Journal of Biochemistry and Molecular Biology, Vol. 37, No. 6, November 2004, pp. 635-641

JOURNAL OF
Biochemistry and Molecular Biology

Review

Mechanisms of Type-I Interferon Signal Transduction

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Received 15 September 2004

Interferons regulate a number of biological functions including control of cell proliferation, generation of antiviral activities and immumodulation in human cells. Studies by several investigators have identified a number of cellular signaling cascades that are activated during engagement of interferon receptors. The activation of multiple signaling cascades by the interferon receptors appears to be critical for the generation of interferon-mediated biological functions and immune surveillance. The present review summarizes the existing knowledge on the multiple signaling cascades activated by Type I interferons. Recent developments in this research area are emphasized and the implications of these new discoveries on our understanding of interferon actions are discussed.

Keywords: Interferons, IRS/PI3' kinase pathway, Jak/Stat pathway, Signal transduction

Interferons (IFNs) are a group of cytokines that possesses a variety of biological functions including inhibition of proliferation, induction of differentiation, modulation of immune system, and inhibition of angiogenenesis. (Petska *et al*., 1987; Pfeffer *et al*., 1998; Stark *et al*., 1998; Uddin *et al*., 1999; Brierley and Fish, 2002). Although initially IFNs were discovered through their antiviral activities, they are now known to exert pleiotropic effects on important cellular functions through multiple signaling pathways (Fig. 1). Because of their important biological activities, IFNs are used in the treatment of various illnesses, including tumors, viral infections, autoimmune disorders and neurological syndromes (Pamar and Platanias, 2003). Despite the widespread use of IFNs as effective pharmacological agents, their exact mechanisms of action in the various diseases still remains elusive. Such difficulty in understanding the mechanisms by which these cytokines act may reflect the complexity of the interferon system in humans. The goal of this review is to summarize the recent findings on the signaling functions engaged by Type I IFNs on target cells.

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Classification of interferons and interferon receptors

IFNs are classified in two major groups: the Type I (IFNα, IFNβ, IFNω) and Type II (IFNγ) interferons. The genes for the different Type I IFNs are all located together, on chromosome 9 (Dias *et al*., 1994). IFNα, IFNβ and IFNω have very significant structural homology with each other, but they have only minimal homology with IFNγ (Petska *et al*., 1987; Stark *et al*., 1998), which is structurally a very different cytokine. All interferons initiate their biological effects by binding to specific receptors present on the cell surface. The type I interferons (IFNα, IFNβ, IFNω) bind to the Type I IFN receptor, while IFNγ binds to the Type II IFN receptor (Petska *et al*., 1987). The Type I IFN receptor (IFNR) has a multisubunit structure in haematopoietic cells (Colamonici *et al*., 1992; Platanias *et al*., 1992; Platanias *et al*., 1993; Colamonici and Domanski, 1994; Uze *et al*., 1995) and at least two distinct chains form its structure: the IFNAR1 and the IFNAR2 subunits (Uze *et al*., 1990; Novick *et al*., 1994; Colamonici *et al*., 1994; Lutfalla *et al* 1995; Domaniski *et al*., 1995). IFNAR1 has a relative molecular mass of 110 kDa, while the IFNAR2 protein occurs in two different forms that are formed during differential splicing of the same gene. These include the IFNAR2c form, a protein with a relative molecular mass of 90-100 kDa and the IFNAR2b form, a protein with a relative molecular mass of 51 kDa. Although the Type II IFN (IFNγ) receptor is completely distinct and different than the Type I, it has a similar structure. The type II IFN receptor is composed of two subunits named as IFNGR1 and IFNGR2. IFNGR1 has a relative molecular mass of 90 kDa and is the major binding subunit. IFNGR2 is a 62 kDa

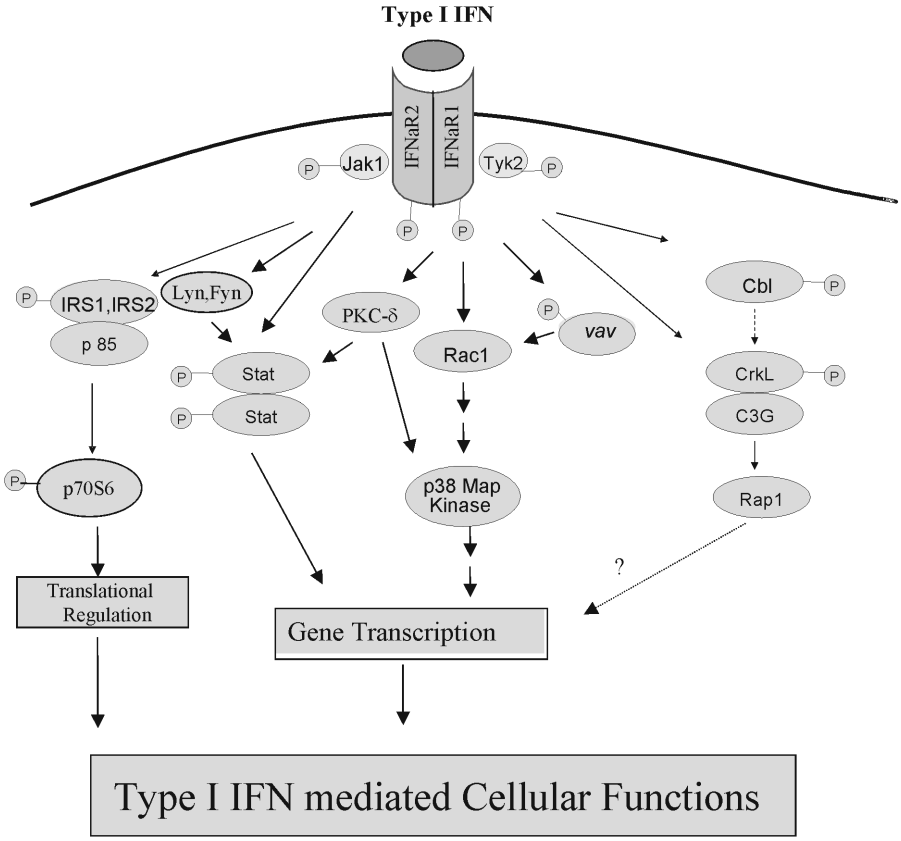

Fig. 1. Multiple signaling pathways mediated by Type I IFN.

glycoprotein that plays a minimal role in ligand binding, but participates in the generation of and transmits IFNγ-signals (Hemmi *et al*., 1994; Bach *et al*., 1997; Stark *et al*., 1998).

The Jak-Stat pathway

The Janus family is composed of non-receptor protein tyrosine kinases (Jaks) that are located proximate to membrane cytokine receptor and are activated by phosphorylation following receptor-ligand binding (Darnell *et al*., 1994; Schindler and Drenell, 1995). Among the four mammalian Jak members (Jak1, 2, 3, and Tyk2) only three have been found to mediate the activity of IFNs. These are the Jak1, Jak2 and Tyk-2 kinases. Jak kinases play a very important role in the generation of interferon signals, as they are responsible for and regulate the tyrosine phosphorylation of Stat-proteins (signal transducers and activators of transcription). Tyk2 and Jak1 are associated with the Type I IFN receptor and are activated when different Type I interferons engage the receptor (Muller *et al*., 1993; Silvenoinen *et al*., 1993; Colamonici *et al*., 1994; Colamonici *et al*., 1995; Uddin *et al*., 1995; Domanaski *et al*., 1997). Then, the activated Jaks induce tyrosine phosphorylation of the receptor subunits (Platanias and Colamonici, 1992; Platanias *et al*., 1994; Abramovich *et al*., 1994; Platanias *et al*., 1996) and positively control activation of several Stat-proteins (Stat-1, Stat-2, Stat-3, and Stat-5). These Stat-members then form homo- and/or hetero-dimmers and translocate to the nucleus to regulate gene transcription. Such regulatory effects on transcription occur via their ability to bind specific sequences present in the promoters of interferon regulated genes, with a consequence the induction of expression of such genes (Meineki *et al*., 1996; Stark *et al*., 1998; White, 1998). Stat1 and Stat2 form a dimer that associates with another protein, p48, a member of the IRF family of proteins (Stark *et al*., 1998) (also called IRF-9). This results in the formation of the interferon stimulated gene factor-3 (ISGF3) complex, which moves to the nucleus to bind to interferon stimulated response elements (ISREs) to induce gene transcription (Darnell, 1997; Uddin *et al*., 1999). In a similar manner, Type I interferons induce the formation of Stat 3:3 homodimers and Stat 3:1 heterodimers (SIF complexes) (Beadling *et al*., 1994) and Stat 5:5 homodimers (Meinke *et al*., 1996) that bind to GAS elements in the promoters of certain interferon regulated genes. Thus, the Jak-Stat pathway can directly transmit signals from the cell surface to the nucleus, without multiple intermediate pathways.

The Vav proto-oncogene in interferon mediated signal transduction

The Vav proto-oncogene product participates in the signaling pathways activated by various cell-surface receptors, including the type I IFN receptor (Platanias and Sweet, 1994). p95vav also forms a stable complex with the IFN-receptor-associated Tyk-2 kinase *in vivo*, and it appears that this kinase regulates its phosphorylation on tyrosine (Uddin *et al*., 1997c). Interestingly, other studies have shown that the phosphorylated p95Vav under IFN-alpha treatment, associates with both chains that constitute the functional type I IFN receptor and contributes to the antiproliferative effects of IFN-alpha (Micouin *et al*., 2000**)**. It has been also reported that IFN treatment triggers translocation of Vav from its nuclear compartment to the cytoplasm where it forms a complex with Tyk2 (Adam *et al*., 2000). Furthermore, IFN induces the formation of a complex between Tyk-2, Vav and Ku-80 in the cytoplasm (Adam *et al*., 2000). Vav has been shown to promote the GDP/GTP exchange activity of small GTPase Rac1 and which activates its downstream signaling. Rac1 has been shown regulate activation of P38 during IFN generated signal (Uddin *et al.*, 2000) suggesting tyrosine phosphorylated Vav may regulate MAP kinase p38 signaling pathway in the interferon-system.

The role of Src-kinases in IFN mediated signal transduction

In addition to Jak-kinases, there is some evidence that Src kinases participate in the induction of IFN-responses. IFN alpha has been shown to induced association of p59 (fyn) with the Type I IFNR-associated Tyk-2 kinase in several human hematopoietic cell lines (Uddin *et al*., 1997d). This interaction is direct, and is mediated by the SH2 domain in p59 (fyn). Furthermore, in response to IFN alpha-treatment of cells, Fyn interacts with the Tyk-2-associated c-cbl proto-oncogene product. In a similar manner, during Type II IFN (IFNγ) stimulation, p59fyn associates via its SH2 domain with the activated form of the IFN gamma-dependent Jak-2 kinase. Altogether, these findings suggest that p59 (fyn) is a common element in IFN alpha and IFN gamma signaling, and is engaged in interferon signaling via specific interactions with distinct Jak kinases. Type I IFNs also activate Lyn, another member of src family of kinases in a variety of B cells, (Uddin *et al.*, 1998) via a similar mechanism involving association of the Lyn src homology 2 (SH2) domain with the Janus family tyrosine kinase Tyk2 (Uddin *et al*., 1998). The precise functions of Src kinases in IFN-signaling remain to be defined, but it is possible that these kinases participate in the phosphorylation of Stat-proteins and/or other signaling substrates, downstream of Jak kinases.

The insulin receptor substrate (IRS)/PI-3' kinase cascade and the engagement of mTOR and the p70 S6 kinase pathway in IFN-signaling

Another important pathway that is activated by Type I IFNs involves members of the insulin receptor substrate (IRS)-system of docking proteins. These proteins are substrates for Jaks and other tyrosine kinases and via phosphorylation of tyrosines in their structure provide docking sites for the src homology 2 (SH2)-domains of downstream signaling elements (White, 1998). Insulin receptor substrate-1 (IRS-1) is the most extensively studied member of this family of proteins. IRS-1 is tyrosine phosphorylated by IFNα or IFNβ treatment of various cell lines of diverse origin (Uddin *et al*., 1995). Then, its tyrosine-phosphorylated form associates with the p85 regulatory subunit of the PI 3'-kinase, resulting in activation the catalytic domain of the PI 3'-kinase (Uddin *et al*., 1995). The serine kinase activity of the PI 3´-kinase is also induced during its interaction with IRS-1 (Uddin *et al*., 1997a), resulting in the phosphorylation of downstream proteins to transduce interferon signals. It should be pointed out that activation of the PI3'-kinase is not required for Type I IFN-dependent formation of the Stat-complexes, such as the ISGF3 complex (Uddin *et al*., 1997a). Furthermore, the Stat-pathway operates distinctively from the IRS-PI3'-kinase pathway and does not require IRS-proteins for its activation (Uddin *et al.*, 1997b). In addition to IRS-1, another member of the family, IRS-2, is also phosphorylated/activated by IFNs and regulates downstream engagement of the PI 3'-kinase pathway (Platanias *et al.*, 1996). The precise role of the IRS-PI 3'-kinase cascade in the generation of IFN-responses was until recently unknown. However, recent studies have shown that the IFN-activated PI 3'-kinase regulates downstream phosphorylation of both p70S6 kinase and the 4E-BP1 repressor of translation, events that regulate initiation of mRNA translation in mammalian cells (Ferrari and Thomos, 1994; Chou and Blenis, 1995). This regulation has been shown to involve the activity of mTOR while the p70 S6 kinase is a serine kinase that regulates the phosphorylation of the 40S ribosomal S6 protein and plays an important roles in the regulation of cell-cycle progression, cell survival, as well as regulation of mRNAs translation (Ferrari and Thomos 1994; Chou and Blenis, 1995). In the interferon system it has been shown that both Type I and II IFNs induce phosphorylation of p70S6 kinase, in a PI3-kinase and mammalian target of rapamycin (mTOR)-dependent manner (Lekmine *et al*., 2003, Lekmine *et al*., 2004). There is also mTOR-dependent phosphorylation of 4E-BP1 on Ser65, Thr70, and Thr37/46, events required for its dissociation from eIF4E and the initiation of mRNA translation. The functional relevance of this cascade in the interferon system was further established by a recent study that demonstrated its requirement in IFN-dependent apoptosis **(**Thyrell *et al.*, 2004).

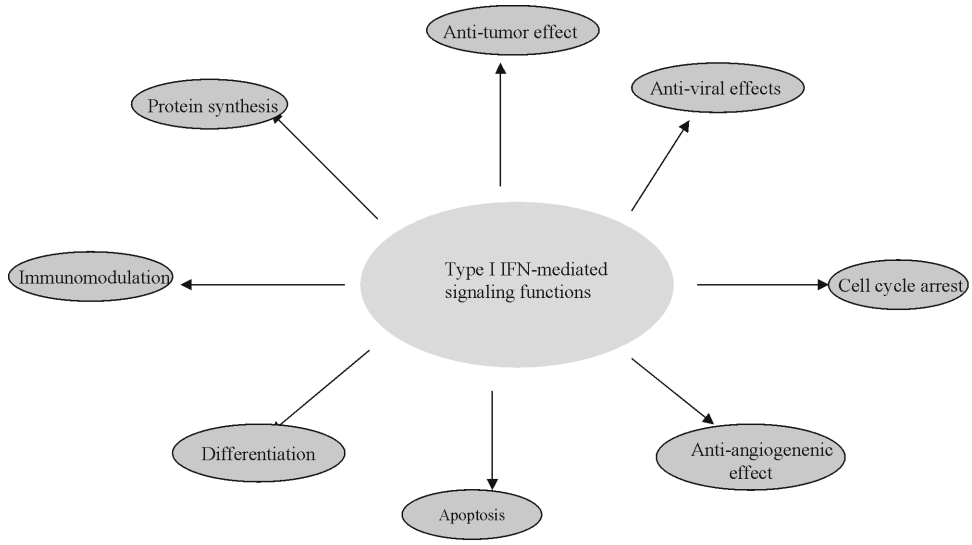

Fig. 2. Multiple biological functions mediated by Type I IFN signal transduction.

Role of PKC-δ in Type I IFN signal transduction

Jak dependent tyrosine phosphorylation of Stat proteins plays a critical role in IFN mediated gene transcription. It is well known that phosphorylation of Stat1 and Stat3 on Ser727 in their C-terminal domains, is required for full IFN-inducible transcriptional activation (Wen and Darnell, 1997; Zhu *et al* 1997; Zhang *et al*., 1998; Yeh and Pellegrini, 1999). Despite the well documented role of serine 727 phosphorylation in the generation of interferon responses, the identity of the serine kinase that regulates this event has been until recently unknown. Recently, we have shown that PKC-δ is activated by Type I and II IFNs to regulate the serine phosphorylation of Stat1. Inhibition of PKC-δ activation by IFN using genetic or pharmacological approaches inhibits serine phosphorylation of Stat1 and gene transcription via ISRE or GAS elements (Uddin *et al*., 2002). In addition inhibition of IFN activated PKC-δ abrogated the activation of p38 suggesting the cross talk of PKC-δ and p38 Mapkinase signaling pathways during IFN generated signals. Further studies to determine the functional contribution of other PKC-isoforms in interferon signaling are currently under way.

The Crk-family of proteins and Type I interferon signaling

CrkL, CrkI and CrkII proteins with SH2 domains, and are human homologues of the v-crk proto-oncogene (Mayer *et al.*, 1998). These proteins play important roles in cytokine signaling, functioning as adapters to link cytokine receptors to downstream signaling elements (Sattler and Salgia, 1998). These proteins are also involved in interferon signaling, as shown in studies demonstrating that Type I interferons induce tyrosine phosphorylation of CrkL and promote the formation of a complex involving CrkL, the CBL proto-oncogene and the kinase Tyk-2 (Ahmad *et al*., 1997, Uddin *et al.*, 1997). The related CrkII protein also is involved in Type I IFN-signaling (Platanias *et al.*, 1999). Both CrkL and CrkII play important roles in the generation of the inhibitory effects of interferons on primitive hematopoietic progenitors. Studies using specific antisense oligonucleotides against CrkL and CrkII demonstrated that the function of these proteins is essential for the generation of the suppressive effects of IFNα on erythroid (BFU-E) and myeloid (CFU-GM) progenitors (Platanias *et al*., 1999). The precise mechanisms by which Crk-proteins mediate growth inhibition in response to interferons also remain to be determined. It is now well established that CrkL forms DNA-binding complexes with a Stat-protein, Stat5 (Fish *et al*., 1999). This CrkL-Stat5 interaction is mediated by IFN-inducible binding of the CrkL-SH2 domain to tyrosine phosphorylated Stat-5, and the resulting CrkL-Stat5 complexes translocate to the nucleus and bind to palindromic sequences in the promoters of IFN-stimulated genes (Fish *et al*., 1999). Thus, CrkL regulates the transcriptional function of Stat-5, acting as a nuclear adaptor for Stat5.

CrkL also interacts via its N-terminus SH3 domain with C3G, a guanine exchange factor for the small G-protein Rap1 (Gotoh *et al*., 1995), which antagonizes the Ras pathway and has tumor suppressor activity (Kitayama *et al*., 1989; Kitayama *et al*., 1990; Cook *et al*., 1993). IFNα activates Rap1, apparently via regulating CrkL phosphorylation, and this small G-protein may be an important mechanism for the generation of IFN-dependent growth inhibition.

Map kinase signaling cascades

Members of the Map family of kinases are also activated by the Type I IFN receptor and participate in the induction of

IFN-responses. The p38 Map kinase pathway appears to play a very important role in the IFN-system. The alfa-isoform of p38 (p38α) is rapidly phosphorylated and activated in a Type I IFN-dependent manner (Uddin *et al.*, 1999). Such an activation is regulated by the small G-protein Rac1, which functions as its upstream effector in a tyrosine kinase-dependent manner (Uddin *et al.*, 2000). The activated form of p38 subsequently results in downstream engagement of a series of serine kinases, including the MapKapK-2 and MapKapK-3 kinases (Uddin *et al.*, 1999). It is now well established that that p38 plays a critical role in Type I IFN-dependent transcriptional regulation, without modifying activation of the Stat-pathway. In particular it has been shown using pharmacological inhibitors of p38 and dominant-negative mutants that the function of p38 is essential for gene transcription via ISRE or GAS elements, independently of any effects on the phosphorylation of Stat-proteins, the formation of Stat-complexes, and their binding to the promoters of IFN-sensitive genes (Uddin *et al*., 1999, Uddin *et al.*, 2000). These phenomena have been more recently confirmed in studies using cells with targeted disruption of the p38-alfa gene (Li *et al*., 2004). Studies using pharmacological inhibitors of p38 or MapKapK-2 knockout cells have established that the p38 pathway is essential for the induction of the antiviral effects of IFNα (Mayer *et al*., 2001, Li *et al.*, 2004). In addition, pharmacological inhibition of p38 reverses the inhibitory effects of IFNs on normal human myeloid and eythroid progenitors, indicating a critical role for this signaling cascade in the regulatory effects of Type I IFNs on hematopoiesis (Verma *et al*., 2002). p38 is also activated during IFNα-treatment of primary leukemia cells from patients with chronic myelogenous leukemia and mediates antileukemic responses (Mayer *et al.*, 2001). Such activation is required for IFNα-dependent suppression of leukemic cell progenitor growth, indicating that this pathway plays a critical role in the induction of the antileukemic effects of IFNα.

Conclusions and translational-therapeutic implications

IFNs are important physiological regulators of various biological functions in humans and excellent pharmacological agents for several diseases, including malignancies and viral infections. These agents have changed the natural history of a variety of malignant and non-malignant disorders and form the basis for the development of other cytokine-based treatments in humans. At this point it is unclear what the role of IFNs will be in the future management of human diseases. Future advances in IFN-based therapy are likely to be based on the emerging information about the cellular actions of IFN and IFN-related signal transduction pathways (Fig. 1 and 2). The identification of multiple signaling pathways activated by the interferon receptors has dramatically advanced the knowledge on the cellular mechanisms by which interferons mediate their effects. Continuation of these efforts in the future will be important. As the components of these pathways become more clearly understood, new potential therapeutic targets may be identified for the development of novel therapies for cancer and viral infections.

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