
Differential and cell type specific expression of murine alpha-interferon genes is regulated on the transcriptional level

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Received December 7, 1987; Revised and Accepted May 19, 1988

ABSTRACT

In mouse cells induced with virus infection or dsRNA, the relative levels of alpha-4 interferon mRNA were higher than the levels of alpha-1 and alpha-6 mRNAs; the ratio between relative levels of alpha-4 and alpha-1 or alpha-6 mRNA was, however, dependent on the cell type. Recombinant plasmids, in which the expression of chloramphenicol acetyltransferase (CAT) gene was directed by the promoter regions of alpha-1, alpha-4 or alpha-6 interferon genes were constructed and their inducible expression was studied either in transient assay or in permanently transfected mouse cells. The highest levels of CAT activity and CAT mRNA were observed with alpha-4 CAT plasmid, while the expression of alpha-1 CAT was consistently higher than that coded by alpha-6 CAT plasmid; the ratio between CAT activities coded by alpha-4 CAT and alpha-1 CAT was dependent on cell type. However, in heterologous Vero cells, the transfected alpha-1 and alpha-4 genes were expressed constitutively, and the levels of mRNAs were comparable. These results show that the difference in the relative levels of individual alpha-1 and alpha-4 mRNAs reflects the transcriptional inducibility of the respective promoter regions.

INTRODUCTION

Interferons represent a family of cellular proteins with pleiotropic effects. Although initially discovered by their ability to inhibit viral infections in recipient cells, it has become clear that their effects are not limited to infected cells and their anticellular, rather than their antiviral effects may be of primary biological importance.

Of the three major types of interferons (alpha, beta, and gamma), two types of interferon genes (alpha and beta) are encoded in mouse and human by one interferon locus which is localized on chromosome 4 in mouse and chromosome 9 in human (1-4). The alpha-interferon consist of a multiple gene family of at least 10 to 15 genes (5,6), whereas the beta-1 interferon is encoded by a single copy gene (7-9). The expression of alpha- and beta-interferon genes can be induced in human and mouse cells infected with virus or treated with dsRNA, although these genes are not expressed at detectable

levels in uninduced cells. The induction leads to activation of interferon gene transcription and the synthesis of biologically active interferons (10,11). The expression of alpha-interferon genes in human cells is cell type specific and alpha genes can be most effectively induced by viral infection in cells of lymphoid origin, while the beta-1 interferon gene can be induced in a variety of cell types.

The inducibility of the human alpha-and beta-interferon genes is determined by the sequences present in the 5' flanking regions of these genes (12-18). Both types of genes contain in this region regulatory elements or enhancer-like sequences that confer inducibility to homologous and heterologous genes (16-18). However, interferon synthesis was shown also to be regulated at the post-transcriptional level; thus, the transient synthesis of beta-1 interferon in human fibroblasts is due to the low stability of beta-1 interferon mRNA, which is rapidly degraded in cytoplasm, during the ongoing transcription of the beta-1 interferon gene (19). Although the alpha- and beta-interferon genes are induced coordinately (20,21), quantitative differences in the relative levels of induced individual alpha mRNAs were detected in both human and mouse cells (22-26).

In this study, we addressed the question whether the differential expression of alpha-interferon genes in mouse cells reflects differences in the rate of transcription, processing or stability of the mRNAs. We constructed plasmids in which the transcription of the bacterial chloramphenicol acetyl-transferase (CAT) gene was directed by promoters of mouse alpha-interferon genes, which were either well expressed (alpha-4) or poorly expressed (alpha-1 and alpha-6) in virus-infected mouse fibroblasts (24,25). The inducible expression of alpha-CAT plasmids was examined either in mouse fibroblasts or lymphoid cells and compared to the expression of the corresponding endogenous alpha interferon genes. The results indicate that the difference between the expression of the endogenous (alpha-4 and alpha-1) interferon genes examined reflects mainly the transcriptional activity of the corresponding promoter region in the particular type of the cell.

MATERIALS AND METHODS

Construction of CAT plasmids

The construction of pRB49 (alpha-1 CAT) and pRB22 (alpha-4 CAT) was done by fusion of either the 242-bp promoter (-189 nt to +52 nt) fragment of interferon alpha-1 or the 487-bp promoter (-464 nt to +22 nt) fragment of alpha-4 to the CAT gene containing the SV40 polyadenylation sequence that

was isolated from the plasmid SV₂CAT and subsequently cloned into pBR322.

PFB5 (alpha-6 CAT) was constructed by ligation of the 451-bp alpha-6 promoter (-428 nt to +22 nt) fragment to pCATB' plasmid (27).

Cell culture and transfection studies

Mouse fibroblast L929 cells were routinely kept in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf serum, unless otherwise indicated. Mouse granulocytes-macrophage FDC-1 cells which require Il-3 for growth, were maintained in RPMI with 10% calf serum and 10% conditioned medium from WEHI-3 cells (source of Il-3).

Transfections were done as follows: I. L-cells were seeded at 1.5×10^6 cells/100-mm plate for transient studies and 5×10^5 cells/60-mm plate for long term studies. Plasmid DNA (10 ug) was added as the calcium phosphate precipitate to the medium, which was changed 3 hr before transfection (28). Cells were then incubated for 4 hr, washed with serum-free DMEM, subjected to treatment for 60 sec with 15% glycerol (in serum-free DMEM), washed twice with phosphate buffered saline (PBS), incubated overnight in DMEM supplemented with 5 mM sodium butyrate, and then induced. For long term experiments, cells were transferred to a selective medium (DMEM with G418, 1 mg/ml) (29) 3 days after transfection, as described previously (28). Single clones and pools of transfected cells were isolated at 10 to 14 days after transfection. II. Granulocyte-macrophage FDC-1 cells were seeded at 10^7 cells/60 mm plates. The next day, cells were washed 3X with RPMI serum-free medium, once with Tris-buffered saline (TBS), and suspended in 500 ul of 0.5 mg/ml DEAE dextran in TBS with 40 ug of plasmid DNA (30). After a 30-min incubation at 37°C, cells were washed once with TBS and once with RPMI containing 10% serum. Cells were then seeded into plates at a density of 10^6 /ml of medium, incubated for 48 hr, and induced.

Assay for CAT activity

CAT activity was determined by a modification of the method of Gorman et al. (31). Sixteen hours after induction, cells from 100-mm dishes were trypsinized, collected by centrifugation and washed with phosphate-buffered saline. Cells were then resuspended in 60 ul of 0.25 M Tris hydrochloride, pH 7.6, and lysed by three freeze-thaw cycles. The cell debris were centrifuged in a microfuge and the supernatant fraction was analyzed for proteins by the Bio-Rad protein assay. For CAT activity, a given amount of [¹⁴C]-chloramphenicol (54.3 mCi/mmol; New England Nuclear Corp), 38 ug of acetyl CoA (lithium salt, Pharmacia) and cellular extract (up to 50 ul) were assayed in a final volume of 150 ul as described previously (32).

Induction of Cells

Cells were induced with either Newcastle Disease virus (NDV) New Jersey LaSota strain or polyinosinic-polycytidylic acid (poly I.rC) as described previously (33). Confluent cultures were infected with NDV at an moi of 1 for 90 min in serum-free DMEM. Medium was then replaced with DMEM supplemented with 3% serum. For poly I.rC inductions, confluent cultures were treated with 20 ug/ml of poly I.rC, 600 ug/ml of DEAE dextran and 5 ug/ml of cycloheximide in serum-free medium. After a 4-hr incubation at 37°C, the cells were washed twice with PBS, and further incubated in DMEM supplemented with 3% serum. In transient assays, cells were induced 48 hr after the transfection.

RNA preparation and analysis

Total RNA was prepared at various times after induction. Cells were washed twice with PBS and lysed with the guanidinium isothiocyanate buffer and RNA was isolated by centrifugation over CsCl cushion (34). For S1 analysis, 10 ug of total RNA were hybridized overnight at 50°C with an RNA probe (33). The samples were then treated with S1 nuclease (35,36) or with RNase A (40 ug/ml) for 30 min at 37°C. The RNase A treated samples were then digested with proteinase K and extracted with phenol and chloroform before further analysis. Protected fragments were identified by electrophoresis on 5% polyacrylamide gels. The labeled fragments were visualized by autoradiography.

Preparation of DNA and RNA probes

For construction of the riboprobes pRB79, pRB32, and pFB24 (Fig. 4), detecting the CAT mRNA the corresponding alpha-CAT plasmids were first digested with an appropriate restriction endonuclease cutting upstream in the alpha promoter region. Appropriate linkers were added at this step when needed and then plasmids were restricted with EcoRI, which cuts 250-bp into the CAT gene. The resulting fragment, which contained part of the corresponding alpha promoter and 250 bp of the CAT gene, was inserted into the appropriate site in the polylinker region of pSP65 (37) and the probes were prepared as described previously (33). The sizes of the probes used and of the protected fragment are shown in Figure 4. The riboprobes for the detection of the alpha-1, alpha-4, and alpha-6 interferon mRNAs (Fig. 1) were constructed in a similar manner. The EcoRI and MstII fragments of alpha-1 and alpha-6 genes and the EcoRI and BglIII fragment of alpha-4 gene were cloned into Sp64 vector. The plasmids were linearized with EcoRI before transcription.

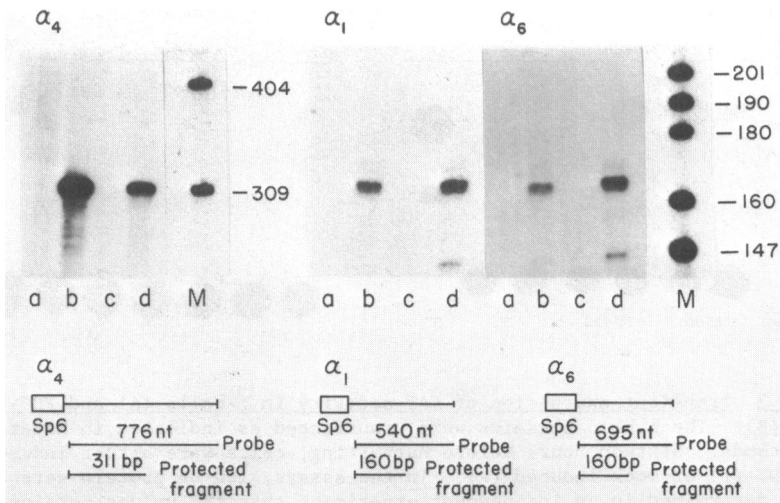


Figure 1 Expression of IFN alpha-1, alpha-4 and alpha-6 genes in mouse cells induced by NDV. L-cells and FDC-1 cells were induced with NDV and total RNA was isolated 8 hr after induction as described in Methods. For each experiment, 10 ug of total RNA were hybridized to the specific ^{32}P labeled probes. Construction of specific probe is given in details in materials and methods and shown schematically in diagram. Lanes a and b are from L-cells and c and d from FDC-1 cells. Lanes a and c represent RNA from uninduced cells and b and d from induced cells. The length of the probes are given in nucleotides (nt) and protected fragments in basepairs (bp).

RESULTS

Expression of alpha-interferon genes

Differential expression of individual murine alpha-interferon genes was previously observed in NDV-infected L-cells (24,25) and an ascites tumor cell line (38) and it was shown that the relative levels of alpha-4 mRNA were much higher than were those of the other alpha mRNAs examined. To determine if the same phenomenon exists in lymphoid cells, we measured the levels of alpha-1, alpha-4 and alpha-6 mRNAs by RNaseA protection assay in L-cells and FDC-1 cells using riboprobes similar to those used by Kelley and Pitha (24). Figure 1 shows that the alpha-4 interferon gene is expressed efficiently in infected L-cells, while the other two alpha genes are expressed in both types of cells at much lower levels. Thus, while the levels of alpha-4 mRNA in L-cells were at least 10- to 50-fold higher than the levels of alpha-1 and alpha-6 mRNAs, in FDC-1 cells both alpha-1 and alpha-6 genes were expressed more efficiently and the relative levels of alpha-4 mRNA were about 2-fold higher than the levels of the other mRNA

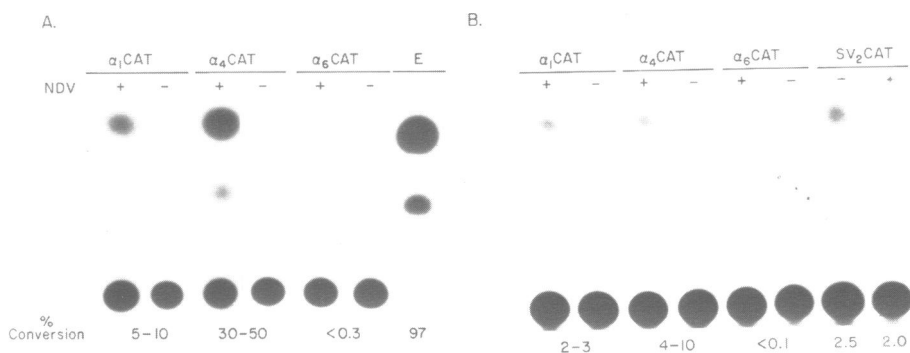


Figure 2 Transient expression of CAT activity in L-cells (A) and FDC-1 cells (B). The hybrid plasmids were transfected as indicated in Materials and Methods. Sixteen hours before harvesting, cells were either induced with NDV (+) or mock induced (-). In the assays, 100 ug protein were used. It was determined in an independent experiment that the values obtained were in linear range of CAT conversion (activity). The controls for the CAT assay were purified CAT enzyme or SV₂ CAT plasmid. The percent conversion given below the autoradiogram represents an average value from several independent experiments.

measured. The levels of actin mRNA that was used as a standard was the same in induced and uninduced L-cells and FDC-1 cells. These results show that the level of the induced expression of the alpha-1, alpha-4, and alpha-6 interferon genes is cell type dependent.

Expression of CAT gene from the recombinant plasmids

To determine whether the differential expression of the alpha-interferon genes in induced mouse cells is related to the difference in the strength of the respective promoter or to the stability of the respective mRNA, we constructed plasmids in which the promoter region of the alpha-interferon gene (242, 487 and 451 nt fragments of alpha-1, alpha-4 and alpha-6, respectively) was inserted 30 nt upstream from the ATG codon of the CAT gene. These plasmids were introduced into L-cells or FDC-1 cells and the levels of CAT activity induced by NDV infection or poly rI.rC treatment (L-cells) were determined in transient assays 48 hr after the transfection. Figure 2 shows that in both types of cells alpha-4 CAT is expressed most efficiently, but in L-cells the CAT activity coded by the alpha-4 CAT is 5- to 6-fold higher than that coded by alpha-1 CAT, whereas in FDC-1 cells, it is only 2- to 3-fold higher. However, both in L-cells and FDC-1 cells, we repeatedly have not observed any CAT expression from alpha-6 CAT plasmid.

The inability of alpha-6 CAT to code for the CAT activity was not a

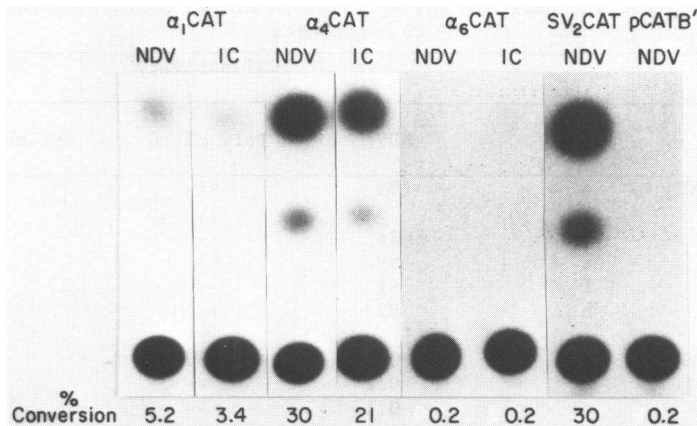


Figure 3 CAT activity from stably transfected cell line pools. Cells were collected 16 hr after induction with either NDV or poly rI.rC (IC). Each assay contained 25 ug protein from all the pools except for SV₂CAT and pCATB', where 100 ug was used. Mock inductions showed no detectable activity (data not shown). Percentage conversion to the acetylated forms for the respective assay is given below the picture.

cloning artifact. This was true even when we prepared recombinant alpha-6 CAT plasmid using an alternative cloning strategy (by fusing the alpha-6 promoter region to the CAT gene containing the SV40 polyadenylation site and inserting it into pBR322) (data not shown). To determine whether the inability to induce the CAT expression from the alpha-6 CAT plasmid was limited to the transient assay, the cells were cotransfected with the alpha-CAT hybrid plasmids together with neomycin gene and inducible expression of CAT gene was tested both in a pool of selected transformants and in individual clones. The pool of transfected cells induced either with poly rI.rC or NDV showed the levels of CAT activity expressed from alpha-4 CAT, alpha-1 CAT and the alpha-6 CAT similar to those detected in the transient assay (Fig. 3); the expression of alpha-4 CAT was about 7-fold higher than that of alpha-1 CAT both in poly rI.rC and NDV induced cells and no CAT activity was induced from the alpha-6 CAT plasmid. Neither the expression of the SV₂CAT (in which the transcription of CAT gene is directed by the SV40 early promoter region) nor the pCATB' (CAT gene without an enhancer region) was enhanced by NDV infection. To exclude the possibility that CAT activity induced in the pool of the long-term cultures represents induction of only a few clones, we isolated 8 individual clones from the pool of cells transfected either with alpha-6 CAT or alpha-4 CAT and examined their

Table I: Levels of inducible CAT activity in clones containing the alpha-CAT constructs.

Plasmid	Clone	CAT activity*			
		NDV	Poly rI.rC	Cycloheximide	
alpha-4 CAT	a	93	92	<0.1	
	b	70	--	--	
	c	86	--	--	
	e	89	81	<0.1	
	f	91	--	--	
	h	82	--	--	
	alpha-6 CAT	a	0.4	3.4	<0.1
		b	0.2	--	--
c		0.1	--	--	
e		0.2	2.5	<0.1	
f		0.2	--	--	
h		0.1	--	--	

*Levels of CAT activity are determined as percentage conversion; it was calculated by dividing the radioactivity (cpm) in the 3-acetyl-chloramphenicol spot by the sum of the radioactivity present in the chloramphenicol and 3-acetyl-chloramphenicol spots.

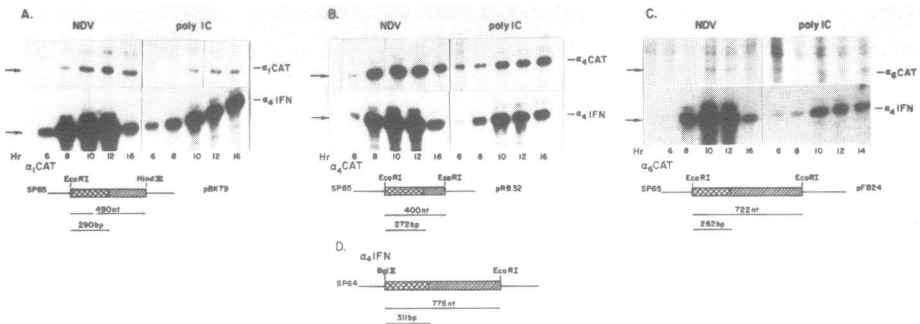


Figure 4 S1 analysis of the accumulated transcripts (for endogenous alpha-4 gene and hybrid alpha CAT plasmids) as function of time in NDV and poly rI.rC induced stably transfected L-cells. RNA from pools of stably transfected cells was extracted 6, 8, 10, 12 and 16 hr after induction. For hybridization both with a riboprobe detecting alpha-4 endogenous transcripts, and with the probes detecting the CAT mRNA, 20 ug RNA were used. A. Represents RNA isolated from cells transfected with alpha-1 CAT. B. From alpha-4 CAT and C. from alpha-6 CAT, respectively. D. Shows a probe for endogenous alpha-4 mRNA. Construction of the specific probes and the size of the protected fragment after hybridization and S1 digestion are shown in the diagram. The cross-hatched bars represent the first 250 bp of CAT coding sequences or 311 bp of alpha-4 gene coding sequences. The hatched bars represent promoter sequences for alpha-1, alpha-4 and alpha-6. The lengths of the probes are given in nucleotides (nt) and protected fragments in basepairs (bp).

inducibility. Table 1 shows that in contrast to the pooled cells, in isolated clones, we were able to detect low levels of expression from the alpha-6 CAT plasmid; however, all clones containing alpha-4 CAT constructs, showed much higher induced CAT activity than did the clones containing the alpha-6 CAT plasmid. Interestingly, while the levels of CAT activity induced by NDV or poly rI.rC in clones containing alpha-4 CAT plasmid were comparable (Table 1), in clones containing alpha-6 CAT plasmid, poly rI.rC was about 10-fold better inducer than NDV.

Kinetics of inducibility

Since all the assays were performed 8 hr after the induction, we needed to verify that the difference in the expression truly reflected the transcriptional strength of the alpha promoter and was not due to the difference in the expression kinetics of individual alpha-interferon genes. Cultures from pools of the L-cells transfected with alpha CAT hybrid plasmids were therefore induced either with NDV or poly rI.rC and RNA was isolated at different times after induction. The pool cells rather than an isolated clone was chosen for this experiment to obtain more representative results. The induced expression of transfected alpha CAT plasmid was compared to the expression of the endogenous alpha-4 interferon gene. Probes specific to alpha-1 CAT, alpha-4 CAT and alpha-6 CAT were used, as well as a probe detecting the endogenous alpha-4 interferon. It can be seen in Figure 4 that the correctly initiated alpha-4 mRNA was detected in the cells as soon as 6 hr after induction with both poly rI.rC and NDV, and that maximal relative levels of alpha-4 mRNA were reached at 10 to 12 hr after induction. In NDV-induced cells the relative levels of alpha-4 mRNA declined by 16 hr, whereas in poly rI.rC-induced cells the levels of alpha-4 mRNA seemed to be constant up to 16 hr. These results indicate that both in poly rI.rC and NDV induced L-cells alpha-4 mRNA is stable. In addition to the correctly initiated alpha-4 mRNA, a small amount of alpha-4 mRNA initiated several nucleotides upstream from the cap site was detected in all NDV-induced samples. On a prolonged exposure, the second initiation site was also detected in alpha-4 mRNA isolated from poly rI.rC-induced cells.

The expression of the transfected alpha CAT hybrid plasmids showed kinetics similar to that of the endogenous alpha-4 interferon gene. The levels of alpha-4 and alpha-1 CAT mRNA were higher in NDV than in poly rI.rC induced cells, and reached maximal levels at 10 to 12 hr after induction in NDV induced cells. The levels of alpha-4 CAT mRNA were about 5- to 10-fold higher than were the levels of alpha-1 CAT mRNA. The alpha-6 CAT mRNA could

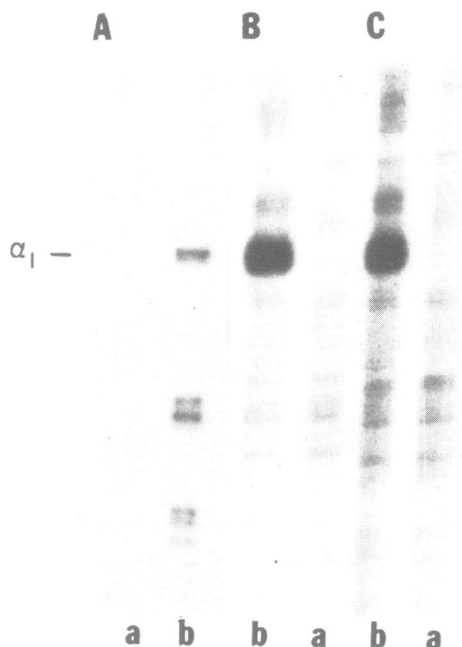


Figure 5 Analysis of endogenous and exogenous alpha-1 mRNA in L-cells. The control L-cells (A) and L-cells transfected with murine alpha-1 interferon gene (pFB-1) (B) or murine alpha-1 interferon gene with SV40 poly(A) site (pFB-3) (C) were induced with NDV (24 hr after transfection) and RNA was isolated 8 hr after the induction. 10 ug of total RNA was analyzed by RNase protection assay as described in methods using an alpha-1 probe identical to that described in Figure 1. a. uninduced cells, b. induced cells.

be detected as a weak band, however, only after a prolonged exposure and the mRNA levels in NDV- and poly rI.rC-induced cells were comparable.

Stability of alpha mRNAs

We and others have previously shown that the stability of the human beta-1 interferon mRNA in induced cells plays a major role in the regulation of interferon synthesis in the poly rI.rC induced cells (19,26) and we have shown that the instability of the beta-1 mRNA resides in the coding or 3' untranslated region of this mRNA (28). The 3' untranslated region of alpha-1 interferon gene, unlike alpha-4 and alpha-6 genes, does not contain a proper polyadenylation signal (TATAAA instead of AATAAA); in addition, this mRNA contains multiple copies of AU rich clusters that are also present in the 3' untranslated region of beta-1 interferon gene and other unstable mRNAs (39). To determine if the low levels of alpha-1 mRNA in the cells

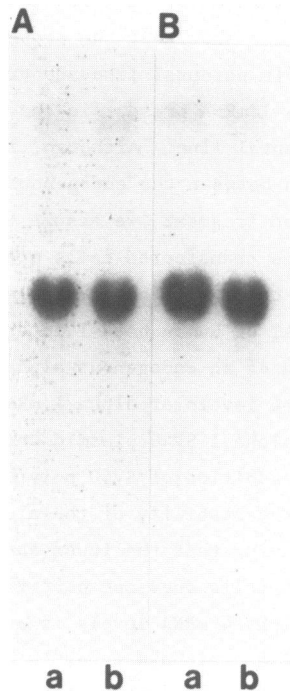


Figure 6 Analysis of murine alpha-1 and alpha-4 mRNA in Vero cells. Vero cells were transfected with 10 ug of murine alpha-1 (pFB-1) (A) and alpha-4 (pkk12) (B) interferon genes and 24 hr after the transfection cells were induced with Sendai virus (moi 10) and RNA was isolated 8 hr later. Ten micrograms of total RNA were electrophoresed through 1.1% agarose, 1.1 M formaldehyde gels, transferred to nitrocellulose and hybridized with the alpha-4 RNA probe described in Figure 1. Lane a represents RNA from control cells and lane b from induced cells.

could be due to its low stability, we examined whether the insertion of strong poly(A) site into 3' end of alpha-1 mRNA would affect its stability. Hybrid genes, in which we inserted a polyadenylation region of SV40 gene beyond the 3' end (1215 nt from the cap site) of the alpha-1 gene, were constructed. The alpha-1 SV40 hybrid gene was then inserted into pBR322 and the resulting plasmid (pFB3) was transfected into L-cells. In parallel, cells were transfected with plasmid (pFB1) containing the 1520-kb fragment of alpha-1 gene cloned in pBR322. Both plasmids contain 305 nt of alpha-1 promoter region and are therefore, inducible with NDV or poly rI.rC. Transfected cells were induced with NDV 24 hr after transfection and the levels of properly initiated alpha-1 mRNA in transfected cells and parental

line were determined 8 hr after induction. The results of RNase A protection analysis in Figure 5 show that the properly initiated alpha-1 mRNA could be detected both in parental line and transfected cells. However, the levels of alpha-1 mRNA present in the transfected cells were much higher than in the parental line. Although, in this experiment, we were not able to distinguish between the endogenous and exogenous alpha-1 mRNA by size of the protected fragment, we assume that the high levels of alpha-1 mRNA expressed in the transfected cells are coded by the plasmid DNA. Transfection of the L-cells with numerous plasmids (e.g., SV₂ CAT, alpha-1, alpha-4, alpha-6 CAT or human beta-1 interferon gene) has never lead to increased expression of an endogenous alpha-interferon gene (data not shown). The size and the levels of alpha-1 mRNA coded by the alpha-1 interferon gene and by the alpha-1 SV40 plasmid were comparable (data not shown), suggesting that the addition of SV40 poly(A) region did not alter either the termination and the stability of the alpha-1 mRNA.

Additional evidence showing that the lower stability of alpha-interferon mRNA in induced L-cells does not contribute to the observed difference in alpha-1 and alpha-4 mRNA levels is suggested from the studies on the expression of alpha-1 and alpha-4 genes in heterologous, simian cells. We have previously shown that Vero cells do not contain either alpha or beta, interferon genes (28 and Diaz, et al. in preparation); however, the transfected, integrated beta-1 interferon gene could be effectively induced in these cells (28). In difference, Vero cells that were transiently transfected with the alpha-1 or alpha-4 interferon genes expressed alpha-genes constitutively (Fig. 6), and no enhancement in the mRNAs levels could be detected after the induction. However, when expressed constitutively, the relative levels of alpha-1 and alpha-4 mRNAs were comparable. We conclude from these results that there is no major difference in the stability of the alpha-1 and alpha-4 mRNAs and that the difference in the relative levels of alpha-1 and alpha-4 mRNAs observed in the induced cells is entirely due to the transcriptional strength of the induced promoter.

DISCUSSION

In the present study, we analyzed the inducibility of three mouse alpha-interferon genes (alpha-1, alpha-4 and alpha-6) in mouse fibroblasts (L-cells) and granulocyte-macrophage (FDC-1) cells and compared the inducible expression of endogenous genes and the transfected plasmids in which the expression of CAT gene was directed by the promoter regions of

these three alpha-interferon genes.

In mouse cells, irrespective of the inducer, the alpha-4 gene was more effectively expressed at the RNA level than were the other two alpha-interferon genes examined. The ratios between the relative levels of alpha-4 and alpha-1 or alpha-6 mRNAs in L-cells and FDC-1 cells, however, were not identical; alpha-4 gene was expressed at much higher levels in L-cells than in FDC-1 cells. Similar phenomenon was observed when the recombinant alpha-1 and alpha-4 CAT constructs were transiently expressed in these two types of cells. The cell type-specific variation in the expression that can be seen both with the endogenous alpha-4 gene and with the alpha-4 CAT plasmid indicate that cell type-specific factors play a role in induction. In heterologous-Vero cells, where the transfected alpha-1 and alpha-4 genes were expressed constitutively, the levels of alpha-1 and alpha-4 mRNA were comparable; these results indicate that it is the inducibility of the genes and not the stability of the mRNAs that is responsible for the observed difference. The preferential expression of certain alpha-interferon genes was previously observed in human cells (22), where the alpha-A and alpha-D genes were induced most efficiently in lymphoid cells but the ratio between the relative levels of alpha-D and alpha-A mRNAs in induced cells varied from 1.7 (in leukocyte cells) to 0.4 (in Namalva cells). Whether this difference was due to the rate of transcription or post-transcriptional regulation was not established.

The strong inducibility of the alpha-4 interferon promoter region was also seen when the expression of the alpha CAT plasmid was examined either in a pool of permanently transfected cells or in individually selected transfected clones. The highest levels of CAT activity and its mRNA were observed repeatedly in the alpha-4 CAT constructs, while the levels of CAT activity and CAT mRNA coded by alpha-1 CAT were reproducibly higher than were those coded by alpha-6 CAT plasmid. However, the data on the inducible expression of endogenous alpha-6 gene and the alpha-6 CAT plasmids show an important difference. Neither by CAT assay or by S1 mapping of the CAT mRNA the expression of alpha-6 CAT plasmid could be detected (either in transient or permanent assay) while the endogenous alpha-6 gene was expressed at low levels. The basis of the observed difference between the expression of the endogenous and exogenous alpha-6 promoter region is not know. It is possible, however, that the 428 nt promoter region used in the construction of the alpha-6 CAT hybrid contains negative regulatory sequences similar to those detected previously in the upstream region of beta-1 interferon

promoter (40). We are presently examining whether a shorter promoter region, such as used for construction of alpha-1 CAT, will be inducible in transfected cells.

The 5' sequences of the promoter region of different mouse alpha-interferon genes show a high degree of homology (24,25,41). It was pointed out by Zwarthoff (25) that in all alpha-interferon genes, the region between -130 to -30 nt is rich in purine residues (80% in mouse and 70% in human). Ryals et al. (17) localized the 5' and 3' boundary of the inducible region in the human alpha-D promoter region between -109 and -64 nt and suggested that two sets of repetitive sequences, R1 [AA(T)GGAAAG] and R2 (CAGAA), may be functionally important. Dion et al. (42) identified similar repeats in the 5' flanking region of the murine alpha-7 interferon gene and upon examination of 500 nt preceding the ATG codon of several other murine alpha-interferons, they found that each flanking region contains repetitive octamers with a consensus sequence GTGGA(T)A(T)A(T)G. Fujita et al. (16) identified several hexamer repeats with a consensus sequence of AAG(A)G(T)GA in the -110 to -64 nt region of human beta-interferon promoter and recently showed (43) that chemically synthesized consensus hexanucleotides tandemly repeated, confer virus inducibility to homologous and heterologous promoters. These authors also found that not only the number of repetitive sequences is important for induction but that a single base change may render the repeat inactive.

It is not without interest that the 5' flanking sequence of the murine alpha-4 interferon gene shows several differences from the promoter region of the other alpha-interferon genes present in the interferon gene cluster (41). The alpha-4 promoter region shows the presence of 19 nt long (G)nA repeat (region -154 to -135) and a number of single nucleotide changes that are absent in alpha-1 and alpha-6 interferon promoters. Since the G/C-rich hexanucleotides are part of several viral enhancer regions such as immediate-early genes of the herpes virus group (44) and SV40 21-bp repeats (45), we initially assumed that it is the polypurine cluster that is responsible for the high inducibility of the alpha-4 interferon promoter. However, our preliminary data indicate that the high strength of the alpha-4 promoter is not determined by the polypurine insert, but that the inducible region is localized in the -109 to -75 nt of the 5' flanking region (Raj, Israeli and Pitha, in preparation). None of these three mouse alpha-interferon genes examined contain, in this region, the two copies of the repeated R₁ and R₂ elements that were identified in human alpha-D interferon

promoter and only a single R₂ is present in the alpha-4 promoter. However, the AGTGAA hexamer that can be found in a single copy both in the human beta (-75 to -69 nt) and alpha-A (-82 to -76 nt) is present in the -109 to -75 inducible region in two copies both in alpha-4 and alpha-1 promoter region, but only in a single copy in alpha-6 promoter. Further experiments have to determine whether this repeat has a functional role in the inducibility of murine alpha-interferon genes.

Although little is known about the mechanism by which virus and dsRNA induce the expression of interferon genes, the induction of the human alpha-D interferon gene seems to occur through a positive control (17), while both positive and negative regulation was shown to play a role in the induction of human beta-1 interferon gene (18,46); which type of control plays a role in cell-specific expression of individual alpha-interferon genes remains to be determined.

ACKNOWLEDGEMENT

We wish to thank Mr. M. Kellum for his excellent technical help, Dr. J. Mosca for his initial advice on the CAT assay, Dr. P. Talalay for her critical review of the manuscript and B. Schneider for typing. This work was supported by NIH grants AI19737 and AI10944.

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