Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1369–1373, February 1991
Biochemistry

Multimerization of AAGTGA and GAAAGT generates sequences that mediate virus inducibility by mimicking an interferon promoter element

(α interferon promoter/inducible enhancer/interferon regulatory factor 1/virus)

DIETER NÄF, STEVEN E. HARDIN*, AND CHARLES WEISSMANN

Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland

Contributed by Charles Weissmann, October 10, 1990

ABSTRACT **Multimeric AAGTGA and GAAAGT, when inserted before a minimal promoter, mediate virus-inducible transcription. We have determined that the active sequence within these multimers is TGAAAGTGAAAGT, which is structurally similar to GAGAAGTGAAAGT, a positive response element delineated in the β-interferon gene promoter. Both sequences behave like protoenhancers and are similar as regards induction by virus or interferon regulatory factor 1 when supported by a simian virus 40 enhancer.**

Viral inducibility of promoters of human genes encoding α and β interferons (IFN-α and IFN-β) is mediated by DNA segments of about 100 base pairs (bp) upstream of the cap site (Fig. 1; refs. 1, 2, 5, 7, 16–18). Inspection of the human IFN-β gene promoter led to the suggestion that repeats of AARKGA, where R is G or A and K is T or G, were unusually frequent and might represent the basic element of a virus-inducible enhancer (1). Indeed, tetramers of AAGGGA, AAGTGA, and AAATGA fused to a minimal promoter (16) efficiently mediated virus inducibility. In the case of the IFN-α gene promoter, we considered that the basic inducible element was more likely GAAANN and showed that the tetramer of GAAAGT [a permutation of AAGTGA (16)] and tetramers of several other hexanucleotides of this type confer virus inducibility when linked to a construct comprising a TATA box and a cap site followed by the coding region of the β-globin gene (7, 15).

Maniatis and his colleagues (3, 19, 20) delineated within the IFN-β promoter two positively regulated sequence elements, PRDI and PRDII (for positive regulatory domains I and II) and proposed a negatively regulated element, NRDI (for negative regulatory domain I). PRDII binds NF-κB, a factor that is activated and translocated to the nucleus after virus infection (4, 10–13) as well as other proteins such as PRDII binding factor 1 (PRDII-BF1) (21). PRDI binds several factors (8, 9, 22–25), one of which, IFN regulatory factor 1 (IRF-1), when overexpressed, activates IFN genes in COS cells or embryonal carcinoma cells (6, 26) and elicits $(GAAAGT)_4$-mediated expression. Another PRDI-binding factor, IFN regulatory factor 2 (IRF-2), is believed to inhibit IRF-1-mediated expression in the absence of viral induction (6, 8). Additional PRDI-like sequences, upstream of PRDI, are required for maximal inducibility (1, 4).

Why does tetramerization of certain hexanucleotides generate virus-inducible sequences? Juxtaposition of hexanucleotides may give rise to sequences that are more or less fortuitously similar to longer natural promoter elements (27). Alternatively, a hexanucleotide may represent an "enhanson"—i.e., a basic promoter element that gains activity upon multimerization (28, 29). To approach this question, we determined the shortest sequence within $(GAAAGT)_4$ and $(AAGTGA)_4$ still able efficiently to mediate induced transcript levels. We synthesized oligonucleotides representing various sequences occurring in these two tetramers and tested them for their capacity to mediate virus- and IRF-1-induced transcription. The shortest, still-efficient sequence was TGAAAGTGAAAGT, which closely resembles PRDI. If this sequence consists of enhansons, then the enhanson is more similar to GAAAGT than to AAGTGA.

MATERIALS AND METHODS

Constructions. Constructions were made by standard procedures (30) and verified by sequencing. Double-stranded oligonucleotides were synthesized chemically with 5′ CG and 3′ TCGA overhangs and inserted between the *Cla* I and *Hin*dIII sites of plasmid 42P (no enhancer; ref. 7). Constructions with the simian virus 40 (SV40) enhancer 1291 base pairs (bp) upstream of the insert were prepared as described (15). The reference plasmid was pSTC407-556 (31). The IRF-1 expression plasmid pIRF-L (9) was from T. Taniguchi.

Transient Transformation. L929 cells were transfected with test and reference plasmid and either pIRF-L or the corresponding "empty" vector CDM8 and were induced with Newcastle disease virus (NDV) or were mock-induced 48 hr later (15). RNA was prepared by a slight modification of the procedure of Auffrey and Rougeon (32) 8 hr after induction.

Nuclease S1 Mapping. Nuclease S1 mapping has been described (15). Rabbit globin standard RNA (BRL) was assumed to contain 50% β-globin RNA (33). The internal reference sequence was under the control of the virus-inducible cytomegalovirus promoter (31), which allowed correction for RNA recovery and efficiency of induction. Nuclease S1 mapping yields test and reference signals of 353 nucleotides and 148 nucleotides, respectively. After autoradiography (0.5–5 days), radioactivities were determined. Test signals were expressed relative to normalized reference signals, and strands per cell were calculated (7, 15). Inducibility is the ratio of induced to uninduced transcript levels.

RESULTS

The Assay System. To measure the transcriptional effects of an oligonucleotide, it was inserted 31 bp upstream of the β-globin gene TATA box, and an SV40 enhancer was placed 1291 bp upstream of it to increase the transcriptional response (figure 1*B* in ref. 15). In this setting the enhancer by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN, interferon; SV40, simian virus 40; IRF, IFN regulatory factor; PRDI and -II, positive regulatory domains I and II; NDV, Newcastle disease virus.

*Present address: Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.

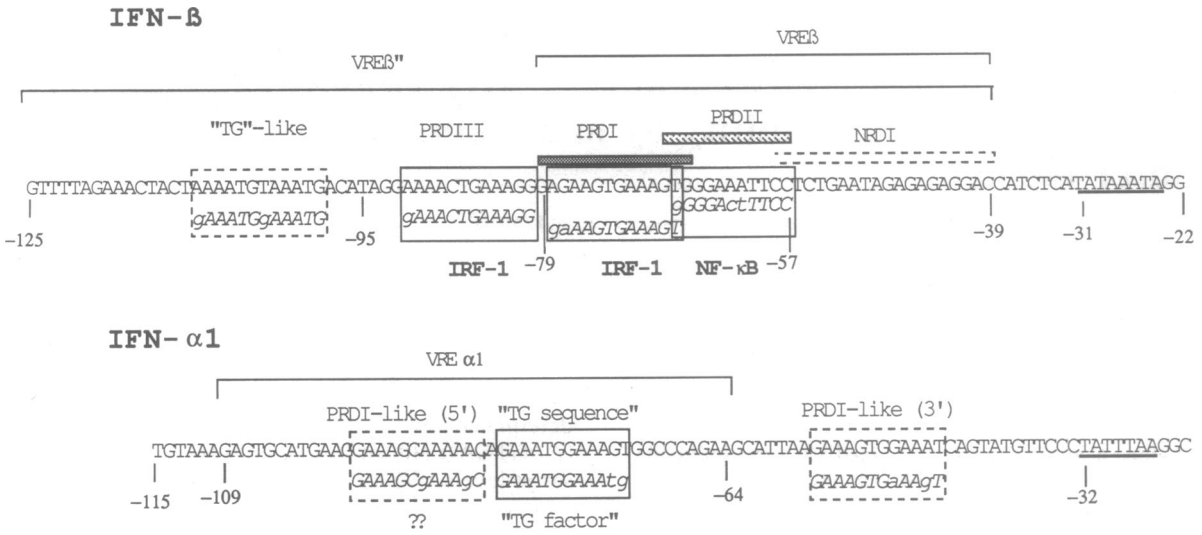

FIG. 1. Sequence of the IFN-α1 (*Lower*) and IFN-β (*Upper*) gene promoters. Established and putative factor binding sites within the IFN-α1 and IFN-β gene promoters. Numbering is from the cap site of the human gene encoding IFN-β (1) and IFN-α1 (2). PRDI, PRDII, and NRDI are delineated as described by Fan and Maniatis (3), and PRDIII is shown as described by Leblanc *et al.* (4). Viral response elements, VREβ and VREβ'' are the minimal inducible sequences determined by Goodbourn *et al.* (5) and Harada *et al.* (6), respectively. VREα1 was described by Kuhl *et al.* (7). Solid frames enclose regions believed to be functionally important for induction; dashed frames enclose sequence similarities to binding sites. (*Upper*) IFN-β gene promoter. Binding to the sites designated "IRF-1" is supported by experiments with recombinant IRF-1 (4, 8, 9). The NF-κB binding site has been characterized (10–13). The short sequences within the "IRF" boxes indicate the match to a $(GAAANN)_4$ oligonucleotide known to respond to IRF-1. The italicized sequence in the "NF-κB" box represents a typical NF-κB element (14); mismatches to the IFN sequences are indicated in small letters. (*Lower*) IFN-α1 gene promoter. The binding of the "TG" factor to the "TG" sequence is based on ref. 15. There is no evidence that IRF-1 binds to either of the "PRDI-like" sequences.

itself does not promote transcription from a minimal promoter but can potentiate activation by upstream elements close to the TATA box (7, 15).

Murine L929 cells were transiently transformed with the test plasmid, a reference plasmid, and, where indicated, with the constitutive IRF-1 expression plasmid pIRF-L (9). Transcript levels were determined 8 hr after induction with NDV or mock induction. A representative autoradiogram is shown in Fig. 2 *Upper*.

Determination of the Sequence in $(GAAAGT)_4$ and $(AAGTGA)_4$ Capable of Mediating Maximal Virus-Induced Transcript Levels. We first determined that $(GAAAGT)_2$ (construct C; see Fig. 2 *Lower* and Table 1 for constructs) was 36% and $T(GAAAGT)_2$ (construct B) was 70% as active in promoting virus-induced transcript levels as the reference sequence $(GAAAGT)_4$ (construct A), while the value for $(AAGTGA)_2$ (construct I) was only 6% (Fig. 2 and Table 1). Therefore, further variations were carried out with $T(GAAAGT)_2$: Replacement of the 5'-proximal G by C (construct F) or removal of the 3' terminal GT (construct G) diminished induced transcript levels to 12% and 6%, respectively, so that the active sequence is described as TG*AAAGTGAAAGT*. The resemblance to the PRDI sequence, GAG*AAGTGAAAGT* (identities italic) is obvious. Virus-induced transcript levels mediated by the (slightly extended) authentic PRDI sequence GGAGAAGTGAAAGTGG (construct K) were marginally less than those by $(GAAAGT)_4$ (construct A) but about 20% greater than by $T(GAAAGT)_2$ (construct B).

The constitutive transcript level given by $(GAAAGT)_4$ was lower than that of most other constructions, so that its inducibility appeared substantially higher than that of PRDI or $T(GAAAGT)_2$. Also, as regards inducibility, GAAAGTGAAAG (construct E) was as effective as the oligonucleotides B, C, and D, which have T residues at either or both termini; however, removal of a G residue from the 3' end of $T(GAAAGT)_2$ or from both ends to give constructs G and H, respectively, caused decreased inducibility. It is not clear why most oligonucleotides give a higher constitutive transcript level than $(GAAAGT)_4$; either $(GAAAGT)_4$ silences some residual effect of the enhancer at a distance (7) or some combination of nucleotides around the shorter oligonucleotides mediates a low level of constitutive activity.

IRF-1 Overexpression Activates Transcription Mediated by Various Oligonucleotides. Tetrameric hexanucleotides of the type $(GAAANN)_4$, where NN is GT, GC, CT, or CC (so-called type I oligonucleotides; ref. 15) mediate efficient transcriptional activation by IRF-1 (generated intracellularly by the expression plasmid pIRF-L), ranging from 20% to 30% of the virus-induced values. In contrast, $(GAAATG)_4$ (construct O, a type II oligonucleotide), while responding about half as well as $(GAAAGT)_4$ to virus induction, does not mediate activation by IRF-1 (ref. 15; Table 1). Also $T(GAAAGT)_2$ (construct B) and the derivatives lacking one or the other terminal T residues (constructs C and D) responded about 20–30% as well to IRF-1 as to virus induction; the corresponding values for $(GAAAGT)_4$ and the PRDI sequence (construct K) were 34% and 14%, respectively (Table 1).

The so-called TG sequence, AGAAATGGAAAGTG (construct N), delineated within the IFN-α gene promoter (15) is similar both to the sequence GAAATGGAAA. . . ., within the type II oligonucleotide $(GAAATG)_4$, and to . . .GAAAGTG, within the type I oligonucleotide $(GAAAGT)_4$ or within PRDI. Table 1 shows that its response to IRF-1 is 7% of that to virus; inasmuch as this weak activity is significant, it may be due to its partial similarity to PRDI.

Two sequences within the IFN-α gene promoter somewhat resemble a type I sequence: GAAAGCAAAAAC (construct L; position −97 to −86) and GAAAGTGGAAAT (construct M; position −56 to −45) (15); however, neither of them mediated virus inducibility (Table 1).

Insertion of Nucleotides Between Hexanucleotide Elements. The introduction of one or more nucleotides between enhansons abolishes transcriptional activation (28). We inserted a varying number of residues between the two GAAAGT elements of TGAAAGTGAAAGT. Table 2 shows that introducing a single residue reduced NDV-induced transcript

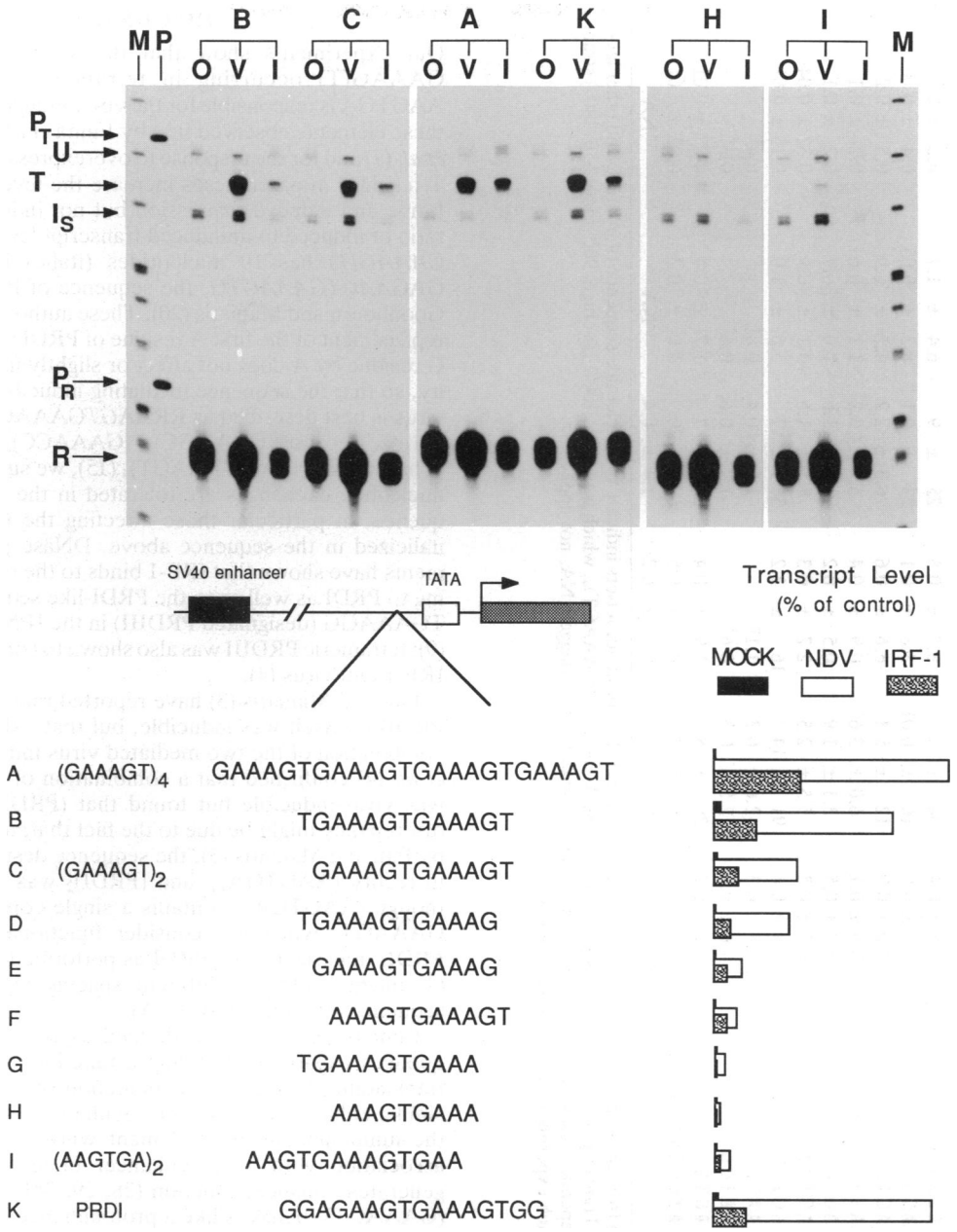

FIG. 2. Determination of transcript levels of various promoter constructions by nuclease S1 analysis. All constructions contained the SV40 enhancer 1291 bp upstream of the oligonucleotide to be tested (see figure 1*B* in ref. 15). Transient transfection of mouse L929 cells and induction by NDV or mock induction were as described. Total RNA was recovered 8 hr after initiation of viral or mock induction, and 40 μg were subjected to nuclease S1 mapping (15). (*Upper*) Autoradiogram after 12 hr at −70°C with an intensifying screen. Lanes: 0, mock induction; V, NDV induction; I, IRF-1 induction; P, probes; M, pBR327 digested with *Msp* I and 5′-^{32}P-labeled. I_S, aberrantly spliced read-through transcripts; P_R, undigested reference probe; P_T, undigested test probe; R, reference signal; T, test signal; U, read-through β-globin transcripts (see figure 1*D* in ref. 7). The capital letters above the lanes correspond to the sequences given in *Lower*. (*Lower*) Summary of results with oligonucleotides derived from $(GAAAGT)_4$. The numerical data are reported in Table 1.

levels to 13% of the unmodified sequence; and insertion of two or more residues, to 3.6% or less.

These findings are compatible with the idea that GAAAGT represents an enhanson, because separation of two enhansons by only one nucleotide might still allow neighboring monomeric proteins to interact. On the other hand, if GAAAGTGAAAGT were the enhanson, then insertions would be in the midst of the recognition sequence, yielding the formal equivalent of four point mutations, as can be seen by comparing GAAAGT*GAAAGT* and GAAAGT*CGAAAG*. However, even these several base changes might not completely abolish activity. Therefore, on the basis of this experiment, it is not possible to decide whether GAAAGT or the dimer represents the enhanson.

Effect of the SV40 Enhancer on the Transcript Levels Mediated by Dimeric GAAAGT and Tetrameric GAAAGT. All experiments described above were performed with an SV40 enhancer upstream to obtain high transcript levels. In Table 3 we compare the activities of $(GAAAGT)_4$ and $(GAAAGT)_2$ with and without enhancer. The enhancer increased virus-induced transcript levels 10-fold in the case of $(GAAAGT)_4$ and 20-fold in the case of $(GAAAGT)_2$ but, remarkably, only 2- to 4-fold in the case of IRF-1-induced stimulation. Perhaps factors mediating the SV40 enhancer effect are activated or become more plentiful after virus infection; a possible candidate would be NF-κB, which could bind to the TC-II/κB motif in the 72-bp repeat (34, 35).

Table 1. Inducibility by NDV and IRF-1 mediated by $(GAAAGT)_4$ and various oligonucleotides derived from it, as well as some naturally occurring IFN promoter sequences

	Oligonucleotide insert	Transcript levels, % of standard				Inducibility*		Ratio of inducibilities* (IRF/NDV)
		Mock	NDV	IRF		NDV	IRF	
A	$(GAAAGT)_4$ atcgaGAAAGTGAAAGTGAAAGTaagctt	0.7 ± 0.3	100 ± 17	36 ± 10		180 ± 87	55 ± 38	0.34 ± 0.09
B	atcgaTGAAAGTGAAAGTaagctt	3.0 ± 0.4	70 ± 20	20 ± 1		23 ± 5	6.6 ± 0.2	0.25 ± 0.02
C	$(GAAAGT)_2$ atcgaaGAAAGTGAAAGTaagctt	1.8 ± 0.6	36 ± 19	10 ± 3.8		21 ± 7	6.1 ± 2.4	0.25 ± 0.1
D	atcgaTGAAAGTGAAAGaagctt	1.5 ± 0.4	38 ± 19	8.7 ± 0.5		25 ± 9	6.4 ± 1.7	0.2 ± 0.1
E	atcgaaGAAAGTGAAAGaagctt	0.7 ± 0.01	16 ± 0.01	5.8 ± 0.1		23 ± 0.1	8.3 ± 0.05	0.4 ± 0.001
F	atcgaTCAAAGTGAAAGTaagctt	2.0 ± 0.9	12 ± 5.4	6.6 ± 0.6		6.3 ± 2.2	4.2 ± 1.9	0.5 ± 0.2
G	atcgaTGAAAGTGAAAaagctt	0.9 ± 0.1	6.0 ± 3.6	0.9 ± 0.4		6.7 ± 3.2	1.1 ± 0.6	0.2 ± 0.1
H	atcgaTCAAAGTGAAAaagctt	1.2 ± 0.5	3.1 ± 1.9	0.5 ± 0.2		3.3 ± 1.6	1.0 ± 0.7	0.2 ± 0.1
I	$(AAGTGA)_2$ atcgatAAGTGAAAGTGAagctt	0.7 ± 0.3	5.5 ± 2.5	2.2 ± 0.3		8.8 ± 1.6	3.1 ± 0.9	0.32 ± 0.04
K	PRDI (IFN-β) atcgatGGAGAAGTGAAAGTGGaagctt	2.9 ± 0.6	91 ± 14	16 ± 3.2		36 ± 7	5.6 ± 1.0	0.15 ± 0.01
L	5′ PRDI-like (IFN-α1) atcgatGGAAAGCAAAAACAagctt	1.8 ± 0.3	2.6 ± 0.3	1.1†		1.5 ± 0.4	0.8†	0.4†
M	3′ PRDI-like (IFN-α1) atcgatAGAAAGTGGAAATCAagctt	1.6 ± 0.6	2.5 ± 0.3	0.6†		1.9 ± 0.6	0.6†	0.3†
N	TG sequence (IFN-α1) atcgatAGAAATGGAAAGTGaagctt	1.2 ± 0.3	29 ± 8	1.9 ± 0.4		25 ± 7	1.7 ± 0.4	0.07 ± 0.01
O	$(GAAATG)_4$ atcgatGAAATGGAAATGGAAATGaagctt	0.8 ± 0.2	54 ± 14	0.9 ± 0.5		67 ± 20	1.0 ± 0.3	0.02 ± 0.01
P	Minimal promoter No insert	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2		1.4 ± 0.4	NA	NA

In all cases, the SV40 enhancer was present 1291 bp upstream from the oligonucleotide insert (see figure 1*B* in ref. 15). Boldface letters indicate the nucleotide sequence of interest, and other letters represent flanking nucleotides up to the *Cla* I and *Hin*dIII sites. Transcript levels were expressed relative to NDV-induced $(GAAAGT)_4$, which was set to 100% [average, 960 (range 550–1400) strands per cell in different experiments]. Averages are from 2–12 independent experiments, except for the values marked with a dagger. NA, not applicable.

*Inducibilities and ratio of inducibilities were calculated for each experiment and averaged thereafter

†Value is from a single experiment.

DISCUSSION

Our experiments show that the sequence (T)GAAAGT-GAAAG(T) occurring in tetramerized GAAAGT and AAGTGA is responsible for the virus-induced transcription by these elements observed first by Fujita *et al.* (16) and by Kuhl *et al.* (7) and for the response to overexpressed IRF-1 (15). The T residues in parentheses increase the levels of both constitutive and induced expression but not inducibility—i.e., the ratio of induced to uninduced transcript levels. (T)G*AAAGT-GAAAG(T)* has 10 nucleotides (italic) in common with GAG*AAGTGAAAGT*G, the sequence of PRDI proposed by Goodbourn and Maniatis (20). These authors have shown that replacement of the first A residue of PRDI by G or the second G residue by A does not affect or slightly increases inducibility, so that the sequence mediating inducibility by IRF-1 and virus is best described as RRAA*GT*GAAAG(T), where R is a purine. Because $(GAAAGC)_4$, $(GAAACC)_4$ and $(GAAACT)_4$ behave similarly to $(GAAAGT)_4$ (15), we surmise that various nucleotide exchanges are tolerated in the IRF-1-binding sequence, in particular those affecting the G and T residues italicized in the sequence above. DNase protection experiments have shown that IRF-1 binds to the region corresponding to PRDI as well as to the PRDI-like sequence GAAAAC-TGAAAGG (designated PRDIII) in the IFN-β gene promoter (9); tetrameric PRDIII was also shown to confer inducibility by IRF-1 and virus (4).

Fan and Maniatis (3) have reported that neither PRDI nor PRDII by itself was inducible, but that a dimer of each or a combination of the two mediated virus inducibility. Leblanc *et al.* (4) confirmed that a combination of PRDI and PRDII was virus-inducible but found that $(PRDI)_2$ was not. This discrepancy might be due to the fact that, in the experiments by Fan and Maniatis (3), the sequence designated PRDI was in reality $(AAGTGA)_4$, and $(PRDI)_2$ was $(AAGTGA)_8$. Although $(AAGTGA)_4$ contains a single copy of TGAAAGT-GAAAGT, which we consider functionally equivalent to PRDI, dimerization of PRDI as performed by Leblanc *et al.* (4) might lead to a different spacing between the active sequences than in $(AAGTGA)_8$.

Enhansons have been defined as the shortest DNA sequences capable of fulfilling a function, such as binding a trans-acting factor. The conjunction of two similar or dissimilar enhansons forms a protoenhancer, which is defined as the minimum enhancer element whose oligomerization or association with other enhancer elements synergistically generates enhancer function (28, 29, 34). By this definition $(GAAAGT)_2$ behaves like a protoenhancer because it shows low IRF-1 or virus-induced activity as a single unit in conjunction with a minimal promoter, and the activity is synergistically increased by duplication or by insertion of the SV40 72-bp repeat at a distance where the enhancer is inactive by itself. However, it has not yet been shown that GAAAGT by itself has any functional properties, as would be required of an enhanson.

We thank Dr. Markus Aebi for advice and T. Taniguchi for critical reading of the manuscript, Mr. Marcel Wehrli for some constructions, Dr. Deborah Maguire for NDV, and Mr. Josef Ecsoedi for synthetic oligonucleotides. The work was supported by the Schweizerische Nationalfonds and the Kanton of Zürich.

1. Fujita, T., Ohno, S., Yasumitsu, H. & Taniguchi, T. (1985) *Cell* **41,** 489–496.
2. Ryals, J., Dierks, P., Ragg, H. & Weissmann, C. (1985) *Cell* **41,** 497–507.
3. Fan, C.-M. & Maniatis, T. (1989) *EMBO J.* **8,** 101–110.
4. Leblanc, J.-F., Cohen, L., Rodrigues, M. & Hiscott, J. (1990) *Mol. Cell. Biol.* **10,** 3987–3993.
5. Goodbourn, S., Zinn, K. & Maniatis, T. (1985) *Cell* **41,** 509–520.
6. Harada, H., Willison, K., Sakakibara, J., Miyamoto, M., Fujita, T. & Taniguchi, T. (1990) *Cell* **63,** 303–312.

Table 2. Effect on transcript levels of inserting one or more nucleotides between the GAAAGT repeats in TGAAAGTGAAAGT

Spacing	Oligonucleotide insert	Transcript levels, % of control: Mock	Transcript levels, % of control: NDV	Inducibility*
None	atcga**TGAAAGTGAAAGT**aagctt	5.0 ± 0.6	100	20 ± 2
+1 bp	atcga**TGAAAGTcGAAAGT**aagctt	1.9 ± 0.8	13 ± 3	7.3 ± 1.3
+2 bp	atcga**TGAAAGTcaGAAAGT**aagctt	1.6 ± 0.2	3.6 ± 0.5	2.3 ± 0.05
+4 bp	atcga**TGAAAGTcagtGAAAGT**aagctt	1.9 ± 1.1	3.1 ± 1.1	2.0 ± 0.6
+8 bp	atcga**TGAAAGTcagtcgatGAAAGT**aagctt	1.7 ± 0.4	2.8 ± 0.1	1.8 ± 0.5
Empty vector	No insert	<0.6†	<0.6‡	NA

All constructs contained the SV40 enhancer 1291 bp upstream of the oligonucleotide to be tested (see figure 1*B* in ref. 15). Values are averages from two independent experiments with expression levels normalized relative to NDV-induced TGAAAGTGAAAGT, which was set to 100% [corresponding to an average of 525 (range, 330–720) strands per cell]. NA, not applicable.

*Inducibilities were calculated for each individual experiment and averaged thereafter.

†No bands were visible on autoradiographs of both experiments.

‡A weak band was visible in one experiment.

Table 3. Response of $(GAAAGT)_4$ and $(GAAAGT)_2$ to the SV40 enhancer

Construction	Enhancer	Transcript levels, % of control: Mock	Transcript levels, % of control: NDV	Transcript levels, % of control: IRF	Inducibility*: NDV	Inducibility*: IRF
atcga(**GAAAGT**)$_4$aagctt	Without	0.6 ± 0.08	10 ± 1.8	9.6 ± 0.7	17 ± 1.8	16 ± 0.7
	With	0.6 ± 0.08	100 ± 3	21 ± 0.2	167 ± 3	35 ± 0.2
atcgaa(**GAAAGT**)$_2$aagctt	Without	0.5 ± 0.04	1.3 ± 0.01	1.3 ± 0.04	2.6 ± 0.04	2.6 ± 0.04
	With	1.5 ± 0.2	27 ± 2.2	6.0 ± 0.08	18 ± 2.2	4 ± 0.2

When present, the SV40 enhancer was 1291 bp upstream of the oligonucleotide to be tested (see figure 1*B* in ref. 15). Boldface letters indicate nucleotides pertaining to the sequence of interest, and other letters represent flanking nucleotides up to the *Cla* I and *Hin*dIII sites. Values are averages from duplicate transfections with expression levels normalized relative to NDV-induced $(GAAAGT)_4$, which was set to 100% (corresponding to 1300 strands per cell).

*Inducibilities are ratios of average transcript levels.

7. Kuhl, D., de la Fuente, J., Chaturvedi, M., Parimoo, S., Ryals, J., Meyer, F. & Weissmann, C. (1987) *Cell* **50**, 1057–1069.
8. Harada, H., Fujita, T., Miyamoto, M., Kimura, Y., Maruyama, M., Furia, A., Miyata, T. & Taniguchi, T. (1989) *Cell* **58**, 729–739.
9. Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T. & Taniguchi, T. (1988) *Cell* **54**, 903–913.
10. Fujita, T., Miyamoto, M., Kimura, Y., Hammer, J. & Taniguchi, T. (1989) *Nucleic Acids Res.* **17**, 3335–3346.
11. Hiscott, J., Alper, D., Cohen, L., Leblanc, J.-F., Sportza, L., Wong, A. & Xanthoudakis, S. (1989) *J. Virol.* **63**, 2557–2566.
12. Lenardo, M. J., Fan, C.-M., Maniatis, T. & Baltimore, D. (1989) *Cell* **57**, 287–294.
13. Visvanathan, K. V. & Goodbourn, S. (1989) *EMBO J.* **8**, 1129–1138.
14. Sen, R. & Baltimore, D. (1986) *Cell* **46**, 705–716.
15. MacDonald, N. J., Kuhl, D., Maguire, D., Näf, D., Gallant, P., Goswamy, A., Hug, H., Büeler, H., Chaturvedi, M., de la Fuente, J., Ruffner, H., Meyer, F. & Weissmann, C. (1990) *Cell* **60**, 767–779.
16. Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K. & Taniguchi, T. (1987) *Cell* **49**, 357–367.
17. Dinter, H. & Hauser, H. (1987) *EMBO J.* **8**, 101–110.
18. Ragg, H. & Weissmann, C. (1983) *Nature (London)* **303**, 439–442.
19. Goodbourn, S., Burstein, H. & Maniatis, T. (1986) *Cell* **45**, 601–610.
20. Goodbourn, S. & Maniatis, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1447–1451.
21. Fan, C.-M. & Maniatis, T. (1990) *Genes Dev.* **4**, 29–42.
22. Fujita, T., Sakakibara, J., Sudo, Y., Miyamoto, M., Kimura, Y. & Taniguchi, T. (1988) *EMBO J.* **7**, 3397–3405.
23. Xanthoudakis, S., Cohen, L. & Hiscott, J. (1989) *J. Biol. Chem.* **264**, 1139–1145.
24. Keller, A. D. & Maniatis, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3309–3313.
25. Driggers, P. H., Ennist, D. L., Gleason, S. L., Mak, W.-H., Marks, M. S., Levi, B.-Z., Flanagan, J. R., Appella, E. & Ozato, K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3743–3747.
26. Fujita, T., Kimura, Y., Miyamoto, M., Barsoumian, E. L. & Taniguchi, T. (1989) *Nature (London)* **337**, 270–272.
27. Raj, N. B. K., Engelhardt, J., Au, W.-C., Levy, D. E. & Pitha, P. M. (1989) *J. Biol. Chem.* **264**, 16658–16666.
28. Ondek, B., Gloss, L. & Herr, W. (1988) *Nature (London)* **333**, 40–45.
29. Fromental, C., Kanno, M., Nomiyama, H. & Chambon, P. (1988) *Cell* **54**, 943–953.
30. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
31. Severne, Y., Wieland, S., Schaffner, W. & Rusconi, S. (1988) *EMBO J.* **7**, 2503–2508.
32. Auffray, C. & Rougeon, F. (1980) *Eur. J. Biochem.* **107**, 121–128.
33. Morrison, M. R., Brinkley, S. A., Gorski, J. & Lingrel, J. (1974) *J. Biol. Chem.* **249**, 5290–5295.
34. Clark, L. & Hay, R. T. (1989) *Nucleic Acids Res.* **17**, 499–516.
35. Kanno, M., Fromental, C., Staub, A., Ruffenach, F., Davidson, I. & Chambon, P. (1989) *EMBO J.* **8**, 4205–4214.