Arch Immunol Ther Exp, 2004, 52, 156–163
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Review

Received: 2003.12.17
Accepted: 2004.02.25
Published: 2004.06.15

Mechanisms of type I interferon signaling in normal and malignant cells

Yongzhong Li, Kishore K. Srivastava and Leonidas C. Platanias

Robert H. Lurie Comprehensive Cancer Center and Division of Hematology-Oncology, Northwestern University Medical School and Lakeside Veterans, Administration Medical Center, Chicago, IL 60611, USA

Summary

Type I interferons (IFNs) are cytokines that induce multiple biological effects on target cells, including antiviral, antiproliferative, and immunomodulatory activities. Consistent with the pleiotropic nature of these cytokines, multiple signaling pathways are activated during binding of IFNs to the type I IFN receptor. An important signaling cascade activated by type I IFNs is the Jak-Stat pathway. Activation of the Tyk-2 and Jak-1 kinases, and downstream formation of various Stat complexes, mediates IFN-dependent gene transcription for IFN-stimulated genes. In addition to the classic Jak-Stat pathway, type I IFNs activate multiple other pathways, including the insulin receptor substrate-phosphatidylinositol 3'-kinase cascade, the CBL-CrkL pathway, and mitogen-activated protein kinase pathways. There is accumulating evidence that non-Stat IFN-regulated signaling pathways play important roles in the generation of the antiproliferative effects of type I IFNs. In this review, the regulation of various signaling cascades by the type I IFN receptor is summarized and an update on recent advances in the field is provided.

Key words: **interferon • cytokines • signaling • malignat cells**

Full-text PDF: http://www.aite-online/pdf/vol_52/no_3/5534.pdf

Author's address: Prof. Leonidas C. Platanias, M.D. Ph.D., Robert H. Lurie Comprehensive Cancer Center, Northwestern University Medical School, 710 North Fairbanks Court, Olson 8250, Chicago, IL 60611, USA, tel.: +1 312 5034267, fax: +1 312 9081372, e-mail: l-platanias@ northwestern.edu

INTRODUCTION

The family of type I interferons (IFNs) includes several cytokine members, all of which are characterized by their ability to induce antiviral responses and growth inhibitory effects on normal and malignant cells[51-54]. There are at least 4 different subtypes of type I IFNs: IFNα, IFNβ, IFNω, and IFNτ, all of which share substantial homology with each other[51-54, 56. 68]. Several different IFNα subtypes exist, while there is only one IFNβ, one IFNω, and one IFNτ[51-54]. The genes encoding the distinct type I IFNs are clustered on chromosome 9 and include 14 distinct IFNα genes, 4 IFNα pseudogenes, one IFNβ and one IFNω gene[21].

All type I IFNs share a common receptor, the type I IFN receptor (IFN-R) [45, 50, 54, 65], whose function is essential for the generation of the biological activities of all the different type I IFNs. The type I IFN-R has a multisubunit structure[10, 11, 59 60, 90] and is composed of at least two different chains: IFNaR1 (previously called the α subunit)[89] and IFNaR2 (previously called the β subunit)[22, 42, 49]. Two different forms of IFNaR2 exist, resulting from differential splicing of the same gene, IFNaR2b[49] and IFNaR2c[22, 42]. After binding of type I IFNs to the type I IFN-R, signaling is initiated by the activation of tyrosine kinases of the Janus family (reviewed in[58, 75]). The two kinases that associate with the type I IFN-R have been identified as the Tyk-2 and Jak-1 tyrosine kinases. Tyk-2 and Jak-1, along with Jak-2 and Jak-3, comprise the family of Janus kinases[33, 34, 66, 92, 95]. During IFNα or IFNβ stimulation of cells, Tyk-2 and Jak-1 are autophosphorylated/activated[12, 13, 46, 73, 79] and regulate phosphorylation of the IFNaR1 and IFNaR2 subunits of the type I IFN-R[1, 57, 62, 63]. It should be pointed out that all type I IFNs (IFNα, IFNβ, IFNω) induce activation of both Tyk-2 and Jak-1 and phosphorylate the receptor subunits[62]. However, IFNβ induces a distinct alteration in the conformation of the type I IFN-R complex, not seen in response to other type I IFNs (IFNα, IFNω). This is evidenced by the detection of an IFNβ-dependent *in vitro* association of IFNaR1 and IFNaR2 in co-immunoprecipitation studies using specific antibodies[17, 63]. Such an interaction is not seen in response to other type I IFNs[17, 63], strongly suggesting that IFNβ-specific signals are generated at the receptor level. Despite this important initial observation, no other clear-cut differences in signaling events by different type I IFNs have been identified thus far. The identification of the signaling mechanisms that define specificity to distinct type I IFNs remains an important outstanding issue in the field of IFN signaling.

SIGNALING PATHWAYS THAT REGULATE TYPE I IFN-DEPENDENT GENE TRANSCRIPTION

The Jak-Stat pathway

The Jak-Stat signaling cascade is a major pathway utilized by the type I IFN-R to initiate transcription of genes for proteins that mediate the biological effects of type I IFNs. The activated Jaks regulate phosphorylation of signal transducer and activator of transcription (Stat) proteins, which subsequently form homodimers or heterodimers with other Stats. Such complexes translocate to the nucleus where they bind to specific sequences present in the promoters of IFN-stimulated genes to initiate transcription[15–17, 32, 40, 58, 75, 78]. There are several Stat-complexes formed during IFNα-stimulation of cells (Table 1). Among them is the IFN-stimulated gene (ISGF3) complex (Stat2:Stat1:IRF-9), which involves the association of tyrosine phosphorylated Stat1 and Stat2 with IRF-9 (also called p48). The mature ISGF3 complex subsequently translocates to the nucleus, where it regulates gene transcription via binding to IFN-stimulated response elements (ISREs) in the promoters of ISGs[15–17, 32, 40, 56, 78]. Several other Stat-containing complexes are formed during engagement of the type I IFN-R, resulting from association of distinct combinations of Stat proteins (Table 1).

Table 1. Different type I IFN-inducible Stat complexes that bind to ISRE or GAS elements

Stat complex	Promoter elements
Stat1:Stat2:IRF-9 (ISGF3)	ISRE
Stat1:Stat1	GAS
Stat2:Stat1	GAS
Stat1:Stat3	GAS
Stat3:Stat3	GAS
Stat5:Stat5	GAS
CrkL:Stat5	GAS

The tyrosine phosphorylated form of Stat3 forms either Stat3:Stat3 homodimers that bind to GAS elements in the promoters of certain ISGs , or associates with the tyrosine phosphorylated form of Stat1 and forms heterodimers that also bind to GAS elements[15–17, 32, 40, 58, 75, 78]. It is of interest that CrkL, a protein with SH2 and SH3 domains[70], undergoes type I IFN-dependent tyrosine phosphorylation[3] and associates with Stat5 to form a signaling complex that translocates to the nucleus[26]. This CrkL: Stat5 complex plays an essential role in the regulation of gene transcription via GAS elements by the type I IFN-R[38, 82]. Altogether, the binding of distinct Stat-containing complexes to different elements in the promoters of

various IFN-regulated genes is essential for gene transcription by type I IFNs.

Tyrosine phosphorylation of Stat proteins plays a critical role in the generation of IFN signals, as such phosphorylation is essential for the translocation of Stat proteins to the nucleus and the binding to the promoters of target genes. In particular, phosphorylation of tyrosine 701 in Stat1 and the corresponding tyrosine 705 in Stat3 have been identified as the critical residues in the C-terminal domains of these proteins[16, 32, 39]. However, there is accumulating evidence that, in addition to tyrosine phosphorylation, Stat proteins require phosphorylation on serine residues in order to be fully transcriptionally activated[93, 94, 96, 97]. Extensive studies over the years have demonstrated that phosphorylation of serine 727 in the C-terminal regions of Stat1 and Stat3 is essential for type I IFN-inducible transcriptional activation[93, 94, 96, 97]. The phosphorylation on this serine residue is not required for nuclear transport of Stat1 and, in fact, does not appear to exhibit any regulatory effects on the translocation of Stat1 to the nucleus. Nevertheless, it is necessary for full transcriptional activation (reviewed in[20]). An outstanding issue in the field of type I IFN-signaling has been the identification of the serine kinase that is activated downstream of Jak kinases to phosphorylate Stat1 and/or Stat3. A recent study from our group established that protein kinase C (PKC)-δ is activated by IFNα or IFNβ and associates with Stat1[85]. Furthermore, it was shown that pharmacological and/or molecular inhibition of PKC-δ-kinase activity results in loss of IFN-dependent serine phosphorylation of Stat1 and gene transcription via ISRE or GAS elements[85]. Based on these observations, it appears that PKC-δ acts as a serine kinase for Stat1 during engagement of the type I IFN-R. Interestingly, the type II IFN-R also activates this serine kinase and utilizes it for the serine phosphorylation of Stat1 on residue 727, suggesting that type I and II IFNs utilize similar or common signals to regulate Stat-serine phosphorylation[19].

It remains to be seen whether other members of the PKC family of proteins also exhibit regulatory effects on IFN-dependent Stat-phosphorylation. The high degree of homology that members of this family have with in their structures, and the relative tissue-specific distribution of distinct isoforms, suggests that this may be the case. It is also likely that additional serine kinases, distinct from the PKC-family, also play roles on the regulation of serine 727 phosphorylation of Stat1, as evidenced by the recent implication of CAMKII on IFNγ-dependent serine 727 phosphorylation of Stat1[48].

The p38 Map kinase pathway

There is strong evidence that members of the mitogen-activated protein (Map) family of kinases play important roles in the generation of biological responses by IFNs. The p38 Map kinase family of kinases includes 4 members (α, β, γ, δ), all of which have significant homology to each other and are all mammalian homologues of the HOG-1 Map kinase in *Saccharomyces cereviciae* (reviewed in[7, 23, 24, 37, 55, 67, 71]).

These kinases mediate important biological responses in a variety of cells and tissues. Their function has been implicated in the regulation of cytokine production, phosphorylation of transcription factors, transcriptional regulation, apoptosis, cell differentiation, and organization of the actin cytoskeleton[7, 23, 24, 37, 55, 67, 71]. There is evidence that diverse stimuli can activate the p38 Map kinase pathway, including stress stimuli, as well as growth factors and other cytokines[7, 23, 24, 37, 55, 67, 71]. However, most of the studies on the role of these kinases and signaling pathways have been examined in response to stress, which is the classic activator and regulator of this pathway.

An interesting observation has been the finding that p38 Map kinase pathway is activated by type I IFNs[29, 84, 86]. Originally, it was shown that IFNα and IFNβ induce phosphorylation of p38 and activation of its kinase domain. This activation was linked to regulatory effects on gene transcription via ISRE elements[29, 84]. Subsequent studies demonstrated that gene transcription via GAS elements is also regulated by p38, as evidenced by the fact that pharmacological inhibitors of p38 or a dominant-negative p38 mutant (p38AGF) inhibit IFN-dependent gene transcription in luciferase promoter assays[86]. One study had originally demonstrated that p38 mediates IFN--dependent serine 727 phosphorylation of Stat1 in HeLa cells[29]. However, subsequent studies failed to confirm such a potential function of p38 in several other cell types[35, 36, 86], indicating that there is a disassociation between the effects of p38 on IFN-dependent gene transcription and activation of Stat proteins.

The mechanisms of activation of the p38 pathway by the type I IFN-R have been under investigation. The small G-protein Rac1 has been identified as an upstream effector of p38 in the IFN system[86]. It has been shown that type I IFN stimulation of cells induces the transition of Rac1 from its GDP-bound form to its GTP-bound/activated form. Rac1 subsequently appears to regulate activation of a MAP kinase kinase kinase, which ultimately mediates p38 activation via intermediate engagement of one or

more MAP kinase kinase. This has been evidenced by experiments in which overexpression of a dominant-negative Rac1 mutant was found to block IFN-dependent activation of p38[86]. It should be pointed out that, although the precise mechanisms by which Rac 1 is activated by the IFN-R are not fully understood, it is likely that, at least in hematopoietic cells, such activation is mediated by the *vav* proto-oncogene product ($p95^{vav}$)[6]. This protein is tyrosine phosphorylated in an IFN-dependent manner by activated Jak kinases[2, 61, 87] and participates in the induction of growth inhibitory responses by IFNα[44]. An important signaling function of Vav appears to be its activity as a guanine exchange factor for Rac1[14, 30, 69], suggesting that during its tyrosine phosphorylation by type I IFNs it regulates Rac1 activation and downstream p38 activation.

An issue of substantial interest has been the identification of the mechanisms by which the p38 Map kinase pathway exerts regulatory effects on IFN--inducible gene transcription without modifying the Stat pathway. There are now three kinases that have been identified as downstream effectors of p38 in the IFN system. These are MapKapK-2, MapKapK-3, and Msk1[41, 84]. MapKapK-2 and the related MapKapK-3 are widely expressed serine threonine kinases whose activation by type I IFNs was originally demonstrated in *in vitro* kinase assay experiments using Hsp25 as an exogenous substrate[84]. Such activation is inhibited by pretreatment of the cells with pharmacological inhibitors of p38, such as SB203580, demonstrating that their activation in the IFN system is p38-dependent[84]. Msk1 is another kinase regulated by the IFN-activated p38 Map kinase pathway[41]. This nucleosomal kinase has been previously shown to be activated in response to stress[18] and to play an important role in stress-dependent serine phosphorylation of histones H3 and HMG-14[9, 74, 76, 77]. Recent studies have established that this kinase is phosphorylated on residues Ser376 and Thr581 in response to IFN-treatment[41]. Furthermore, IFNα induces activation of its kinase domain, as shown by immune-complex kinase assay experiments[41]. As phosphorylation of H3 and HMG-14 has been implicated in the regulation of chromatin remodeling and immediate early gene transcription[9, 77], it is possible that IFNα-dependent activation of this kinase plays a role in p38-dependent transcriptional activation. However, the validity of such a hypothesis remains to be directly established in future studies.

Independently of the precise downstream mechanisms involved, there is now definitive evidence that p38α is essential for IFN-dependent gene transcription independently of activation of Stat proteins. This was recently established using p38α knockout mouse embryonic fibroblasts[41]. In such cells there is a defective induction of gene transcription of ISRE and GAS elements by either IFNα or IFNβ compared with wild-type $p38\alpha^{+/+}$ cells[41]. On the other hand, IFNγ-dependent transcription via GAS elements is intact in the absence of p38α, indicating that the p38 pathway selectively mediates signals important for transcription by the type I IFN-R, but not the type II (IFNγ) IFN-R. The activation of MapKapK-2, MapKapK-3, and Msk1 is also defective in $p38\alpha^{-/-}$ cells, while Stat activation is intact, further demonstrating the lack of a relationship between the effects of p38 on IFN-dependent gene transcription and Stat activation[41].

There have been studies aimed at directly addressing the functional relevance of p38 in the generation of the specific biological activities of type I IFNs on normal and malignant cells. p38 is phosphorylated and activated by IFNα in chronic myologenous leukemia (CML)-derived cell lines and primary granulocytes from patients with CML[43]. Pharmacological inhibition of its activity using SB203580 or SB202190 abrogates the induction of IFN-dependent growth inhibition on CML-derived primary leukemic CFU-GM progenitors[43]. These findings have strongly suggested that activation of p38 is essential for the induction of the antileukemic effects of IFN, underscoring the importance of this pathway in IFN signaling. Other studies have also demonstrated that the p38 Map kinase and MapKapK-2 are activated by IFNα and IFNβ treatment of primary hematopoietic progenitors obtained from the bone marrow of normal donors[91]. Such activation of the p38/MapKapK-2 pathway appears to be essential for normal hematopoiesis, as evidenced by the fact that pharmacological inhibition of p38, using SB203580 or SB202190, abrogates the suppression of erythroid (BFU-E, CFU-E) and myeloid (CFU-GM) precursors by IFNs[91]. On the other hand, pharmacological inhibition of the MEK/Erk pathway does not reverse such suppression, indicating the specificity of the p38 response in normal hematopoietic progenitors.

Another important function of IFNs is the generation of antiviral responses[4, 5, 31, 72]. Because of this activity, IFNs are currently used as agents for the treatment of certain viral infections and syndromes. As a major mechanism by which IFNα mediates its antiviral effects is the induction of transcription of genes for proteins that mediate antiviral responses, it is not surprising that p38 plays a critical role in the induction of such antiviral effects. Recent studies have established that SB203580 reverses the induction of IFN responses against encephalo-myocarditis virus

(EMCV) infection[43]. It also appears that the MapKapK-2 is a downstream mediator of such effects, as MapKapK-2$^{-/-}$ MEFs are resistant to the antiviral activity of IFNα against EMCV[41].

SIGNALING PATHWAYS THAT REGULATE TYPE I IFN-DEPENDENT mRNA TRANSLATION

Despite the substantial advances in the mechanisms that regulate gene transcription of IFN-stimulated genes, the signaling pathways utilized by type I IFNs to initiate mRNA translation for protein products that mediate their responses have not been elucidated. Recent studies have attempted to define whether the IFN-activated phosphatidylinositol (PI)3'-kinase pathway participates in the induction of translational responses. It is well known that IFNα, IFNβ, and IFNω induce phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2[64, 80, 81, 83, 88], which are multisite docking proteins for the SH2 domains of various signaling elements[47]. Previous studies have demonstrated that one of the type I IFN-regulated SH2-containing proteins that bind to IRS-1 and IRS-2 is the p85 regulatory subunit of the PI3'-kinase[64, 81, 83, 88]. Such binding occurs via the interaction of the SH2 domain of p85 and results in activation of the p110 catalytic subunit of the PI3'-kinase. This subunit exhibits both serine and PI kinase activities, both of which are induced in a type I IFN-dependent manner[81, 88]. Despite these important original observations, the precise role that the IRS/PI3'-kinase pathway plays in IFN signaling and the functional responses that it mediates had been unknown until very recently. Recent studies demonstrated that IFNα and IFNβ induce phosphorylation of the p70 S6 kinase and that such activation is PI3'-kinase- and mammalian target of rapamycin (mTOR)-dependent[39]. This finding implicated the IRS/PI3'-kinase pathway in the regulation of mRNA translation, as p70 S6 kinase plays roles in the regulation of mRNA translation in various cellular systems[8, 25]. p70 S6 kinase is a serine kinase that has been shown to phosphorylate the 40S ribosomal S6 protein[8, 25], an important cellular event for translation of mRNAs that have oligopyrmidine tracts in their 5'-untranslated region[8, 25]. Interestingly, phosphorylation/activation of the p70 S6 kinase is rapamycin sensitive, indicating that the FKBP12 rapamycin-associated protein (FRAP)/mTOR is required for such activation[39]. On the other hand, rapamycin did not exhibit any regulatory effects on the induction of Stat-serine phosphorylation or formation of IFN-dependent ISGF3 or SIE DNA-binding complexes[39]. Consistent with the lack of an effect of mTOR inhibition on Stat activation, IFN-dependent gene transcription via ISRE or GAS elements was found to be mTOR independent in luciferase reporter assays[39]. Thus, the IRS/PI3'-kinase-mTOR pathway appears to selectively regulate events required for mRNA translation, but not gene transcription, in response to type I IFNs.

Another major mechanism by which cytokines regulate initiation of mRNA translation is deactivation of repressors of mRNA translation. In particular, the 4E-binding protein 1 (4E-BP1) is deactivated when it is phosphorylated by various cytokine receptors. This results in its disassociation from the eukaryotic initiation factor 4E (eIF4E) complex[27]. In studies to define whether deactivation of 4E-BP1 occurs during IFN treatment of cells, we found that IFNα and/or IFNβ induce the phosphorylation of 4E-BP1 on threonines 37/46 and threonine 70. It should be pointed out that phosphorylation on all these sites is required for the dissociation of the protein from eIF4E and the initiation of translation[28]. As expected, such a type I IFN inducible phosphorylation of 4E-BP1 leads to its dissociation from the eIF4E complex[39], allowing the initiation of mRNA translation. Although the precise sequence of events that lead to the generation of this important signaling event remain to be defined, it appears that PI3'-kinase and FRAP/mTOR play important roles in such responses. This has been demonstrated in experiments in which the effects of the PI3'-kinase inhibitor LY294002 and the FRAP/ /mTOR inhibitor rapamycin on the phosphorylation of 4E-BP1 were studied. Both inhibitors were found to block IFN-dependent phosphorylation of 4E-BP1, demonstrating the requirement of the PI3'-kinase/ /mTOR pathway for the deactivation of this repressor of mRNA translation[39].

CONCLUSIONS

It is becoming evident that the signaling mechanisms by which type I IFNs mediate the induction of their biological activities are complex. In addition to the classic Jak-Stat pathway, multiple additional signaling cascades are activated by the type I IFN-R and appear to play important roles in the generation of IFN signals. Furthermore, there is now evidence for a cross-talk between the Jak-Stat pathway and other cascades, with a prime example being the interaction of the CrkL adapter with activated Stat5 and the binding of the CrkL:Stat5 complex to GAS elements. As it stands now, the Jak-Stat pathway remains the major pathway that regulates transcriptional responses (Fig. 1). However, IFN-dependent transcriptional regulation also requires an intact p38 Map kinase pathway, which appears to function as an auxiliary pathway, likely via modification of nuclear histone phosphorylation and chromatin remodeling. Much

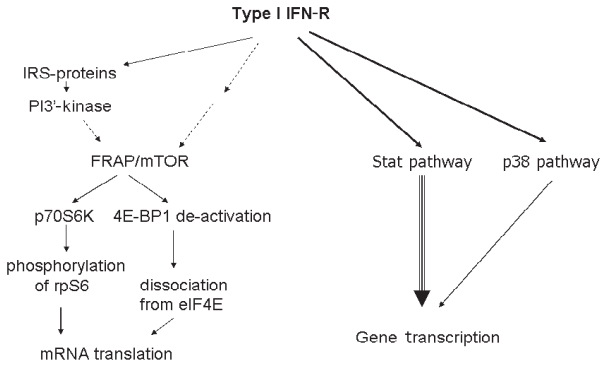

Figure 1. Pathways activated by the type I interferon receptor (IFN-R) to regulate gene transcription and mRNA translation.

less is known regarding the mechanisms of IFN-dependent initiation of mRNA translation, although based on recent studies, the principal regulatory pathway for such effects appears to be the IFN-activated IRS-PI3'-kinase cascade.

Future studies should be directed towards understanding the precise contributions of distinct IFN-induced signals on the generation of the different biological properties of IFNs. Such clarification of the mechanisms of action of IFNs is of high importance, as these cytokines are critical components of the immune response in humans, and have important clinical activities in the treatment of various viral and neoplastic diseases.

ACKNOWLEDGMENT

We thank Melissa Negron at the Robert H. Lurie Comprehensive Cancer Center for expert secretarial assistance.

REFERENCES

1. Abramovich C., Shulman L. M., Ratoviski E., Harroch S., Tovey M., Eid P. and Revel M. (1994): Differential tyrosine phosphorylation of the IFNAR chain of the type I interferon receptor and an associated surface protein in response to IFNα and IFNβ. EMBO. J., 13, 5871–5877.
2. Adam L., Bandyopadhyay D. and Kumar R. (2000): Interferon-α signaling promotes nucleus-to-cytoplasmic redistribution of p95Vav, and formation of a multisubunit complex involving Vav, Ku80, and Tyk2. Biochem. Biophys. Res. Commun., 267, 692–696.
3. Ahmad S., Alsayed Y., Druker B. J. and Platanias L. C. (1997): The type I interferon receptor mediates tyrosine phosphorylation of the CrkL adaptor protein. J. Biol. Chem., 272, 29991–29994.
4. Biron C. A. (1999): Initial and innate responses to viral infections – pattern setting in immunity or disease. Curr. Opin. Microbiol., 2, 374–381.
5. Brierley M. M. and Fish E. N. (2002): IFNα/β receptor interactions to biologic outcomes: understanding the circuitry. J. Interferon Cytokine Res., 22, 835–845.
6. Bustelo X. R. (2000): Regulatory and signaling properties of the Vav family. Mol. Cell. Biol., 20, 1461–1477.
7. Cano E. and Mahadevan L. C. (1995): Parallel signal processing among mammalian MAPKs. Trends Biochem. Sci., 20, 117–122.
8. Chou M. M. and Blenis J. (1995): The 70 kDa S6 kinase: regulation of a kinase with multiple roles in mitogenic signalling. Curr. Opin. Cell Biol., 7, 806–814.
9. Clayton A. L. and Mahadevan L. C. (2003): MAP kinase-mediated phosphoacetylation of histone H3 and inducible gene regulation. FEBS Lett., 546, 51–58.
10. Colamonici O. R. and Domanski P. (1994): Identification of a novel subunit of the type I interferon receptor localized to chromosome 21. J. Biol. Chem., 268, 10895–10899.
11. Colamonici O. R., Pfeffer L., D'Alessandro F., Platanias L. C., Rosolen A., Nordan R., Cruciani R. A. and Diaz M. O. (1992): Multichain structure of the interferon α-2 receptor on hematopoietic cells. J. Immunol., 148, 2126–2132.
12. Colamonici O. R., Uyttendaele H., Domanski P., Yan H. and Krolewski J. J. (1994): p135tyk2 an interferon-dependent tyrosine kinase, is physically associated with an interferon receptor. J. Biol. Chem., 269, 3518–3522.
13. Colamonici O. R., Yan H., Domanski P., Handa R., Smalley D., Mullerman J., Witte M., Krishnan K. and Krolewski J. J. (1994): Direct binding and tyrosine phosphorylation of the a subunit (cloned subunit) of the type I IFN receptor and the p135tyk2 tyrosine kinase. Mol. Cell Biol., 14, 8133–8142.
14. Crespo P., Schuebel K. E., Ostrom A. A., Gutkind J. S. and Bustelo X. R. (1997): Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the *vav* proto-oncogene product. Nature, 385, 169–172.
15. Darnell J. E. Jr. (1997): Stats and gene regulation. Science, 277, 1630–1635.
16. Darnell J. E. Jr. (1998): Studies of IFN-induced transcriptional activation uncover the Jak-Stat pathway. J. Interferon Cytokine Res., 18, 549–554.
17. Darnell J. E. Jr., Kerr I. M and Stark G. R. (1994): Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular proteins. Science, 264, 1415–1420.
18. Deak M., Clifton A. D., Lucocq J. M. and Alessi D. R. (1998): Mitogen and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38 and may mediate activation of CREB. EMBO. J., 17, 4426–4441.
19. Deb D. K., Sassano A., Lekmine F., Majchrzak B., Verma A., Kambhampati S., Uddin S., Rahman A., Fish E. N. and Platanias L. C. (2003): Activation of protein kinase Cδ by IFN-γ. J. Immunol., 171, 267–273.
20. Decker T. and Kovarik P. (2000): Serine phosphorylation of Stats. Oncogene, 19, 2628–2637.
21. Diaz M. O., Pomycala H., Bohlander S., Maltepe E., Malik K., Brownstein B. and Olopade O. I. (1994): Structure of the human type I interferon gene cluster determined from a YAC clone contig. Genomics, 22, 540–552.
22. Domanski P., Witte M., Kellum M., Rubinstein M., Hackett R., Pitha P. and Colamonici O. R. (1995): Cloning and expression of a long form of the β subunit of the interferon α/β receptor that is required for signaling. J. Biol. Chem., 270, 21606–21611.
23. Dong C., Davis R. J. and Flavell R. A. (2002): MAP kinases in the immune response. Annu. Rev. Immunol., 20, 55–72.
24. English J., Pearson G., Wilsbacher J., Swantek J., Karandikar M., Xu S. and Cobb M. H. (1999): New insights into the control of MAP kinase pathways. Exp. Cell. Res., 253, 255–270.
25. Ferrari S. and Thomas G. (1994): S6 phosphorylation and the p70s6k/p85s6k. Crit. Rev. Biochem. Mol. Biol., 29, 385–413.
26. Fish E. N., Uddin S., Korkmaz M., Majchrzak B., Druker B. J. and Platanias L. C. (1999): Activation of a CrkL-Stat5

signaling complex by type I interferons. J. Biol. Chem., 274, 571–573.

27. Gingras A. C., Kennedy S. G., O' Leary M. A., Sonenberg N. and Hay N. (1998): 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt (PKB) signaling pathway. Genes Dev., 12, 502–513.

28. Gingras A. C., Raught B., Gygi S. P., Niedzwiecka A., Miron M., Burley S. K., Polakiewicz R. D., Wyslouch-Cieszynska A., Aebersold R. and Sonenberg N. (2001): Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes Dev., 15, 2852–2864.

29. Goh K. C., Haque S. J. and Williams B. R. (1999): p38 MAP kinase is required for STAT1 serine phosphorylation and transcriptional activation induced by interferons. EMBO. J., 18, 5601–5608.

30. Gringhuis S. I., De Leij L. F., Coffer P. J. and Vellenga E. (1998): Signaling through CD5 activates a pathway involving phosphatidylinositol 3'-kinase, Vav, and Rac1 in human mature T-lymphocytes. Mol. Cell. Biol., 18, 1725–1735.

31. He Y. and Katze M. G. (2002): To interfere and to anti-interfere: the interplay between hepatitis C virus and interferon. Viral Immunol., 15, 95–119.

32. Horvath C. M. (2000): STAT proteins and transcriptional responses to extracellular signals. Trends Biochem. Sci., 25, 496–502.

33. Ihle J. N. (1995): The Janus protein tyrosine kinase family and its role in cytokine signaling. Adv. Immunol., 6, 1–35.

34. Ihle J. N., Witthuhn B. A., Quelle F. W., Yamamoto K., Thierfelder W. E., Kreider B. and Silvennoinen O. (1994): Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends Biochem. Sci., 19, 222–227.

35. Kovarik P., Mangold M., Ramsauer K., Heidari H., Steinborn R., Zotter A., Levy D. E., Muller M. and Decker T. (2001): Specificity of signaling by STAT1 depends on SH2 and C-terminal domains that regulate Ser727 phosphorylation, differentially affecting specific target gene expression. EMBO. J., 20, 91–100.

36. Kovarik P., Stoiber D., Eyers P. A., Menghini R., Neininger A., Gaestel M., Cohen P. and Decker T. (1999): Stress-induced phosphorylation of STAT1 at Ser727 requires p38 mitogen-activated protein kinase whereas IFN-γ uses a different signaling pathway. Proc. Natl. Acad. Sci. USA, 96, 13956–13961.

37. Kyriakis J. M. and Avruch J. (1996): Sounding the alarm: protein kinase cascades activated by stress and inflammation. J. Biol. Chem., 271, 24313–24316.

38. Lekmine F., Sassano A., Uddin S., Majchrzak B., Miura O., Druker B.J., Fish E. N., Imamoto A. and Platanias L. C. (2002): The CrkL adapter protein is required for type I interferon-dependent gene transcription and activation of the Small G-protein Rap1. Biochem. Biophys. Res. Commun., 291, 744–750.

39. Lekmine F., Uddin S., Sassano A., Parmar S., Majchrzak B., Sonenberg N., Hay N., Fish E. N. and Platanias L. C. (2003): Activation of the p70 S6 kinase and phosphorylation of the 4E-BP1 repressor of mRNA translation by type I interferons. J. Biol. Chem., 278, 27772–27780.

40. Levy D. E. and Darnell J. E. Jr. (2002): Stats: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol., 3, 651–62.

41. Li Y., Sassano A., Majchrzak B., Deb D. K., Levy D. E., Gaestel M., Nebreda A. R., Fish E. N. and Platanias L. C. (2004): Role of p38α map kinase in type I interferon signaling. J. Biol. Chem., 279, 970-979.

42. Lutfalla G., Holland S. J., Ginato E., Monneron D., Reboul J., Rogers N. C., Smith J. M., Stark G. R., Gardiner K., Mogensen K. E., Kerr I. M. and Uzé G. (1995): Mutant U5A cells are complemented by an interferon-αβ-receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluster. EMBO. J., 14, 5100- 5108.

43. Mayer I. A., Verma A., Grumbach I. M., Uddin S., Lekmine F., Ravandi F., Majchrzak B., Fujita S., Fish E. N. and Platanias L. C. (2001): The p38 Map kinase pathway mediates the growth inhibitory effects of IFNα in BCR-ABL expressing cells. J. Biol. Chem., 276, 28570–28577.

44. Micouin A., Wietzrbin J., Steunou V. and Martyre M. C. (2000): $p95^{Vav}$ is associated to the IFNα/β receptor and contributes to the antiproliferative effect of IFNα in megakaryocytic cell lines. Oncogene, 19, 387–394.

45. Mogensen K. E., Lewerenz M., Reboul J., Lutfalla G. and Uze G. (1999): The type I interferon receptor: structure, function, and evolution of a family business. J. Interferon Cytokine Res., 19, 1069–1098.

46. Müller M., Briscoe J., Laxton C., Guschin D., Ziemecki A., Silvennoinen O., Harpur A. G., Barbieri G., Witthun B. A., Schindler C., Pellegrini S., Wilks A. F., Ihle J. N., Stark G. R. and Kerr I. M. (1993): The protein tyrosine kinase JAK-1 complements defects in interferon α/β and δ signal transduction. Nature, 366, 129–135.

47. Myers M., Sun X. and White M. F. (1994): The IRS-signaling system. Trends Biochem. Sci., 19, 289–294.

48. Nair J. S., DaFonseca C. J., Tjernberg A., Sun W., Darnell J. E. Jr., Chait B. T. and Zhang J. J. (2002): Requirement of Ca^{2+} and CaMKII for Stat1 Ser-727 phosphorylation in response to IFN-γ. Proc. Natl. Acad. Sci. USA, 99, 5971–5976.

49. Novick D., Cohen B. and Rubinstein M. (1994): The human interferon α/β receptor: Characterization and molecular cloning. Cell, 77, 391–400.

50. Parmar S. and Platanias L. C. (2003): Interferons. Mechanisms of action and clinical applications. Curr. Opin. Oncol., 15, 431–439.

51. Pestka S. (1986): Interferon standards and general abbreviations. Methods Enzymol., 119, 14–23.

52. Pestka S. (1997): The human interferon α species and hybrid proteins. Semin. Oncol., 24, S9-4–S9-17.

53. Pestka S. (2000): The human interferon α species and receptors. Biopolymers, 55, 254–287.

54. Pestka S., Langer J. A., Zoon K. C. and Samuel C. E. (1987): Interferons and their actions. Annu. Rev. Biochem., 56, 727–777.

55. Platanias L. C. (2003): Map kinase signaling pathways and hematologic malignancies. Blood, 101, 4667–4679.

56. Platanias L. C. (2003): The p38 mitogen-activated protein kinase pathway and its role in interferon signaling. Pharmacol. Ther., 98, 129–142.

57. Platanias L. C. and Colamonici O.R. (1992): Interferon α induces rapid tyrosine phosphorylation of the subunit of its receptor. J. Biol. Chem., 267, 24053–24057.

58. Platanias L. C. and Fish E.N. (1999): Signaling pathways activated by interferons. Exp. Hematol., 27, 1583–1592.

59. Platanias L. C., Pfeffer L. M., Barton K., Vardiman J. W., Golomb H. M. and Colamonici O. R. (1992): Expression of the IFNα receptor in hairy cell leukemia cells. Br. J. Haematol., 82, 541–546.

60. Platanias L. C., Pfeffer L. M., Cruciani R. and Colamonici O. R. (1993): Characterization of the α-subunit of the IFNα receptor. Evidence for N- and O-linked glycosylation and association with other surface proteins. J. Immunol., 150, 3382–3388.

61. Platanias L. C. and Sweet M. E. (1994): Interferon α-induces rapid tyrosine phosphorylation of the *vav* proto-oncogene product in hematopoietic cells. J. Biol. Chem., 269, 3143–3146.

62. Platanias L. C., Uddin S. and Colamonici O. R. (1994): Tyrosine phosphorylation of the α and β subunits of the type I interferon receptor. Interferon β selectively induces tyrosine phosphorylation of a α subunit-associated protein. J. Biol. Chem., 27, 17761–17764.

63. Platanias L. C., Uddin S., Domanski P. and Colamonici O. R. (1996): Differences in signaling between interferon α and β. Interferon β selectively induces the interaction of the α and βL subunits of the type I interferon receptor. J. Biol. Chem., 271, 23630–23633.

64. Platanias L. C., Uddin S., Yetter A., Sun X.-J. and White M. F. (1996): The type I interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2. J. Biol. Chem., 271, 278–282.

65. Prejean C. and Colamonici O. R. (2000): Role of the cytoplasmic domains of the type I interferon receptor subunits in signaling. Semin. Cancer Biol., 10, 83–92.

66. Rane S. G. and Reddy E. P. (2000): Janus kinases: components of multiple signaling pathways. Oncogene, 19, 5662–5679.

67. Rincon M., Flavell R. A. and Davis R. J. (2001): Signal transduction by MAP kinases in T lymphocytes. Oncogene, 20, 2490–2497.

68. Roberts R. M., Liu L., Guo Q., Leaman D. and Bixby J. (1998): The evolution of the type I interferons. J. Interferon Cytokines Res., 18, 805–816.

69. Salojin K. V., Zhang J. and Delovitch T. L. (1999): TCR and CD28 are coupled via ZAP-70 to the activation of the Vav/Rac-1-/PAK-1/p38 MAPK signaling pathway. J. Immunol., 163, 844–853.

70. Sattler M. and Salgia R. (1998): Role of the adapter protein CrkL in signal transduction of normal hematopoietic and BCR/ABL--transformed cells. Leukemia, 12, 637–644.

71. Schaeffer H. J. and Weber M. J. (1999): Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol. Cell. Biol., 19, 2435–2444.

72. Sen G. C. (2001): Viruses and interferons. Annu. Rev. Microbiol., 55, 255–281.

73. Silvennoinen O., Ihle J. N., Schlessinger J. and Levy D. E. (1993): Interferon-induced nuclear signaling by Jak protein tyrosine kinases. Nature, 366, 583–585.

74. Soloaga A., Thomson S., Wiggin G. R., Rampersaud N., Dyson M. H., Hazzalin C. A., Mahadevan L. C. and Arthur J. S. (2003): MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. EMBO. J., 22, 2788–2797.

75. Stark G. R., Kerr I. M., Williams B. R. G., Silverman R. H. and Screiber R. D. (1998): How cells respond to interferons. Annu. Rev. Biochem., 67, 227–264.

76. Thompson S., Clayton A. L., Hazzalin C. A., Rose S., Barratt M. J. and Mahadevan L. C. (1999): The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG--14 kinase. EMBO. J., 18, 4779–4793.

77. Thomson S., Mahadevan L. C. and Clayton A. L. (1999): MAP kinase-mediated signaling to nucleosomes and immediate-early gene induction. Semin. Cell Dev. Biol., 10, 205–214.

78. Uddin S., Alsayed Y., Grumbach I., Woodson J. and Platanias L. C. (1999): Cellular pathways activated by interferons in hematopoietic cells. Haema, 2, 192–197.

79. Uddin S., Chamdin A. and Platanias L. C. (1995): Interaction of the transcriptional activator Stat-2 with the type I IFN receptor. J. Biol. Chem., 270, 24627–24630.

80. Uddin S., Fish E. N., Sher D., Gardziola C., Colamonici O. R., Kellum M., Pitha P., White M. F. and Platanias L. C. (1997): The IRS-pathway operates distinctively from the Stat-pathway in hematopoietic cells and transduces common and distinct signals during engagement of the insulin or IFNα receptors. Blood, 90, 2574–2582.

81. Uddin S., Fish E. N., Sher D. A., Gardziola C., White M. F. and Platanias L. C. (1997): Activation of the phosphatidylinositol 3'-kinase serine kinase by IFNα. J. Immunol., 158, 2390–2397.

82. Uddin S., Lekmine F., Sassano A., Rui H., Fish E. N., Platanias L. C. (2003): Role of Stat5 in type I interferon-signaling and transcriptional regulation. Biochem. Biophys. Res. Commun., 308, 325–330.

83. Uddin S., Majchrzak B., Wang P. C., Modi S., Khan M. K., Fish E. N. and Platanias L. C. (2000): Interferon α-dependent activation of the phosphatidylinositol 3-'kinase serine kinase requires engagement of the IRS- but not the Stat-pathway. Biochem. Biophys. Res. Commun., 270, 158–162.

84. Uddin S., Majchrzak B., Woodson J., Arunkumar P., Alsayed Y., Pine R., Young P. R., Fish E. N. and Platanias L. C. (1999): Activation of the p38 Map kinase by type I IFNs. J. Biol. Chem., 274, 30127–30131.

85. Uddin S., Sassano A., Deb D. K., Verma A., Majchrzak B., Rahman A., Malik A. B., Fish E. N. and Platanias L. C. (2002): Protein kinase C-δ (PKC-δ) is activated by type I interferons and mediates phosphorylation of Stat1 on serine 727. J. Biol. Chem., 277, 14408–14416.

86. Uddin S., Sharma N., Majchrzak B., Mayer I., Lekmine F., Young P. R., Bokoch G. M., Fish E. N. and Platanias L. C. (2000): The Rac1/p38 Map kinase pathway is required for interferon α-dependent transcriptional activation but not serine phosphorylation of Stat proteins. J. Biol. Chem., 275, 27634–27640.

87. Uddin S., Sweet M. E., Colamonici O. R., Krolewski J. J. and Platanias L. C. (1997): The *vav* proto-oncogene product interacts with the Tyk-2 tyrosine kinase. FEBS Lett., 403, 31–34.

88. Uddin S., Yenush L., Sun X.-J., Sweet M. E., White M. F. and Platanias L. C. (1995): Interferon α engages the insulin receptor substrate-1 to associate with the phosphatidylinositol 3'-kinase. J. Biol. Chem., 270, 15938–15941.

89. Uzé G., Lutfalla G. and Gresser I. (1990): Genetic transfer of a functional human interferon receptor into mouse cells: cloning and expression of its cDNA. Cell, 60, 225–232.

90. Uzé G., Lutfalla G., Mongensen K. E. (1995): α and β interferons and their receptor and their friends and relations. J. Interferon Res., 15, 3–26.

91. Verma A., Deb D. K., Sassano A., Uddin S., Varga J. Wickrema A. and Platanias L. C. (2002): Activation of the p38 Map kinase pathway mediates the suppressive effects of type I interferons and transforming growth factor β on normal hematopoiesis. J. Biol. Chem., 277, 7726–7735.

92. Verma A., Kambhampati S., Parmar S. and Platanias L. C. (2003): Jak family of kinases in cancer. Cancer Metastasis Rev., 22, 423–434.

93. Wen Z. and Darnell J. E. Jr. (1997): Mapping of Stat3 serine phosphorylation to a single residue (727) and evidence that serine phosphorylation has no influence on DNA binding of Stat1 and Stat3. Nucleic Acids Res., 25, 2062–2067.

94. Wen Z., Zhong Z., Darnell J. E. Jr. (1995): Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell, 82, 241–250.

95. Yeh T. C. and Pellegrini S. (1999): The Janus kinase family of protein tyrosine kinases and their role in signaling. Cell Mol. Life Sci., 55, 1523–1534.

96. Zhang J. J., Zhao Y., Chait B. T., Lathem W. W., Ritzi M., Knippers R. and Darnell J. E. Jr. (1998): Ser727-dependent recruitment of MCM5 by Stat1α in IFN-γ-induced transcriptional activation. EMBO J., 17, 6963–6971.

97. Zhu X., Wen Z., Xu L. Z. and Darnell J. E. Jr. (1997): Stat1 serine phosphorylation occurs independently of tyrosine phosphorylation and requires an activated Jak2 kinase. Mol. Cell. Biol., 17, 6618–6623.