DNA-protein interactions at the interferon-responsive promoter: evidences for an involvement of phosphorylation

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ABSTRACT

Several αβ and γ interferons (IFN) induced genes are regulated transcriptionally via a 29 bp cis acting regulatory element (ICS, Interferon Consensus Sequence). The ICS binding capacity of HeLa cell nuclear extracts was strictly dependent on the presence of nucleoside triphosphate and Mg^{2+}. It increased upon αβ or γ IFN treatment of the cells. Three 50 mer synthetic oligonucleotides containing the ICS or putative ISRE (Interferon Stimulatable Response Element), representing portions of genes inducible by αβ IFN (HLA-A3 and IFI-56K) or by γ (HLA-DR) were used as probes to titrate nuclear factors interacting with the ICS. All three probes were retarded in a mobility shift assay in two bands. Phosphorylation conditions were crucial for demonstrating their existence and/or their relative amounts. A factor whose activity and/or amount was modulated upon IFN treatment, appeared to be involved in phosphorylation dependent post-translational modification(s) of the ISRE binding proteins responsible for altered binding properties.

INTRODUCTION

Interferons (IFN) are cytokines modulating numerous cell functions. The ability to confer cellular resistance to viral infection was the first reported action of IFNs (1). They also exert an antiproliferative action and exhibit immunomodulatory properties (2, 3). IFNs are known to interact with membrane receptors specific of either type I (αβ) or type II (γ) IFN (4–7). The immediate biochemical consequences of these interactions are not clearly defined, almost any known second messengers of hormonal action being potential mediators for their action (8, 9). One of the earliest known consequence of their interaction with the plasma membrane is the transcriptional activation of a number of genes. Some of these induced genes possess either in their transcribed region (10) or in their 5′ non coding region (11–13) a sequence of about 30 bp with a high degree of homology (ICS for Interferon Consensus Sequence) (14). A number of additional IFN inducible genes have now been cloned such as the 2–5A synthetase, Mx, 9–27, 6–16, ISG 54 and ISG 15 (15–20). All these genes possess in their 5′ flanking region a sequence with a certain homology involving 12–13 bp (21). This sequence was referred to as the ISRE (Interferon-Stimulatable Response Element). ICS and ISRE themselves show some homology (17).

Studies on the potential regulation by IFNs of binding activities recognizing the ISRE have been performed; they revealed the existence of both constitutive and inducible factors with differential dependency upon protein synthesis (16, 18, 19, 21, 22). Depending on the cell line used, the time course of appearance of these factors, their number and/or their relative amounts are different. Albeit it seems likely that a common description of the phenomena will arise, most of these data were obtained with probes specific of a single gene.

Our initial study showed that an ICS representing a 29 bp consensus for 9 IFN-modulated genes, was retarded as a single band whose intensity increased after αβ or γ IFN treatment of the cells (23). It could not be decided if it corresponded to an increase in the amount of an initial binding protein or if its affinity was enhanced. The present study shows that the observed increase in the binding capacity was dependent on the phosphorylation conditions used in the assay. Similar results were obtained with three different probes representing no longer a consensus sequence, but part of the 5′ flanking region of genes containing the ICS (see Table I). These genes are inducible by either αβ IFN (HLA-A3 and IFI-56K genes) or γ IFN (HLA-DR gene). In all cases, 2 complexes, B1 and B2, of different mobility were observable. The formation of the faster migrating species (B1) was highly dependent on phosphorylation conditions. As a consequence, the formation of the slower migrating complex (B2) was affected since the formation of both complexes was mutually exclusive. The results suggested that a protein, which does not interact directly with the DNA target, was involved in the modulation of the DNA binding properties of the nuclear extracts. IFN action might thus result in part from a different susceptibility or capacity of the DNA binding proteins to be phosphorylated.

MATERIALS AND METHODS

Cell culture: HeLa cells were subcultured in plastic Petri dishes and grown with RPMI 1640 supplemented with 10% (v/v) foetal calf serum and antibiotics. For nuclear extracts preparation, the cells were grown in suspension in MEM medium. Cells were seeded at an initial density of $0.2 \approx \times 10^6$ cells/ml and used 5 days later at a density of 0.8×10^6/ml. Nuclear extracts were prepared according to Dignam *et al.* (24). Type I (αβ) IFN originating from Namalva cells (1.2×10^6 IU/mg) was donated by Merieux (Lyon, France) and recombinant human type II (γ) IFN (10^7 IU/mg) was given by Roussel-Uclaf. Cells were

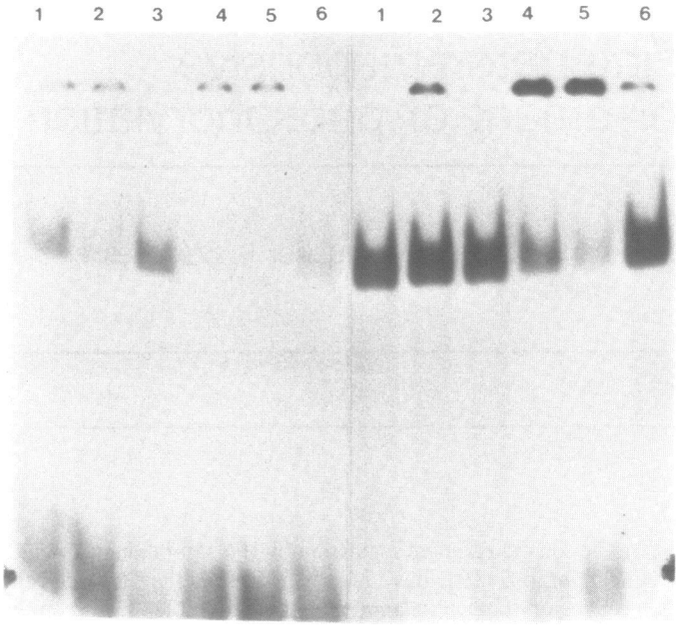

Figure 1. Effects of Mg^{2+} and nucleoside triphosphates on the ICS binding capacity of HeLa cell nuclear extracts. Nuclear extracts from untreated HeLa cells were incubated with labeled ICS and either 2 mM (left panel) or 12 mM (right panel) Mg^{2+}. When present, each nucleotide was added at 0.5 mM final concentration. Lane 1: ATP, CTP, GTP and UTP; lane 2: AMPPNP, GTP, CTP and UTP; lane 3: ATP; lane 4: AMPPNP; lane 5: no nucleotide added; lane 6: CTP, GTP and UTP.

treated with 500 U/ml IFN for 1 h. Proteins were assayed according to Bradford (25).

Oligonucleotides: Oligonucleotides were synthesized on a Biosearch DNA synthesizer and purified by electrophoresis on a 12% (w/v) acrylamide-8.3 M urea sequencing gel. The material of the appropriate size was extracted and purified on Sep-Pak cartridge (Waters). Oligonucleotides were labeled using (α-^{32}P) ddATP (3000 Ci/mM) with terminal transferase (Pharmacia). Each strand was hybridized to the other in 500 mM NaCl by boiling for 2 min and lowering slowly the temperature to 42°C. After ethanol precipitation, the unincorporated ddATP was eliminated with a NACS column (BRL).

Gel retardation assay: Nuclear extracts were preincubated at 30°C under 30 μl in a medium containing: 20 mM Hepes-KOH pH 7.9, 10% (v/v) glycerol, 0.25 mM dithiothreitol, 0.1 mM EDTA, 50 mM KCl, 0.5 mM of each ATP, CTP, GTP, UTP, 2 mM $MgCl_2$, 0.5 μg *E. coli* DNA, 0.5 μg poly (dI) poly (dC) and nuclear proteins as indicated in the legends. At the end of the preincubation period, $12-20\times10^3$ cpm of the probe (0.03−0.1 ng) were added and the incubation continued for an additional 10 min. period. Bound and free probes (20 μl) were separated on polyacrylamide gels (acrylamide/bisacrylamide: 60/1) containing 6% (v/v) glycerol, 10 mM Tris-HCl pH 7.4 and 2 mM $MgCl_2$. The gels were prerun at 4°C for 2 h at 10 volts/cm using 10 mM Tris HCl pH 7.4 and 2 mM $MgCl_2$ as a running buffer. Samples were loaded on the gel after adding 0.1% (v/v) Triton X100 (final concentration).

DNase I footprinting experiments: Incubations were performed as described above with 8 μg of nuclear extract protein. Samples were then incubated for 3 min at 30°C with 40 μg/ml DNase I (Sigma) and processed for analysis on a sequencing gel (12% acrylamide-8.3 M urea).

DMS interference assay: Labeled strands were treated with 1 μl dimethyl sulfate (DMS) for 5 min as described by Maxam and Gilbert (26). After ethanol precipitation, the labeled strand was annealed to the complementary one. 30 μg nuclear extract proteins were incubated with 5×10^5 cpm of the DMS-treated probe in a reaction mixture scaled up 5 fold with respect to that described above. After electrophoresis (3 mm thick gels), the gels were transferred for 2 h in 0.5× TBE on DEAE paper using a semi-dry blotting technique. The dried DEAE paper was autoradiographed and the bands corresponding to the bound and free probes were eluted in 1.25 M NaCl overnight at 42°C. Samples were extracted with phenol, precipitated with ethanol and washed in 70% (v/v) ethanol. The final pellets were resuspended in 100 μl piperidine, heated at 90°C for 30 min and processed for gel electrophoresis analysis.

RESULTS

The results presented in Fig. 1 show that the ability of nuclear extracts to bind the ICS is highly dependent on both Mg^{2+} and ATP. In the absence of any added nucleoside triphosphate (lane 5), the binding was almost undetectable. On the contrary, the binding was the highest whether all 4 NTPs (lane 1) or ATP (lane 3) alone were present. CTP, GTP and UTP (lane 6) in combination were less effective than ATP alone for promoting ICS binding albeit they could substitute to some extent for ATP. AMPPNP, a non-hydrolysable analogue of ATP, was ineffective in promoting ICS binding (lane 4); it did not antagonize the effects of other nucleotides (lane 2). In the presence of higher Mg^{2+} concentrations (12 mM), similar observations could be made but with lower quantitative differences due to the enhancement of binding and an almost complete binding of the probe. In the presence of EDTA (1 mM) or in the absence of added Mg^{2+} no binding could be detected (not shown).

The addition of AMPPNP abolished the binding with nuclear extracts from control cells and lowered that from IFN-treated cells. Vanadate (0.5 mM), an inhibitor of phosphatases (27−29), appeared to have little or no effect on the ability of nuclear extracts to bind the ICS except when AMPPNP was present (not shown). AMPPNP may act as a competitive inhibitor of endogenous ATP; extracts originating from untreated cells were either already more dephosphorylated or they had a lower ability to be phosphorylated.

In order to further analyse the effects of IFNs on the ability of nuclear proteins to interact with the ICS depending on the phosphorylating conditions, we used DNA fragments containing the ICS from three different IFN-modulated genes. Two of them corresponded to genes inducible by $\alpha\beta$ IFN (IFI-56K and HLA-A3), the other being inducible by γ IFN (HLA-DR). The data presented in Fig. 2 show the following: 1/ the 50-mer oligonucleotides were retarded in 2 bands, the slowest migrating species (B2) being the more abundant under the conditions used; 2/ all probes including the ICS can compete with each other with the same efficiency; 3/ the amounts of protein in the nuclear extract binding each probe were similar as deduced from the amount of binding detected in the absence of competitor, the probes being labeled with the same specific activity; 4/ the relative displacement of B2 by the unlabeled oligonucleotides was higher

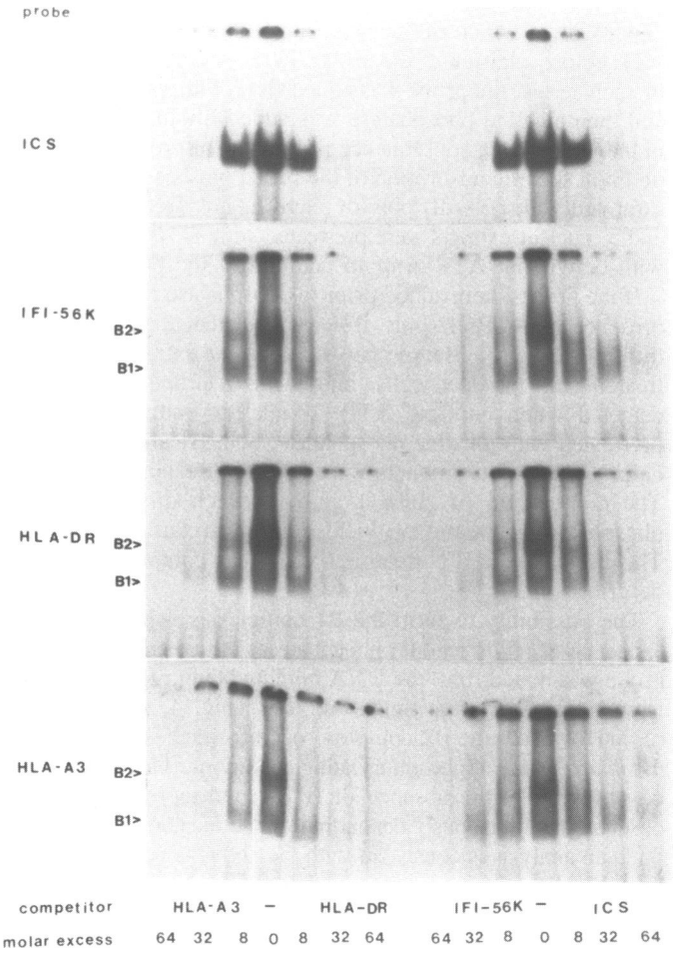

Figure 2. Cross specificity of the probes used. Each probe was labeled at the same specific activity (see Materials and Methods) and incubated with control nuclear cell extract (6 μg) either alone (no competitor added) or with increasing molar excess of each of the oligonucleotides used. 20,000 cpm were used in each assay and the exposure time was the same in each case.

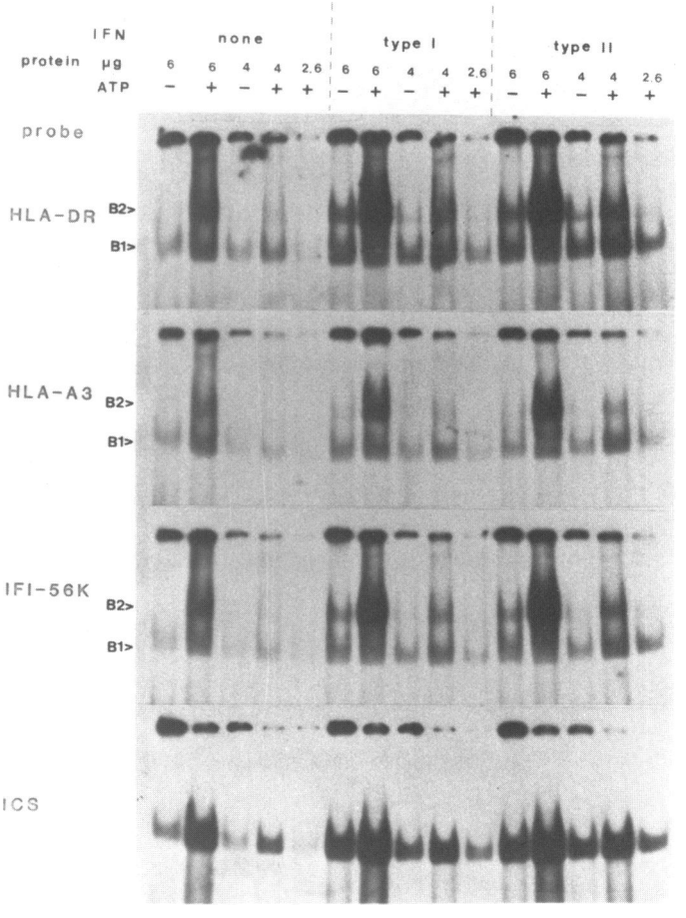

Figure 3. Dependence on the protein concentration and ATP of the ability of nuclear extracts to bind the ICS- and ISRE-containing sequences: effects of IFN treatment. Nuclear extracts were prepared from untreated cells or from cells treated for 1 h with either type I or type II IFN. The binding capacity of the extracts was tested at three protein concentrations (2.6, 4 and 6μg) with or without ATP (at 4 and 6μg protein). Each probe was labeled at the same specific activity and 20,000 cpm added per assay.

than that observed with B1, suggesting that its amount was lower and its affinity higher than those of B1. It should be noticed that the labeled ICS (which is a 29-mer instead of a 50-mer oligonucleotide) was retarded as a single band, with a mobility shift similar to that of B1. Finally, it can be noted that the amount of radioactivity which did not enter the gel was displaced in a way similar to that entering the gel.

The results presented in Fig. 3 were obtained with all 4 probes in the presence or absence of added ATP using nuclear extracts prepared from control cells (left) or from cells treated for 1 h with αβ IFN (center) or γ IFN (right). The protein concentration was also varied using 6, 4 and 2.6 μg nuclear protein for testing each extract. The rationale for doing so was that if ATP is a cofactor of the ICS binding protein(s), the amount of retarded probe should be directly proportional to the amount of added protein. If, on the contrary, ATP was the substrate of a protein distinct from the ICS binding protein(s) (a kinase for instance) but modifying their binding properties, ICS binding should be highly protein concentration dependent.

The amount of probe bound was the lowest when ATP was omitted from the incubation medium as mentioned above. Only B1 was detected with nuclear extracts from untreated cells. Under these conditions, an effect of IFN (αβ or γ) treatment was detectable as seen by the higher amount of material retarded in B1 and the appearance of B2. The differences in the pattern observed (B2 versus B1) and the signal intensities detected when comparing the data obtained with 6 and 4 μg nuclear extract protein suggest a cooperation of factors in the extracts for allowing the formation of the B2 complex.

In the presence of ATP, the binding was enhanced at any protein concentration whatever the source of the nuclear extract. It should however be pointed out that: 1/ the binding was always the highest with nuclear extracts originating from cells treated with IFN; 2/ B2 was detectable in the extracts originating from control cells at the highest protein concentration only; 3/ even at this protein concentration, the amount of material bound was lower with extracts from untreated cells and 4/ at the lowest protein concentration, B1 was detectable only with extracts from IFN-treated cells. Similar results were obtained with the HLA-A3, HLA-DR and IFI-56K probes. The same conclusions can be drawn with the consensus oligonucleotide except for the absence of the second retarded band. Taking into account the behaviour of the extracts as a function of protein concentration,

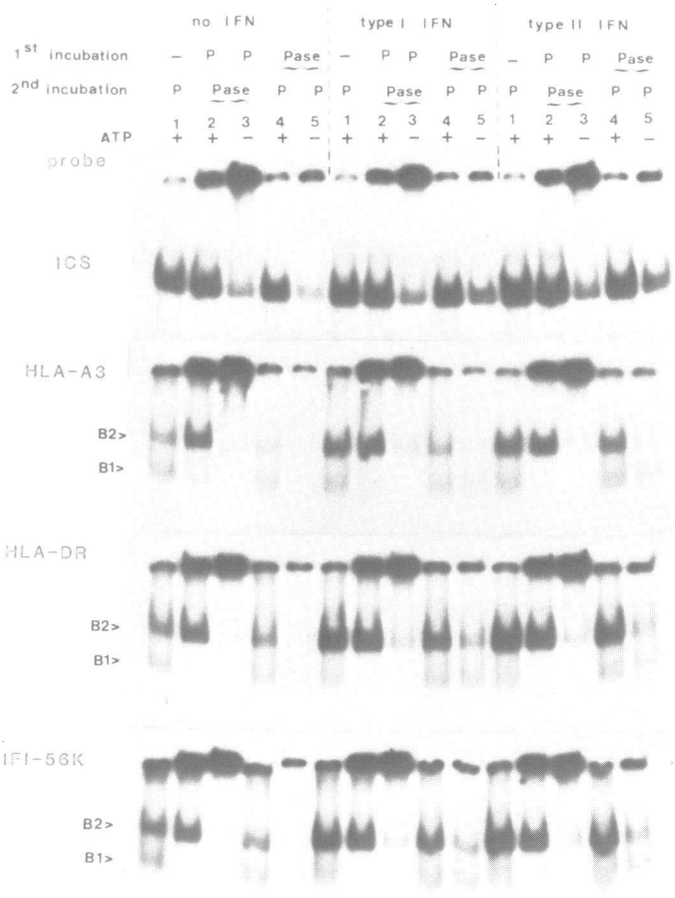

Figure 4. Modulation by phosphorylation conditions of ICS and ISRE nuclear binding proteins properties. Cellular extracts (6 μg) originating from untreated (left), αβ (center) or γ (right) IFN-treated cells were tested for their ability to bind the indicated probes. IFN treatment was for 1 h. The procedure involving two sequential incubations is described at the top of the figure. When present (lanes 1, 2 and 4) ATP was added at 0.5 mM. Key to symbols: P: probe; Pase: alkaline phosphatase (1 Unit).

assay conditions (plus or minus ATP) or cell treatment (plus or minus IFN), it is likely that this single retarded band corresponds to the sum of B1 and B2. This lack of separation would be consistent with differences of lower amplitude since summing the effects detected on B1 (which were of minimal amplitude) and on B2.

The specificity of nucleotides for promoting the binding was studied with the 50 mer in an experiment (not shown) similar to that described in Fig. 1. Three sources of nuclear extracts were used: control ones and those originating from IFN αβ or IFN γ treated cells. With all extracts, omitting ATP or substituting AMPPNP for ATP led to a decreased binding. IFN treatment led to a larger retardation of the probes when compared to control extracts. Whatever the pretreatment of the cells, ATP was as effective as the mixture of all 4NTPs for promoting the binding. The simultaneous addition of GTP, UTP and CTP was less efficient than ATP alone for eliciting a maximal binding in control extracts.

The influence of phosphorylation conditions was further investigated as shown in Fig. 4. The first lane of each series of 5 samples corresponds to the conditions used so far e.g. conditions in which the nuclear extract was preincubated with ATP before addition of the probe. In the second and third lanes of each series, the probe was added first, with or without ATP, and then alkaline phosphatase was added (the material stacked in the wells was a consequence of the lack of preincubation and of a non specific adsorption of the probe on sites not previously saturated with poly(dI) poly(dC) and *E. coli* DNA). In lanes 4 and 5, nuclear extracts were pretreated with alkaline phosphatase with or without ATP prior to addition of the probe.

If the probes were added prior to phosphatase treatment in the presence of ATP: 1/ only B2 was detected, 2/ no significant differences were observed between nuclear extracts from IFN-treated and untreated cells, and 3/ no retarded complex was detected when omitting ATP. If nuclear extracts were first pretreated with alkaline phosphatase, both B1 and B2 could be detected, but their amounts were lower in the absence of ATP. The comparison of lanes 1 and 4 (which differed only by phosphatase pretreatment) yielded similar retardation patterns. The effects of the IFN treatment were then comparable to those described in Fig. 3.

The possibility to form the B1 complex is dependent on the existence of (endogenous) phosphatases which cannot act if the probe was added first, the DNA binding being rapid and almost irreversible within the time scale used (Fig. 4, lanes 2 and 3) (23). However, the B2 complex, once formed, did not require the maintenance of phosphorylating conditions. Therefore B2 can be observed in the absence of B1 or both complexes can be observed simultaneously depending on the incubation conditions. Such an analysis is consistent with the above conclusions drawn from the data obtained with vanadate (in addition, vanadate was able to block the action of exogenously added alkaline phosphatase).

Qualitatively similar but much less pronounced results were obtained with the ICS, most probably for the reasons evoked above. The same analysis could not therefore be carried out on this sole basis, but the way of variation of the binding detected as a function of the assay conditions used, did not conflict with the results obtained with probes specific for a given gene.

Previously published results indicated that a significant protection from DNase I digestion was observed on the non coding strand of the ICS (23). The DNase footprinting patterns obtained with both coding and non coding strands of all three 50 mer probes are shown in Fig. 5 and summarized in Table I. As with the ICS (23), a differential pattern was observed with the strand containing the AAACT motif upon IFN treatment, these differences being rather subtle. Mainly two regions of protection from DNase action could be defined on each strand of the DNA (see also Table I). If the sequences of all three probes are centered on the AAACT motif, it is striking to notice that the patterns of DNase footprinting resemble each other. This occurred despite the fact that the sequences involved differed to a significant extent in particular on their 3′ end (AAACT strand): 12−15 nucleotides were protected from DNase action on its 5′ end and 5−8 on its 3′ end depending on the probe used. On the other strand, 12−15 nucleotides were protected at the 5′ end, and 5−7 at the 3′ end. The regions protected on each strand thus correspond partially to each other. At lower protein concentration (4 μg), the 3′ end of the AAACT strand was no longer protected and correspondingly, this was the case for the 5′ end of the complementary strand (not shown). This observation leads to a proposal for the location of both B1 and B2 complexes.

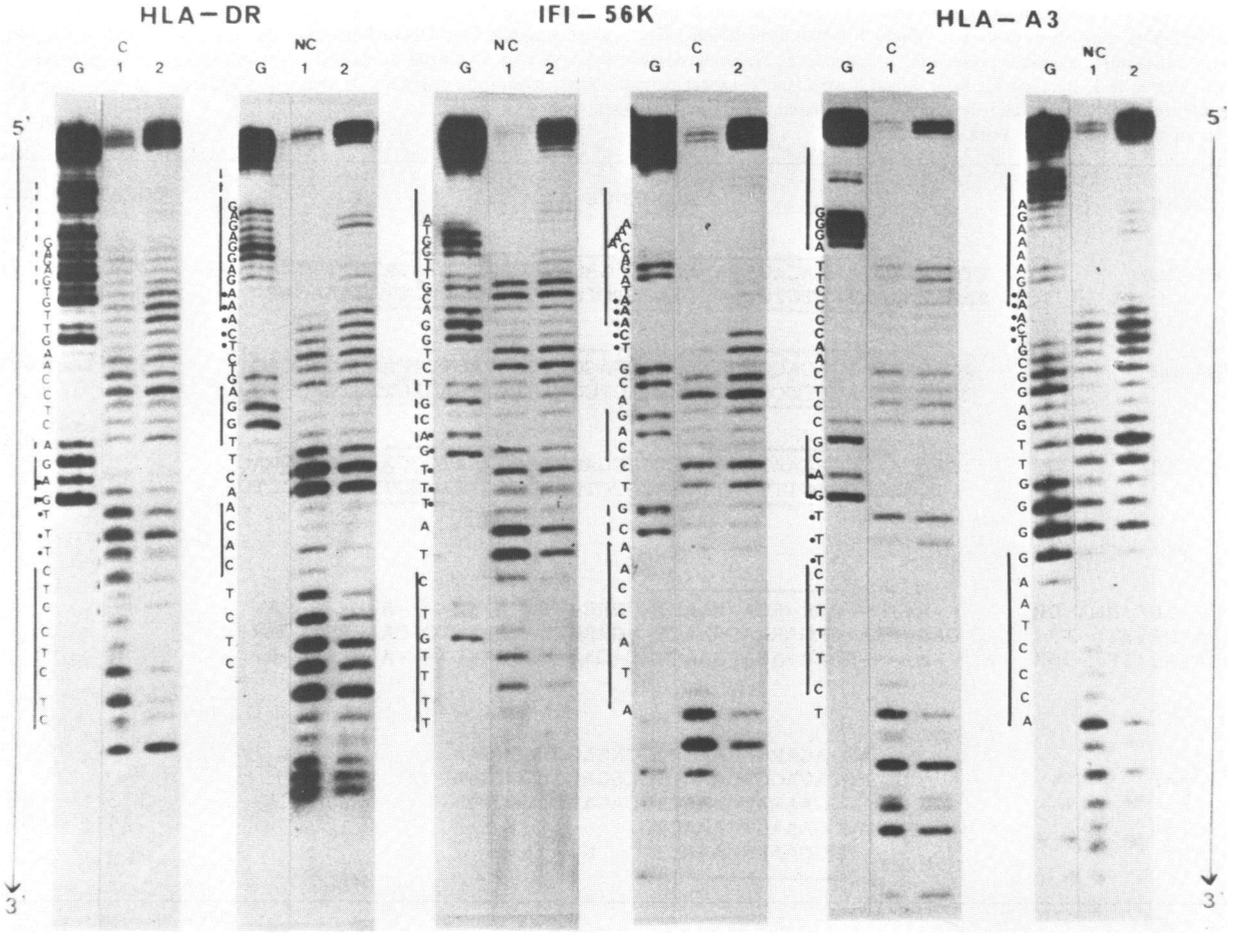

Figure 5. DNase I footprinting analysis. DNase footprinting was performed as described under Materials and Methods. Each strand constituting the 50-mer oligonucleotide was labeled with terminal transferase and annealed with the complementary strand. Nuclear extracts were prepared from either untreated cells (lanes 1) or $\alpha\beta$ IFN-treated cells (lanes 2). NC (non coding) and C (coding) refer to complementary strands of each double stranded oligonucleotide. The sequences are indicated along the gel from the 10th to the 40th position. The AAACT motif (boxed by dashed lines in Table 1) and its complementary sequence are indicated by dots. The regions protected from DNase I digestion are indicated by solid lines, dashed lines indicating a weaker protection. These results are summarized in the first part of Table I. Under the same experimental conditions (40 μg/ml, 3 min at 30°C), DNase I digestion of the probes without protein addition yielded an oligonucleotide ladder extending up to 12–16 bases depending on the probe used, indicating therefore an almost complete destruction of the probes. G, marker track showing cleavage at G residues after DMS treatment and piperidine cleavage.

Since at low protein concentration, mainly B1 was detected with nuclear extracts from control cells, the binding site for B2 must be on the 3′ end of the AAACT strand.

Methylation interference and DMS protection experiments were carried out (not shown). The following observations could be made: 1/ the nuclear extracts were able to modify some of the probes (deletion of one base and altered cleavage reaction) which precluded the utilization of the non incubated probes as internal standards; 2/ the bases for which either a protection or an enhancement of DMS action was evident were all within the regions protected from DNase digestion; 3/ the probe retarded either in B1 or B2, depending on the gene under study, did not exhibit similar sensitivity to methylation interference. This is consistent with the fact that albeit a consensus could be defined, the bases which are involved in the recognition of the proteins were not necessarily those which reacted with DMS or those whose methylation would alter DNA-protein interactions. It should also be noticed that the conditions during the gel retardation were necessarily different from those used during the incubation. It is therefore difficult to ascertain that the characteristics of the interaction were not altered during the migration.

CONCLUSIONS

The synthetic ICS was able to recognize the same proteins as did the parts of the natural genes containing the ICS. However despite the fact that in this latter case two retarded bands were observable, with the ICS a single band could be detected with apparently the properties of both B1 and B2 complexes. The specificity of the interaction detected indicates that each probe used was able to compete with each other. The competition was complete in every case and the amounts of binding proteins detected were similar. These observations indicated therefore that similar amounts of identical nuclear binding proteins were detected with each of the probes.

The increase in binding capacity upon IFN treatment as well as the susceptibility of DNA binding to phosphorylation conditions were similar whether the cells were treated with type I or type II IFN. Similarly the binding detected was identical

Table 1. Comparison of the sequences of the probes used and those of ICS and ISRE: patterns of DNase I digestion. Upper part: The sequences of the ICS-containing regions of the HLA-A3 genes were compared. Every 10th base is underlined. The regions protected from DNase I digestion are shown by the lines along the sequences, the dashed lines indicating a weaker protection. All sequences are centered on the AAACT motif (boxed by dashed lines). Middle part: 50 mers were compared between each other with R standing for G or A and S for C or G. Lower part: the sequences of the originally described ICS (14), the 29 mers used in this study, the 6−16 gene, the ISG-54 gene and the ISRE are compared. The core motif is boxed.
*The cap site of this gene has not been determined.

GENE	SEQUENCE	LOCATION, nt
IFI-56K	TTCTATTTTAAAACAGATAAACTGCAGACCTGCAACCATAGATGTTTCTA AAGATAAAATTTTGTCTATTTGACGTCTGGACGTTGGTATCTACAAAGAT	-219 to -170
HLA-DR	AGAGAAAATGAGAGGAGAAACTCTGAGGTTCAACACTCTCAACACTGGAG TCTCTTTTACTCTCCTCTTTGAGACTCCAAGTTGTGAGAGTTGTGACCTC	-557 to -606
HLA-A3	GAGCAGGGGAGAAAAGAAACTGCGGAGTTGGGGAATCCCAAGGCTGGGAC CTCGTCCCCTCTTTTCTTTGACGCCTCAACCCCTTAGGGTTCCGACCCTG	*
IFI-56K/HLA-DR	--R-----RAR-RGA-AAACT--RRR--T--A--C----A-------A-	
HLA-DR/HLA-A3	GAG-ARR-GAGARRAGAAACT--GRRGTT-RR-A-TC-CAA-RCTGGRR	
HLA-A3/IFI-56K	--R----RRAR-ARA-AAACTGC-GA--TG-RR------A-G--T--R-	
ICS	AG-AGARGAGAAACTGCNGAGGTNSNGAA	
29 mer	AGTAGAGGAGAAACTGCGGAGGTGGTGAA	
6-16	GGGGAAAATGAAACTGCAGAGTGCAC	-113 to -88
ISG-54	AGGGAAAGTGAAACTG	-85 to -99
ISRE	RGGAARNGAAACT N = 1 or 2	

whether the probes used corresponded to part of genes exclusively inducible by γ IFN (HLA-DR) or by $\alpha\beta$ IFN (IFI-56K and HLA-A3). (It should be noted that the functional significance of the HLA-DR probe used had not been proven at variance with the sequence lying within −160 nucleotides of the transcription initiation site (30). Our choice was dictated by the original observation of Friedman and Stark (14)). The specificity of IFN action exists at the level of the IFN-receptor interaction and at the level of the final response observed. The enhanced ability of nuclear extracts to bind the ICS irrespective of the IFN type underlines that this sequence is implicated in the action of both types of IFNs. The specificity at the transcriptional level probably results from other interactions to be defined either with other DNA regulatory regions or with proteins altering the activity of transcriptional factors.

The comparison of the sequences involved in the DNA/protein interaction shows that a substantial degree of divergence could be compatible with the existence of such an interaction. The highest degree of homology was observed when comparing the sequences of the HLA-A3 and HLA-DR genes (see Table I). This homology was reduced when comparing the HLA-A3 and IFI-56K genes and minimal when comparing the HLA-DR and IFI-56K genes. However it is striking to notice that the regions protected from DNase digestion, on either strands, had similar length and were distributed almost equidistally around the core motif AAACT. This observation suggests that either distinct flanking sequences may help in defining a DNA binding protein binding site or that a short highly conserved motif is sufficient to promote the binding on divergent adjacent sequences (31, 32). Methylation interference assays or DMS protection experiments can thus yield patterns which were not necessarily the same when comparing one probe to another.

Studies concerning the interaction of DNA binding proteins following the IFN treatment of cells with the ISRE showed that three factors were involved in such interactions (16, 18, 19, 21, 22). One of them was present in nuclear extracts of both control and IFN-treated cells, the two others being only detectable consecutively to the IFN action. In our case, only two retarded complexes were detectable, their amount and/or affinity being larger after the IFN treatment of the cells. The lack of detection of factors which could be apparented to either the ISGF3 (33, 34) or E (35, 36) factors might be a consequence of the choice of the sequences used. However, it must be emphasized that the binding which was detected in our study, was Mg^{2+} dependent and highly modulated by the phosphorylation conditions. Whether the assay conditions used in these studies and the choice of the cellular system used made all the differences has to be determined. However, using the 39 bp ISRE containing probe of the 6−16 gene described in reference 35, preliminary results showed two retarded bands with mobilities similar to those observed for B1 and B2. Their dependency as a function of the protein concentration was similar to that observed with the 50 mer probes used in this paper.

Upon IFN treatment of the cells, the binding capacity of the extracts was enhanced. If ATP was omitted from the incubation medium, only B1 could be detected with extracts originating from untreated cells. This was also the case, but at low protein concentration with nuclear extracts from IFN treated cells. Anyhow, even under these conditions the amount of B1 was larger with these extracts. Increasing the protein concentration,

especially in the presence of ATP, increased the amount of B2 much more than that of B1, suggesting a multifactorial interaction between factors to enable the formation of the B2 complex.

The ability to change the binding properties appeared not to be intrinsic to the protein being part of the B2 complex but rather under the control of a protein susceptible to activate this protein directly by phosphorylation or via the release of an inhibitor. Modulation of this activity can alter the amount of the B2 protein and/or its affinity. Therefore, at variance with the cytoplasmic latent DNA binding protein whose amount first increases in the cytoplasm and then migrates to the nucleus (34, 36), it is likely that the IFN-dependent increase in DNA binding activity observed here results from an alteration of a DNA binding protein effector (34) whose activity and/or nuclear localization is mediated by IFN treatment.

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