MECHANISMS OF TYPE-I- AND TYPE-II-INTERFERON-MEDIATED SIGNALLING

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Abstract | Interferons are cytokines that have antiviral, antiproliferative and immunomodulatory effects. Because of these important properties, in the past two decades, major research efforts have been undertaken to understand the signalling mechanisms through which these cytokines induce their effects. Since the original discovery of the classical JAK (Janus activated kinase)–STAT (signal transducer and activator of transcription) pathway of signalling, it has become clear that the coordination and cooperation of multiple distinct signalling cascades — including the mitogen-activated protein kinase p38 cascade and the phosphatidylinositol 3-kinase cascade — are required for the generation of responses to interferons. It is anticipated that an increased understanding of the contributions of these recently identified pathways will advance our current thinking about how interferons work.

Interferons (IFNs) are widely expressed cytokines that have potent antiviral and growth-inhibitory effects. These cytokines are the first line of defence against viral infections and have important roles in immunosurveillance for malignant cells. The IFN family includes two main classes of related cytokines: type I IFNs and type II IFN[1,2]. There are many type I IFNs, all of which have considerable structural homology. These include IFN-α (which can be further subdivided into 13 different subtypes, IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8, -α10, -α13, -α14, -α16, -α17 and -α21), IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ and IFN-ω[1–3]. IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω exist in humans, whereas IFN-δ and IFN-τ have been described only for pigs and cattle, respectively, and do not have human homologues[2]. The genes that encode type I IFNs are clustered on chromosome 9 in humans[2] and on chromosome 4 in mice[4]. All type I IFNs bind a common cell-surface receptor, which is known as the type I IFN receptor[1–3] (FIG. 1). By contrast, there is only one type II IFN, IFN-γ[1–3]. The gene that encodes this cytokine is located on chromosome 12 in humans and chromosome 10 in mice, and the protein does not have marked structural homology with type I IFNs[1–6]. IFN-γ binds a different cell-surface receptor, which is known as the type II IFN receptor[7,8] (FIG. 1). IFN-γ is a markedly different cytokine than the type I IFNs, but it was originally classified in the IFN family because of its ability to 'interfere' with viral infections, which is consistent with the original definition of IFNs[2,9]. Recently, a new class of IFNs or IFN-like molecules[2] has emerged, the IFN-λ molecules: IFN-λ1, -λ2 and -λ3, which are also known as interleukin-29 (IL-29), IL-28A and IL-28B, respectively[10]. They also have antiviral properties[10], but they are distinct from the type I and type II IFNs and bind a different cell-surface receptor, which is composed of two chains, IFNLR1 (also known as IL-28 receptor-α, IL-28Rα) and IL-10Rβ[10]. IFN-λ molecules might ultimately be classified and accepted as type III IFNs. They are not discussed further in this Review because they do not transduce signals through the classical type I or type II IFN receptors and because we do not know much about the signalling pathways that they mediate.

Both the type I IFN receptor and the type II IFN receptor have multichain structures, which are composed of at least two distinct subunits: IFNAR1 and

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IFNAR2 for the type I IFN receptor, and IFNGR1 and IFNGR2 for the type II IFN receptor (FIG. 1). Each of these receptor subunits interacts with a member of the Janus activated kinase (JAK) family[11,12]. In the case of the type I IFN receptor, the IFNAR1 subunit is constitutively associated with tyrosine kinase 2 (TYK2), whereas IFNAR2 is associated with JAK1 (REFS 4,11–13) (FIG. 1). In the case of the type II IFN receptor, the IFNGR1 subunit associates with JAK1, whereas IFNGR2 is constitutively associated with JAK2 (REFS 4,8) (FIG. 1). The initial step in both type-I- and type-II-IFN-mediated signalling is the activation of these receptor-associated JAKs, which occurs in response to a ligand-dependent rearrangement and dimerization of the receptor subunits, followed by autophosphorylation and activation of the associated JAKs. As well as activation of classical JAK–STAT (signal transducer and activator of transcription)-signalling pathways (discussed later), activation of IFN-receptor-associated JAKs seems to regulate, either directly or indirectly, several other downstream cascades. Such diversity of signalling is consistent with the pleiotropic biological effects of IFNs on target cells and tissues.

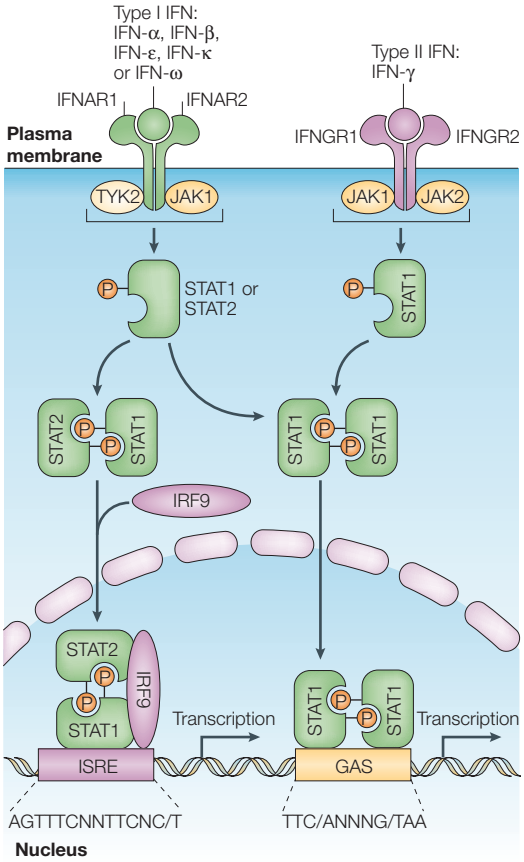


Figure 1 | **Interferon receptors and activation of classical JAK–STAT pathways by type I and type II interferons.** All type I interferons (IFNs) bind a common receptor at the surface of human cells, which is known as the type I IFN receptor. The type I IFN receptor is composed of two subunits, IFNAR1 and IFNAR2, which are associated with the Janus activated kinases (JAKs) tyrosine kinase 2 (TYK2) and JAK1, respectively. The only type II IFN, IFN-γ, binds a distinct cell-surface receptor, which is known as the type II IFN receptor. This receptor is also composed of two subunits, IFNGR1 and IFNGR2, which are associated with JAK1 and JAK2, respectively. Activation of the JAKs that are associated with the type I IFN receptor results in tyrosine phosphorylation of STAT2 (signal transducer and activator of transcription 2) and STAT1; this leads to the formation of STAT1–STAT2–IRF9 (IFN-regulatory factor 9) complexes, which are known as ISGF3 (IFN-stimulated gene (ISG) factor 3) complexes. These complexes translocate to the nucleus and bind IFN-stimulated response elements (ISREs) in DNA to initiate gene transcription. Both type I and type II IFNs also induce the formation of STAT1–STAT1 homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements that are present in the promoter of certain ISGs, thereby initiating the transcription of these genes. The consensus GAS element and ISRE sequences are shown. N, any nucleotide.

It is well established that IFNs induce the expression of hundreds of genes, which mediate various biological responses[14]. Some of these genes are regulated by both type I and type II IFNs, whereas others are selectively regulated by distinct IFNs. For example, expression of the *9-27* gene (also known as *IFITM1*) is induced by all IFNs, whereas expression of the gene encoding 2′,5′-oligoadenylate synthetase 1 is selectively induced by IFN-α and IFN-β but not IFN-γ[14]. Also, expression of the IFN-regulatory factor 1 (*IRF1*) gene is preferentially induced by IFN-γ, whereas expression of the gene that encodes hypoxia-inducible factor 1 is relatively selectively induced by IFN-β[14].

The first signalling pathway shown to be activated by IFNs was the JAK–STAT pathway[15–18]. The original discovery of this pathway in the 1990s (REFS 15–18) provided a simple model for IFN-mediated signalling, involving rapid nuclear translocation and initiation of gene transcription by STATs that have been activated at the plasma membrane in response to JAK-mediated phosphorylation. This model remains important, and it is clear that this mechanism is required for the induction of many of the effects of IFNs. In fact, the JAK–STAT-signalling cascade is the most extensively studied IFN-dependent pathway, and its functional relevance in the IFN system has been firmly established by many experimental approaches. However, it has now become apparent that the activation of JAK–STAT pathways alone is not sufficient for the generation of all of the biological activities of IFNs. There is accumulating evidence that several other IFN-regulated signalling elements and cascades are required for the generation of many of the responses to IFNs. Some of these pathways operate independently of the JAK–STAT pathway, whereas others cooperate with STATs to optimize the transcriptional regulation of target genes. This article provides a brief overview of recent discoveries and developments regarding IFN-activated JAK–STAT pathways, but the main focus is the mechanisms of activation and the functional roles of non-STAT pathways in IFN-mediated signalling, an area in which important developments have occurred in the past few years.

IFN-activated STATs and their cellular partners

The importance of JAK–STAT pathways in both type-I- and type-II-IFN-mediated signalling continues to ignite research efforts that aim to better understand

the mechanisms that regulate the activation of JAKs and STATs by IFNs. Recent studies have uncovered some additional regulatory mechanisms that are required for optimal activation and function of JAK–STAT pathways. Such developments are discussed in the next section, after a brief review of the well-established knowledge about classical JAK–STAT pathways.

Classical pathways. The binding of IFN-α or other type I IFNs to the type I IFN receptor results in the rapid autophosphorylation and activation of the receptor-associated JAKs TYK2 and JAK1 (REFS 10,18), which in turn regulate the phosphorylation and activation of STATs[11,19]. The STATs that are activated in response to type I IFNs include STAT1, STAT2, STAT3 and STAT5 (REFS 11,19–22). The activation of such STATs is a common response to different type I IFNs, consistent with all of these IFNs binding the same receptor and thereby activating a common pathway that involves the same JAKs, TYK2 and JAK1 (REFS 11,19–22). STAT4 and STAT6 can also be activated by IFN-α, but such activation seems to be restricted to certain cell types, such as endothelial cells or cells of lymphoid origin[23–26]. After phosphorylation by JAKs, the activated STATs form homodimers or heterodimers that translocate to the nucleus and initiate transcription by binding specific sites in the promoters of IFN-stimulated genes (ISGs)[4,11,19–22].

An important transcriptional complex that is induced by type I IFNs is the ISG factor 3 (ISGF3) complex[5,6,19–21] (FIG. 1). The mature ISGF3 complex is composed of the phosphorylated (activated) forms of STAT1 and STAT2, together with IRF9, which does not undergo tyrosine phosphorylation[19–21]. This complex is the only complex that binds specific elements known as IFN-stimulated response elements (ISREs) that are present in the promoters of certain ISGs, thereby initiating their transcription. Other STAT complexes that are induced by type I IFNs include STAT1–STAT1, STAT3–STAT3, STAT4–STAT4, STAT5–STAT5 and STAT6–STAT6 homodimers, as well as STAT1–STAT2, STAT1–STAT3, STAT1–STAT4, STAT1–STAT5, STAT2–STAT3 and STAT5–STAT6 heterodimers[5,6,19–21]. Such IFN-induced complexes bind another type of element — known as an IFN-γ-activated site (GAS) element— that is present in the promoter of ISGs[5,19–21,25,26]. Of the hundreds of known ISGs, some have only ISREs or only GAS elements in their promoters, whereas others have both elements; therefore, combinations of different STAT-containing complexes might be required for the optimal transcriptional activation of a particular gene. There is also emerging evidence that modulation of the function of distinct STATs might account for specific responses. For example, a recent report showed that IFN-α- or IFN-β-mediated activation of STAT4 is required for IFN-γ production during viral infections[27], whereas surprisingly, STAT1 negatively regulates IFN-α-dependent induction of IFN-γ expression[27]. So, it seems that different combinations of STATs can be induced by type I IFNs to target the transcription of functionally distinct genes, but the mechanisms defining such differential STAT usage and specificity are not understood.

The transcription of type II IFN (IFN-γ)-dependent genes is regulated by GAS elements, and STAT1 is the most important IFN-γ-activated transcription factor for the regulation of these transcriptional responses. After engagement of the type II IFN receptor by IFN-γ, JAK1 and JAK2 are activated and regulate downstream phosphorylation of STAT1 on the tyrosine residue at position 701 (Tyr701)[19–21,28] (FIG. 1). Such phosphorylation results in the formation of STAT1–STAT1 homodimers, which translocate to the nucleus and bind GAS elements to initiate transcription[19–21,28]. In contrast to type I IFNs, IFN-γ does not induce the formation of ISGF3 complexes and thereby cannot induce the transcription of genes that have only ISREs in their promoter.

Serine phosphorylation of STATs. The phosphorylation of tyrosine residues in STATs by activated JAKs is a crucial step in IFN-mediated signalling, as it is required for the formation of the various STAT complexes and for their translocation to the nucleus. However, several other events that involve biochemical modification of STATs, or the interaction of STATs with other proteins that function as transcriptional co-activators, are also required for optimal IFN-regulated gene transcription. Both type I and type II IFNs induce phosphorylation of STAT1 and STAT3 on the serine residue at position 727 (Ser727), which is located in their carboxy (C)-terminal domain[29,30]. Such phosphorylation is not required for their translocation to the nucleus or for their binding to the promoters of ISGs, but it is essential for full transcriptional activation[29,30]. The biological relevance of such phosphorylation was recently established by the generation of gene-targeted mice expressing a mutant STAT1 in which Ser727 was replaced with an alanine residue[31]. These mice had increased mortality after infection with *Listeria monocytogenes*, and induction of expression of ISGs in macrophages was considerably reduced, establishing that serine phosphorylation of STAT1 has an important role in IFN-γ-dependent innate immune responses[31].

The identification of the serine kinase(s) that regulates the phosphorylation of Ser727 has been an area of considerable research effort in the past few years. One serine kinase that interacts with STAT1 (REFS 32–34) and regulates Ser727 phosphorylation in response to either type I (REF. 32) or type II (REF. 33) IFNs is a member of the protein kinase C (PKC) family, PKC-δ. There is also evidence that additional IFN-dependent serine kinases might be activated in a cell-type-restricted manner and that these kinases are involved in the regulation of STAT1 Ser727 phosphorylation. This has been implied by studies showing that PKC-ε[35] and calcium/calmodulin-dependent protein kinase II (REF. 36) can also regulate IFN-γ-dependent STAT1 Ser727 phosphorylation.

Interactions of STATs with co-activators. STATs interact, in the nucleus, with several co-activator proteins that have important roles in the regulation of transcription. These include p300 and CBP (cAMP-responsive-element-binding protein (CREB)-binding

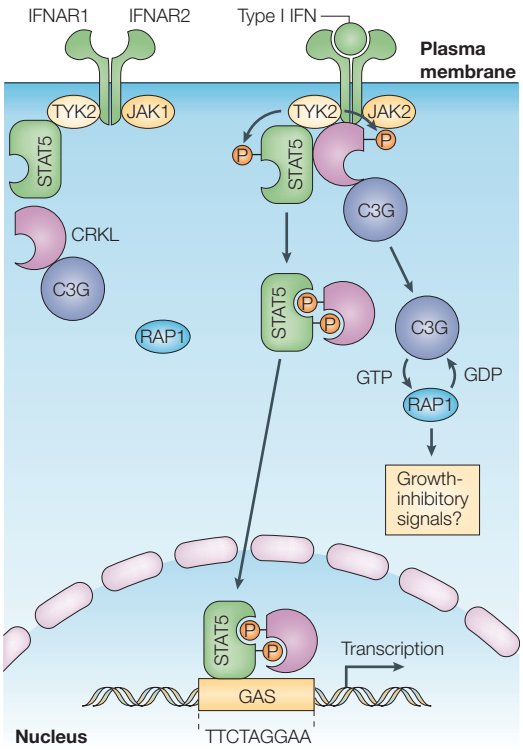


Figure 2 | **Activation of CRKL during engagement of the type I interferon receptor, and the role of CRKL in type-I-interferon-mediated signalling.** CRKL is present in a latent cytoplasmic form that constitutively associates with the guanine-nucleotide-exchange factor (GEF) C3G. A member of the STAT (signal transducer and activator of transcription) family of proteins, STAT5, is associated with tyrosine kinase 2 (TYK2) that is bound to the type I interferon (IFN) receptor subunit IFNAR1. After engagement of the type I IFN receptor by an IFN, CRKL associates with TYK2 and undergoes rapid tyrosine phosphorylation. The activated form of CRKL forms a signalling complex with STAT5, which also undergoes TYK2-dependent tyrosine phosphorylation. The CRKL–STAT5 complex translocates to the nucleus and binds specific GAS (IFN-γ-activated site) elements that are present in the promoters of certain IFN-stimulated genes (ISGs), which initiates transcription of these genes. The specific GAS-element sequence that is bound by CRKL–STAT5 complexes is shown. The IFN-dependent phosphorylation (activation) of CRKL also results in induction of the GEF activity of C3G. C3G subsequently regulates guanine-nucleotide exchange of the small G-protein RAP1, resulting in activation of this GTPase, which might then promote growth-inhibitory responses. JAK, Janus activated kinase.

protein)[37,38], and minichromosome maintenance deficient 5 (MCM5), and the recruitment of each of these depends on STAT1 Ser727 phosphorylation[31,39,40]. p300 and CBP are co-activators that have HISTONE-ACETYLTRANSFERASE activity[41], which is important for the regulation of chromatin remodelling that increases IFN-α- or IFN-γ-inducible transcription[39,40].

IFN-activated STATs have also been shown to interact with other proteins that might increase their transcriptional capacities. These other proteins include the transcriptional co-activator general control non-depressible 5 (GCN5)[42] and the chromatin-remodelling factor brahma-related gene 1 (BRG1)[43], which interact with STAT2, and they also include NMI (nMYC and STAT interactor), which interacts with all STATs, except STAT2 (REF. 44), and enhances their association with the co-activators CBP or p300. Surprisingly, recent studies have also shown that histone-deacetylase activity is required for IFN-dependent gene transcription[45–47], and histone deacetylase 1 has been shown to associate with both STAT1 and STAT2 (REF. 45). It seems that the function of enzymes that promote or impede histone acetylation can modify the transcriptional capacity of STATs, underscoring the complexity of the process. Further work in this area is necessary to clarify the precise sequence of events that regulates IFN-dependent histone acetylation and chromatin remodelling in the promoters of STAT-regulated ISGs.

It is probable that additional interactions of STATs with co-activator molecules, and possibly co-repressor molecules, will be identified in future studies, and these efforts should lead to a more complete understanding of the mechanisms of STAT regulation and function. Nevertheless, at present, there is accumulating evidence that certain pathways that do not involve STATs have important roles in IFN-mediated signalling (discussed in the following sections).

CRK proteins in IFN-mediated signalling

The CRK family of adaptor proteins has three members — CRKL, CRKI and CRKII — all of which are cellular homologues of VIRAL CRK[48]. CRKI and CRKII result from differential splicing of the same gene, whereas CRKL is encoded by a distinct gene[5,49]. These proteins contain SRC HOMOLOGY 2 (SH2) DOMAINS and SH3 DOMAINS, and they function as adaptors, facilitating the formation of various signalling complexes in response to diverse stimuli[5,49]. Both CRKL and CRKII contain one SH2 domain and two SH3 domains (one in the amino (N)-terminus and one in the C-terminus), whereas CRKI lacks one of the SH3 domains[5,49]. The SH2 domains of these proteins interact either with upstream protein-tyrosine-kinase-receptor substrates, such as p130^{CAS}, paxillin, insulin-receptor substrate (IRS) proteins, CBL (Casitas B-lineage lymphoma) or CBL-B[49], or with non-receptor tyrosine kinases, such as TYK2 (REF. 5). By contrast, their SH3 domains interact with downstream GUANINE-NUCLEOTIDE-EXCHANGE FACTORS (GEFs) and other signalling components[5,49].

The first evidence for an involvement of CRK proteins in IFN-mediated signalling was the finding that CRKL interacts with TYK2 and is tyrosine phosphorylated in response to treatment of cells with IFN-α, IFN-β or IFN-ω[50] (FIG. 2). Subsequent studies showed that CRKL is also tyrosine phosphorylated during IFN-γ-mediated signalling[51]. This provided evidence for a link between the IFN receptors and the GEF C3G, with which CRKL associates through its N-terminal SH3 domain[5,49]. C3G is a GEF for RAP1, a small GTPase that is related to RAS[52,53]. In response to hormones and cytokines, RAP1 participates in the regulation of a broad spectrum of biological activities, including cellular proliferation, differentiation and

HISTONE ACETYLTRANSFERASE
A protein that acetylates core histones, which results in important regulatory effects on chromatin structure and assembly, and on gene transcription.

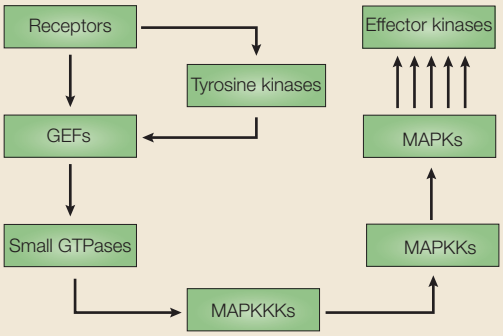
VIRAL CRK
(vCRK). The product of an oncogene encoded by the CT10 avian sarcoma virus. The oncogene transforms cells and can induce tumours in newborn chickens.

SRC-HOMOLOGY-2 DOMAINS
(SH2 domains). Protein domains that are commonly found in signal-transduction molecules. They specifically interact with phosphotyrosine-containing protein sequences.

SH3 DOMAINS
(SRC-homology-3 domains). Protein domains that are commonly found in signal-transduction molecules. They specifically interact with certain proline-containing protein sequences. Classically, they contain either (Arg/Lys)-X-X-Pro-X-X-Pro or Pro-X-X-Pro-X-Arg motifs, where X denotes any amino acid.

GUANINE-NUCLEOTIDE-EXCHANGE FACTORS
Proteins that stimulate the exchange of guanine diphosphate (GDP) for guanine triphoshate (GTP) in small GTPases, resulting in activation of the GTPase.

Box 1 | **Mitogen-activated protein kinases**



Mitogen-activated protein kinases (MAPKs) are widely expressed serine/threonine kinases that mediate signals for the regulation of important cellular functions, including gene transcription, post-transcriptional regulation, apoptosis and cell-cycle progression[13,66–70]. These kinases have been conserved throughout evolution and are classified into three main groups[66–70]: the extracellular-signal-regulated kinases (ERKs), ERK1 and ERK2; the p38 family, p38α, p38β, p38γ and p38δ; and the JUN amino-terminal kinases (JNKs), JNK1, JNK2 and JNK3. There are also additional MAPKs, such as ERK3, ERK5, ERK7 and ERK8 (REFS 66–70), which are atypical and cannot be classified into any of these groups. The activation of the different MAPKs is regulated by upstream dual-specificity kinases, which are known as MAPK kinases (MAPKKs; also known as MKKs) (see figure), and these phosphorylate MAPKs on both threonine and tyrosine residues in Thr-X-Tyr motifs that are specific for the distinct MAPK families: Thr-Glu-Tyr for ERKs, Thr-Pro-Tyr for JNKs and Thr-Gly-Tyr for p38-family members[66–70]. The activation of MAPKKs is regulated by other upstream kinases, known as MAPKK kinases (MAPKKKs; also known as MKKKs). Activation of MAPKKKs or MAPKKs usually occurs downstream of small GTPases, the function of which is regulated by guanine-nucleotide-exchange factors (GEFs), which in turn are frequently substrates for receptor or non-receptor tyrosine kinases. Through their various downstream effectors, MAPKs regulate diverse functional responses, depending on the stimulus and cellular context.

adhesion[52,53]. Interestingly, RAP1 was originally identified as a small G protein that opposes the function of RAS and blocks RAS-mediated transformation of cells[54], and there is some direct evidence that RAP1 can mediate suppression of cell growth[55,56]. However, it can also promote cellular proliferation, so it seems that its effects on cell growth depend on the stimuli and cell type that are involved[57].

Both type I and type II IFNs have been shown to rapidly and transiently activate RAP1 (REF. 51) in a CRKL-dependent manner[58]. The activation of RAP1 downstream of CRKL by both type I and type II IFNs implies that this is a mechanism by which IFNs can generate growth-inhibitory responses. Consistent with this, it has been shown that CRKL and the related protein CRKII are required for the growth-inhibitory effects of IFN-α and IFN-γ on human haematopoietic progenitors[59]. However, the precise mechanisms by which RAP1 could augment the induction of IFN-regulated antiproliferative responses remain to be determined. In other systems, RAP1 has been shown to regulate the activation of mitogen-activated protein kinase (MAPK)-signalling cascades[52,53], including the p38-signalling pathway[60], which transmits signals that are essential for the generation of the antiproliferative effects of IFNs (discussed later). It is therefore possible that the CRKL–RAP1 pathway mediates growth-inhibitory responses through regulating the activation of p38, but this needs to be examined directly. It is also of interest to determine whether RAPL, a downstream effector of RAP1 that was recently shown to have crucial roles in lymphocyte and dendritic-cell trafficking[61], is involved in the generation of the immunomodulatory effects of IFNs.

As well as the downstream activation of RAP1, CRKL has additional important roles in type-I-IFN-mediated signalling (FIG. 2). In response to treatment of cells with IFN-α or IFN-β, the SH2 domain of CRKL binds to the IFN-activated form of STAT5, resulting in the formation of a CRKL–STAT5 signalling complex that translocates to the nucleus and binds a GAS element (TTCTAGGAA) that is present in the promoter of certain ISGs[62] (FIG. 2). Such a function for CRKL, as a nuclear adaptor for STAT5, has been also shown in chronic myeloid leukaemia (CML)-derived, *BCR–ABL*-ONCOGENE-expressing, IFN-sensitive cell lines[63]. CRKL–STAT5 complexes were also detected by gel-shift assays using elements from the promoter of the promyelocytic leukemia (*PML*) gene[63], an IFN-α-inducible gene that mediates growth-inhibitory responses[64]. The formation of CRKL–STAT5 complexes seems to be crucial for gene transcription regulated by GAS elements, as shown by defective transcription of type-I-IFN-inducible genes by CRKL-deficient mouse embryonic fibroblasts (MEFs)[58]. MEFs that are deficient in both STAT5α and STAT5β have similar defects in IFN-α-inducible, GAS-element-driven gene transcription[65], underscoring the functional relevance of CRKL–STAT5 complexes in the generation of responses to type I IFNs.

MAPKs in IFN-mediated signalling

p38 and type-I-IFN-inducible transcription. Of the various MAPK pathways (BOX 1), it seems that the p38-signalling cascade has the most important role in the generation of IFN-mediated signals. This group of kinases includes several isoforms (p38α, p38β, p38γ and p38δ), which are encoded by distinct genes[13] but have considerable structural homology[13,66–70]. It was initially shown that p38α is phosphorylated and activated in a type-I-IFN-dependent manner in several IFN-sensitive cell lines[71,72]. In addition, in studies using SB203580, a pharmacological inhibitor of p38, or overexpression of a kinase-inactive p38α mutant, it was shown that inhibition of p38 activity blocks IFN-α-dependent transcription of genes that are regulated by ISREs[71]. However, inhibition of p38 activity did not block tyrosine phosphorylation of STAT1 or STAT2, or formation of the mature ISGF3 complex and the binding of this complex to ISREs, indicating that mechanisms that are independent of STAT activation account for the effects on ISRE-driven transcription[71]. In further studies, it was established that p38 is also required for type-I-IFN-driven gene transcription through GAS elements[73]. As in the case of ISRE-driven transcription, this was unrelated to effects on the tyrosine phosphorylation of STAT1, STAT3 or STAT5, or the formation of sis-inducible factor (SIF) complexes (that is, STAT1–STAT1, STAT1–STAT3 and STAT3–STAT3) or CRKL–STAT5 DNA-binding complexes[73]. In addition, although Ser727 in both STAT1 and STAT3 is in

BCR–ABL ONCOGENE
A fusion gene that results from an abnormal chromosomal translocation in which the breakpoint-cluster region (BCR) is fused to the Abelson leukaemia virus (ABL) tyrosine-kinase gene. In humans, this oncogene is involved in the pathogenesis of chronic myeloid leukaemia and some cases of acute lymphoblastic leukaemia.

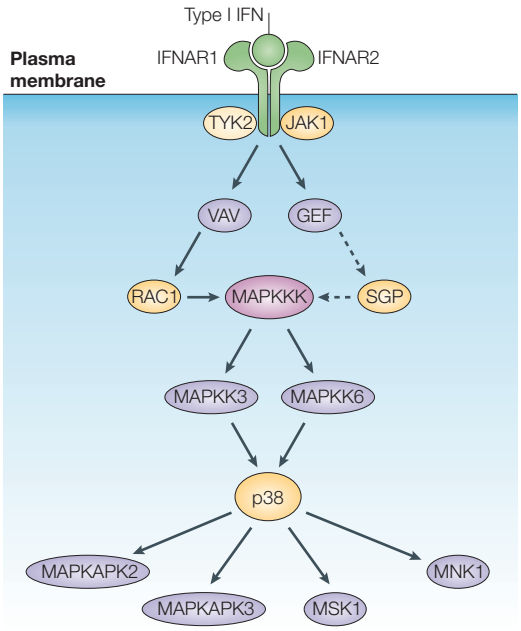


Figure 3 | **Mechanisms of activation of the mitogen-activated protein kinase p38 and its downstream effectors by type I interferons.** Interferon (IFN)-activated Janus activated kinases (JAKs) regulate the phosphorylation (activation) of VAV or other guanine-nucleotide-exchange factors (GEFs), resulting in the downstream activation of RAC1 and, possibly, other small G proteins (SGPs) that can regulate the signalling pathway of the mitogen-activated protein kinase (MAPK) p38. A MAPK kinase kinase (MAPKKK) is subsequently activated and regulates downstream activation of the MAPK kinases MAPKK3 and MAPKK6, which directly phosphorylate p38, resulting in its activation. Activated p38 subsequently regulates activation of multiple downstream effectors, including MAPK-activated protein kinase 2 (MAPKAPK2), MAPKAPK3, mitogen- and stress-activated kinase 1 (MSK1) and MAPK-interacting protein kinase 1 (MNK1). IFNAR1, type I IFN receptor subunit 1; IFNAR2, type I IFN receptor subunit 2; TYK2, tyrosine kinase 2.

a consensus phosphorylation motif for MAPKs, extensive studies have established that p38 does not function as a serine kinase towards STAT1 in response to type I (REF. 73) or type II (REF. 74) IFNs. Other studies have shown that disruption of the *p38α* gene results in defective transcription of genes that are regulated by ISREs and/or GAS elements[75] but that IFN-α-dependent serine phosphorylation of STAT1 and formation of DNA-binding complexes are intact in p38α-deficient cells[75], which firmly establishes that the regulatory effects of the p38-signalling pathway on type-I-IFN-dependent transcriptional regulation are not linked to any direct effects on the function of STATs. In contrast to type I IFNs, gene transcription induced by IFN-γ, driven by GAS elements, is not defective in p38α-deficient cells[75,76], indicating that p38α does not have direct regulatory effects on type-II-IFN-dependent transcription.

Upstream effectors of the type-I-IFN-activated p38-signalling pathway. The important role of p38 in type-I-IFN-dependent transcriptional regulation has prompted extensive studies attempting to identify the mechanisms of its activation by signalling through the type I IFN receptor. The small GTPase RAC1 is activated by type I IFNs[73], and overexpression of a dominant-negative RAC1 mutant blocks IFN-dependent activation of p38 (REF. 73). Although this finding does not exclude the possibility of involvement of other small GTPases as upstream regulators of p38, it indicates that RAC1 has a crucial role in the activation of this pathway by IFNs. Interestingly, it is known that RAC1 is a substrate for the guanine-nucleotide-exchange activity of VAV[77], an SH2- and SH3-domain-containing protein[78] that is activated by type I IFNs[79] and mediates IFN-induced growth-inhibitory responses[80]. So, it is probable that the initial step in the activation of the p38-signalling pathway by type I IFNs is tyrosine phosphorylation of VAV by TYK2 (REF. 81), followed by VAV-dependent exchange of GDP for GTP in RAC1, ultimately leading to activation of downstream MAPK kinases (MAPKKs; also known as MKKs) that phosphorylate p38 (FIG. 3). Indeed, recent studies have shown that both MAPKK3 and MAPKK6 are rapidly activated during treatment of cells with type I IFNs, which regulates downstream activation of p38 (REF. 82). Interestingly, the IFN-dependent activation of p38 is preserved in MEFs that are deficient in either MAPKK3 or MAPKK6, indicating that these kinases have redundant roles in IFN-mediated signalling[82].

Downstream effectors of the type-I-IFN-activated p38-signalling pathway. The identification of effector kinases that are activated downstream of p38 and mediate its regulatory effects on gene transcription and on the generation of IFN-induced biological properties is of considerable interest. There is evidence that, during treatment of cells with type I IFNs, the kinase MAPK-activated protein kinase 2 (MAPKAPK2) is rapidly activated in a p38-dependent manner[71]. Such activation seems to be important for IFN-dependent transcriptional activation, as shown by the requirement for this kinase in IFN-dependent transcription of *Isg15* (IFN-stimulated protein of 15 kDa)[82] and in induction of the antiviral properties of type I IFNs[75]. The potential role of the related kinase MAPKAPK3, which is also activated by type I IFNs[71], in the generation of responses to IFNs remains to be examined. It should be pointed out that studies with mice that are deficient in MAPKAPK2 have established that this kinase has an important role in the post-transcriptional regulation of expression of various cytokine genes at the level of mRNA stability[83]. It will be interesting to examine whether MAPKAPK2 also has such a role in IFN-mediated signalling and, if this is the case, to dissect which IFN-mediated responses require its transcriptional regulatory effects compared with its post-transcriptional regulatory effects.

Other kinases that might be activated by the p38-signalling pathway in response to type I IFNs include mitogen- and stress-activated kinase 1 (MSK1) and MSK2 (REFS 84,85). These kinases are activated in response to stress and are engaged downstream of

both the p38- and the extracellular-signal-regulated kinase (ERK)-signalling cascades[85]. The kinase activities of MSK1 and MSK2 are required for phosphorylation of histone H3, which is important for immediate early gene expression[86]. Recent studies have established that MSK1 is activated by IFN-α, and such activation is decreased in cells that have a targeted disruption of the *p38α* gene[75]. It is therefore possible that the regulatory effects of the p38-signalling pathway on the transcription of ISGs are, at least in part, mediated by MSK1- and/or MSK2-dependent phosphorylation of histone H3, but this remains to be clarified. Finally, another putative p38-dependent effector for the generation of responses to IFNs is the MAPK-interacting protein kinase 1 (MNK1) (FIG. 3), which is also phosphorylated in a type-I-IFN-dependent manner (Y. Li and L.C.P., unpublished observations). This kinase, and the related MNK2, are serine kinases that phosphorylate the eukaryotic translation-initiation factor 4E (EIF4E), an important regulator of mRNA translation, and they are activated downstream of MAPK-signalling cascades[87]. Interestingly, MNK1 and MNK2 have been implicated in the negative regulation of CAP-DEPENDENT TRANSLATION[88], and it remains to be seen whether they have similar roles in regulating IFN-mediated signalling.

Role of the p38-signalling pathway in type-I-IFN-mediated biological responses. After the original study showing that p38 is activated by IFNs, extensive studies were carried out to determine the functional relevance of this signalling cascade in the generation of the biological effects of type I IFNs on normal and malignant cells. p38 was found to be activated in a type-I-IFN-dependent manner in BCR–ABL-expressing cell lines and in primary cells from patients with CML[89]. Importantly, pharmacological inhibition of p38 was found to reverse the suppressive effects of IFN-α on leukaemic progenitors from the bone marrow of patients with CML[89], showing that p38 is essential for the antileukaemic properties of this cytokine. Other studies have also established that p38 and its downstream effector MAPKAPK2 are activated in a type-I-IFN-dependent manner in primitive haematopoietic precursors, to mediate haematopoietic-suppressive signals[90].

In addition to the apparent requirement of the p38-signalling pathway for the growth-inhibitory effects of type I IFNs, there is evidence that its function is essential for the induction of antiviral responses by type I IFNs. Pharmacological inhibition of p38 (REF. 89) or overexpression of a dominant-negative p38 mutant[72] has been found to abrogate the antiviral properties of IFN-α against vesicular stomatitis virus and encephalomyocarditis virus. Similarly, p38 mediates IFN-α-dependent antiviral activities against hepatitis C virus (HCV) *in vitro*[91], indicating that the p38-signalling pathway has an important role in the induction of the therapeutic effects of IFN-α in patients who have HCV-induced hepatitis[92].

Roles of other MAPKs in type-I- and type-II-IFN-mediated signalling. In addition to the induction of the p38-signalling pathway, there is evidence that other MAPK-signalling pathways are activated by IFNs. Previous studies have shown that the MEK–ERK pathway is activated by IFN-α, IFN-β[93] and IFN-γ[94], and there is also evidence that this pathway is activated in response to viral infection and regulates activation of IRF3 and production of type I IFNs[95]. An important function of ERK cascades in IFN-mediated signalling is the regulation of IFN-γ-dependent transcription by CCAAT/enhancer-binding protein-β (C/EBP-β)[96], a transcription factor that binds response elements known as IFN-γ-activated transcriptional elements (GATEs), which are present in the promoters of particular ISGs[96,97]. There is also evidence that the IFN-activated MEK–ERK-signalling pathway mediates additional signals, such as phosphorylation of peroxisome-proliferative-activated receptor-γ (PPAR-γ), which increases the targeting of PPAR-γ for degradation by the ubiquitin–proteasome system[98]. However, in certain situations, IFNs can also block the constitutive activation of the MEK–ERK-signalling pathway. For example, IFN-α blocks the MEK–ERK-signalling cascade in basal-cell-carcinoma cells, which results in induction of CD95 (also known as FAS) expression and apoptosis[99].

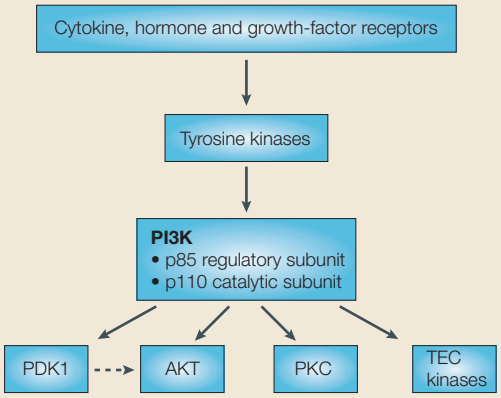
Regarding the JUN N-terminal kinase (JNK) pathway, little is known about the potential involvement of this group of MAPKs in IFN-mediated signalling. Recent evidence indicates that JNKs regulate the induction of signals during the IFN response to viral infection, leading to elimination of virus-infected cells by apoptosis[100]. However, at present, it is unclear whether JNKs are directly activated by ligation of IFN receptors and whether they have direct roles in IFN-mediated signalling.

PI3K in IFN-mediated signalling

Mechanisms of IFN-dependent activation of PI3K. The first evidence implicating the phosphatidylinositol 3-kinase (PI3K)-signalling pathway (BOX 2) in IFN-mediated signalling was the finding that several type I IFNs (IFN-α, IFN-β and IFN-ω) induce tyrosine phosphorylation of IRS1 and that the p85 regulatory subunit of PI3K associates, through its N- and C-terminal SH2 domains, with IRS1 in an IFN-dependent manner[101]. Such interaction was found to result in activation of the p110 catalytic subunit of PI3K[101]. IRS1 is a member of the IRS family of multi-site docking proteins, a group of proteins with several tyrosine-phosphorylation sites. These tyrosine residues are present in specific motifs such that, when phosphorylated, they function as binding sites for various signalling proteins that contain SH2 domains, including the p85 regulatory subunit of PI3K[5,102]. Other members of this family include IRS2, IRS3, IRS4, GAB1 (growth-factor-receptor-bound protein 2 (GRB2)-associated binding protein 1) and GAB2 (REFS 5,102). In addition to IRS1, IRS2 was subsequently shown to undergo phosphorylation in a type-I-IFN-dependent manner and to provide docking sites for PI3K[103,104]. So, type I IFNs activate the

CAP-DEPENDENT TRANSLATION
An important step for the initiation of mRNA translation. It involves a complex process in which the Met-tRNAi initiator and the 40S and 60S ribosomal subunits are all assembled by eukaryotic translation-initiation factors into the 80S ribosome at the start codon of a specific mRNA.

Box 2 | **The phosphatidylinositol 3-kinase signalling pathway**



The main function of the phosphatidylinositol 3-kinase (PI3K)-signalling pathway is the phosphorylation of phosphatidylinositol lipids at the D3 position of the inositol ring[134,135]. Such phosphorylation occurs in response to various stimuli, including cytokines, growth factors and hormones[134,135] (see figure). It is regulated only by class Ia PI3Ks and not by class Ib, class II or class III PI3Ks[134]. The class Ia PI3Ks are composed of a regulatory subunit, which contains SRC homology 2 (SH2) and SH3 domains, thereby allowing interactions with other signalling proteins, and an associated catalytic subunit, which has enzymatic activity that regulates phosphorylation of the D3 position of the inositol ring[134,135]. There are several isoforms of the regulatory subunit (p85α, p85β, p55α, p55γ and p50α), which are encoded by three genes, and there are three isoforms of the catalytic subunit (p110α, p110β and p110δ), which are each encoded by a separate gene[135]. The induction of PI3K activity results in the phosphorylation of the membrane lipid phosphatidylinositol-4,5-bisphosphate to generate phosphatidylinositol-3,4,5-trisphosphate, to which PLECKSTRIN-HOMOLOGY DOMAINS of signalling proteins bind[134,135]. Binding of 3-phosphoinositides also occurs through PHOX-HOMOLOGY DOMAINS, which are present in certain proteins[134,135]. Several downstream effectors of the PI3K-signalling pathway have been identified, and these lead to the activation of various downstream signalling cascades. These effectors include the serine/threonine kinases AKT and 3-phosphatidylinositol-dependent protein kinase 1 (PDK1), various isoforms of protein kinase C (PKC), and members of the TEC family of tyrosine kinases, including TEC, BTK (Bruton's tyrosine kinase), ITK (interleukin-2-inducible T-cell kinase) and RLK (resting lymphocyte kinase)[135].

PI3K-signalling pathway downstream of JAKs, in an IRS-dependent[101,103,104] but STAT-independent[105,106] manner. In addition to induction of phosphatidylinositol-kinase activity, activation of PI3K results in induction of serine-kinase activity[103], and one substrate for such serine-kinase activity is IRS1 itself[107,108].

In contrast to type I IFNs, which use IRS proteins to activate the PI3K pathway, IFN-γ does not induce phosphorylation of IRS1 or IRS2 (REF. 103). However, PI3K activity can be detected in IFN-γ-treated cells[106], indicating that other unknown phosphoproteins have analogous roles in type-II-IFN-mediated signalling to those of IRS proteins in type-I-IFN-mediated signalling. One candidate for having such activity is CBL, which has binding sites for the SH3 domain of p85 and is phosphorylated in an IFN-γ-dependent manner[51].

Regulation of serine phosphorylation of STAT1 by PI3K. The activation of PI3K by IFN-γ seems to have important functional consequences in IFN-γ-inducible transcriptional regulation. It was originally shown that the pharmacological inhibition of PI3K blocks the IFN-γ-dependent phosphorylation of STAT1 on Ser727 and reduces STAT1-driven transcription[109], indicating that a downstream effector of PI3K phosphorylates STAT1 on Ser727. Subsequent studies showed that a member of the PKC family of proteins, PKC-δ, is activated by treatment of cells with either type I (IFN-α, IFN-β or IFN-ω)[32] or type II (IFN-γ)[33] IFNs and associates with STAT1 (REFS 32,33). Inhibition of the kinase activity of PKC-δ was found to block phosphorylation of STAT1 on Ser727, as well as STAT1-mediated gene transcription through ISREs or GAS elements, indicating a crucial role for this kinase in IFN-dependent gene transcription. Moreover, it was shown that the IFN-γ-dependent activation of PKC-δ is PI3K dependent[33], strongly indicating that PKC-δ is a downstream effector of the PI3K pathway that functions as a serine kinase towards STAT1. Interestingly, after the original finding that PKC-δ is an IFN-activated serine kinase for STAT1 (REF. 32), other studies showed that such PKC-δ-mediated phosphorylation of STAT1 on Ser727 mediates chemotherapy-induced apoptosis, indicating that this event is required for the transcription of pro-apoptotic genes[110].

So, consistent with its growth-inhibitory and tumour-suppressor properties[111,112], PKC-δ seems to have an important role in IFN-mediated signalling, by functioning as a serine kinase for STAT1 (FIG. 4). It should be pointed out that the relative tissue-specific distribution of various PKC isoforms indicates that, in certain situations, other PKC isoforms might also contribute to the regulation of serine phosphorylation of STAT1 and/or of IFN-dependent transcriptional activity. Consistent with this hypothesis, recent studies have shown that PKC-ε is activated downstream of PI3K and functions as a serine kinase towards STAT1 in mesangial cells[35]. Other studies have shown that PKC-θ, which is closely related to PKC-δ, is activated by type I IFNs in T cells and is required for optimal IFN-α-dependent transcriptional activation[113].

Regulation of apoptosis by IFN-activated PI3K. As well as its effects on the regulation of IFN-dependent transcriptional activation by STAT1, the PI3K-signalling pathway seems to have other roles in IFN-mediated signalling. It is well established that AKT, a downstream effector of PI3K, mediates anti-apoptotic and pro-survival signals[114]. Therefore, it is not surprising that the IFN-inducible PI3K–AKT-signalling pathway can also mediate survival signals in a cell-type-restricted manner. For example, IFN-β has been shown to promote survival of primary astrocytes through activation of the PI3K–AKT-signalling pathway[115]. Such a function of PI3K might be important for the therapeutic effects of IFN-β in the setting of multiple sclerosis, because such effects depend, in part, on the ability of IFN-β to protect astrocytes from the apoptotic cell death that is seen in the early stages of this disease[115]. Similarly, activation of this pathway seems to promote IFN-α-dependent survival of primary B cells[116], neutrophils[117] and lymphoblastoid cell lines[118]. Conversely, there is also evidence that activation of the PI3K-signalling pathway is required for IFN-α-induced apoptosis of U266 myeloma cells[119]. Taken together, these findings indicate that, during responses to IFNs, the PI3K-signalling pathway can mediate either pro-apoptotic or anti-apoptotic signals, depending on

PLECKSTRIN-HOMOLOGY DOMAINS
Amino-acid sequences that are present in several signalling proteins that mediate their function through binding phosphatidylinositols. A subset of these domains selectively binds phosphatidylinositol 3-kinase products. Pleckstrin-homology domains also anchor proteins to membranes by binding membrane lipids.

PHOX-HOMOLOGY DOMAINS
Amino-acid sequences that are present in certain signalling proteins and that target these proteins to organelle membranes. Such targeting occurs through interactions between conserved motifs in the PHOX-homology domains and specific phosphoinositides. These domains have a structure that is distinct from pleckstrin-homology domains.

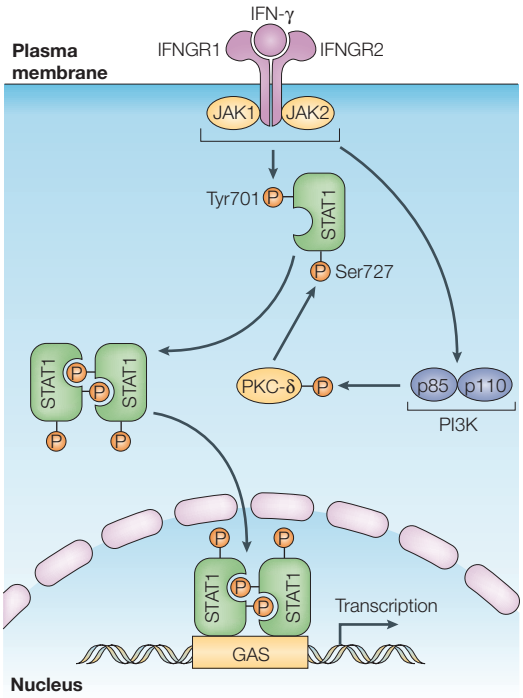


Figure 4 | **Activation of phosphatidylinositol 3-kinase and protein kinase C-δ by the type II interferon receptor and crosstalk with the STAT-signalling pathway.** After binding of interferon-γ (IFN-γ) to the type II IFN receptor, Janus activated kinase 1 (JAK1) and JAK2 are activated and phosphorylate STAT1 (signal transducer and activator of transcription 1) on the tyrosine residue at position 701 (Tyr701). The tyrosine-phosphorylated form of STAT1 forms homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements, which are present in the promoters of IFN-γ-regulated genes. The IFN-γ-activated JAKs also regulate, through as-yet-unknown intermediates, activation of the catalytic subunit (p110) of phosphatidylinositol 3-kinase (PI3K). The activation of PI3K ultimately results in downstream activation of protein kinase C-δ (PKC-δ), which in turn regulates phosphorylation of STAT1 on the serine residue at position 727 (Ser727). The phosphorylation of Ser727 is not essential for the translocation of STAT1 to the nucleus or for the binding of STAT1 to DNA, but it is required for full transcriptional activation. IFNGR1, IFN-γ receptor subunit 1; IFNGR2, IFN-γ receptor subunit 2.

the cellular context and, probably, the simultaneous activation or absence of activation of other IFN-dependent signalling pathways.

Other functions of PI3K in IFN-mediated signalling. Finally, several other IFN-dependent biological effects have been ascribed to PI3K, underscoring the importance of this cascade in IFN-mediated signalling. These include IFN-γ-stimulated expression of inducible nitric-oxide synthase by microglial cells[120], IFN-stimulated adhesion of monocytes[121] and regulation of IFN-β-dependent phosphorylation of the p65 subunit (also known as REL-A) of nuclear factor-κB (NF-κB)[122]. Importantly, as discussed in the next section, activation of the PI3K-signalling cascade also regulates IFN-inducible activation of the mammalian target of rapamycin (MTOR), which mediates the initiation of mRNA translation.

IFN-mediated signals for mRNA translation

It is well established that IFNs inhibit the translation of viral mRNAs, and such inhibition is an important mechanism by which IFNs mediate their antiviral effects[123]. However, little is known about the mechanisms by which type I and type II IFNs regulate the initiation of mRNA translation for specific ISGs in IFN-sensitive cells and thereby regulate the generation of proteins that mediate the biological effects of IFNs. The activation of MAPKAPK2 and MNK1 indicates a role for p38 in the regulation of IFN-dependent mRNA translation. There is also evidence that, in bovine myometrial cells, the p38-signalling pathway regulates the IFN-τ-dependent induction of prostaglandin G/H synthase 2 at the post-transcriptional level[124]. So, downstream effectors of p38 might be regulating signals for the initiation of mRNA translation, but their precise roles are unknown.

It has recently been shown that MTOR is activated during treatment of cells with either type I (REF. 125) or type II (REF. 126) IFNs (FIG. 5). Such IFN-dependent activation of MTOR was found to result in downstream activation of p70 S6 kinase (p70-S6K)[125,126] and phosphorylation of the S6 ribosomal protein[126], indicating that there is an IFN-mediated pathway for the regulation of 5′-terminal oligopyrimidine tract (TOP) mRNA translation[127]. The IFN-inducible activation of MTOR and/or p70-S6K was inhibited by pharmacological inhibition of PI3K[125,126]. It was also defective in MEFs that were deficient in both the α- and β-subunits of the p85 regulatory subunit of PI3K, establishing that this MTOR-signalling pathway requires PI3K activity to be induced by IFN-mediated signalling[125,126]. Treatment of cells with IFN-α or IFN-β was also found to result in phosphorylation of the repressor of mRNA translation EIF4E-binding protein 1 (4EBP1; also known as EIF4EBP1) at multiple sites, including on the threonine residue at position 37 (Thr37) and/or Thr46, Ser65 and Thr70 (REF. 125), leading to the de-activation of 4EBP1 and its dissociation from EIF4E, and the initiation of translation[127–129] (FIG. 5). Similar findings were observed for the type II IFN (IFN-γ) receptor[126], and the phosphorylation of 4EBP1 in response to both type I and type II IFNs was shown to be PI3K and MTOR dependent[125,126]. Activation of the MTOR-signalling pathway by IFNs had no effect on the phosphorylation of STATs or on gene transcription[118,125,126], indicating that it selectively regulates IFN-induced mRNA translation but not gene transcription.

A role for MTOR in the induction of apoptosis by IFNs has already been described[114], but its potential roles in the induction of antiviral responses and in the regulation of cell-cycle progression remain to be defined. Further studies using additional biochemical and genetic approaches are necessary to firmly establish the roles of this pathway in the generation of the biological effects of IFNs. Nevertheless, the finding that MTOR is involved in IFN-mediated signalling[125,126], together with the more recent finding that it is involved in all-*trans*-retinoic-acid-mediated signalling in leukaemia cells[130], raises questions about whether the original perception of the MTOR-signalling pathway as a

Box 3 | **Clinical uses of interferons**

Interferons (IFNs) have been used in various clinical settings[6,136–138]. The diseases or syndromes in which different IFNs have shown clinical activity are summarized here.

IFN-α

Haematological malignancies. **Chronic myeloid leukaemia | cutaneous T-cell lymphoma | hairy-cell leukaemia | multiple myeloma**

Solid tumours. **Malignant melanoma | renal-cell carcinoma | AIDS-related Kaposi's sarcoma**

Viral syndromes. **Hepatitis C | hepatitis B | severe acute respiratory syndrome**

IFN-β

Multiple sclerosis

IFN-γ

Chronic granulomatous disease | severe malignant osteopetrosis

'mitogenic' signalling cascade is accurate. There is no doubt that the PI3K–AKT–MTOR-signalling pathway is activated by growth factors and other mitogenic stimuli to transduce pro-survival and growth-promoting signals[127–129]. However, because this signalling cascade is also activated by IFNs, which suppress growth and can mediate pro-apoptotic effects[120], this indicates that differential regulation of the cascade by various stimuli can lead to divergent biological responses. The precise mechanisms that might determine such specificity remain to be identified, so caution should be taken in the development of new anticancer treatments that target MTOR[131], particularly in cases in which such drugs might be administered together with IFNs.

Conclusions

There is accumulating evidence that multiple signalling pathways are activated by IFNs and that the cooperative function of several signalling cascades is required for the generation of responses to these IFNs. In addition to the signalling pathways described here, the list of newly identified IFN-regulated signalling pathways is rapidly growing and includes elements that seem to have important roles in the induction of biological responses, despite their precise biochemical functions in IFN-mediated signalling not yet being determined. For example, there is evidence that IFN-γ-inducible transcription of certain genes is STAT1 independent[132], and recent studies have shown that expression of a subset of IFN-γ-regulated genes requires the function of the IKK (inhibitor of NF-κB (IκB) kinase) complex, as shown by experiments using MEFs that were deficient in both IKK-α and IKK-β[133]. Although the IKK complex is a regulator of the NF-κB-signalling pathway, its effects on IFN-γ-inducible gene transcription are independent of NF-κB[133], indicating that these kinases have additional signalling functions.

It is probable that the list of new elements involved in IFN-mediated signalling will continue to grow during the next few years, whereas the contributions of known pathways might need to be re-evaluated. At present, it seems that activation of more than one signalling pathway is required for the generation of the different biological properties of IFNs, and no signalling cascade alone is sufficient for the generation of any given biological end-point. For example, the functions of both the STAT- and p38-signalling pathways are required for the antiviral effects of IFNs, but activation of either pathway alone is not sufficient to elicit an antiviral response[13]. Such a requirement for multiple signalling pathways also seems to be the case for IFN-dependent antiproliferative responses, and it might reflect the synergistic effects of various signals at the levels of gene transcription and mRNA translation.

The complexity of the IFN system leaves no doubt that extensive effort will be required to precisely define the hierarchical structure of the IFN-mediated signalling machinery. IFNs have had a considerable impact on clinical medicine, and their use has changed the outcome of various malignancies, viral infections and autoimmune disorders (BOX 3). Filling in the gaps in our understanding of IFN-mediated signalling mechanisms should provide important insights for developing new agents that target similar pathways and have similar biological properties but are more potent and more specific.

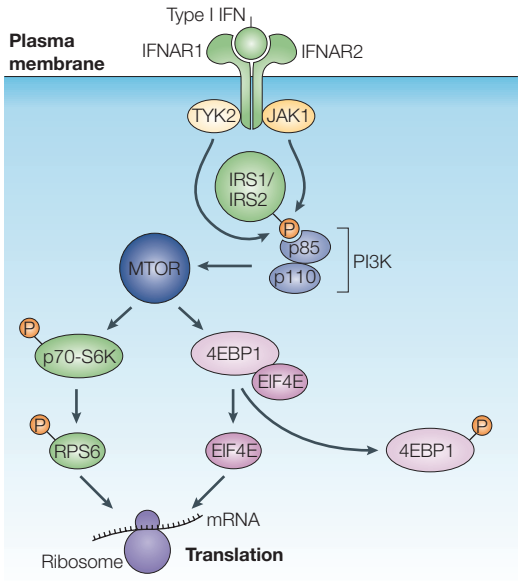


Figure 5 | **Type-I-interferon-activated signalling pathways that mediate initiation of mRNA translation.** Activated tyrosine kinase 2 (TYK2) and Janus activated kinase 1 (JAK1) regulate tyrosine phosphorylation of insulin-receptor substrate 1 (IRS1) and IRS2, which provide docking sites for the SRC homology 2 (SH2) domains of the regulatory subunit (p85) of phosphatidylinositol 3-kinase (PI3K). PI3K is subsequently activated and regulates downstream activation of mammalian target of rapamycin (MTOR). In turn, MTOR regulates phosphorylation (activation) of p70 S6 kinase (p70-S6K), which then phosphorylates ribosomal protein S6 (RPS6), resulting in the initiation of mRNA translation. MTOR also regulates phosphorylation of the translational repressor 4EBP1 (eukaryotic translation-initiation factor 4E (EIF4E)-binding protein 1). Such phosphorylation results in its de-activation and subsequent dissociation from EIF4E, allowing the initiation of cap-dependent mRNA translation. IFN, interferon; IFNAR1, type I IFN receptor subunit 1; IFNAR2, type I IFN receptor subunit 2.

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Competing interests statement
The author declares no competing financial interests.

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