Protein Kinase C-βII Is an Apoptotic Lamin Kinase in Polyomavirus-transformed, Etoposide-treated pyF111 Rat Fibroblasts*

Anna Chiarini‡, James F. Whitfield§, Ubaldo Armato‡, and Ilaria Dal Pra‡

From the ‡Histology and Embryology Unit, Department of Biomedical and Surgical Sciences, Medical School, University of Verona, Verona I-37134, Italy and §Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada

The role of protein kinase C-βII (PKC-βII) in etoposide (VP-16)-induced apoptosis was studied using polyomavirus-transformed pyF111 rat fibroblasts in which PKC-βII specific activity in the nuclear membrane (NM) doubled and the enzyme was cleaved into catalytic fragments. No PKC-βII complexes with lamin B1 and/or active caspases were immunoprecipitable from the NM of proliferating untreated cells, but large complexes of PKC-βII holoprotein and its catalytic fragments with lamin B1, active caspase-3 and -6, and inactive phospho-CDK-1, but not PKC-βI or PKC-δ, could be immunoprecipitated from the NM of VP-16-treated cells, suggesting that PKC-βII is an apoptotic lamin kinase. By 30 min after normal nuclei were mixed with cytoplasms from VP-16-treated, but not untreated, cells, PKC-βII holoprotein had moved from the apoptotic cytoplasm to the normal NM, and lamin B1 was phosphorylated before cleavage by caspase-6. Lamin B1 phosphorylation was partly reversed, but its cleavage was completely prevented, despite the presence of active caspase-6, by adding a selective PKC-β inhibitor, hispidin, to the apoptotic cytoplasms. Thus, a PKC-βII response to VP-16 seems necessary for lamin B1 cleavage by caspase-6 and nuclear lamina dissolution in apoptosing pyF111 fibroblasts. The possibility of PKC-βII being an apoptotic lamin kinase in these cells was further suggested by lamin B1-bound PKC-δ being inactive or only slightly active and by PKC-α not combining with the lamin.

A growing body of evidence suggests that the commitment to and execution of apoptosis are mediated through the phosphorylation of specific proteins by several protein kinases (1). A number of PKCδ isoforms appear to be among these protein kinases (2, 3). Apoptogens may activate or inactivate and cause the translocation of various PKC isoforms from the cytosol onto cytoskeletal components, cytoplasmic membranes, mitochondria, and/or the nuclear envelope (4–11); induce their migration from such subcellular structures to nucleoplasmic and/or cytosolic fractions (8, 12); or cause the PKC holoenzymes to be cleaved into N-terminal regulatory and C-terminal catalytic fragments (CFs) by several proteases, including caspases (11, 13–15). Despite suggestions that a particular PKC isoform (e.g. the novel PKC-δ and/or its CFs) might play a pivotal role in apoptosis (13, 14), the available evidence indicates the involvement and cleavage of novel PKCs (e.g. PKC-ε and PKC-θ), the classical Ca²⁺-stimulable PKCs (e.g. PKC-β), atypical PKCs (e.g. PKC-γ), and PKC-related (e.g. PKR) kinases in apoptosis (2, 3, 15).

This is the third report from a continuing study of the roles of protein kinase C isozymes in drug-induced apoptosis using a polyomavirus-transformed embryo rat fibroblast, the pyF111 cell, as a model (10, 11). We chose this fibroblast, since it is prone to apoptosis because it cannot make the antiapoptotic Bcl-2 and Bcl-XL proteins but can make the proapoptotic Bax protein (10, 11). We have shown that whereas a surge of the activity of PKC-δ holoprotein anchored in the cytoplasmic particulate fraction is a common component of the signal given by diverse apoptogenic drugs to pyF111 cells, only the DNA-damaging topoisomerase-II inhibitors cause a prompt, large, and irreversible fall of PKC-δ’s specific activity at the nuclear envelope, despite the accumulation and proteolysis of PKC-δ holoproteins at the envelope (10, 11). Thus, according to the apoptogen used, a PKC isoform can be activated, inactivated, or unaffected at the same time in different parts of the pyF111 cell.

The present experiments focused on the behavior and activities of other PKC isoforms at the nuclear envelope of apoptosing VP-16-treated pyF111 cells. The results of these experiments indicate that during the execution phase of apoptosis, catalytically competent PKC-βII holoproteins move onto the nuclear envelope, where they target specific substrates and are cleaved into CFs.

In our search for the nuclear substrates of PKC-βII and PKC-δ, we focused on the lamins. The nuclear lamina is a network of lamins attached to the inner nuclear membrane (16, 17). Lamins are type V intermediate filament proteins with a conserved, central α-helical rod domain flanked by non-α-helical N- and C-terminal domains (16–18). The nuclear lamina can be reconfigured by phosphorylation while operating as an important anchoring site for specific chromatin regions and contributing to the higher order chromatin organization in interphase.

sine 15-phosphorylated; SN1, whole cytoplasm fraction; VP-16, etoposide; CHO, Chinese hamster ovary.

* This work was supported by research grants from the Ministry for University and Scientific and Technological Research (40 and 60% allotment funds, Rome) and the “Progetto Sanità ` 1996/97” of the Fondazione della Cassa di Risparmio di Verona, Verona I-37134, Italy and ”Progetto Sanità ` 1996/97” of the Fondazione della Cassa di Risparmio di Verona, Vicenza, Belluno & Ancona (Italy). 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 1734 solely to indicate this fact.

† To whom correspondence and reprint requests should be addressed: Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada. Tel.: 1-613-993-9090; Fax: 1-613-941-4475; E-mail: pthosteo@rogers.com.

§ This is the third report from a continuing study of the roles of protein kinase C isozymes in drug-induced apoptosis using a polyomavirus-transformed embryo rat fibroblast, the pyF111 cell, as a model (10, 11). We chose this fibroblast, since it is prone to apoptosis because it cannot make the antiapoptotic Bcl-2 and Bcl-XL proteins but can make the proapoptotic Bax protein (10, 11). We have shown that whereas a surge of the activity of PKC-δ holoprotein anchored in the cytoplasmic particulate fraction is a common component of the signal given by diverse apoptogenic drugs to pyF111 cells, only the DNA-damaging topoisomerase-II inhibitors cause a prompt, large, and irreversible fall of PKC-δ’s specific activity at the nuclear envelope, despite the accumulation and proteolysis of PKC-δ holoproteins at the envelope (10, 11). Thus, according to the apoptogen used, a PKC isoform can be activated, inactivated, or unaffected at the same time in different parts of the pyF111 cell.

The present experiments focused on the behavior and activities of other PKC isoforms at the nuclear envelope of apoptosing VP-16-treated pyF111 cells. The results of these experiments indicate that during the execution phase of apoptosis, catalytically competent PKC-βII holoproteins move onto the nuclear envelope, where they target specific substrates and are cleaved into CFs.

In our search for the nuclear substrates of PKC-βII and PKC-δ, we focused on the lamins. The nuclear lamina is a network of lamins attached to the inner nuclear membrane (16, 17). Lamins are type V intermediate filament proteins with a conserved, central α-helical rod domain flanked by non-α-helical N- and C-terminal domains (16–18). The nuclear lamina can be reconfigured by phosphorylation while operating as an important anchoring site for specific chromatin regions and contributing to the higher order chromatin organization in interphase.

sine 15-phosphorylated; SN1, whole cytoplasm fraction; VP-16, etoposide; CHO, Chinese hamster ovary.
PKC-βII as Apoptotic Lamin Kinase in Transformed Fibroblasts

Isolation of Cytoplasms and Nuclei for Cell-free Reconstituted System—We basically followed the procedure of Shimizu et al. (26). Untreated or VP-16-treated (1.0 μM 1 for 6, 18, or 24 h) pyF111 cells were harvested by scraping them into cold (4 °C) phosphate-buffered saline and centrifuging the suspension at 200 × g for 10 min. The sedimented cells were carefully resuspended in a solution containing 10 mM Hepes, pH 7.9, 10 mM KCl, 1 mM MgCl₂, 1.0 mM dithiothreitol, 20 μM sodium orthovanadate, and complete EDTA-free protease inhibitor mixture (Roche Molecular Biochemicals). The cells were then chilled on ice for 15 min and gently lysed by adding 0.6% (v/v) Nonidet P-40. The lysate was centrifuged at 200 × g for 10 min to produce the whole nuclei fraction, pellet, and the supernatant as the whole cellular fraction. The supernatant was boiled in sample buffer (0.0625 M Tris-HCl, pH 7.4, 10 mM Na₂HPO₄, 1.0 mM dithiothreitol, 20 mM magnesium orthovanadate, and complete EDTA-free protease inhibitor mixture (Roche Molecular Biochemicals), containing 7.4, 200 mM NaCl), 1.0 mM dithiothreitol, 20 mM magnesium orthovanadate, and complete EDTA-free protease inhibitor mixture (Roche Molecular Biochemicals). The separated proteins were blotted onto a nitrocellulose membrane (0.45 μm; Bio-Rad) (10, 11). To immunodetect PKC-α, PKC-βII, PKC-βI, and PKC-δ, the blots were probed with iso-specific rabbit IgG polyclonal antibodies (final dilution 1.0 μg ml⁻¹), which recognized the carboxyl-terminal amino acid sequences of each isoform (PKC-α and Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The staining of the particular band pertaining to each PKC isoform could be suppressed by preincubating each antibody with the relevant peptide against which the antibody had been raised (Santa Cruz Biotechnology). The blots were also probed with antibodies (final dilution 1.0 μg ml⁻¹) to detect cytochrome c, lamin B1, tyrosine 15-phosphorylated Tyr(P)³⁷, i.e. inactive) CDK-1 kinase or total “pan,” i.e. both active and Tyr(P)³⁷, i.e. inactive) CDK-1 kinase (Santa Cruz Biotechnology), and serine-phosphorylated (Ser(P)) proteins (Calbiochem). Blots were next incubated with alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgG (Santa Cruz Biotechnology), and stained with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent (Sigma). The developed blots were photographed with an Olympus 3300™ digital camera, and the determination of the M₀ and the densitometric analysis of each specific band were carried out with ImageQuant™ software (Jandel Corp., Erkrath, Germany).

Immunoprecipitation of PKC Isoforms, Lamin B1, and Ser(P) Proteins—The same amount of protein (300 μg) from each NM sample was used for the immunoprecipitation experiments. Protein samples were incubated at 4 °C for 3 h with antibodies directed against PKC-α, PKC-βI, and PKC-δ (anti-PKC-α from Sigma and anti-PKC-βI and anti-PKC-δ from Santa Cruz Biotechnology), lamin B1 (Chemicon International, Temecula, CA), or Ser(P)-proteins (Calbiochem) conjugated to 4-μm-diameter Dynabeads (Dynal). The immunocomplex-bearing beads were collected by centrifugation at 2500 rpm for 5 min at 4 °C and washed five times with Tris-buffered saline (20 mM Tris, pH 7.4, 200 mM NaCl), 1.0 mM dithiothreitol, 20 μM sodium orthovanadate, and complete EDTA-free protease inhibitor mixture (Roche Molecular Biochemicals). After a final wash, the immunocomplex-bearing beads were resuspended in Tris-buffered saline to measure PKC activity or in sample buffer for Western immunoblotting.

Assay of Immunopurified Native PKC-βI or PKC-δ Specific Activity—A colorimetric PKC activity assay kit, the Spinzyme Format™ (Pierce) was used (10, 11). This kit includes as a PKC-specific substrate, the e-peptide (i.e. EKRMRPRKRGVSRRVR, where X is lissamine rhodamine B). To measure the specific activity per μg of protein of an immunopurified PKC isoform, the assay mixture (20 mM Tris-HCl, pH 7.4, 0.1 mM EDTA (or alternatively 0.5 mM calcium), 2.0 mM ATP, 10 mM MgCl₂, 0.002% (v/v) Triton X-100, 0.36 mM dye-labeled e-peptide) without any cofactor was added to the immunocomplex-bearing beads. All of the assay mixtures were incubated for 30 min at 30 °C, and the amounts of phosphorylated e-peptide were determined as recommended by the supplier. The results were expressed in arbitrary units calculated for each sample as the ratios between optical density values and μg of the corresponding immunoprecipitated protein.

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
cells or apoptotic cytoplasmic fractions from cells incubated with VP-16 for 6, 18, and 24 h. Nuclei isolated from the untreated cells were next washed twice by centrifugation/resuspension in the lysis buffer without Triton X-100 and finally suspended at a density of 1.0–2.0 × 10^7 nuclei ml⁻¹. Control or apoptotic cytoplasts (4 volumes) were then incubated at 30 °C for 30 min with nuclei (1 volume) from untreated cells either with or without hispin (5.0 μM) (Calbiochem), a PKC-β, but not PKC-δ, inhibitor (45–47). Equal amounts of protein (i.e. 300 μg) from the NM fractions of these reconstituted nucleus-cytoplasm (N-C) mixtures were immunoprecipitated with an anti-Ser(P) antibody, and such precipitates were blotted and challenged with anti-PKC-βII or anti-lamin B1 antibody.

Assay of Caspase-3 and -6 Activity—The activities of caspase-3 and -6 were measured in various subcellular fractions and in immunoprecipitates from the NM fractions with the specific fluorometric substrates Ac-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin (Ac-DEVD-AMC) and Ac-Val-Glu-Ile-Asp-7-Amino-4-methylcoumarin (Ac-VEID-AMC), respectively (both from Alexis Corp., San Diego, CA). Base-line activities were determined in samples from untreated pyF111 cultures at 0 h (48). The results pertaining to immunoprecipitates were expressed in arbitrary units calculated for each sample as the ratios between fluorescence values and μg of the corresponding immunoprecipitated protein.

Statistical Analysis—Statistical significance was assessed with Student’s t-test for unpaired samples; only differences with p < 0.05 were regarded as significant.

RESULTS

Apoptosis Induction by VP-16 in pyF111 Cells—The number of substrate-adhering cells in untreated pyF111 cultures increased by 9.8-fold on the average between 0 h (i.e. 24 h after planting in F-160 flasks and a fresh medium change) and 72 h, when more than 99.7% of the cells were viable as indicated by their exclusion of trypan blue or EB fluorochrome (Fig. 1A; see also Refs. 10 and 11).

Both at 0 and 48 h later (i.e. when they were exponentially growing), the fractions of untreated pyF111 cells in the G1, S, and G2-M phases of the cell cycle did not significantly change, and the apoptotic hypodiploid (sub-G1) cell population remained negligible (Fig. 1B).

As expected and as previously shown (10, 11), VP-16 (1.0 μg ml⁻¹ of culture medium) caused cells to stop and accumulate in the G2-M phase (i.e. from around 15–50% by 48 h) with a corresponding drop in the G1 and S fractions (Fig. 1B) and triggered apoptosis, the execution of which started 24 h after the drug was added to the cultures and had killed 50% of the cells by 48 h and 75% by 72 h (Fig. 1A; see also Refs. 10 and 11). The size of the hypodiploid or sub-G1 fraction had also risen greatly by 48 h (Fig. 1B). Between 24 and 72 h, the cells lost their high molecular weight DNA, which had been cleaved into a characteristic ladder of oligonucleosomal DNA fragments (data not shown; see Refs. 10 and 11 for identical results). As previously shown (10, 11), PKC-δ specific activity at the nuclear envelope soon began dropping dramatically, while cytochrome c escaped from the mitochondria into the cytosol, where its level peaked by 24 h and stayed at that level for the next 48 h (data not plotted).

Effects of VP-16 on the Translocation, Specific Activity, and Cleavage of Activated PKC-βII Holoproteins at the NM—As revealed by immunoblotting, at both 0 and 48 h there were nearly undetectable amounts of 80-kDa PKC-βII holoproteins in the NM fraction of untreated, actively proliferating pyF111 cells (Fig. 2, A and B). And no PKC-βII CFs were detected in such NM samples even when the amount of protein loaded into each lane was 30 μg instead of the usual 10 μg. The specific activity of the trace amounts of Ca²⁺-stimulable PKC-βII immunoprecipitated by anti-PKC-βII-specific antibody from the NM of untreated cells was unchanged between 0 and 48 h (Fig. 2C) and could not be increased by adding 0.5 mM Ca²⁺ to the assay mixtures (Fig. 2C).

During the first 18 h of exposure to VP-16 (1.0 μg ml⁻¹), the amount of PKC-βII holoproteins in the NM changed only slightly (Fig. 2, A and B), but the specific activity of the PKC-βII immunoprecipitated from the NM rose by 44% (p < 0.05 by 18 h) (Fig. 2C). Between 18 and 72 h, the amount of PKC-βII holoproteins increased 4 times (p < 0.001) in the NM fraction, where it also underwent a massive proteolysis that resulted in large accumulations of 47- and 40-kDa PKC-βII CFs (Fig. 2, A and B).

The PKC-βII specific activity immunoprecipitated from the NM reached twice its starting level (p < 0.001) between 18 and 48 h but then dropped to only 57% of the starting activity (p < 0.01) during the next 24 h in the VP-16-treated cells (Fig. 2C). Adding 0.5 mM Ca²⁺ to the immunoprecipitated test mixtures from the NM fractions of the VP-16-exposed cells between 18 and 72 h did not further increase the PKC-βII specific activity, indicating that all of the PKC-βII holoproteins loaded into the NM fraction were active (Fig. 2C).

![Fig. 1. The G₂-M block and increased size of the apoptotic sub-G₁ fraction caused by VP-16 in pyF111 cells. A, pyF111 fibroblasts (1.2 × 10⁶/flask) were either untreated (0 h; left) or treated with VP-16 (1.0 μg ml⁻¹) (right). The cells were doubly stained with AO-EB (46) and observed under the fluorescence microscope. In untreated actively proliferating cells, only the membrane-permeable AO stains the nuclei yellow-green and the cytoplasmas pale green. After a 72-h exposure to VP-16, most of the cells have died, and the remaining AO-stained ones appear to be shrinking or have just been converted into apoptotic bodies (solid arrow), whose clumps of chromatin emit a dazzling yellow fluorescence. Conversely, aged apoptotic bodies have lost their membrane integrity and emit a red fluorescence due to penetration of the otherwise membrane-impermeable EB (dashed arrow). Magnification, ×120. B, pyF111 cells (1.2 × 10⁶/flask) were either untreated (0 and 48 h) or treated (48 h) with VP-16 (1.0 μg ml⁻¹). Cellular DNA contents and sizes of the several cell cycle fractions and of the sub-G₁ (hypodiploid) fraction were assessed with a fluorescence-activated cell sorter as previously detailed (11). Bars represent mean values from three independent experiments. S.E. values (not shown) were within 11% of the mean values, *p < 0.05.](http://www.jbc.org/)
It should be noted that the PKC-β_{II} holoproteins and their C-terminal fragments were held in the NM fraction. At no time after VP-16 addition did any detectable immunoprecipitable PKC-β_{II} activity appear in the NP fractions (not shown).

These results demonstrate that, during the execution phase of VP-16-induced apoptosis, active PKC-β_{II} holoproteins were massively loaded onto the nuclear envelope and cleaved into 47- and 40-kDa PKC-β_{II} CFs, which remained attached to the envelope.

PKC-β_{II} Associates with Lamin B1 in the NM of pyF111 Cells during VP-16-Induced Apoptosis but Not during the G_{2} Build-up to Mitosis—These observations prompted us to search for possible substrates of the PKC-β_{II} collecting at the NM of pyF111 cells.

Lamin B1 is a PKC-β_{II} substrate during the G_{2} build-up to mitosis in human promyelocytic leukemia HL-60 cells (49, 50) and human erythroleukemia K562 cells (51). To find out whether this also applies to the pyF111 fibroblasts, we treated NM fractions from untreated 0 h and from logarithmically proliferating 48-h cells with anti-lamin B1 or anti-PKC-β_{II} antibodies, but neither PKC-β_{II} holoproteins nor PKC-β_{II} CFs coimmunoprecipitated with lamin B1 from the normal NM samples (Fig. 3, A–D). Therefore, PKC-β_{II} is probably not a significant mitotic lamin kinase in the untreated fibroblasts, because if it were, the more than 15% of the cell population that was in G_{2}-M (Fig. 1B) should have had at least a detectable amount of coimmunoprecipitable lamin B1, PKC-β_{II} holoprotein, and PKC-β_{II} CFs. And, as noted above, there was still no measurable PKC-β_{II} in triple the amount of NM protein from the proliferating cells.

In contrast, a substantial amount of lamin B1 could be immunoprecipitated from the NM fractions with the anti-PKC-β_{II} antibody by 6 h after adding VP-16, and it continued rising up to 24 h (Fig. 3, A and B) but then began falling very slowly (Fig. 3B). Conversely, no PKC-β_{II} could be immunoprecipitated with the anti-PKC-β_{II} antibody from the NM of either untreated or VP-16-treated cells (not shown). PKC-β_{II} holoproteins and their 47- and 40-kDa CFs were also strongly immunoprecipitated along with lamin B1 by the anti-lamin B1 antibody from the NM fractions of the VP-16-treated pyF111 cells (Fig. 3, C and D). There were both 80- and 77-kDa, PKC-β_{II} holoprotein bands in the anti-lamin B1 antibody-precipitated fractions after both 24 and 48 h of treatment. The faster holoprotein band might have been a dephosphorylated form of the catalytically competent enzyme (52). Immunoprecipitated PKC-β_{II} holoprotein-lamin B1 complexes predominated during the first 24–48 h, but by 72 h nearly equal amounts of PKC-β_{II} holoprotein-lamin B1, 47-kDa PKC-β_{II} CF-lamin B1, and 40-kDa PKC-β_{II} CF-lamin B1 complexes had appeared (Fig. 3, C and D). These results suggest that a part of the VP-16-triggered apoptotic mechanism is the formation at the NM of PKC-β_{II}-lamin B1 complexes, in which the lamin B1 is marked by the PKC for cleavage by caspase-6 (53, 54–57). Moreover, it appears that, unlike human cells (49–51, 54, 55), VP-16-ex-

![Image](http://www.jbc.org/)

**FIG. 3. PKC-β_{II} coimmunoprecipitates with lamin B1 only from the NM fractions of VP-16-treated cells.** A, NM fractions from untreated (0 h and 48-h proliferating (48p)) and VP-16 (1.0 μg ml^{-1})-exposed cells were isolated as indicated. PKC-β_{II} was immunoprecipitated from equal aliquots (300 μg of protein) of the NM fractions, and the whole immunoprecipitate samples were subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and challenged with an anti-lamin B1 antibody. The immunoblot is typical of those from five separate experiments. Molecular mass is indicated on the right. B, densitometric analysis of the 69-kDa lamin B1 bands coimmunoprecipitated with PKC-β_{II} from the NM fractions. C, lamin B1 was immunoprecipitated from the same NM fractions as in A, and the whole immunoprecipitated samples were subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and challenged with an anti-PKC-β_{II} antibody. The immunoblot shown is a representative one out of five separate experiments. Points on the curves in B and D represent means ± S.E. from five independent experiments. I.P., immunoprecipitation.
posed pyF111 rat cells use PKC-βII as an apoptotic rather than a mitotic lamin kinase.

**Active Caspase-3 and -6 Asociate with Lamin B1-PKC-βII Complexes in the NP Fraction of VP-16-Treated Cells**— The VP-16-induced rapid surge of cytosolic cytochrome c (see Ref. 10) triggered a cascade of events including a relatively early activation of the cytoplasmic “executioner” or “effector” caspase, caspase-3, the activity of which, as detected by using the selective fluorogenic substrate Ac-DEVD-AMC, increased 3.25-fold \((p < 0.001)\) to a peak between 6 and 18 h and then fell back to the starting level by 72 h (Fig. 4A). The nuclear caspase-3 activity rose gradually during the first 24 h but then sharply rose 3.2-fold \((p < 0.02)\) to a peak at 48 h and then fell (Fig. 4B). It was this burst of nuclear caspase-3-like activity that probably started the execution phase of apoptosis (10, 11). It has been reported that the nuclear lamina is broken down during the execution phase of apoptosis by caspase-6 activated by caspase-9 and/or caspase-3 (48, 53, 56–60). Using the caspase-6-specific substrate, Ac-VEID-AMC (48), we found that this protease was only marginally active in both the whole cytoplasmic (SN1) and NP fractions of untreated (0-h) pyF111 cells (Fig. 4, A and B), but the caspase-6 specific activity in the cytoplasm (the SN1 fractions) had increased 2.4 times \((p < 0.001)\) by 6 h after adding VP-16, although it took 18 h for this activity to double \((p < 0.001)\) in the NP fractions (Fig. 4, A and B). By 48 h, the cytoplasmic caspase-6 activity was 19.7 times higher than the starting value \((p < 0.001)\) and then slowly dropped (Fig 4A). At the same time, the nucleoplasmic activity was 20.3 times higher than the starting value \((p < 0.001)\), and then it too began dropping (Fig. 4B).

The caspase-6 activity immunoprecipitated from the NM fractions by either the anti-lamin B1 or anti-PKC-βII antibody was about 5–10 times higher than in the NP fractions (Fig. 4, compare B with C), because of the enzyme’s accumulation in lamin B1-PKC-βII complexes in the nuclear envelope. The caspase-6 activity was relatively low in both the anti-lamin B1 and anti-PKC-βII immunoprecipitates from the NM fractions of untreated 0-h cells. The immunoprecipitable activity increased only 1.3–1.5-fold \((p > 0.05)\) during the first 24 h after adding VP-16, but between 24 and 48 h the activity shot up 4.9-fold in the anti-lamin B1 antibody precipitates \((p < 0.001)\) and 5.6-fold in the anti-PKC-βII antibody precipitates \((p < 0.001)\). Between 48 and 72 h, the caspase-6 activity rose another 2.9 times \((p < 0.001)\) in the anti-lamin B1 antibody precipitates, but by then anti-PKC-βII antibody precipitates with caspase-6 activity had begun disappearing (Fig 4C). Thus, since up to 48 h the caspase-6 could be increasingly precipitated with either anti-lamin B1 or anti-PKC-βII and since lamin B1 could be precipitated with anti-PKC-βII antibody and since PKC-βII could be precipitated with anti-lamin B1 antibody, active caspase-6 was probably contained in increasing numbers of caspase-6-lamin B1-PKC-βII complexes during the execution phase of apoptosis.

Trace amounts of caspase-3 activity were also immunoprecipitated by anti-PKC-βII antibody from the NM fraction of untreated cells (Fig. 4D), and PKC-βII-bound caspase-3 activity changed relatively little during the first 24 h of VP-16 exposure but surged 5-fold between 24 and 48 h only to drop thereafter (Fig. 4D). Thus, active caspase-3 was also a part of a large complex containing PKC-βII, PKC-βII CFs, active caspase-6, and lamin B1.

While PKC-βII holoprotein or any of its CFs were never found in the NP of untreated or VP-16-treated pyF111 cells (not shown), the surge of caspase-6 activity in the NP (Fig. 4B) was associated, especially between 24 and 48 h, with the appearance in the NP of 45-kDa fragments of lamin B1, which is normally restricted to the NM fraction (17) (Fig. 4E).

**VP-16 Causes PKC-βII to Rapidly Act as an Apoptotic Lamin Kinase in a Cell-free Reconstituted N-C System**—After having identified PKC-βII as a nucleus-seeking apoptotic lamin kinase, we set out to show that this active, hence serine-phosphorylated (49, 61), PKC-βII originated in the cytoplasm in response to VP-16’s apoptogenic signal. To do this, we used a cell-free N-C model consisting of normal, intact nuclei from untreated (0-h) cells mixed with either untreated normal cytoplasm from 0-h cells or apoptotic cytoplasm from cells that had been exposed to VP-16 for 6, 18, or 24 h. These N-C mixtures were incubated at 37 °C for only 30 min before isolating their NM...
fractions. And the experiments were carried out either in the presence or in the absence of hispidin, a PKC-β (but not PKC-δ) inhibitor (45–47). Hispidin was used at a concentration of 5.0 μM, which inhibited by 90% the peak specific activity of the PKC-βII immunoprecipitated from the NM fractions of untreated cells (10) (Fig. 5). Moreover, hispidin (up to 10 μM) must not have affected the cells’ other PKCs because it did not affect the total PKC activity assayable in NM fractions isolated from untreated cells, which contained very small amounts of β-isoforms (not shown). The NM fractions isolated from the cell-free N-C mixtures were immunoprecipitated with an anti-Ser(P) antibody, and Western immunoblots of the components of these immunoprecipitates were probed with either the antilamin B1 antibody or the anti-PKC-βII antibody.

Only trace amounts of phosphorylated PKC-βII could be precipitated by the anti-Ser(P) antibody from the NM fractions of untreated nuclei mixed with control cytoplasms for 30 min (Fig. 6, A and B). But the bands of Ser(P)-PKC-βII were more than 11-fold thicker (p < 0.001) when the NM fractions were from untreated nuclei that had been mixed for 30 min with the 6- or 18-h apoptotic cytoplasms (Fig. 6, A and B). Thus, VP-16-pretreated cytoplasms can load the NM fractions of untreated nuclei with Ser(P)-PKC-βII. The apparently more rapid rise in the amount of holoenzyme at the NMs of the N-Cs as compared with that observed in the NMs from the whole cells (cf. Fig. 2A) is probably due to the immunoprecipitation by the anti-Ser(P) antibody and, possibly, to the arbitrarily set N/C volumetric ratio (i.e. 1:4) used for the N-Cs. Moreover, two bands of the Ser(P)-PKC-βII holoprotein instead of one were clearly visible in the NM fraction when the 24-h apoptotic cytoplasms were added to the nuclei from untreated cells (Fig. 6, A and B), which was the same as when PKC-βII was immunoprecipitated in complexes with lamin B1 from whole cells (Fig. 3C). Moreover, in both kinds of specimens (cf. Figs. 3C and 6A), no PKC-δII- CFs could be detected when the exposure to VP-16 did not exceed 24 h. Thus, the apoptotic signal from VP-16 produced active, serine-phosphorylated, PKC-βII holoproteins in the cytoplasms that massively translocated to the nuclear envelopes of the nuclei from untreated cells.

A sizable amount of 69-kDa Ser(P)-lamin B1 could be immunoprecipitated from the NM fractions of the nuclei from untreated cells mixed with untreated control cytoplasms (Fig. 7, A and C). The amount of immunoprecipitable Ser(P)-lamin B1 nearly doubled when 18- or 24-h apoptotic cytoplasms were used (Fig. 7, A and C). Importantly, a 45-kDa fragment of Ser(P)-lamin B1 also appeared in the NM fractions when untreated nuclei were mixed with the 18- or 24-h apoptotic cytoplasms (Fig. 7E). This was accompanied by a significant increase in caspase-6 activity in the nuclei from untreated cells that had been mixed with the 18- or 24-h apoptotic cytoplasms (Fig. 7E). These data are consistent with there having been a prompt (i.e. within 30 min) phosphorylation of lamin B1 in-
duced in the normal nuclei by contact with the 18- or 24-h apoptotic cytoplasms followed by lamin B1 cleavage by an active caspase-6 from these cytoplasms.

To better define the relevance of PKC-\(\beta\)II action in this lamin phosphorylation and proteolysis, we added the PKC-\(\beta\) inhibitor hispidin (45–47) to the apoptotic cytoplasms. The hispidin totally prevented any increase above basal (0-h) values in the amount of 69-kDa Ser(P)-lamin B1 at the NM when the 6-h apoptotic cytoplasms were used (Fig. 7, B and C), but another protein kinase may have phosphorylated the lamin at later times because the level of 69-kDa serine-phosphorylated lamin B1 was reduced by only 30% when the normal nuclei were mixed with hispidin-treated 18- or 24-h apoptotic cytoplasms. However, hispidin did not stop caspase-6 activity from increasing in the nuclei of the N-C systems (Fig. 7E).

Thus, inhibiting PKC-\(\beta\)II activity with hispidin only partially

**FIG. 7.** When added to apoptotic cytoplasms, the PKC-\(\beta\) inhibitor hispidin reduces lamin B1 phosphorylation and totally prevents lamin B1 proteolysis. N-C mixtures were prepared as indicated and incubated for 30 min at 30 °C either with or without the PKC-\(\beta\) inhibitor hispidin (5.0 \(\mu\)M). NM fractions were next extracted, and Ser(P) (pSer)-proteins were immunoprecipitated from equal aliquots (i.e. 500 \(\mu\)g of protein) of the fractions. Each sample was processed as in Fig. 6 and challenged with an anti-lamin B1 antibody, and the antigen-antibody complexes were visualized as indicated. A and B, representative immunoblots out of three distinct experiments. Molecular masses are indicated between the panels. C and D, densitometric analyses of the bands pertaining to Ser(P)-lamin B1 holoprotein (69-kDa) (C) and its fragments (45-kDa) (D) obtained either in the absence or the presence of hispidin (H). Absolute densitometric values pertaining to the changes in protein amounts are reported in C and in D. Bars represent mean values ± S.E. for three distinct experiments. E, the activity of caspase-6 in whole nuclei from untreated cells mixed with either control or apoptotic cytoplasms was not significantly changed by adding hispidin (5.0 \(\mu\)M) to the cytoplasmic fractions. Samples of whole nuclei were taken from the various N-C mixtures to which hispidin had or had not been previously added. Caspase-6 activity associated with such nuclei was assessed as described in the legend to Fig. 4. Bars represent mean values ± S.E. from three separate experiments.
reduces the serine phosphorylation of 69-kDa lamin B1, but it completely prevents the cleavage of the lamin into 45-kDa fragments despite the presence of an increased nuclear caspase-6 activity. Therefore, caspase-6 specifically needs PKC-βII activity to depolymerize lamin B1 in VP-16-treated fibroblasts.

Other Possible Lamin Kinases in VP-16-treated pyF111 Cells—To further gauge how crucial the activity of PKC-βII at the NM is for the execution of apoptosis in the VP-16-treated pyF111 cells, we determined the possible apoptogenic contributions of other lamin kinases. For this we immunoprecipitated the NM fractions from both untreated (proliferating) and VP-16-exposed (apoptosing) pyF111 cells with the anti-lamin B1 antibody and then probed the immunoblots of the precipitated components with specific anti-kinase antibodies.

PKC-α could have phosphorylated lamin B1 (26), because there were substantial amounts of the holoprotein and its 40-kDa CF in the NM fractions of both proliferating and apoptosing cells (not shown). But neither PKC-α nor its CF immunoprecipitated with lamin B1 the NM fractions of either the proliferating or apoptosing cells (not shown).

CDK-1 is the principal prophase-triggering protein kinase in eukaryotic cells (reviewed in Refs. 50, 54, 55). The results in Fig. 8 (A and B) demonstrate that CDK-1 kinase immunoprecipitated with lamin B1 from the NM fractions both of proliferating and apoptosing cells as revealed by a pan-antibody that did not distinguish between CDK-1’s active dephosphorylated form and its inactive phosphorylated form. Clearly, this pan-antibody revealed that the amounts of CDK-1-lamin B1 (presumably cyclin B-CDK-1-lamin B1) complexes increased both with the entry of the untreated cultures into their logarithmic growth phase (i.e. by 48 h) and after adding the G2/M-blocking VP-16 (Fig. 8, A and B). Using an anti-Tyr(P)15-CDK-1 antibody, which bound to the inactive form of the enzyme, we found, as would be expected, that the CDK-1 immunoprecipitated by anti-lamin B1 antibodies from the NM fractions of the untreated, proliferating (both 0 and 48 h) cells did bind the anti-Tyr(P)15-CDK-1 antibody and was therefore unphosphorylated and active (Fig. 8, C and D). In contrast, the Tyr(P)15 residues of the increasing amounts of CDK-1 that immunoprecipitated with lamin B1 from the NM fractions of the apoptosing and G2/M-blocked (Fig. 1B) VP-16-treated cells did bind the anti-Tyr(P)15-CDK-1 antibody and were therefore phosphorylated and inactive (Fig. 8, C and D). Thus, the CDK-1 accumulating in the VP-16-treated cells was inactive and could not have phosphorylated lamin B1. To find out whether the CDK-1-lamin B1 complexes were or were not different from the caspase-3-caspase-6-lamin B1-PKC-βII complexes, we looked for the presence or absence of CDK-1 in NM immunoprecipitates obtained with the anti-PKC-βII antibody. Our results show that 48 h after the addition of VP-16, mainly inactive Tyr(P)15-CDK-1 is significantly associated with and hence yet another part of the large caspase-3-caspase-6-lamin B1-PKC-βII complex (Fig. 8E).

Finally, PKC-δ could also be a lamin kinase in the pyF111 cells, because the 80-kDa PKC-δ holoprotein and its 40-kDa CF coimmunoprecipitated with lamin B1 from the NM fractions of 0-h proliferating cells (Fig. 9, A and C). The amount of holoprotein-δ-lamin B1 complexes had doubled by 48 h when the cells were in their logarithmic growth phase, while the 40-kDa PKC-δ CF-lamin B1 complexes had dropped by 38% (Fig. 10, A and C). PKC-δ, like CDK-1 kinase, was also immunoprecipitated from the NM fractions of the apoptosing cells by anti-lamin B1 antibody, but, instead of increasing like the CDK-1 in CDK-1-lamin B1-PKC-βII complexes, the PKC-δ holoprotein-lamin B1 complexes dropped by 38–43% between 24 and 48 h after the drug was added (Fig. 9, B and D). The 40-kDa PKC-δ CF-lamin B1 complexes dropped slightly by 24 h but then rose 40% by 48 h after VP-16 was added (Fig. 9, B and D). It must be stressed here that the fall of PKC-δ-holoprotein-lamin B1 complexes and the rise in 40-kDa PKC-δ CF-lamin B1 complexes coincided with the persistent 77–83% drop in the PKC-δ specific activity at the NM that we have previously shown to happen soon after adding VP-16 (10). It must be noted that unlike CDK-1, but like PKC-βII, PKC-δ was not immunoprecipitated by anti-PKC-βII antibody from the NM fractions of VP-16-treated cells (data not shown). This preliminary finding suggests that there is a PKC-δ-lamin B1 complex that is separate from the caspase-3-caspase-6-lamin B1-PKC-βII-CDK-1 complex. In conclusion, while PKC-δ could have served along with CDK-1 kinase for the phosphorylation of lamin B1 at the G2-M transition in the proliferating cells, in no way could it have approached the lamin-phosphorylating action of the massively surging PKC-βII in the NM fractions of the VP-16-treated cells. Therefore, PKC-βII is an important, if not the major, apoptotic lamin kinase in etoposide-treated pyF111 cells.

**DISCUSSION**

The present results are consistent with the hypothesis that at the onset of the execution of the VP-16-triggered apoptosis another PKC isoform, PKC-βII, is converted into an activable form by phosphorylation and induced to move from the cytosol to the nuclear envelope. There, the phospho-PKC-βII holoproteins become anchored to the inner membrane where they are activated and phosphorylate lamin B1 to which active caspase-6 then binds and cleaves. These findings are the first evidence of PKC-βII being the apoptotic lamin kinase in VP-16-treated transformed fibroblasts, although it appears to be the mitotic lamin kinase in erythroleukemia K562 cells and human promyelocytic leukemia HL-60 cells (49, 51, 62, 63). But other PKC isoforms, PKC-α and PKC-δ, seem, respectively, to be the main lamin kinases in camptothecin-induced and Ara C-induced apoptosis in HL-60 cells (26, 39).

In various cell models (i.e. COS-7, HL-60, NIH 3T3, SF-9, etc.), when the cells near the end of the G2 build-up to mitosis, the PKC-βII holoproteins are sequentially phosphorylated at three positions (i.e. first at Thr545 on the activation loop by the PDK-1 kinase and then at Thr544 and Ser660 on the turn and hydrophobic motifs near the C terminus by autophosphorylation) (52, 61, 64, 65). Thus locked in a catalytically competent, but still inactive, conformation, they move from the cytosol and accumulate at the nuclear envelope, where they are selectively captured when their C-terminal V5 regions bind to a phosphatidylycerol in the membrane (49, 51, 62, 66). The phosphatidylglycerol-tethered kinases are then activated by perinuclear Ca2+ transients and diacylglycerols released from the nuclear envelope by phosphatidylinositol-specific phospholipase C (2, 62). The active PKC-βII then triggers the disassembly of the nuclear lamina that is needed for the breakdown of the nuclear envelope and entry into prophase by phosphorylating lamin B’s Ser655 and Ser665 residues (49). Thus, PKC-βII activity has been considered essential for cell proliferation (29, 54) and consequently would be expected to be the lamin B1 phosphorylator in untreated proliferating pyF111 cells.

But it is not. In logarithmically growing pyF111 cultures, when at least 15% of the cells are in the G2-M phase at 48 h (Fig. 1B), there were only tiny amounts of activated PKC-βII holoproteins in the nuclear envelope fraction, and their specific activities were very low when immunoprecipitated from this fraction. Furthermore, there were no detectable PKC-βII CFs in the nuclear envelope fractions of the untreated cells, and no lamin B1-PKC-βII complexes could be immunoprecipitated...
from the nuclear envelope fractions from actively proliferating (both 0- and 48-h) pyF111 cells. Instead of PKC-βII, we found two other active protein kinases, PKC-βI and CDK-1, complexed with lamin B1 in the proliferating cells. Furthermore, although PKC-βI is constitutively hyperexpressed and has the highest immunoprecipitable specific activity at the NM of the untreated pyF111 cells (10), it seems likely from the large amount of evidence from other cells, that it is only a minor mitotic lamin kinase compared with active CDK-1 kinase.

On the other hand, although PKC-βII did not collect in the nuclear membrane of the proliferating pyF111 fibroblasts, VP-16 caused it to become functionally competent and move from the cytosol to the nuclear envelope at the onset of the execution phase of apoptosis (i.e. between 24 and 48 h of exposure). Although lamin B1/PKC-βII complexes could be immunoprecipitated from the NM fraction even at early time points (e.g. 6 h of VP-16 exposure), they peaked around 48 h when strong caspase-3 and -6 activities could be immunoprecipitated with them.

The assumption that the apoptogenic signal from VP-16 induced PKC-βII to become the apoptotic lamin kinase was validated by the results obtained with reconstituted nucleus-cytoplasmic mixtures. As in the whole fibroblasts, only nearly undetectable or trace amounts of PKC-βII holoproteins were in the NM fraction of untreated nuclei mixed with control cytoplasms from untreated cells. On the other hand, apoptotic
PKC-βII as Apoptotic Lamin Kinase in Transformed Fibroblasts

Fig. 9. PKC-δ is mainly a mitotic lamin kinase in pyF111 cells. Lamin B1 was immunoprecipitated from 300-μg protein aliquots of the NM fractions isolated from untreated (0 and 48 h) or VP-16 (1.0 μg/ml)-exposed cells. Each sample was subjected to SDS-PAGE, transferred onto nitrocellulose membranes, and probed with an anti-PKC-δ antibody as indicated. Shown are the bands of PKC-δ coimmunoprecipitated with lamin B1 from the NM fractions of untreated, proliferating cells (A) and of VP-16 (1.0 μg/ml)-incubated cells (B). The immunoblots are representative of the blots from three distinct experiments. Molecular masses are indicated between the panels. C and D, densitometric analyses of the bands of PKC-δ holoprotein and its CFs immunoprecipitated by anti-lamin B1 antibody from the NM fractions of untreated nuclei mixed with control cytoplasms and probed with an anti-PKC-δ antibody as indicated. Shown are the means ± S.E. values of three separate experiments.

Lamin B1•PKC-δ complexes

A Controls
0 48 h kDa
80 40

B VP-16-treated
0 24 48 h kDa
80 40

C Lamin B1-bound PKC-δ in control
0 48 h proteins

D Lamin B1-bound PKC-δ in VP-16-treated pyF111 cells
0 48 h proteins

Cytoplasms from treated cells had catalytically competent Ser(P)-PKC-βII molecules that rapidly (within 30 min) migrated to the NM. Moreover, our data suggest that, as in the whole cells, the serine-phosphorylated lamin B1 immunoprecipitated from the NM fractions of untreated nuclei mixed with control cytoplasms was the substrate of PKC-δ and CDK-1 kinases as well as PKC-βII. However, since adding the PKC-βII (but not PKC-δ) inhibitor hispidin (45–47) to the apoptotic cytoplasms only partially reduced the serine phosphorylation of lamin B1 but totally suppressed the lamin’s proteolysis, PKC-βII activity is essential for the apoptotic dissolution of the nuclear lamina in VP-16-treated pyF111 cells. The specificity of the role of PKC-βII as apoptotic lamina kinase is further validated by the observation that the only other hispidin-inhibitable PKC isoform, PKC-δII, was not in the large caspase-3/caspase-6-lamin B1/PKC-βII-Tyr(P)15-CDK-1 complexes recovered from the NM of VP-16-treated cells.

In keeping with reports from other laboratories (29, 65), the present results indicate that, when catalytically competent PKC-βII holoproteins reached the nuclear envelope of VP-16-treated cells, they formed tight complexes with lamin B1 that could be precipitated with either anti-lamin B1 or anti-PKC-βII antibody. According to our previous study (10), while these PKC-βII-related events were happening at the nuclear envelope, cytochrome c was being released from the mitochondria into the cytosol, where it triggered a caspase cascade (reviewed recently and briefly by Finkel (67)) that resulted in active caspases-3 and -6 moving into the nuclear membrane and forming the caspase-3/caspase-6-lamin B1/PKC-βII-Tyr(P)15-CDK-1 complexes. Therefore, it could be argued that between 24 and 72 h, lamin B1 phosphorylation by PKC-βII was immediately followed by proteolysis. However, while the number of caspase-6-containing complexes precipitable by the anti-lamin B1 antibody was still rising, the number of caspase-6-containing complexes that could be immunoprecipitated by the anti-PKC-βII antibody was still bound to and phosphorylating the lamin. In other words, caspase-6-lamin B1 complexes could be formed and persist, whereas caspase-6-lamin B1-active PKC-βII complexes are short lived because of cleavage of lamin B1 by the caspase depending upon the activity of PKC-βII. Indeed, inhibiting PKC-βII with hispidin totally suppressed lamin B1 cleavage (Fig. 7, B and D). The PKC-βII-dependent lamin B1 cleavage would destroy the nuclear lamina, its associated structures and ultimately the nuclear membrane (17, 33, 62, 68–70).

While caspase-6 cleaved lamin B1, the question of whether it also cleaved PKC-βII has not been answered by the present results. From a preliminary analysis of PKC-βII’s primary se-
PKC-βII as Apoptotic Lamin Kinase in Transformed Fibroblasts

FIG. 10. The possible interactions of PKC-βII, lamin B1, and caspase-6 at the NM of VP-16-exposed transformed fibroblasts are crucial for the execution of apoptosis. Exposure to VP-16 renders cytosolic PKC-βII catalytically competent, first via the PDK-1 kinase and then via autophosphorylation. The now functionally competent phospho-PKC-βII molecules migrate to the NM, where they bind to the inner membrane, become active, and serine-phosphorylate lamin B1. Concurrently, caspase-6 is activated downstream from the release of cytochrome c from the mitochondria into the cytosol, the formation of the apoptosome, and the activation of executioner caspase-9. Active caspase-6 travels to the NM and the nucleoplasm (NP). At the NM, active caspase-6 binds to phosphorylated lamin B1-active PKC-βII complexes and cleaves the lamin into 45-kDa fragments. PKC-βII holoproteins and its CFs of PKC-βII are never found in the NP, but active caspase-6 complexes do get into the NP, where they cleave phosphorylated lamin B1. It should be noted that caspase-3 also reaches the nucleus and joins lamin B1 and PKC-βII in a complex on the nuclear envelope, but it would not cleave lamin B1 (34–37).) The interactions of PKC-βII, lamin B1, and caspase-6 in a large complex lead to the dissolution of the nuclear lamina and to the structural and functional disruption of the chromatin, events that are required for the fragmentation of the nucleus (karyorexis) and the formation of apoptotic bodies. α, phosphorylated form of the molecule; *, activated form of the enzyme; NM, nuclear envelope; NP, nucleoplasm.

...one could find in the nuclear membrane fraction of the VP-16-treated pyF111 cells but not in other cells such as Chinese hamster ovary (CHO) cells. Thus, VP-16 blocks the CHO cells in G2 phase without triggering apoptosis by preventing the Tyr15 phosphorylation and activation of CDK-1 (78). Also, a transient initial activation followed by a Tyr15 phosphorylation-associated inactivation of CDK-1 kinase (with no change in cyclin B levels) and by rapid apoptosis occurs in VP-16- or camptothecin-treated human HL-60 cells (23). These findings suggest the notion that an exposure to a topoisomerase-II inhibitor prevents the activation of CDK-1 kinase in various kinds of human, rat, and hamster cells. However, whether this induces apoptosis probably depends on cell-specific differences in the response to DNA damage.

PKC-βII and CDK-1 were not the only possible lamin kinases one could find in the nuclear membrane fraction of the VP-16-treated pyF111 cells. PKC-α and PKC-δ were also there. However, PKC-α did not form complexes with lamin B1, although it does this in camptothecin-treated human HL-60 cells (26). Therefore, PKC-α is not a lamin kinase in either untreated or etoposide-treated pyF111 fibroblasts, although a less direct role of this PKC isoform in apoptotic nuclear membrane dissolution cannot be ruled out at present. Then again, in these same fibroblasts, 79-kDa PKC-δ holoprotein-lamin B1 complexes dropped between 24 and 48 h after the addition of VP-16, whereas the 40-kDa PKC-δ CFs-lamin B1 complexes increased only slightly. Thus, since PKC-δ activity has been found to drop promptly by around 77–83% at the NM of VP-16-treated pyF111 cells (10), it probably did not substantially contribute to the lamin B1 phosphorylation necessary for nuclear lamina dissolution.

Several signals are known to be transduced via the translocation of PKCs to the nucleus (28, 61, 79, 80). Phosphorylation of nuclear lamins by PKCs conveys the signaling evoked by either mitogenic or apoptotic stimuli. An important question is whether the same PKC isotype(s) is invariably involved in such transductions (80). The available evidence (26, 38, 39) and the results of the present experiments would support the view that no PKC isoform is the universal eukaryotic mitotic or apoptotic lamin phosphorylator. Thus, the PKC isoforms involved in functions such as mitotic and apoptotic lamin phosphorylation must be identified for each type of stimulant and...
PKC-βII as Apoptotic Lamin Kinase in Transformed Fibroblasts

each type of cell in each animal species.

Last, our results suggest the scenario in Fig. 10 for the disassembly of the nuclear lamina and the disruption of the nuclear chromatin in the VP-16-treated apoptosing pyF111 fibroblasts. PKC-βII binds to the nuclear envelope, where it is activated and binds to and starts phosphorylating lamin B1. Back in the cytosol, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases as such, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated and binds to and starts phosphorylating lamin B1. The activated and binds to and starts phosphorylating lamin B1.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.

In the cytoplasm, the release of mitochondrial cytochrome c starts a caspase cascade, one of the results of which is the arrival at the nuclear envelope of active caspase-6 that joins the lamin B1-phospho-PKC-βII/CDK-1 complex in which it cleaves lamin B1. The caspase-6 (but not the NM-restricted PKC-βII) also gets into the nucleoplasm, where it cleaves the lamins in matrix-associated foci, where they are working in DNA replication factories (19) or being farnesylated for insertion into the nuclear envelope (17). PKC-βII is also cleaved in the complex probably by other yet to be defined proteases such as, perhaps, calpains (70, 74). Meanwhile, the cells in the G2 phase have made the mitotic lamin kinase CDK-1 and have phosphorylated its Thr14 and Tyr15 residues with the dual function PKC and PKC-δ, activated.
PKC-βII as Apoptotic Lamin Kinase in Transformed Fibroblasts

73. Varghese, J., Radhika, G., and Sarin, A. (2001) *Eur. J. Immunol.* 31, 2035–2041
74. Blomgren, K., Zhu, C., Wang, X., Karlsson, J. O., Leverin, A. L., Bahr, B. A., Mallard, C., and Hagberg, H. (2001) *J. Biol. Chem.* 276, 10191–10198
75. Chua, B. T., Guo, K., and Li, P. (2000) *J. Biol. Chem.* 275, 5131–5135
76. Solomon, M. J. (1993) *Curr. Opin. Cell Biol.* 5, 180–186
77. O’Connor, P. M., Ferris, D. K., Hoffmann, I., Jackman, J., Draetta, G., and Kohn, K. W. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 9480–9484
78. Lock, R. B. (1992) *Cancer Res.* 52, 1817–1822
79. Rogue, P., Labourdette, G., Masmoudi, A., Yoshida, Y., Huang, F. L., Huang, K. P., Zwiller, J., Vincendon, G., and Malviya, A. N. (1990) *J. Biol. Chem.* 265, 4161–4165
80. Hocevar, B. A., and Fields, A. P. (1991) *J. Biol. Chem.* 266, 28–33
Protein Kinase C-βII Is an Apoptotic Lamin Kinase in Polyomavirus-transformed, Etoposide-treated pyF111 Rat Fibroblasts
Anna Chiarini, James F. Whitfield, Ubaldo Armato and Ilaria Dal Pra

J. Biol. Chem. 2002, 277:18827-18839.
doi: 10.1074/jbc.M111921200 originally published online March 18, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111921200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 76 references, 40 of which can be accessed free at http://www.jbc.org/content/277/21/18827.full.html#ref-list-1