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Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity

David E Levy*, Isabelle Marié and Arun Prakash

The genes for type I IFNs (IFN-α and IFN-β) are rapidly induced in response to viral infection. IFN regulatory factor (IRF) proteins are key to the regulation of IFN gene expression; the early response to virus results in secretion of a subset of IFN genes through the action of IRF3 in conjunction with additional transcription factors, such as NF-κB and AP-1 (c-jun–ATF2). This early IFN acts in an autocrine manner to stimulate the production of IRF7, a transcription factor capable of activating the many additional members of the IFN-α gene family. The dependence of IRF7 on virus-induced phosphorylation for its activity ensures that IFN production is limited to virus-infected cells. Additional members of the IRF family may provide additional levels of control, in both a cell-type and virus-specific manner, particularly in dendritic cells that serve as major producers of IFN and a key interface between innate and adaptive immunity.

Addresses
Molecular Oncology and Immunology Program, Department of Pathology and New York University Cancer Institute, New York University School of Medicine, New York, NY 10016, USA
*e-mail: levyd01@med.nyu.edu

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Abbreviations

DC	dendritic cell
ds	double stranded
IFN	interferon
IPC	IFN-producing cell
IRF	IFN-regulatory factor
TLR	Toll-like receptor

Introduction

Since the discovery of interferons (IFNs) in 1957 as endogenous inhibitors of viral infection [1], considerable evidence has accumulated revealing that these host defense molecules play a vital role in innate resistance to a wide variety of infectious agents through the induction of direct, cell-autonomous resistance to viral and microbial pathogens. In addition, they have been increasingly recognized as essential early warning molecules signaling the presence of pathogens, providing a pivotal function at the interface between innate and adaptive responses. Thus, they provide potent regulatory interactions with the adaptive immune system, for example, by activating the microbiocidal functions of macrophages, upregulating antigen processing by professional antigen-presenting cells, and inducing cytokine expression that modulates lymphocyte and myeloid cell function (for recent reviews, see [2–8]). Given the potency of IFN-mediated host defense, it is understandable that this family of cytokines is tightly regulated, being expressed at nearly undetectable levels in uninfected individuals, but rapidly induced to high concentrations following infection.

It is also understandable that essentially all cells of the body are capable of producing and responding to IFN, as nearly all cell and tissue types are susceptible to infection. However, not all cells are created equal in this regard. In particular, a specialized type of dendritic cell (DC) capable of producing extremely large amounts of IFN following viral infection was identified in human blood, and designated the natural IFN-producing cell, or IPC [9,10]. More recently, a similar cell type characterized by plasmacytoid morphology and the absence of haematopoietic lineage markers has been identified in the T cell zones and inflamed lymph nodes of mice [11••,12••]. These cells appear to be a key cell type for translating the detection of invading pathogens into signals that can be read by the adaptive immune system. DCs are the major antigen-presenting cells of the adaptive immune response, but are also poised to directly detect incoming pathogens by virtue of a series of pattern-recognition receptors (PRRs), which recognize a diverse array of nonself features expressed by pathogens [13]. Intriguingly, DCs are a heterogeneous population of cells, not only in terms of the PRRs that they express, allowing them to recognize distinct families of pathogens, but also in terms of the effector molecules they produce. Thus, different DCs may be specialized to respond to distinct pathogens and do so in an individualized manner to produce an appropriately polarized response.

Transcriptional regulation of type I IFN genes

IFN expression in response to virus infection is regulated primarily by changes in gene transcription. In this regard, induction of the human and mouse IFN-β genes has been most intensely studied. It is clear that immediate-early gene induction (that is, new transcription that does not rely on ongoing protein synthesis) depends on the coordinated action of a series of transcription factors, each of which is activated by phosphorylation from a latent state in response to viral infection. These transcription factors

include the classically inducible transcription factor complex NF-κB, which is activated by phosphorylation-dependent destruction of its cytoplasmic inhibitor, IκB. Other activated transcription factors include the AP-1 complex (composed of c-jun and ATF2) and one or more IFN-regulatory factor (IRF) proteins, all of which are activated by virus-induced phosphorylation. When activated, these three transcription factor complexes interact with the IFN-β gene transcriptional control region in a concerted and highly cooperative fashion, leading to the efficient recruitment of transcriptional coactivators, the basal transcriptional machinery and RNA polymerase holoenzyme [14].

But IFN-β is not the only type I IFN gene. In addition, this family is composed of a large number of IFN-α subtypes plus the additional family members δ, κ, λ, τ, and ω, all of which are clustered on human chromosome 9 and mouse chromosome 4. The multitude of type I IFN genes conserved throughout vertebrate evolution has remained a puzzle, especially as they all interact with a single cell-surface receptor complex that appears to couple to a uniform signal transduction cascade [15]. Hints of distinct functions for the individual IFN subtypes have come from two areas. First, it appears that the subtypes are not fully equivalent, at least from a functional point of view. Thus, different subtypes display distinct bioavailabilities and pharmacokinetics. More intriguingly, they may also signal in subtly distinct manners, the best example of this is IFN-β. The common type I IFN receptor complex couples to the tyrosine kinases Jak1 and Tyk2; however, IFN-β is capable of some residual signaling even in the absence of Tyk2, although all other type I IFNs are significantly impaired [16]. Moreover, whereas different type I IFN isotypes induce the same set of target genes, IFN-β also induces some unique genes [17]. The mechanism for the unique action of IFN-β remains unclear [18], but the existence of genes uniquely induced by IFN-β underscores the possibility of additional isotype-specific functions for individual type I IFN species.

A second suggestion of distinct functions for IFN isotypes comes from the unique regulation of IFN-α gene expression. IFN-α gene expression bears superficial similarities with the kinetics and pattern of IFN-β induction, but this similarity contradicts the absence of some of the mechanistic aspects critical for IFN-β transcription. For example, many of the well-characterized IFN-β enhanceosome proteins are dispensable for IFN-α transcription (e.g. there is no characterized role for NF-κB or AP-1). However, similar to the IFN-β gene, IFN-α genes rely on activated IRF proteins for their inducible expression [19]. Significantly, IFN-α genes are not regulated as a cohesive block but rather show differential, subtype-specific patterns of expression due to differential requirements for individual IRF proteins. Specifically, some subtypes rely on IRF3 whereas others require IRF7 for their induction [20]. For example, in the mouse IFN-α4 and IFN-β can be induced by IRF3 alone, whereas many other IFN-α subtypes, such as IFN-α2, -α5, -α6 and -α8, require the presence of IRF7.

IRF family members and the differential expression of IFN-α genes

The IRF family consists of nine mammalian proteins characterized by an amino-terminal DNA-binding domain that contains a tryptophan repeat motif distantly related to c-myb [21]. IRF3 and IRF7 are closely related to each other and share several structural and functional characteristics. Both proteins exist in a monomeric latent state and rely on intramolecular interactions that are disrupted following virus-induced phosphorylation to induce dimerization and activation. Both proteins also bind similar DNA sequences, and their activity and subcellular localization are similarly governed by virus-induced phosphorylation at a related, carboxy-terminal regulatory domain. Although other regions of both IRF3 and IRF7 are phosphorylated, it is phosphorylation within this common regulatory domain that is dependent on virus infection and is, therefore, essential for the full activity of the proteins. Phosphorylated IRF3 and IRF7 activity can be mimicked by substitution of individual serine residues with phosphomimetic aspartate residues [22]; however, the exact sites of virus-induced phosphorylation within the regulatory domains have yet to be definitively established. It appears that a single virus-activated kinase cascade targets both IRF3 and IRF7 activation [23•–25•].

A major distinction between IRF3 and IRF7 is that IRF3 is constitutively expressed in most cell types, whereas IRF7 is expressed only following exposure of cells to IFN. This requirement for IFN-dependent induction of IRF7 was key to understanding its role in differential IFN-α gene expression. Because IFN is synthesized and secreted during viral infection, IRF7 is induced and can contribute to further IFN gene expression. In the absence of IFN signaling (e.g. in cells lacking IFN receptors or the STAT1 transcription factor), IFN production is solely dependent on pre-existing components, such as IRF3. Using mouse cells deficient in IFN signaling, not only was the response to IFN impaired but the production of IFN, particularly IFN-α, was also severely curtailed [20,26]. The reduced quantity of IFN produced and additional qualitative differences in the pattern of IFN-α subtype distribution also characterized the absence of IRF7 [20]. Specifically, only IFN-β and IFN-α4 are induced by virus infection of STAT1-deficient or IFN receptor-deficient mouse cells [20]. These findings led to the hypothesis that induction of IFN-β and IFN-α4 occurs through an immediate-early signaling pathway dependent entirely on pre-existing protein components, such as IRF3 [27–29]. By contrast, induction of other species of IFN-α, such as

IFN-α2, -α5, -α6 and -α8, requires the additional induction and activation of IRF7 [20]. The induced accumulation of IRF7 appears to be solely responsible for the augmenting effects of autocrine IFN, as ectopic expression of recombinant IRF7 is fully capable of substituting the requirement of IFN signaling for the induction of the delayed class of IFN-α genes [20,26,30]. This model has been recently validated by the analysis of IRF3-deficient mice [31].

The distinct utilization of trans-acting factors by IFN-β and IFN-α4 in comparison to the requirements of other IFN-α genes reflects the distinct DNA-binding specificities of IRF3 and IRF7 [32]. Although both IRF3 and IRF7 contain a typical IRF DNA-binding domain, they each exhibit distinct patterns of interaction with target sequences. IFN-α genes display variability within their promoters; some are targets for IRF7 but not for IRF3, whereas others can bind both transcription factors [33]. It was also discovered that IRF3 efficiently recruits coactivator proteins, such as CBP, although IRF7 does not. It is possible that the concerted recruitment of coactivator proteins also modulates promoter selection by cooperative interaction with other promoter factors.

These observations led to the model of IFN-α gene expression divided into three distinct phases (Figure 1). First, the immediate-early or sensitization phase involves the induction of IFN-β and IFN-α4 through the activation of IRF3 and other latent transcription factors following virus-induced phosphorylation (Figure 1a). Rapid but low-level synthesis of IFN-α4 and -β results in positive autocrine feedback by the induction of IRF7 protein production, due to IFN-dependent JAK-STAT signaling during a second, IRF7 induction phase (Figure 1b). Continuing viral infection leads to the phosphorylation of newly synthesized IRF7, resulting in its activation and the induction of additional members of the IFN-α gene family during a delayed early or amplification phase of the IFN response (Figure 1c). This model provides a mechanism for the tight regulation of IFN production. The initial production of IFN requires the sensing of a viral infection by the innate immune system. If the low levels of primary IFN initially produced early during infection prove sufficient to successfully block viral replication, further IFN production is curtailed because IRF7 protein accumulated in this phase remains latent. However, with continued viral infection, the newly synthesized, latent IRF7 can now undergo phosphorylation and subsequent activation, resulting in the potent induction of numerous IFN-α species and the secretion of high levels of IFN-α.

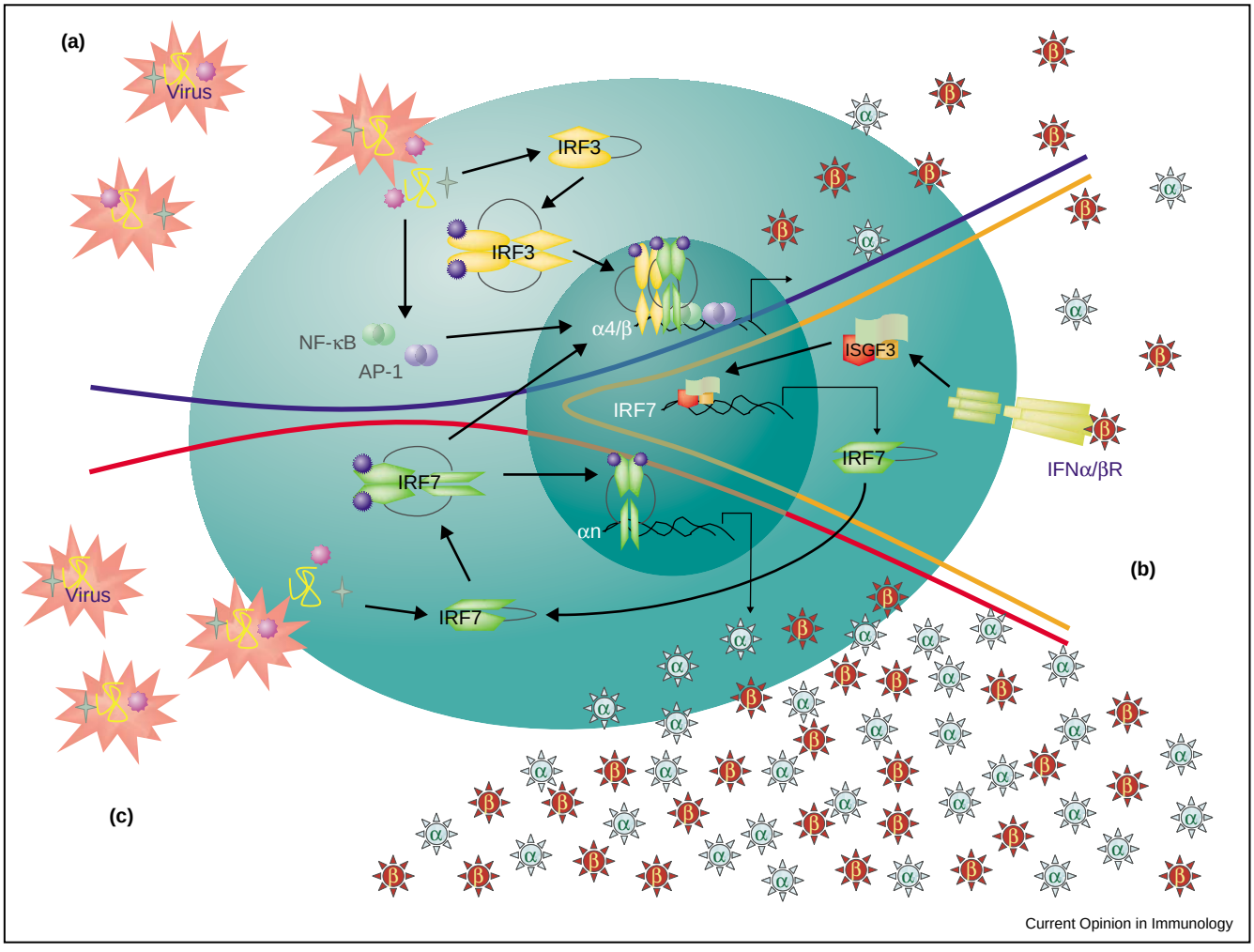
Differential regulation of the multiple IFN-α genes suggests possible reasons for the conservation of the many members of this multigene family. One hypothesis is that the maintenance of a battery of individual genes is maintained as a means of modulating different levels of IFN when needed. Rather than the perhaps more complex mechanism of regulating the level of expression of a single gene by several distinct rates of transcription, increasing amounts of IFN production can be achieved by activating different numbers of individual genes, each as a relatively simple on/off switch. As the activation of IFN gene transcription is always linked to the continued presence of virus through IRF phosphorylation, IFN is only produced when needed. The constitutive production of IRF3 in most cell types allows the rapid induction of small amounts of IFN following virus infection. Induction of IRF7 as an autocrine response provides efficient amplification, as required.

Further support for this idea has come from the characterization of an additional IRF family member, IRF5. Similar to IRF7, this protein also targets a subset of IFN-α genes, although a different subset from that activated by IRF7 [34••]. Moreover, IRF5 is also expressed in a limited set of cells but is inducible in response to viral infection and autocrine IFN [34••]. Intriguingly, IRF5 is differentially sensitive to viral infection, as it is targeted for phosphorylation only by some viruses and not others. Unlike IRF7, which is phosphorylated in cells infected by a variety of virus types, IRF5 is phosphorylated in cells infected by Newcastle disease virus (NDV), vesicular stomatis virus (VSV) and herpes simplex virus, but not in cells infected with Sendai virus [35]. Moreover, the IFN-α genes activated by phosphorylated IRF5 are virus specific, with different patterns of IFN-α production induced in response to VSV than those induced by NDV. This result raises the interesting possibility that different IFN-α isotypes have evolved to fight infection by distinct pathogens, mirroring their pattern of regulation. There is still one additional IRF family member, IRF6, whose biological function remains to be elucidated (GenBank accession number U73029). Perhaps IRF6 also targets a subset of IFN-α genes that are activated by specific viruses. Interestingly, the *Xenopus* homologue of mouse IRF6 is expressed in a temporally and tissue-specific manner during development, raising the possibility of additional complexity [36]. It is also possible that other IRF proteins, although initially characterized with distinct functions, could also play a role in IFN production under select conditions.

IFN synthesis and the dendritic cell connection

The model for differential regulation of IFN gene expression described above has been predominantly elucidated in fibroblasts and epithelial cells. Such cells are found at sites of primary viral infection, such as in the respiratory tract, and are important sentinels of pathogen attack. Historically, however, IFN-α synthesis was first detected in leukocytes, and the IPC subset of DCs appears to be the major IFN producer during infection [9,10,11••,12••]. DCs form an important interface between innate and

Figure 1



Multiphasic induction of murine type I IFN genes. Type I IFN genes are differentially regulated, dependent on the abundance of distinct transcription factors, and can be divided into three phases. **(a)** The immediate early (sensitization) phase. Early induction of type I IFN gene expression is governed by an immediate-early, sensitization phase dependent exclusively on pre-existing cellular components. Virus infection stimulates a phosphorylation cascade, leading to the activation of at least three families of transcription factors, including NF-κB, AP-1 and IRF3. Activation of the IFN-β promoter requires all three transcription factors, whereas the mouse IFN-α4 promoter appears to require only activated IRF3. Additional members of the IFN-α gene family (α_n) are insensitive to these transcription factors and therefore remain dormant. **(b)** IRF7 induction phase. Secretion of early IFN produces an autocrine response through stimulation of the JAK-STAT pathway by the IFN receptor system, leading to activation of the trimeric transcription factor complex, ISGF3. Among the genes activated by ISGF3 is IRF7, leading to accumulation of this protein only in response to IFN production. **(c)** Delayed early (amplification) phase. IRF7 is produced as a latent protein; however, the continued presence of viral infection leads to its phosphorylation and subsequent activation. Many members of the IFN-α gene family (α_n) possess promoter binding sites for activated IRF7 and become transcriptionally active during this late phase of delayed-early IFN induction. Additional IRF family members, such as IRF5, may similarly differentially regulate subsets of the IFN-α gene family in response to distinct viruses.

acquired immunity, both as producers of essential immunomodulatory cytokines and as major antigen-presenting cells required for activation of T cell maturation and proliferation [37,38]. These cells are also essential guardians against pathogen attack, as they are resident in most tissues and therefore ideally situated to encounter invading organisms.

Recent work has revealed the increasingly complex nature of DCs. They can be crudely divided into myeloid-like and lymphoid-like on the basis of cell surface proteins, internal markers, and unique patterns of gene expression [39•], with the IPC falling in the lymphoid-like class. However, considerable microheterogeneity of DCs exists. A major distinction between different DCs is the cytokine profile that they produce, and their differential ability to respond to distinct stimuli. For example, they can be classified by the subsets of Toll-like receptors (TLRs) they express, and there is a good correlation between TLR expression and pathogen recognition

[40]. Interestingly, TLR3, which is a dsRNA receptor and therefore possibly involved in viral recognition [41••], is found not on IPCs or plasmacytoid DCs but rather on myeloid DCs. By contrast, TLR9, a receptor for bacterial DNA [42], is found on IPCs [43]. Indeed, bacterial DNA is a potent activator of IFN-α production by IPCs, whereas dsRNA is not, although it is an effective IFN-α inducer in myeloid DCs [44••]. Viral infection potently induces IFN-α production in IPCs as expected, raising a question as to the relevance of the dsRNA-specific TLR3 for viral infections. IPCs also express TLR7, which is capable of signaling the induction of IFN-α gene expression in response to synthetic IFN inducers, although the natural ligand for this receptor remains undefined [45••], making it difficult to judge its importance for viral recognition. Adding to the potential complexity of TLRs as markers of DC subsets, the pattern of TLR expression is itself modulated in response to IFN and viral infection, with TLR1, TLR2, TLR3 and TLR7 expression induced by IFN-α treatment [46••]. Therefore, it remains unclear whether TLRs serve as sentinels for virus in a similar manner to their clearly defined role in detecting bacterial and fungal infection.

An intriguing, but as yet largely unexplored, question is whether differential IRF protein expression resulting in the ability to express distinct isotypes of IFN-α might also be a characteristic of DC subtypes. For example, IFN-α production by DCs is much less sensitive to positive autocrine feedback than IFN production by peripheral cells; DCs also produce a different spectrum of IFN-α subtypes than fibroblasts [47••]. In addition, there is a question of whether IFN-α production (or indeed synthesis of other cytokines) is a stable characteristic of DC subsets or if it is dynamically regulated during the maturation of a response. Indeed, the typical cytokines produced in response to infection (e.g. TNF-α, lymphotoxin, IL-6 and IL-12) set up an interacting network that shapes the ongoing response. For example, IFN-α is an inhibitor of IL-12 production during viral infection, effectively limiting its production to IPCs by negatively regulating its synthesis by other cell types [48•]. At the same time, significant synergy exits between TNF family members and virus-induced IFN production [49•]. TNF is also an effective inducer of IRF7 expression [50•] and might therefore further modify the pattern of specific IFN production. At least one IRF protein, IRF4, is differentially regulated among DC subtypes and is highly expressed in IPCs [39•]. Further characterization of possible differential IRF protein expression and consequent unique patterns of IFN-α isotype expression by distinct DC subsets may be a fruitful avenue for future research.

Conclusions

The induction of type I IFN gene expression is an essential component of innate immunity to viruses and other microbes. Precise molecular mechanisms have been defined, which allow rapid and robust induction of type I IFN genes in response to infection. Cross-talk between the induction of IFN gene expression and subsequent IFN-mediated induction of IRF7 accumulation is one example of this precise control, using positive feedback and multiple IFN-α target genes to achieve highly regulated levels of active IFN. Most cells are capable of producing and responding to IFN, but molecular mechanisms in individual cell types differ. In particular, DCs are a major source of IFN and constitute an essential guardian against infection by acting at the interface between innate and acquired immunity. Microheterogeneity between DC subtypes creates the potential for considerable signaling cross-talk and specificity in cytokine production and response, enabling a coordinated, acutely controlled and individualized response to infection.

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