Activation of SAPK/JNK Signaling by Protein Kinase Cδ in Response to DNA Damage*

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The cellular response to genotoxic stress includes activation of protein kinase Cδ (PKCδ). The functional role of PKCδ in the DNA damage response is unknown. The present studies demonstrate that PKCδ is required in part for induction of the stress-activated protein kinase (SAPK/JNK) in cells treated with 1-β-D-arabinofuranosylcytosine (araC) and other genotoxic agents. DNA damage-induced SAPK activation was attenuated by (i) treatment with rottlerin, (ii) expression of a kinase-inactive PKCδ(3-KR) mutant, and (iii) down-regulation of PKCδ by small interfering RNA (siRNA). Coexpression studies demonstrate that PKCδ activates SAPK by an MKK7-dependent, SEK1-independent mechanism. Previous work has shown that the nuclear Lyn tyrosine kinase activates the MEKK1 → MKK7 → SAPK pathway but not through a direct interaction with MEKK1. The present results extend those observations by demonstrating that Lyn activates PKCδ, and in turn, MEKK1 is activated by a PKCδ-dependent mechanism. These findings indicate that PKCδ functions in the activation of SAPK through a Lyn → PKCδ → MEKK1 → MKK7 → SAPK signaling cascade in response to DNA damage.

The mechanisms by which DNA damage is converted into intracellular signals that control the mammalian genotoxic stress response are largely unknown. Certain insights were derived from the finding that cells respond to agents that arrest DNA replication or damage DNA with induction of c-jun and other early response genes (1–5). Subsequent work showed that DNA damage is associated with activation of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) (6–12). Phosphorylation of the c-Jun N terminus by SAPK activates the c-Jun transcription function and thereby autinduction of the c-jun gene (13, 14). SAPK also activates early response gene expression by phosphorylation of the ATF2 and Elk1 transcription factors (15–17). The available evidence indicates that genotoxic stress activates a nuclear complex of the c-Abl and Lyn protein-tyrosine kinases (18, 19) and that this complex regulates SAPK activation (7, 20). c-Abl functions directly upstream to MEKK1 and activates SAPK by a SEK1-dependent mechanism (8, 21). By contrast, whereas Lyn activates the MEKK1 → MKK7 → SAPK pathway, the direct downstream effector of Lyn is not known (20).

The protein kinase C (PKC) family of serine/threonine kinases has been subdivided into the following: (i) the conventional PKCs (α, β, and γ), which are calcium-dependent and activated by diacylglycerol, (ii) the novel PKCs (δ, θ, and μ), which are calcium-independent and activated by diacylglycerol, and (iii) the atypical PKCs (ζ and λ), which are calcium-independent and not activated by diacylglycerol (22). The ubiquitously expressed novel PKC, PKCδ, is tyrosine-phosphorylated and activated by c-Abl in the response to DNA damage (23). As found for c-Abl and Lyn (24, 25), PKCδ interacts with the nuclear DNA-dependent protein kinase catalytic subunit (DNA-PKcs) (26). Phosphorylation of DNA-PKcs by PKCδ inhibits the function of DNA-PKcs to form complexes with DNA and to phosphorylate downstream targets (26). Other studies have demonstrated that the nuclear complex of c-Abl and Lyn includes the protein-tyrosine phosphatase, SHPTP1 (27, 28), and that PKCδ phosphorylates and inactivates SHPTP1 in the response to DNA damage (29). In cells that respond to genotoxic stress with the induction of apoptosis, PKCδ is cleaved by caspase-3 to a constitutively active catalytic fragment (PKCδCF) (30, 31). The finding that PKCδCF induces nuclear condensation and DNA fragmentation has indicated that cleavage of PKCδ contributes to the apoptotic response (32).

The present studies have addressed the involvement of PKCδ in the activation of stress signals in response to arrest of DNA replication and to DNA damage. The results demonstrate that PKCδ is required in part for activation of SAPK. The results also demonstrate that PKCδ transduces Lyn-mediated signals to MEKK1 in a Lyn → PKCδ → MEKK1 → MKK7 → SAPK pathway.

MATERIALS AND METHODS

Cell Culture—Human U-937 myeloid leukemia cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. MCF-7 cells, HeLa cells, and 293T embryonal kidney cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and antibiotics. Cells were treated with araC (Sigma-Aldrich), rottlerin (Calbiochem), or Go6976 (Calbiochem). Irradiation was performed at room temperature with a Gammasell 1000 (Atomic Energy of Canada) and a 137Cs source emitting at a fixed rate of 0.21 gray/min.

Cell Transfections—The PKCδ(K-R) cDNA (29) was cloned into a pcDNA3.1/myc-His vector (Invitrogen) and introduced stably into U-937 cells by electroporation (Gene Pulser; Bio-Rad) and selection in geneticin (Roche Molecular Biochemicals). U-937(Lyn-K-R) cells were prepared as described previously (20, 28). 293T cells were transiently transfected with pGFP, pGFP-PKCδCF, pGFP-PKCδCF(K-R) (29),...
Fig. 1. DNA damage-induced activation of SAPK, but not ERK or p38 MAPK, is attenuated by rottlerin. A and B, U-937 cells were pretreated with 5 μM rottlerin or 50 ng Go6976 for 30 min. The cells were then treated with 10 μM araC (A) or 15 gray of IR (B), harvested at the indicated times, lysed, and subjected to immunoprecipitation (UP) with anti-SAPK. The immunoprecipitates were incubated with GST-c-Jun and [γ-32P]ATP. GST-c-Jun phosphorylation was assessed by SDS-PAGE and autoradiography (upper panels). The lysates were also subjected to immunoblot analysis (IB) with anti-SAPK (lower panel). C and D, cells were pretreated with rottlerin and then exposed 10 μM araC. Anti-ERK (C) or anti-p38 MAPK (D) immunoprecipitates were subjected to immune complex kinase assays with MBP or GST-ATF2, respectively, as substrate (upper panels). The lysates were also subjected to immunoblot analysis with anti-ERK (C) or anti-p38 MAPK (D) (lower panels).

pFLAG, pFLAG-SEK1(K-R), pFLAG-MKK7(K-R), or pFLAG-MEK1(K-M) (20) by the calcium phosphate method. DNA concentrations were adjusted with empty vector.

siRNA Transfections—siRNA duplexes (siRNAs) were synthesized and purified by Japan Bio Service (Saitama, Japan). The siRNA sequences for targeting PKCδ were 5'-GAUAAAGGAGCCUCAGTT-3' for PKCδsiRNA1 and 5'-GCGUGAGUUCGGCUGGATT-3' for PKCδsiRNA2. GFPsiRNA was used as a negative control (33). Transfection of siRNAs was performed as described (34).

Immunoprecipitations—Cell lysates were prepared as described (20, 28) and cleared by centrifugation at 14000 rpm for 15 min. Soluble proteins were incubated with anti-JNK1 (sc-474; Santa Cruz Biotechnology, Inc.), anti-ERK2 (sc-154; SCBT), anti-p38 MAPK (sc-535; SCBT), anti-PKCδ (sc-937; SCBT), anti-Lyn (sc-15; SCBT), or anti-MEK1 (21) antibodies for 2–6 h at 4 °C followed by 1 h of incubation with protein A/G-Sepharose beads (SCBT). The immune complexes were washed three times with lysis buffer and separated by SDS-PAGE.

In Vitro Kinase Assays—In vitro kinase assays for SAPK, ERK, and p38 MAPK were performed as described (29, 30). Anti-PKCδ immunoprecipitates were incubated with histone H1 as a substrate (29).

Immunoblot Analyses—Proteins were separated by SDS-PAGE and transferred to nitrocellulose filters. After blocking with 5% dry milk in phosphate-buffered saline containing 0.05% Tween 20, the filters were incubated with anti-JNK1/SAPK, anti-ERK2, anti-p38 MAPK, anti-PKCδ, anti-Lyn (Transduction Laboratories), anti-MEK1, anti-tubulin (Sigma-Aldrich), anti-GFP (Roche Molecular Biochemicals), anti-GST (Upstate Biotechnology Inc.), anti-FLAG (Sigma-Aldrich), or anti-phospho-Tyr (4G10; Upstate Biotechnology, Inc.) antibodies for 1–4 h at room temperature. The antigen-antibody complexes were visualized by chemiluminescence (PerkinElmer Life Sciences).

Glycerol Gradient Sedimentation Analysis—Cosedimentation analysis of PKCδ and Lyn by glycerol gradient ultracentrifugation was performed as described (35).

Direct Binding Assays—Assays for direct binding of PKCδ and Lyn in vitro were performed as described previously (35).

RESULTS

PKCδ Functions in Activation of SAPK in Response to DNA Damage—To investigate whether PKCδ functions as an upstream effector of the SAPK response to genotoxic agents, U-937 cells were pretreated with PKCδ inhibitor, rottlerin, followed by exposure to araC. Anti-SAPK immunoprecipitates were subjected to in vitro kinase assays by phosphorylating GST-c-Jun. The results demonstrate that pretreatment with rottlerin attenuates araC-induced activation of SAPK (Fig. 1A). By contrast, there was no detectable effect of pretreatment with the PKCα and PKCβ inhibitor, Go6976 (Fig. 1A). Similar results were obtained with U-937 cells exposed to ionizing radiation (IR) (Fig. 1B). To determine whether PKCδ is required for the induction of other MAPK family members, ERK and p38 MAPK, cells exposed to rottlerin and then araC or IR were subjected to immunoprecipitation with anti-ERK2 and anti-p38 MAPK. Analysis of the immunoprecipitates for phosphorylation of myelin basic protein (MBP) or ATF2 demonstrated that rottlerin has less of an inhibitory effect on araC- or IR-induced p38 MAPK activity and no apparent effect on ERK activation (Fig. 1, C and D and data not shown). These results suggest that activation of SAPK is at least in part dependent on PKCδ in the response to genotoxic agents.

Attenuation of SAPK Activation in Cells Expressing a Kinase-inactive PKCδ Mutant—To further assess involvement of PKCδ in SAPK activation, a Myc-tagged kinase-inactive PKCδ(K-R) mutant was introduced stably in U-937 cells. In contrast to U-937 cells expressing the empty vector, two separate clones of transfected cells expressed Myc-PKCδ(K-R) (Fig. 2A). Anti-PKCδ immunoprecipitates from control and araC-treated U-937/neo and U-937/PKCδ(K-R) cells were analyzed for phosphorylation of histone H1. The results demonstrate that activation of PKCδ by araC is abrogated in U-937 cells expressing PKCδ(K-R) (Fig. 2B). To confirm the involvement of PKCδ in DNA damage-induced activation of SAPK, U-937/neo and U-937/PKCδ(K-R) cells were exposed to araC and were analyzed for SAPK activation. The results demonstrate that activation of SAPK is attenuated, but not completely inhibited, in araC-treated cells expressing kinase-negative PKCδ(K-R) (Fig. 2C). Similar results were obtained in IR-treated cells (Fig. 2D). By contrast, DNA damage-induced ERK and p38 MAPK activation were unaffected in U-937/PKCδ(K-R) cells, as compared with that in U-937/neo cells (data not shown). These findings indi-
icate that PKCδ is involved in SAPK activation in the cellular response to diverse genotoxic agents.

**Attenuation of SAPK Activation in Cells Transfected with PKCδ siRNAs or a Kinase-inactive M KK7(K-R) Mutant**—To further assess the role of PKCδ in SAPK activation, 293T cells were treated with siRNA duplexes that target PKCδ. The results demonstrate that the PKCδsiRNA1 down-regulates PKCδ expression (Fig. 3A). Less pronounced results were obtained with PKCδsiRNA2 (Fig. 3A). As a control, treatment with a GFPsiRNA had little if any effect (Fig. 3A). Importantly, treatment with PKCδsiRNA, but not GFPsiRNA, attenuated araC-induced SAPK activation (Fig. 3B). To determine whether the kinase function of PKCδ is sufficient for SAPK activation, 293T cells were transfected with PKCδCF. Analysis of anti-SAPK immunoprecipitates for phosphorylation of GST-c-Jun demonstrated that expression of PKCδCF induces SAPK activity (Fig. 3C). By contrast, expression of kinase-inactive PKCδ(CF)(K-R) had little effect on SAPK activity (Fig. 3C). Similar results were obtained in HeLa cells expressing PKCδCF or PKCδCF(K-R) (data not shown). To define the effectors downstream of PKCδ in the SAPK pathway, 293T cells were co-transfected with PKCδCF and kinase-inactive SEK1(K-R) or M KK7(K-R). Analysis of anti-SAPK immunoprecipitates demonstrated that expression of M KK7(K-R), but not SEK1(K-R), is associated with attenuation of SAPK activity by PKCδ (Fig. 3D). These results indicate that PKCδ induces SAPK activation by an M KK7-dependent, SEK1/M KK4-independent mechanism.

**Lyn Tyrosine Kinase Is an Upstream Effector of PKCδ in Response to Genotoxic Stress**—Previous studies have demonstrated that the Lyn tyrosine kinase is activated in the response to genotoxic stress and that Lyn regulates SAPK activation by an M KK7-dependent pathway (20). Whereas the present results indicate that the PKCδ-mediated SAPK signaling pathway is also M KK7-dependent, we asked whether Lyn is involved in PKCδ → SAPK signaling. To determine whether PKCδ binds to Lyn, we incubated glutathione beads containing GST or GST-PKCδ with purified Lyn. Analysis of the adsorbrates demonstrated binding of Lyn with GST-PKCδ and not GST (Fig. 4A). To determine whether PKCδ phosphorylates Lyn in vitro, heat-inactivated purified Lyn was incubated with recombinant PKCδ. Analysis of the reaction products demonstrated no detectable phosphorylation of Lyn by PKCδ (Fig. 4B, lane 2). By contrast, incubation of heat-inactivated PKCδ with purified Lyn showed that Lyn phosphorylates PKCδ (Fig. 4B, lane 5). These findings provide support for a direct interaction between PKCδ and Lyn.

To determine whether Lyn contributes to the regulation of PKCδ in vivo, anti-Lyn immunoprecipitates from control and araC-treated cells were analyzed by immunoblotting with anti-PKCδ. The results demonstrate that Lyn associates constitutively with PKCδ and that araC treatment has little if any effect on the extent of the interaction (Fig. 5A). These results were confirmed when anti-PKCδ immunoprecipitates were subjected to immunoblotting with anti-Lyn (Fig. 5A). A constitutive association between Lyn and PKCδ was also observed in MCF-7 and HeLa cells (data not shown). To confirm these findings with another approach, lysates from U-937 cells were separated by sedimentation in a glycerol gradient. Analysis of the gradient fractions by immunoblotting with anti-Lyn and anti-PKCδ demonstrated cosedimentation of Lyn and PKCδ.

**Fig. 2. Kinase-inactive PKCδ(K-R) mutant attenuates SAPK activation in response to DNA damage.** A, lysates from U-937/neo and U-937/PKCδ(K-R) (clone 1 and 2) cells were analyzed by immunoblotting (IB) with anti-PKCδ (upper panel), anti-Myc (middle panel), or anti-tubulin (lower panel). B, cells were left untreated or treated with 10 µM araC for 1 h. Immunoprecipitates (IP) prepared with the anti-PKCδ antibody (sc-937) were assayed for phosphorylation of histone H1. C and D, cells were treated with 10 µM araC (C) or 15 gray of IR (D) and harvested at the indicated times. Anti-SAPK immunoprecipitates were assayed for phosphorylation of GST-c-Jun (upper panels). Lysates were also subjected to immunoblot analysis with anti-SAPK (lower panels).
To extend the analysis, anti-PKC\u03b9/H9254 immunoprecipitates from araC-treated U-937/neo and U-937/Lyn(K-R) cells were analyzed for tyrosine phosphorylation of PKC\u03b9/H9254 by immunoblotting with anti-phospho-Tyr. The results demonstrate that expression of Lyn(K-R) blocks tyrosine phosphorylation of PKC\u03b9/H9254 in response to araC (Fig. 5C). In concert with these findings and the demonstration that PKC\u03b9/H9254 is activated by tyrosine phosphorylation (23), Lyn(K-R) also attenuated the induction of PKC\u03b9/H9254 activity by araC (Fig. 5D, left). Conversely, to determine whether PKC\u03b9/H9254 regulates Lyn in the DNA damage response, anti-Lyn immunoprecipitates from U-937/neo and U-937/PKC\u03b9/H9254(K-R) cells were analyzed for Lyn activity by assessing autophosphorylation and transphosphorylation of enolase. The finding that araC-induced activation of Lyn is comparable in U-937/neo and U-937/PKC\u03b9/H9254(K-R) cells (Fig. 5D, right) suggests that Lyn may function as an upstream effector of PKC\u03b9.

**PKC\u03b9 is an Upstream Effector of MEKK1**—Our previous studies showed that Lyn-induced SAPK activation is MEKK1-dependent (20). To investigate the possibility that PKC\u03b9 interacts with MEKK1, anti-MEKK1 immunoprecipitates were analyzed by immunoblotting with anti-PKC\u03b9. The results show that complexes of PKC\u03b9 and MEKK1 are detectable in control and araC-treated cells (Fig. 6A, left panel). araC-induced increases in the association of PKC\u03b9 and MEKK1 were detectable at 0.5 h and maximal at 2 h of treatment (Fig. 6A, right panel). To further assess the interaction between PKC\u03b9 and MEKK1, we first performed in vitro kinase assays by incubating recombinant PKC\u03b9 with GST or GST-MEKK1 in the presence of,
Regulation of SAPK/JNK by PKCδ

DISCUSSION

PKCδ Is Activated by Different Mechanisms in DNA Damage Response—Involvement of PKCδ in the genotoxic stress response has been supported by the finding that both arrest of DNA replication and induction of DNA lesions are associated with PKCδ activation (23, 29). The available evidence indicates that full-length PKCδ is activated as an early event within 1 h of exposure to genotoxic agents (23, 29). Phosphorylation of PKCδ on tyrosine as a mechanism for PKCδ activation by DNA-damaging agents is mediated in part by the c-Abl kinase (23, 36). The results of the present study demonstrate that Lyn also phosphorylates PKCδ and that Lyn-mediated tyrosine phosphorylation of PKCδ contributes to PKCδ activation.

PKCδ is also activated as a later event (3−6 h) in the DNA damage response by caspase-3-mediated proteolytic cleavage (22, 30, 31, 37). The resulting C-terminal 40-kDa fragment contains the catalytic domain, which, in the absence of the N-terminal regulatory domain, is constitutively active (30−32). The finding that tyrosine phosphorylation of PKCδ is required for activation of caspase-3 and thereby PKCδ cleavage has supported a link between both mechanisms of PKCδ activation (36). Whereas expression of PKCδCF induces characteristics of apoptosis (32), the precise events responsible for this response are unknown but may involve an interaction between PKCδCF and DNA-PKcs (26). In contrast to the activation of PKCδ by tyrosine phosphorylation, cleavage of PKCδ to the constitutively active catalytic fragment is irreversible and thus may function in the prolonged stimulation of multiple pro-apoptotic pathways.

Role for PKCδ in SAPK Activation—SAPK is activated in diverse cell types by agents, such as araC, that block DNA replication and by others, such as IR, that induce DNA lesions (7−12). Like SAPK, PKCδ is also activated by both arrest of DNA replication and DNA damage (23, 29). The present results demonstrate that treatment of cells with the PKCδ inhibitor, rottlerin, attenuates activation of SAPK in response to genotoxic stress. In concert with these findings, expression of the kinase-inactive PKCδ(K-R) mutant attenuated activation of both PKCδ and SAPK in response to araC and IR. Moreover, down-regulation of PKCδ expression by siRNA was associated with attenuation of DNA damage-induced SAPK activation. These findings provided support for the involvement of PKCδ as an upstream effector in the regulation of SAPK activation. The results also demonstrate that inhibition of PKCδ signaling attenuates the early (<1 h) and later periods of SAPK activation. Thus, SAPK is activated by signals transduced by PKCδ and possibly, after activation of caspase-3, by PKCδCF. In this context, the results show that expression of PKCδCF is also associated with SAPK activation.

Previous studies have shown that nuclear c-Abl is an upstream effector of the SAPK response to both arrest of DNA replication and DNA damage (7, 8). Other work has demonstrated that activated forms of Abl induce SAPK activity (5, 38, 39). Lyn forms a nuclear complex with c-Abl and, like c-Abl, also contributes to SAPK activation in response to genotoxic stress (20). Whereas c-Abl activates SAPK by a SEK1-dependent mechanism, Lyn → SAPK signaling is mediated by MKK7 (8, 20). The respective roles of the c-Abl → SEK1 → SAPK and the Lyn → MKK7 → SAPK pathways in DNA damage response may vary in different cell types or under different growth conditions. Nonetheless, the finding that inhibition of PKCδ attenuates SAPK activation in response to araC and IR indicates that PKCδ affects one or both of the pathways.

Evidence for a Lyn → PKCδ → MEKK1 → MKK7 → SAPK Cascade—c-Abl interacts directly with MEKK1 and activates MEKK1 in response to DNA damage (21). In turn, MEKK1

**FIG. 5.** Lyn is an upstream effector of PKCδ. A, U-937 cells were left untreated or treated with 10 μM araC for 1 h. Cell lysates were subjected to immunoprecipitation with anti-Lyn, pre-immune rabbit serum (PIRS), or anti-PKCδ. The immunoprecipitates were analyzed by immunoblotting (IB) with anti-Lyn or anti-PKCδ. B, lysates from U-937 cells were layered onto a 10 to 35% glycerol gradient. After centrifugation, the indicated fractions (fr.) were subjected to SDS-PAGE and immunoblotting with anti-PKCδ and anti-Lyn. C, U-937/neo and U-937/Lyn(K-R) cells were treated with 10 μM araC for 1 and 2 h. Anti-PKCδ immunoprecipitates were subjected to immunoblot analysis with anti-phospho-Tyr (upper panel) or anti-PKCδ (lower panel). D, lysates from control and araC-treated U-937/neo and U-937/Lyn(K-R) cells were subjected to immunoprecipitation with anti-PKCδ (left panel). Lysates from control and araC-treated U-937/neo and U-937/Lyn(PKCδ(K-R)) cells were subjected to immunoprecipitation with anti-Lyn (right panel). Immune complex kinase assays were performed by incubating the precipitates with histone H1 for PKCδ activity (left panel) and with enolase for Lyn activity (right panel).

[γ-32P]ATP. Analysis of the products demonstrated that PKCδ phosphorylates MEKK1 in vitro (Fig. 6B). To further define whether PKCδ activates MEKK1, GST-MKK7(K-R) was co-incubated with or without recombinant PKCδ and kinase-active MEKK1 (yeast-derived) in the presence of [γ-32P]ATP. The results demonstrated that MEKK1 phosphorylates GST-MKK7(K-R) and that the addition of recombinant PKCδ increases MEKK1-mediated MKK7(K-R) phosphorylation (Fig. 6C, lane 3). As a control, there was no detectable phosphorylation of MKK7(K-R) by PKCδ (Fig. 6C, lane 1). To extend these findings, anti-SAPK immunoprecipitates from 293T cells expressing PKCδCF and FLAG-tagged kinase-inactive MEKK1(K-M) were analyzed for phosphorylation of GST-c-Jun. The results demonstrate that expression of MEKK1(K-M) decreases PKCδ-induced SAPK activation in a dose-dependent manner (Fig. 6D). These results collectively indicate that MEKK1 is a downstream effector of PKCδ in the SAPK signaling pathway.
Transduces c-Abl-mediated signals to SEK1 and SAPK (8, 21) (Fig. 7). The finding that kinase-inactive MEKK1(K-M) blocks Lyn-mediated activation of SAPK provided support for the Rho family GTPases (41), TRAF family members (42), the mixed-lineage protein kinases, ASK1 (20). The present findings demonstrate that Lyn associates with PKCδ and that tyrosine phosphorylation and activation of PKCδ is mediated in part by a Lyn-dependent mechanism. The results further demonstrate that PKCδ associates with MEKK1 and that the interaction between PKCδ and MEKK1 is increased in response to DNA damage. Moreover, phosphorylation of MEKK1 by PKCδ stimulated MEKK1 activity. These findings thus collectively support a model in which PKCδ functions downstream to Lyn and as an upstream effector of the MEKK1 → MKK7 → SAPK pathway (Fig. 7).

SAPK is activated in the response of cells to environmental stress (40). MKK7 is a specific activator of SAPK, whereas SEK1 (also known as MKK4) activates both SAPK and p38 MAPK. In turn and depending on the type of stress, upstream effectors of MKK7 and SEK1 include MEKK1–4 and the mixed-lineage protein kinases, ASK1–2, TAK1, and TPL2 (40). The available evidence indicates that MEKK1 is activated by the Rho family GTPases (41), TRAF family members (42), the ECSIT adapter protein (43), protein kinase G (44), and c-Abl (21). The results of the present studies provide support for PKCδ as yet another effector of MEKK1 activation. PKCδ is activated in response to growth factor receptor stimulation (45, 46), DNA-damage (23), and oxidative stress (47). PKCδ is also activated by caspase-3-mediated cleavage in the apoptotic response. Thus, although the present work has focused on involvement of PKCδ in DNA damage-induced signaling, the findings do not exclude the possibility that PKCδ or PKCδCF contributes to SAPK activation in response to other types of stress.

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