Rapid induction of type I interferon (IFN) expression is a central event in the establishment of the innate immune response against viral infection and requires the activation of multiple transcriptional proteins following engagement and signaling through Toll-like receptor-dependent and -independent pathways. The transcription factor interferon regulatory factor-3 (IRF-3) contributes to a first line of defense against viral infection by inducing the production of IFN-β that in turn amplifies the IFN response and the development of antiviral activity. In murine knock-out models, the absence of IRF-3 and the closely related IRF-7 ablates IFN production and increases viral pathogenesis, thus supporting a pivotal role for IRF-3/IRF-7 in the development of the host antiviral response.

Upon recognition of specific molecular components of viruses, the host cell activates multiple signaling cascades that stimulate an innate antiviral response, resulting in the disruption of viral replication and the mobilization of the adaptive arm of the immune system (1, 2). Central to the host antiviral response is the production of type I interferons (IFNs), a large family of multifunctional immunoregulatory proteins (3). Multiple Toll-like receptor (TLR)-dependent (TLR-3, -4, -7, and -9) and -independent (RIG-I and Mda5) pathways are involved in the cell-specific regulation of type I IFNs, with evidence accumulating that cooperation between different pathways is required to ensure a robust and controlled activation of antiviral response (1). Signal-induced activation of latent transcription factors such as nuclear factor κB (NF-κB) and interferon regulatory factors (IRF) via post-translational modifications, primarily phosphorylation events, leads to the recruitment of these factors to the type I IFN promoters in a temporally and spatially coordinated manner. This review will focus on the mechanisms of activation and the physiological functions of IRF-3 and the closely related IRF-7, whereas other articles in this series from Drs. Akira and Fujita will detail aspects of TLR-dependent and -independent signaling pathways that trigger innate antiviral responses.

The best understood example of a virus-inducible transcription unit is the IFN-β promoter. A 60-bp DNA fragment, located −110 and −36 relative to the transcription start site, is a virus-inducible molecular switch composed of four positive regulatory domains (PRDI–IV). The IFN-β gene is activated by the cooperative binding of three transcription factor families (NF-κB, IRFs, and ATF-2/c-Jun) and an architectural protein (HMG I(Y)) to the nucleosome-free PRD regions of the promoter to form an enhanceosome (4). The enhanceosome modifies and repositions a nucleosome that blocks the formation of a transcriptional preinitiation complex on the IFN-β promoter; this is accomplished by the ordered recruitment of histone acetyltransferases, SWI/SNF, and basal transcription factors. Acetylation of the nucleosome by the GCN5 histone acetyltransferase-containing complex is followed by the recruitment of the CBP-PolII holoenzyme. Next, nucleosome structure is altered by the SWI/SNF remodeling machine, thus permitting the recruitment of TFIIID to the TATA element (5, 6). The DNA bending induced upon TFIIID binding to the promoter causes sliding of the SWI/SNF-modified nucleosome to a new position 36 bp downstream, thus allowing the initiation of transcription (5).

IRF-3

Specificity of type I IFN induction is achieved by members of the IRF transcription factor family. In all, nine human IRFs have been identified (IRF-1–IRF-9); each member shares extensive homology in the N-terminal DNA binding domain (DBD), characterized by five tryptophan repeat elements located within the first 150 amino acids of the protein. The IRF DNA binding domain mediates specific binding to GAAANN and AANNNGAA sequences, termed the IFN-stimulated regulatory element in IFN-stimulated genes (ISGs). In addition to their role in immune regulation, IRFs are also involved in regulation of cell cycle, apoptosis, and tumor suppression (3). Each IRF contains a unique C-terminal domain, termed the IRF association domain (IAD); the unique function of a particular IRF is accounted for by the ability of the IAD to interact with other members of the IRF family and other factors, its intrinsic transcriptional potential, and cell type-specific expression of the IRFs.

IRF-3, a critical player in the induction of type I IFNs following virus infection, is a constitutively expressed phosphoprotein of 427 amino acids (7). Transcriptional activity of IRF-3 is controlled by virus and dsRNA-induced, C-terminal phosphorylation events on serines 385 and 386, as well as the serine/threonine cluster between amino acids 396 and 405 (8, 9), mediated by the IKK-related kinases TBK-1 and IKKe (10, 11) (Fig. 1). Based on available biochemical data, a model for IRF-3 activation proposes that
C-terminal phosphorylation induces a conformational change in IRF-3 that allows homo- and heterodimerization, nuclear localization, and association with the co-activator CBP/p300 (12). Inactive IRF-3 constitutively shuttles into and out of the nucleus, whereas phosphorylation-dependent association with CBP/p300 retains IRF-3 in the nucleus and induces transcription of IFN-β and other genes (13).

**IRF-7**

IRF-7 was first described to bind and repress the Epstein-Barr virus Qp promoter regulating Epstein-Barr virus nuclear antigen I (14), but its importance in virus-induced IFN-α gene regulation was quickly recognized (15–17). IRF-7 is a multifunctional protein with transcriptional activity that, like IRF-3, depends on C-terminal phosphorylation (15, 18). However, constitutive IRF-7 expression is restricted to B cells and dendritic cells; in other cells, IRF-7 is virus- and IFN-inducible. Also distinct from IRF-3, IRF-7 has a half-life of ~30 min, which may represent a mechanism that ensures transient IFN induction (15–17).

In addition to the DBD, IRF-7 contains multiple regulatory domains in the C-terminal region that regulate its activity (Fig. 1) (19). In particular, the C-terminal region between amino acids 471 and 487 (Fig. 1) is the target of virus-induced phosphorylation. Whereas C-terminal phosphorylation modulates the transactivation function of IRF-7 (18, 20), IFN and lipopolysaccharide treatment modulates transcription of the IRF-7 gene (21), making IRF-7 regulation dually dependent on stimuli. Serines 471/472 and 477/479 appear to be important residues of activation (15, 22); substitution of the Ser-477/479 with the phosphomimetic Asp also appears to be important residues of activation (15, 22); substitution of the Ser-477/479 with the phosphomimetic Asp also

**Target Gene Activation by IRF-3 and IRF-7**

Both IRF-3 and IRF-7 play distinct and essential roles in the IFN-α/β response to virus infection (3, 23). Biochemical and gene profiling studies have delineated dsRNA and virus-induced genes (24, 25) and have attempted to dissect IRF-3 and IRF-7 target genes (26, 27). Human IFN-β, IFN-α1, and RANTES (regulated on activation normal T cell expressed and secreted) promoters are stimulated by IRF-3 coexpression, whereas IFN-α4, -α7, and -α14 promoters are preferentially induced by IRF-7 only (19). DNA binding site selection studies demonstrated that IRF-3 and IRF-7 bound to the 5′-GAAAN-NGAAANN-3′ consensus motif found in many virus-inducible genes; however, single nucleotide substitutions in either of the GAAA half-site motifs will eliminate IRF-3 binding and transactivation activity but do not affect IRF-7 interaction or transactivation (19, 28). IRF-3 possesses a restricted DNA binding site specificity and interacts with CBP, whereas IRF-7 has a broader DNA binding specificity that contributes to its capacity to stimulate IFN-α subtype expression.

A mRNA profiling study of 8556 genes in Jurkat cells expressing a constitutively active form of IRF-3 identified IRF-3 target genes, including transcripts for several known ISGs, indicating that in addition to its role in IFN-β regulation, IRF-3 discriminated among IFN-stimulated regulatory element-containing genes involved in the establishment of the antiviral state (26). A microarray analysis of infected BJAB cells overexpressing IRF-7 revealed that IRF-7 also activates a subset of antiviral, inflammatory, and pro-apoptotic proteins, as well as a group of mitochondrial genes and genes affecting the DNA structure (27).

**In Vivo Requirements for IRF-3 and IRF-7 in the IFN Antiviral Response**

The importance of IRF-3 and IRF-7 in regulating the early and late phases of IFN expression (17, 19) was demonstrated through the generation of IRF-3 and IRF-7 knock-out mice (30). IRF-3−/− mice were more susceptible to virus infection, and serum IFN levels from encephalomyocarditis virus-infected mice were significantly lower in the IRF-3−/− mice than wild type mice. Additionally, cells defective in the expression of both IRF-3 and IRF-7 completely failed to induce type I IFNs in response to infection by many viruses; the lack of IFN responsiveness was recuperated by coexpressing both proteins, clearly demonstrating that IRF-3 and IRF-7 have essential and distinct roles in ensuring transcriptional efficiency of the IFN-α/β genes (30).

Using IRF-7−/− mice, Honda et al. (23, 31) demonstrated that IRF-7 is essential for the induction of type I IFN via virus-mediated and TLR-dependent signaling pathways. In IRF-7−/− mice, induction of IFN-α mRNA was completely inhibited, and the levels of IFN-β mRNA were reduced; in IRF-3/IRF-7 double knock-out MEFs, IFN-β levels were completely abrogated. Also, the serum level of IFN was significantly lower in IRF-7−/− MEFs. In contrast, MyD88−/− MEFs induced IFN-α/β mRNA to similar levels as wild-type MEFs, indicating type I IFN induction was IRF-7-dependent but MyD88-independent (3, 31).

Regulation of IFN production in plasmacytoid dendritic cells (pDCs) is completely distinct (1, 32). pDCs are known as professional IFN-producing cells and stand out among other cells
for their ability to produce high amounts of IFN-α/β after TLR-7 or TLR-9 engagement and signaling (33–36). pDCs, unlike fibroblastic, epithelial, and most hematopoietic cells, utilize a TLR- and MyD88-dependent pathway of IFN-α/β induction, which is controlled exclusively by IRF-7 (33–36) (see Fig. 3). Comparison between IRF-3−/− pDCs and IRF-7−/− pDCs revealed that IFN induction by TLR-7, -8, or -9 was normal in IRF-3−/− cells but completely ablated in IRF-7−/− cells, indicating that IRF-7 is essential and IRF-3 dispensable for MyD88-dependent induction of IFN-α/β genes in pDCs (31). Furthermore, Honda and Taniguchi (23) demonstrated that induction of CD8+ T cell responses in pDCs were completely dependent on IRF-7. Thus, IRF-7 is important in the development of innate and adaptive immunity in pDCs.

**Triggering the IFN Antiviral Response through the IKK-related Kinases**

An important breakthrough in the understanding of innate immune response signaling was the demonstration that IRF-3 and IRF-7 are the primary in vivo targets of the IKK-related kinases, TBK-1/NAK/T2K and IKKe/IKKi (10, 11). Both kinases directly phosphorylate IRF-3 and IRF-7 in their C-terminal signal-responsive domain (22, 37), and the serine residues targeted for phosphorylation during virus infection (12) are also targets of TBK-1 and IKKe in vitro (22, 38). Alignment of the primary sequence of the C-terminal domains of IRF-3 and IRF-7 revealed an extended SXSXXXS consensus motif that appears to be the in vitro target for TBK-1 and IKKe (38).

Although initially characterized as homologues of the 1kB kinases (IKKe and IKKβ) based on limited sequence homology and the potential to stimulate NF-κB signaling, TBK-1 and IKKe have distinct functions and activities (39). TBK-1 is ubiquitously expressed in a wide variety of cells, whereas IKKe expression is relegated to cells of the immune compartment (40) but is inducible in non-hematopoietic cells by stimulation with activating agents such as tumor necrosis factor, phorbol 12-myristate 13-acetate, lipopolysaccharide, and virus infection through an NF-κB-dependent mechanism (41). Importantly, expression of the IKK-related kinases is essential to initiate IRF signaling in response to de novo Sendai, vesicular stomatitis virus, or measles virus infection; RNAi directed against either IKKe or TBK-1 reduces both vesicular stomatitis virus–inducible IRF-3 phosphorylation and development of the antiviral state (10, 22).

Subsequent analysis of the response to virus infection in TBK-1−/− and IKKe−/− mice demonstrated that TBK-1 is principally involved in downstream signaling to IRF-3 and IRF-7 phosphorylation and development of the antiviral response (42, 43), with an accessory role associated to date with IKKe (42, 43). Recent studies have demonstrated that TBK-1 and IKKe are not redundant but that IKKe selectively regulates a subset of interferon-responsive antiviral genes during virus infection (44). In addition, other kinases are likely to influence signaling to the IRF-3-dependent antiviral response. For example, Sarkar et al. (45) demonstrated that another kinase, phosphatidylinositol 3-kinase, is necessary to mediate full IRF-3 activation following TLR-3 activation by dsRNA.

**Figure 2. Summary of the structural interactions within the C-terminal region of IRF-3.** The ribbon diagrams illustrate the interactions between the IAD of IRF-3 and the IBiD region of CBP. Left, intramolecular interactions between the IAD of IRF-3 (in green) and the flanking autoinhibitory structures (in red). Phosphorylation sites are in yellow. Right, intermolecular interactions between the IAD of IRF-3 (in green) and the IBiD region of CBP (in blue). The figure was provided by Dr. Kai Lin.

**Structural Studies on the IRF-3 C-terminal Domain**

The three-dimensional crystal structures of the IRF-3 DNA binding domain and C-terminal domain have been independently reported (46, 47). These studies describe an important mechanism for the regulation of IRF-3 activity, whereby N- and C-terminal autoinhibitory domains within the C-terminal IAD interact to form a highly condensed hydrophobic core. This interaction buries several key residues within the IAD involved in IRF-3 dimerization and are therefore residues required for nuclear accumulation, DNA binding, and transactivation. It is proposed that virus-inducible, C-terminal phosphorylation events abolish autoinhibitory interactions by introducing charge repulsions within this region that unmask the IAD active site and realign the DNA binding domain to form active IRF-3 with the capacity to recruit the CBP histone acetyltransferase (46, 47). Subsequently, the crystal structure of a complex between the IAD of IRF-3 and a portion of CBP/p300 was solved (Fig. 2) (48). The IRF-3-binding domain (IBiD) of CBP/p300 maps to a 46-residue segment in the C-terminal glutamine-rich region of CBP and binds to a hydrophobic surface on IAD, which is buried by intramolecular interactions in latent IRF-3 (46) (Fig. 2). The IBiD covers the same surface as the autoinhibitory elements in latent IRF-3 indicating that the condensed conformation of latent IRF-3 and the interaction with IBiD are mutually exclusive (48); consequently, these autoinhibitory elements must be displaced during activation to allow CBP interactions, consistent with phosphorylation-induced unfolding of the C-terminal segment (Fig. 2). Interestingly, the C-terminal domain of IRF-3 exhibits sequence similarity to the Mad homology 2 (MH2) domain of the Smad family of transcriptional proteins, suggesting a common evolutionary relationship between the IRF and Smad proteins (46, 47).

**Negative Regulation of IRF-3 Activity**

Several recent studies have focused on mechanisms that limit IRF-3 activity. IRF-3 is targeted for proteasomal degradation (9) following virus infection, and polyubiquitination is required for IRF-3 degradation (49). IRF-3 is recruited to Cullin1 following virus infection, and expression of a dominant-negative Cullin1 inhibits IRF-3 degradation (49).
IRF-3 is subject to direct negative regulation as well by the peptidyl-prolyl isomerase Pin1 (50). After stimulation by dsRNA, phosphorylation of the Ser339-Pro340 motif of IRF-3 results in the interaction with Pin1, polyubiquitination, and then proteasome-dependent degradation of IRF-3. Suppression of Pin1 by RNA interference or genetic deletion resulted in enhanced IRF-3-dependent IFN-β production and reduced virus replication, thus demonstrating a novel role for Pin1 peptidyl-prolyl isomerization in the negative regulation of IRF-3 responses.

In contrast, IRF-3 can also be stabilized in infected cells; both IFN treatment and RNA virus infection increase conjugation of the IFN-induced, ubiquitin-like protein ISG15 to IRF-3. ISGylation subverts ubiquitin-mediated degradation of IRF-3 and enhances IRF-3-dependent gene activity. In this case, proteolysis of IRF-3 is counteracted by induction of ISG15 expression, and ISGylation provides a feedback mechanism that enhances the host innate antiviral response via IRF-3 stabilization (51).

The activity of IRF-3 and IRF-7 may also be indirectly downregulated through a number of emerging mechanisms that act at the level of TLR-dependent and/or RIG-1-dependent signaling. For example, the NF-κB-inducible, anti-apoptotic protein A20 is a candidate negative regulator of the signaling cascade to IRF-3 activation that efficiently blocks RIG-1-mediated activation of NF-κB- and IRF-3-induced genes (52). The level of A20 inhibition appears to be upstream of TBK-1/IKKe kinases, downstream of RIG-1 itself, and may affect the formation of independently of dsRNA or 5′-triphosphate RNA by engaging in a protein complex with IPS-1 and competing with IKKe for a common interaction site on IPS-1. LGP2 may act as part of a negative feedback mechanism through protein interactions with the RIG-MAVS-IKK complex (65) and by sequestering dsRNA from RIG-1 (54). In a related study, LGP2 was shown to interact directly with RIG-1 to block RIG-1 dimerization and downstream signaling to the IFN response (66).

**Perspectives**

IRF-3 and IRF-7 are now regarded as master regulators of type I IFN activation and are firmly integrated within the TLR-dependent and -independent pathways of the innate immune response to viral pathogens. Despite a wealth of knowledge about these important transcription proteins, many unanswered questions remain regarding IRF-3/IRF-7 biology, structure, and function. The full range of target genes triggered by IRF-3 and IRF-7 remains to be elucidated; co-activators of IRF-7 have not been identified; no structural information is yet available for IRF-7 domains; how other signaling pathways may converge on IRF-3 and IRF-7 requires further investigation; understanding the negative regulation of IRF-3 and IRF-7 is just beginning; how NF-κB and IRF pathways cross-talk is unknown. The answers to these and many other questions will undoubtedly have important therapeutic implications for immune response modulation.
