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Repression of the murine interferon α11 gene: identification of negatively acting sequences

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ABSTRACT

The uninducible murine interferon α11 gene (Mu IFN-α11) shows strong homology with the highly inducible Mu IFN-α4 gene in the promoter region. Negative regulatory sequences located between positions −470 and −145 were characterized in the Mu IFN-α11 promoter. The removal of these sequences leads to virus-inducibility of Mu IFN-α11 while their insertion in Mu IFN-α4 corresponding region significantly reduced the inducibility of Mu IFN-α4 promoter. On the other hand, the virus-responsive element (VRE) of the Mu IFN-α11 differs by a single nucleotide substitution at position −78 from the VREα4. Constructions carrying either VREα11 or VREα4 upstream a heterologous promoter displayed different virus inducibilities. The −78 A/G substitution affects the inducibility by decreasing the affinity of VRE-binding *trans*-regulators. Our results suggest that the combined effect of the negative regulatory sequences and of the mutation in the VREα11, completely silences the Mu IFN-α11 gene.

INTRODUCTION

The analysis of the expression of murine interferon α genes revealed that the levels of individual IFN-α mRNAs are very different and cell type specific (1−5). These genes are shown to be most efficiently induced in fibroblast-like cells, such as the murine L929 cell line, upon viral infection with Newcastle Disease virus (a double-stranded RNA virus) or treatment with synthetic double stranded poly rI-rC, as well as in NDV-infected bone marrow macrophages grown in the presence of M-CSF (macrophage colony stimulating factor). They differ from Hu IFN-α genes which are poorly expressed in induced fibroblasts and HeLa cells (where IFN-β mRNA is predominant) and which are expressed as well as IFN-β in Sendai virus-induced leukocytes and lymphoblastoid Namalwa cells or myeloblastoid KG-1 cell line (6).

Although differences of expression exist between the murine and human IFN-α genes, yet, the differential expression as well as the cell-type specificity of Mu IFN-α genes were shown to reflect the transcriptional inducibility of their respective promoter regions (7).

Cell transfection by chimeric plasmids containing IFN promoter regions upstream a reporter gene revealed that virus-induced transcription of Hu IFN-α1 gene is mediated by a 46-bp fragment of the promoter extending from −109 to −64 relative to the cap site (8). Similarly, it has been reported that a 35-bp fragment located between position −109 and −75 was able to confer inducibility to Mu IFN-α4 gene (9). Attention was focused on these fragments respectively called 'Virus Responsive Element' (VRE) and 'Inducible Element' (IE) in order to examine their effect in the differential expression of IFN-α genes. These elements show a high level of homology and contain repeats of AGTGAA motif or the variants of its permutated form GAAANN. The multimerization of AGTGAA repeats contained in the inducible element of Mu IFN-α4 gene was shown to confer both inducible and constitutive expression to a minimal promoter while its multimerized mutant AATGAA was preferentially expressed in induced cells (10). Recently in Hu IFN-α1, the properties of different types of tetrameric hexanucleotides (GAAANN) which mediate virus-inducibility have been characterized (11): type I (where NN = GT, GC, CT or CC) causes silencing, and is interferon-inducible, type II (NN =TG) neither silences nor responds to IFN, and type III (NN = CG) shows the properties of type II but in addition is constitutively active. However, the synthetic hexamers did not show cell type specific restriction, indicating that their interaction does not reflect the complexity of differential expression observed for the interferon α genes.

Within a 40-kb murine genomic fragment, we isolated (C.Coulombel, G.Vodjdani and J.Doly, in press) three new Mu IFN-α genes, namely α7, α8, α11 which encode for biologically active proteins and yet are not virus-inducible in L929 and C243 cells. We also confirmed that Mu IFN-α4 gene in the same conditions of induction is highly inducible in L929 cells (7) and we showed its inducibility in C243 cells. Our aim was to determine if modifications within their promoter region may be crucial for virus-inducibility.

The comparison of the 5′-flanking sequences of the murine interferon α genes isolated so far and particularly of their VRE-corresponding region revealed a striking homology between the highly inducible Mu IFN-α4 and the uninducible Mu IFN-α11 (Fig. 1A). They differ, indeed, in the VRE, only by a single A/G

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substitution at position −78. In this paper, we examined if this difference in the VRE could explain the uninducibility of Mu IFN-α11 gene. VREα11 failed to respond to virus induction in C243 cell line, thus revealing the importance of the −78 substitution. Since this element was able to confer inducibility to the heterologous *tk* promoter in L929 cells, further investigation in the Mu IFN-α11 promoter extending up to −470 led us to show the existence of negative *cis*-acting sequences located upstream the VRE. Investigation of such an uninducible gene will give a better understanding of the mecanisms which control the repression of some murine IFN-α genes with regard to the other members of the family upon virus induction.

MATERIAL AND METHODS

Plasmid constructions

pVRE11 and pVRE4 were obtained by insertion of the synthetic VREα11 and VREα4 elements at the HindIII and BamHI sites in pBLCAT2 (12). They are composed of the Mu IFN-α4 or Mu IFN-α11 49-bp (−112 to −64) promoter region flanked on either side by HindIII and BamHI linkers (Fig. 1B). The partial HinfI hydrolysis the Mu IFN-α11 HaeIII segment (−470 to +174) yielded two fragments (−470 to +19) and (−261 to +19) which were inserted at the XbaI site in pBLCAT3 (12) to obtain pIF11T and pIF11A respectively. pIF11B corresponds to the DraI-BamHI fragment of pIF11T which was inserted at the XbaI-BamHI sites in pBLCAT3. The pIF11C, D, E and F plasmids were obtained by Bal 31 exonuclease digestion of the Mu IFN-α11 promoter from the DraI site (−213). The 5′ and 3′ end points of the different inserts are indicated on Fig. 1B. Mu IFN-α4 promoter (−477 to +19) is the NlaIII-HinfI fragment of α4EE plasmid provided by Dr. Zwarthoff.

Oligonucleotide site-directed mutagenesis of the Mu IFN-α promoters was carried out by the method of Carter et al. (13). The template was M13mp19AmIV containing the 500-bp HindIII-BamHI of the promoter fragments excised from pIF4T and pIF11T. The mutagenic oligonucleotides used were V′α11 (5′-CCCCTTGCTTCCCAATTCTCT-3′) and V′α4 (5′-CCC-CTTGCTTTCCAATTCTCT-3′). The mutated promoters were cloned in HindIII-BamHI digested pBLCAT3, providing pIF4T(11) and pIF11T(4) respectively. The hybrid promoters were obtained by the *in vivo* homologous recombination method of Weber et al. (14). The junction regions of the resulting plasmids are shown in Fig. 1B. All constructions were verified by sequencing double-stranded DNA (15).

Cell transfection and induction

L929 or C243 cells were seeded at 10^6 cells per 10 cm dish in DMEM/7.5% FCS the day before transfection. Cells were transfected by the calcium phosphate precipitation method (16) with plasmid DNA (10 μg) and the internal control pCH110 plasmid (17). Cells were then incubated for 24 h, washed with serum free DMEM, and reincubated 18 h before induction with NDV (New Jersey, La Jolla strain) at a m.o.i. of 1 in serum free medium for 1 hour. After treatment for 5 h with cycloheximide (2 μg/ml) and the last 2 hours with actinomycine D (0.2 μg/ml) the medium was replaced with fresh DMEM supplemented with 5% fetal calf serum. Cells were harvested 16 h postinduction, and cytoplasmic extracts were prepared by four freeze-thaw cycles and separated from cellular debris by centrifugation. Cellular extracts were assayed for 1 h or 3 h for CAT activity at 37°C as described by Nordeen et al. (18). The protein concentration was determined with the Coomassie G250 procedure, using bovine serum albumin as standard (19). The β-galactosidase activity was measured according to Casadaban et al. (20). The CAT values were normalized to the β-

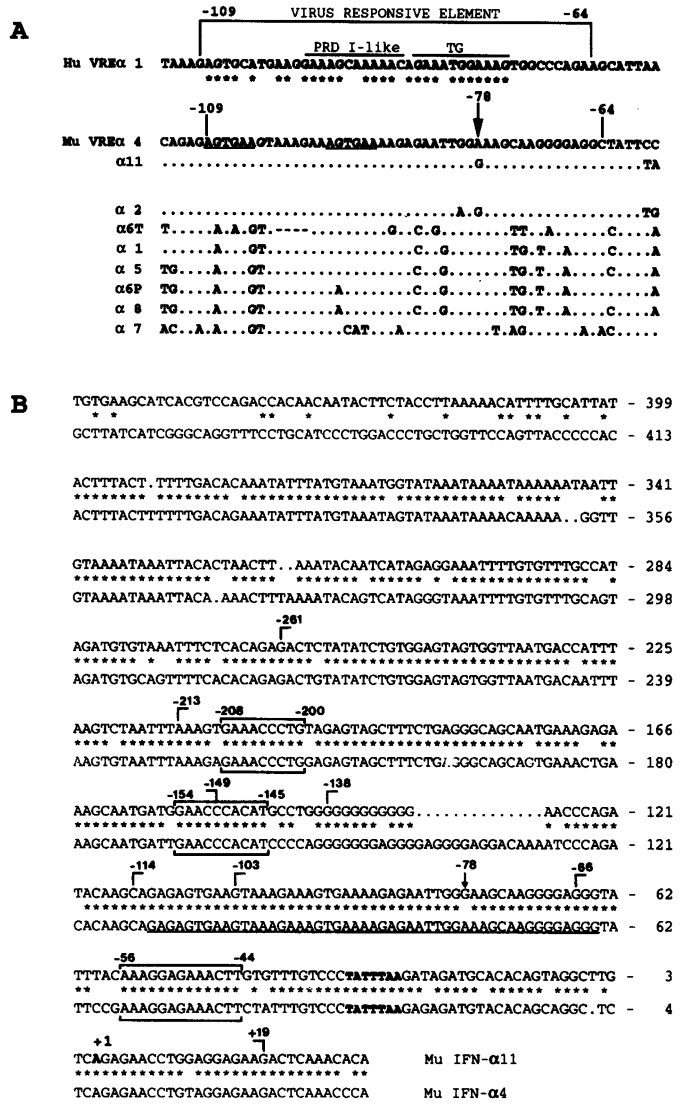

Figure 1A. Comparison of the Virus Responsive Element (VRE) sequences of the different murine IFN-α genes (1, 2, 25−27) with the human IFN-α1 gene (8). Dots correspond to those nucleotides which are identical to the Mu VREα4. Dashed lines denote gaps introduced to achieve the best alignment. Numbering is from the cap site of either human or murine IFN-α genes. Asterisks indicate the nucleotide homology in the VRE between Hu IFN-α1 and Mu IFN-α4. The TG sequence, the PRDI-like sequence (11) are overlined and the hexamer motifs (24) are underlined. The arrowhead at position −78 indicates the A/G substitution in Mu VREα4/α11. **B.** Promoter sequences of Mu IFN-α11 and Mu IFN-α4 extending respectively up to −457 and −470. Homology between the two sequences is denoted by asterisks; gaps indicated by dots are introduced to achieve the best alignment. Numbering is from the cap site (+1) of either gene. The plasmids pIF11T and pIF4T contain the promoter from −470 to +19 and −477 to +19, respectively. The position of 5′-borders of the deleted mutants (described in Material and Methods) is indicated by a broken line : pIF11A (−261), B (−213), C (−138), D (−114), E (−103) and F (−66). The underlined sequence indicates the oligonucleotide which has been synthetized corresponding to the VRE. The position of the A/G substitution is denoted by an arrow. Horizontal brackets (−208/−200; −154/−145 and −56/−44) indicate the junction regions in the α11/α4 hybrid promoters, namely plasmids : pIFH65, pIFH64 and pIFH66. The broken line at position −149 indicates the 3′-border of the repressive region of Mu IFN-α11 promoter.

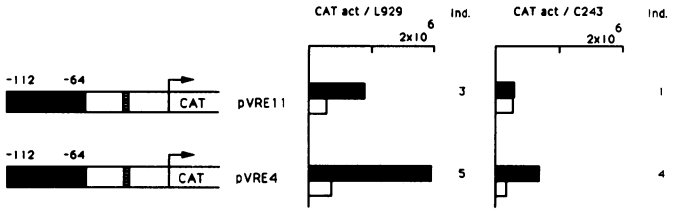

Figure 2. Analysis of Mu IFN-α11 and IFN-α4 VRE sequences. pVRE4 and pVRE11 were obtained by insertion of the synthetic HindIII-BamHI VREα4 and VREα11 elements in the corresponding sites in pBLCAT2. L929 and C243 cells were transfected with 10 μg of the test plasmid containing the CAT reporter gene. Cells were induced by NDV or mock-induced. 24 hours after induction, cells were harvested and subjected to the CAT activity assay. CAT activity is expressed as cpm/mg of protein-hour. Values of inducibility (Ind) correspond to the ratio of NDV-induced (black bars) to mock-induced (white bars) CAT activities.

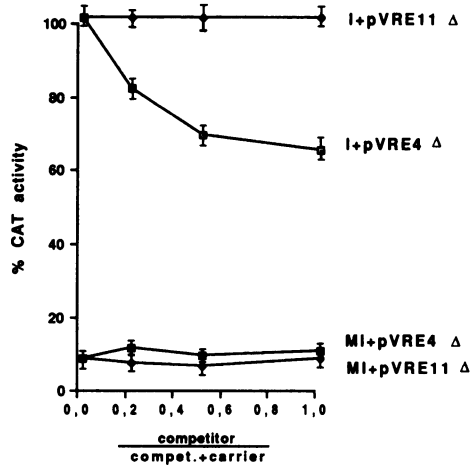

Figure 3. *In vivo* competition experiments. L929 cells were transfected with a mixture of indicator plasmid pIF4T, competitor plasmid pVRE4Δ or pVRE11Δ (0,10 μg, 25 μg and 50 μg, ie : 0, 20, 50 and 100 fold molar ratios), and carrier plasmid pUC18 up to 50 μg. Transcription from the Mu IFN-α4 promoter in the indicator plasmid was monitored by CAT enzyme measurements in extracts of NDV-induced (I) or mock-induced (MI) L929 cells. The horizontal axis shows the ratio of the amount of competitive plasmid versus the total amount of DNA used in each transfection. The variation between duplicate assays is indicated.

galactosidase activity. In L929 cells, when the detection of the β-galactosidase activity needed extended incubation time (up to 12 hours) the CAT values were only corrected with respect to the protein concentration. Inducibility values derive from the results obtained in three independent transfection experiments with different preparations of the same plasmid.

In vivo competition experiments

L929 cells were cotransfected with 50 μg of DNA containing the indicator plasmid pIF4T (0,5 μg), the CAT-gene-deleted competitor plasmid pVRE4Δ or pVRE11Δ (20, 50 and 100 fold molar excess) and the carrier plasmid pUC18. These experiments were performed according to Séguin et al. (21). CAT activity was monitored in extracts from NDV-induced or mock-induced L929 cells.

RESULTS

Effect of the nucleotide −78 substitution on the uninducibility of Mu IFN-α 11 gene

Virus-induced activation of transcription of interferon type I genes is mediated by the 5'-flanking sequences of the genes extending up to −120-bp (22−24). This region is also highly conserved among different murine IFN-α genes. A 46-bp fragment of the promoter region, located between positions −109 and −64 in Hu IFN-α1 gene, called 'VRE' was shown to confer maximal inducibility to an heterologous promoter (8). The VREα1-corresponding region of Mu IFN-α11 gene which is uninducible in fibroblastic cell lines upon viral infection shows striking homology with the highly inducible Mu IFN-α4 gene VRE region, they differ indeed only by a single nucleotide substitution at position −78 (Fig. 1A). In order to examine whether the uninducibility of Mu IFN-α11 gene is due to this mutation, synthetic oligonucleotides corresponding to VREα11 and VREα4 were inserted in pBLCAT2 containing the heterologous Herpes simplex virus thymidine kinase (*tk*) promoter fused to the bacterial chloramphenicol acetyl transferase (CAT) gene. The resulting constructions, namely pVRE11 and pVRE4 were transfected into both L929 and C243 cell lines (Fig. 2). The results indicate that VREα11 isolated from its genomic context is able to confer virus-inducibility to a heterologous promoter in L929 cells, but in a lesser extent in comparison to VREα4. The inducibility value of VREα4 obtained in our conditions in L929 cells, is comparable to those obtained when the inducible element of Mu IFN-α4 (−109 to −75) was placed upstream other heterologous promoters (10). In the C243 cell line, VREα4 is inducible in a comparable level as in L929 cells, whereas VREα11 failed to respond to virus induction. The differences in the inducibility values obtained with each cell line suggest that virus-induced activation of transcription is cell-specific. This variation between the inducibilities confered by VREα11 and VREα4 upstream of the *tk* promoter may be underestimated since the constitutive level of transcription from the intact *tk* promoter is high (unpublished data). A similar effect was also observed with the interferon β regulatory element (IREβ) placed upstream *tk* promoter (28). However, the low inducibility of VREα11 in L929 cells and its uninducibility in C243 cells can only account for the A to G substitution at position −78, but this mutation is not sufficient to explain the uninducibility of Mu IFN-α11 gene, at least in L929 cells.

In order to determine, for a given cell type, the effect of the −78 substitution on the affinity of potential VRE-binding factors, *in vivo* competition experiments were carried out using pIF4T as the reporter plasmid. pVRE11Δ and pVRE4Δ were respectively cotransfected in increasing amounts as competitor plasmids. Since VREα11 was efficient in L929 cells, they were used as the recipient cell line. Figure 3 shows that VREα4 competed rather strongly with pIF4T in a 20:1 molar excess whereas VREα11 did not compete with the same promoter fragment up to a 100:1 molar excess. These results indicate the importance of the A/G substitution on the interaction of the VRE with its potential *trans*-regulators.

The effect of A/G substitution was then tested in the context of each promoter extending up to −470 with plasmids pIF4T(11) and pIF11T(4) obtained by site-directed mutagenesis of pIF4T and pIF11T respectively (Fig. 4). The comparison of CAT values monitored after transfection with these recombinant plasmids suggests that the A to G substitution has no significant effect on the induced level of the Mu IFN-α4 promoter but causes an increase in its constitutive level, thus leading to a 50% decrease

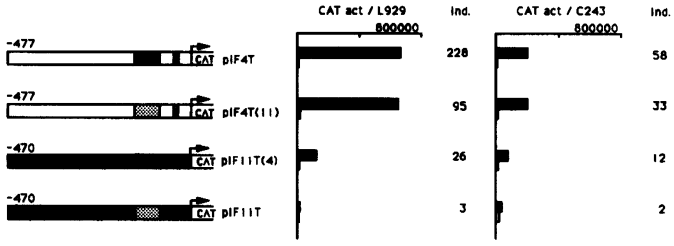

Figure 4. Effect of nucleotide −78 substitution on the expression of IFN-α11 and IFN-α4 promoters. L929 and C243 cells were transfected with the constructions symbolized in the diagram where the light doted box correspond to VREα11, the black box to VREα4. The hatched region corresponds to the TATA box. The white and darken rectangles correspond to the Mu IFN-α4 and Mu IFN-α11 promoters respectively. Induction with NDV was carried out 48 hr after transfection. Cells were harvested after 24 hr post induction. CAT activities were determined, and inducibility values are given as the ratio of induced/mock induced CAT activities.

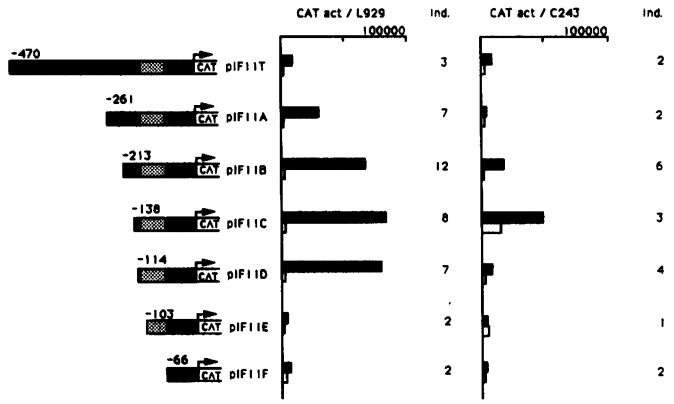

Figure 5. Evidence for negatively acting sequences in the Mu IFN-α11 promoter. Different 5' extentions (see Fig. 1B) of the Mu IFN-α11 promoter were inserted into the pBLCAT3 vector. The further procedure was as in Fig 4.

in inducibility. In contrast the G to A mutagenesis which placed the VREα4 in Mu IFN-α11 promoter exerted a considerable effect in both cell lines. Indeed, the Mu IFN-α11 promoter became virus-inducible. However, the inducibility ratios obtained with pIF11T(4) were approximately 5−8 times lower in comparison to pIF4T, and those obtained with pIF4T(11) were 15 to 30 times higher than pIF11T respectively in C243 and L929 cells. These results suggest the existence of VRE-surrounding *cis*-acting sequences which modulate the regulation of the Mu IFN-α11 gene expression.

Presence of negatively cis-acting sequences in the Mu IFN-α11 promoter

The modulatory effect of VRE-flanking sequences can be explained either by the presence of cooperating enhancers in the Mu IFN-α4 promoter, their effect being abolished by nucleotide changes in that of Mu IFN-α11 (Fig. 1B), or by the presence of repressor sequences in Mu IFN-α11 promoter. Since VREα11 responded to virus induction in L929 cells, and the Mu IFN-α11 promoter extending up to −470 was ineffective, we investigated on the presence of negatively acting sequences in

Figure 6. Effect of the 5'-flanking sequences of Mu IFN-α11 on the expression of Mu IFN-α4. The IFN-α11/α4 hybrid promoters were obtained by *in vivo* homologous recombination. The 500-bp HindIII-BamHI fragment of pIF11T was ligated to the HindIII-digested pIF4T construction. After transformation in *E. coli*, the resulting hybrid plasmids were sequenced. The portions corresponding to the IFN-α11 promoter are in dark and those of α4 are in white. Nucleotide positions of the crossover regions are detailed on Fig. 1B. VREα11 is light doted whereas VREα4 is black.

VREα11-surrounding promoter regions. Thus transfection experiments with pBLCAT3 containing 5'-deleted fragments of Mu IFN-α11 promoter were carried out and the results are shown in Figure 5. Inducibility was recovered in L929 cells when the −470 end point was deleted up to −261 (pIF11A). Deletion extending up to −213 increased inducibility and the further deletions maintained it. When deletion reached −103 (pIF11E) within the VREα11, inducibility was abolished. The comparable inducibility ratios obtained with pIF11C and pIF11D suggest that the poly(G) stretch (−140 to −129) has no significant effect on inducibility. The effect of deletions was less apparent in C243 cells; however, inducibility is observed only when deletions extend up to −213. A higher induced level was obtained with pIF11C which also showed an increase in constitutive expression. These results suggest that negative domains are located between positions −470 and −213. Since, in both L929 and C243 cell lines, a significant increase in inducibility was observed when the −261 to −213 region was removed and since pIF11C gave the highest induced values, the −261 to −149 fragment of Mu IFN-α11 promoter was inserted upstream VREα4 in pVRE4. This fragment displayed a 50% decrease on the constitutive level of *tk* promoter in uninduced conditions. A comparable decrease was also obtained on the inducibility confered by VREα4 to the same promoter thus confirming the negative role of this fragment (unpublished data).

To determine if this negative effect can also influence the IFN-α4 expression, hybrid promoters carrying different portions of IFN-α11 and IFN-α4 were constructed by homologous recombination in *E. coli* (Fig. 6). pIFH65 plasmid containing the −470 to −200 fragment of IFN-α11 showed a significant decrease of the inducibility in L929 cells and C243 cells. The induced level in C243 cells was drastically reduced when hybrid promoter contained the −470 to −145 IFN-α11 promoter fragment. These results suggest that some negative effects due to Mu IFN-α11 sequences are cell-specific, and that they can also exert their effect on a highly inducible truncated IFN-α4 promoter. The α11/α4 hybrid promoter containing the −470 to −44 sequence of Mu IFN-α11 showed no inducibility in C243 cells, while in L929 cells a faint inducibility was still detectable. Comparison of the inducibility values of pIFH66 and pIF11T suggests that the sequences located downstream the VRE (−44 to +19) may also have an effect on inducibility in L929 cells.

These data indicate that in Mu IFN-α11 promoter, a negative element is located between position −470 and −200, and that the −200 to −145 segment shows an additional negative effect in C243 cells.

DISCUSSION

In order to explain the absence of viral inducibility of the Mu IFN-α11 gene, we studied the different segments of its promoter. We showed that VREα11 when placed in front of a heterologous promoter was able to confer virus inducibility in L929 cells but with a lower efficiency than VREα4. This decrease can only be attributed to the A/G substitution at position −78. This substitution, at least in the VRE isolated from its genomic context, seems also to contribute to define the cell-specificity since VREα11 did not confer any inducibility in C243 cell line while VREα4 responded to induction. We have shown that the Mu IFN-α genes which are highly expressed in L929 cells have a lower expression in C243 cells (C. Coulombel, G. Vodjdani and J. Doly in press). The latter cell line known to produce high levels of type I interferons is transformed by moloney sarcoma virus (29, 30). The inability of VREα11 to respond to virus induction may be related to this transformed state. By *in vivo* competition experiments VREα4 competed efficiently with the Mu IFN-α4 promoter while VREα11 even in large excess did not. These data which indicate that A/G substitution alters the affinity of VRE-dependent *trans*-regulators in L929 cells were also confirmed *in vitro* , by gel shift analysis (unpublished results). These results suggest that the differences observed in the inducibility of VREα11 and VREα4 do not arise from their commonly shared (−109 to −88) region, but from their 3′-portion. Therefore, even if *trans*-regulators such as IRF1, IRF2 and PRDI-BF (31−33) which have a potential binding site in the common region should have a role in Mu IFN-α gene regulation, they might not account for these differences. They rather seem to be explained by the presence of a TG-binding site in the 3′ portion of VREα11 and α4. Indeed, it has been reported that the TG sequence GAAATGGAAA, located in the Hu VREα1 between −84 and −75, is involved in virus inducibility and binds a new protein. The A/C substitution at position −76 or at −82 greatly reduces the inducibility of Hu VREα1; furthermore, the binding of the TG protein is strongly impaired by methylation of the GG doublet (11). In mouse, this sequence exists with a single mutation in Mu VREα4, two mutations in Mu VREα1, α6P, α5, α7, α8 and α11 and three mutations in α2 and α6T (Fig. 1A). If a TG-like protein is also involved in the regulation of Mu IFN-α genes, the −78 A/G substitution of Mu VREα11 could affect the binding of this putative *trans*-activator. The relative inefficiency of Mu VREα11 may not be due to the −78 A/G mutation itself, but to the GGG triplet it creates. It can be pointed out that Mu IFN-α2 has the same mutation in its VRE, still no GGG triplet is formed since a second G/A mutation occurs at position −80, and this gene was shown to be relatively inducible (2, 25).

However, the discrepancy between the inducibility confered by VREα11 and the uninducibility of the natural Mu IFN-α11 promoter indicate that the substitution in the VRE region is not sufficient to explain the absence of expression of the Mu IFN-α11 gene.

By site-directed mutagenesis at position −78 we reciprocally converted VREα4 to VREα11 in the Mu IFN-α4 and VREα11 to VREα4 in the Mu IFN-α11 promoters, in order to verify this assumption. Our results indicate that A/G mutation caused a 50% decrease in the inducibility of the Mu IFN-α4 promoter as a consequence of an increase in the constitutive level of expression in both cell lines. In contrast, the G/A mutation led to significant inducibility of Mu IFN-α11 promoter. The comparison of inducibility values obtained with pIF4T and pIF11T(4) on one hand, pIF11T and pIF4T(11) on the other hand clearly demonstrate that VRE-surrounding *cis*-acting sequences modulate the regulation of the two promoters (Fig. 4). We show, by progressive 5′ deletions of the Mu IFN-α11 promoter that in L929 cells inducibility is recovered once the 5′ border reaches position −261 and increases when deletion extends to −213. Despite the very low levels of expression in C243 cells, inducibility was also recovered only when the deletions extend up to −213. These results suggest that negatively acting sequences between −470 and −213 modulate the Mu IFN-α11 gene expression in both cell lines. The sequence between −213 and −138 seems also to have an additional repressive effect. The −138 deleted promoter caused an increase in the induced but also in the constitutive expression levels which are more pronounced in C243 cells. The removal of the upstream negatively acting sequences may release the cooperative action of inducible and constitutive enhancer in the Mu VREα11. Hybrid α11/α4 promoters obtained by *in vivo* recombination showed that the repressor sequences of Mu IFN-α11 are also able to exert their effect on highly inducible Mu IFN-α4 promoter. The −470 to −200 region of Mu IFN-α11 showed a 50% decrease of inducibility in both cell lines. Addition of the −201 to −149 region abolished the inducibility in C243 cells, whereas it had no effect in L929 cells. To examine the negative effect of the −261 to −149 Mu IFN-α11 promoter segment, we inserted this fragment upstream a VREα4 element and the *tk* promoter. This region reduced the inducibility confered by the VRE to the heterologous promoter and it also exerted its effect on the constitutive level of the *tk* promoter in uninduced or mock induced cells.

These results, taken together, indicate that the negatively acting sequences of the Mu IFN-α11 promoter are located between positions −470 and −145, the −200 to −145 segment showing a specificity for C243 cell line. Furthermore, the inducibility values obtained with the natural Mu IFN-α11 promoter and the α11/α4 hybrid promoter pIFH66 suggested that the −44 to +19 segment of the Mu IFN-α11 has also a negative effect in comparison to Mu IFN-α4 promoter. We propose that the VRE-surrounding negatively acting sequences and the mutation in the VRE region when individually tested affect the inducibility, but that their additional effect completely silences the Mu IFN-α11 gene expression.

Such repressor sequences were neither described in murine nor in human interferon α gene promoters. However, it has been mentioned that the natural Hu IFNα1 promoter extending from −675 to −64 displays less activity than the −109 to −64 segment joined to a minimal β globin promoter. This suggests that the Hu IFN-α1 promoter also contains inhibitory sequences (8, 11). In the case of Mu IFN-α4 gene, the −464 to +19 natural promoter shows 20% higher activity than the −109 to +19 fragment, suggesting the absence of negative elements in this promoter (9). Surprisingly, the regulation of Mu IFN-α11 shows more similarities with that of the Hu IFN-α1 than the Mu IFN-α4.

Recently it has become obvious that control of eukaryotic gene transcription besides positive regulation also involves negative regulation (for a review see 34). The insulin gene promoter was shown to contain a negative regulatory element (NRE) which

exerts its effect only in insulin producing cells (35). This sequence, as in our case also caused a 50% decrease in *tk* promoter activity in the sense orientation. Another similarity with the insulin NRE is the fact that the −261 to −149 Mu IFN-α11 promoter exerts its effect without requirement of a functional enhancer (i.e. VRE). Further investigations are in progress for the determination of interaction sites with potential repressors of Mu IFN-α11 gene. We suggest that nucleotide substitutions occuring in the VRE region at critical positions affect the affinity of VRE dependent *trans*-regulators and thus modulate the cell-type specific expression of Mu IFN-α genes. Moreover the negative regulation shows also cell specificity, therefore the NDV-uninducible Mu IFN-α genes can be expressed in cells where repressors are absent or inefficient. Alternatively the mutations occuring in these sequences may affect the affinity of repressors which can lead to optimal induction of highly expressed Mu IFN-α genes.

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