Protein Kinase C-δ (PKC-δ) Is Activated by Type I Interferons and Mediates Phosphorylation of Stat1 on Serine 727*

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It is well established that engagement of the Type I interferon (IFN) receptor results in activation of JAKs (Janus kinases), which in turn regulate tyrosine phosphorylation of STAT proteins. Subsequently, the IFN-dependent tyrosine-phosphorylated/activated STATs translocate to the nucleus to regulate gene transcription. In addition to tyrosine phosphorylation, phosphorylation of Stat1 on serine 727 is essential for induction of its transcriptional activity, but the IFNα-dependent serine kinase that regulates such phosphorylation remains unknown. In the present study we provide evidence that PKC-δ, a member of the protein kinase C family of proteins, is activated during engagement of the Type I IFN receptor and associates with Stat1. Such an activation of PKC-δ appears to be critical for phosphorylation of Stat1 on serine 727, as inhibition of PKC-δ activation diminishes the IFNα- or IFNβ-dependent serine phosphorylation of Stat1. In addition, treatment of cells with the PKC-δ inhibitor rottlerin or the expression of a dominant-negative PKC-δ mutant results in inhibition of IFNα- and IFNβ-dependent gene transcription via ISRE or GAS elements. Interestingly, PKC-δ inhibition also blocks activation of the p38 MAP kinase, the function of which is required for IFNα-dependent transcriptional regulation, suggesting a dual mechanism by which this kinase participates in the generation of IFNα responses. Altogether, these findings indicate that PKC-δ functions as a serine kinase for Stat1 and an upstream regulator of the p38 MAP kinase and plays an important role in the induction of Type I IFN-biological responses.

Type I IFNs* (IFN α, β, ω) are pleiotropic cytokines that exhibit antiproliferative, antiviral, and immunomodulatory effects in vitro and in vivo (1–5). For Type I interferons to elicit their biological effects on target cells, binding to the multisubunit Type I interferon receptor is required (1–5). This results in activation of the receptor-associated Tyk-2 and Jak-1 kinases (reviewed in Refs. 2–5), the activation of which regulates phosphorylation of multiple signaling elements and engagement of several downstream pathways, including the STAT pathway (reviewed in Refs. 1–5), the IRS-PI3'-kinase pathway (6–9), the Crk pathway (10–12), and pathways involving mitogen-activated protein (MAP) kinases (13–16). Thus, multiple signaling cascades are regulated by the Type I IFN receptor, a finding consistent with the pleiotropic biological effects of Type I interferons in vitro and in vivo.

JAK-STAT pathways play critical roles in interferon-dependent gene regulation. The activated JAK kinases regulate tyrosine phosphorylation of STAT proteins and the formation of different STAT complexes that translocate to the nucleus to initiate gene transcription via binding to distinct elements in the promoters of IFN-activated genes. There is strong evidence that, in addition to tyrosine phosphorylation, phosphorylation on serine is required for the transcriptional properties of Stat1 and Stat3 (reviewed in Ref. 17). Stat1 has a phosphorylation site in its C terminus, serine 727, which plays a critical role in the induction of gene transcription. Previous studies have established that phosphorylation of Ser-727 in Stat1 is essential for Type II IFN (IFNγ)-dependent transcriptional activation (18–22). Similarly, phosphorylation of Stat3 on Ser-727 is required for the full transcriptional activity of this protein without modifying its DNA-binding properties (21). The functional relevance of serine phosphorylation of Stat1 has been demonstrated in studies in which it was shown that complementation of Stat1-deficient cells with a Ser-727 mutant fails to restore induction of the antiproliferative and antiviral properties of IFNγ, whereas re-expression of the wild type protein restores such defects (23, 24). Most of the studies evaluating the functional relevance of serine phosphorylation of Stat1 have been performed in the Type II IFN-system. However, there is evidence that Stat1 is also phosphorylated on serine during engagement of the Type I IFN (IFNα) receptor (16, 25), suggesting a role for such phosphorylation of Stat1 in the generation of Type I IFN responses.

The mechanisms regulating Type I IFN-inducible phosphorylation of Stat1 on serine 727 have not been elucidated, and the serine kinase regulating such phosphorylation remains unknown. A good candidate kinase would have been the p38 MAP kinase, as the STAT-serine phosphorylation site is in a conserved motif, which is a potential site for phosphorylation by proline-directed kinases of the MAP kinase family (17). Furthermore, previous studies had shown that pharmacologi-
cal or molecular inhibition of the p38 MAP kinase pathway blocks interferon-dependent gene transcription (14, 15). However, extensive studies by us and others have established that p38 does not function as a serine kinase for Stat1 in response to IFNα (16) or IFNγ (26) and that its regulatory effects on Type I IFN-dependent gene transcription are unrelated to modification of components of the STAT-pathway (16).

In the present study we provide evidence that a member of the PKC family of proteins, PKC-δ, is phosphorylated during engagement of the Type I IFN receptor, and its kinase domain is induced. Our data demonstrate that PKC-δ interacts with Stat1 in an IFNα-dependent manner and regulates its phosphorylation on serine 727. In addition, specific pharmacological inhibitors of PKC-δ, or a dominant-negative PKC-δ mutant, inhibit IFNα-dependent gene transcription in luciferase reporter assays. Interestingly, engagement of PKC-δ also appears to be required for downstream activation of the p38 MAP kinase, suggesting the existence of a dual mechanism by which this PKC isoform participates in the regulation of IFN-dependent responses.

**Experimental Procedures**

**Cells and Reagents**—The U-266 and Molt-4 cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics. U2OS cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. Human recombinant IFNα2 was provided by Hoffmann-La Roche. Human recombinant consensus IFNα was provided by Amgen Inc. Human recombinant IFNγ was provided by Biogen Inc. Antibodies against the phosphorylated forms of p38 and Erk-2 were obtained from New England Biolabs and were used for immunoblotting. Polyclonal antibodies against PKC-δ, p38, and Stat1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against the phosphorylated/activated form of PKC-δ at threonine 505 and against the phosphorylated form of the p38 MAP kinase at threonine 180 and tyrosine 182 were obtained from New England Biolabs (Beverly, MA). Antibodies that specifically recognize the phosphorylated forms of Stat1 at serine 727 and tyrosine 701 and an anti-phosphotyrosine monoclonal antibody (4G-10) were obtained from Upstate Biotechnology Inc. and were used for immunoblotting. The pan-PKC inhibitor H7, the PKC-δ inhibitor rottlerin, and the p38 MAP kinase inhibitor SB203580 were purchased from Calbiochem.

**Cell Lysis, Immunoprecipitation, and Immunoblotting**—Cells were stimulated with 1 × 10^6 units/ml of the indicated interferons for the indicated times and lysed in phosphorylation lysis buffer as described previously (6–9). Immunoprecipitations and immunoblotting using an ECL (enhanced chemiluminescence) method were performed as described (10). In the experiments in which pharmacological inhibitors of PKC-δ or p38 were used, the cells were pretreated for 60 min with the indicated concentrations of the inhibitors and subsequently treated for the indicated times with interferons prior to lysis in phosphorylation lysis buffer.

**PKC-δ Kinase Assays**—Immune complex kinase assays to detect PKC-δ activation were performed as described previously (14, 27). Briefly, cells were treated for the indicated times with IFNα or IFNγ, and the cells were lysed in phosphorylation lysis buffer. Cell lysates were immunoprecipitated with an anti-PKC-δ antibody, and immunoprecipitates were washed three times with phosphorylation lysis buffer and two times with kinase buffer (25 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 0.5 mM EDTA, 1 mM dithiothreitol, 20 μg of phosphatidylycerine, 20 μg ATP) and were resuspended in 30 μl of kinase buffer containing 5 μg of histone H1 as an exogenous substrate, to which 20–30 μCi of [γ-^32P]ATP was added. The reaction was incubated for 15–30 min at room temperature and was terminated by the addition of SDS-sample buffer. Proteins were analyzed by SDS-PAGE, and the phosphorylated form of histone H1 was detected by autoradiography. In some experiments recombinant active PKC-δ kinase (obtained from Upstate Biotechnology Inc.) was added directly in the kinase buffer together with Stat1 immunoprecipitated from cell lysates of U-266 cells, and after completion of the in vitro kinase assay reaction, the phosphorylation of Stat1 was detected by SDS-PAGE analysis followed by immunoblotting with an anti-Ser-727 Stat1 antibody.

**p38 MAP Kinase Assays**—The activation of the p38 kinase in response to IFNα was evaluated by in vitro kinase assays as described previously (14).

**Results**

We first determined whether during IFNα treatment of sensitive cells, PKC-δ is phosphorylated/activated. U-266 or Molt-4 cells were incubated in the presence or absence of IFNα, and cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of PKC-δ on threonine 505. As shown in Fig. 1, treatment of cells with IFNα resulted in strong phosphorylation of the protein, whereas there was no change in the amount of protein detected prior to and after IFNα treatment (Fig. 1, A–D). Similarly, treatment of cells with IFNβ also resulted in strong phosphorylation of PKC-δ (Fig. 2), suggesting that this kinase is a common element in the signaling pathways of all different Type I IFNs.

We subsequently determined whether the kinase domain of PKC-δ is activated by IFNα stimulation. Cells were incubated in the presence or absence of IFNα, and after cell lysis and immunoprecipitation with an anti-PKC-δ antibody, in vitro kinase assays were carried out on the immunoprecipitates using histone H1 as an exogenous substrate. IFNα treatment resulted in strong induction of the kinase activity of PKC-δ as evidenced by the phosphorylation of histone H1 (Fig. 3). Such phosphorylation of histone H1 in the kinase assay was blocked by pretreatment of cells with rottlerin, a pharmacological inhibitor that selectively blocks activation of PKC-δ (30), but not by either of the PKC isoforms (27, 30–33) (Fig. 3). On the other hand, pretreatment of cells with SB203580 (an inhibitor of the p38 MAP kinase) or LY379186 (a selective inhibitor of PKC-β) had no effects on the activation of PKC-δ and phosphorylation of histone H1 in the kinase assays (data not shown), further demonstrating the specificity of the process. Thus, during engagement of the Type I IFN receptor, PKC-δ is phosphorylated...
and its kinase activity is induced, strongly suggesting that this member of the PKC family of proteins plays a role in the generation of signals by the Type I IFN receptor.

It is well known that PKC-δ/H9254 exhibits serine kinase activity in other systems. Our data that this serine kinase is activated during engagement of the Type I IFN receptor raised the possibility that it may function as a STAT kinase and regulate phosphorylation of Stat1 on serine 727. To investigate such a hypothesis, experiments were performed in which cells were pretreated in the presence or absence of PKC inhibitors, and the IFNα/H9251-induced phosphorylation of Stat1 on serine 727 was examined by immunoblotting with an antibody against the phosphorylated form of PKC-δ. D, the blot shown in C was stripped and reprobed with an antibody against PKC-δ.

Fig. 1. IFNα induces phosphorylation of PKC-δ. A, Molt-4 cells were treated with IFNα for the indicated times. The cells were lysed, and equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of PKC-δ. B, the blot shown in A was stripped and reprobed with an antibody against PKC-δ. C, U-266 cells were treated with IFNα for 30 min as indicated. The cells were lysed, and equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of PKC-δ. D, the blot shown in C was stripped and reprobed with an antibody against PKC-δ.

PKC-δ-specific inhibitor rottlerin (27, 30–34). Pretreatment of cells with rottlerin also blocked the IFNα-induced Stat1 serine phosphorylation (Fig. 5 A, B, D, and E), whereas it had no
effects on the IFN-dependent tyrosine phosphorylation of Stat1 on Tyr-701 (Fig. 5, C and F).

Similarly, the IFNβ-inducible phosphorylation of Stat1 on serine 727 was also inhibited by pretreatment of cells with H7 or rottlerin (Fig. 6). On the other hand, treatment of cells with LY379196, a selective inhibitor of PKC-β but not PKC-δ, had no effects on the serine phosphorylation of Stat1 (Fig. 7), further establishing the specificity of these findings. Thus, the function of PKC-δ appears to be essential for the IFNα- and IFNβ-dependent phosphorylation of Stat1 on Ser-727, suggesting that either this PKC isoform functions as the Type I IFN-dependent serine kinase for Stat1 or regulates activation of a downstream serine kinase that directly phosphorylates Stat1.

The data using the pharmacological inhibitors of PKC-δ strongly suggested that this kinase regulates phosphorylation of Stat1 on serine 727. To directly determine whether the protein phosphorylates Stat1, in vitro kinase assays experiments were performed in which exogenous recombinant active PKC-δ protein was added to Stat1, immunoprecipitated from lysates of untreated cells. As shown in Fig. 8, A and B, the addition of the active PKC-δ protein resulted in strong phosphorylation of Stat1 on serine 727. Similarly, in studies in which a GST-Stat1 fusion protein was used as a substrate for PKC-δ immunoprecipitated from lysates of IFNα-treated cells, we found that Stat1 acts as a substrate for the kinase activity of PKC-δ (Fig. 8, C and D).

To obtain further information on the role that PKC-δ plays in Stat1 serine phosphorylation in vivo, we examined whether it interacts with Stat1 in intact cells. U-266 cells were incubated in the presence or absence of IFNα, and the cells were lysed in phosphorylation lysis buffer. Cell lysates were immunoprecipitated with an anti-Stat1 antibody and, after SDS-PAGE analysis, immunoblotted with an anti-PKC-δ antibody. PKC-δ was clearly detectable in anti-Stat1 immunoprecipitates after IFNα treatment of cells (Fig. 9, A and B), suggesting that it associates with PKC-δ to act as a substrate for its kinase activity. Consistent with this finding, in experiments in which cell lysates from IFNα-treated cells were immunoprecipitated with an anti-PKC-δ antibody and immunoprecipitates were immunoblotted with an anti-Stat1 antibody, we found that Stat1 protein can be detected in anti-PKC-δ immunoprecipitants in an IFNα-dependent manner (Fig. 9, C and D). We also determined whether the IFNα-inducible association of Stat1 with PKC-δ and its subsequent phosphorylation on serine 727 plays any role in its nuclear translocation and DNA binding activity. Molt-4 cells were preincubated in the presence or absence of rottlerin and then treated with IFNα in the continuous presence or absence of the PKC-δ inhibitor. Nuclear extracts were then obtained and analyzed by GDAC. As shown in Fig. 9E, Stat1 translocated to the nucleus and bound DNA in an IFNα-dependent manner. Rottlerin had no effect on the DNA binding of Stat1, indicating that the PKC-δ-mediated serine 727 phosphorylation of the protein does not affect its DNA binding activity (Fig. 9E).

In subsequent studies, we sought to determine the functional consequences of the IFNα-induced PKC-δ-dependent Stat1 serine phosphorylation. We examined whether inhibition of PKC-δ activation has negative regulatory effects on IFNα-dependent gene transcription via ISRE or GAS elements. Cells were transfected with ISRE or 8X-GAS-luciferase constructs and treated with IFNα in the presence or absence of the PKC-δ inhibitor rottlerin. Luciferase activity was subsequently measured. IFNα induced strong luciferase activity via either ISRE or GAS elements, but preincubation with rottlerin significantly decreased such activities (Fig. 10). In parallel studies in which IFNβ was used instead of IFNα, rottlerin, and also H7, inhibited the IFNβ-induced luciferase activity, whereas the PKC-β inhibitor LY379196 did not (Fig. 11). To further establish the role of PKC-δ in Type I IFN-dependent transcriptional regulation, we determined the effects of a dominant-negative PKC-δ mutant, created by the substitution of arginine 376 with lysine and therefore lacking a functional catalytic domain (27, 29), on IFNα-induced transcriptional activity in luciferase promoter assays. As shown in Fig. 12, overexpression of the dominant-negative PKC-δ mutant diminished IFNα-dependent induction of luciferase activity, using either the ISRE-Luc or the 8X-GAS-Luc constructs (Fig. 12, A and B). On the other hand, overexpression of a dominant-negative/kinase-inactive PKC-ε mutant, created by substitution of arginine 437 to lysine (27, 29), had no effects on IFNα-dependent luciferase promoter activity.
suggesting that this PKC isoform plays no role in IFN/H9251-induced transcriptional activation and further demonstrating the specificity of these findings.

Recent work from our group has demonstrated that the p38 MAP kinase pathway is activated by IFN/H9251 and that its function is essential for IFN/H9251-dependent gene transcription, independently of STAT activation (14, 16). We have also recently shown that PKC-6 regulates downstream activation of p38 in response to thrombin (27). This prompted us to determine whether the regulatory effects of PKC-6 on transcriptional activation of interferon-sensitive genes are mediated in part via effects on the IFNα- or IFNβ-inducible activation of p38. Cells were treated with IFNα or IFNβ in the presence or absence of the PKC-6 inhibitor rottlerin, and total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody that recognizes the phosphorylated/activated form of p38 (14, 16). As shown in Fig. 13, rottlerin inhibited activation of p38 in response to either IFNα (Fig. 13, A and B) or IFNβ (Fig. 13, C and D) treatment, suggesting that PKC-6 functions as an upstream regulator of p38 activation by IFNα. Consistent with this find-
FIG. 9. Stat1 associates with PKC-δ in an IFNα-dependent manner, but the nuclear translocation and DNA binding of Stat1 is PKC-δ-independent. A, U-266 cells were incubated in the presence or absence of IFNα for 20 min. The cells were lysed, and cell lysates were immunoprecipitated with an antibody against Stat1. Immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against PKC-δ. B, the blot shown in A was stripped and reprobed with an antibody against Stat1 to control for loading. C, U-266 cells were incubated in the presence or absence of IFNα for 15 min. The cells were lysed, and cell lysates were immunoprecipitated with an antibody against PKC-δ. Immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against Stat1. D, the blot shown in C was stripped and reprobed with an antibody against PKC-δ to control for loading. E, Molt-4 cells were pretreated for 30 min with rottlerin as indicated and subsequently treated with IFNα for 30 min as indicated. Nuclear extracts were subsequently prepared and analyzed by GDAC. GDAC eluates were resolved by SDS-PAGE and after Western blotting probed with an anti-Stat1 antibody.

ing, pretreatment of cells with rottlerin also inhibited the activation of the MAPKapK-2 kinase (Fig. 14A), which we have previously shown to be activated downstream of p38 in response to IFNα (14). To exclude the possibility that rottlerin has nonspecific effects on the kinase domain of p38, the effect of rottlerin on the kinase domain of p38 was directly determined. The addition of rottlerin directly to anti-p38 immunoprecipitates from IFNα-treated cells had no effect on the kinase activity of p38 (Fig. 14B), whereas as expected, addition of the p38-inhibitor SB203580 inhibited such an activation (Fig. 14B). These data strongly suggest that activation of PKC-δ is essential for Type I IFN-dependent activation of p38 and are consistent with the findings of a recent study (27) demonstrating that the thrombin-dependent activation of p38 is PKC-δ-dependent.

DISCUSSION

Our data provide the first evidence that PKC-δ is activated during engagement of the Type I IFN receptor and functions as a serine kinase for Stat1. They also demonstrate that the function of this PKC isomor is essential for transcriptional regulation of interferon-sensitive genes. PKC-δ is a member of the PKC family of serine-threonine kinases, which play important roles in signaling for various cytokine receptors (reviewed in Refs. 32–34). The different protein kinase C isozymes are classified based on their requirements for activation. The first group includes the conventional PKC (cPKC) isozymes (PKC-α, β, γ), which require increases in both intracellular calcium and phorbol esters for their activation (32–34). The second group, in which PKC-δ is included, is the group of novel PKCs (nPKC), which do not require Ca2+ for their activation (PKC-δ, ε, η, θ, μ) but are activated by phorbol esters (32–34). Finally, a third group of atypical PKCs (aPKC) has been recently identified (PKC-ζ, λ), which are not activated in response to phorbol esters, the typical PKC activators (32–34).

Members of the PKC family have previously been shown to participate in the regulation of several important cellular responses such as differentiation, cell growth, and apoptosis (32–34). Interestingly, different PKC isoforms appear to exhibit opposing effects on cell growth and proliferation. For instance, PKC-ε promotes cell growth and functions as an oncogene (35), whereas PKC-δ exhibits antiproliferative effects and suppresses cell growth in various systems (35–37). Our finding that PKC-δ is activated by the Type I IFN receptor to participate in the generation of IFN-signals is consistent with the fact that this kinase mediates antiproliferative responses (35–37), as Type I IFNs are potent inhibitors of normal and neoplastic cell growth.

Although it is well known that the kinase domains of members of the PKC family exhibit serine-threonine kinase activity, very little is known about their ability to function as serine kinases for STAT proteins. Prior to the present study, evidence had been provided that PKC-δ plays a role in IL-6-dependent phosphorylation of Stat3 on serine 727 (30). That study demonstrated that PKC-δ associates with Stat3 in an IL-6-dependent manner and that pharmacological inhibition of PKC-δ with rottlerin abrogates the IL-6-induced phosphorylation of serine 727 in Stat3 (30). In addition, another study demonstrated that during engagement of the IL-6 receptor, PKC-δ is activated downstream of Rac1 and SEK1/MKK-4 to regulate Stat3 phosphorylation on serine 727 (38). Interestingly, our previous studies have shown that the small GTPase Rac1 is also activated by the Type I IFN receptor to regulate downstream engagement of
suggesting that activation of PKC-δ/H9254 by IFN-H9251 may occur downstream of Rac1. Thus, it is likely that the Type I IFN receptor regulates activation of a Rac1-PKC-δ/p38 signaling cascade, which plays a critical role in the induction of gene transcription.

An important and outstanding issue in the field of cytokine signaling, which is required to complete our understanding of the IFN-activated JAK-STAT pathway, is the identification of the Type I IFN-dependent serine kinase for Stat1. Serine 727 in Stat1 is located in the C terminus of the protein in a PSP motif. Previous studies have established that the phosphorylation of this site during engagement of the IFNγ receptor requires upstream activation of the Jak-2 tyrosine kinase (19), and the IFNγ-activated Pyk-2 tyrosine kinase has been also
implicated (40). A more recent study has provided evidence that the IFN-γ-dependent serine phosphorylation of Stat1 on serine 727 is regulated by a serine kinase downstream of the PI3-kinase pathway (41). Thus, it is possible that the IFN-γ-dependent serine phosphorylation of Stat1 on serine 727 is modulated by a serine kinase downstream of the PI3-kinase pathway (41).

Such phosphorylation appears to be dependent on upstream activation of the Jaks (41), which is associated with the Type I IFN receptor and plays an important role in the generation of IFN-γ-dependent biological responses. Although the Type I IFN receptor also induces serine phosphorylation of Stat1 (16), the serine kinase that mediates such effects remains unknown. In fact, the Pyk-2 tyrosine kinase, which regulates IFN-γ-inducible phosphorylation of Stat1 on serine 727, does not mediate IFN-γ-dependent phosphorylation of the protein (40). We have previously also established that the p38 MAP kinase does not function as a serine kinase for Stat1 in a large number of cell lines (16), indicating that it is not the IFN-γ-activated serine kinase that phosphorylates Stat1. Also, although the Type I IFN receptor activates the PI3-kinase pathway (6–8), it does not appear to induce the kinase activity of Akt (42), which is the downstream effector for PI3-kinase-dependent Stat1 serine phosphorylation by the IFN-γ receptor (41). Thus, it is possible that the Type I and II interferon receptors utilize different pathways to regulate serine phosphorylation of Stat1, a finding that is not surprising when the heterogeneity of the pathways that regulate STAT serine phosphorylation in response to other cytokines and extracellular stimuli is taken into account (43–47). However, it is possible that IFN-γ also activates PKC-δ or another member of the PKC superfamily to act as a Stat1 serine kinase downstream of PI3-kinase, especially when the regulatory effects that the PI3-kinase pathway exhibits on the activation of members of the PKC family in other systems are taken into account (48–54); this remains to be determined in future studies. Nevertheless, it is possible that, in contrast to the Type II IFN system, the positive regulatory effects of PKC-δ on the Type I IFN activation of p38 may be more important than the phosphorylation of Stat1 on serine 727 for the generation of some Type I IFN biological responses. This is because p38 exhibits strong regulatory effects on IFN-γ-dependent gene transcription via the promoters of essentially all Type I IFN-dependent genes, as all of them contain ISRE or GAS elements or both in their promoters. On the other hand, serine phosphorylation of Stat1 is important for Type I IFN-dependent gene transcription via GAS elements but may not be essential for ISGF3-dependent gene transcription, in which case the Stat2 transcription via GAS elements may be mediated primarily via ISRE elements may be mediated primarily via isotype blockade of downstream activation of the p38 pathway, and this remains to be determined in future studies.

Future studies should also define the motifs in Stat1 that are required for its interaction with PKC-δ during IFN-γ-stimulation, as well as the role that the Type I IFN-receptor-associated JAK kinases play in the induction of such events. A recent study has demonstrated that PP2, a Src kinase inhibitor, blocks the IFN-α/β-induced serine phosphorylation of Stat1 (25), suggesting that an Src kinase is involved in the pathway that ultimately regulates PKC-δ activation and Stat1 serine phosphorylation. Other studies have shown that two members of the Src family of kinases, Fyn (55) and Lyn (56), interact via
their SH2 (Src homology 2) domains with the Type I IFN receptor associated Tyk-2 kinase to be engaged in IFNα signaling. Thus, it is possible that the regulation of PKC-δ is PKC-ε kinase-inactive mutant cDNAs.

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