Engagement of Protein Kinase C-θ in Interferon Signaling in T-cells*

Kishore K. Srivastava‡, Sandeep Batra‡, Antonella Sassanoč, Yongzhong Li, Beata Majchrzakš, Hiroaki Kiyokawah, Amnon Altman, Eleanor N. Fishš, and Leonidas C. Platanias**

From the ¶Robert H. Lurie Comprehensive Cancer Center and Division of Hematology-Oncology, Northwestern University Medical School and Lakeside Veterans Administration Medical Center, Chicago, Illinois 60611, the ¶Department of Molecular Genetics, University of Illinois at Chicago, Chicago, Illinois 60607, the ¶Division of Cell Biology, La Jolla Institute for Allergy and Immunology, San Diego, California 92121, and the ¶Division of Cell & Molecular Biology, Toronto Research Institute, University Network and Department of Immunology, University of Toronto, Toronto, Ontario M5G 2M1, Canada

Protein kinase C-theta (PKC-θ) plays important roles in the activation and survival of lymphocytes and is the predominant PKC isoform expressed in T-cells. Interferons (IFNs) are pleiotropic cytokines that exhibit important biological effects in cells and tissues, binding to specific cell-surface receptors is required. Type I IFNs (IFN-α and IFN-β) bind to the Type I IFN receptor, whereas Type II IFN (IFN-γ) utilizes the IFN-γ receptor (1, 2, 4, 5). The engagement of the interferon receptors by different IFN subtypes results in activation of common and distinct signaling cascades that ultimately mediate generation of the biological properties of interferons. These include JAK-STAT pathways (6–13), the Type I IFN receptor (1, 2, 3, 4), and the Type II IFN receptor (1, 2, 3, 4, 5). The engagement of T-cells. Both Type I (α, β) and Type II (γ) IFNs induce phosphorylation of PKC-θ in human T-cell lines and primary human T-lymphocytes. Such phosphorylation of PKC-θ resulted in activation of its kinase domain, suggesting that this kinase plays a functional role in interferon signaling. Consistent with this, inhibition of PKC-θ protein expression using small interfering RNAs (siRNA) abrogated IFN-α and IFN-γ-dependent gene transcription via GAS elements. Similarly, blocking of PKC-θ kinase activity by overexpression of a dominant-negative PKC-θ mutant also blocked GAS-driven transcription, further demonstrating a requirement for PKC-θ in IFN-dependent transcriptional activation.

The effects of PKC-θ on IFN-dependent gene transcription were not mediated by regulation of the IFN-activated STAT pathway, as siRNA-mediated PKC-θ knockdown had no effects on STAT1 phosphorylation and binding of STAT1-containing complexes to SIE/GAS elements. On the other hand, siRNA-mediated PKC-θ inhibition blocked phosphorylation/activation of MKK4, suggesting that interferon-dependent PKC-θ activation regulates downstream engagement of MAP kinase pathways. Altogether, these findings demonstrate that PKC-θ is an interferon-inducible kinase and strongly suggest that it plays an important role in the generation of interferon-responses in T-cells.

Interferons (IFNs) are pleiotropic cytokines that exhibit important biological effects in vitro and in vivo, including antiviral, antiproliferative, and immunomodulatory activities. For interferons to exhibit their biological effects in cells and tissues, binding to specific cell-surface receptors is required. Type I IFNs (IFN-α and IFN-β) bind to the Type I IFN receptor, whereas Type II IFN (IFN-γ) utilizes the IFN-γ receptor (1, 2, 4, 5). The engagement of the interferon receptors by different IFN subtypes results in activation of common and distinct signaling cascades that ultimately mediate generation of the biological properties of interferons. These include JAK-STAT pathways (6–13), the Type I IFN receptor (1, 2, 3, 4, 5) and the Type II IFN receptor (1, 2, 3, 4, 5). The engagement of PKC-θ in IFN-dependent gene transcription was not mediated by regulation of the IFN-activated STAT pathway, as siRNA-mediated PKC-θ knockdown had no effects on STAT1 phosphorylation and binding of STAT1-containing complexes to SIE/GAS elements. On the other hand, siRNA-mediated PKC-θ inhibition blocked phosphorylation/activation of MKK4, suggesting that interferon-dependent PKC-θ activation regulates downstream engagement of MAP kinase pathways. Altogether, these findings demonstrate that PKC-θ is an interferon-inducible kinase and strongly suggest that it plays an important role in the generation of interferon-responses in T-cells.

* This work was supported by National Institutes of Health Grants CA78716 and CA90479 (to L. C. P.), a Merit Review Grant from the Department of Veterans Affairs (to L. C. P.), and Canadian Institutes of Health Research Grant MOP15094 (to E. N. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

** To whom correspondence should be addressed: Robert H. Lurie Comprehensive Cancer Center, Northwestern University Medical School, 710 North Fairbanks Ave., Olson Pavilion 8250, Chicago, IL 60611. Tel.: 312-503-4287; Fax: 312-908-1372; E-mail: l-platanias@northwestern.edu.

† The abbreviations used are: IFN, interferon; STAT, signal transduction and activator of transcription; PKC, protein kinase C; MAP, mitogen-activated protein; MKK4, mitogen-activated protein kinase kinase 4; GAS, interferon-γ-activated site; PI 3′-kinase, phosphatidylinositol 3′-kinase; ISG, interferon stimulated gene; siRNA, small interfering RNA; JNK, c-Jun NH2-terminal kinase.

This paper is available on line at http://www.jbc.org 29911

Received for publication, February 24, 2004, and in revised form, May 6, 2004
Published, JBC Papers in Press, May 18, 2004, DOI 10.1074/jbc.M401997200

The Journal of Biological Chemistry Vol. 279, No. 29, Issue of July 16, pp. 29911–29920, 2004
Printed in U.S.A.
members of the PKC family exhibit, as well as the tissue-specific distribution of several PKC isoforms, suggest that more than one PKC isoform may be activated by interferons to play distinct or complementary roles in the generation of IFN responses. In the present study we determined whether PKC-θ participates in Type I and II interferon signaling in T-cells. Our data provide the first evidence that this PKC isoform is activated in an interferon-dependent manner in cell lines of T-cell origin and primary human T-lymphocytes. Such activation appears to play an important functional role in IFN signaling, as it is required for IFN-γ-dependent gene transcription via GAS elements and regulates downstream engagement of MAP kinase cascade 4 (MKK4).

EXPERIMENTAL PROCEDURES

Cells and Reagents—The Molt-4 and Jurkat acute T-cell leukemia cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics. U2OS cells were grown in McCoy medium, supplemented with 10% fetal bovine serum and antibiotics. Human recombinant IFN-α2 was provided by Hoffmann-La Roche. Human recombinant IFN-β was provided by Biogen Inc. Polyclonal antibodies against PKC-θ, PKC-α, PKC-δ, PKC-ε, MKK4, and STAT1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against the phosphorylated form of PKC-θ on Thr538, the phosphorylated form of MKK4 at Thr261, and the phosphorylated form of mTOR on Ser2448 were obtained from Cell Signaling Inc. (La Jolla, CA).

Cell Lines, Immunoprecipitations, and Immunoblotting—Cells were incubated in the presence or absence of 1 × 10^5 IU/ml of the indicated interferons for the indicated times, and lysed in phosphorylation lysis buffer as previously described (40, 41). In the experiments in which the effects of pharmacological inhibitors on the phosphorylation of PKC-θ were examined, the cells were preincubated for 30 min in the presence or absence of LY294002 (50 μM) or rapamycin (40 nM), prior to treatment with the indicated interferons. Immunoprecipitations and immunoblotting using an enhanced chemiluminescence (ECL) method were performed as previously described (40, 41).

PKC-θ and MKK4 Kinase Assays—Cells were incubated in the presence or absence of the indicated interferons for the indicated times at 37 °C. The cells were subsequently lysed in phosphorylation lysis buffer (40, 41), and lysates were immunoprecipitated with an antibody against PKC-θ, using protein G-Sepharose. The immune complexes were subsequently washed three times with phosphorylation lysis buffer containing 0.1% Triton X-100 and 2 times with kinase buffer (25 mM HEPES, 25 mM MgCl2, 25 mM β-glycerophosphate, 2 mM dithiothreitol, 0.1 mM Na3VO4, and 20 μM ATP) and resuspended in 30 μl of kinase buffer containing 10 μCi of [γ-32P]ATP and 5 μg of histone H1 protein. The reaction was incubated for 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. For MKK-4 kinase assays, lysates were immunoprecipitated with an anti-MKK4 antibody, and 1 μg of recombinant p38 (UBI) was used as an exogenous substrate.

Isolation and Expansion of Normal Lymphocytes from Peripheral Blood—Peripheral blood was collected from healthy volunteers, after obtaining informed consent approved by the Institutional Review Board (IRB) of Northwestern University. Peripheral blood mononuclear cells were separated by Ficoll-Hypaque gradient sedimentation and the lymphocyte interface band was aspirated and washed in RPMI, 10% fetal bovine serum. The cells were stimulated with phytohemagglutinin (2.5 μg/ml) for 72 h in RPMI supplemented with 10% fetal bovine serum and were subsequently incubated with the indicated doses of interferons for the indicated times.

siRNA Transfections—siRNA duplexes (siRNAs) were synthesized and purified by Qiagen Inc. The PKC-θ target sequences were: siRNA1

![Image](http://www.jbc.org/Downloaded from)
(5'-AAACCCACCGCCTTGACTACT-3') and siRNA 2 (5'-AAGAGCCCGACCTTCTGTGAA-3'). Molt-4 cells were transfected with a mixture of the PKC-θ-specific siRNAs or control scrambled siRNA by electroporation. 48 h after transfection, the cells were treated with the indicated interferons for the indicated times and processed for immunoblotting studies or luciferase promoter assays.

Luciferase Reporter Assays—Cells were transfected with a β-galactosidase expression vector and a luciferase reporter gene containing eight GAS elements linked to a minimal prolactin promoter (8X-GAS) by electroporation. The 8X-GAS construct (42) was kindly provided by Dr. Christofer Glass (University of California, San Diego). The pAP-1-luc construct was obtained from Stratagene. In the experiments in which the effects of overexpression of wild-type PKC-θ, a kinase-defective PKC-θ mutant (PKC-θ-K409R) (43), or a constitutively active mutant (PKC-θ-A148E) (43) were determined, the cells were co-transfected with either control empty vector or the indicated cDNAs. Forty-eight hours after transfection, triplicate cultures were either left untreated or treated with IFN, as indicated. The cells were washed twice with cold phosphate-buffered saline and after cell lysis, luciferase activity was measured using the protocol of the manufacturer (Promega). The measured luciferase activities were normalized for β-galactosidase activity for each sample.

Quantitative TaqMan Reverse Transcriptase-PCR—Molt-4 cells were electroporated with either scrambled or PKC-θ-specific siRNA. After 24 h incubation, cells were treated with IFN-α for 5 h. RNA was isolated using the RNeasy kit (Qiagen), and 1 μg of total RNA was reverse transcribed to cDNA using the OmniScript reverse transcriptase kit and Oligo(dT) primer (Qiagen). Real-time reverse transcriptase-PCR of human IRF-9 and ISG15 genes was carried out by an ABI 7900 Sequence Detection System (64) using FAM-labeled probes and primers (Applied Biosystems, TaqMan Assays on Demand). Relative quantitation of mRNA levels was plotted as -fold change compared with untreated cells. 18S ribosomal RNA was used for normalization (33). Briefly, ΔCT values (target gene CT - 18S CT) for each triplicate sample were averaged, and ΔΔCT was calculated as previously described. mRNA amplification was determined by the formula 2^-ΔΔCT (33).

**Fig. 2.** Type I and II IFNs induce PKC-θ kinase activity. Molt-4 (A and B) and Jurkat (D and E) cells were treated with IFN-α, -β, and -γ as indicated. Cell lysates were immunoprecipitated with either an anti-PKC-θ antibody or with control nonimmune rabbit immunoglobulin (R IgG), and subjected to an in vitro kinase assay using histone H1 as an exogenous substrate. Proteins were analyzed by SDS-PAGE and transferred to Immobilon. The phosphorylated proteins were detected by autoradiography (A and D). The membranes were subsequently immunoblotted with anti-PKC-θ antibody to control for protein loading (B and E). The signals for the different bands were quantitated by densitometry and the intensity of histone H1 phosphorylation relative to immunoprecipitated PKC-θ protein was calculated. C, the ratios of H1 activity to PKC-θ protein levels for the different conditions of the experiment of A and B are shown. F, the ratios of H1 activity to PKC-θ protein levels for the different conditions of the experiment of D and E are shown.
FIG. 3. **The activation of PKC-θ is PI 3′-kinase- and mTOR-dependent.** A, Molt-4 cells were preincubated for 30 min in the presence or absence of the PI 3′-kinase inhibitor LY294002 or the mTOR inhibitor rapamycin, as indicated. The cells were subsequently treated with IFN-γ for 40 min, and equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with the antiphospho-PKC-θ antibody. B, the blot shown in A was stripped and re-probed with the anti-PKC-θ antibody, to control for loading. C, Molt-4 cells were treated with IFN-α or IFN-γ, as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of mTOR on serine 2448. D, the blot shown in C was stripped and re-probed with an anti-mTOR antibody, to control for protein loading. E, the signals for the different bands shown in blots C and D were quantitated by densitometry, and the ratio of the signal for phospho-mTOR to the signal for total mTOR protein for each experimental condition was calculated. F, Molt-4 cells were treated with IFN-α or IFN-γ for 10 min, as indicated. Total cell lysates were immunoprecipitated with either an anti-PKC-θ antibody, or control non-immune rabbit immunoglobulin (R IgG), as indicated. Immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted with anti-phospho-mTOR antibody. G, the blot shown in E was stripped and re-probed with an anti-PKC-θ antibody.

**RESULTS**

We initially determined whether treatment of T-cell-derived human leukemia cell lines with interferons results in phosphorylation and activation of PKC-θ. Molt-4 cells were incubated in the presence or absence of IFN-α, IFN-β, or IFN-γ, and after cell lysis, equal amounts of lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of PKC-θ on threonine 538. Both Type I (α and β) and II (γ) IFNs induced strong phosphorylation of PKC-θ, whereas there was no change in the amount of PKC-θ protein detected prior to and after IFN stimulation (Fig. 1, A and B). Similar results were obtained when the Jurkat acute T-cell leukemia cell line was studied (Fig. 1, C and D). To examine whether phosphorylation of PKC-θ by IFNs occurs under more physiologically relevant conditions, we performed studies using primary normal T-lymphocytes, obtained from the peripheral blood of normal donors. Phytohemagglutinin-expanded T-lymphocytes were treated with either IFN-α or IFN-γ, and total cell lysates were resolved by SDS-PAGE and immunoblotted with the anti-PKC-θ antibody. IFN treatment resulted in phosphorylation of the PKC-θ (Fig. 1, E and F), strongly suggesting that this kinase is activated and plays a role in the generation of IFN responses in T-cells.

In subsequent studies, we sought to determine directly whether the kinase domain of PKC-θ is activated in an interferon-dependent manner. Cells were treated with IFN-β or IFN-γ, and after immunoprecipitation of cell lysates with an anti-PKC-θ antibody, in vitro kinase assays were performed on the immunoprecipitates using histone H1 as an exogenous substrate. IFN-β or IFN-γ treatment of Molt-4 cells resulted in strong induction of the kinase activity of PKC-θ, as demonstrated by the phosphorylation of histone H1 used as an exogenous substrate (Fig. 2, A–C). Similar results were obtained when Jurkat cells were stimulated with IFN-α or IFN-γ (Fig. 2, D–F). Thus, both Type I and II IFNs induce activation of the kinase domain of PKC-θ, indicating a functional engagement of this kinase in IFN signaling.

In efforts to understand the mechanisms of upstream regulation of PKC-θ during its engagement by the Type I IFN receptor, we determined the effects of pharmacological inhibition of the PI 3′-kinase pathway, which is activated by interferons and has been previously shown to regulate the phosphorylation/activation of PKC-θ (37). Molt-4 cells were preincubated in the presence or absence of the PI 3′-kinase-specific inhibitor LY294002, and were subsequently treated with IFN-γ. Total cell lysates were subsequently analyzed by SDS-
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Fig. 4. Inhibition of IFN-γ-dependent gene transcription via GAS elements by a dominant-negative PKC-θ-mutant. A, Molt-4 cells were co-transfected with an 8X-GAS-luciferase construct and with either empty vector or the PKC-θ kinase-defective mutant (PKC-θK409R). 24 h after transfection, the cells were treated with IFN-γ, and luciferase activity was measured. Data are expressed as -fold increase in response to IFN-γ treatment over control untreated samples for each condition. Mean ± S.E. values of three independent experiments are shown. B, U2OS cells were co-transfected with 8X-GAS plasmid and with empty vector, or wild-type PKC-θ, or a constitutively active PKC-θ mutant (PKC-θ-A148E). 48 h after transfection the cells were treated with IFN-γ for 6 h, and luciferase activity was measured. Data are expressed as -fold increase in response to IFN-γ treatment over control untreated samples for each condition. Mean ± S.E. values of three independent experiments are shown. C, Molt-4 cells were transfected with either an AP-1 luciferase construct or an 8X-GAS luciferase construct. The cells were subsequently treated with IFN-γ, and luciferase activity was measured. Data are expressed as -fold increase in response to IFN-γ treatment over control untreated samples for each condition. Mean ± S.E. values of two independent experiments are shown.

PAGE and immunoblotted with anti-phospho-PKC-θ antibody. Treatment of cells with LY294002 blocked the IFN-γ-dependent phosphorylation of PKC-θ (Fig. 3, A and B). Such phosphorylation was also blocked when cells were pretreated with rapamycin, an inhibitor of mTOR, which has been recently shown to be phosphorylated/activated by Type I (19) and II (44) IFNs (Fig. 3, A and B). Thus, in addition to regulating activation of the p70 S6 kinase pathway (19, 44), activation of the PI 3-′ kinase/FRAP/mTOR pathway by interferons appears to exhibit regulatory effects on the activation of PKC-θ in T-cells. To better understand the mechanisms by which such regulation occurs, we determined whether PKC-θ interacts with mTOR in intact cells, to possibly act as a substrate for its kinase activity. As expected (19, 44), mTOR was phosphorylated on serine 2448 in response to interferon treatment of Molt-4 cells (Fig. 3, C–E). When lysates from IFN-α- or IFN-γ-treated cells were immuno-precipitated with an anti-PKC-θ antibody, the activated form of mTOR could be detected in anti-PKC-θ immunoprecipitates (Fig. 3, F and G), suggesting mTOR associates directly or indirectly with PKC-θ to regulate its phosphorylation and activation by interferons.

In subsequent studies, we sought to obtain information on the functional role that PKC-θ plays in the generation of IFN responses. A major mechanism by which interferons mediate their biological effects on target cells is the induction of gene transcription via GAS elements, present in the promoters of ISGs (6–8). To examine whether PKC-θ regulates induction of IFN-dependent transcriptional activation in T-cells, we examined the effects of overexpression of a kinase-inactive/dominant-negative PKC-θ mutant (PKC-θK409R). Molt-4 cells were co-transfected with the 8X-GAS luciferase construct and either control vector alone or the dominant-negative PKC-θ mutant. IFN-γ treatment induced strong promoter activity, but overexpression of the kinase-inactive PKC-θ mutant exhibited dominant-negative effects on the induction of IFN-γ-dependent luciferase activity (Fig. 4A), suggesting a requirement for PKC-θ activity on IFN-γ-dependent transcriptional activation. Consistent with this, overexpression of wild-type PKC-θ or a constitutively active mutant (PKC-θ-A148E) (43) in U2OS cells, which are not of T-cell origin and therefore lack PKC-θ expression, enhanced IFN-γ inducible gene transcription (Fig. 4B). As activation of PKC-θ has been previously implicated in gene transcription via AP-1 responsive elements, we also sought to determine whether there is IFN-dependent induction of AP-1 activity in T-cells, and if so, whether such activity is regulated by the IFN-activated form of PKC-θ. However, as shown in Fig. 4C, IFN treatment did not induce luciferase activity in Molt-4 cells transfected with an AP-1-luc construct. Thus, IFN-de-
pendent activation of PKC-\(\theta\) in T-cells appears to selectively regulate IFN-responsive elements, but not AP-1 elements.

The studies using a dominant-negative PKC-\(\theta\) mutant for the first time implicated this PKC isoform in IFN-dependent transcriptional regulation in T-cells. To further confirm the validity of such a hypothesis and establish the putative role of PKC-\(\theta\) in IFN-dependent transcriptional activation, we performed studies in which the expression of endogenous PKC-\(\theta\) in Molt-4 cells was blocked, using the siRNA methodology. For this purpose, siRNA duplexes to specifically target PKC-\(\theta\) were designed and used. Transfection of PKC-\(\theta\)-specific siRNA, but not scrambled siRNA, in Molt-4 cells resulted in significant down-regulation of endogenous PKC-\(\theta\) levels (Fig. 5, A and B), but had no effects on the expression of other PKC isoforms, including PKC-\(\alpha\), PKC-\(\beta\), PKC-\(\epsilon\), and PKC-\(\delta\) (Fig. 5, C–J). Subsequently, Molt-4 cells were transfected with PKC-\(\theta\) siRNA, together with an 8X-GAS luciferase construct. The cells were then treated with different interferons, and luciferase activity was measured. Inhibition of PKC-\(\theta\) expression resulted in abrogation of gene transcription via GAS elements in response to IFN-\(\alpha\) (Fig. 6A), IFN-\(\beta\) (Fig. 6B), or IFN-\(\gamma\) (Fig. 6C).

Altogether, the luciferase reporter assays provided strong evidence for a role for PKC-\(\theta\) in IFN-dependent transcriptional activation. To directly determine whether PKC-\(\theta\) regulates the transcription of genes that are involved in the generation of IFN responses, the effects of siRNA-mediated inhibition of PKC-\(\theta\) expression on IFN-inducible transcription of the IRF-9 and ISG15 genes were determined. Molt-4 cells were transfected with either scrambled siRNA or PKC-\(\theta\)-specific siRNA, and IFN-\(\gamma\) was examined by quantitative reverse transcriptase-PCR. As shown in Fig. 7, inhibition of PKC-\(\theta\) expression blocked transcription for both the IRF-9 and ISG15 genes, demonstrating that the function of PKC-\(\theta\) is essential for IFN-dependent expression of these genes in cells of T-cell origin.

In attempts to identify the mechanisms by which PKC-\(\theta\) may be regulating IFN-dependent transcriptional activation, we examined whether it is required for STAT activation and formation of DNA-binding complexes. A key STAT protein for the regulation of gene transcription via GAS elements is STAT1. This STAT family member undergoes phosphorylation in an IFN-dependent manner and forms either homodimeric, or heterodimeric complexes with other STAT proteins, which translocate to the nucleus and bind to GAS elements in the promoters of IFN-responsive genes (6–13). Molt-4 cells were transfected with either scrambled siRNA or PKC-\(\theta\)-specific siRNA, and the induction of STAT1 phosphorylation by IFN-\(\alpha\) or IFN-\(\gamma\) was examined. As shown in Fig. 8, inhibition of PKC-\(\theta\) expression did not affect phosphorylation of STAT1 on tyrosine 701 (Fig. 8, A and B), an event required for the translocation of the protein to the nucleus and DNA binding (6–13). In addition, when gel-shift assays were performed using an SIE element, we found that inhibition of PKC-\(\theta\) expression did not affect the formation of STAT1:1 and STAT1:3 DNA-binding complexes (Fig. 8C).

Previous studies have shown that in response to calcineurin (45) and during activation of T-lymphocytes (46), PKC-\(\theta\) regulates downstream activation of MKK4, a MAP kinase kinase that acts as an upstream effector for JNK and p38 MAP ki-
nases (47–49). We examined whether MKK4 is activated in an IFN-γ-dependent manner in T-cells, and, if so, whether the function of PKC-θ is required for such activation. Molt-4 cells were treated for 30 min with IFN-α or IFN-γ, and phosphorylation was detected by immunoblotting with an anti-phospho-MKK4 antibody. Both interferons induced phosphorylation of MKK4, whereas there was no change in the levels of MKK4 expression (Fig. 9, A–C). Moreover, in experiments in which in vitro kinase assays were performed on anti-MKK4 immunoprecipitates from IFN-stimulated cell lysates, we found interferon-inducible MKK4 kinase activity, evidenced by the phosphorylation of recombinant p38 used as an exogenous substrate (Fig. 9D). The phosphorylation/activation of MKK4 was diminished in cells transfected with PKC-θ siRNA (Fig. 9, E and F), indicating that the IFN-activated MKK4 is a downstream effector of PKC-θ.

**DISCUSSION**

Despite the significant advances in the field of IFN signaling, the precise mechanisms by which interferons generate their diverse biological effects in target cells remain to be elucidated. There is now accumulating evidence that non-STAT pathways are engaged and activated by the interferon receptors and play important roles in interferon signaling. Such pathways either modify STAT activation or, in some cases, act independently of STATs (3, 9, 10). In the present report, we provide the first evidence that PKC-θ is activated by Type I and II IFNs and participates in IFN signaling. PKC-θ is a member of the PKC family of serine-threonine kinases, which play important roles in the generation of biological responses for several cellular receptors (reviewed in Refs. 49–55). The members of the PKC family of proteins are classified in three groups, based on the mechanisms regulating their activation in response to different stimuli. The first group is comprised of the conventional PKC isoforms (PKC-α, -β, and -γ). These PKC isoforms are activated in response to the classic PKC activators, the phorbol esters, and require increases in intracellular calcium for their activation (54–56). The second group is the group of novel PKCs, in which PKC-θ belongs. This is the group of novel PKCs, which do not require Ca2+ for their activation (PKC-δ, -ζ, -θ, -η, and -μ), but are activated by phorbol esters (54–56). Finally, a third group of atypical PKCs has been recently identified (PKC-ζ and -λ), which are not activated in response to phorbol esters, the typical PKC activators (54–56).

Our data demonstrate that PKC-θ is phosphorylated in an IFN-dependent manner in cells of T-cell origin and that its kinase activity is induced. Importantly, our findings establish...
that PKC-\(\theta\) positively regulates IFN-dependent gene transcription, as evidenced by the fact that siRNAs targeting PKC-\(\theta\) expression or overexpression of a dominant-negative PKC-\(\theta\) mutant, result in inhibition of Type I and II IFN-dependent transcriptional regulation.

It is well established that interferons generate their biolog-
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PKC-θ and PKC-δ is mediated by the PI 3'-kinase pathway (Ref. 37 and current report), whereas the function of both isoforms appears to be essential for IFN-dependent gene transcription. However, their effects are mediated by distinct and non-overlapping mechanisms, as selective siRNA-mediated down-regulation of PKC-θ blocks IFN-dependent transcriptional activation without affecting PKC-δ expression. On the other hand, and consistent with this hypothesis, siRNA targeting of PKC-δ does not affect PKC-θ expression, or Type I- or II-interferon dependent phosphorylation of PKC-θ. Further studies to define the potential engagement of other members of the PKC family in interferon signaling are warranted and may provide important insights on the mechanisms by which these cytokines generate their effects on normal and neoplastic cells.

Acknowledgment—We thank Dr. Christopher Glass for providing us with the 8X-GAS luciferase construct.

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J. Biol. Chem. 2004, 279:29911-29920.
doi: 10.1074/jbc.M401997200 originally published online May 18, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M401997200

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