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Review

Type I interferons: expression and signalization

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Abstract. Type I interferon (IFN-A and IFN-B) genes encode a large family of multifunctional secreted proteins involved in antiviral defence, cell growth regulation and immune activation. These cytokines, as a consequence of their biological activities, have been established as effective therapeutic molecules for malignant and viral diseases. Virus infection is the main inducer leading to transient expression of type I IFN (A and B) and the antiviral response appears to proceed through a two-step pathway requiring, first, induction of type I IFN gene expression and, second, transcriptional activation by the synthesized IFN proteins, binding to their specific cell surface receptors, of a large number of genes. The proteins they encode are responsible, in part, for the pleiotropic multiple biological activities of the IFN. In this two-step pathway, the virus-induced IFN genes and the IFN-stimulated gene (ISG) expression seem to share common factors. Even if IFN-A genes are structurally related and very often coordinately induced in virus-infected cells, differences in the expression of the individual IFN-A messenger RNAs of the multigenic IFN-A gene family are observed in human as well as in murine cells, reflecting, in a particular cell type, the transcriptional activity of the corresponding promoter regions. Important studies on interferon regulatory factors and ISG factors have been made in the last decade. However, some factors involved in IFN-A gene regulation remain to be identified. Our goal has been to review the factors involved in the control of the type I IFN gene expression to understand the mechanisms of induction and repression of their transcription and to explain the properties of these cytokines through their signal transduction pathway.

Key words. Type I interferons; regulation expression; transcription factors; activators; repressors; signal transduction; interferon receptors.

Introduction

Interferons (IFNs) constitute a family of secreted cytokines expressed as an early response to various stimuli, and in particular to viral infection, in eukaryotic cells. They confer cellular resistance to virus, act as negative growth factors in normal and transformed cell types, and affect cell growth and differentiation. IFNs also have modulatory effects on the immune system, such as the activation of the major histocompatibility complex (MHC) gene expression and stimulation of natural killer (NK) cells to mediate antibody-dependent cytotoxicity via other cytokines [1].

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Induction of type I IFN gene expression

Type I IFN genes were known to exist in two families, A and B with several characteristics such as subtype genes and pseudogenes, and an absence of introns in mammalian IFN genes. In humans, one B-type and more than 20 subtypes of A-type IFN were discovered through advances in molecular biology and gene technology [2, 3], and more than 70% homology on amino acid sequence has been shown in subtypes of human IFN-α (table 1). In 1985, a new family of type I IFN was reported by several groups independently [4, 5]. This new type of IFN was designated omega (ω) or IFN-αII. The IFN-ω gene family was found to be constituted of multiple genes in humans, cattle and horses. In humans, only one out of five IFN gene sequences is functional; all the others are pseudogenes [6].

The classical, acid-stable human IFN proteins, IFN-α and IFN-β, consist of 166 amino acids (with one exception, IFN-α2, which has 165 amino acids); the single human IFN-ω protein, IFN-ω1, is a basic protein consisting of 172 amino acids. IFN-ω is more closely related to IFN-α than to IFN-β (protein sequence identity 50–60% vs. 29%), but IFN-ω is not neutralized by antisera to human IFN-α, -β or -γ [7].

IFN-τ, previously named trophoblastin or trophoblast protein type 1 is a new class of type I IFN that has been described in sheep, goats and cows [8, 9]. In these species, IFN-τ is physiologically secreted by trophoblast as an antiluteolytic protein responsible for maternal recognition of pregnancy [10]. IFN-τ exhibits antiviral activity, is weakly cytotoxic and has immunosuppressor effects towards human CD4-positive lymphocytes [11]. IFN-τ shares a 70% amino acid sequence homology with IFN-ω and a 55% homology with IFN-α [12–14].

As for IFN-τ, the role of animal equivalents to human IFN-ω in maternal recognition of pregnancy points to a physiological role of these proteins which is at least in part distinct from that of IFN-α.

Viral infection of eukaryotic cells induces early and transient expression of various cytokine and chemokine genes, including those of type I IFN (IFN-A and IFN-B). The secreted IFNs then transduce signals through the cognate receptor in an autocrine or paracrine manner to activate IFN-stimulated genes (ISG) some of which are responsible for the pleiotropic biological activities attributed to the IFN, including their antiviral activity.

After virus infection, type I IFN genes encoding IFN-α and IFN-β proteins are expressed in a large variety of human and murine cells. Type II IFN (the immune IFN-γ) is secreted mainly by Th-1 lymphocytes and NK cells. The early steps of virus entry in cells, such as the binding of virions to their cellular receptors and membrane fusion events, are poorly understood as opposed to the next step of infection, which consists of the release of viral genome and glycoprotein envelope into the cytoplasm of the infected cells. The double-stranded RNA contained in the viral genome or produced during virus replication is suggested to be the main inducer signal for IFN and ISG gene transcription [15, 16]; however, glycoproteins from the virion envelope have also been shown to induce IFN [17].

Although the transduction pathways triggered by different viruses remain to be elucidated, transcriptional activation mechanisms of target genes such as IFN have been well studied.

Table 1. Characteristics of different types of IFN in humans and mice.

Interferons	Type I interferons			Type II interferons
	IFN-α	IFN-β	IFN-ω	IFN-γ
Main productive cells	leucocytes fibroblasts macrophages epithelial cells		leucocytes	lymphocytes T
Inducing agents	virus dsRNA mitogenes		virus physiological stimuli	mitogens antigens interleukin 2
Chromosomal localization		human chr9 murine chr 4		human chr 12 murine 10
Number of genes	>15 human genes >10 murine genes	1 single gene	1 human gene and 4 pseudogenes	1 single gene
Introns	0	0	0	3
kDa	16–27	28–35	24.5	20–25
Amino acids (mature protein)	166	166	172	143

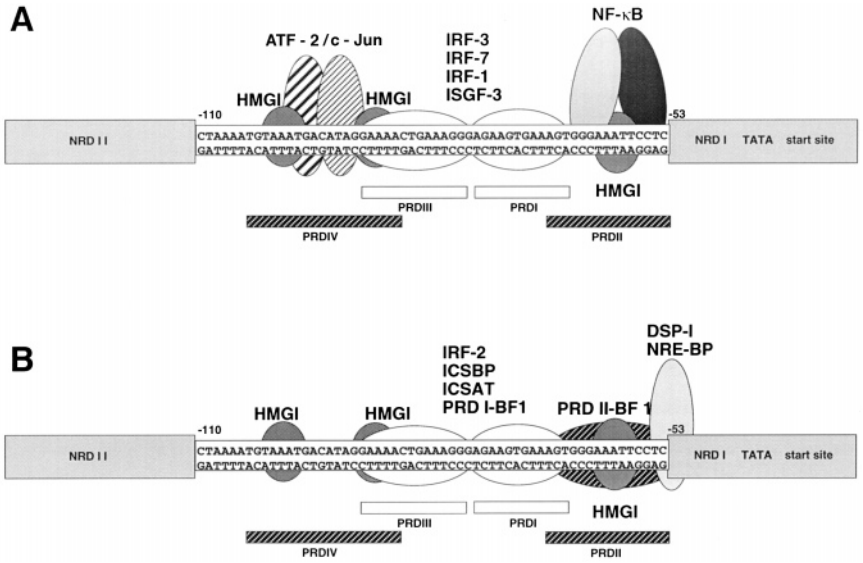

Figure 1. (*A*) Interaction of regulatory activators with the human IFN-B gene promoter after virus induction: NFκB binds to PRDII; IRF-1, IRF-3, IRF-7 and ISGF-3 bind to PRDI and PRDIII; ATF-2/c-Jun binds to PRDIV; HMGI binds to AT-rich regions within PRDII and sites flanking the PRDIV. HMGI binding is constitutive. (*B*) Interaction of constitutive and post-inductional repressors of the human IFN-B gene promoter: DSP-I, NRE-BP and PRDII-BF 1 bind to PRDII; IRF-2, ICSBP, ICSAT and PRDI-IRF-1 bind to PRDI and PRDIII; HMGI binds to AT-rich-regions within PRDII and sites flanking the PRDIV.

Type I IFNs are clustered on human chromosome 9 and murine chromosome 4 [18–20]. IFN-A genes are structurally related and very often coordinately induced in virus-infected cells, but differences in the expression of the individual IFN-A messenger RNAs (mRNAs) have been observed in human and in murine cells, reflecting, in a particular cell type, the transcriptional activity of the corresponding promoter regions. The ratio of IFN-A to IFN-B transcripts as well as the proportion of individual IFN-A mRNAs varies with the cell type and with the inducer [21, 22]. This differential virus inducibility has been demonstrated to occur at the level of gene transcription [23, 24], although regulation also occurs at other levels including mRNA stability [25, 26].

Virus-induced expression of IFN-A and IFN-B genes is mediated by regulatory sequences located within 200 bp upstream of the transcription start site of their promoters [27–30]. IFN-B gene expression is regulated by multiple factors which interact with positive regulatory domains (PRDs) located within the virus responsive element B (VRE-B) (fig. 1): IRF-1 (interferon regulatory factor-1), which recognizes both the PRDI and PRDIII domains, also known as IRF elements [31, 32]; nuclear factor NF-κB, which recognizes the PRDII domain [33–35]; and the ATF-2/c-Jun heterodimer, which binds to the PRDIV domain and is required for maximal virus inducibility in mouse L929 and human HeLa cells [36]. Binding of both NF-κB and ATF-2/c-Jun complexes is facilitated by HMGI(Y), members of the high mobility group of proteins that bend DNA and allow VRE-B-binding factors to assemble in a higher-order nucleoprotein complex called 'enhanceosome', thus promoting virus-induced transcription of the IFN-B gene [37, 38].

Factors specifically involved in regulating IFN-A genes are less well defined. The VRE of the human IFN-A1 gene (VRE-A1) and the inducible element of the murine IFN-A4 gene have been shown to contain a PRDI-like site, thus involving IRF-1 in the induced expression of IFN-A genes [39–41]. A comparative study of IFN-A4 and IFN-A6 gene promoters displaying virus inducibility has led to the detection by electrophoretic mobility shift assay (EMSA) of binding activity denoted as AF-1. It has been proposed that AF-1-forming proteins cooperate with IRF-1 in the virus-induced transcription of IFN-A genes [42, 43]. Another factor possibly specifically involved in IFN-A gene regulation is the TG protein, which binds to the hexameric GAAATG repeats that confer virus inducibility through an IRF-1-independent pathway. The so-called 'TG sequence' is adjacent to the PRDI-like motif in human VRE-A1 and is conserved in most human or murine IFN-A [39, 44]. However, AF1-related and TG-binding proteins have yet to be characterized.

Interestingly, deletion of the gene coding for IRF-1 does not affect virus-inducible expression of type I IFN genes, thus raising questions about its role [45, 46]. Another member of the IRF family, IFN-stimulated gene factor 3γ (ISGF3γ), has been suggested to participate in the regulation of the IFN-B gene promoter, both alone or as a heteromeric complex (ISGF3) formed with Stat1α and Stat2, which are members of the signal transducers and activators of transcription family [47–50]. In fact, ISGF3 has been shown to be induced secondarily by the virus and involved in the amplification of type I IFN gene expression triggered initially by viral infection of cells [51]. However, the primary transcription factor directly activated by the virus and mediating the PRDI-dependent initiation of transcription of type I IFN genes remains to be identified.

Together with previously published data [39], we reported insight into the modular architecture of murine IFN-A gene promoters [52, 53]. We suggested that the VRE of IFN-A4 is composed of four enhancer motifs that exhibit different properties: the [A] motif, represented by the [−103 to −93] GTAAAGAAAGT sequence, which is not virus-inducible even in multiple copies and requires a juxtaposition with the [B] domain to respond to virus induction [43], the [B] and [C] motifs, corresponding respectively to the [−98 to −87] GAAAGTGAAAAG and [−85 to −74] GAATTG-GAAAGC sequences, are virus-responsive once multimerized or in combination with each other; and finally the [D] motif represented by the [−57 to −46] GAAAG-GAGAAAC sequence is identified and shown to cooperate with the [B] and [C] domains to confer maximal Newcastle disease virus (NDV) inducibility to the IFN-A4 promoter in L929 cells (fig. 2).

Comparative analysis of the murine IFN-A4 and IFN-A11 gene promoters, including their inducible elements, was carried out, and a virus-induced factor (VIF) stimulated within 1 h of virus contact with cells [52] was identified [53] on the IFN-A4 promoter.

VIF was shown to specifically recognize the PRDI-like domain shared by the inducible elements (IE-A4) and the TG-like domain of IE-A4 of virus-inducible murine IFN-A genes. The binding affinity of VIF is considerably reduced by −78 A/G substitution, which disrupts the TG-like binding site (module C) in the poorly expressed IFN-A11 promoter. Moreover, a synergy between multiple VIF-binding elements has been shown to be involved in the differential expression of IFN-A genes [53].

The similarity of the results obtained in transfection experiments in HeLa cells with different IFN-A4 and IFN-A11 constructs and the detection of VIF-binding activity in nuclear extracts obtained from NDV-induced HeLa S3 cells suggest that the model ascribing a role for VIF on the initiation of transcription of IFN-A genes may also be valid for human cell lines. The current proposition for IFN-A gene virus-induced transcription would involve direct activation by NDV of a primary transcription factor, possibly VIF [53], within 1 h of contact between the cells and the virions and without requiring de novo protein synthesis.

VIF was demonstrated to be different from IRF-1 and IRF-2. The absence of reactivity between VIF and anti-ISGF3γ antibodies indicated that VIF was also different from ISGF3.

A new member of the IRF family, IRF-3 [54], identified on binding to ISRE and ISG15 promoter, is expressed constitutively in a variety of tissues, and its mRNA level does not change in virus-infected or IFN-treated cells. Virus-inducible phosphorylation causes a posttranslational modification of IRF-3 leading to the nuclear translocation of phosphorylated IRF-3, stimulation of DNA-binding and transcriptional activity, and association with the transcriptional coactivator CBP/p300 [55].

Recent data suggest that IRF-3 and CBP/p300 may represent components of virus or double-stranded RNA (dsRNA)-inducible activated complexes such as VA-IRF [56] or DRAF-1 [57]. Wathelet et al. [58] report that virus infection may result in the phosphorylation of both IRF-3 and IRF-7 in the cytoplasm. The virus-activated IRF-3/IRF-7 complex may undergo a conformational change allowing specific DNA binding and association with the transcriptional coactivators p300 and CBP to form the virus-activated factor (VAF). In the case of the IFN-B promoter, virus infection leads to the coordinate activation of ATF-2/c-Jun, NFκB and VAF. The relationship between VAF and previously identified virus-inducible proteins that recognize regulatory elements in virus-inducible genes remains to be determined [52, 59, 60].

The IFNs subsequently produced bind to type I IFN receptor to elicit signal-inducing ISGF-3, which requires de novo synthesis. The ISGF-3 complex, once accumulated, can more efficiently mediate not only IFN-induced gene expression but also IFN induction by viruses or poly(rl)poly(rC) [51].

Negative regulation of IFN type I gene expression

Studies carried on type I IFN promoters could also serve as a model system leading to the understanding of the negative control of eukaryotic gene expression.

In fact, IFN type I gene expression requires not only the appropriate activation of these genes but also their repression before and after virus induction. Some *cis*-acting elements of the IFN-B promoter, such as negative regulatory domain (NRD) I and NRDII, have been characterized as being involved in the constitutive repression of the gene, whereas PRD elements were shown to mediate both induction and repression [25, 61,

62]. It clearly appears that the interplay of the binding of activator as well as repressor factors is necessary in regulating the expression of type I IFN genes as well as for the differential expression of the IFN-A gene family.

The first IFN gene transcriptional repressor discovered was IRF-2, isolated by its homology to IRF-1 [32]. IRF-2 binds PRDI and PRDIII within the VRE-B of the IFN-B gene and also recognizes the IRF elements present in other virus-inducible genes such as IFN-A [42, 43]. The role of IRF-2 as a negative regulatory factor in type I IFN gene expression was confirmed by obtaining IRF-2-deficient mice. Inded, in IRF-$2^{-/-}$ mice, the level of IFN-A and

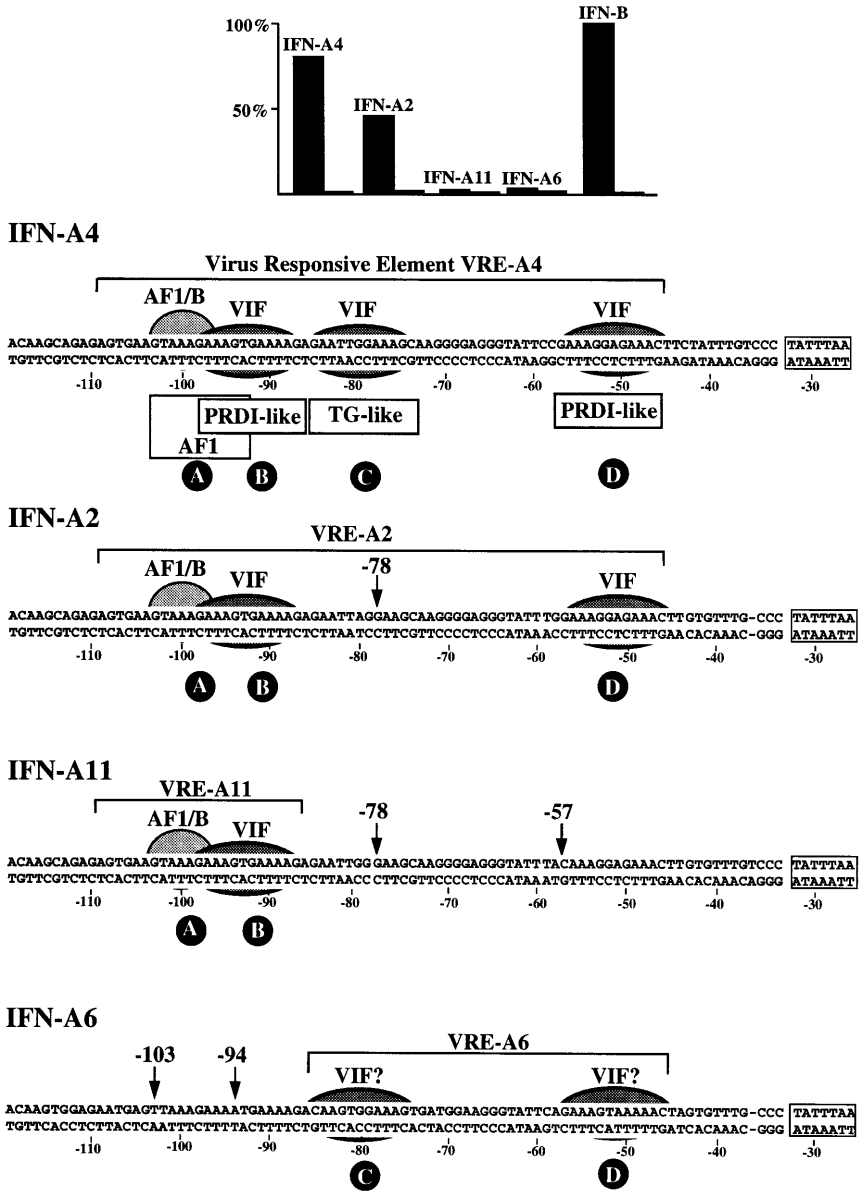

Figure 2. Model for differential virus-induced activation of transcription of murine IFN-A genes. In the first panel, mRNA levels of four murine IFN-A genes detected in L929 cells induced by NDV (solid bars) or noninduced (open bars) are represented relative to the levels of expression of murine IFN-B mRNA. IFN-A4, IFN-A2, IFN-A11 and IFN-A6 promoter sequences are indicated below; the numbering is relative to the transcription start of the promoter, the TATA box being indicated in each case. Nucleotides distinguishing these sequences are represented by larger characters, the −78A-G and the −57G-C mutations disrupting, respectively, the C and D motifs are indicated by arrows. Localization of the binding sites for potential *trans*-activators, namely AF1 sequence (the A motif), PRDI-like domain (the B and D motifs) and TG-like domain (the C motif) used in the text and the factors binding to these domains are indicated. The combination of these domains in the promoters of the IFN-A genes defines the VREs which are indicated above each sequence.

IFN-B mRNA following virus induction is higher than the level observed in wild-type mice [45]. IRF-2 was described as a repressor of transcription because of its ability to antagonize IRF-1 by competing for the IRF-binding site. Protein IRF-2 is expressed constitutively in many cell types and plays a role in maintaining the gene repressive state before virus induction occurs. IRF-2 is induced after virus infection as well as by IFN [32] and may be considered as a postinduction repressor of type I IFN gene expression [62, 63].

The IFN consensus sequence-binding protein (ICSBP) is a member of the IRF family and has a negative effect, similar to that of IRF-2, on IRF-1-mediated induction of PRDI-containing promoters [64–66]. Like IRF-2, ICSBP is constitutively expressed but exhibits a cell-type-restricted pattern since ICSBP is expressed in cells of the lymphoid/macrophage lineage. The use of ICSBP-deficient mice has demonstrated that, in some tissues, IFN-A and IFN-B gene expression was not markedly modified after virus induction, suggesting that ICSBP has a limited role in the repression of IFN genes [67].

The PU.1 interaction partner (Pip)/lymphoid-specific IRF (LSIRF) in adult T-cell leukaemia cell lines or activated T cells (ICSAT) is presently called IRF-4 [68–71]. Just like IRF-2 and ICSBP, human ICSAT protein exhibits a negative effect on IRF-1- or IFN-mediated induction, but the murine Pip/LSIRF protein associated with the PU.1 protein acts as an activator of transcription. The effect of Pip/LSIRF knockout on the expression of IFN genes has not been examined [71]. IRF-7 is associated with Epstein-Barr virus latency and is also able to repress transcriptional activation by both IFN and IRF-1 of an element containing an ISRE [72].

Another repressor factor that binds the PRDI of VRE B – PRDI-BF1 – has been isolated [73]. PRDI-BF1 is not related to the IRF family; it is constitutively expressed at low level and, like IRF-2, is a virus-inducible gene. PRDI-BF1 is considered to be a postinduction repressor of the IFN-B gene (fig. 1B). NF-κB/Rel activity on PRDII-dependent virus induction of the IFN-B gene is modulated by the κB inhibitory proteins (IκB) [34, 74–77]. After induction, de novo synthesis of IκBα restores the level of this inhibitor protein and contributes to postrepression of the IFN-B gene by sequestering NF-κB in the cytoplasm. The kinetics of virus induction of another isolated repressor, PRDII-BF1, suggests that it might be a PRDII-postrepressor of the IFN-B gene, but its function in inhibition of virus induction has not been clearly demonstrated [78].

Another protein may repress NF-κB/Rel activity on the PRDII of the IFN-B promoter. The transcriptional corepressor dorsal switch protein (DSP-1) has been isolated from *Drosophila* and contains two HMG domains [79]. DSP-1 can convert NF-κB from a transcriptional activator to a repressor. This effect requires the negative regulatory element (NRE) present in NRDI, which partially overlaps PRDII [80]. However, the mammalian equivalent DSP-1-like protein has not yet been isolated.

Much of the recent work in the study of the differential expression of IFN-A genes after virus induction has been devoted to the substitutions present in the VRE as well as to the characterization of the corresponding DNA-binding factors of the related activators [23, 57, 58, 81–83].

On the other hand, in the distal portion of the promoter of the IFN-A11 gene upstream of VRE-A, a negative acting region called DNRE (distal negative regulatory element) has been defined [84, 85]. This sequence displays an inhibitory effect on the inducibility of a VRE-A4- containing promoter independent of its orientation or its position and is considered to be a silencer region. The presence of this novel element, located upstream of the VRE-A of the IFN-A11 promoter, may also exert a more general modulatory effect on the transcriptional regulation of the promoters of other IFN-A genes. Indeed, in murine and human cell lines, the same distal silencer active element, DNRE, is present in both murine IFN-A11 and IFN-A4 promoters, and similar constitutive DNRE-binding proteins could be implicated in negative regulation. Until now, two DNRE-binding factors have been described. One of them has been identified as related or identical to the HMGI(Y) protein. The other has not yet been identified and has a molecular mass of 38 kDa. This protein may be related to the silencer activity. Binding of the HMGI(Y)-related factor does not seem to modulate the binding to DNRE of the 38-kDa protein, which is present in uninduced as well as in virus-induced nuclear extracts from murine and human cell lines (fig. 3).

The presence of a *cis*-acting region located between the DNRE and the VRE-A of the IFN-A4 promoter appears to overcome the DNRE silencer activity and therefore is considered to be an antisilencer [85]. The presence or absence of silencer and antisilencer elements could play a role in the differential expression of IFN-A genes.

Signal transduction at the type I IFN receptor level

The IFNAR1 chain, the first component of the type I IFN receptor, was cloned in 1990 [86]. It is a 557-amino acid precursor with a predicted 27-amino acid leader sequence and a single transmembrane segment of 21 amino acids. The predicted molecular weight of human IFNAR1 is 63,000; but the molecule is heavily glyco-

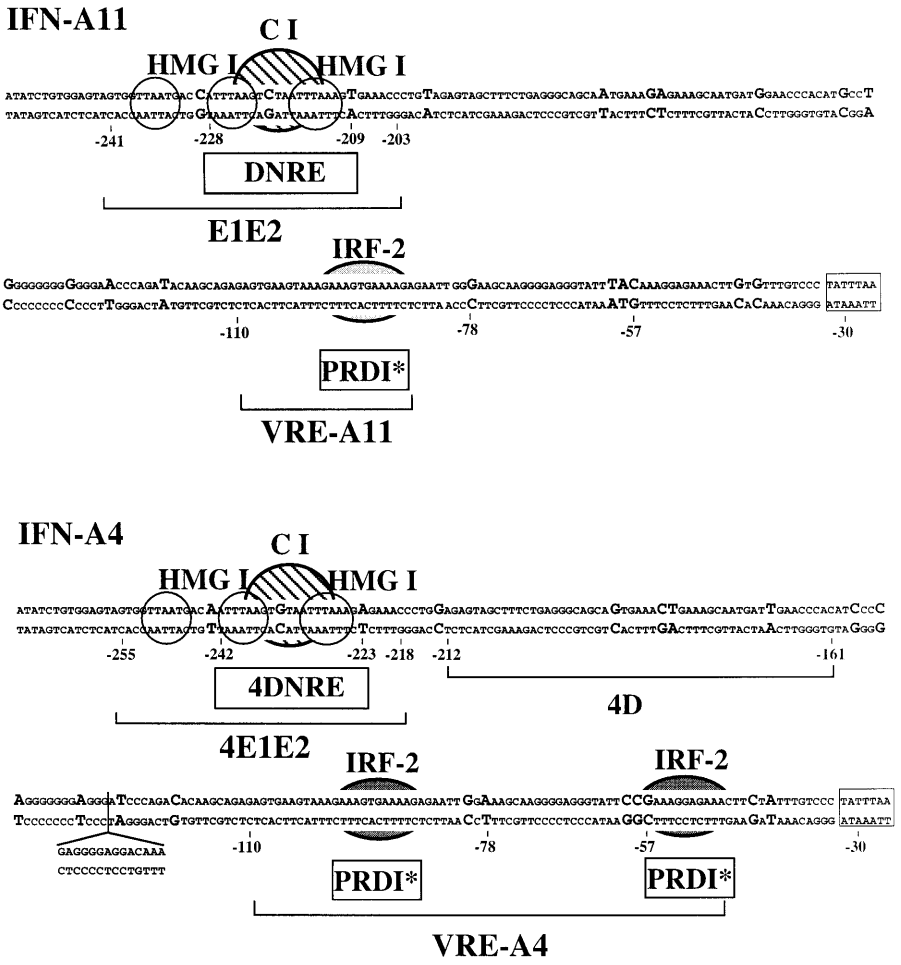

Figure 3. Model for the interaction of constitutive and postinductional repressor with the murine IFN-A11 and -A4 gene promoters. IFN-A11 and -A4 promoter sequences are indicated; the numbering is relative to the transcription start of the promoter, the TATA box being indicated in each case. Nucleotides distinguishing these sequences are represented by larger characters. Localization of the binding sites for potential *trans*-repressors and *trans*-activators, namely DNRE and 4DNRE within E1E2 and 4E1E2, respectively, PRDI-like domain (represented by PRDI*) and the factors binding to these domains are indicated: IRF-2 binds to PRDI* within the VRE-A11 and -A4; CI binds to DNRE and 4DNRE; HMGI binds to AT-rich regions within E1E2 and 4E1E2.

sylated, and in western blot, anti-IFNAR1 antibodies recognize a molecule of around 110 K [87]. The 409-amino acid extracellular portion is organized into two domains of about 200 amino acids (D200), each showing an internal duplication. This structure appears to be a duplication of D200, characteristic of the extracellular portion of receptors belonging to the helical cytokine receptor family [88]. The 100-amino acid intracellular portion of IFNAR1 does not carry any recognized enzymatic activity, but it has an essential role in interaction with signal transduction components. Quite early it was suspected that cloning IFNAR1 was not the end of the story, and in 1992 the requirement for an additional component which would bind IFN, together with IFNAR1, was demonstrated by studying the behaviour of two murine IFN-α subtypes in murine IFNAR1-transfected homologous or heterologous cells [89]. In addition, cross-linking studies showed that IFN was also in close contact with another protein, antigenically distinct from IFNAR1 [90].

Meanwhile, the three-dimensional (3D) crystal structure of murine IFN-β was determined [91]. The four-α-helical bundle structure of murine IFN-α definitively put the IFN in the helical cytokine class, as the basic structural framework is shared by many molecules that interact with receptors from the cytokine receptor family [92]. Together with the elucidation of the crystal structure of the growth hormone-receptor complex [93], the 3D information is now making new sense of the considerable structure-function work that had been performed on IFN during, and even prior to, the 1980s. All these results were constructively reviewed by Mitsui

[94]. New human IFN mutants were constructed and expressed, and interactions between IFNAR1 and part of the IFN-α molecule, containing elements of the A and C α helices, were described. It was proposed that the putative second component of the receptor (IFNAR2) likely binds to a site on the opposite side of the IFN molecule (the side composed by α helix D and the large loop between α helix A and B), where a number of residues were known to be particularly sensitive to mutational changes [95]. More recently, the crystal structures of both human IFN-α2 (96) and -β [97] have been elucidated. While their 3D structures superimpose well on that of the murine IFN-β, which crystallized as a monomer, the asymmetric unit of both human forms turned out to be a zinc-linked dimer.

For a time, IFNAR2 remained a functional concept, but thanks to the work of M. Rubinstein's laboratory, a soluble IFN-binding protein was purified from human urine and a complementary DNA (cDNA) encoding a transmembrane protein with a short 67-amino acid intracytoplasmic portion was cloned [98]. It was later shown that the corresponding gene produces several alternatively spliced transcripts encoding a soluble form and two transmembrane forms, one with the short intracytoplasmic tail and one with a 251-amino acid long intracytoplasmic portion [99]. The physiological function of both the soluble and short transmembrane forms is still unknown, but the long transmembrane form meets the requirements for a functional IFNAR2 component [99, 100]. Like IFNAR1, IFNAR2 belongs to the helical cytokine receptor family. The two genes are clustered on human chromosome 21, together with CRFB-4 [an orphan cytokine receptor now recognized as a component of the interleukin (IL)-10 receptor] and IFNGR2 (a component of the IFN gamma receptor) [99]. The extracellular domain of IFNAR2 is a single D200 domain (243 amino acids, including a 26-amino acid long predicted leader sequence). Functional IFNAR2 is detected as a band of about 95 K in western blot [101].

Knockout mice lacking the IFNAR2 gene have not yet been described. However, two strains of mice with a disturbed IFNAR1 gene have been studied [102, 103]. These mice are more or less normal: they show only minor differences in their immune responses compared with wild-type mice. However, they die rapidly if they are infected with a virus such as vesicular stomatitis virus (VSV) at normally nonlethal doses.

Study of the signalling mechanisms of IFN has led to characterization of genes transcriptionally induced by IFN and of their DNA response elements, to purification of IFN-regulated transcription factors, to cloning of the Stat proteins and to the discovery that Jak tyrosine kinases play a central role in IFN signal transduction analogous in a sense to that of the intrinsic intracellular kinase domains of certain growth factor receptors [104]. Excellent reviews by Darnell et al. [105], Schindler and Darnell [106], Levy [107], Pellegrini and Dusanter-Fourt [108] have been published.

The immediate early response of a cell following binding of IFN to its receptor is the transcriptional activation of a set of genes containing a conserved response element called ISRE (IFN-stimulated response element) in their promoter. The transcription of a subset of these IFN-inducible genes is also activated by virus infection [109]. Recent experimental evidence shows that IRF-3 and IRF-7 play an essential role both in the induction of the IFN-B gene and in this subset of IFN-inducible genes [110]. ISRE binds the ISGF-3 transcription factor, which is composed of Stat1, Stat2 and p48, a DNA-binding protein belonging to the IRF family. Stat1 and Stat2 are present in untreated cells. Following IFN treatment, they are activated by phosphorylation on a single tyrosine in a set of reactions which are likely to occur at the IFN-receptor complex level. The phosphorylated Stats then dimerize through their SH2 domains and translocate to the nucleus to bind the ISRE sequences that participate in transcription induction.

Tyrosine phosphorylation of Stats by type I IFN requires the Jak kinases Tyk2 and Jak1, which are likely to interact with the intracytoplasmic portions of IFNAR1 and IFNAR2, respectively. The more recent model for the mechanisms of receptor activation of Stat proteins presented by Li et al. [110] suggests that Stat1, Stat2 and Jak1 are preassociated on IFNAR2. When IFNAR1 and IFNAR2 aggregate on binding of IFN, the tyrosine 466 of IFNAR1 becomes phosphorylated by Tyk2 or Jak1 to provide a docking site for the SH2 domain of Stat2, which is then phosphorylated on a tyrosine by Tyk2 or Jak1, allowing contact with the SH2 domain of Stat1. Stat1 and Stat2 then dissociate from the receptor-Jak complex and translocate to the nucleus. There is very little information on the transport mechanism of Stat1 and Stat2.

The description of the sequential events leading to the regulation of IFN-induced transcription is certainly not complete without mentioning a recently discovered family of cytokine-inducible inhibitors of Jak kinases and Stats [111–113]. Other enzymatic activities such as that of phospholipase A2 or protein tyrosine phosphatase [114, 115] may also be implicated in regulation of the Jak/Stat pathway. Other signalling pathways may also contribute to IFN action: MAP kinase (ERK2) [116]; the protein arginine methyl transferase [117], which has been found bound to IFNAR1; phosphatidylinositol-3-kinase, which has been shown to interact with IFNAR1 through Stat3 [118]. Moreover, an essential factor for IFN signalling, distinct from receptor components, has been mapped on the distal part of human chromosome 21 between the 10;21 and r21 cytogenetic breakpoint

[119–121]. Cells lacking Stat2 do not respond to type I IFN [122]. Cells lacking Stat1, as well as mice carrying a disrupted Stat1 gene, are also completely resistant to type I IFN but also to type II IFN, since the IFN-γ-induced transcription factor is a dimer of Stat1 [123]. To sustain type I IFN actions, the tyrosine kinase activity of Jak1 is an essential activity [124, 125]. This is not so for the other tyrosine kinase, Tyk2, since, while cells lacking Tyk2 cannot indeed sustain the activity of IFN-α, they nevertheless present reduced sensitivity towards IFN-β [126].

The structure of Tyk2, like that of other Jak kinases, can be subdivided into three major domains: an amino-terminal domain of about 600 amino acids (N), a carboxy-terminal tyrosine kinase domain of about 250 amino acids (TK) and a central domain called kinase-like (KL) because it presents a high degree of homology with the TK domain but is devoid of some of the essential amino acids needed for tyrosine kinase enzymatic activity [127]. Cells lacking Tyk2 do not bind type I IFN like wild-type cells: the dynamics of binding of both IFN-α and -β are changed, and the binding affinity is lower for IFN-α but not for IFN-β [128]. Moreover, it has been found that cells lacking Tyk2 express only a low level of IFNAR1 compared with wild-type cells [129]. The level of IFNAR1 expression is restored by the introduction of the N domain of Tyk2; the high-affinity binding of IFN-α requires N plus KL domains; and wild-type behaviour for binding and biological activities of both IFN-α and -β calls for a full-length Tyk2 and its tyrosine kinase activity [128–130]. Tyk2 is also implicated in the functioning of other cytokine receptors, such as IL6-R, IL10-R and IL12-R [108]. It would be interesting to know if, in these receptor systems, a similar partition between domains and receptor expression, high-affinity binding and signal transduction is also found.

The type I IFN receptor system has been a valuable and informative experimental system for studying the functioning of cytokine receptors. Many aspects are still intriguing, in particular the functional relationship between ligand binding on the extracellular portions of receptor components and the intracytoplasmic kinases. Although there has been considerable success in identifying and ordering the elements of the signal transduction pathway, there are still many open questions concerning the up- and downregulation of the system.

Concluding remarks

In the 40 years since the discovery of interferon, the IFN family of genes and proteins has grown in the number and diversity of its functions.

Much is now known about the mechanism of transcriptional activation of IFN-B gene expression with the protein-protein interaction between the transactivators on an enhanceosome structure. Nevertheless, IFN-A gene virus induction, from the cell surface to the nuclear activation of the primary factor required to start IFN-A gene transcription, remains to be precisely defined. Although progress in isolating and cloning transcriptional factors (activators or repressors) have led to a better functional scheme, the production of deficient mice for the different factors involved in IFN expression has already revealed, and will continue to reveal, the real involvement of these factors in vivo, their role, and their dependent and independent pathways for type I IFN gene regulation as well as for type I ISGs. Important studies on the IRF family and on the factors implicated in ISG transcription, as well as on IFN receptors, have already been conducted, and have been discussed in this review. Isolation and identification of the Stats and of the Jak and Tyk kinase involvement as well as the structure of the IFN receptors (IFNAR1, IFNAR2) has not only clarified the signal transduction pathway of IFNs but has also contributed to defining the complex and crucial roles of the Stats for a greater understanding of the diverse effects of IFNs.

The new concept arising from the discoveries of some of these factors is that of identical factors involved in signal transduction of virus and of IFNs. A complete dissection of the mechanism of regulation of IFN gene expression would help in understanding the consequences of dysfunctional expression of these genes and of their negative and positive transcriptional factors. This is crucial for the development of clinical applications of members of the IFN family in viral and immunopathogenic diseases.

IFN-α and its related compounds have indeed been used for more than 10 years in the treatment of a number of diseases, including viral illnesses, childhood haemangiomas, various cancers and leukaemia. Thus, IFN-α is used mainly as a standard therapy or in combination with other antineoplastic agents for hairy cell leukaemia [131], for chronic myelogenous leukaemia [132], metastazing renal carcinoma and acquired immunodeficiency syndrome (AIDS) associated Kaposi sarcomas [133]. However, the use of IFNs is limited by severe side effects. Recently, the ability of IFN-τ to inhibit human immunodeficiency virus (HIV) replication at noncytotoxic doses was described and interest in its role in the design of future HIV-derived diseases is increasing [134].

Whereas IFNα has emerged as a promising treatment for chronic viral hepatitis B and C, with a 30–40% chance of viral clearance for the hepatitis B carrier, IFN-B (IFNβ1b) was the first therapeutic intervention shown to convincingly reduce multiple sclerosis (MS)

relapse rates and to retard disability [135]. MS is an inflammatory neurological disease resulting from immune attack and demyelination of nerve fibres. Roughly 15 different viruses have been associated with MS without a single one emerging so far as the strongest candidate. These viruses could initiate the immune disorders leading to demyelination of MS via a common mechanism, and the effect of IFN-β may correspond to its antiviral as well as its immunomodulatory properties [135, 136].

The exact mechanism of IFN action remains to be demonstrated, and studies on the regulation both of IFN gene expression and of ISG expression are currently providing new clues for understanding IFN biological activity.

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