JOURNAL OF VIROLOGY, July 1983, p. 89–95
0022-538X/83/070089-07$02.00/0

Vol. 47, No. 1

Cycloheximide Induces Expression of the Human Interferon β_1 Gene in Mouse Cells Transformed by Bovine Papillomavirus-Interferon β_1 Recombinants

LUC MAROTEAUX,[1] LOUISE CHEN,[1] STELLA MITRANI-ROSENBAUM,[2] PETER M. HOWLEY,[2] AND MICHEL REVEL[1]*

Virology Department, Weizmann Institute of Science, Rehovot, Israel,[1] and Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20205[2]

Received 9 September 1982/Accepted 30 March 1983

Mouse cells transformed by a bovine papillomavirus recombinant vector containing the human interferon (IFN) β_1 (IFN-β_1) gene could be induced to produce human as well as mouse IFNs. The optimal conditions for induction of human IFN and of its mRNA in these transformants resembled those needed for mouse IFN: high concentrations of DEAE-dextran and low concentrations of polyriboinosinic acid-polyribocytidylic acid. Superinduction by inhibitors of protein synthesis which strongly stimulate IFN-β_1 induction in human cells had only a small effect on human IFN induction in bovine papillomavirus IFN-β_1-transformed mouse cells. In contrast, cycloheximide without double-stranded RNA could induce significant levels of human IFN in the bovine papillomavirus IFN-β_1 mouse transformants. After cycloheximide treatment, these cells contained IFN-β_1 mRNA whose 5′ ends originated in the authentic start site of the human IFN-β_1 gene, as shown by S1 nuclease mapping. The transferred human gene, propagated extrachromosomally in the mouse cells, was, therefore, inducible under conditions different from those in human cells. The results also confirmed that the inhibitor of protein synthesis, cycloheximide, can induce expression of a human IFN gene.

Expression of the interferon (IFN) β_1 (IFN-β_1) gene, coding for the major IFN species in human fibroblasts, is induced by double-stranded (ds) RNA or by virus infection (2, 22, 28). When cycloheximide or other inhibitors of protein synthesis are added to ds RNA, superinduction is observed, and more of this IFN is produced after removal of the inhibitor (11, 29, 34). This IFN superinduction is accompanied by a stabilization of the IFN-β_1 mRNA (4, 22, 26).

Cycloheximide alone, without ds RNA, is not usually considered to be an IFN inducer (28), but there are indications that in some cells, this inhibitor of translation can induce IFN. Thus, Tan and Berthold (30) and Tan et al. (31) reported that in one human aneuploid cell line derived from simian virus 40-transformed WI38 fibroblasts, cycloheximide and other inhibitors of protein synthesis triggered some IFN production even in the absence of ds RNA. In human fibroblasts, although the IFN-β_1 mRNA is not induced without ds RNA, another mRNA apparently coding for a minor species, IFN-β_2 (27, 36), is induced by cycloheximide. In the present work we demonstrate that cycloheximide alone can also induce the synthesis of IFN-β_1 and its mRNA in mouse cell clones in which the cloned human IFN-β_1 gene (6, 9, 13, 15, 19, 20) is propagated as part of a bovine papillomavirus (BPV) extrachromosomal vector (24). This IFN-β_1 induction by cycloheximide is compared with the induction by ds RNA which was previously demonstrated in such cells (18, 38).

MATERIALS AND METHODS

Cells. The BPV IFN-β_1-transformed mouse cells have been described previously (18). In brief, a 1.6-kilobase (kb) *Hin*dIII fragment of IFN-β_1/pBR322 clone IFN-C631 (19) was inserted in both possible orientations at the *Hin*dIII site of the recombinant plasmid pBPV$_{69T}$ consisting of the 69% transforming region of BPV DNA type I (5.4 kb). Transformed foci were isolated, and the two IFN-producing cell lines SR-117-13C and SR-117-21E were derived and characterized as described previously (18). Line ID-13 was derived from C127 cells transformed by BPV type 1 DNA (14).

IFN inductions and assays. Cultures of 0.4×10^6 cells in 6-cm plates were induced 3 to 4 days after seeding with 2 ml of medium containing polyriboinosinic acid-polyribocytidylic acid [poly(rI):poly(rC)] (PL Biochemicals), cycloheximide (Sigma Chemical Co.), and 0.5×10^6 M_r DEAE-dextran (Pharmacia Fine Chemicals, Inc.) at various concentrations. Unless otherwise stated, induction medium was removed at 4 h, the cells were washed, and 2 ml of fresh medium was added for

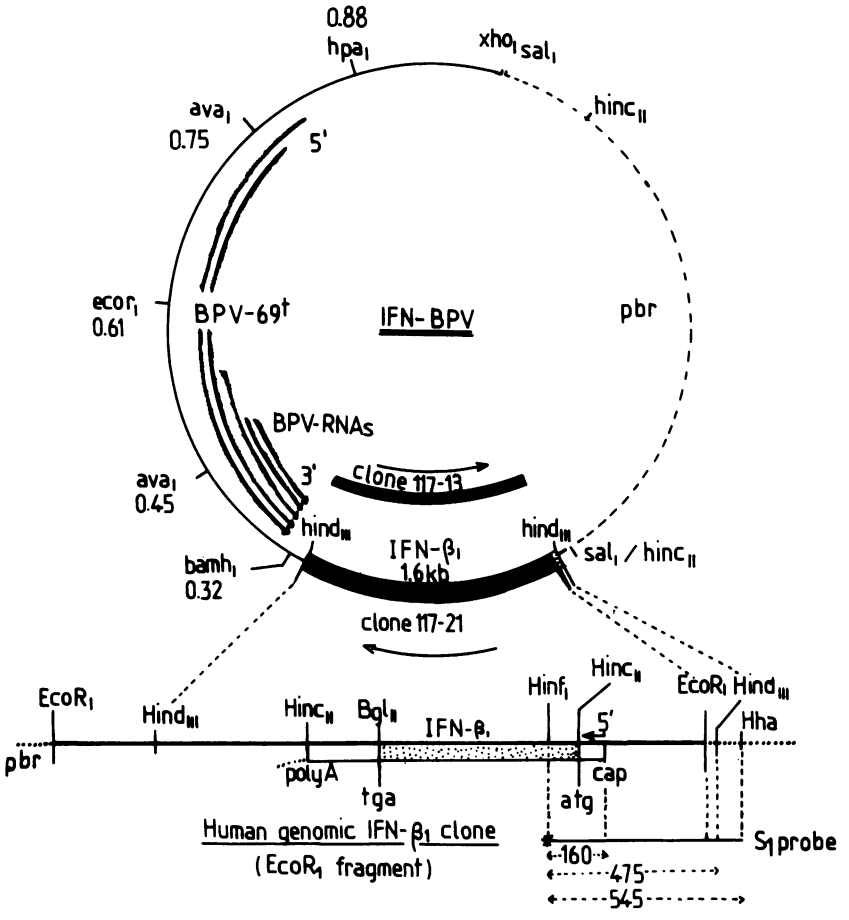

FIG. 1. BPV vector containing the human IFN-β_1 gene. (——) 69% transforming fragment of BPV DNA with the coordinates of the main restriction sites (12); (– – – –) modified pBR322 sequences. The lower portion shows the human genomic *Eco*RI fragment containing the IFN-β_1 gene (19) subcloned in pBR322. The gene was introduced into the *Hin*dIII site of the BPV vector on a *Hin*dIII fragment (▭) consisting of 1.6 kb of human genomic DNA (——) and 29 nucleotides of pBR (·····). The cap and polyadenylation sites of IFN-β_1 mRNA are indicated, as is the coding region (▥). In clone 117-13, the gene was inserted in the same orientation as that of BPV RNAs (arrow)(12), whereas in clone 117-21, the orientation was opposite. The 545-nucleotide S1 probe (*Hin*fI-*Hha*I) is indicated, as are the protected fragments expected if cap-initiated (160 nucleotides) or readthrough transcripts (475 nucleotides) are present.

24 h. In some experiments, 0.2×10^6 cells were seeded in 3.5-cm cluster plates (six wells; Costar Co.), and 1 ml of medium was used. The vesicular stomatitis virus cytopathic assay was used to measure human IFN activity (19, 28). The media to be tested and an IFN-β standard (100 U/ml) were serially diluted twofold in 50 μl of minimal essential medium containing 5% fetal calf serum and 0.5% gentamicin in a 96-well microplate, and 3×10^4 human diploid FS11 fibroblasts in 50 μl of medium were added per well. After 18 h, the medium was removed, and the cells were infected with 5×10^3 PFU of vesicular stomatitis virus in 0.1 ml of minimal essential medium containing 2% fetal calf serum. The last dilution inhibiting the vesicular stomatitis virus cytopathic effect was recorded 24 to 30 h after infection, and units were calculated with a National Institutes of Health IFN-β standard (G023-902-527). Data were calculated from at least two assays. The same procedure, but with L929 cells, was used to measure mouse IFN activity. Human poly(rI):poly(rC)-induced IFN gave only 0.15% cross-activity on mouse L cells, and mouse poly(rI):poly(rC)-induced IFN gave 1% cross-activity on human FS11 cells. These values were low enough to allow the measure of human and mouse IFN titers without further corrections.

ss nuclease analysis of IFN-β_1 RNA. As a DNA probe for single-stranded (ss) S1 nuclease mapping (1, 8, 35) we used a 545-base-pair (bp) *Hin*fI-*Hha*I fragment of IFN-β_1/pBR322 clone C631 (see Fig. 1) which was labeled at the 5'-protruding end of the *Hin*fI site, annealed to total cell RNA, and S1 nuclease treated as described elsewhere (16a, 18). The relative abundance of the protected fragments was quantitated after auto-

TABLE 1. Induction of human IFN by Sendai virus and ds RNA in BPV IFN-β_1-transformed mouse cells

Cell line	Human IFN produced (U/ml) by:	
	Non-induced cells	Sendai virus[a]
ID-13 (BPV DNA)	<4	<4
SR-117-13C (BPV IFN-β_1) (viral orientation)	196	1,125
SR-117-21E (BPV IFN-β_1) (opposite orientation)	12	2,500

[a] Sendai virus (600 HAU/ml) was added for 1 h. IFN produced was measured at 24 h.

TABLE 2. Effect of ds RNA and DEAE-dextran concentrations on IFN production by BPV IFN-β_1-transformed mouse cells[a]

Poly(rI):poly(rC) (μg/ml)	DEAE-dextran (μg/ml)	IFN produced (U/ml)	
		Human	Mouse
50	50	625	600
50	200	875	1,600
50	400	1,500	3,500
50	600	1,400	7,000
50	800	1,000	10,000
0	400	750	64
25	400	3,000	2,000
100	400	2,250	3,000
200	400	1,500	4,000

[a] SR-117-21E cells were induced for 4 h, and IFN was measured at 24 h. Cycloheximide (50 μg/ml) was present during induction.

radiography by spectrophotometric scanning of the X-ray films.

RESULTS

Conditions for human IFN induction in BPV IFN-β_1-transformed mouse cells. We studied two cell clones that were previously isolated by transformation of C127 mouse cells by BPV IFN-β_1 recombinant DNA (18). In clone SR-117-21E (117-21), the 1.6-kb IFN-β_1 genomic segment is oriented opposite to the direction of BPV transcription (12), whereas in clone SR-117-13C (117-13), the IFN-β_1 gene is in the direction of BPV transcription and located downstream from the 3' ends of BPV RNAs (Fig. 1). When tested for production of human-specific IFN, noninduced 117-13 cells showed some constitutive level of activity which was not seen in 117-21 cells (Table 1). In both BPV IFN-β_1 transformants, the synthesis of human IFN was strongly inducible by Sendai virus infection (Table 1). No human IFN was made in cells transformed only by the BPV vectors.

Induction of IFN by a ds RNA such as poly(rI):poly(rC) depends critically on experimental conditions (2, 11, 28). Thus, in mouse cells, high concentrations of DEAE-dextran have to be present for mouse IFN induction (7, 33). Similarly, human IFN synthesis in the BPV IFN-β_1 mouse transformants was optimal when the DEAE-dextran concentration was raised to 400 to 600 μg/ml for 25 μg of poly(rI):poly(rC) (Table 2). In all these experiments, 50 μg of cycloheximide per ml was added, and under these conditions the yields of human IFN became comparable to those obtained with Sendai virus. We noted, however, that a significant amount of human IFN was obtained even in the absence of ds RNA (Table 2), and we decided to investigate this effect in greater detail.

Cycloheximide induction of human IFN in BPV IFN-β_1-transformed mouse cells. When 117-21 cells were exposed for 4 h to 50 μg of cycloheximide per ml, a 10-fold stimulation of IFN synthesis was observed (Table 3), whereas DEAE-dextran had no such effect. Cycloheximide induction was also observed in 117-13 cells, although in these cells the stimulation was consistently lower (Table 3). Thus, in 117-21 cells

TABLE 3. Effect of cycloheximide on IFN induction in BPV IFN-β_1-transformed mouse cells[a]

Poly(rI):poly(rC) (μg/ml)	Cycloheximide (μg/ml)	IFN produced (U/ml)			
		SR-117-21E		SR-117-13C	
		Human	Mouse	Human	Mouse
0	0	60	80	210	150
0	50	890 (760)[b]	690 (225)[b]	495	625
25	0	2,060	26,000	5,500	60,000
25	50	2,730	30,000		
25	50 (Plus actinomycin D)	4,510	30,000		

[a] Induction was for 4 h, and IFN production was measured at 24 h. DEAE-dextran (600 μg/ml) was added. Where indicated, actinomycin D (0.5 μg/ml) was added for the last 30 min of induction. Results are from an average of three experiments.

[b] Without DEAE-dextran.

the yield of human IFN produced by cycloheximide was 40% that produced by poly(rI):poly(rC), whereas in 117-13 cells it was about 10% that produced by poly(rI):poly(rC). A stimulation of mouse IFN production by cycloheximide was also observed (Table 3), but the yields of mouse IFN with the antibiotic alone were 2 to 3% of those with ds RNA.

To confirm these results, we studied a series of subclones derived from 117-21 cells (Table 4). The amount of human IFN produced by the subclones was variable. Two subclones, D1 and D2, which appeared morphologically as flat, nontransformed cells, produced no human IFN but did produce mouse IFN. DNA analysis indicated that these two subclones had lost the recombinant BPV vector (data not shown). In all the other subclones, human IFN was produced after induction with cycloheximide alone as well as after induction with poly(rI):poly(rC). The cycloheximide induction in some subclones was almost as high as that with ds RNA (Table 4, clones A1, A2, and C1), whereas in others (Table 4, clones C2 and C3), it was much lower than that with ds RNA. All subclones produced mouse IFN, but in several clones (A2 and C1), cycloheximide strongly induced human but not mouse IFN. These results confirmed the cycloheximide-mediated induction of human IFN in BPV IFN-β_1-transformed mouse cells and prompted us to compare the IFN-β_1 mRNAs produced by these cells under different induction conditions.

Analysis of IFN-β_1 RNA in BPV IFN-β_1-transformed mouse cells. To detect the presence of specific transcripts of the IFN-β_1 gene and to map its 5′ ends, we used ss nuclease analysis (1, 35). The DNA probe used was a 545-bp *Hinf*$_1$-*Hha*I fragment of plasmid IFN-C631 (19) which contains the 1.83-kb IFN-β_1 *Eco*RI genomic fragment in pBR322 (Fig. 1). The DNA labeled at the 5′ end of the *Hinf*I site was annealed with total cell RNA and digested with S1 nuclease, and the protected fragments were analyzed as described previously (16a, 18). If the BPV IFN-β_1-transformed cells contained IFN-β_1 mRNA with 5′ ends originating at the same position as in human fibroblasts (6, 20), a 160-nucleotide-long DNA fragment would be protected. If transcription originated from the BPV DNA outside the human gene, a 475-nucleotide-fragment would be protected (Fig. 1). In line with previous results (18), no protection of the 160-nucleotide fragment was seen in the noninduced 117-21 or 117-13 cells (Fig. 2, lanes 1 and 5). However, in 117-13 cells in which the IFN-β_1 gene is oriented as BPV transcription, protection of the 475-nucleotide fragment was observed in noninduced cells (Fig. 2, lane 1), suggesting readthrough from the BPV-transcribed region (Fig. 1). These externally initiated transcripts may account for the constitutive levels of human IFN produced by the 117-13 cells (Table 1). These externally initiated transcripts were never seen in either induced or noninduced 117-21 cells (Fig. 2, lanes 5 through 8). In both types of mouse cell transformants, after induction with optimal concentrations of poly(rI):poly(rC) and DEAE-dextran with or without cycloheximide, a strong 160-nucleotide-long protected fragment (Fig. 2, lanes 3, 7, and 8) identical to that protected by authentic IFN-β_1 mRNA from human fibroblasts was seen (Fig. 2, lane 4). The

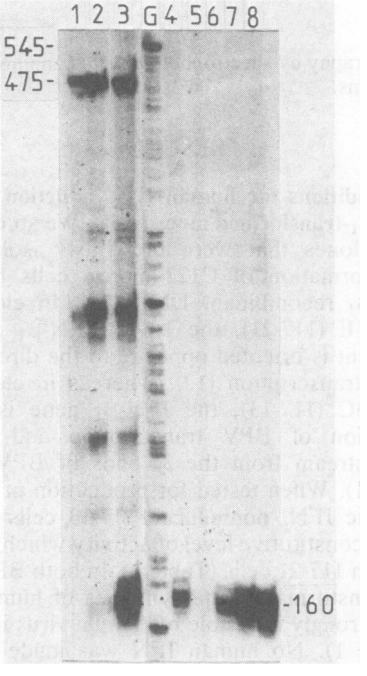

FIG. 2. S1 nuclease analysis of human IFN-β_1 RNAs in BPV IFN-β_1-transformed mouse cells. Each lane represents about 30 μg of total cell RNA hybridized to 2×10^4 cpm of the 5′-end-labeled [^{32}P]DNA probe (545 nucleotides), as described in Materials and Methods. After S1 nuclease treatment, the protected fragments were electrophoresed on a sequencing gel (17). The autoradiography is shown. Lanes: 1, noninduced 117-13 cells; 2, 117-13 cells treated for 4 h with 50 μg of cycloheximide and 600 μg of DEAE-dextran per ml; 3, same as in lane 2 but with 25 μg of poly(rI):poly(rC) per ml; 4, RNA from induced human diploid FS-11 fibroblasts; 5, noninduced 117-21 cells; 6, 117-21 cells treated with cycloheximide and DEAE-dextran; 7, same as in lane 6 but with poly(rI):poly(rC) and DEAE-dextran; 8, same as in lane 7 but with poly(rI):poly(rC), cycloheximide, and DEAE-dextran; G, guanine-specific chemical degradation (17) of the DNA S1 probe used as a marker. Protected fragments are as indicated in Fig. 1. For scanning, a shorter exposure was used.

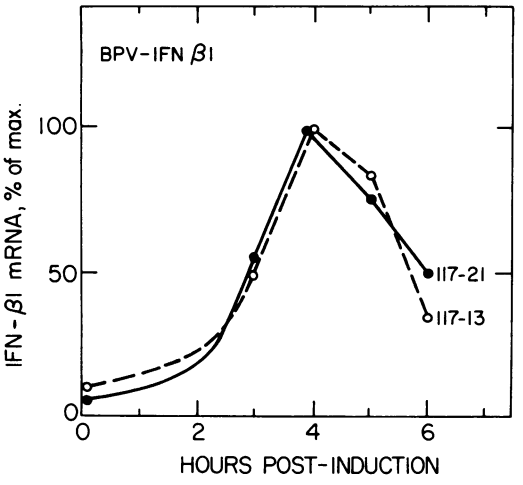

FIG. 3. Time course of human IFN-β_1 RNA induction in BPV IFN-β_1-transformed mouse cells. 117-21 cells were induced for 2 h with 50 μg of poly(rI):poly(rC) and 50 μg of DEAE-dextran per ml and for 4 h with 50 μg of cycloheximide per ml. Total cell RNA was extracted at the indicated times and analyzed by the S1 nuclease procedure described in Materials and Methods and in the legend to Fig. 2. The IFN-β_1 mRNA, quantitated by measuring the amount of 160-nucleotide-long fragments protected, is shown as the percentage of maximum induction. 117-13 cells were also induced as described above. The 475-bp band of the 117-13 cells was not affected by these treatments (data not shown).

RNA annealing to this 160-nucleotide fragment was maximal at 4 h and nearly disappeared by 6 h after induction (Fig. 3).

The main questions were whether human IFN-β_1 mRNA is induced in mouse transformants treated with cycloheximide alone and whether this RNA originates from the authentic 5′ cap site in the IFN-β_1 gene or represents some abnormal transcript. S1 nuclease analysis showed that cycloheximide induced IFN-β_1 RNA with authentic 5′ ends in 117-21 cells (Fig. 2, lane 6). Scanning of the autoradiography indicated that the level of RNA induction reflects the amount of human IFN activity produced (30 to 40% that with ds RNA). In 117-13 cells, cycloheximide also induced IFN-β_1 RNA with authentic 5′ ends, and as for IFN activity, the level induced seemed lower than that in 117-21 cells (5 to 10% of the ds RNA-induced level). We conclude that in these mouse cells, cycloheximide induces a genuine expression of the IFN-β_1 gene propagated on the BPV vector.

DISCUSSION

The mechanisms which control the expression of the IFN genes in humans and of the IFN-β_1 gene in particular are still poorly understood. In several systems, transfer of the IFN gene to foreign cells (3, 10, 16, 16a, 18, 21, 23, 32, 38; Maroteaux et al, in press) has demonstrated that inducibility by ds RNA, a common feature for all type I IFN genes, is retained by the transferred gene. Even when the IFN gene is linked to external promoters, induction is required to observe the accumulation of mRNA whose 5′ ends map near the putative promoter of the IFN gene (16, 16a). This was clearly seen in the 117-13 cells studied here. Induction may activate a transcription process initiating at the IFN gene cap site, but the possibility that stabilization of short-lived transcripts made continuously causes the accumulation of the IFN mRNA has not been excluded. Among the different gene transfer systems available for studying the mechanisms of induction, use of the extrachromosomal BPV vector (14, 24) to propagate the human IFN-β_1 gene in mouse cells (18, 38) presents some advantages. The structure of the DNA in which the gene is inserted can be better controlled than in cotransformation experiments (37), in which the human DNA is integrated at different sites of the recipient cell genome (25). The BPV IFN-β_1-transformed mouse cell lines are relatively stable and can be cloned and

TABLE 4. Human IFN induction in subclones of BPV IFN-β_1-transformed mouse cells[a]

IFN	Poly(rI):poly(rC)	Cycloheximide	IFN produced (U/ml) by SR-117-21E subclone:						
			A_1	A_2	C_1	C_2	C_3	D_1	D_2
Human	−	−	350	190	64	190	24	<4	<4
	−	+	6,000	1,000	1,000	750	625	8	32
	+	+	8,000	2,500	1,500	5,000	3,000	12	48
Mouse	−	−	240	<40	<40	<40	<40	<40	<40
	−	+	1,000	40	50	160	220	60	50
	+	+	30,000	5,000	2,500	12,500	20,000	5,000	4,500

[a] Induction was for 4 h with 25 μg of poly(rI):poly(rC) or 50 μg of cycloheximide per ml as indicated. All cultures received 600 μg of DEAE-dextran per ml, and IFN produced was measured at 24 h. Clones D_1 and D_2 were revertants with nontransformed phenotypes.

grown for long periods of time, therefore allowing the comparison of various conditions of induction on the same cells. This cannot be done as easily with simian virus 40 vectors replicating in monkey cells (16a, 32) because only transient expression is obtained and because monkey IFN and human IFN cross-react. In the BPV mouse cell system, the human IFN produced during induction can be differentiated from mouse IFN by its species specificity; it also does not act on the host cells. From the study of many mouse cell clones transformed by BPV IFN-β_1 DNA (18, 38), it is clear that the regulation of the IFN-β_1 gene by ds RNA is retained in these episomal genomes present in 50 to 100 copies per cell (14). These cells have the additional feature that the IFN-β_1 gene is now also inducible by a 4-h treatment with cycloheximide.

Cycloheximide and other inhibitors of protein synthesis do not induce the IFN-β_1 gene in most human cells, although when added together with ds RNA, they superinduce and stabilize IFN mRNA (4, 22, 26). Tan and Berthold (30) and Tan et al. (31) have found that in an aneuploid human fibroblast cell line (line 108), cycloheximide and other inhibitors of protein synthesis can induce some IFN. These authors suggested that this effect may be characteristic of high-IFN-producing cells and may result from an unstable hypothetical repressor (31). In the different BPV IFN-β_1 subclones, the extent of induction by cycloheximide varied, but there was no clear correlation with the IFN-producing capacity of the cells. There was also no correlation between the effect of cycloheximide on human and mouse genes which would be expected if an unstable repressor were present. The effect of cycloheximide on the BPV IFN-β_1 transformants could be related to the presence of multiple copies of the human gene, as could also be the case for human cell line 108 (30). Cycloheximide could indeed act by inhibiting the synthesis of some regulatory protein, but it could also have an effect on the stability of the IFN-β_1 mRNA, as found in superinduction (4, 22). Preliminary results with isolated nuclei indicate that, in contrast to human fibroblasts, the mouse BPV IFN-β_1 transformants show some transcription of the IFN-β_1 gene even before induction, but these RNA transcripts do not accumulate in the cell (U. Nir, B. Cohen, and M. Revel, unpublished data). The effect of cycloheximide may help clarify the different mechanisms which prevent IFN synthesis in noninduced cells. Furthermore, the fact that cycloheximide can be a genuine inducer of IFN-β_1 mRNA may have important implications, since it suggests that IFN can be made by cells even without accumulation of ds RNA from viral origin. In human diploid fibroblasts, although cycloheximide does not induce IFN-β_1 mRNA, it induces another mRNA, IFN-β_2, to the extent of about 10% that induced by ds RNA (36). This induction by cycloheximide has raised the suspicion that IFN-β_2 mRNA is not the product of a real active IFN gene (5). An antiviral activity is, however, found associated with the IFN-β_2 protein (A. Zilberstein, A. Nissim, and M. Revel, submitted for publication), and the present work demonstrates that in some cells expression of the major IFN-β_1 gene can also be regulated by cycloheximide. There is, therefore, much similarity between the regulation of the IFN-β_1 and IFN-β_2 genes in human cells.

ACKNOWLEDGMENTS

We thank Y. Mory and Y. Groner for helpful discussions.
This work was supported in part by InterYeda, Rehovot, Israel.

LITERATURE CITED

1. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S_1 endonuclease-digested hybrids. Cell **12**:721–732.
2. **Burke, D. C.** 1980. The type I human interferon gene system, chromosomal location and control of expression, p. 47–64. *In* I. Gresser (ed.), Interferon 2. Academic Press, Inc., New York.
3. **Canaani, D., and P. Berg.** 1982. Regulated expression of human interferon-β_1 gene after transduction into cultured mouse and rabbit cells. Proc. Natl. Acad. Sci. U.S.A. **79**:5166–5170.
4. **Cavalieri, R. L., E. A. Havell, J. Vilcek, and S. Petska.** 1977. Induction and decay of human fibroblast interferon mRNA. Proc. Natl. Acad. Sci. U.S.A. **74**:4415–4419.
5. **Content, J., L. DeWit, D. Pierard, R. Derynck, E. DeClercq, and W. Fiers,** 1982. Secretory proteins induced in human fibroblasts under conditions used for the production of interferon-β. Proc. Natl. Acad. Sci. U.S.A. **79**:2768–2773.
6. **Degrave, W., R. Derynck, J. Tavernier, G. Haegeman, and W. Fiers,** 1981. Nucleotide sequence of the chromosomal gene for human fibroblast (β_1) interferon and of the flanking regions. Gene **14**:137–143.
7. **Dianzani, F., P. Cantagalli, S. Gagnoni, and G. Rita.** 1968. Effects of DEAE-dextran on production of interferon induced by synthetic double-stranded RNA in L cell cultures. Proc. Soc. Exp. Biol. Med. **128**:708–710.
8. **Favaloro, J., R. Treisman, and R. Kamen.** 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S_1 gel mapping. Methods Enzymol. **65**:718–749.
9. **Gross, G., U. Mayr, W. Bruns, F. Grosveld, M. H. M. Dalil, and J. Collins.** 1981. The structure of a thirty-six kilobase region of the human chromosome including the fibroblast interferon gene IFN-β. Nucleic Acids Res. **9**:2495–2507.
10. **Hauser, H., G. Gross, W. Bruns, H. K. Hochkeppel, U. Mayr, and J. Collins.** 1982. Inducibility of the human interferon β gene is maintained in mouse L cell clones. Nature (London) **297**:650–654.
11. **Havell, E. A., and J. Vilcek.** 1972. Production of high-titered interferon in cultures of human diploid cells. Antimicrob. Agents Chemother. **2**:476–484.
12. **Heilman, C. A., L. Engel, D. R. Lowy, and P. M. Howley.** 1982. Virus-specific transcription in bovine papillomavirus-transformed mouse cells. Virology **119**:22–34.
13. **Houghton, M., I. J. Jackson, A. G. Porter, S. M. Doel,**

C. H. Catlin, C. Barber, and N. H. Carey. 1981. The absence of introns within a human fibroblast interferon gene. Nucleic Acids Res. **9**:247–266.

14. **Law, M. F., D. R. Lowy, I. Dvoretsky, and P. M. Howley.** 1981. Mouse cells transformed by bovine papillomavirus contain only extrachromosomal viral DNA sequences. Proc. Natl. Acad. Sci. U.S.A. **78**:2727–2731.
15. **Lawn, R. M., J. Adelman, A. E. Franke, C. M. Houck, M. Gross, R. Najarian, and D. V. Goeddel.** 1981. Human fibroblast interferon gene lacks introns. Nucleic Acids Res, **9**:1045–1052.
16. **Mantei, N., and C. Weissman.** 1982. Controlled transcription of a human α interferon gene introduced into mouse cells. Nature (London) **297**:128–132.

16a. **Maroteaux, L., C. Kahana, Y. Mory, Y . Groner, and M. Revel.** 1983. Sequences involved in the regulated expression of the human interferon-β_1 gene in recombinant SV40 DNA vectors replicating in monkey cells. EMBO J. **2**:325–332.

17. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base specific chemical cleavages. Methods Enzymol. **65**:499–560.
18. **Mitrani-Rosenbaum, S., L. Maroteaux, Y. Mory, M. Revel, and P. M. Howley.** 1983. Inducible expression of the human interferon β_1 gene linked to a bovine papilloma virus DNA vector and maintained extrachromosomally in mouse cells. Mol. Cell. Biol. **3**:233–240.
19. **Mory, Y., Y. Chernajovsky, S. I. Feinstein, L. Chen, U. Nir, J. Weissenbach, Y. Malpiece, P. Tiollais, D. Marks, M. Ladner, C. Colby, and M. Revel.** 1981. Synthesis of human interferon-β_1 in *Escherichia coli* infected by a lambda phage recombinant containing a human genomic fragment. Eur. J. Biochem. **120**:197–202.
20. **Ohno, S., and T. Taniguchi.** 1981. Structure of a chromosomal gene for human interferon-β. Proc. Natl. Acad. Sci. U.S.A. **78**:5305–5309.
21. **Ohno, S., and T. Taniguchi.** 1982. Inducer-responsive expression of the cloned human interferon-β_1 gene introduced into cultured mouse cells. Nucleic Acids Res. **10**:967–977.
22. **Raj, N. B. K., and P. M. Pitha.** 1981. Analysis of interferon mRNA in human fibroblast cells induced to produce interferon. Proc. Natl. Acad. Sci. U.S.A. **78**:7426–7430.
23. **Reyes, G. R., E. R. Gavis, A. Buchan, N. B. K. Raj, G. S. Hayward, and P. M. Pitha.** 1982. Expression of human-β interferon cDNA under the control of a thymidine kinase promoter from herpes simplex. Nature (London) **297**:598–600.
24. **Sarver, N., P. Gruss, M.-F. Law, G. Khoury, and P. M. Howley.** 1981. Bovine papilloma virus deoxyribonucleic acid: a novel eucaryotic cloning vector. Mol. Cell. Biol. **1**:486–496.
25. **Scangos, G., and F. H. Ruddle.** 1981. Mechanisms and applications of DNA mediated gene transfer in mammalian cells: a review. Gene **14**:1–10.
26. **Sehgal, P. B., D. S. Lyles, and I. Tamm.** 1978. Superinduction of human fibroblast interferon production: further evidence for increased stability of interferon mRNA. Virology **89**:186–198.
27. **Sehgal, P. B., and A. D. Sagar.** 1980. Heterogeneity of poly(I)poly(C)-induced human fibroblast interferon mRNA species. Nature (London) **288**:95–97.
28. **Stewart, W. E., II.** 1979. The interferon system. Springer-Verlag, New York.
29. **Tan, Y. H., J. A. Armstrong, Y. H. Ke, and M. Ho.** 1970. The regulation of cellular interferon synthesis. Proc. Natl. Acad. Sci. U.S.A. **67**:464–471.
30. **Tan. Y. H., and W. Berthold.** 1977. A mechanism for the induction and regulation of human fibroblastoid interferon genetic expression. J. Gen. Virol. **34**:401–411.
31. **Tan, Y. H., C. Tan, and W. Berthold.** 1977. Genetic control of the interferon system. Tex. Rep. Biol. Med. **35**:63–68.
32. **Tavernier, J., D. Gheysen, F. Derinck, J. Van der Heyden, and W. Fiers.** 1983. Deletion mapping of the inducible promoter of human IFN-β gene. Nature (London) **301**:634–636.
33. **Trapman, J.** 1979. A systematic study of interferon production by mouse L-929 cells induced with poly I:poly C and DEAE-dextran. FEBS Lett. **98**:107–110.
34. **Vilcek, J., and M. H. Ng.** 1971. Post-transcriptional control of interferon synthesis. J. Virol. **7**:588–594.
35. **Weaver, R. F., and C. Weissmann.** 1979. Mapping of RNA by a modification of the Berk and Sharp procedure: the 5′ termini of 15S β-globin mRNA precursor and mature 10S β-globin mRNA have identical map coordinates. Nucleic Acids Res. **7**:1175–1193.
36. **Weissenbach, J., Y. Chernajovsky, M. Zeevi, L. Shulman, H. Soreq, U. Nir, D. Wallach, M. Perricaudet, P. Tiollais, and M. Revel.** 1980. Two interferon mRNAs in human fibroblasts: *in vitro* translation and *E. coli* cloning studies. Proc. Natl. Acad. Sci. U.S.A. **77**:7152–7156.
37. **Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel.** 1979. Transformation of mammalian cells with genes from prokaryotes and eukaryotes, Cell **16**:777–785.
38. **Zinn, K., P. Mellon, M. Ptashne, and T. Maniatis.** 1982. Regulated expression of an extrachromosomal human β-interferon gene in mouse cells. Proc. Natl. Acad. Sci. U.S.A. **79**:4897–4901.