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Regulated expression of human interferon β_1 gene after transduction into cultured mouse and rabbit cells

[plasmid transducing vectors/dominant selectable marker (*neo*)/transduction of animal cells/interferon induction]

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ABSTRACT **The human interferon β_1 gene has been inserted into simian virus 40 hybrid plasmid vectors carrying the bacterial phosphotransferase gene (*neo*), and introduced into cultured mammalian cells by DNA transfection. A majority of the transformants resistant to the antibiotic G418 were capable of synthesizing and secreting biologically active human interferon. The *neo*/interferon transformants contain several copies of the transfecting DNA integrated into cellular DNA sequences. In most transformants the production of human interferon and its mRNA is induced by the addition of poly(rI)·poly(rC); by contrast, the level of *neo* mRNA is not increased under the same conditions. The 5′ end of the human interferon mRNA produced after induction was indistinguishable from the interferon mRNA induced in human fibroblasts. This indicates that information enabling human β_1 interferon gene to be induced by poly(rI)·poly(rC) is localized to sequences within, or 5′-proximal to, the coding sequence.**

The induction of interferon in mammalian cells is an attractive model for studying regulation of gene expression. In contrast to hormonal stimulation, which is limited to specific cell types (1), almost all cells can be induced to produce interferons characteristic of the particular cell (2). Moreover, interferons have defined host range specificities and high specific biological activities; hence, they can be readily identified and assayed.

Most fibroblast cells do not produce detectable quantities of interferon constitutively (2). But shortly after virus infection or exposure to inducers—e.g., poly(rI)·poly(rC) (2)—fibroblast interferons appear in the culture medium. Because actinomycin D blocks the induction of interferon synthesis, it has been surmised that either *de novo* transcription of interferon genes or stabilization of constitutively produced interferon mRNA is responsible for the inducible phenotype. Furthermore, because inhibitors of protein synthesis do not inhibit the induction of interferon mRNA (2, 3), induction is probably a primary response, much as mouse mammary tumor virus gene expression is induced by glucocorticoids (4), or ecdysone induces gene expression in Drosophila (5).

A cDNA clone corresponding to the mRNA encoding the major human fibroblast interferon (hIFN-β_1) has been obtained and characterized (6–8). Recently, a genomic DNA segment specifying hIFN-β_1 has been cloned and its sequence has been determined (9–14). Comparison of the two DNA sequences established that the genomic segment lacks intervening sequences. But examination of the nucleotide sequence of the hIFN-β_1 gene does not permit us to discern what regions are needed for transcription, processing, and regulation.

As a prelude to such an analysis, the expression and inducibility of the hIFN-β_1 gene has been examined after transduction into cultured mammalian cells. This has been achieved by cotransduction with a plasmid vector carrying a dominant selectable gene, *neo*, that encodes a bacterial phosphotransferase (15). A high proportion of the cells selected for expression of *neo* also contain and express, inducibly or constitutively, the co-transduced interferon gene. This result demonstrates that nucleotide sequences involved in interferon induction by poly(rI)·poly(rC) are contained within the 0.9-kilobase (kb) segment containing the hIFN-β_1 gene. While this paper was being readied, similar observations were reported by Ohno and Taniguchi (16).

METHODS

Cell Culture and Selection of Transductants. Mouse L cells (obtained from T. C. Merigan, Stanford University) were maintained in Dulbecco's modified Eagle's medium containing penicillin, streptomycin, and 5% newborn calf serum. Human foreskin fibroblasts (from T. C. Merigan) and rabbit kidney cells (RK13, from R. H. L. Pang and J. Vilček, New York University) were maintained in the same medium containing 5% fetal calf serum. The antibiotic G418 (provided by P. J. L. Daniels of Schering) was stored in Dulbecco's modified Eagle's medium (4 mg/ml) and diluted into culture medium as needed.

Supercoiled plasmid DNA was introduced into tissue culture cells (10 μg for about 5×10^6 cells) as a calcium phosphate precipitate (17) followed by a glycerol shock after 4 hr (18). About 48 hr later, the cells were trypsinized and replated at various dilutions, the lowest being 1:20. Selection for G418 resistance was instituted 12–16 hr later by the addition of G418 at 400 μg/ml (15). Transformed cells were subsequently maintained with G418 at 200 μg/ml.

Interferon Induction. Mouse L cells and their transformants were induced by incubation with poly(rI)·poly(rC) (10 μg/ml) in the presence of DEAE-dextran (100 μg/ml) in phosphate-buffered saline for 1 hr at 37°C. RK13 and human foreskin fibroblasts were induced with poly(rI)·poly(rC) at 50 and 100 μg/ml, respectively; noninduced control plates were treated similarily but without poly(rI)·poly(rC). The cells were washed twice with phosphate-buffered saline, covered with Dulbecco's modified Eagle's medium (8 ml for a 100-mm plate) containing penicillin, streptomycin, and the appropriate serum, and incubated at 37°C. The medium was collected 24 hr later and assayed for interferon activity.

Assay of Interferon. Interferon titers were determined in microtiter plates by inhibition of the cytopathic effect on human foreskin cells infected with vesicular stomatitis virus (VSV) (2) and are expressed in reference standard units. Reference hIFN-β was supplied by the National Institute of Allergy and Infectious Diseases. Analogous measurements for mouse interferon were made by using L cells challenged with VSV.

Analysis of hIFN mRNA. Cytoplasmic poly(A)$^+$RNA was extracted (19) from confluent cultures of induced or control L

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Abbreviations: SV40, simian virus 40; hIFN-β_1, human fibroblast interferon; kb, kilobase(s); VSV, vesicular stomatitis virus.

cell transformants 8 hr after induction. RNA was also obtained from nontransformed L cells subjected to the same induction or control procedures and from human foreskin fibroblasts 6 hr after they had been superinduced (20) for interferon production. The position of the 5′ end of the interferon mRNA was determined by using end-labeled DNA probes (21) and the S1 nuclease method described by Berk and Sharp (22). The end-labeled hybridization probes (indicated in the text) were prepared by labeling the appropriate 5′ ends in restriction fragments with [γ-^{32}P]ATP and polynucleotide kinase (23). After hybridizing the RNA preparations with excess labeled DNA probe, the DNA·RNA hybrids were digested with S1 nuclease and the protected fragments were analyzed by electrophoresis on neutral 1.5% agarose gels (21, 22).

RESULTS

A 1.8-kb chromosomal DNA fragment containing the hIFN-β_1 gene has been characterized (9–14) and shown to contain the entire transcribed region for hIFN-β_1 as well as 283 and 714 nucleotides in the 5′ and 3′ flanking sequences, respectively. *Eco*RI cleavage of the recombinant clone pgHFIF-4 (10, 11) (provided by W. Fiers) followed by electrophoretic fractionation on agarose gel yielded the 1.8-kb hIFN-β_1 segment. This was inserted into the unique *Eco*RI restriction site of pSV2–*neo** (15) (Fig. 1) and the resulting recombinant plasmids were screened by restriction enzyme analysis to recover those whose interferon gene orientation was opposite to that of *neo*. This particular plasmid, designated pSV2–*neo*–ghIFN-β_1, contains two tandem transcription units: one expresses *neo* by using simian virus 40 (SV40) transcription and processing signals, and the other is the putative hIFN-β_1 genomic transcription unit.

A second plasmid contained, in addition to the same *neo* transcription unit, a hybrid transcription unit in which the 5′ transcription control sequence and coding sequence of hIFN DNA were joined to a segment containing the SV40 small tumor antigen intervening sequence and the SV40 early region polyadenylylation site (25) (pSV2–*neo*–hIFN-β_1) (Fig. 1). This was achieved by removing the sequences distal to the interferon termination codon by digestion with *Bgl* II and insertion of the 0.9-kb 5′ end fragment between the *Pvu* II and *Bgl* II restriction sites of pSV2 (25). Then, a fragment containing the *neo* transcription unit, with its ends appropriately modified, was inserted at the single *Bam*HI restriction site of pSV2 to yield the desired pSV2–*neo*–hIFN-β_1 recombinant.

Synthesis of hIFN in Transformants. Two types of cells—L (mouse) and RK13 (rabbit)—were transformed to G418 resistance by each of the two plasmids. The frequency of transformation to G418 resistance ranged between 10^{-3} and 10^{-4}, with no consistent differences between the two types of recipient cells or the transfecting DNA. Because human cells are not protected against virus infection by rabbit interferon and only poorly by mouse interferon (2), the detection and quantitation of hIFN production by these transformants was simplified.

Several randomly selected G418-resistant rabbit and mouse clones were assayed for hIFN production before and after induction with poly(rI)·poly(rC). Only moderate levels of hIFN were detected in induced RK13 transformants (648 units/5 × 10^6 cells) and little or none in noninduced transformants or after induction of nontransformed RK13 cells. Because higher levels of hIFN were detected among the G418-resistant L cell clones, these were chosen for more detailed analysis.

Of 18 G418-resistant L cell transformants, 15 produced hIFN after exposure to poly(rI)·poly(rC). By contrast, the parental L cells treated with poly(rI)·poly(rC) did not produce detectable amounts of hIFN as judged by the lack of protection of human fibroblast cells against the cytopathic effect of VSV. Poly(rI)·poly(rC) also induced mouse interferon (17,500 units/10^7 cells) but there was no difference in the amounts produced by transformed and nontransformed L cells.

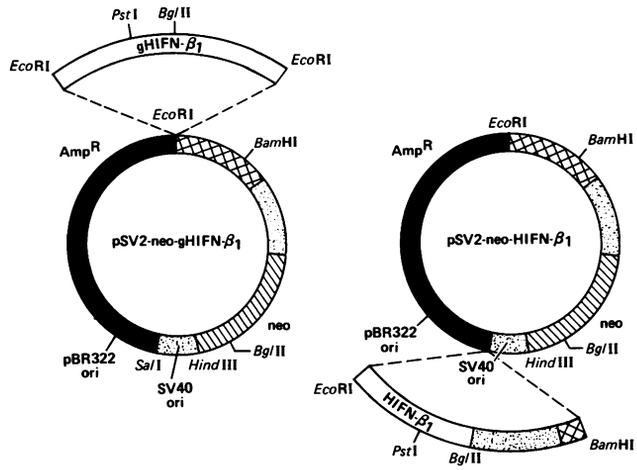

FIG. 1. Structure of hIFN DNA transducing vectors. The vectors are derivatives of pSV2–*neo* (22): pBR322 DNA is represented by the solid black segment and contains the pBR322 origin of DNA replication and the β-lactamase gene; the hatched segment represents the 1.4-kb *neo* gene; one stippled region specifies the SV40 origin of DNA replication and early promoter (*ori*) and the joined stippled and cross-hatched segment contains the SV40 small tumor antigen intervening sequence and site at which the SV40 early transcript is polyadenylylated; the open arc represents either the 1.8-kb *Eco*RI fragment that contains the entire hIFN gene or the 0.9-kb *Eco*RI–*Bgl* II subfragment that contains all but the 3′ untranslated region of the hIFN gene.

Two of the 18 G418-resistant L cell transformants examined failed to produce hIFN before or after treatment with poly(rI)·poly(rC) (clones A5 and A6, Table 1). Two of the transformants (clones B4 and B5) produced substantially the same levels of interferon before and after induction with poly(rI)·poly(rC); thus, although producing different amounts of interferon constitutively, both are noninducible. Four clones (A4, A7, A8, and B6) produced no interferon constitutively but were inducible to varying levels after exposure to poly(rI)·poly(rC). The remainder form a group that is partially constitutive for hIFN formation—that is, each produced some hIFN in the absence of poly(rI)·poly(rC) and increased levels of hIFN after treatment with poly(rI)·poly(rC). Generally, those clones that produced the most hIFN after induction also produced the highest levels constitutively; and clones that failed to make hIFN without induction produced relatively low amounts after induction. Thus, the transformants are heterogeneous with respect to the way they express the transduced hIFN gene, only a few showing the fully inducible phenotype characteristic of human fibroblasts.

The level of hIFN produced by several clones was quite high—1,944 units/10^7 cells in clones B3, B8, and B9 and 5,832 units/10^7 cells in clones A1 and A3. By comparison, human foreskin fibroblast cultures treated with poly(rI)·poly(rC) produce about 1,000 units/10^7 cells. However, the conditions for induction of the mouse transformants were optimal for induction of mouse interferon (for example, in the presence of DEAE-dextran) and these conditions may not be optimal for induction of hIFN synthesis by the same cells.

Production of hIFN-β_1 mRNA. L cell transformants that produced hIFN after exposure to poly(rI)·poly(rC) also contained the corresponding hIFN mRNA (Fig. 2). This was established by using Weaver and Weissmann's modification (21) of the S1 nuclease procedure of Berk and Sharp (22). Cytoplasmic poly(A)$^+$RNA was hybridized to an excess of a 5′-end

* This particular plasmid has the *Eco*RI–*Sal* I fragment derived from pML, a deletion mutant of pBR322 (provided by M. Botchan) (24).

Table 1. hIFN synthesis in L cell transformants

Clone	hIFN produced, units/10^7 cells: Noninduced	Induced
A. pSV2–*neo*–ghIFN-β_1 transformed:		
1	648	5,832
2	72	216
3	648	5,832
4	<8	216
5	<8	<8
6	<8	<8
7	<8	216
8	<8	72
B. pSV2–*neo*–hIFN-β_1 transformed:		
1	72	216
2	216	648
3	216	1,944
4	648	648
5	216	216
6	<8	72
7	<8	ND*
8	216	1,944
9	216	1,944
10	72	648

* ND, not done.

^{32}P-labeled DNA fragment that spans the hIFN coding and 5′ noncoding regions [the 918-nucleotide fragment bounded by the *Eco*RI and *Bgl* II restriction sites (Fig. 1)], the hybrid was digested with S1 nuclease, and the products were electrophoresed in neutral agarose gel (Fig. 2). With RNA obtained from human foreskin fibroblasts treated with poly(rI)·poly(rC), two ^{32}P-labeled fragments are detectable in the gel. One band (918 nucleotides), present in variable quantities, derives from reassociated probe; the second band (640 nucleotides) corresponds to 569 nucleotides encoding hIFN and 71 nucleotides 5′-proximal to the coding sequence. However, no 640-nucleotide fragment was detected with RNA from uninduced cells (Fig. 2). The same analysis performed with RNA isolated from L-cell transformant clones B8, B9, B10, and A1 (Table 1) confirmed that, with these cells as well, poly(rI)·poly(rC) induces the accumulation of mRNA that protects the same 640-nucleotide fragment (Fig. 2). Such a RNA species was not present in normal L cells treated with poly(rI)·poly(rC). A faint band (790 nucleotides) corresponding to a RNA whose 5′ end is 221 nucleotides upstream from the start of the hIFN coding sequence was detected in L-cell transformant clone A1, but the level of this RNA did not change upon addition of poly(rI)·poly(rC).

Because the transfecting DNA contained a SV–*neo* transcription unit physically linked to the hIFN-β_1 gene and selection of the transformants was for expression of *neo*, we tested whether *neo* expression is also inducible by poly(rI)·poly(rC). This was determined by the same S1 nuclease procedure but with a ^{32}P-end-labeled probe corresponding to the *neo* transcription unit; this consisted of a 1.5-kb DNA fragment labeled with ^{32}P at the 5′ end generated by *Bgl* II cleavage of the *neo* sequence in the plasmid DNA (Fig. 3). Transcription of pSV2–*neo* yields a *neo* mRNA that protects a 380-nucleotide DNA fragment, indicating that the 5′ end of the RNA occurs at the SV40 early region transcription start, 70 nucleotides upstream from the start of the *neo* segment (15). RNA from each of the hIFN-producing clones, protected the expected 380-nucleotide fragment, but there was no discernible increase in the amount of that fragment with RNA from cells previously treated with poly(rI)·poly(rC). Indeed, there was a consistent decrease in the amount of *neo* mRNA in the induced cells; the explanation

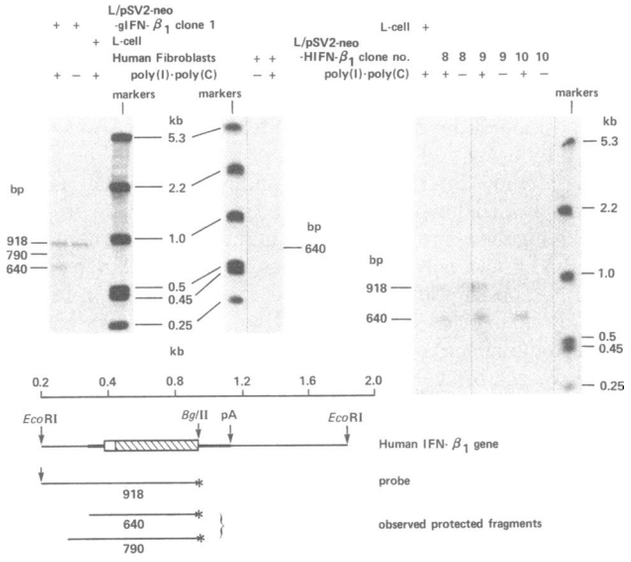

FIG. 2. hIFN mRNA produced by transformed clones. Cytoplasmic poly(A)$^+$RNA was extracted from selected G418-resistant mouse L cell clones 8 hr after poly(rI)·poly(rC) or mock treatment and analyzed by the Weaver–Weissmann modification (21) of the S1 nuclease procedure of Berk and Sharp (22). The end-labeled DNA probe used to hybridize with the mRNA preparations was 918 nucleotides and it, as well as the protected fragments, are indicated by solid lines. The distance (in nucleotides) between the ^{32}P-labeled 5′ end, marked with an asterisk, to either a restriction site or the terminus of the protected fragment is indicated. The diagram of the hIFN gene structure depicts the regions coding for the mature protein and signal peptide as hatched and open boxes, respectively; the nontranslated 5′ and 3′ regions are represented by thickened solid lines. The polyadenylylation site is indicated by pA. RNA was extracted from 1–2 × 10^7 pSV2–*neo*–ghIFN-β_1, or pSV2–*neo*–hIFN-β_1-transformed L cells or from 4 × 10^6 human fibroblasts. Size markers are *Hin*dIII-cleaved PM2 phage DNA labeled with ^{32}P at the ends. bp, Base pairs.

of this is not apparent. Nevertheless, it seems clear that, although poly(rI)·poly(rC) induces the expression of the transduced hIFN-β_1 gene, it does not increase the expression of the physically linked *neo* transcription unit.

State of the Transduced DNA in Transformed Cells. Cells that express *neo* and hIFN were examined for the content and organization of the corresponding gene sequences in the cell DNA. Electrophoresis of low molecular weight DNA present in Hirt extracts (26) of transformed clones A1, A3, B8, and B9 failed to detect any (less than one copy per cell) of the transfecting DNA (data not shown). Thus, as in previous experiments with cells transformed with analogous plasmids (27, 15), the transfecting DNA does not appear to be maintained in the stably transformed cells as an autonomously replicating plasmid.

However, the high molecular weight, ostensibly chromosomal, DNA of each of the transformed clones contained transfecting DNA sequences. Fig. 4 shows Southern blots (30) of *Bgl* II-cleaved DNA from different transformed clones after hybridization with ^{32}P-labeled hIFN DNA (the fragment between the *Eco*RI and *Bgl* II restriction sites shown in Fig. 1); this analysis detects only those segments containing the 5′-proximal and coding sequences of hIFN.

DNA from the parental L cells did not hybridize with the labeled probe, indicating that mouse and human IFN-β sequences are not homologous. However, cell DNA from three clones transformed with pSV2–*neo*–hIFN-β_1 (clones A1, A7, and A3) contained the hIFN DNA sequence. With clones A1 and A3 the hIFN sequence occurs mostly as a 3.9-kb fragment corresponding to the intact segment between the two *Bgl* II restriction sites in the transfecting plasmid (the pBR322-con-

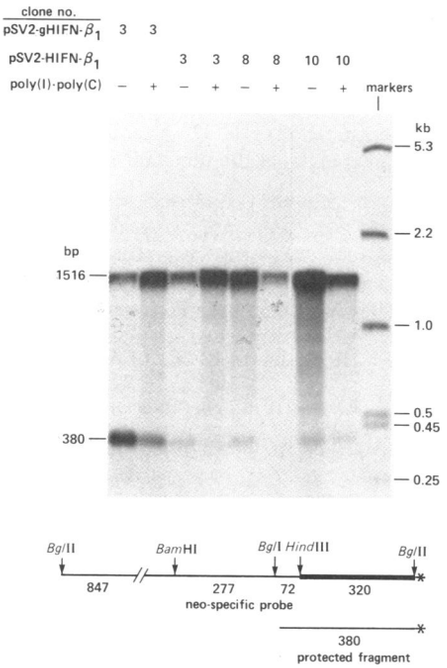

FIG. 3. *neo*-specific RNA produced by transformed clones. Poly(rI)·poly(rC) induction, extraction of RNA (from 1×10^7 cells), and S1 nuclease analysis were as in Fig. 2. The 1.5-kb end-labeled fragment obtained by *Bgl* II cleavage of pSV2–*neo*–hIFN-β_1 DNA was used as the hybridization probe; this segment is shown in the diagram with thick and thin lines representing the *neo* and SV40 DNA segments, respectively. The distance, in nucleotides, between endonuclease restriction sites or from the labeled 5′ end, marked with an asterisk, to the terminus of an observed protected fragment is indicated. Size markers are the same as in Fig. 2.

taining segment between the *Bgl* II sites shown in Fig. 1). Probing these same blots with a ^{32}P-labeled 3.5-kb DNA segment obtained from the remaining portion of the original transfecting plasmid revealed the occurrence of equivalent amounts of a 3.5-kb DNA fragment (data not shown). Thus, it appears that clones

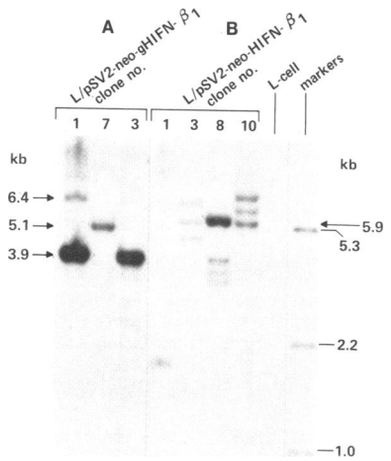

FIG. 4. DNA sequences derived from the transducing plasmids in the high molecular weight DNA extracted from G418-resistant mouse L cells. DNA (20 μg) from selected clones of transformed cells was prepared according to Wigler *et al.* (28), digested with excess *Bgl* II, electrophoresed in 0.7% agarose, and, after mild depurination (29), transferred to nitrocellulose paper (30). The blots were hybridized with a ^{32}P-labeled nick-translated (31) segment containing the hIFN DNA segment between the *Eco*RI and *Bgl* II restriction sites (Fig. 1). The size markers are labeled fragments produced by cleavage of PM2 phage DNA with *Hin*dIII.

A1 and A3 contain mostly "head-to-tail" tandemly arranged oligomers of the transfecting plasmid DNA. Clone A1 and clone A7 contain hIFN DNA segments >3.9 kb long (6.4 and 5.1 kb, respectively) (Fig. 4*A*). The larger fragments are consistent with integration of the plasmid DNA within the 3.9-kb segment, yielding hIFN DNA fragments whose lengths reflect the spacing between *Bgl* II restriction sites in the hIFN and flanking cell DNA. This interpretation anticipates that, as with clones A1 and A3, the blot of clone A7 DNA should contain an intact 3.5-kb fragment corresponding to the other portion of the transfecting plasmid, a prediction that was confirmed in the appropriate hybridization (data not shown).

The comparable analysis of three transformants produced with pSV2–*neo*–hIFN-β_1 (clones B3, B8, and B10) revealed a pattern indicating multiple integrations (Fig. 4*B*). Fragments smaller and larger than 5.9 kb, the size of the hIFN DNA segment between the two *Bgl* II restriction sites in the plasmid (see Fig. 1), would arise if integration occurred within this segment and the *Bgl* II sites in the flanking cell DNA sequence were spaced close to or far from the sites of integration, respectively. Except for clone B1, DNA from each of the transformed cells of the B series yielded a fragment of about 5.9 kb. Clone B8 is distinctive among this group in having a stretch of intact head-to-tail tandemly repeated copies of the transfecting plasmid DNA. It would be anticipated that each of the copies of the tandemly repeated plasmid DNA sequences, as well as those that appear to have integrated within the 5.9-kb segment, should yield a 1.5-kb DNA band when the Southern blot is hybridized with the ^{32}P-labeled small fragment (1.5 kb) generated by *Bgl* II cleavage of the original transfecting plasmid DNA. This was found (data not shown).

In summary, each of the G418-resistant transformants that produced hIFN and hIFN mRNA contained the sequence corresponding to the intact or reconstructed hIFN transcription units. In some transformants these sequences were clustered as tandem arrays; in others the hIFN DNA sequence occurred as a single copy or in very short clusters. The data suggest that, in stably transformed cells, the transfecting DNA is recombined with host DNA sequences and probably does not occur as autonomously replicating DNA; however, our experiments cannot exclude the occurrence of very large episomal forms. Among the limited number of transformants so far analyzed, it appears that those with substantial quantities of integrated hIFN DNA—e.g., clones A1, A3, and B8—produced the highest levels of hIFN after induction with poly(rI)·poly(rC). However, clone B3 produced the same quantity of hIFN as clones B8 and B9, yet it contained only half to a third the amount of hIFN DNA. Further studies are needed to explore this stoichiometric relationship and to ascertain if flanking cell DNA sequences or localized chromosomal conformations influence the expression of associated hIFN genes.

DISCUSSION

Transduction of mouse L cells with plasmids containing the selectable marker G418 phosphotransferase (*neo*) (15) and the hIFN-β_1 gene yielded G418-resistant clones that produce and secrete hIFN-β_1. Two types of construction were used: one contained the entire hIFN-β_1 transcription unit as well as 5′ and 3′ genomic flanking sequences; in the other, immediately downstream from the hIFN translation termination codon there was a SV40 DNA segment containing the small tumor antigen intervening sequence and early region polyadenylylation signal (25). G418-resistant transformants that produce hIFN were obtained at about equal frequency with both transducing DNAs. However, because of the variability in the hIFN DNA copy number among the transformants and the possible effect of adjacent mouse DNA sequences on the expression of acquired

hIFN genes, specific inferences about the effect of splicing on hIFN expression cannot be made.

Transformation of L cells to G418 resistance and hIFN production is accompanied by the incorporation of the transduced genes into the cellular genomes. Our data indicate that the exogenous DNA is most probably integrated into the cellular DNA and not maintained as an autonomously replicating plasmid. Most of the transformants examined contain several copies of the transfecting DNA per genome. Comparison of the intensity of the bands produced by A1, A3, and B8 DNAs with known quantities of plasmid DNA indicates that these transformants contain about 5–10 copies per genome, many of which are arranged in one or more tandem head-to-tail arrays. Perhaps, it is the multiplicity of acquired DNA copies that accounts for the high frequency of cotransformants for *neo* and the hIFN gene.

One purpose of these experiments was to learn if the transduced hIFN gene could be expressed in heterologous cells, if the resulting hIFN would be secreted into the medium, and if the production of hIFN and the corresponding hIFN mRNA were inducible by poly(rI)·poly(rC). To this end, expression of the transduced hIFN gene in the G418-resistant L cells was measured in two ways: (*i*) appearance of material in the culture medium that can protect cultured human cells against infection with VSV, and (*ii*) accumulation of cytoplasmic polyadenylylated hIFN mRNA. Because the assay for hIFN activity distinguishes mouse and human interferons, we infer that the transduced hIFN gene can be expressed and that in some transformants its expression is inducible by poly(rI)·poly(rC). Independent assays with mouse cells challenged with VSV infection indicate that, after treatment with poly(rI)·poly(rC), the same transformants produce 17,500 units of mouse interferon per 10^7 cells. Induction of human foreskin fibroblasts with poly(rI)·poly(rC) results in the appearance in the medium of about 1,000 units/10^7 cells compared to about 2–5 times that amount made by the highest producers among the transformants.

The second assay shows clearly that hIFN mRNA, which is distinguishable from the mouse counterpart by hybridization to a hIFN DNA probe, is detectable in the transformants only after induction with poly(rI)·poly(rC). The apparent absence of hIFN mRNA prior to induction, even in those cells that produce hIFN constitutively, may be due to the greater sensitivity of the assay for hIFN activity or to rapid turnover and a low steady-state level of hIFN mRNA in uninduced cells. Our analysis of the hIFN mRNA maps the 5′ end to a location 71 nucleotides upstream from the initiator codon; this compares to the previously estimated position of the 5′ end of hIFN-β_1 mRNA as 74 ± 2 nucleotides upstream from AUG (14). Another hIFN mRNA present at very low levels in cells transduced with pSV2–*neo*–ghIFN-β_1 but not influenced by treatment of the cells with poly(rI)·poly(rC) was not further investigated. Conceivably, it is transcribed from either further upstream in the hIFN DNA segment, adjacent plasmid sequences, or the SV40 late region promoter. The regulated response of pSV2–*neo*–hIFN-β_1 transformants to poly(rI)·poly(rC) addition identifies the *Eco*RI–*Bgl* II DNA fragment, which contains the hIFN-β_1 coding region and 357 base pairs upstream, as sufficient for induction of this gene.

Although the expression of the transduced hIFN gene is increased by exposure of the cells to poly(rI)·poly(rC), there is no concomitant increase in the expression of the cotransduced *neo*. This is especially significant in those transformants that contain both genes arranged in tandem, as they exist in the original transducing plasmid. Induction in this case, therefore, probably does not activate an extensive region of the genome but rather acts on the hIFN gene itself or on some post-transcriptional product or step in the pathway. This would imply that genes that are induced concomitantly with interferon in human fibroblasts (32, 33) may have their own recognition sequences for the induction phenomenon.

The availability of a cloned DNA segment that can express hIFN and is responsive to induction by poly(rI)·poly(rC) should facilitate the identification of the DNA sequence needed for the induction and an analysis of the mechanism of that response.

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1. Yamamoto, K. R. & Alberts, B. M. (1976) *Annu. Rev. Biochem.* **45**, 721–746.
2. Stewart, W. E., II (1979) *The Interferon System* (Springer, Vienna).
3. Raj, N. B. K. & Pitha, P. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7426–7430.
4. Ringold, G. M., Yamamoto, K. R., Tomkins, G. M., Bishop, J. M. & Varmus, H. E. (1975) *Cell* **6**, 299–305.
5. Ashburner, M. (1974) *Dev. Biol.* **39**, 141–157.
6. Derynck, R., Content, J., DeClercq, E., Volckaert, G., Tavernier, J., Devos, R. & Fiers, W. (1980) *Nature (London)* **285**, 542–547.
7. Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. & Muramatsu, M. (1980) *Gene* **10**, 11–15.
8. Houghton, M., Eaton, M. A. W., Stewart, A. G., Smith, J. C., Doel, S. M., Catlin, G. H., Lewis, H. M., Patel, T. P., Emtage, J. S., Carey, N. H. & Porter, A. G. (1980) *Nucleic Acids Res.* **8**, 2885–2894.
9. Houghton, M., Jackson, I. J., Porter, A. G., Doel, S. M., Catlin, G. H., Barber, C. & Carey, N. H. (1981) *Nucleic Acids Res.* **9**, 247–266.
10. Tavernier, J., Derynck, R. & Fiers, W. (1981) *Nucleic Acids Res.* **9**, 461–471.
11. Degrave, W., Derynck, R., Tavernier, J., Haegeman, G. & Fiers, W. (1981) *Gene* **14**, 137–143.
12. Gross, M., Najarian, R. & Goeddel, D. V. (1980) *Nucleic Acids Res.* **9**, 1045–1052.
13. Gross, G., Mayr, U., Bruns, W., Grosveld, F., Dahl, H. M. & Collins, J. (1981) *Nucleic Acids Res.* **9**, 2495–2507.
14. Ohno, S. & Taniguchi, T. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5305–5309.
15. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
16. Ohno, S. & Taniguchi, T. (1982) *Nucleic Acids Res.* **10**, 967–977.
17. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
18. Parker, B. A. & Stark, G. R. (1979) *J. Virol.* **31**, 360–369.
19. Villarreal, L. P., White, R. T. & Berg, P. (1979) *J. Virol.* **29**, 209–219.
20. Vilček, J. & Havell, E. A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3909–3913.
21. Weaver, R. F. & Weissmann, C. (1979) *Nucleic Acids Res.* **7**, 1175–1193.
22. Berk, A. J. & Sharp, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1274–1278.
23. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
24. Lusky, M. & Botchan, M. (1981) *Nature (London)* **293**, 79–81.
25. Mulligan, R. C. & Berg, P. (1980) *Science* **209**, 1422–1427.
26. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–369.
27. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2072–2076.
28. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lucy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
29. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
30. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
31. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
32. Raj, N. B. K. & Pitha, P. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4918–4922.
33. Gross, G., Mayr, U. & Collins, J. (1981) in *The Biology of the Interferon System*, eds. DeMaeyer, E., Gallasso, G. & Schellekens, H. (Elsevier/North-Holland, Amsterdam), pp. 85–90.