor (cI-ts). We describe here how plasmids containing P_L in front of the HF–IF coding sequence direct the synthesis of polypeptides with human fibroblast interferon activity in *E. coli*.

Construction of plasmids allowing expression of HF–IF

Construction of the different plasmids containing HF–IF DNA under the control of lambda P_L is schematically represented in Fig. 1. The formation and use of acceptor plasmids pPLa2311, pPLa8 and pPLc24 will be published in detail elsewhere (E. Remaut *et al.*, in preparation).

None of the chimaeric plasmids previously described contains an uninterrupted HF-IF gene[10]. A complete and continuous coding sequence for HF–IF was reconstituted by inserting an *Eco*RI-*Pst*I fragment from pHFIF-6 and a *Pst*I-*Hae*II fragment from pHFIF-7 into the plasmid pPLa2311. From the resulting plasmid, designated pPLa-HFIF-67-1, a *Bgl*II fragment was excised and ligated into the *Bam*HI site of the β-lactamase region of pPLa8 in the sense orientation with respect to the P_L promoter. The known nucleotide sequence around the *Bam*HI-*Bgl*II junction in this plasmid, pPLa-HFIF-67-12, predicts that a polypeptide initiated at the AUG of the β-lactamase part will terminate on a double amber stop codon in the 5′-untranslated region of the HF–IF gene, 23 nucleotides before the HF–IF initiating AUG (data not shown).

pPLa-HFIF-67-12Δ19 was derived from pPLa-HFIF-67-12 by deleting a *Hin*dII fragment starting within the β-lactamase gene and extending up to three nucleotides before the HF–IF initiating AUG (Fig. 1). From the known nucleotide sequence of the β-lactamase gene (as determined on the progenitor pBR322)[13] and of the HF–IF gene[10], a continuous reading frame starting at the initiating AUG of the β-lactamase gene and running up to the terminating UGA of the HF–IF gene is predicted. The expected fusion polypeptide consists of 82 amino acid residues of the β-lactamase protein, one amino acid coded for at the fused *Hin*dII site, and the complete polypeptide (including the putative signal sequence) specified by the HF–IF gene. The predicted sequence around the junction is: β-lactamase gene moiety-GUU.AAC. AUG-HFIF gene, where the GUU triplet codes for amino acid 82 of the β-lactamase protein.

Alternatively, a hybrid plasmid with the controllable lambda P_L promoter in the clockwise orientation was constructed. The acceptor plasmid was pPLc24, which contains the P_L promoter followed by an *Eco*RI-*Bam*HI fragment (derived from pMS2-7 [ref. 14]) containing the ribosome binding site and part of the MS2 polymerase gene. The pPLa-HFIF-67-1 *Bgl*II fragment containing the HF–IF gene was inserted into the *Bam*HI site of pPLc24, resulting in loss of *Bam*HI and *Bgl*II sensitivity but formation of *Sau*3AI sites at the joints (Fig. 1). In this new plasmid, pPLc-HFIF-67-8, a continuous reading frame starting at the initiating AUG of the MS2 polymerase gene and terminating at the UGA of the HF–IF gene, can be predicted on the basis of the known nucleotide sequences of the MS2 polymerase gene[15] and of pHFIF-6 and pHFIF-7 (ref. 10). The expected fusion protein consists of the N-terminal 98 amino acids of the MS2 polymerase moiety, 27 amino acids coded for by sequences between the *Bgl*II site and the initiating AUG of the HF-IF gene, followed by the complete HF–IF coding region,

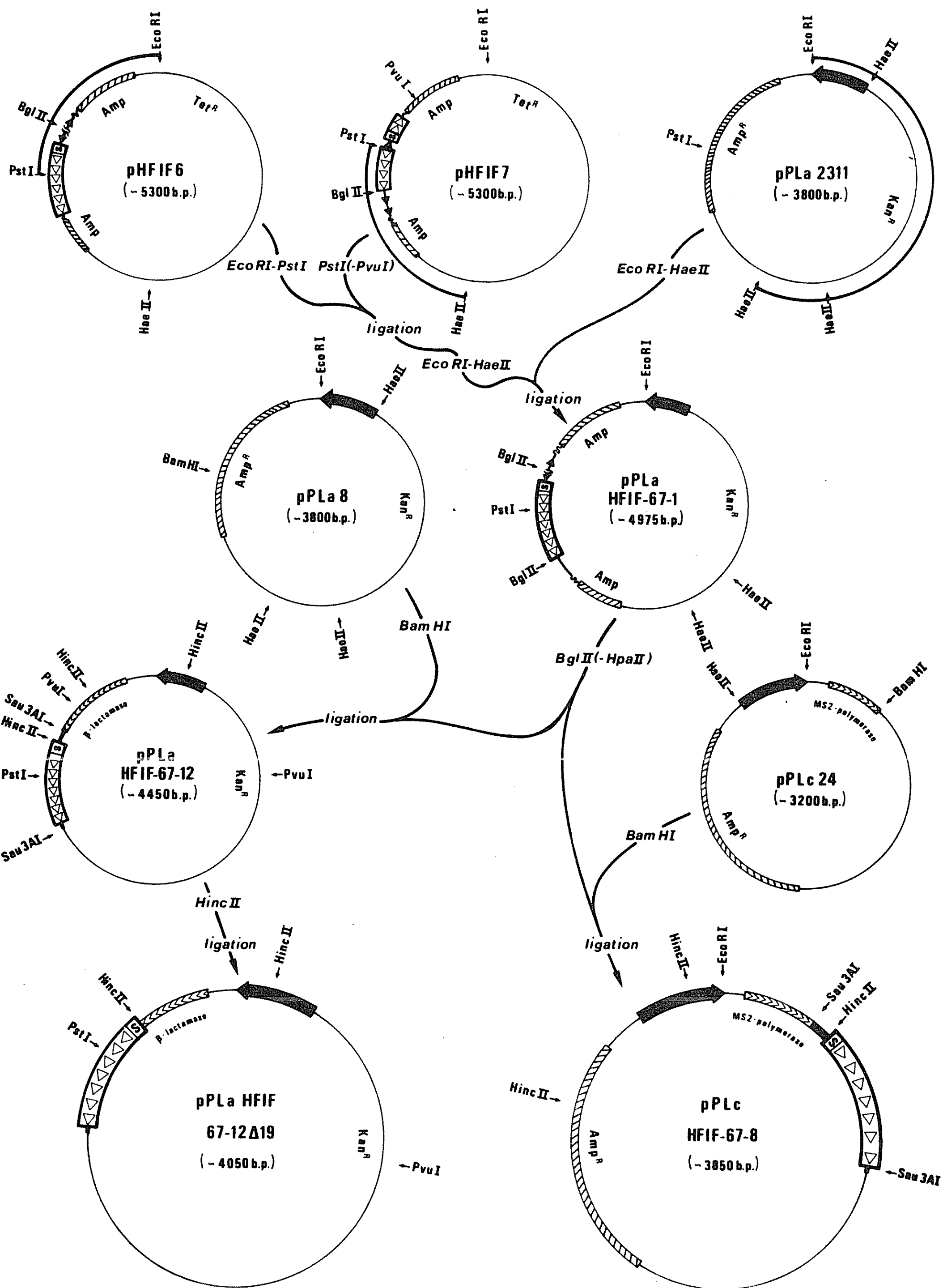

Fig. 1 Schematic outline of the construction of the different plasmids. The HF–IF DNA is indicated by a heavy line, the boxed area being the coding region with the putative signal peptide (S); the triangles indicate the $5' \rightarrow 3'$ direction of the insert. The heavy arrow indicates the lambda P_L-promoter control element. The ampicillin-resistance region is shown as a shaded area. An interruption of the base line in the insert shows the cross-over region of the inversions of our original HF–IF cDNA-containing plasmids[10]. dA·dT tails are indicated by a wavy line. Amp, β-lactamase region coding for resistance to ampicillin and carbenicillin; Kan, region coding for kanamycin resistance. To construct pPLa-HFIF-67-1, pHFIF-6 DNA was cleaved with *Eco*RI and *Pst*I and ligated to pHFIF-7 which had been cleaved with *Pst*I and *Pvu*I. Following ligation, the mixture was digested with *Eco*RI and *Hae*II and ligated to pPLa2311 which had been digested with *Eco*RI and partially with *Hae*II. The segments of the outer circles indicate the fragments retained in the pPLa-HFIF-67-1 construction: only those restriction sites relevant to the constructions are indicated. Transformants were obtained in $C600r_K^-m_K^+(\lambda)$ with selection for kanamycin resistance and screened for sensitivity to carbenicillin. The structure of a representative plasmid, pPLa-HFIF-67-1, was confirmed by digestions with *Eco*RI, *Pst*I, *Bgl*II and *Hinc*II (=*Hind*II). To construct pPLa-HFIF-67-12, the HF–IF gene was excised from pPLa-HFIF-67-1 with *Bgl*II, followed by fragmentation of the remaining part of the plasmid with *Hpa*II, which does not cut the HF–IF gene. The DNA was ligated to pPLa8, which had been opened with *Bam*HI. After ligation, the mixture was redigested with *Bam*HI to eliminate recircularized pPLa8 acceptor plasmids. Transformants were obtained in $C600r_K^-m_K^+(\lambda)$ by selection for kanamycin resistance. The structure of pPLa-HFIF-67-12 was examined by analysis with *Pst*I and *Hinc*II. To construct pPLa-HFIF-67-12Δ19, pPLa-HFIF-67-12 was partially digested with *Hinc*II and ligated at a low DNA concentration ($0.01\ \mu g\ ml^{-1}$). The mixture was then digested with *Xor*II, an isoschizomer of *Pvu*I producing 3′-protruding ends[26], and religated at low DNA concentration, thereby substantially reducing the probability of religation of the original pPLa-HFIF-67-12 plasmids. The structure of pPLa-HFIF-67-12Δ19 was examined by digestion with *Hinc*II and *Pvu*I. To construct pPLc-HFIF-67-8, the DNA of pPLa-HFIF-67-1 was digested with *Bgl*II and ligated into the *Bam*HI-opened pPLc-24 DNA. The ligation mixture was redigested with *Bam*HI to eliminate religated parental pPLc-24 plasmids. Transformants were selected for resistance to carbenicillin. The insertion and orientation of the *Bgl*II HF–IF fragment was established by *Hinc*II digestion. Restriction enzymes (Biolabs, Boehringer or BRL) were used according to the manufacturer's specifications. Ligations were carried out at 22 °C with T4 DNA ligase in Tris-Cl (50 mM, *p*H 7.6), $MgCl_2$ (5 mM), β-mercaptoethanol (7 mM), ATP (50 μM), (ref. 27). All plasmids were subsequently transferred into *E. coli* M5219 (K12 M72 lac_{am} trp_{am} Sm^R [λCI857ΔHI bio 2521])[16]. Enzymatic reactions were stopped by heating at 65 °C for 10 min.

including the signal peptide. The predicted sequence around the junction is

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                              -Trp-Asp-Leu-Gln-Phe-Arg-Arg-Gln-Pro-
MS2 polymerase gene moiety-UGG.GAU.CUU.CAG.UUU.CGG.AGG.CAA.CCU.

Phe-Glu-Ala-Phe-Ala-Leu-Ala-Gln-Gln-Val-Val-Gly-Asp-Thr-Val-Arg-
UUC.GAA.GCC.UUU.GCU.CUG.GCA.CAA.CAG.GUA.GUA.GGC.GAC.ACU.GUU.CGU.

Val-Val-Asn-Met-
GUU.GUC.AAC.AUG-HFIF coding region
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The first amino acid, tryptophan, corresponds to position 98 of the MS2 polymerase.

All constructed chimaeric plasmids were transformed into *E. coli* C600$r_K^- m_K^+(\lambda)$ and, after characterization, transferred into *E. coli* M5219 (ref. 16), allowing the temperature-dependent controlled expression of the lambda P_L (E. Remaut *et al.*, in preparation).

Detection of IF activity in bacterial extracts

Transcription from the P_L promoter on the plasmids can be turned on by shifting the growing culture from 28 to 42 °C. The synthesis of IF-related product(s) was examined by assaying an S100 extract of the bacteria for antiviral activity (Table 1). The cells were lysed either by lysozyme treatment followed by freeze-thawing or by heating in 1% SDS, 1% β-mercaptoethanol, 5 M urea. The extracts of temperature-induced *E. coli* M5219 containing pPLa-HFIF-67-12Δ19 or pPLc-HFIF-67-8 showed a clear antiviral activity, which was reproducibly higher with the latter plasmid. The same non-induced strains as well as induced M5219 containing a reference plasmid (pPLa8) did not show any detectable activity. In M5219 containing pPLa-HFIF-67-12, trace amounts of antiviral activity were occasionally detected after temperature shift (data not shown), presumably due to a rare reinitiation event. The much higher activity obtained after lysis with the SDS, β-mercaptoethanol, urea mixture indicates a possible nonspecific sticking of the antiviral product(s) to bacterial components, for example, cell membranes or nucleic acids. In parallel experiments in which authentic HF–IF was added to a control bacterial extract obtained by lysozyme treatment, only 10–40% of the activity was recovered.

Low but significant amounts of antiviral activity were detected in the supernatant after osmotic shock of induced M5219 transformed with pPLc-HFIF-67-8 (Table 1). When a more severe method of periplasmic extraction was used (that is, spheroplast formation), some activity was also detected with induced M5219 transformed by pPLa-HFIF-67-12Δ19. These results suggest that at least some of the bacterial HF–IF may be secreted into the periplasmic compartment, perhaps concomitantly with the removal of the signal peptide; other explanations, however, cannot be excluded.

Characterization of the bacterial IF activity

The antiviral activity detected in the above-mentioned extracts of induced bacteria was tested for several biological and physical properties characteristic of HF–IF (Table 2). First, the antiviral activity was non-dialysable; after dialysis for 16 h at neutral *p*H the antiviral activity was retained, albeit often at reduced levels (which was also the case for authentic HF–IF preparations). The observed decrease is presumably due to nonspecific sticking to the dialysis membranes, as HF–IF is known to be rather hydrophobic[17], and the unglycosylated bacterial form may be even more so. The antiviral activity could be recovered after precipitation with 67% saturated ammonium sulphate, a concentration known to precipitate HF–IF[18].

When tested for stability at *p*H 2, a common property of fibroblast and leukocyte interferon[1], bacterial HF–IF proved to remain active (Table 2), although again there was often partial loss of activity, but this was also the case with reconstituted authentic HF–IF controls.

The sensitivity of the bacterial HF–IF activity to protease was tested by treating the diluted bacterial extracts with increasing amounts of trypsin. The activity was abolished at the same concentration of trypsin that abolished the activity of authentic HF–IF added to an inactive control lysate.

HF–IF, in contrast to leukocyte interferon, is stable after heating in 1% SDS, 1% β-mercaptoethanol, 5 M urea[19], although we only obtained 10–20% recovery of activity with authentic HF–IF, either alone or in the presence of an inactive bacterial extract (data not shown). The bacterial HF–IF activity remained active in these conditions, as lysis of induced bacteria in this solution resulted in extracts with the highest antiviral titre (Table 1).

The antigenic properties of the *E. coli* IF activity were compared with those of authentic HF–IF. Serial dilutions of goat anti-HF–IF antiserum were incubated with diluted extracts containing bacterial HF–IF activity and with control HF–IF preparations in the presence or absence of an inactive bacterial lysate. The bacterial IF activity was neutralized by the specific antiserum, but some differences were noted in the neutralizing antibody titres for bacterial IF and authentic HF–IF (Table 2). Small differences in neutralization titre were also reported for bacterial leukocyte IF when compared with authentic leukocyte IF[11]. This can be explained by a difference either in antigenicity or in specific IF activity of these bacterial proteins relative to authentic IF.

Table 1 Interferon activity in extracts of *E. coli* M5219 transformed by expression plasmids containing the HF–IF coding sequence

		Interferon activity (units per ml extract) in			
Plasmid	Temperature	S100 extracts after lysis by lysozyme and freeze–thawing (I)	S100 extracts after lysis with SDS, β-mercaptoethanol, urea (II)	Periplasmic fraction: osmotic shock (III)	Periplasmic fraction: spheroplast formation (IV)
pPLa-HFIF-67-12Δ19	28 °C	<3; <2	<30; <100	<2	2
	42 °C	200; 20	200; 2000	<2	10
pPLc-HFIF-67-8	28 °C	<3; <2	<100; <100	<2	<2
	42 °C	200; 50	2,000; 3,000	30	30
pPLa8	42 °C	<3; <2	<100; <100	<2	<2

LB medium (150 ml) was inoculated with 1/500 volume of a fresh seed culture, saturated at 28 °C, and maintained with vigorous shaking at 28 °C until a cell concentration of 2×10^8 ml^{-1} was reached. Induction was by shifting the temperature to 42 °C and incubation of the cultures for 3 h to a final concentration of $4–6\times10^8$ cells ml^{-1}. The cells were collected and washed with Tris-HCl (50 mM, *p*H 7.4), NaCl (30 mM) and repelletted. Several different extraction procedures were used: I, the bacterial pellet was resuspended in a final volume (4 ml) with HEPES-NaOH (50 mM, *p*H 7.0), NaCl (30 mM) 3% calf serum, β-mercaptoethanol (3 mM), to which lysozyme (Sigma) was added to 1 mg ml^{-1}. After incubation at 0 °C for 30 min, the suspension was subjected to one or two freeze–thawing cycles. The S100 fraction was prepared by ultracentrifugation at 60,000 r.p.m. for 1 h in a Beckman SW60 Ti rotor. II, The cells were resuspended as in I and lysed in HEPES-NaOH (50 mM, *p*H 7.0), NaCl (30 mM), 3% calf serum, 1% SDS, 1% β-mercaptoethanol, urea (5 M) at 90 °C for 1–2 min. Clearing by ultracentrifugation was as in I. III, Osmotic shock procedure[21]: the bacterial cell pellet was resuspended in 20% sucrose, EDTA (100 mM), Tris-HCl (100 mM, *p*H 7.4), to a cell concentration of 1×10^{10} ml^{-1}. After 10 min at 0 °C, the suspension was centrifuged for 10 min at 10,000 r.p.m. The pellet was resuspended in water to a cell concentration of 1×10^{10} ml^{-1}. After 10 min on ice, the suspension was again cleared for 10 min at 10,000 r.p.m. To this osmotic shock supernatant was added HEPES-NaOH (50 mM, *p*H 7.0), NaCl (30 mM), β-mercaptoethanol (3 mM) and 3% calf serum. IV, Cells were resuspended in 3.6 ml of 0.1 M Tris-HCl, *p*H 8.0, 20% sucrose, to which 0.4 ml of lysozyme (5 mg ml^{-1} in EDTA [20 mM]) was added. After incubation for 30 min at 0 °C, the suspension was centrifuged for 10 min at 10,000 r.p.m. The supernatant was adjusted to 3% calf serum. IF activity was measured by a cytopathic effect (CPE)-inhibition assay on human fibroblasts trisomic for chromosome 21 in microtitre trays. The cells were challenged with vesicular stomatitis virus (Indiana strain) and the CPE was recorded at 24 h. All assays included an internal HF–IF reference which was calibrated against the NIH HF–IF reference G023-902-527. The limit of detection, normally 1 unit ml^{-1}, was often elevated due to toxicity of certain samples (for example, for the samples obtained with method II, the limit of detection was 30–100 units ml^{-1}). The titres are expressed in units ml^{-1}, although it should be noted that they were obtained by 0.5 $\log_{10}$ dilutions.

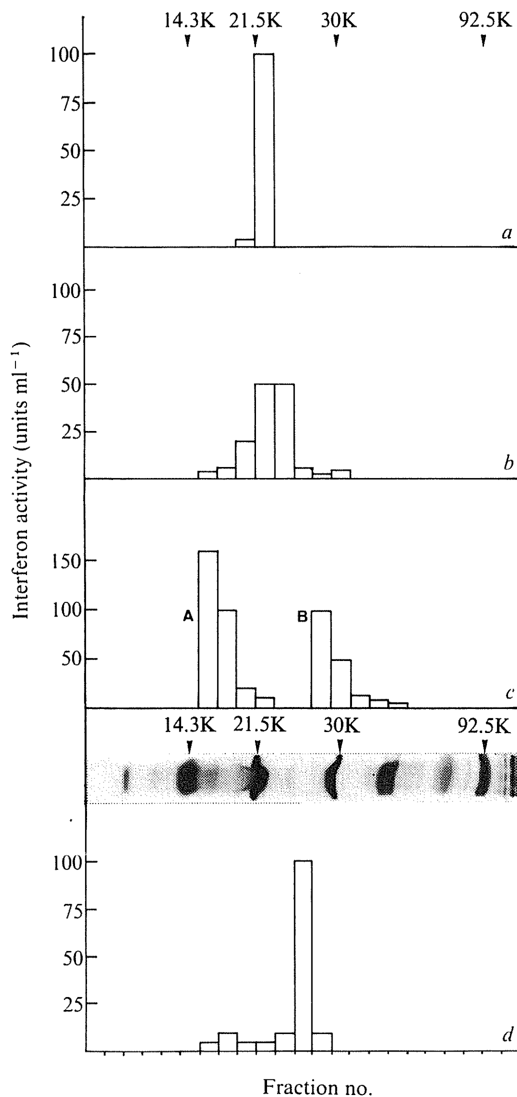

Fig. 2 Polyacrylamide gel patterns of bacterial HF–IF and authentic HF–IF. The following 100-μl samples were loaded on slots of 2 cm width: *a*, authentic HF–IF in Eagle's minimal essential medium, 10% calf serum; *b*, authentic HF–IF in control bacterial extract of M5219/pPLa8 (42 °C); *c*, bacterial extract of M5219/pPLc-HFIF-67-8 (42 °C); *d*, bacterial extract of M5219/pPLa-HFIF-67-12Δ19 (42 °C). ^{14}C-labelled protein markers electrophoresed in an equivalent amount of bacterial extract of M5219/pPLc-HFIF-67-8 (42 °C) are shown as an insertion in *d*. Bacterial extracts were prepared by treatment with SDS, β-mercaptoethanol, and urea (compare with procedure II in legend to Table 1). All samples were boiled for 1 min before electrophoresis, which was run according to Laemmli[28] in 12.5% polyacrylamide gel. IF activity profiles (*a, b, c, d*) were obtained after elution of successive 0.5 cm gel slices for 15 h in 500 μl of 0.5% bovine serum albumin in Tris (0.0125 M), glycine (0.096 M), 0.05% SDS followed by the antiviral IF assay (compare with legend to Table 1). Arrows with molecular weight corresponding to ^{14}C-labelled markers are indicated on top.

HF–IF is largely species specific, exhibiting little (if any) antiviral effect on heterologous cells[1]. Consistent with this property, bacterial IF showed no detectable antiviral protection on cells of monkey, feline, rabbit or mouse origin (Table 3). With respect to the feline cells, bacterial and authentic HF–IF behave differently from human leukocyte interferon.

Further evidence substantiating the presence of active HF–IF in induced *E. coli* extracts was provided by demonstrating the induction of 2′, 5′-oligoadenylate (2–5A) synthetase. Kerr *et al.*[20] first reported that interferon increases the level of this enzyme in susceptible cells. As shown in Table 4, appropriate bacterial extracts were able to enhance the incorporation of [α-^{32}P]ATP in ppp5′A2′p5′A2′p5′A. The 2–5A synthetase-inducing activity of the bacterial extracts was proportional to their antiviral activity. This bacterial IF activity was likewise neutralized by anti-HF–IF antiserum.

Size estimations of bacterial HF–IF

To estimate the molecular weight of the bacterial IF activity in comparison with authentic HF–IF, bacterial extracts were fractionated by polyacrylamide gel electrophoresis in denaturing conditions and the antiviral activity determined for eluates from successive gel slices (Fig. 2). Whilst authentic HF–IF showed a single peak of activity after electrophoresis, the bacterial IF activity appeared in two different peaks. Very accurate molecular weights could not be assigned because of insufficient resolution of the gel; this was mainly due to an overloading effect

Table 2 Characterization of the bacterial HF–IF activity

	Interferon titre (units ml⁻¹)	
	Before	After
a, Dialysis at neutral *p*H:		
M5219/pPLc-HFIF-67-8 (42 °C) (I)	200	200
(II)	1,000	200
(III)	30	20
M5219/pPLa-HFIF-67-12Δ19 (42 °C) (I)	200	20
Control HF–IF in M5219/pPLa8 (42 °C) (I)	200	100
	200	10
b, Precipitation with $(NH_4)_2SO_4$ at 67% saturation:		
M5219/pPLc-HFIF-67-8 (42 °C) (I)	100	100
	100	200
M5219/pPLa-HFIF-67-12Δ19 (42 °C) (I)	100	100
Control HF–IF in M5219/pPLa8 (42 °C) (I)	20	20
	30	20
c, *p*H 2 treatment:		
M5219/pPLc-HFIF-67-8 (42 °C) (I)	100	20
(II)	5	5
M5219/pPLa-HFIF-67-12Δ19 (42 °C) (I)	100	10
Control HF–IF in M5219/pPLa8 (42 °C) (I)	1,000	100
d, Heat treatment in 1% SDS, 1% β-mercaptoethanol, 5 M urea (see Table 1)		
	Inactivating end point concentration (mg ml⁻¹)	
e, Trypsin digestion:		
M5219/pPLa-HFIF-67-12Δ19 (42 °C) (II)	0.03	
M5219/pPLc-HFIF-67-8 (42 °C) (II) (1,000 units ml⁻¹)	0.03	
Control HF–IF in M5219/pPLa8 (42 °C) (II) (1,000 units ml⁻¹)	0.03	
M5219/pPLc-HFIF-67-8 (42 °C) (III) (30 units ml⁻¹)	0.03	
Control HF–IF in M5219/pPLa8 (42 °C) (III) (30 units ml⁻¹)	0.03	
	Neutralization titre (units ml⁻¹)	
f, Neutralization by antiserum		
pPLc-HFIF-67-8 (42 °C) (I)	$10^{5.2}$	
(II)	$10^{5.0}$	
(III)	$10^{5.3}$	
pPLa-HFIF-67-12Δ19 (42 °C) (I)	$10^{4.5}$	
Control HF–IF in extract of M5219/pPLa8 (42 °C)	$10^{5.7}$	
pPLc-HFIF-67-8 (42 °C) (II): elution peak A	$10^{4.5}$; $10^{4.2}$	
elution peak B	$10^{4.5}$; $10^{4.7}$	
Control HF–IF eluted from polyacrylamide gel	$10^{5.0}$	
Control HF–IF	$10^{5.7}$	
Control leukocyte IF	<10	
g, Anti-viral activity in heterologous cells (see Table 3)		
h, 2–5A synthetase induction (see Table 4)		

The following experimental methods were used for the characterization: *a*, dialysis at neutral *p*H took place overnight at 4 °C against phosphate buffered saline (PBS). *b*, Two volumes of a saturated $(NH_4)_2SO_4$ solution were added to one volume of extract. After 30 min on ice, the pellet was centrifuged at 12,000*g* for 10 min and redissolved in PBS. *c*, The bacterial extracts were either dialysed for 15 h against glycine-HCl (50 ml, *p*H 2.2), followed by dialysis against PBS for 3 h, or acidified with HCl, followed by neutralization with NaOH. After removal of the precipitate the antiviral activity was determined. *e*, Trypsin digestion was for 1 h at 37 °C with serial dilutions of the enzyme added to the diluted extract. The lowest trypsin concentration that completely abolished the antiviral activity is indicated. *f*, The antibody neutralization assays were carried out essentially as described by Havell *et al.*[22]. About 10 IF units ml⁻¹ of the preparations were incubated for 1 h at 37 °C with serial dilutions of goat anti-HF–IF antiserum, after which the residual antiviral activity was determined. Values are presented as neutralizing titres, that is, the highest dilution of antiserum which neutralized the protective effect of IF by 50% multiplied by the interferon titre of the sample assayed. Roman numerals in parentheses refer to the extraction methods described in Table 1.

Table 3 Antiviral protection of bacterial IF activity and authentic interferons

	Interferon activity (units per ml), assayed on					
	Human T-21	Human VGS	Monkey BSC-1	Rabbit primary kidney	Feline lung	Mouse L-929
M5219/pPLc-HFIF-67-8 (42 °C) (II)	3,000	300	<100	<100	<100	ND
(III)	30	<10	<10	<10	<10	ND
M5219/pPLc-HFIF-67-8 (42 °C) (II) elution peak A	2,000	200	<10	<10	<10	<10
M5219/pPLc-HFIF-67-8 (42 °C) (II) elution peak B	2,000	500	<10	<10	<10	<10
Human fibroblast interferon	3,000	500	30	30	<3	<2
Human leukocyte interferon	5,000	500	30	10	1,000	30
Human immune (type II) interferon	3,000	1,000	10	<3	<3	<2
Mouse L-929 interferon	<2	<2	<2	<2	<2	500

The antiviral activity was assayed as described in the legend to Table 1, except that the titres were directly determined from the dilution end points. T-21 are human fibroblasts trisomic for chromosome 21; VGS are normal human diploid fibroblasts[23]. Feline lung cells were obtained from Flow Laboratories (cat. no 0-10907). ND, not determined.

which resulted in a different migration of the proteins, as revealed by internal ^{14}C-labelled protein markers. Both peaks were neutralized to the same extent with anti-HF–IF antiserum (Table 2) and did not show detectable IF activity on heterologous cells (Table 3). The first peak, corresponding to an approximate molecular weight (MW) of 15,000–18,000, may have arisen by haphazard proteolytic cleavage of the fusion protein, or by limited *bona fide* processing at the now internal signal peptide, or by a combination of both processes. As shown previously, the absence of the carbohydrate moieties results in a protein which migrates in polyacrylamide gel to a position of about 4,000 MW below the authentic glycosylated HF–IF[10]. The 15,000–18,000-MW component, clearly present in *E. coli* M5219/pPLc-HFIF-67-8 extract, could also be detected at low but still significant levels in M5219/pPLa-HFIF-67-12Δ19 extracts. The second peak, with an apparent higher molecular weight, could correspond to the fused prokaryotic HF–IF polypeptide, or a slightly processed form. The tentative identification of the slower moving HF–IF activity peak as the fusion protein is strengthened by a different migration of the activity in the extract of M5219/pPLa-HFIF-67-12Δ19 (with a predicted fusion protein of about 28,000 MW) compared to the M5219/pPLc-HFIF-67-8 extract (with a predicted fusion protein of about 33,000 MW) (Fig. 2). The fusion proteins may themselves have some activity or be processed to an active product at the time when the bacterial extract (or gel eluate) is applied onto the human cells for the antiviral assay.

Table 4 2–5A synthetase inducing activity of bacterial lysates and its neutralization by anti-HF–IF antiserum

	^{32}P incorporation (in c.p.m.) into the 2–5A trimer (background subtracted)
a, M5219/pPLc-HFIF-67-8 (42 °C) (III) (30 units ml^{-1})	3,618
Control HF–IF added to M5219/pPLa8 (42 °C) (III) (30 units ml^{-1})	3,695
M5219/pPLa8 (42 °C) (III) (<2 units ml^{-1})	(−1,360)
Control HF–IF (18 units ml^{-1})	1,120
(60 units ml^{-1})	4,338
(180 units ml^{-1})	10,273
(600 units ml^{-1})	21,698
b, Control HF–IF (100 units ml^{-1})	12,468
M5219/pPLa8 (42 °C) (I)	1,011
M5219/pPLa-HFIF-67-12Δ19 (42 °C) (I)	42,193
plus 1 n.u. per ml anti-HF–IF	29,260
plus 100 n.u. per ml anti-HF–IF	727
M5219/pPLc-HFIF-67-8 (42 °C) (I)	17,478
plus 1 n.u. per ml anti-HF–IF	12,115
plus 100 n.u. per ml anti-HF–IF	7,992
plus 10000 n.u. per ml anti-HF–IF	2,517

All samples were diluted sixfold (*a*) or tenfold (*b*) before assay. Antiviral titres (before dilutions) are given in parentheses. Neutralization with goat anti-HF–IF antiserum was at 37 °C for 1 h; n.u. neutralizing units. Roman numerals in parentheses refer to the extraction methods described in Table 1 legend. The obtained values are listed after subtraction of the endogenous background activity: 3,342 c.p.m. in *a* and 2,073 c.p.m. in *b*. The 2–5A synthetase assay was modified from Kimchi *et al.*[24] and Minks *et al.*[25]. Confluent monolayers of HeLa cells in microtitre plates (96 wells) were treated with the diluted bacterial extract or with control HF–IF for 20 h. After cooling and washing with NaCl (140 mM), Tris-HCl (35 mM, *p*H 7.5), the cultures were lysed in 5 μl (*a*) or 10 μl (*b*) of 0.5% NP40, PMSF (1 mM), NaCl (140 mM), Tris-HCl (35 mM *p*H 7.5). After shaking vigorously for 20 min at 0 °C, the lysates were collected and centrifuged for 20 min at 10,000*g*. 3.5 μl of the supernatant was incubated for 2 h at 31 °C in 6 μl of KOAc (100 mM), $Mg(OAc)_2$ (25 mM), HEPES-KOH (10 mM, *p*H 7.4), ATP (5 mM), fructose-1,6-bis-phosphate (4 mM), DTT (1 mM), poly I·C (20 μg ml^{-1}) and 2 μCi of lyophilized [α-^{32}P]ATP (400 Ci mmol^{-1}). The reaction was stopped by heating for 3 min at 95 °C and the samples were treated with 150 units ml^{-1} of calf intestine alkaline phosphatase (Boehringer) at 37 °C for 1 h. After clearing, 1 μl was spotted on PEI-cellulose thin-layer plates and chromatographed in 1 M acetic acid for 2–3 h. The plates were autoradiographed and the incorporation of ^{32}P in the 2–5A trimer was determined.

Conclusion

We have demonstrated the expression of HF–IF activity in *E. coli*. This synthesis depends on the presence of the HF–IF cDNA gene in the appropriate orientation and of the controlled induction of transcription from the P_L promoter. The antiviral activity obtained from *E. coli* is due to the presence of polypeptide(s) which for all physicochemical, biological and immunological characteristics tested closely resembles authentic HF–IF. Polyacrylamide gel electrophoresis resolved the bacterial IF activity into two different size classes; the smaller component presumably resulted from a post-translational cleavage of the fusion proteins.

The HF–IF produced in *E. coli* is still low in titre (about 100 units per 5×10^8 cells ml^{-1}), but undoubtedly this can be improved by better plasmid constructions. Thus, we hope to produce sufficient quantities of bacterial HF–IF to compare its biological and pharmacological properties with those of authentic glycosylated HF–IF and of bacterial leukocyte IF, and perhaps to evaluate its potential clinical applications.

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