Clonal Derivatives of the RD-114 Cell Line Differ in Their Antiviral and Gene-Inducing Responses to Interferons

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The human rhabdomyosarcoma cell line RD-114 is partially responsive to interferons (IFNs). In these cells, alpha interferon (IFN- α) or gamma interferon (IFN- γ) inhibits the replication of some viruses but not of others. Similarly, some of the IFN-inducible mRNAs are induced poorly, whereas others are induced well. Here we report the isolation of clonal derivatives of this line which display different spectra of responses to IFNs. Among the eight extensively characterized clonal lines, one, C10, did not respond to IFN- α or IFN- γ at all. Retrovirus production by each of the seven other lines was inhibited by both IFN- α and IFN- γ . Replication of vesicular stomatitis virus was inhibited strongly by IFN- α in clone B1 but not in others, whereas it was not appreciably affected by IFN- γ in any clone. Replication of encephalomyocarditis virus was inhibited strongly by IFN- γ in clones A1, A2, A3, B3, and B8 and by IFN- α in clone A2. Neither IFN inhibited the multiplication of these clones greatly, although their doubling times were slightly increased. Five mRNAs were induced by IFNs to varying degrees in the seven clones. mRNA 2A was most strongly induced by IFN- γ in clone A3. mRNA 1-8 was strongly induced by IFN- α in clone A1 and by either IFN in clones A2 and A3. The highest concentrations of 2',5'-oligoadenylate synthetase mRNA, mRNA 561, and mRNA 6-16 were in IFN- α -treated clones A1 and A2. These results demonstrated the existence of clonal heterogeneity in IFN responses in a cell line and strengthened the view that IFN treatment of cells generates multiple signals leading to a variety of IFN-induced phenotypes.

Interferons (IFNs) have a variety of biological activities, including actions against viruses which use different replication strategies (7, 13). We have been using cell lines which are partially responsive to IFNs for studying their mechanisms of action (1, 3, 6, 14, 15). Replication of some viruses but not of others is inhibited by IFNs in these cell lines.

RD-114 is a cell line which is partially responsive to both alpha interferon (IFN- α) and gamma interferon (IFN- γ). Either class of IFN impairs the production of the resident retrovirus (RD-114) but does not inhibit appreciably the replication of vesicular stomatitis virus (VSV) or encephalomyocarditis virus (EMCV) (3). Neither IFN has a strong anticellular effect on these cells. The double-stranded RNA-dependent enzymes 2',5'-oligoadenylate [2-5(A)] synthetase and protein kinase are induced poorly by IFNs in these lines (15). We recently compared the induction patterns of seven IFN-inducible mRNAs in RD-114 and HeLa cells. mRNAs 561, 6-16, 1-8, 2A, and 6-26 have similar induction patterns in the two cell lines, whereas 2-5(A) synthetase mRNA and MTII mRNA are poorly induced in RD-114 cells as compared with HeLa cells (5, 6).

In the course of our studies with RD-114 cells, we observed that the IFN responses of the cell line were changing with continued culturing. Cell cultures of early passage which were partially responsive to IFNs became unresponsive after continued passage for a long time. There are two possible explanations for this observed phenomenon, namely, that the entire cell population was gradually losing some component involved in an early step of the IFN response (such as IFN receptors) or that the original cell population was heterogenous in its IFN response and the unresponsive cells were becoming the predominant popula-

tion with continued culturing because they could replicate faster. To distinguish between these possibilities, we isolated clonal derivatives of the RD-114 cell line. In the studies reported here we demonstrate that the original cell population was heterogenous. The isolated clonal derivatives displayed remarkable dissimilarities in their IFN responses.

MATERIALS AND METHODS

We have described in earlier publications the sources of IFNs (4, 6), procedures for cell culturing (3, 6), methods for VSV and EMCV yield reduction assays (3, 14), methods for measuring retrovirus production (3, 14), characteristics of the cDNA probes used, and the methods for determining the concentration of a specific mRNA by dot blot analysis (4, 6). Geneticin (G418 sulfate) was purchased from Gibco Laboratories.

RD-114 cells at a density of 10^6 cells per 100-mm-diameter plate were transfected with plasmid DNA by calcium phosphate precipitation procedures (16). A mixture of two plasmids was used for the experiments. Plasmid pbCAT (11) is a pSVCAT-derived vector in which the promoter and enhancer regions of the IFN-responsive mouse gene 202 have been inserted upstream of the chloramphenicol acetyltransferase gene (a gift from Peter Lengyel), and pIPB₁ is a ptk-derived (8) vector containing the bacterial neomycin phosphotransferase gene driven by the promoter of the herpes virus thymidine kinase gene (a gift from Richard Axel). pbCAT (10 μ g) and pIPB₁ (1 μ g) were used for transfection of each plate. Cells were kept in contact with the DNA for 4 h, after which they were subjected to a 15% glycerol shock for 2 min. At 24 h after transfection, selection medium containing 1 mg of G418, a neomycin analog, per ml was added. Seven to ten days of culturing in this medium killed most of the cells, but a few colonies of cells, one to five per plate, grew. Well-separated colonies were harvested by

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FIG. 1. Antiretroviral effects of IFN- α and IFN- γ in different RD-114 clones. Cells were cultured in 100-mm plates. Nearly confluent cultures were treated with IFNs for 18 h, and the medium was changed and collected after another 6 h. RD-114 virions present in the culture medium were centrifuged, suspended, and assayed for virion-associated reverse transcriptase activity as described previously (3, 14). The amounts of virus produced by different clones were different. Data were calculated by normalizing virus production by each untreated culture as 100%. Virus production by each clone as measured by the radioactivity (counts per minute) incorporated into newly synthesized DNA by the virion-associated enzyme was as follows: A1, 36,200; A2, 105,451; A3, 301,752; B1, 30,827; B3, 157,319; B6, 38,826; B8, 72,605; and C10, 5,076. Open bars, Untreated cells; solid bars, cells treated with 500 U of IFN- α per ml; hatched bars, cells treated with 100 U of IFN- γ per ml.

trypsinization in cloning cylinders and propagated in the presence of G418. Once a stable cell line from each clone had been established, the drug was removed from the culture medium. The clonal lines have been maintained in drug-free medium since then.

RESULTS

Isolation of clonal derivatives of RD-114 cells. During the course of our previous studies of antiviral and gene-inducing activities of IFNs in RD-114 cells, we observed that the properties of the cell line were changing with time. A systematic study indicated that the line was responding to IFNs less and less as the passage number increased. However, all responses to IFNs were not affected similarly. This suggested to us that the cell population might be heterogeneous and that the relative proportions of different members might be changing with continued cell passage. To test this hypothesis, we decided to isolate clonal derivatives of the cell line and to test their individual characteristics.

We were unsuccessful in our repeated attempts to clone these cells by plating them at a low density and allowing individual well-separated colonies to grow. However, cell clones were readily obtained when densely plated cells were transfected with DNA containing a drug resistance gene and the transfectants were selected for drug resistance. The cell clones used in the experiments described here were isolated in the following way. RD-114 cells of early (A series), middle (B series), or late (C series) passage numbers were transfected with vector pIPB₁, containing the neomycin resistance gene. G418-resistant cells grew as isolated colonies, which were picked, cultured, and subcloned. Three isolates from the A series (A1, A2, and A3), four isolates from the B series (B1, B3, B6, and B8), and one isolate from the C series (C10) were used for the detailed studies described here. These clones have now been cultured continuously for about 6

months, and their individual properties appear to be stable so far.

Antiretroviral effects of IFN- α and IFN- γ . We reported previously that both IFN- α and IFN- γ inhibit the production of the resident retrovirus (RD-114) by the RD-114 cell line (3). In our first set of experiments, we tested whether the same was true for the eight clones isolated from this line (Fig. 1). All clones except C10 were responsive to the antiretroviral effects of IFNs. IFN- γ (100 U/ml) strongly inhibited virus production by the seven clones; less inhibition was observed when 10 U of IFN- γ per ml was used (data not shown). The extents of inhibition in response to 500 U of IFN- α per ml varied from clone to clone, the maximum being for clone B3 and the minimum being for clone A2. It should be noted that the absolute amount of virus production varied from clone to clone, but there does not appear to be any direct relationship between the amount of virus production and the extent of IFN-mediated inhibition of production.

Effects of IFNs on replication of VSV and EMCV. Replication of VSV and EMCV is relatively insensitive to IFNs in the uncloned RD-114 cell line (3). However, our clones displayed a wide variety of responses. In response to 500 U of IFN- α per ml, VSV replication was inhibited 2,000-fold in clone B1, but in the other clones, little inhibition was observed (Fig. 2). On the other hand, EMCV replication was most strongly inhibited by IFN- α in clone A2 (1,000-fold), although there was some inhibition (100-fold) in clone B1 as well (Fig. 2). IFN- γ (100 U/ml) did not inhibit VSV replication appreciably in any of the clones (Fig. 3). However, EMCV replication was highly sensitive to IFN- γ in several

FIG. 2. Anti-VSV and anti-EMCV effects of IFN- α in RD-114 clones. Data are presented for each clone as the fold reduction of virus yield in cells treated with 500 U of IFN- α per ml as compared with untreated cells. VSV yields in untreated cells were in the range of 10^{12} to 10^{13} PFU/ml, and EMCV yields in untreated cells were in the range of 10^{11} to 10^{12} PFU/ml. Solid bars, Fold reduction of VSV yields; hatched bars, fold reduction of EMCV yields.

clones. In the most sensitive clone, A2, EMCV replication was inhibited 10,000-fold in response to 100 U of IFN- γ per ml, but it was barely affected in clone B6 (Fig. 3), although retrovirus production by clones A2 and B6 was equally diminished by IFN- γ (Fig. 1).

Experiments described in Fig. 2 and Fig. 3 were done with single doses of IFNs. To examine more carefully whether differences in antiviral effects observed in different clones could be abolished if other IFN doses were used, experiments shown in Fig. 4 and Fig. 5 were done.

The effects of increasing doses of IFN- α and IFN- γ on VSV replication were examined in the most sensitive clone, B1, and the least sensitive clone, A3 (Fig. 4). In clone B1, there was a dose-dependent decrease in VSV yield, reaching up to 10,000-fold at 1,000 U of IFN- α per ml, whereas in clone A3, there was only a 10-fold decrease at this concentration of IFN- α . IFN- γ (1 U/ml) decreased VSV yield 20-fold in clone B1, but the yield did not decrease further when the dose was increased up to 100 U/ml. In clone A3, IFN- γ barely affected VSV yield.

Clones A2 and B6 were chosen as the most and least responsive lines, respectively, for determining the dose-response curves for anti-EMCV effects. IFN- α decreased EMCV yield in a dose-dependent manner up to 10,000-fold in A2 but only about 15-fold in B6. Similarly, EMCV replication was very sensitive to IFN- γ in clone A2 but not in clone B6 (Fig. 5).

The above-described pairs of clones chosen for studying in detail the anti-VSV and anti-EMCV effects exemplified most convincingly the existence of the differential antiviral effects

FIG. 3. Anti-VSV and anti-EMCV effects of IFN- γ in RD-114 clones. Data are presented for each clone as the fold reduction of virus yield in cells treated with 100 U of IFN- γ per ml as compared with untreated cells. VSV yields in untreated cells were in the range of 10^{12} to 10^{13} PFU/ml, and EMCV yields in untreated cells were in the range of 10^{11} to 10^{12} PFU/ml. Solid bars, Fold reduction of VSV yields; hatched bars, fold reduction of EMCV yields.

FIG. 4. IFN dose response of VSV replication in RD-114 clones B1 and A3. RD-114 clones B1 and A3 were treated with increasing doses of IFN and subsequently infected with VSV at a multiplicity of infection of 10. Virus yields were determined by a plaque assay in L929 cells. Open circles, Clone B1, IFN- α ; solid circles, clone B1, IFN- γ ; open triangles, clone A3, IFN- α ; solid triangles, clone A3, IFN- γ .

of IFNs. As measured by antiretroviral effects, A2 and B6 were equally responsive to IFN- α and IFN- γ . The same conclusions could be drawn for the pair B1 and A3. However, EMCV replication was exquisitely sensitive to IFN- α and IFN- γ in clone A2 but not in clone B6. Similarly, VSV replication was sensitive in clone B1 but not in clone A3. However, even in clone B1 VSV replication was much more sensitive to IFN- α than to IFN- γ . Thus, the sensitivity of a virus was dependent on the IFN class as well as on the host cell.

Gene induction by IFNs in RD-114 clones. We have reported that mRNAs 561, 6-16, and 1-8 can be normally induced in RD-114 cells by IFN- α and that mRNAs 1-8 and 2A can be induced by IFN- γ . However, the induction of 2-5(A) synthetase mRNA by IFN- α and that of MTII mRNA by IFN- γ are poorer in RD-114 cells than in HeLa cells (6). We were interested in examining the induction patterns of these mRNAs in the clonal derivatives of the RD-114 cell line to determine whether they were equally responsive. For this purpose, cells were treated with IFN- α for 6 h or with IFN- γ for 12 h. These lengths of treatment were chosen to allow optimum induction of mRNAs by the two IFN classes (6).

Each clone had a unique response pattern (Fig. 6). None of the five mRNAs tested was induced in clone C10. The level of mRNA 2A was high in untreated clone A1 and was increased further by IFN treatment; in other clones, except for B3, this mRNA was induced effectively by IFN- γ from a low constitutive level. mRNA 1-8 (2) was induced to high

FIG. 5. IFN dose response of EMCV replication in RD-114 clones A2 and B6. RD-114 clones A2 and B6 were treated with increasing doses of IFN and subsequently infected with EMCV at a multiplicity of infection of 10. Virus yields were determined by a plaque assay in L929 cells. Open circles, Clone A2, IFN- α ; solid circles, clone A2, IFN- γ ; open triangles, clone B6, IFN- α ; closed triangles, clone B6, IFN- γ .

levels in clones A2 and A3 by both IFNs but only by IFN- α in clone A1. 2-5(A) synthetase mRNA (12) was induced best in clone A1 in response to IFN- α . This mRNA and mRNA 6-16 were better induced by IFN- α in clones A1 and A2, but in clone A3 both IFNs induced these mRNAs equally well. mRNA 561 was induced exclusively by IFN- α , and the highest level was attained in clone A1. In Fig. 6 we have presented the data in a manner convenient for comparing the responses of different clones. However, the absolute values of concentrations of the different IFN-inducible mRNAs are not directly comparable; thus, IFN- α -treated A1 cells did not have the same levels of mRNAs 1-8, 561, and 2-5(A) synthetase. Relative to IFN- α -treated HeLa cells, for example, IFN- α -treated A1 cells had three times less 2-5(A) synthetase mRNA and three times more 6-16 mRNA. In general, clones A1 and A2 were high responders to IFN- α , clones A3, B1, and B6 were intermediate responders, and clones B3 and B8 were low responders (Table 1). Similarly, clone A3 was a high responder to IFN- γ , clones A2, B1, and B6 were intermediate responders, and clones A1, B3, and B8 were poor responders (Table 2). These generalizations were based on fold induction over the concentrations in untreated cells which were in general low but different in different clones. However, since uninduced levels were low, their estimations were relatively inaccurate, introducing some uncertainty into calculations of fold stimulation.

Anticellular effects of IFNs. We tested whether IFNs have anticellular effects on the RD-114 clones by culturing the cells in the presence of IFNs. About 10^5 cells were plated in

FIG. 6. Induction of mRNAs by IFNs in RD-114 clones. Cytoplasmic levels of five mRNAs in different RD-114 clones were determined by using the dot blot hybridization procedures described before (4, 6). For estimation of the relative level of each mRNA, the maximum level of that mRNA attained in any one of the IFN-treated clones was assigned an arbitrary value of 100 units. The values are therefore comparable only within the context of one mRNA. Open bars, Concentrations in untreated cells; solid bars, concentrations in cells treated with 500 U of IFN- α per ml for 6 h; hatched bars, concentrations in cells treated with 100 U of IFN- γ per ml for 12 h. 2-5(A) SYN, 2-5(A) Synthetase.

100-mm plates; after the cells had attached to the surface, 500 U of IFN- α or 100 U of IFN- γ per ml or no IFN was added, and culturing was continued for 6 days. Every day, cell numbers in duplicate plates of untreated, IFN- α -treated, and IFN- γ -treated cell cultures were determined by trypsinization and cell counting. All clones replicated even in the presence of IFNs, although their generation times were slightly lengthened. The percent decrease in cell numbers on day 6 as compared with untreated cell numbers for each clone is shown in Tables 1 and 2. All clones except for C10 had similar doubling times, which were increased at most 20% by IFN-treatment. Clone C10, whose doubling time was much shorter, was unaffected by IFN treatment.

DISCUSSION

Isolation of the cell variants. The procedures developed for isolating the clones studied here evolved from experiments designed for an unrelated purpose. Samanta et al. (11) reported the identification of a region of the mouse type I IFN-inducible 202 gene which renders IFN inducibility to a chloramphenicol acetyltransferase transcription unit when attached to the latter and transfected into mouse Ehrlich ascites tumor cells. We were interested in learning whether

TABLE 1. Antiviral, anticellular, and gene induction activities of IFN- α in RD-114 clones^a

Clone	Activity					mRNA ^c			
	Anti-VSV ^b	Anti-EMCV ^b	Anti-retroviral ^c	Anti-cellular ^d	2A	1-8	2-5(A) synthetase	6-16	561
A1	20	10	51	37	1	10	4	17	20
A2	5	1,000	44	30	2	9	6	20	16
A3	4	30	64	66	3	8	8	10	9
B1	2,000	100	69	50	1	5	4	13	10
B3	5	10	86	5	2	3	6	5	6
B6	10	4	49	65	2	7	9	14	11
B8	80	10	74	27	1	3	2	6	4
C10	2	2	0	5					

^a IFN- α at 500 U/ml was used for these estimations.^b Data represent the fold reduction in virus yield.^c Data represent the percent reduction in virus yield as measured by a reverse transcriptase assay.^d Data represent percent differences in numbers of cells between cultures grown in the presence or absence of IFNs for 6 days.^e Data represent ratios of mRNA concentrations in IFN-treated and untreated cells.

human IFN- α could elevate the level of chloramphenicol acetyltransferase in RD-114 cells that had been transfected with pbCAT, a eucaryotic expression vector in which the chloramphenicol acetyltransferase-coding region is attached to the promoter and enhancer regions of the 202 gene. For doing this type of experiment, we decided to isolate permanent transfectants in which pbCAT DNA had been integrated into the genome. To select for such transfectants, we cotransfected pIPB₁ and selected transfectants on the basis of their resistance to G418. It should be kept in mind, therefore, that each RD-114 clone studied here has been transfected with both pbCAT and pIPB₁. Although it is not apparent how the presence of these DNA vectors in the genome may affect the IFN responses of the isolates, such a possibility cannot be ruled out. However, irrespective of whether the differentially responsive sister clones already existed before transfection or whether they were created by the transfection procedure, these clones can be profitably used for understanding the mechanisms of action of IFNs, as illustrated by the studies reported here.

Heterogeneity of cell lines in IFN responses. The studies reported here demonstrated the inherent heterogeneity in IFN responses among cells in a cell line that has been propagated continuously in tissue culture in the laboratory. In our experience, this type of heterogeneity is not uncommon. We reported (14) differences in IFN responses of two mouse cell lines, NIH/MOL.B and NIH/MOL.C, in only one

of which VSV replication was inhibited by IFNs. The history of the cell lines indicated that they were of identical origin. Subsequently, we were able to isolate clonal derivatives of the NIH/MOL.C line which had different IFN responses (unpublished data). Similarly, Salzberg et al. (10) isolated two clones of NIH 3T3 cells in both of which retrovirus replication was inhibited by IFNs but in only one of which IFNs inhibited the replication of VSV and mengovirus. In another mouse cell line, JLSV-11, VSV replication was strongly inhibited but EMCV replication was marginally inhibited by IFNs (1, 14). Recently, by systematic screening of clonal derivatives of this cell line, we were able to identify clones in which neither VSV nor EMCV replication was inhibited by IFNs and clones in which both viruses were inhibited strongly (R. Kumar and G. C. Sen, unpublished observations).

The basis for differences in IFN responses among sister clones from the same cell line is unclear. It remains to be established whether they differ genetically or whether the differences are due to epigenetic factors. Long-term culturing of the isolated clones and examination for maintenance of their respective IFN response phenotypes will definitely be necessary for resolution of these issues.

Use of the cell variants. The existence of these cell variants suggests that IFN responsiveness is not an all-or-none proposition: some effects of IFNs may be manifested in the absence of others. There was a class of variants, whose

TABLE 2. Antiviral, anticellular, and gene induction activities of IFN- γ in RD-114 clones^a

Clone	Activity					mRNA			
	Anti-VSV	Anti-EMCV	Anti-retroviral	Anti-cellular	2A	1-8	2-5(A) synthetase	6-16	561
A1	2	1,000	87	36	2	3	2	4	2
A2	50	10,000	99	50	8	9	3	9	2
A3	0	7,000	85	45	25	10	11	13	2
B1	10	45	96	37	11	4	1	5	2
B3	20	1,000	94	33	2	4	3	2	1
B6	10	10	92	47	8	3	3	4	2
B8	3	2,000	90	42	3	7	1	1	1
C10	0	0	0	0					

^a IFN- γ at 100 U/ml was used for these estimations. Data are reported exactly as described in Table 1, footnotes *b* through *e*.

prototype member was the clone C10, in which IFNs did not elicit any of the tested responses. It is conceivable that a very early step in IFN action, such as receptor binding, is defective in these variants. The underlying causes for the defects in IFN responses among the other clones appear to be more complex.

It is curious to note that clone C10, which was totally unresponsive to IFNs, multiplied more rapidly than the others. This explains the loss of IFN response of the uncloned cell line with continued passage. The high growth rate of clone C10 could be due to its release from the natural antigrowth effects of endogenous IFNs.

Our previous studies with partially responsive cell lines and the present one with clonal derivatives having different IFN responses are useful for understanding both the mode of induction and the function of IFN-inducible genes. These clones can be used for classifying signals generated by IFN-receptor interactions, with the basic premise that if two genes are not coinduced, they must be induced by different signals. In cell variants the full repertoire of signals is not produced, and hence all the genes are not induced. The consequence of the lack of expression of all IFN-inducible genes is that only some terminal phenotypes are expressed. These cells therefore display only a partial antiviral state upon IFN treatment. This in turn offers the opportunity of correlating the products of specific IFN-inducible genes with the inhibition of replication of specific groups of viruses. Since the full list of IFN-inducible genes is yet to be assembled (9), such correlations at this moment are of a negative nature by necessity. For example, from the results presented here it seems unlikely that the products of mRNAs 561, 1-8, and 6-16 are sufficient for the inhibition of VSV replication, since these mRNAs are induced by IFN- α more in clone A1 than in clone B1 and since VSV replication is inhibited only in the latter clone. Similarly, EMCV replication is strongly inhibited by IFN- γ in several clones in the virtual absence of the induction of 2-5(A) synthetase mRNA, suggesting that this enzyme is not necessary for the inhibition of EMCV replication. Further studies are needed to rigorously examine the validity of the above suggestions.

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LITERATURE CITED

1. Belkowski, L., and G. C. Sen. 1987. Inhibition of vesicular stomatitis viral mRNA synthesis by interferons. *J. Virol.* **61**:653-660.
2. Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. R. Stark. 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. *Cell* **38**:745-755.
3. Herz, R. E., B. Y. Rubin, and G. C. Sen. 1983. Human interferon- α and - γ -mediated inhibition of retrovirus-production in the absence of an inhibitory effect on vesicular stomatitis virus and encephalomyocarditis virus replication in RD-114 cells. *Virology* **125**:246-250.
4. Kusari, J., and G. C. Sen. 1986. Regulation of synthesis and turnover of an interferon-inducible mRNA. *Mol. Cell. Biol.* **6**:2062-2067.
5. Kusari, J., and G. C. Sen. 1987. Transcriptional analyses of interferon-inducible mRNAs. *Mol. Cell. Biol.* **7**:528-531.
6. Kusari, J., R. K. Tiwari, R. Kumar, and G. C. Sen. 1987. Expression of interferon-inducible genes in RD-114 cells. *J. Virol.* **61**:1524-1531.
7. Lengyel, P. 1982. Biochemistry of interferons and their actions. *Annu. Rev. Biochem.* **51**:251-282.
8. McKnight, S. L. 1980. The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. *Nucleic Acids Res.* **8**:5949-5963.
9. Revel, M., and J. Chebath. 1986. Interferon-activated genes. *Trends Biochem. Sci.* **11**:166-170.
10. Salzberg, S., D. H. Wreschner, F. Oberman, A. Panet, and M. Bakhanashvili. 1983. Isolation and characterization of an interferon-resistant cell line deficient in the induction of (2'-5') oligoadenylate synthetase activity. *Mol. Cell. Biol.* **3**:1759-1765.
11. Samanta, H., D. A. Engel, H. M. Chao, A. Thakur, M. A. Garcia-Blanco, and P. Lengyel. 1986. Interferons as gene activators: cloning of the 5' terminus and the control segment of an interferon activated gene. *J. Biol. Chem.* **261**:11849-11858.
12. Saunders, M. E., D. R. Gewert, M. E. Tugwell, M. McMahon, and B. R. G. Williams. 1985. Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure. *EMBO J.* **4**:1761-1768.
13. Sen, G. C. 1984. Biochemical pathways in interferon action. *Pharmacol. Ther.* **24**:235-257.
14. Sen, G. C., and R. E. Herz. 1983. Differential antiviral effects of interferon in three murine cell lines. *J. Virol.* **45**:1017-1027.
15. Sen, G. C., R. Herz, and B. Y. Rubin. 1983. Antiviral, anticellular and enzyme-inducing activities of interferons in RD-114 cells. *J. Gen. Virol.* **64**:2213-2220.
16. Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc. Natl. Acad. Sci. USA* **76**:1373-1376.