c-Jun Is a Downstream Target for Ceramide-activated Protein Phosphatase in A431 Cells*

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Stimulation of [3H]serine-labeled A431 cells with tumor necrosis factor-α (TNF-α) or bacterial sphingomyelinase (SMase) resulted in a rapid decrease (–50% by 15 min) in cellular [3H]sphingomyelin content and generation of the lipid moiety [3H]ceramide, which remained elevated 60 min later. Sphingomyelin hydrolysis in response to TNF-α or bacterial SMase resulted in a time-dependent decrease in the phosphorylation state of c-Jun protein, an effect that was also observed in cells treated with the membrane-permeable ceramide analogue N-hexanoylsphingosine (C_{6}-ceramide). The rapid dephosphorylation of the c-Jun protein in response to TNF-α, SMase, or C_{6}-ceramide was not observed in A431 cells treated with the serine-threonine phosphatase inhibitor okadaic acid. After the initial steps of previously described methods for the purification of a ceramide-activated protein phosphatase termed CAPP (Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C., and Hannun, Y. A. (1993) J. Biol. Chem. 268, 15523–15530), we obtained a cytosolic fraction from A431 cells that specifically dephosphorylated 32P-labeled c-Jun protein used as substrate in an immunocomplex phosphatase assay. Phosphatase activity in vitro was apparent only in the presence of ceramide (5 μM) and was specifically abrogated when okadaic acid (1 nM) was included in the immunocomplex phosphatase assay. These results provide strong evidence for c-Jun as a downstream target for CAPP activated in response to post-TNF signaling in A431 cells.

After having long been considered just as structural components of the plasma membrane, sphingolipids and their metabolites have recently emerged as important messengers of cell-regulatory molecules involved in more complex cellular processes, such as cellular proliferation, differentiation, and cell death (reviewed in Refs. 1–10). Ceramide (N-acetylphosphosine) generated in response to agonist-induced sphingomyelinase (N-acetylphosphosine-phosphohydrolase) hydrolysis seems to mediate cellular responses induced by 1α,25-dihydroxy vitamin D_{3}, interferon-γ, interleukin 1β, and nerve growth factor (3–10). Many of the pleiotropic actions of TNF-α are also specifically mediated by the positive coupling of the activated 55-kDa TNF receptor (p55TNFR) to one or more sphingomyelinases (SMase), which stimulate the phosphodiesterase cleavage of sphingomyelin to phosphocholine and the cell-regulatory moiety ceramide (11–16).

Efforts aimed at delineating a sphingolipid-dependent pathway for signal transduction led to the characterization of different protein kinases that include a ceramide-activated and proline-directed protein kinase first identified by its ability to phosphorylate Thr-669 of the epidermal growth factor receptor in A431 cells (herein referred to as ceramide-activated protein kinase (12, 13, 17)), components of the mitogen-activated protein kinase cascade (16, 18), and a non-phorbol ester-stimulable and diacylglycerol-independent protein kinase C_{ζ} isotype (19, 20). In addition, a cytosolic Ser/Thr protein phosphatase of the 2A-type specifically activated by ceramide, and thus termed CAPP, has also been reported (21–25).

Although regulation of transcription factors by posttranslational modification via protein phosphorylation plays a paramount role in cellular regulation (reviewed in Refs. 25–29), only a reduced number of observations support a role for a ceramide-activated multicomponent system involving kinases and phosphatases as a mechanism whereby signals emanating from the plasma membrane are transduced to the nucleus to affect the transcriptional machinery of the target cell (3–10). These evidence include CAPP-mediated down-regulation of c-myc gene expression (11), protein kinase C_{ζ}-induced translocation of preformed nuclear factor-κB from the cytosol to the nucleus (13, 14, 19, 20), and more recently, ceramide-mediated activation of JNK (c-Jun N-terminal kinase, also referred to as stress-activated kinase) and the subsequent augmented expression of c-jun gene (30–32) and AP-1 activity (31, 33).

Members of the c-Jun gene family encode components that can either homodimerize or heterodimerize with proteins of the Fos family to form AP-1 (25–29), a sequence-specific transcription factor that is strictly dependent for its DNA binding and transcriptional activity of the site-specific phosphorylation status of the preexisting and/or newly synthesized c-jun un component (29, 34, 35).

Although JNK/SAPK-induced phosphorylation of sites at the N-terminal transactivation domain of c-jun is important for functional AP-1 activation (36–40), in unstimulated fibroblastic and epithelial cells c-jun is constitutively phosphorylated near the DNA binding region, and removal of these phosphates...
is also important for transcriptional activity in quiescent cells (41–46).

Because ceramide activates JNK/SAPK (30–32), which in turn results in enhanced transcription of c-jun and DNA binding activity of AP-1 (31, 33), we investigated whether activation of sphingomyelin metabolism may also affect c-jun phospho-
rylation in resting epithelial A431 cells. Results presented here strongly suggest that ceramide generated in response to TNF-α-induced sphingomyelin hydrolysis stimulates a cytosolic OA-sensitive phosphatase activity in A431 cells that specifically dephosphorylates c-jun in intact cells or in an immunocomplex phosphatase assay.

MATERIALS AND METHODS

Reagents and Hormones—The human recombinant TNF-α was purchased from Calbiochem. Bacterial SMase from Bacillus cereus, phospholipase A2 from Crothalus adamanteus, phospholipase C from Clostridium perfringens, phospholipase D from Streptomyces chromofuscus, phospholipase C from Clostridium perfringens, and bacterial SMase from Bacillus cereus were purchased from Calbiochem. Bacterial SMase from Bacillus cereus was obtained from Biomol Research Laboratories (Plymouth, PA). Dulbecco’s modified Eagle’s medium, Ham’s F-12 medium, fetal calf serum, and other tissue culture reagents were purchased from Life Technologies, Inc.

Cell Culture—The A431 human epithelial tumor cells (kindly provided by Dr. F. Casanueva, University of Santiago de Compostela, Santiago de Compostela, Spain) were grown in a mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (1:1) supplemented with 10% fetal calf serum, 2 mM l-glutamine, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). Cultures were maintained at 37 °C under a water-saturated atmosphere of 5% CO2 and 95% air, and cells were passaged twice weekly by trypsinization before reaching confluence.

For experiments, cells were harvested by trypsinization and were inoculated (1–3 × 10⁵ cells/well) into multidish (6 × 34-mm wells or 24 × 16-mm wells) Falcon plastic culture plates (Becton & Dickinson, Oxford, CA) and were grown in the same medium until 60% confluence.

Quantitation of Sphingomyelin Metabolites in A431 Cells—Serum-starved subconfluent cultures of A431 cells (4–5 × 10⁵ cells/well) were labeled for 24 h in serum-free medium containing 2 μCi/ml of [6-³H]serine (DuPont NEN), the time period necessary to achieve isotopic steady state in these cells (results not shown). Labeled cells were rinsed twice with fresh medium supplemented with 25 mM serine, were allowed to equilibrate for 30 min in this medium, and thereafter were treated with TNF-α (10 ng/ml), SMase (0.3 unit/ml), or an equivalent volume of diluent (50 μl) of diluent. At the indicated time periods, the culture medium was replaced by 1 ml of ice-cold methanol and transferred to clean glass tubes containing 2 ml of chloroform (47). The organic phases were washed three times by vigorous mixing with 3 ml of chloroform:0.1 M KCl (1:1, v/v), were separated from the aqueous phases by centrifugation, and were dried under nitrogen. The organic phases were redi-
solved in 100 μl of chloroform and were spotted on activated silica Gel 60 G chromatography plates (Merck), and lipids were separated by sequential one-dimensional chromatography in the solvent system chloroform:benzene:ethanol (80:40:75, v/v/v) followed by another run in the basic mobile phase chloroform:methanol:28% ammonium hydroxide (65:25:5, v/v/v, as described (48). Lipids were visualized with molibde-
num blue spray or iodine vapors, and fractions that comigrated with the same retention factors as the unlabeled standards were scraped into scintillation vials; the associated radioactivity was determined by liquid scintillation counting as described (49, 50).

³²P orthophosphate Labeling of A431 Cells—Subconfluent cultures (4–5 × 10⁵ cells/well) were rinsed twice with phosphate-free Dulbecco’s modified Eagle’s medium and were labeled containing 4 h with 50 μCi/ml carrier-free ³²P orthophosphate (DuPont NEN). All experimental agents were freshly diluted in sterile culture medium and were added in 50-μl aliquots with control incubations, receiving the same volume of medium and a similar final concentration of diluent (less than 0.1% diluent of the appropriate stock solutions). After incubation, the cul-
tures were treated with 1 M NaOH and 1% (w/v) SDS and were precipitated and washed with 100 μl of 70% ethanol. The precipitate was precipitated by sonication from the appropriate stock solutions, with control incubations receiving an equivalent amount of diluent (final concentra-
tion in the assay of less than 0.5% ethanol). Diphosphorylation reac-
tions were initiated by adding equal amounts of the pooled ³²P-immuno-
precipitated substrate (40 μl of a 50% slurry in assay buffer), and diphosphorylation reactions were typically conducted for 15 min at 37 °C.

To terminate reactions, each incubation was quenched with 4 vol-
umes of ice-cold assay buffer centrifuged at 14,000 rpm for 5 min at 4 °C, and the immunocomplexes were washed three times, boiled in SDS-sample buffer, and resolved and analyzed as described above.

RESULTS

TNF-α and Bacterial SMase Stimulate Sphingomyelin Hy-
drosis in A431 Cells—In A431 cells labeled to isotopic steady state with [³H]serine, treatment with TNF-α (10 ng/ml) or ex-

orthovandate, 30 mM sodium pyrophosphate, and protease inhibitors (5 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfon fluoride, and 15 mM iodoacetamide).

The lysates were preincubated by centrifugation in a refrigerated Beck-
dman table-top centrifuge (14,000 × g for 20 min at 4 °C), and suitable aliquots of the supernatants were used to determine trichloroacetic acid-precipitable radioactivity or total protein content by a commercial BCA method (Sigma).

Immunoprecipitation of c-jun—Equal amounts of cell proteins were immunoprecipitated at 4 °C by an overnight incubation with 1 μg of a purified rabbit polyclonal antibody raised against a peptide spanning the 15 C-terminal residues of the human c-jun protein (Oncogene Science, Cambridge, MA) that was preadsorbed to protein A-Sepharose CL4B (Pharmacia Biotech Inc.). In some experiments, the immunospecificity of the antibody was assessed by preadsorbing the antisemur (2 h at 4 °C) before addition to the samples with different concentra-
tions (1–5 μg) of the synthetic peptide used to generate anti-jun antibody.

Labeled immunocomplexes were collected by centrifugation (14,000 × g for 2 min at 4 °C) and were washed four times with 1 ml of lysis buffer, and protein complexes were eluted from the beads by boiling in 2 × concentrated sample buffer (10% [v/v] glycerol, 1 mM dithiothreitol, 1% [w/v] SDS, 50 mM Tris-HCl, pH 6.8, and 0.02% bromophenol blue). After 75% SDS-polyacrylamide gel electrophoresis (51), the gels were dried under vacuum and were recorded as autora-
diographs by photostimulable storage imaging (52), followed by helium-
neon laser scanning in a Molecular Dynamics 400A PhosphorImager (Molecular Dynamics, Sunnydale, CA). Volume integrations with sub-	raction of appropriate backgrounds were performed with software pro-
vided by the manufacturer of the system.

Extraction of a CPP-like Activity from the Cytosol of A431 Cells—In Vitro c-jun Dephosphorylation—An enriched preparation of c-jun was obtained from A431 cells essentially by the methods as described by Okazaki et al. (23) and Wolf et al. (24). Cells were harvested by tryp-
inization and were resuspended in homogenization buffer (20 mM HEPES, pH 7.4) supplemented with 1 mM EDTA, 0.1 mM EGTA, 0.5 mM dithiothreitol, and protease inhibitors (5 μg/ml aproti-
in, 25 μg/ml leupeptin, 1 mM phenylmethylsulfon fluoride, and 2 μg/ml pepstatin A). Cell suspensions were sonicated (at 4 °C for 10 s at 50% setting) in a Braun-Labsonic 2000 (Barcelona, Spain) ultrasonicator, and complete cell lysis was assessed by light microscopy. Cell homogenates were centrifuged (100,000 × g for 60 min at 4 °C), and the cytosol was passed through a column of Sephadex G-50 (Pharmacia) equilibrated with the same buffer. The clarified cytosol was mixed for 60 min at 4 °C with DEAE-Sephalose preequilibrated with buffer A (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.1 mM EGTA, 1 mM benzam-
dine, 0.5 mM dithiothreitol, and 10% glycerol), and the slurry was poured over a 4-ml plastic column and washes with 10 ml of buffer A.

The columns were washed with buffer A supplemented with 150 mM NaCl until A₂₈₀ Returned to base line; thereafter, columns were eluted at a flow rate of 0.5 ml/min with a linear (200–400 mM) sodium chloride gradient in the same buffer. Fractions (1 ml) were collected, snap-frozen with liquid nitrogen, and stored frozen (−80 °C). Phosphatase activity was assayed essentially as described by Okazaki et al. (23) and Wolf et al. (24), except that pooled c-jun immunoprecipitates (obtained from three to four ³²P-labeled 150-mm dishes of A431 cells) were used as a substrate.

Briefly, proteins (1–5 μg) of each fraction were diluted to 75 μl with assay buffer (50 mM Tris-HCl, pH 7.4, and 1 mM EDTA) and were incubated in parallel (5 min at 30 °C) with C₆⁻Cer (5 μM), OA (1 μM), or a combination thereof. 1 μl of each sample was added in 5-μl aliquots freshly di-
luted by sonication from the appropriate stock solutions, with control incubations receiving an equivalent amount of diluent (final concentra-
tion in the assay of less than 0.5% ethanol). Diphosphorylation reac-
tions were initiated by adding equal amounts of the pooled ³²P-immuno-
precipitated substrate (40 μl of a 50% slurry in assay buffer), and diphosphorylation reactions were typically conducted for 15 min at 37 °C.

To terminate reactions, each incubation was quenched with 4 vol-
umes of ice-cold assay buffer centrifuged at 14,000 rpm for 5 min at 4 °C, and the immunocomplexes were washed three times, boiled in SDS-sample buffer, and resolved and analyzed as described above.

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Ceramide-activated Protein Phosphatase

Fig. 1. Effect of TNFα and bacterial SMase on sphingomyelin hydrolysis in A431 cells. Serum-starved A431 cells (4 x 10⁶ viable cells/well) were labeled for 24 h in serum-free medium supplemented with [³²P]orthophosphate (500 μCi/ml) and were treated for the time periods indicated with 10 ng/ml TNFα (●) or 0.3 unit/ml of bacterial SMase (▲) to induce the direct hydrolysis of sphingomyelin, with control cells (○) receiving an equivalent volume (50 μl) of vehicle. Cellular lipids were extracted and separated by sequential thin-layer chromatography as described under “Materials and Methods,” and the radioactivity associated with sphingomyelin (upper panel) or ceramide (lower panel) was quantitated by liquid scintillation counting. Results represent the mean (bars, S.E.) of triplicate or quadruplicate cultures from an experiment representative of three others. In parallel experiments, no effect on sphingomyelin hydrolysis was observed when cells were treated with equivalent amounts of other phospholipases (phospholipase C, phospholipase A₂, or phospholipase D), heat-inactivated SMase, or TNFα (results not shown).

Fig. 2. Time course of c-Jun dephosphorylation in A431 cells treated with TNFα, bacterial SMase, or membrane-permeable C6-cer. A431 cells (4 x 10⁶ cells/well) were labeled for 4 h in phosphate-free medium in the presence of ³²P]orthophosphate (500 μCi/ml) and were treated for the time periods indicated with 10 ng/ml of TNFα (○), 0.3 unit/ml bacterial SMase (▲), or 5 μM of cell-permeant C6-cer (△). All experimental agents were added in 50-μl aliquots freshly diluted in sterile culture medium, with control incubations receiving the same volume of medium and a similar final concentration (less than 0.1%) of diluent. To terminate experiments, the medium was aspirated, and cell lysates were obtained and immunoprecipitated overnight with 1 μg of c-Jun polyclonal antiserum, as described under “Materials and Methods.” The immunospecificity in TNFα-treated cells was determined by preadsorbing the antiserum (2 h at 4 °C) with the synthetic peptide used to generate anti-c-Jun antibody, and similar results were obtained with cells treated with SMase or C6-cer (results not shown).

Effect of OA on c-Jun Unphosphorylation in A431 Cells Treated with TNFα, Bacterial SMase, or C6-cer. In an effort to determine the mechanism(s) associated with a ceramide-induced decrease in preexisting ³²P-labeled J un protein levels, ³²P-labeled cells were treated for 30 min with TNFα, SMase, or C6-cer in the presence or absence of 10 nM OA (Fig. 3). Whereas OA alone slightly reduced c-Jun phosphorylation levels, the cell-permeant phosphatase inhibitor effectively blocked a TNFα-induced decrease in ³²P-labeled c-Jun levels in A431 cells. Although OA has been reported to induce down-modulation and shedding of the p55-type TNFα receptor in A431 and other cell types (53), it seems unlikely that the antagonistic effect of OA on TNFα-induced c-Jun dephosphorylation may simply reflect a nonspecific consequence of receptor transmodulation because a similar, albeit less pronounced, effect of the phosphatase inhibitor on ³²P-labeled c-Jun unlevels was observed in cells treated with exogenous SMase or C6-cer analogue. No differences were observed in c-Jun phosphorylation after

Effect of OA on c-Jun phosphorylation in A431 cells treated with TNFα, bacterial SMase, or membrane-permeable C6-cer. A431 cells were labeled for 4 h with ³²P]orthophosphate and were treated for the time periods indicated with 10 ng/ml of TNFα (○), 0.3 unit/ml bacterial SMase (▲), or 5 μM of cell-permeant C6-cer (△). All experimental agents were added in 50-μl aliquots freshly diluted in phosphate-free medium, with control incubations receiving the same volume of medium and a similar final concentration (less than 0.1%) of diluent. To terminate experiments, the medium was aspirated, and cell lysates were obtained and immunoprecipitated overnight with 1 μg of c-Jun polyclonal antiserum, as described under “Materials and Methods.” The immunospecificity in TNFα-treated cells was determined by preadsorbing the antiserum (2 h at 4 °C) with the synthetic peptide used to generate anti-c-Jun antibody, and similar results were obtained with cells treated with SMase or C6-cer (results not shown).
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Dephosphorylation of c-Jun by a Phosphatase Activity Extracted from A431 Cells Treated with TNFα, Bacterial SMase, or C6-cer. A431 cells were labeled for 4 h with [32P]orthophosphate (500 μCi/ml) and were treated thereafter for 30 min with 10 ng/ml of TNFα, 0.3 unit/ml bacterial SMase, or the membrane-permeable C6-cer (5 μM) alone or in the simultaneous presence of 10 nM OA (●). After this time period, cell lysates were obtained and were immunoprecipitated with c-Jun polyclonal antiserum as described under “Materials and Methods.” Proteins were separated by SDS-polyacrylamide electrophoresis, and densitometric analysis of the dried gels was performed on a Molecular Dynamics 400A PhosphorImager. Results show an experiment representative of two other experiments.

DISCUSSION

The multitude of biological responses induced by TNFα relies on the activation of two dissimilar receptors of 55 kDa (p55TNFR) and 75 kDa (p75TNFR) that are coexpressed on the membranes of virtually all cell types (reviewed in Refs. 54–56). Although the mechanism of signal transduction has not been fully elucidated, early biochemical events detectable after TNFα-stimulation include GTP-binding proteins, activation of phospholipases (phospholipase A2 and phosphatidylcholine-specific phospholipase C), and different sphingomyelin phosphodiesterases (SMases) characterized by its differential pH optimum and cellular localization (54, 55). Although most cell types coexpress both types of TNFα receptors, the 55-kDa TNFR is responsible for the majority of cytokine actions, being the contribution of the larger receptor species explained, at least in part, by the so-called ligand passing model in which the pp75TNFR presents TNFα to the signal-transducing pp55TNFR molecule (56). In the present study, the demonstration that TNFα induces sphingomyelin hydrolysis to sphingosine and a ceramide moiety in human A431 cells complements earlier observations placing the positive coupling of the activated p55TNFR to a neutral and/or an acidic SMase as an early transmembrane event in other cell types (3–10). Although a complex pattern of target proteins, which include ceramide-activated protein kinase (12, 13, 17), mitogen-activated protein kinases (16, 18), protein kinase Cζ (19, 20), and protein phosphatase 2A-type CAPP (21–24), may account, at least in part, for enhanced phosphorylation of cellular proteins observed after TNFα stimulation (54, 55), only few data support a role for...
ceramide-mediated activation of gene transcription (3–10).

Ligand-stimulated sphingomyelin hydrolysis has been recently reported to activate JNK/SAPK (30–32), a proline-directed kinase that phosphorylates the c-Jun component of AP-1 at its N-terminal transactivation domain (36–40). Because AP-1 plays a major role as a convergence point coupling extracellular signals from the membrane receptor to the nucleus (25–29), the above-mentioned results provide an important link for the sphingomyelin pathway in TNF α-induced phenotypic responses.

Ceramide-activated JNK contributes to cellular responses via phosphorylation of c-Jun in hepatocytes (30), HL-60 cells (31), and bovine aortic endothelial cells (32), and in the present study, we present strong evidence supporting the notion that TNF α-induced generation of ceramide activates an OA-sensitive phosphatase that specifically dephosphorylates the c-Jun component of AP-1 in resting A431 epithelial cells (Figs. 2 and 3). Moreover, after the initial steps described by Okazaki et al. (23) and Wolff et al. (24) for CAPP purification from rat brain and cultured T9 rat glioma cells, we show (Fig. 4) that cytosolic extracts of A431 cells incubated with ceramide (5 μM) dephosphorylated 32P-labeled c-Jun used as a substrate in an immunocomplex phosphatase assay, an effect that was specifically abrogated by concentrations of OA (1 nM) compatible with protein phosphatase 2A inhibition in cell-free systems (reviewed in Ref. 57). As shown in Fig. 5, a similar OA-sensitive effect on 32P-labeled c-Jun unphosphorylation was also observed after incubation of cytosolic extracts obtained from TNF α, SMase, or short-chain ceramide-treated A431 cells. Because the cytosolic heterotrimeric CAPP shares several biochemical properties with protein phosphatase 2A, including its sensitivity to OA in a range (1-10 μM) similar to concentrations used in this study (21–24), it seems reasonable to conclude that c-Jun unphosphorylating activity in A431 cells is mediated by CAPP.

Functional activation of AP-1 requires two events that are frequently, but not always, coordinately induced in resting cells: phosphorylation by JNK/SAPK of serine proximal to the DNA binding domain of c-Jun, resulting in reduced AP-1 association to DNA (41–46).

Although a physiological role for these kinases in AP-1 regulation derives from the observation that overexpression of glycogen synthase kinase-3 (45) or microinjection of CKII results in c-Jun dephosphorylation in intact cells (46), the mechanisms whereby the activity of these kinases is regulated in vivo are not fully understood (25–29, 34, 35). Both kinases are constitutively active, tonically phosphorylating important cellular proteins that are normally activated by dephosphorylation reactions involving protein phosphatases 2A and 2B; thus far, in vivo studies have failed to detect measurable changes in CIK1 or glycogen synthase kinase-3 in response to extracellular agents (25–28, 58). In addition, although JNK/SAPK is activated by phosphorylation and is activated by a mitogen-activated protein kinase phosphatase-1 (59), it is presently unknown whether dephosphorylation of c-Jun at its C-terminal domain in intact cells is mediated by protein kinase C-induced phosphorylation and inactivation of glycogen synthase kinase-3 (42, 44) and/or direct activation of an as yet unidentified J un-phosphatase (29).

Results showing the ability of ceramide-treated cytosolic extracts to dephosphorylate c-Jun can be a faithful measure of the enzymatic activity of CAPP and support the notion that c-Jun unphosphorylation may be a downstream target for CAPP under in vivo conditions. Although this possibility is reinforced by the observation that extracts obtained from TNF α, SMase, or ceramide-treated cells are also endowed with a similar phosphatase activity (Fig. 5), the cascade of events that may ultimately result in c-Jun unphosphorylation in intact cells is complex; nevertheless, the present findings provide a framework for future studies, which are currently under way.

Nevertheless, the results presented here are important by themselves, and together with previous reports showing a role for CAPP in c-myc transmembrane (11), constitute a significant advance toward understanding the role of CAPP as a downstream target of ceramide-induced nuclear events.

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