

Innate Immune Receptors and IRF Family Transcription Factors

Takashi Fujita* and Mitsutoshi Yoneyama

Department of Tumor Cell Biology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo, Tokyo 113-8613, Japan

Abstract: Toll like receptors (TLRs) function as signaling receptors for pathogen-derived molecules and provoke innate immune responses, which are preparatory for initiating acquired immunity. Each TLR triggers both common and unique signals, resulting in the activation of a specific set of transcription factors and hence the activation of common and specific target genes. Some members of the Interferon Regulatory Factor (IRF) family of transcription factors are specifically activated by TLR signaling and participate in the critical processes of innate immunity. Recently, a non-TLR receptor that recognizes viral double stranded RNA and participates in the antiviral innate responses was identified.

INTRODUCTION

Interferon- α and - β (type I IFN, IFN-I) were the first cytokines identified as functioning in antiviral innate responses [1]. Large amounts of IFN-I are produced in virus-infected cultured cells of many different types. Extensive searches revealed that synthetic or virus-derived double-stranded RNA (dsRNA) is the critical molecule that directly triggers the expression of IFN-I. Bacterial infection also induces relatively low levels of IFN-I, and bacterial endotoxin is the direct trigger for the induction. More than 40 years after the discovery of IFN-I, its induction mechanisms have been elucidated at molecular levels. Toll like receptors (TLRs) recognize a group of molecules derived from infecting agents and trigger “danger” signals [2]. These signals result in the production of various cytokines, including IFN-I that activate innate responses to the infection. Furthermore, the signals result in the activation of dendritic cells (DCs) to initiate acquired immune responses [3,4]. In this review, we focus on the pathways that activate Interferon Regulatory Factors (IRFs) and particularly on IRF-3, which plays critical roles in these reactions.

IRF-3 AND IRF-7

Using cultured cells and cloned IFN-I genes, the *cis*-acting promoter elements necessary for virus-induced activation were identified [5,6]. Multiple IRF binding sites were found in IFN-I gene promoters as indispensable elements for maximal gene induction. There are 9 genes encoding IRFs in human and mouse genomes. Each IRF contains a conserved DNA binding domain at the N-terminal region that recognizes the IRF motif (also called Interferon Stimulation Response Element (ISRE) or Interferon Response Element (IRE)). The C-terminal regulatory domains diverge significantly among the family members, reflecting the functional diversity of each member [7]. Recent gene disruption studies demonstrated that IRF-3 and

IRF-7 are critical in the production of IFN-I [8]. IRF-3 and IRF-7 exhibit limited primary structure homology and are considered to be the closest relatives in the family. IRF-3 is expressed ubiquitously, and accumulates in the cytoplasm as an inactive form. IRF-7 is virtually absent in most cells and its expression is inducible by IFN treatment [9,10]. These differential expression patterns suggest that IRF-3 initiates and IRF-7 maintains and amplifies IFN-I gene expression. Like IRF-3, the IFN-induced IRF-7 is cytoplasmic and inactive. Upon induction, such as by virus infection, both IRF-3 and IRF-7 undergo phosphorylation at specific serine residues and then form active dimers [11-13]. In the case of IRF-3, its critical phosphorylation site is Ser386 [14]. IRF-7 shares a similar Ser residue at roughly equivalent position, and the mutagenesis of this Ser abolished the activation [10]. Recently, the crystal structure of the regulatory domain of IRF-3 (175C) was determined, providing insight into the activation mechanisms of IRF-3 and IRF-7 at an atomic level [15,16]. Unexpectedly, the overall structure of 175C is similar to that of the regulatory domain (Mad homology domain 2) of Smad, a critical transcription factor for TGF- β /BMP signaling. This structural similarity suggests not only an evolutionary relationship but also a mechanistic resemblance for the activation. The 175C protein crystallized as a dimer in the asymmetric unit. At the interface of the dimer, a loop that contains the phospho-acceptor Ser386 interacted with a pocket structure with basic residues from the other molecule. Although the crystallized 175C protein was not phosphorylated, the structure strongly suggested that signal-induced phosphorylation dramatically increases the interaction between the phosphorylated residues and the pocket structure. Mutagenesis of the basic amino acids in the pocket abolished dimer formation by the full-length protein, which had been produced in cultured cells and phosphorylated, supporting the validity of this model [16]. This strategy of dimer formation resembles that of Smad, although the phosphorylation occurs at the flexible tail rather than at the structured loop. IRF-3 requires the histone acetyl transferases, CBP and p300, as coactivators, not only for transactivation but also for its DNA binding activity [17,18]. Interestingly, one side of the dimer in the crystal was rich in acidic residues. Mutagenesis of these residues in the context

*Address correspondence to this author at the Department of Tumor Cell Biology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo, Tokyo 113-8613, Japan; Email: fujita@rinshoken.or.jp

of the full-length protein abolished the activation-dependent interactions with CBP and p300, suggesting that the acidic surface is an interface with the coactivators [16].

THE REGULATORY PROTEIN KINASES TBK-1 AND IKK-I ACTIVATE IRF-3 AND IRF-7

TANK Binding Kinase-1 (TBK-1) and inducible I B kinase (IKK-i) (alternatively named as IKK-) were initially identified as protein kinases structurally related to the catalytic subunits (IKK- and IKK-) of the conventional IKK complex [19-23]. Over-expression of TBK-1 or IKK-i in human 293 cells results in the specific phosphorylation and dimerization of IRF-3 [24,25]. Mice with disrupted gene for TBK-1 are embryonic lethal; however, the double knockout with *Tnfrsf1a* is viable, indicating its critical functions in other pathways besides innate immunity [26]. Macrophages derived from *Tbk-1*, *Tnfrsf1a* double knockout mice exhibited defects in dsRNA- or LPS-induced activation of IRF-3 (Fig. 1). Embryonic cells derived from the *Tbk-1* knockout mouse exhibited severely reduced activation of IRF-3 upon virus infection. The reduced response can be restored by the expression of wild type IKK-i, but not by a kinase-deficient IKK-i. Furthermore, cells from the *Tbk-1*, *Ikk-i* double knockout mouse showed virtually no activation of the pathway [27]. These results indicate that TBK-1 and IKK-i regulate IRF-3 (and possibly IRF-7) at least in a partially redundant fashion. The exact activation mechanism of these kinases and the nature of the hypothesized signalosome remain to be solved.

ACTIVATION OF IRF-3 BY TLR3 STIMULATION

Analyses of TLR3-deficient cells revealed that TLR3 functions as a receptor for dsRNA, which results in the activation of cytokine genes and the maturation of DCs [28] (Fig. 1). TLR3 is expressed on the lysosomal membrane, and its recognition of endocytosed dsRNA requires acidification of the lysosome [29]. Recently a novel dsRNA receptor, distinct from TLR3, was identified (see below). Stimulation of TLR3 results in the activation of NF- B and IRF-3, as well as the p38 MAP kinase pathway and its downstream transcription factors. IRF-3 activation and DC maturation are independent of MyD88, a common adaptor for TLR signaling [30]. Part of the NF- B activation by TLR3 is independent of MyD88, and MyD88 deficient cells exhibit NF- B activation with delayed kinetics [31]. Another adaptor, the TIR domain-containing adaptor inducing IFN- (TRIF) [32], also called TIR-containing adaptor molecule-1 (TICAM-1) [33], is responsible for the alternative pathway [34,35]. TRIF interacts with TLR3 and IRF-3 [33]. TRIF can transmit two independent signals for the activation of NF- B and IRF-3, through its distinct interfaces for the respective effectors [36,37].

ACTIVATION OF IRF-3 BY TLR4 STIMULATION

The activation of IRF-3 by LPS stimulation was first demonstrated using the glioma cell line U373 [38]. Later it was shown that TLR4 signaling activates a MyD88-independent pathway including the activation of IRF-3,

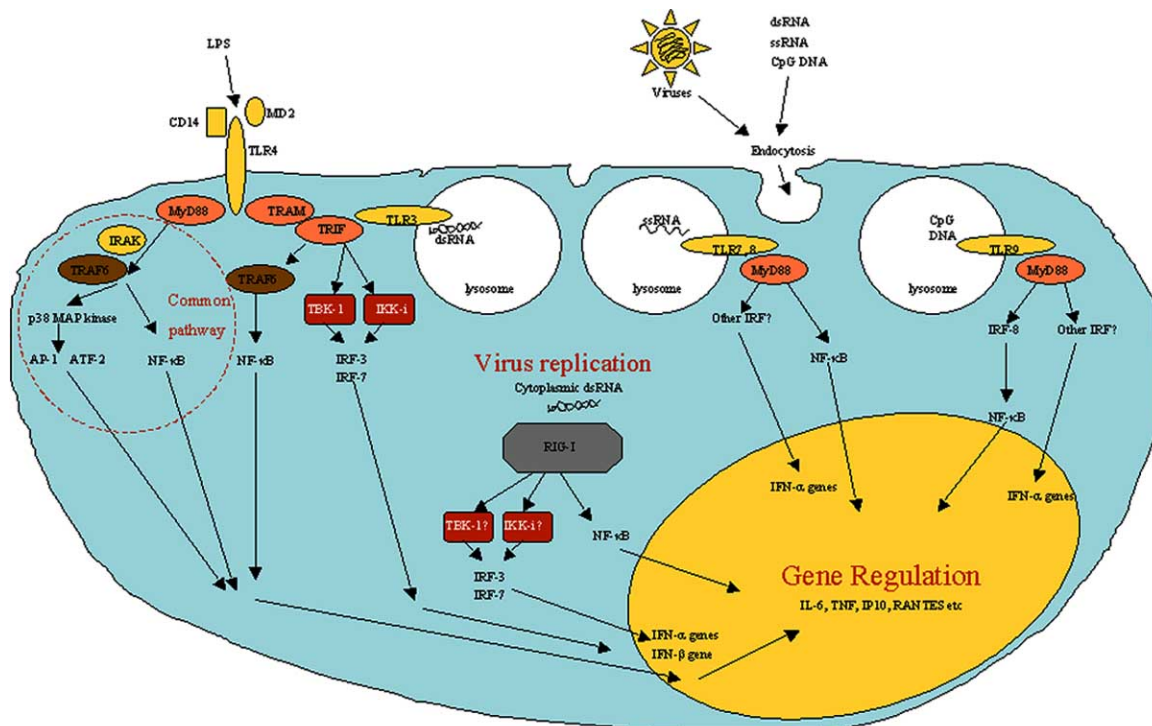


Fig. (1). Innate immune receptors and the IRF family of transcription factors

Signaling by representative TLRs is summarized. TLR3 and TLR4 activate MyD88-dependent and MyD88-independent pathways, which involve the specific signaling adaptor molecules indicated. TLR3, TLR7, TLR8 and TLR9 recognize their respective ligands in the lysosomal compartment and trigger signals to the cytoplasm. A novel pathogen receptor, RIG-I, recognizes cytoplasmic dsRNA and induces the activation of IRF-3, 7 and NF- B. These signals are integrated in the nucleus, resulting in the activation of specific target genes.

using monocytes and macrophages derived from knockout mice [31]. Although TLR4 signaling requires TRIF [34,35], they do not interact directly [39]. Another adaptor, TRIF-related adaptor molecule (TRAM), also called TIR-containing adaptor molecule-2 (TICAM-2), mediates the signal from TLR4 to TRIF [39-41]. Thus, TLR3 and TLR4 activate two pathways, dependent on MyD88 or TRIF, (Fig. 1).

SIGNAL TRANSDUCTION BY TLR7 AND TLR8

Low molecular compounds, imidazoquinoline and a guanosine derivative, were shown to function as ligands for TLR7, using peritoneal cells derived from knockout mice [42]. Imidazoquinoline was already known to induce the production of cytokines, including IFN-I. However this signaling is dependent on MyD88. Searches for natural ligands for TLR7 using plasmacytoid dendritic cells (PDCs) revealed that single stranded (ss) RNA, particularly poly U, and particles of RNA viruses are recognized in the lysosome compartment after endocytotic incorporation into the cells [43-45]. Irrespective of the homology between TLR7 and TLR8 derived from human and mouse, these receptors function differently. Functional complementation experiments using human 293 cells, which express neither TLR7 nor TLR8, revealed that human TLR7 and TLR8 can each confer the reactivity to the imidazoquinoline derivative R-848, but only TLR8 confers the reactivity to ssRNA [44]. These results indicate that mouse TLR7 and human TLR8 function as receptors for ssRNA (Fig. 1). PDCs produce a large amount of IFN- upon stimulation with ssRNA; however, its activation mechanism, and particularly the activation of the IRF family, remains to be elucidated.

SIGNALING BY TLR9 AND IRF-8

TLR9 recognizes CpG DNA and transmits signals that result in cytokine production including IFN- and DC maturation [46,47]. IRF-8-deficient mice have fewer DCs, suggesting that IRF-8 is critical in the generation of DCs [48-51]. The generated DCs are mostly non-PDCs and are non-responsive to CpG for the activation of NF- B and the downstream cytokine genes. Expression of IRF-8 in these cells restored the activation of NF- B [52], suggesting that IRF-8 plays dual roles in the development of DCs and the activation of NF- B in TLR9-induced signaling.

ACTIVATION OF IRF-3 AND CYTOKINE PRODUCTION TRIGGERED BY NON-TLR INNATE PATHOGEN RECEPTORS

Virus infection induces the production of IFN-I in cells from a wide range of tissues and in cultured cells. The embryonic fibroblasts derived from TLR3 deficient mice normally induce IFN-I upon stimulation by virus infection or dsRNA transfection. These results led to the hypothesis that a cytoplasmic receptor for dsRNA other than TLR3 exists. Recently, expression cloning experiments identified an RNA helicase, termed Retinoic acid Inducible Gene-I (RIG-I), as a critical molecule in dsRNA-induced antiviral responses [53] (Fig. 1). Expression of RIG-I is IFN-inducible; however, without an appropriate trigger, the protein is biologically inert. RIG-I is composed of a helicase domain of the DExH/D RNA helicase family and a short domain homologous to the Caspase recruitment domain (CARD)(Fig. 2). The helicase domain directly recognizes dsRNA, which results in the activation of its ATPase activity. The helicase domain alone or the full-length RIG-I

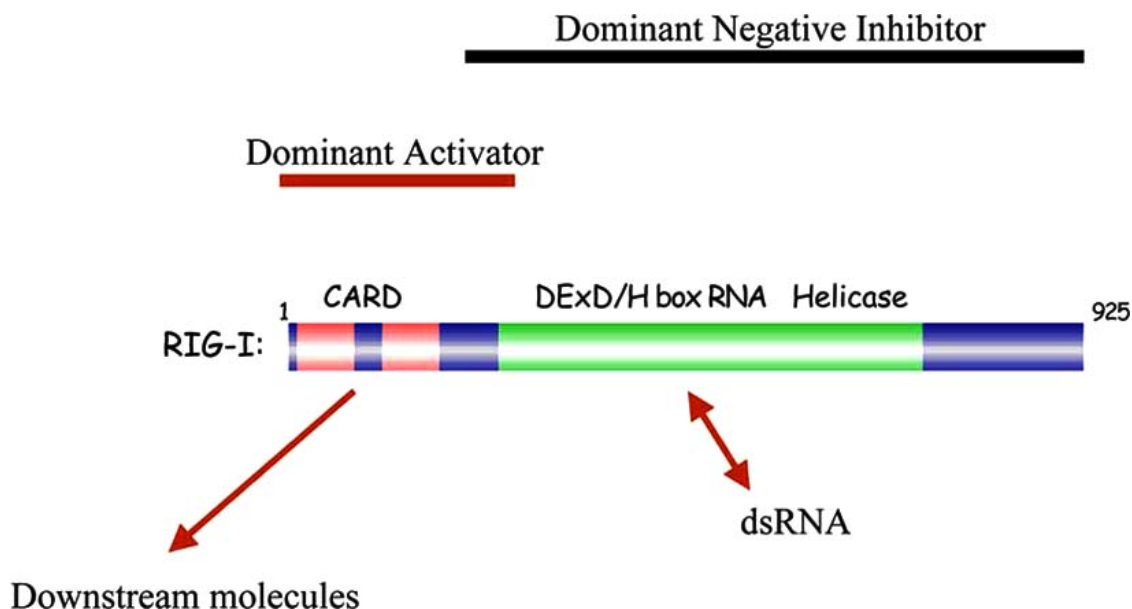


Fig. (2). Domain structure of RIG-I

Schematic representation of RIG-I. RIG-I contains CARD and Helicase domains at the N-terminal and C-terminal regions, respectively. When isolated, each domain functions as a dominant activator and a dominant inhibitor of the signaling, respectively. The helicase domain is responsible for the interaction with dsRNA.

with a disrupted ATPase functions as a dominant inhibitor for virus-induced gene activation. The expression of CARD alone activates the downstream transcription factors NF- κ B and IRF-3, resulting in the induction of type I IFN. Analyses using gene knockdown and biological assays strongly suggested its critical role in antiviral responses. Thus, RIG-I is the first non-TLR molecule that senses cytoplasmic dsRNA by its helicase domain and promotes the activation of its effector domain, CARD to further the innate antiviral signals.

We have presented an overview of the pathogen recognition and activation of IRF transcription factors. However, there are many molecules still missing from the picture, particularly the IRFs other than IRF-3 and 7, as well as the growing members of the non-TLR pathogen receptors.

NOTE ADDED TO THE PROOF

After we completed the review, an important paper was published by S. Akira's group (Kawai *et al.*, *Nat. Immunol.*, **2004**, 5, 1061) that IRF-7 plays an important role at downstream of MyD88 signaling.

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