Published in final edited form as:
Biochimie. 2007 ; 89(6-7): 744–753.

The IRF family, revisited

A. Paun[1] and **P.M. Pitha**[1,2,*]

1 *The Division of Viral Oncology at the Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins School of Medicine Baltimore, Maryland 21231*

2 *Department of Molecular Biology and Genetics, The Johns Hopkins School of Medicine Baltimore, Maryland 21231*

Abstract

Since the discovery of interferon 50 years ago a great deal of progress has been made in understanding how interferons work and how and why they are induced. Key factors in interferon induction are the interferon regulatory factors (IRF). In this review of IRF we aim to show you not only the historical side of the IRF but also the integral, anti-viral and hematopoetic roles of these transcription factors, as well as the sometimes surprising and even forgotten roles that these proteins play, not only in interferon signaling but throughout the immune system and the body as a whole. Further research will no doubt expand the repertoire of these multifunctional proteins even more.

Introduction

It has been 50 years since the antiviral protein interferon (IFN) was first recognized. The molecular mechanism of the virus-mediated induction of Type I IFN has been under intensive investigation for the last 30 years. Remarkable progress has been made in recent years in the identification of cellular receptors detecting the invading pathogens as well as in understanding the signaling pathways leading to the induction of Type I *IFN* genes. This chapter will focus on the transcription factors of the IRF family, which play a critical role in the antiviral response. We would like this review to be both a historical and a future perspective.

The expression of the Type I IFN genes is strongly regulated and the IFN synthesis, induced by viral infection is generally transient. Deregulated production of IFN is associated with some of the autoimmune diseases. The expression of the IFN*A* and *B* genes is regulated both on the transcriptional and posttranscriptional levels. In a context of this chapter we will discuss only the transcriptional regulation. Initially the virus-mediated activation of IFN-*B* gene transcription served as a model for the study of inducible transcription. The sequence domain in the 5′ region of the IFN genes, termed the virus responsive elements (VRE), contains multiple GAAANN repeats which are highly conserved in both IFN-*A* and IFN-*B* gene promoters [1–4]. The stimulation of IFN-*B* gene transcription by viral infection or dsRNA is mediated by a ternary complex-enhanceosome consisting of NFκB, interferon regulatory factors (IRF), activated protein 1 (AP-1), JUN and the high mobility protein HMG-1, which are recruited to the VRE of the IFN-*B* promoter [5,6] This enhanceosome further recruits histone acetyl transferases (HAT) and the CREB binding protein (CREB).

*Corresponding author: Phone: +1 410 955 8871, Fax: +1 410 955 0840, Email: parowe@jhmi.edu.

The expression of the IFN-*A* gene subtypes is also regulated at the transcriptional level [7], however the VRE of IFN-*A* promoters do not contain an NFκB site, but have multiple AANNGAAA repeats which can bind members of IRF family [8–11]. IRF-1, IRF-3 and IRF-7 together with histone transacetylases are part of the transcriptionally active IFN-*A* enhanceosome [12]. The differential expression of individual IFN-*A* subtypes was shown to be due to distinct nucleotide substitutions in these domains [12–15] as well as the presence of negative regulatory sequences (DNRE) located in the upstream promoter regions of some IFN-A subtypes [16]. Thus while the activation of IFN-*B* gene transcription is regulated by both NFκB and IRF-3, activation of the IFN-*A* genes depends mostly on IRF.

The IRF-family

The IRF are transcription mediators of virus-, bacteria- and IFN-induced signaling pathways and as such play a critical role in antiviral defense, immune response, cell growth regulation and apoptosis. To date, nine human cellular *IRF* genes (*IRF-1, IRF-2, IRF-3, IRF-4/Pip/ICSAT, IRF-5, IRF-6, IRF-7, ICSBP/IRF-8* and *ISGF3γ/p48/IRF-9)* as well as virus-encoded analogues of cellular IRF have been identified [17,18]. These factors all share significant homology in the N-terminal 115 amino acids, which contains the DNA-binding domain and is characterized by five tryptophan repeats. Three of these repeats contact DNA with specific recognition of the GAAA and AANNNGAA sequences [19]. However, the unique function of a particular IRF is accounted for by a combination of cell type-specific expression, its intrinsic transactivation potential, and an ability to interact with other members of the IRF family or other transcription factors and co-factors [20]. All IRF but IRF-1 and IRF-2 contain the IRF associated domain (IAD) which mediates these interactions, in the, 3′ terminal part of the protein. The availability of genetically modified mice, which have distinct IRF deleted revealed that the function of IRF is not limited to the induction of Type I IFN genes (Table 1).

The first IRF: IRF-1 and IRF-2

The first IRF, IRF-1 and IRF-2 were identified through their ability to bind to the positive regulatory domain 1 (PRDI) in the VRE of the IFN-*B* gene and were assumed to function as an activator and repressor of the IFN-*B* gene, respectively [22]. However, homozygous deletion of IRF-1 in mice did not impair activation of IFN-*A* or IFN-*B* genes in infected MEFs, while dsRNA-mediated induction of TypeI IFN was down-regulated [23,24]. Subsequent studies have revealed that IRF-1 is involved in a broad spectrum of the antiviral defense mediated by IFN-γ. The induction of nitric-oxide synthetase (iNOS), guanylate binding protein and 2′,5′-OAS was impaired in IFN-γ-treated IRF-1 deficient MEFs [25,26]. Induction of iNOS and IL-12p35 was also inhibited in IRF-1 null myeloid DC. It was than shown that IRF-1 is effectively induced by IFN-γ and IFN-γstimulated expression of NO synthetase genes is mediated by IRF-1 [27,28]. While IRF-1 doesn't have a critical role in the virus stimulation of Type I *IFN* genes, the presence of IRF-1 was detected in the IFN-*B* enhanceosome binding to the IFN-*B* promoter region [29] as well as in the IFN-*A* enhanceosome [12]. Furthermore, analysis of the repertoire of lymphoid cells from IRF-1 null mice has shown defects in the maturation of $CD8^+$ T cells as well as a defective Th1 response, impaired production of IL-12 in macrophages and defective NK cell development [30]. These data indicate that IRF-1 has essential functions in the development and activation of various immune cells. In addition, IRF-1 also plays a critical role in the inducible expression of MHC class I and apoptosis; cells from IRF-1 deficient mice are resistant to UV- and drug-induced apoptosis [24].

While both IRF-1 and IRF-2 bind to the PRDI domain in the VRE of the IFN-*B* gene, this region also binds a protein named Blimp-1 which has an important role in the late stages of the B cells differentiation [31]. It was later shown that Blimp-1 recognizes the same DNA binding domain as IRF-1 and IRF-2, but not that of IRF-4 or IRF-8 [32]. It would therefore be interesting to examine whether IRF-1 or IRF-2 have any role in final stages of B cell

differentiation. In humans, polymorphisms in IRF-1 were shown to be associated with a predisposition to asthma in the pediatric population [33], and deletion of IRF-1 has been observed in myelodisplastic syndrome and leukemia [34].

IRF-2 was identified as a factor binding to the same recognition site as IRF-1, which suppresses its transcriptional activity. Over-expression of IRF-2 in NIH/3T3 cells resulted in oncogenic transformation of these cells. This effect has been attributed to the IRF-2 mediated inhibition of the proapototic and growth regulatory function of IRF-1. However IRF-2 was also shown to activate transcription of the *histone 4* gene [35] and inhibit N-Ras. In primary hematopoetic cells and myeloid cells, N-Ras functions as a growth inhibitor and over-expression of IRF-2 in a myeloid cell line reversed N-Ras-induced growth suppression [36]. The role of IRF-2 in the innate antiviral defense has not be yet clearly established, however IRF-2 null mice exhibit NK cell deficiency and IRF-2 deficient NK cells show an immature phenotype and compromised receptor expression, indicating that IRF-2 deficiency results in a defect in the late stages of NK cells maturation [37]. IRF-2 was also found to have a role in the development of myeloid DC [38]. A possible role for IRF-2 in adaptive immunity is indicated by the observation that IRF-2, together with STAT1, stimulates expression of the transporter of antigenic peptides to MHC class I (TAP1) by directly binding to the cytokine responsive region of TAP1 promoter [39].

The antiviral IRF: IRF-3 and IRF-7

While ectopic over-expression of IRF-1 in undifferentiated embyrionic stem (ES) cells stimulated the expression of Type I *IFN* genes, IRF-1 failed to bind the VRE of IFN-*A*. However mutations that disrupted the IRF binding site in the IFN-*A* promoter abolished its inducibility [40,41]. The search for a new IRF which could activate the promoters of IFN-*A* and -*B* genes led to identification of IRF-3 and IRF-7. The identification of these two IRF and their role in the transcriptional activation of Type IFN genes had a major impact on the understanding of the molecular mechanism of the pathogen induced innate antiviral response [8,42–44]. It became quite obvious that although pathogen recognition may be mediated by distinct cellular receptors and signaling pathways, they all lead to the activation of IRF-3 or IRF-7 which are critical for the transcriptional activation of Type I IFN genes [45,46].

The ubiquitously expressed IRF-3 [8,42] is activated in infected cells upon recognition of dsRNA, which has been considered the common signature of virus infected cells. Toll like receptor 3 (TLR3) or the cytoplasmic RNA helicases RIG-I and MDA-5, which are characterized by the presence of caspase recruitment domains (CARD) are important for the recognition of most RNA virus infections [47](rev in [45,48]. The TLR-3 and RIG-I/MDA5 signaling pathways lead to the phosphorylation of IRF-3 at the C' terminal region, where serine 386 is critical for activation by the two noncanonic IκB kinases; TBK-1 and IKKε [49–51]. Crystal structure analysis shows that, phosphorylation results in the structural changes which allow IRF-3 activation [52,53]. The activated IRF-3 then homo- or heterodimerizes with IRF-7 and translocates to the nucleus, where it associates with the CREB binding proteins CBP/p300. [54–56] and stimulates transcription of IFN-*B*, as well as of some interferon stimulated genes (ISG), such as *RANTES* and *ISG54* [57–59,60,]. While expression of IRF-3 alone is sufficient to activate the promoter of the IFN-*B* gene [60,61], the IFN-*B* enhanceosome contains not only IRF-3 but also IRF-7 [62,63]. The phosphorylated IRF-3 is under negative regulation by ubiquitin-mediated degradation [54] and it was shown that the propyl isomerase Pin1 targets activated IRF-3 for ubiquitin mediated degradation [64]. In contrast the IFN-induced ubiquitin-like protein ISG15 subverted the ubiquitin mediated degradation of IRF-3, stabilized IRF-3 in infected cells and increased its nuclear retention thus contributing to the enhancement of the host antiviral response [65]

Mice with a homozygous deletion of IRF-3 show impairment in the encephalomyocarditis virus (EMCV)-mediated induction of Type I IFN, while the antiviral response of IRF-3 null MEF against VSV was normal. However the expression levels of Type I IFN in NDV-infected MEF were substantially decreased, though IFN expression could be rescued by ectopic IRF-7 [63]. In contrast, in a recent study, the virus-mediated induction of Type I IFN was not significantly decreased either in IRF-3 null MEFs or pDC [66]. Despite these conflicting data, there is little doubt that IRF-3 plays a critical role in the antiviral response. Firstly its ubiquitous expression allows stimulation of the antiviral response and synthesis of IFNβ in all variety of infected cells [67] and secondly, even low levels of autocrine or paracrine IFNβ stimulates expression of IRF-7 and IRF-5 and triggers the amplification of the antiviral response [44, 68].. Finally the observation that many viruses prevent the induction of Type I IFN by targeting the function of IRF-3, and consequently the induction of Type I *IFN* genes, underlines the importance of IRF-3 in the induction of the antiviral response [69,70].

IRF-7, was initially identified as a factor binding to the Oq promoter of the Epstein Barr virus (EBV) and a splice variant of IRF-7 was recognized as a factor that plays a critical role in the induction of IFN-*A* genes [43,71]. IRF-7 is localized on human chromosome 11p15.5 in a region that is CpG rich. These clusters are methylated in some cancers and silencing of the IRF-7 promoter by methylation was observed in cancer cells [72]. IRF-7 expression can be induced not only by Type I IFN but also by TNFα [73], however IRF-7 is also constructively expressed in some lymphoid cells and especially in pDC, which are high producers of IFNα in response to TLR7/8 and TLR9 activation [74].

Reconstitution of IRF-7 expression in infected human fibroblasts, which expressed only IFNβ conferred, expression of several IFN-*A* genes [11]. Mice with a homozygous deletion of IRF-7 were unable to express Type I *IFN* genes upon viral infection or activation of TLR9 by CpG-rich DNA, indicating that IRF-7 is a master regulator of Type I IFN expression [66]. Like IRF-3, IRF-7 is phosphorylated by the TLR3-, TLR7/8- and TLR9-mediated signaling pathways where serines 477 and 479 appear to be critical targets for activation by TBK-1 [75]. In contrast, TLR7-and TLR9-stimulated phosphorylation of IRF-7 is dependant not on TBK-1 but rather on MyD88 and, IκB [76] and involves formation of ternary complex containing MyD88, IRAK-4, IRAK-1 and TRAF6 [77]. Virus-induced expression of distinct IFN-*A* subtypes is determined by the organization of the IRF-3 and IRF-7 recognizing domains in the VRE of the IFN-*A* promoters. Distortion in the GAAA core sequence of these binding domains affects the cooperativity of IRF-3 and IRF-7 binding and their synergistic activation. The differential expression of the individual IFN-*A* subtypes has been shown to be due to a distinct nucleotide substitution in these domains [12,13,15,78] and by the presence of negative regulatory sequences (DNRE) located in the upstream regulatory region of some IFN-*A* subtypes [16]. IRF-3 and IRF-7, together with histone transacetylases, have been shown to be part of the transcriptionally active human IFN*A1* enhanceosome [12], whereas the murine IFN*A11* promoter, which is not activated by IRF-3, binds only IRF-7 homodimers [15]. These data indicate that the relative levels of IRF-3 and IRF-7 in cells determine the levels of expression of individual IFN-*A* subtypes. IRF-7 was shown to have a short half life which may play a role in the regulating the transient expression of IFN-*A* genes [63]. IRF-7 expression also has a role in differentiation of monocytes to macrophages [79] and ectopic expression of a constitutively active IRF-7 in macrophages increased tumoricidal activity of these macrophages [80]

The hematopoetic IRF: IRF-4 and IRF-8

IRF-4 and IRF-8 show a high degree of homology. They are expressed primarily in lymphocytes, macrophages, B cells and DC [81,82]. These two proteins demonstrate only a weak DNA binding affinity, which can be increased by association with other transcription

factors[83,84]. IRF-4 binding is stabilized upon heterodimerization with the transcription factor PU.1, and this heterodimer was shown to bind to the IgG enhancer and activate expression of the immunoglobulin (Ig) light-chain in B cells [81]. IRF-4 has also been shown to be a natural antagonist of both IRF-1 and IRF-5 transactivation. The dominant negative action of IRF-4 was observed on IRF-1-mediated transactivation of the TRAIL promoter, while the inhibition of IRF-5 activation was due to the competition for binding to MyD88 [85].

IRF-4 null mice have a deficiency in mature T and B cells indicating that IRF-4 has a critical role in the maturation of B and T cells [86] and possibly also in the development of $CD4^+$DC, which are absent in these mice.[87]. IRF-4 null mice have a developmental block at several steps of T cells and B cells differentiation, indicating that IRF-4 is critical for both function and homeostasis as B cells do not form germinal centers in the spleen and lymph nodes [88]. IRF-4 is also required for differentiation of B lymphocytes into plasma cells, which is induced by antigen and T cells. This differentiation requires antibody class switching recombination and the processing of the membrane-expressed antibodies to secrete the antibodies. IRF-4 is required for the induction of the cytidine deaminase (AID), which together with Pax5, induces class switch recombination, and somatic hypermutation in antigen-activated B cells [89]. IRF-4 is also required for development of antibody producing plasma cells that are controlled by Blimp-1, as Blimp-1 is unable to induce differentiation to plasma cells in the absence of IRF-4 [90]. The function of IRF-4 can be also abrogated by an inability to associate with PU.1, such as observed in the primary effusion lymphoma (PEL), characteristic by arrests in B cell differentiation [91]. These data indicate that any defect in IRF-4 expression or function will lead to immune deficiency and an inability to produce antibodies. Interestingly, in multiple myeloma cells, IRF-4 was found to be translocated near the immunoglobulin heavy chain locus and consequently over-expressed, suggesting the deregulated expression of IRF-4 may contribute to the phenotype of multiple myeloma [92].

IRF-8 shares a number of properties with IRF-4; it binds DNA after interaction with the transcription factors of the IRF family, including IRF-1 and IRF-2 as well as PU.1 and E47 [82]. While the IRF-8/IRF1 complex generally functions as a suppressor of transcription, the IRF-8/IRF-4 heterodimer activates transcription of ISG15 [93]. The IRF-8/IRF-1 complex also induces numerous genes that are important for macrophage differentiation and macrophage-induced inflammation [94,96] and IRF-8 null mice develop chronic immunodeficiency and a myelogenous-like syndrome [97]. IRF-8 null mice also show major defects in $CD8^+$DC and pDC and the Flt3L-induced differentiation of mouse bone cells to pDC-like cells is dependant on IRF-8 [98,99]. IRF-8 is also critical for the expression of PML in Myeloid cells [21]. IRF-8 null mice also displayed increased susceptibility to infection, which was shown to be due to a defect in Th1 immune response and inability to express IL-12 and IRF-8 was consequently shown to stimulate the transcription of IL-12p40 [100]. The fact that IRF-8 affects differentiation of the high IFN producing pDC indicates its importance in the innate antiviral response as well as in TLR- MyD88 antiviral signaling pathway.

The surprising IRF: IRF-5 and IRF-6

IRF-5 and IRF-6 are another pair of IRF that show a completely different functions. While IRF-5 seems to have a role in apoptosis and the immune response to pathogens, IRF-6 is a key regulator of the switch from keratinocyte proliferation to differentiation [101]. IRF-6 null mice are embryonic lethal and the embryos showed abnormal external morphology; with abnormal skin, short forelimbs and a lack of ears, hind limbs and tails. These mice have also had abnormal craniofacial morphogenesis and skeletal defects. The major histological change detected in these mice was the absence of a normal stratified epidermis as in IRF-6 null epidermis the keratrinocytes did not stop proliferating and failed to differentiate [102]. In humans the IRF-6 gene is localized in the critical region of the Van der Woulde syndrome locus. This disorder

is associated with an autosomal dominant form of cleft lip and palate and a nonsense mutation in IRF-6. [103]. IRF-6 mutations are also associated with Popliteal Pterygium Syndrome (PPS) which is characterized by a similar orofacial phenotype skin lesions and genital abnormalities. [104]. The known functions of the other IRF seem to be associated with the immune response, and apoptosis or growth regulation, generally in lymphoid cells. These unexpected properties of IRF-6 indicate that IRF may also have a basic role unrelated to the immune response and that IRF functions may be not limited to the cells of the immune system. Furthermore, the fact that none of the other IRF null mice are embryonic lethal indicates that there is a redundancy of some of the IRF functions.

In contrast to IRF-6, IRF-5 has been implicated in the innate inflammatory response, although its role in the antiviral response has been recently challenged [105]. Human IRF-5 cDNA (AY 504946), cloned from DC, shows some properties that are distinct from IRF-3 and IRF-7. The IRF-5 polypeptide contains two nuclear localization signals, whereas IRF-3 or IRF-7 have only one, and consequently nuclear IRF-5 could be detected in uninfected cells [17,106]. The activation and phosphorylation of IRF-5 by viral infection could be detected in cells infected with NDV or VSV but not in cells infected with Sendai virus or treated with polyI:C, indicating that the activation may be virus specific [17]. While both Type I IFN and viral infection stimulate expression of IRF-5 gene [107], IRF-5 can be also induced by the tumor suppressor p53 [108] suggesting a connection between IRF-5 and p53 induced pro-apoptotic pathways [108]. Like p53, IRF5 stimulates the cyclin-dependent kinase inhibitor p21 while repressing Cyclin B1; stimulates the expression of the proapoptotic genes Bak1, Bax, caspase 8 and DAP kinase 2, [109] and promotes cell cycle arrest and apoptosis independently of p53. [110]

In vitro experiments have shown that in infected cells IRF-5, like IRF-7, binds to the VRE of IFN-*A* genes and activates expression of these genes, however the subtypes of IFN-*A* induced by IRF-5 and IRF-7 were distinct. While IFN-*A1* was the major subtype induced by NDV in IRF-7-expressing cells, IRF-5-expressing cells expressed IFN-*A8* as the major subtype, further suggesting that not all IFN-*A* genes are induced by IRF-7 [17]. Furthermore, the transcriptional signatures of IRF-5 and IRF-7 in NDV-infected B cells was both overlapping and distinct [111]. Gene array analysis revealed a significant increase in the transcription of a number of *ISG* in NDV-infected B cells over-expressing IRF-5 which were not expressed in IRF-7 expressing cells. Interestingly, IRF-5 over-expression specifically up-regulated several of the early inflammatory genes including RANTES, MIP-1β, I-309, MCP-1 and IL-8, indicating that IRF-5 has an important role in the transcriptional regulation of the early inflammatory cytokines and chemokines [109,111]. These results indicated that IRF-5 and IRF-7 have both overlapping and non-redundant functions in infected cells. Distinction also exists between the activation of IRF-3 and IRF-5; the TLR-3 mediated TRIF pathway that activates both IRF-3 and IRF-7 does not activate IRF-5, while however the MyD88-dependent pathway activates IRF-5 but not IRF-3. In vitro experiments also indicated that MyD88 activation of huIRF-5 is dependant on IRAK1 and TRAF6 (Schoenemeyer, 2005 #112}.

Human IRF-5 is expressed in multiple spliced variants, some of which are transcriptionally inactive or may function as dominant negative mutants [107]. The activation and nuclear transport of individual variants of IRF-5 also seems to be distinct, which may help to explain the variation in results from different laboratories [112,113]. Mutations in the hu*IRF-5* gene confers a predisposition to autoimmune disease; systemic lupus erythromatosis (SLE) is characterized by constitutive IFNα production and polymorphisms in the *tyrosine kinase 2* and *IRF-5* genes, with elevated expression of multiple spliced variants of IRF-5. This observation indicates a connection between IRF-5 expression, IFNα production and autoimmunity [114].

While the critical role of IRF-7 in the induction of Type I IFN genes has been confirmed in *vivo* [66], IRF-5 null mice did not show any defect in CpG- or polyI:C-mediated induction of

Type I IFN, however the response to viral infection in these mice has not yet been analyzed [105]. In correlation with the *in vitro* results seen for huIRF-5, the expression of inflammatory cytokines TNFα, IL-6 and IL-12 was significantly down regulated in IRF-5 null mice [105, 109]. Based on these results it is now generally assumed that IRF-5 participates in the induction of inflammatory cytokines rather than in Type I IFN [48,105]. However it may be premature to discard all the results on huIRF-5, before the muIRF-5 and its functions are characterized and the mouse experiments extended to viral infections. Our preliminary results indicate several differences in the properties of huIRF-5 and muIRF-5 (NM_012057) isolated from splenocytes of CB57BL/6J mice. Firstly, unlike huIRF-5 which is expressed in multiple spliced variants [107], we have detected only one muIRF-5 splice variant which is expressed at very low levels in the bone marrow of C57BL/6J mice. Furthermore, muIRF-5 is activated by MyD88 and TBK-1 but not by NDV infection, properties shared with huIRF-5 variant 5, which is also activated by TBK-1 but not by NDV infection [113]. MyD88-activated muIRF-5 stimulates the promoters of both IFN-*A* and *IL-6/12* genes using a luciferase reporter assay (unpublished results). Thus the discordant effects of muIRF-5 on the activation of *IFN* genes in cells expressing ectopic muIRF-5 and mouse cells lacking IRF-5 expression are unexpected. This difference indicates that in the presence of high levels of MyD88-activated IRF-7, the contribution of IRF-5 to the induction of *IFN* genes is negligible, and its role is limited to the induction of inflammatory chemokines and cytokines which are not stimulated by IRF-7. The MyD88-mediated activation of both IRF-5 and IRF-7 involves formation of a tertiary complex consisting of MyD88, IRAK-4, TRAF6 and IRF-5 and it is likely that this complex preferentially assembles with IRF-7 than with IRF-5 [105,115]. It was also shown that IRF-4 competes the binding of IRF-5 to MyD88 and therefore in cells expressing IRF-4 such as pDC or B cells, IRF-5 may be not efficiently activated [116]. Thus the role of IRF-5 in the stimulation of Type I *IFN* genes may be limited to those cells which do not express IRF-4 or activated IRF-7 and may depend on a distinct, concentration-dependent activation of IRF-5 and IRF-7. Further analyses of the role of IRF-5 in the antiviral and autoimmune induction of Type I IFN are clearly warranted.

The forgotten IRF; IRF-9

IRF-9 plays a major role in the antiviral effect of Type I IFN. It is a component of the tertiary complex ISGF3 that is formed in IFN-treated cells and binds to the ISRE elements of ISG, stimulating their transcription [117–119]. In this complex, which also contains STAT1 and STAT2, IRF-9 is the major DNA binding component. IRF-9 can also form a DNA binding complex with the STAT1 homodimer and with STAT2 alone, with these complexes binding to DNA with the same specificity as ISGF3 [120]. MEFs from IRF-9 null mice are deficient in both Type I and Type II IFN responses [121,122]. Furthermore the MEFs from these mice show an impaired induction of IRF-7 expression and inhibition of IFN-*A* genes transcription. The IRF-9 mice showed impairment in both Type I and Type II IFN signaling [95]. Hovewer the phenotype of the IRF-9 mice has not been characterized yet in details.

The viral IRF: KSHV-encoded viral IRF

Kaposi's sarcoma-associated herpes virus (KSHV) is a member of the γ herpes virus family and is genetically similar to EBV and monkey Herpes Virus Saimiri (HVS) [123]. Sequence analysis of the KSHV genome revealed the presence of about 80 open reading frames (ORFs) and a number of ORFs showing homology to cellular genes that regulate cell growth, immune functions, inflammation and apoptosis [123,124]. These include a cluster of four ORFs with homology to the cellular transcription factors of IRF family [125].

Three vIRF have been cloned and characterized. The K9-encoded vIRF-1, expressed in PEL cells treated with TPA, has been studied most extensively and was shown to inhibit both, the virus-mediated induction of Type I *IFN* genes and IFN-induced genes (ISG) and its over-

expression in NIH/3T3 confers tumorigenicity when injected into nude mice [126–129]. However targeted expression of vIRF-1 in B cells or endothelial cells failed to induce tumor formation in transgenic mice (Lubyova and Pitha, unpublished results). vIRF-1 binds to CBP/p300 and inhibits its acetyltransferase activity resulting in the global inhibition of histones H3 and H4 acetylation [130,131]. The anti-apoptotic effect of vIRF-1 has been related to the binding of vIRF-1 to p53, IRF1 and GRIM19 [126,132,133].

vIRF-2 (ORF K11.1) encodes a small nuclear protein (163 aa) that is constitutively expressed in PEL cells [130]. Unlike the cellular IRF, vIRF-2 binds to an oligodeoxynucleotide corresponding to the NFκB site and specifically associates with several cellular IRF and with p300 [130]. In addition, vIRF-2 binds also dsRNA-activated protein kinase (PKR), inhibits its kinase activity and blocks the phosphorylation of the PKR substrate, eukaryotic translation initiation factor 2α [134]. Additional transcripts encompassing vIRF-2 spliced to K11ORF have also been identified. This transcript can be detected only in TPA-treated PEL cells and the corresponding vIRF-2 protein was shown to inhibit IFN signaling [135,136].

vIRF-3/LANA2, encoded by ORFs K10.5 and K10.6,[137,138] is a multifunctional nuclear protein constitutively expressed in KSHV-positive PEL and Castleman's disease tumors, but not in Kaposi's sarcoma spindle cells [135,137,138],[139]. The vIRF-3 protein binds to IRF-3, IRF-7 and CBP/p300 and stimulates IRF-3/IRF-7-mediated transcription of Type I *IFN* genes [139]. Furthermore, interaction of vIRF-3 with p53 results in inhibition of p53-mediated transcriptional activation and p53-induced apoptosis [138]. vIRF-3 also interacts with another tumor suppressor gene MM-1(Lubyova et al., manuscript submitted for publication). Inhibition of IKKβ kinase activity and down-modulation of NFκB-dependent transcription by vIRF-3 was also reported[140].

Considerations and future perspectives

The essential role of IRF-3 and IRF-7 in the antiviral response to pathogens has been clearly established. While the activation of IRF-3 is associated with MyD88-independent or RIG-I pathways, IRF-7 is activated by both MyD88-dependant and independent pathways, possibly by two different kinases. Whether these distinct pathways target the phosphorylation of identical or distinct IRF-7 residues remains to be determined. The role of IRF-1 and IRF-5 in the antiviral response is yet to be evaluated, but it is necessary to further consider the reasons for the observed differences between the results using the IRF-5 over-expressing and IRF-5 null cells. It is conceivable that such a differences point to the functional redundancy as well as the critical importance of a balance between the activated IRF in different signaling pathways and cell types. Further insight into a functional deregulation of IRF activation in autoimmune disease, as observed for IRF-5, highlights how changes in IRF activation may result in a deleterious host immune response. The determination of the cause of the modulation of IRF functions may eventually lead to therapeutic interventions for these disorders. Future investigations will also reveal whether IRF have a basic role in embryogenesis or regulation of cell growth and differentiation that is unrelated to the immune response, as there are already indications that a block in IRF-6 expression results in a defect in embryonic development. Research to date serves to highlight the integral and varied role of the IRF in both the development and function of the immune system and further studies will no doubt uncover further important roles for these multifunctional proteins.

Acknowledgements

Work in P.M.P laboratory was supported by NIAID grant R01 AI19737-19. We apologize to authors whose work we were unable to reference due to the space limitation.

References

1. Goodbourn S, Maniatis T. Overlapping positive and negative regulatory domains of the human beta-interferon gene. Proc Natl Acad Sci U S A 1988;85:1447–1451. [PubMed: 3422743]
2. Raj NB, Engelhardt J, Au WC, Levy DE, Pitha PM. Virus infection and interferon can activate gene expression through a single synthetic element, but endogenous genes show distinct regulation. J Biol Chem 1989;264:16658–16666. [PubMed: 2550451]
3. Ryals J, Dierks P, Ragg H, Weissmann C. A 46-nucleotide promoter segment from an IFN-alpha gene renders an unrelated promoter inducible by virus. Cell 1985;41:497–507. [PubMed: 3986909]
4. Fujita T, Ohno S, Yasumitsu H, Taniguchi T. Delimitation and properties of DNA sequences required for the regulated expression of human interferon-beta gene. Cell 1985;41:489–496. [PubMed: 2985279]
5. Kim TK, Maniatis T. The mechanism of transcriptional synergy of an in vitro assembled interferon-beta enhanceosome. Mol Cell 1997;1:119–129. [PubMed: 9659909]
6. Merika M, Williams AJ, Chen G, Collins T, Thanos D. Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. Mol Cell 1998;1:277–287. [PubMed: 9659924]
7. Bisat F, Raj NB, Pitha PM. Differential and cell type specific expression of murine alpha-interferon genes is regulated on the transcriptional level. Nucleic Acids Res 1988;16:6067–6083. [PubMed: 3399385]
8. Au WC, Moore PA, Lowther W, Juang YT, Pitha PM. Identification of a member of the interferon regulatory factor family that binds to the interferon-stimulated response element and activates expression of interferon-induced genes. Proc Natl Acad Sci U S A 1995;92:11657–11661. [PubMed: 8524823]
9. Fujii Y, Shimizu T, Kusumoto M, Kyogoku Y, Taniguchi T, Hakoshima T. Crystal structure of an IRF-DNA complex reveals novel DNA recognition and cooperative binding to a tandem repeat of core sequences. Embo J 1999;18:5028–5041. [PubMed: 10487755]
10. Lin R, Genin P, Mamane Y, Hiscott J. Selective DNA binding and association with the CREB binding protein coactivator contribute to differential activation of alpha/beta interferon genes by interferon regulatory factors 3 and 7. Mol Cell Biol 2000;20:6342–6353. [PubMed: 10938111]
11. Yeow WS, Au WC, Juang YT, Fields CD, Dent CL, Gewert DR, Pitha PM. Reconstitution of virus-mediated expression of interferon alpha genes in human fibroblast cells by ectopic interferon regulatory factor-7. J Biol Chem 2000;275:6313–6320. [PubMed: 10692430]
12. Au WC, Yeow WS, Pitha PM. Analysis of functional domains of interferon regulatory factor 7 and its association with IRF-3. Virology 2001;280:273–282. [PubMed: 11162841]
13. Au WC, Su Y, Raj NB, Pitha PM. Virus-mediated induction of interferon A gene requires cooperation between multiple binding factors in the interferon alpha promoter region. J Biol Chem 1993;268:24032–24040. [PubMed: 8226947]
14. Lopez S, Reeves R, Island ML, Bander MT, Christelt N, Doly J, Navarro S. Silencer activity in the interferon A gene promoter. J Biol Chem 1997;272:22789–22799.
15. Civas A, Genin P, Morin P, Lin R, Hiscott J. Promoter organization of the interferon-A genes differentially affects virus-induced expression and responsiveness to TBK1 and IKKepsilon. J Biol Chem 2006;281:4856–4866. [PubMed: 16380379]
16. Lopez S, Island ML, Drouin J, Bandu MT, Christeff N, Darracq N, Barbey R, Doly J, Thomas D, Navarro S. Repression of virus-induced interferon A promoters by homeodomain transcription factor Ptx1. Mol Cell Biol 2000;20:7527–7540. [PubMed: 11003649]
17. Barnes B, Lubyova B, Pitha PM. On the role of IRF in host defense. J Interferon Cytokine Res 2002;22:59–71. [PubMed: 11846976]
18. Nguyen H, Hiscott J, Pitha PM. The growing family of interferon regulatory factors. Cytokine Growth Factor Rev 1997;8:293–312. [PubMed: 9620643]
19. Escalante CR, Yie J, Thanos D, Aggarwal AK. Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. Nature 1998;391:103–106. [PubMed: 9422515]
20. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 2001;19:623–655. [PubMed: 11244049]

21. Dror N, Rave-Harel N, Burchert A, Azriel A, Tamura T, Tailor P, Neubauer A, Ozato K, Levi BZ. IRF-8 is indispensable for the expression of PML and the formation of nuclear bodies in myeloid cells. J Biol Chem. 2006in press
22. Fujita T, Kimura Y, Miyamoto M, Barsoumian EL, Taniguchi T. Induction of endogenous IFN-alpha and IFN-beta genes by a regulatory transcription factor, IRF-1. Nature 1989;337:270–272. [PubMed: 2911367]
23. Matsuyama T, Kimura T, Kitagawa M, Pfeffer K, Kawakami T, Watanabe N, Kundig TM, Amakawa R, Kishihara K, Wakeham A, et al. Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. Cell 1993;75:83–97. [PubMed: 8402903]
24. Reis LF, Ruffner H, Stark G, Aguet M, Weissmann C. Mice devoid of interferon regulatory factor 1 (IRF-1) show normal expression of type I interferon genes. EMBO J 1994;13:4798–4806. [PubMed: 7957048]
25. Kamijo R, Harada H, Matsuyama T, Bosland M, Gerecitano J, Shapiro D, Le J, Koh SI, Kimura T, Green SJ, et al. Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. Science 1994;263:1612–1615. [PubMed: 7510419]
26. Kimura T, Nakayama K, Penninger J, Kitagawa M, Harada H, Matsuyama T, Tanaka N, Kamijo R, Vilcek J, Mak TW, et al. Involvement of the IRF-1 transcription factor in antiviral responses to interferons. Science 1994;264:1921–1924. [PubMed: 8009222]
27. Coccia EM, Stellacci E, Marziali G, Weiss G, Battistini A. IFN-gamma and IL-4 differently regulate inducible NO synthase gene expression through IRF-1 modulation. Int Immunol 2000;12:977–985. [PubMed: 10882409]
28. Saura M, Zaragoza C, McMillan A, Quick RA, Hohenadl C, Lowenstein JM, Lowenstein CJ. An antiviral mechanism of nitric oxide: inhibition of a viral protease. Immunity 1999;10:21–28. [PubMed: 10023767]
29. Thanos D, Maniatis T. Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. Cell 1995;83:1091–1100. [PubMed: 8548797]
30. Duncan GS, Mittrucker H-W, Kagi D, Matsuyama T, Mak TW. The transcription factor interferon regulatory factor-1 is essential for natural killer cell function in vivo. J Exp Med Journal of Experimental Medicine 1996;184:2043–2048.
31. Keller AD, Maniatis T. Identification and characterization of a novel repressor of beta-interferon gene expression. Genes Dev 1991;5:868–879. [PubMed: 1851123]
32. Kuo TC, Calame KL. B lymphocyte-induced maturation protein (Blimp)-1, IFN regulatory factor (IRF)-1, and IRF-2 can bind to the same regulatory sites. J Immunol 2004;173:5556–5563. [PubMed: 15494505]
33. Wang TN, Chu YT, Chen WY, Feng WW, Shih NH, Hsiang CH, Ko YC. Association of interferon-gamma and interferon regulatory factor 1 polymorphisms with asthma in a family-based association study in Taiwan. Clin Exp Allergy 2006;36:1147–1152. [PubMed: 16961714]
34. Willman CL, Sever CE, Pallavicini MG, Harada H, Tanaka N, Slovak ML, Yamamoto H, Harada K, Meeker TC, List AF, et al. Deletion of IRF-1, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia. Science 1993;259:968–971. [PubMed: 8438156]
35. Vaughan PS, Aziz F, van Wijnen AJ, Wu S, Harada H, Taniguchi T, Soprano KJ, Stein JL, Stein GS. Activation of a cell-cycle-regulated histone gene by the oncogenic transcription factor IRF-2. Nature 1995;377:362–365. [PubMed: 7566094]
36. Passioura T, Shen S, Symonds G, Dolnikov A. A retroviral library genetic screen identifies IRF-2 as an inhibitor of N-ras-induced growth suppression in leukemic cells. Oncogene 2005;24:7327–7336. [PubMed: 16007130]
37. Taki S, Nakajima S, Ichikawa E, Saito T, Hida S. IFN regulatory factor-2 deficiency revealed a novel checkpoint critical for the generation of peripheral NK cells. J Immunol 2005;174:6005–6012. [PubMed: 15879093]
38. Honda K, Mizutani T, Taniguchi T. Negative regulation of IFN-alpha/beta signaling by IFN regulatory factor 2 for homeostatic development of dendritic cells. Proc Natl Acad Sci U S A 2004;101:2416–2421. [PubMed: 14983024]

39. Rouyez MC, Lestingi M, Charon M, Fichelson S, Buzyn A, Dusanter-Fourt I. IFN regulatory factor-2 cooperates with STAT1 to regulate transporter associated with antigen processing-1 promoter activity. J Immunol 2005;174:3948–3958. [PubMed: 15778351]
40. MacDonald NJ, Kuhl D, Maguire D, Naf D, Gallant P, Goswamy A, Hug H, Bueler H, Chaturvedi M, de la Fuente J, et al. Different pathways mediate virus inducibility of the human IFN-alpha 1 and IFN-beta genes. Cell 1990;60:767–779. [PubMed: 2107026]
41. Au WC, Raj NB, Pine R, Pitha PM. Distinct activation of murine interferon-alpha promoter region by IRF-1/ISFG-2 and virus infection. Nucleic Acids Res 1992;20:2877–2884. [PubMed: 1614874]
42. Ronco LV, Karpova AY, Vidal M, Howley PM. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev 1998;12:2061–2072. [PubMed: 9649509]
43. Au WC, Moore PA, LaFleur DW, Tombal B, Pitha PM. Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon A genes. J Biol Chem 1998;273:29210–29217. [PubMed: 9786932]
44. Marie I, Durbin JE, Levy DE. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. Embo J 1998;17:6660–6669. [PubMed: 9822609]
45. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783–801. [PubMed: 16497588]
46. Honda K, Takaoka A, Taniguchi T. Type I interferon [correction of inteferon] gene induction by the interferon regulatory factor family of transcription factors. Immunity 2006;25:349–360. [PubMed: 16979567]
47. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 2006;441:101–105. [PubMed: 16625202]
48. Honda K, Taniguchi T. IRF: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol 2006;6:644–658. [PubMed: 16932750]
49. Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol 2003;4:491–496. [PubMed: 12692549]
50. Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. Triggering the interferon antiviral response through an IKK-related pathway. Science 2003;300:1148–1151. [PubMed: 12702806]
51. McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. Proc Natl Acad Sci U S A 2004;101:233–238. [PubMed: 14679297]
52. Takahasi K, Suzuki NN, Horiuchi M, Mori M, Suhara W, Okabe Y, Fukuhara Y, Terasawa H, Akira S, Fujita T, Inagaki F. X-ray crystal structure of IRF-3 and its functional implications. Nat Struct Biol 2003;10:922–927. [PubMed: 14555995]
53. Qin BY, Liu C, Lam SS, Srinath H, Delston R, Correia JJ, Derynck R, Lin K. Crystal structure of IRF-3 reveals mechanism of autoinhibition and virus-induced phosphoactivation. Nat Struct Biol 2003;10:913–921. [PubMed: 14555996]
54. Lin R, Heylbroeck C, Pitha PM, Hiscott J. Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. Mol Cell Biol 1998;18:2986–2996. [PubMed: 9566918]
55. Yoneyama M, Suhara W, Fukuhara Y, Fukuda M, Nishida E, Fujita T. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. Embo J 1998;17:1087–1095. [PubMed: 9463386]
56. Weaver BK, Kumar KP, Reich NC. Interferon regulatory factor 3 and CREB-binding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. Mol Cell Biol 1998;18:1359–1368. [PubMed: 9488451]
57. Lin R, Mamane Y, Hiscott J. Structural and functional analysis of interferon regulatory factor 3: localization of the transactivation and autoinhibitory domains. Mol Cell Biol 1999;19:2465–2474. [PubMed: 10082512]

58. Grandvaux N, Servant MJ, tenOever B, Sen GC, Balachandran S, Barber GN, Lin R, Hiscott J. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J Virol 2002;76:5532–5539. [PubMed: 11991981]
59. Peters KL, Smith HL, Stark GR, Sen GC. IRF-3-dependent, NFkappa B- and JNK-independent activation of the 561 and IFN-beta genes in response to double-stranded RNA. Proc Natl Acad Sci U S A 2002;99:6322–6327. [PubMed: 11972054]
60. Juang YT, Lowther W, Kellum M, Au WC, Lin R, Hiscott J, Pitha PM. Primary activation of interferon A and interferon B gene transcription by interferon regulatory factor 3. Proc Natl Acad Sci U S A 1998;95:9837–9842. [PubMed: 9707562]
61. Schafer SL, Lin R, Moore PA, Hiscott J, Pitha PM. Regulation of type I interferon gene expression by interferon regulatory factor-3. J Biol Chem 1998;273:2714–2720. [PubMed: 9446577]
62. Wathelet MG, Lin CH, Parekh BS, Ronco LV, Howley PM, Maniatis T. Virus infection induces the assembly of coordinately activated transcription factors on the IFN-beta enhancer in vivo. Mol Cell 1998;1:507–518. [PubMed: 9660935]
63. Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, Nakao K, Nakaya T, Katsuki M, Noguchi S, Tanaka N, Taniguchi T. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity 2000;13:539–548. [PubMed: 11070172]
64. Saitoh T, Tun-Kyi A, Ryo A, Yamamoto M, Finn G, Fujita T, Akira S, Yamamoto N, Lu KP, Yamaoka S. Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1. Nat Immunol 2006;7:598–605. [PubMed: 16699525]
65. Lu G, Reinert JT, Pitha-Rowe I, Okumura A, Kellum M, Knobeloch KP, Hassel B, Pitha PM. ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation. Cell Mol Biol (Noisy-le-grand) 2006;52:29–41. [PubMed: 16914094]
66. Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, Shimada N, Ohba Y, Takaoka A, Yoshida N, Taniguchi T. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 2005;434:772–777. [PubMed: 15800576]
67. Lowther WJ, Moore PA, Carter KC, Pitha PM. Cloning and functional analysis of the human IRF-3 promoter. DNA Cell Biol 1999;18:685–692. [PubMed: 10492399]
68. Sato M, Hata N, Asagiri M, Nakaya T, Taniguchi T, Tanaka N. Positive feedback regulation of type I IFN genes by the IFN-inducible transcription factor IRF-7. FEBS Lett 1998;441:106–110. [PubMed: 9877175]
69. Katze MG, He Y, Gale M Jr. Viruses and interferon: a fight for supremacy. Nat Rev Immunol 2002;2:675–687. [PubMed: 12209136]
70. Weber F, Kochs G, Haller O. Inverse interference: how viruses fight the interferon system. Viral Immunol 2004;17:498–515. [PubMed: 15671747]
71. Zhang L, Pagano JS. IRF-7, a new interferon regulatory factor associated with Epstein-Barr virus latency. Mol Cell Biol 1997;17:5748–5757. [PubMed: 9315633]
72. Lu R, Au WC, Yeow WS, Hageman N, Pitha PM. Regulation of the promoter activity of interferon regulatory factor-7 gene. Activation by interferon snd silencing by hypermethylation. J Biol Chem 2000;275:31805–31812. [PubMed: 10924517]
73. Lu R, Moore PA, Pitha PM. Stimulation of IRF-7 gene expression by tumor necrosis factor alpha: requirement for NFkappa B transcription factor and gene accessibility. J Biol Chem 2002;277:16592–16598. [PubMed: 11877397]
74. Izaguirre A, Barnes BJ, Amrute S, Yeow WS, Megjugorac N, Dai J, Feng D, Chung E, Pitha PM, Fitzgerald-Bocarsly P. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. J Leukoc Biol 2003;74:1125–1138. [PubMed: 12960254]
75. tenOever BR, Sharma S, Zou W, Sun Q, Grandvaux N, Julkunen I, Hemmi H, Yamamoto M, Akira S, Yeh WC, Lin R, Hiscott J. Activation of TBK1 and IKKvarepsilon kinases by vesicular stomatitis virus infection and the role of viral ribonucleoprotein in the development of interferon antiviral immunity. J Virol 2004;78:10636–10649. [PubMed: 15367631]

76. Hoshino K, Sugiyama T, Matsumoto M, Tanaka T, Saito M, Hemmi H, Ohara O, Akira S, Kaisho T. IkappaB kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. Nature 2006;440:949–953. [PubMed: 16612387]
77. Uematsu S, Sato S, Yamamoto M, Hirotani T, Kato H, Takeshita F, Matsuda M, Coban C, Ishii KJ, Kawai T, Takeuchi O, Akira S. Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-{alpha} induction. J Exp Med 2005;201:915–923. [PubMed: 15767370]
78. Morin P, Braganca J, Bandu MT, Lin R, Hiscott J, Doly J, Civas A. Preferential binding sites for interferon regulatory factors 3 and 7 involved in interferon-A gene transcription. J Mol Biol 2002;316:1009–1022. [PubMed: 11884139]
79. Lu R, Pitha PM. Monocyte differentiation to macrophage requires interferon regulatory factor 7. J Biol Chem 2001;276:45491–45496. [PubMed: 11585813]
80. Romieu-Mourez R, Solis M, Nardin A, Goubau D, Baron-Bodo V, Lin R, Massie B, Salcedo M, Hiscott J. Distinct roles for IFN regulatory factor (IRF)-3 and IRF-7 in the activation of antitumor properties of human macrophages. Cancer Res 2006;66:10576–10585. [PubMed: 17079482]
81. Eisenbeis CF, Singh H, Storb U. Pip, a novel IRF family member, is a lymphoid-specific, PU.1-dependent transcriptional activator. Genes Dev 1995;9:1377–1387. [PubMed: 7797077]
82. Politis AD, Sivo J, Driggers PH, Ozato K, Vogel SN. Modulation of interferon consensus sequence binding protein mRNA in murine peritoneal macrophages. Induction by IFN-gamma and down-regulation by IFN-alpha, dexamethasone, and protein kinase inhibitors. J Immunol 1992;148:801–807. [PubMed: 1730873]
83. Marecki S, Atchison ML, Fenton MJ. Differential expression and distinct functions of IFN regulatory factor 4 and IFN consensus sequence binding protein in macrophages. J Immunol 1999;163:2713–2722. [PubMed: 10453013]
84. Tailor P, Tamura T, Ozato K. IRF family proteins and type I interferon induction in dendritic cells. Cell Res 2006;16:134–140. [PubMed: 16474425]
85. Yoshida K, Yamamoto K, Kohno T, Hironaka N, Yasui K, Kojima C, Mukae H, Kadota J, Suzuki S, Honma K, Kohno S, Matsuyama T. Active repression of IFN regulatory factor-1-mediated transactivation by IFN regulatory factor-4. Int Immunol 2005;17:1463–1471. [PubMed: 16172134]
86. Lu R, Medina KL, Lancki DW, Singh H. IRF-4,8 orchestrate the pre-B-to-B transition in lymphocyte development. Genes Dev 2003;17:1703–1708. [PubMed: 12832394]
87. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, Singh H, Ozato K. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. J Immunol 2005;174:2573–2581. [PubMed: 15728463]
88. Mittrucker HW, Matsuyama T, Grossman A, Kundig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS, Mak TW. Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. Science 1997;275:540–543. [PubMed: 8999800]
89. Gonda H, Sugai M, Nambu Y, Katakai T, Agata Y, Mori KJ, Yokota Y, Shimizu A. The balance between Pax5 and Id2 activities is the key to AID gene expression. J Exp Med 2003;198:1427–1437. [PubMed: 14581609]
90. Klein U, Casola S, Cattoretti G, Shen Q, Lia M, Mo T, Ludwig T, Rajewsky K, Dalla-Favera R. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol 2006;7:773–782. [PubMed: 16767092]
91. Arguello M, Sgarbanti M, Hernandez E, Mamane Y, Sharma S, Servant M, Lin R, Hiscott J. Disruption of the B-cell specific transcriptional program in HHV-8 associated primary effusion lymphoma cell lines. Oncogene 2003;22:964–973. [PubMed: 12592383]
92. Iida S, Rao PH, Butler M, Corradini P, Boccadoro M, Klein B, Chaganti RS, Dalla-Favera R. Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. Nat Genet 1997;17:226–230. [PubMed: 9326949]
93. Meraro D, Gleit-Kielmanowicz M, Hauser H, Levi BZ. IFN-stimulated gene 15 is synergistically activated through interactions between the myelocyte/lymphocyte-specific transcription factors, PU. 1, IFN regulatory factor-8/IFN consensus sequence binding protein, and IFN regulatory factor-4: characterization of a new subtype of IFN-stimulated response element. J Immunol 2002;168:6224–6231. [PubMed: 12055236]

94. Dror O, Nussinov R, Wolfson HJ. The ARTS web server for aligning RNA tertiary structures. Nucleic Acids Res 2006;34:W412–415. [PubMed: 16845038]

95. Kimura T, Kadokawa Y, Harada H, Matsumoto M, Sato M, Kashiwazaki Y, Tarutani M, Tan RS, Tkasugi T, Matsuyama T, Mak TW, Noguchi S, Taniguchi T. Essential and non-redundant role of p48 (ISGF3 gamma) and IRF-1 in both type I and typeII interferon responses, as revealed by gene targeting studies. Gene To cells 1996;1:115–124.

96. Liu J, Guan X, Tamura T, Ozato K, Ma X. Synergistic activation of interleukin-12 p35 gene transcription by interferon regulatory factor-1 and interferon consensus sequence-binding protein. J Biol Chem 2004;279:55609–55617. [PubMed: 15489234]

97. Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, Lou J, Knobeloch KP, Gabriele L, Waring JF, Bachmann MF, Zinkernagel RM, Morse HC 3rd, Ozato K, Horak I. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell 1996;87:307–317. [PubMed: 8861914]

98. Tamura T, Ozato K. ICSBP/IRF-8: its regulatory roles in the development of myeloid cells. J Interferon Cytokine Res 2002;22:145–152. [PubMed: 11846985]

99. Tamura T, Thotakura P, Tanaka TS, Ko MS, Ozato K. Identification of target genes and a unique cis element regulated by IRF-8 in developing macrophages. Blood 2005;106:1938–1947. [PubMed: 15947094]

100. Meraro D, Hashmueli S, Koren B, Azriel A, Oumard A, Kirchhoff S, Hauser H, Nagulapalli S, Atchison ML, Levi BZ. Protein-protein and DNA-protein interactions affect the activity of lymphoid-specific IFN regulatory factors. J Immunol 1999;163:6468–6478. [PubMed: 10586038]

101. Richardson RJ, Dixon J, Malhotra S, Hardman MJ, Knowles L, Boot-Handford RP, Shore P, Whitmarsh A, Dixon MJ. Irf6 is a key determinant of the keratinocyte proliferation-differentiation switch. Nat Genet 2006;38:1329–1334. [PubMed: 17041603]

102. Ingraham CR, Kinoshita A, Kondo S, Yang B, Sajan S, Trout KJ, Malik MI, Dunnwald M, Goudy SL, Lovett M, Murray JC, Schutte BC. Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (Irf6). Nat Genet 2006;38:1335–1340. [PubMed: 17041601]

103. Mostowska A, Wojcicki P, Kobus K, Trzeciak WH. Gene symbol: IRF6. Disease: Van der Woude syndrome. Hum Genet 2005;116:534. [PubMed: 15988826]

104. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet 2002;32:285–289. [PubMed: 12219090]

105. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, Kano S, Honda K, Ohba Y, Mak TW, Taniguchi T. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 2005;434:243–249. [PubMed: 15665823]

106. Barnes BJ, Moore PA, Pitha PM. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon alpha genes. J Biol Chem 2001;276:23382–23390. [PubMed: 11303025]

107. Mancl ME, Hu G, Sangster-Guity N, Olshalsky SL, Hoops K, Fitzgerald-Bocarsly P, Pitha PM, Pinder K, Barnes BJ. Two discrete promoters regulate the alternatively spliced human interferon regulatory factor-5 isoforms. Multiple isoforms with distinct cell type-specific expression, localization, regulation, and function. J Biol Chem 2005;280:21078–21090. [PubMed: 15805103]

108. Mori T, Anazawa Y, Iiizumi M, Fukuda S, Nakamura Y, Arakawa H. Identification of the interferon regulatory factor 5 gene (IRF-5) as a direct target for p53. Oncogene 2002;21:2914–2918. [PubMed: 11973653]

109. Barnes BJ, Field AE, Pitha-Rowe PM. Virus-induced Heterodimer Formation between IRF-5 and IRF-7 Modulates Assembly of the IFNA Enhanceosome in Vivo and Transcriptional Activity of IFNA Genes. J Biol Chem 2003;278:16630–16641. [PubMed: 12600985]

110. Hu G, Barnes BJ. Interferon regulatory factor-5-regulated pathways as a target for colorectal cancer therapeutics. Expert Rev Anticancer Ther 2006;6:775–784. [PubMed: 16759167]

111. Barnes BJ, Richards J, Mancl M, Hanash S, Beretta L, Pitha PM. Global and distinct targets of IRF-5 and IRF-7 during innate response to viral infection. J Biol Chem 2004;279:45194–45207. [PubMed: 15308637]

112. Lin R, Yang L, Arguello M, Penafuerte C, Hiscott J. A CRM1-dependent nuclear export pathway is involved in the regulation of IRF-5 subcellular localization. J Biol Chem 2005;280:3088–3095. [PubMed: 15556946]

113. Cheng TF, Brzostek S, Ando O, Van Scoy S, Kumar KP, Reich NC. Differential activation of IFN regulatory factor (IRF)-3 and IRF-5 transcription factors during viral infection. J Immunol 2006;176:7462–7470. [PubMed: 16751392]

114. Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, Bauer JW, Ortmann WA, Koeuth T, Gonzalez Escribano MF, Pons-Estel B, Petri M, Daly M, Gregersen PK, Martin J, Altshuler D, Behrens TW, Alarcon-Riquelme ME. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nat Genet 2006;38:550–555. [PubMed: 16642019]

115. Kawai T, Sato S, Ishii KJ, Coban C, Hemmi H, Yamamoto M, Terai K, Matsuda M, Inoue J, Uematsu S, Takeuchi O, Akira S. Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 2004;5:1061–1068. [PubMed: 15361868]

116. Negishi H, Ohba Y, Yanai H, Takaoka A, Honma K, Yui K, Matsuyama T, Taniguchi T, Honda K. Negative regulation of Toll-like-receptor signaling by IRF-4. Proc Natl Acad Sci U S A 2005;102:15989–15994. [PubMed: 16236719]

117. Veals SA, Schindler C, Leonard D, Fu XY, Aebersold R, Darnell JE Jr, Levy DE. Subunit of an alpha-interferon-responsive transcription factor is related to interferon regulatory factor and Myb families of DNA-binding proteins. Mol Cell Biol 1992;12:3315–3324. [PubMed: 1630447]

118. Darnell JEJ, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. [Review]. Science 1994;264:1415–1421. [PubMed: 8197455]

119. Improta T, Schindler C, Horvath CM, Kerr IM, Stark GR, Darnell JE Jr. Transcription factor ISGF-3 formation requires phosphorylated Stat91 protein, but Stat113 protein is phosphorylated independently of Stat91 protein. Proc Natl Acad Sci U S A 1994;91:4776–4780. [PubMed: 8197134]

120. Kraus TA, Lau JF, Parisien JP, Horvath CM. A hybrid IRF9-STAT2 protein recapitulates interferon-stimulated gene expression and antiviral response. J Biol Chem 2003;278:13033–13038. [PubMed: 12574168]

121. Harada H, Matsumoto M, Sato M, Kashiwazaki Y, Kimura T, Kitagawa M, Yokochi T, Sok-PinTan R, Takasugi T, Kadokawa Y, Schindler C, Schreiber RD, Noguchi S, Taniguchi T. Regulation of IFN-à/á genes: evidence for a dual function of the transcription factor complex ISGF3 in the production and action of IFN-à/á. Genes to Cells 1996;1:995–1005. [PubMed: 9077462]

122. Kimura T, Kadokawa Y, Harada H, Matsumoto M, Sato M, Kashiwazaki Y, Tarutani M, Tan RS, Takasugi T, Matsuyama T, Mak TW, Noguchi S, Taniguchi T. Essential and non-redundant roles of p48 (ISGF3 gamma) and IRF-1 in both type I and type II interferon responses, as revealed by gene targeting studies. Genes Cells 1996;1:115–124. [PubMed: 9078371]

123. Moore PS, Chang Y. Antiviral activity of tumor-suppressor pathways: clues from molecular piracy by KSHV. Trends Genet 1998;14:144–150. [PubMed: 9594662]

124. Boshoff C, Endo Y, Collins PD, Takeuchi Y, Reeves JD, Schweickart VL, Siani MA, Sasaki T, Williams TJ, Gray PW, Moore PS, Chang Y, Weiss RA. Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. Science 1997;278:290–294. [PubMed: 9323208]

125. Cunningham C, Barnard S, Blackbourn DJ, Davison AJ. Transcription mapping of human herpesvirus 8 genes encoding viral interferon regulatory factors. J Gen Virol 2003;84:1471–1483. [PubMed: 12771416]

126. Burysek L, Yeow WS, Lubyova B, Kellum M, Schafer SL, Huang YQ, Pitha PM. Functional analysis of human herpesvirus 8-encoded viral interferon regulatory factor 1 and its association with cellular interferon regulatory factors and p300. J Virol 1999;73:7334–7342. [PubMed: 10438822]

127. Flowers CC, Flowers SP, Nabel GJ. Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor confers resistance to the antiproliferative effect of interferon-alpha. Mol Med 1998;4:402–412. [PubMed: 10780883]

128. Gao SJ, Boshoff C, Jayachandra S, Weiss RA, Chang Y, Moore PS. KSHV ORF K9 (vIRF) is an oncogene which inhibits the interferon signaling pathway. Oncogene 1997;15:1979–1985. [PubMed: 9365244]

129. Li M, Lee H, Guo J, Neipel F, Fleckenstein B, Ozato K, Jung JU. Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor. J Virol 1998;72:5433–5440. [PubMed: 9620998]

130. Burysek L, Yeow WS, Pitha PM. Unique properties of a second human herpesvirus 8-encoded interferon regulatory factor (vIRF-2). J Hum Virol 1999;2:19–32. [PubMed: 10200596]

131. Li XL, Blackford JA, Judge CS, Liu M, Xiao W, Kalvakolanu DV, Hassel BA. RNase-L-dependent destabilization of interferon-induced mRNAs. A role for the 2–5A system in attenuation of the interferon response. J Biol Chem 2000;275:8880–8888. [PubMed: 10722734]

132. Nakamura H, Li M, Zarycki J, Jung JU. Inhibition of p53 tumor suppressor by viral interferon regulatory factor. J Virol 2001;75:7572–7582. [PubMed: 11462029]

133. Seo T, Park J, Lee D, Hwang SG, Choe J. Viral interferon regulatory factor 1 of Kaposi's sarcoma-associated herpesvirus binds to p53 and represses p53-dependent transcription and apoptosis. J Virol 2001;75:6193–6198. [PubMed: 11390621]

134. Burysek L, Pitha PM. Latently expressed human herpesvirus 8-encoded interferon regulatory factor 2 inhibits double-stranded RNA-activated protein kinase. J Virol 2001;75:2345–2352. [PubMed: 11160738]

135. Jenner RG, Alba MM, Boshoff C, Kellam P. Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. J Virol 2001;75:891–902. [PubMed: 11134302]

136. Fuld S, Cunningham C, Klucher K, Davison AJ, Blackbourn DJ. Inhibition of interferon signaling by the Kaposi's sarcoma-associated herpesvirus full-length viral interferon regulatory factor 2 protein. J Virol 2006;80:3092–3097. [PubMed: 16501120]

137. Lubyova B, Pitha PM. Characterization of a novel human herpesvirus 8-encoded protein, vIRF-3, that shows homology to viral and cellular interferon regulatory factors. J Virol 2000;74:8194–8201. [PubMed: 10933732]

138. Rivas C, Thlick AE, Parravicini C, Moore PS, Chang Y. Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. J Virol 2001;75:429–438. [PubMed: 11119611]

139. Lubyova B, Kellum MJ, Frisancho AJ, Pitha PM. Kaposi's sarcoma-associated herpesvirus-encoded vIRF-3 stimulates the transcriptional activity of cellular IRF-3 and IRF-7. J Biol Chem 2004;279:7643–7654. [PubMed: 14668346]

140. Seo T, Park J, Lim C, Choe J. Inhibition of nuclear factor kappaB activity by viral interferon regulatory factor 3 of Kaposi's sarcoma-associated herpesvirus. Oncogene 2004;23:6146–6155. [PubMed: 15208654]

Table 1

Phenotypic changes in IRF null mice.

IRF	Defects	Reference
IRF-1	Apoptosis, iNOS, IL-12	23,24,25,26
IRF-2	NK cells deficiency and inhibition of NK cells maturation; development of myeloid DC	37,38
IRF-3	Down modulation of type I IFN induction; increased susceptibility to infection	63,66,
IRF-4	T, B cells maturation, B cells differentiation Th2 response	87,88
IRF-5	Induction of inflammatory cytokines, IL-6, TNF α and IL12	105
IRF-6	Embryonic lethal, differentiation of keratinocytes	101,102
IRF-7	Block in Type I IFN induction	66
IRF-8	Differentiation to pDC, induction of IL-12, IL-23	97,98,99,100
IRF-9	Type I and II IFN signaling, induction of IRF-7 and IFNα and ISG	95,121