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Molecular Characterization of Protein Kinase C-α Binding to Lamin A

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Abstract Previous results from our laboratory have identified lamin A as a protein kinase C (PKC)-binding protein. Here, we have identified the regions of PKC-α that are crucial for this binding. By means of overlay assays and fusion proteins made of glutathione-S-transferase (GST) fused to elements of rat PKC-α, we have established that binding occurs through both the V5 region and a portion of the C2 region (i.e., the calcium-dependent lipid binding (CaLB) domain) of the kinase. In particular, we have found that amino acid 200–217 of the CaLB domain are essential for binding lamin A, as a synthetic peptide corresponding to this stretch of amino acids prevented the interaction between the CaLB domain and lamin A. We also show that the presence of four lysine residues of the CaLB domain (K205, K209, K211, and K213) was essential for the binding. We have determined that binding of elements of PKC-α to lamin A does not require the presence of cofactors such as phosphatidylserine (PS) and Ca^{2+}. We have also found that the binding site of lamin A for the CaLB domain of PKC-α is localized in the carboxyl-terminus of the lamin, downstream of amino acid 499. Our findings may prove to be important to clarify the mechanisms regulating PKC function within the nucleus and may also lead to the synthesis of isozyme-specific drugs to attenuate or reverse PKC-dependent nuclear signaling pathways important for the pathogenesis of cancer. J. Cell. Biochem. 86: 320–330, 2002. © 2002 Wiley-Liss, Inc.

Key words: PKC-binding proteins; overlay assays; nucleus; fusion proteins; PKC regulation

Protein kinase C (PKC) is a serine/threonine protein kinase, which has originally been identified as a phospholipid-sensitive kinase [Nishizuka, 1995]. Subsequent molecular cloning of PKC revealed the existence of a family of related but distinct enzymes [Hug and Sarre, 1993]. The isozymes occupy critical nodes in the complex cellular signal transduction networks that regulate diverse cellular processes, including gene expression, proliferation, differentiation, apoptosis, tumor promotion, migration, hypertrophy, secretion, and permeability [Jaken, 1996; Mellor and Parker, 1998; Toker, 1998; Dempsey et al., 2000; Musashi et al., 2000; Naruse and King, 2000]. The PKC isozymes can be divided into three subclasses: conventional cPKCs (−α,−βI, βII,−γ) that are activated by diacylglycerol, PS and Ca^{2+}; novel nPKCs (−δ,−ε,−η,−θ) that are dependent on PS and diacylglycerol; and atypical aPKCs (−ζ,−ι/−λ) that only respond to PS. Each PKC isozyme has a modular structure with unique or variable (V) regions. In addition, there are regions common (C) to all the isozymes. The subclasses differ from each other in the common regions localized in their regulatory domain. The regulatory

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domain of cPKC contains two common regions, C1 and C2. The C1 region possesses two cysteine-rich loops (Cys1 and Cys2) that mediate diacylglycerol and phorbol-ester binding. The C2 region is peculiar to cPKCs and mediates Ca^{2+} binding. Moreover, the C2 domain mediates PS binding through the domain referred to as CaLB [Hug and Sarre, 1993]. PKC isozymes display only limited differences in substrate specificity or sensitivity to activators. Since, there usually are various isozymes within a given cell type, the differential subcellular localization has been proposed to explain isoform specificity [Ron and Kazanietz, 1999]. The differential localization appears to depend, at least in part, on interactions of each PKC isozyme with specific anchoring proteins, referred to as PKC-binding proteins. Over the last 10 years, several PKC-binding proteins have been identified [e.g., Hyatt et al., 1994; Liao et al., 1994; Chapline et al., 1996, 1998; Izumi et al., 1997]. These proteins are referred to as STICKs (substrates interacting with C-kinase), RICKs (receptors for inactive C-kinase), or RACKs (receptors for activated C-kinase) [Mochly-Rosen and Gordon, 1998; Jaken and Parker, 2000]. All STICKs are phospholipid-binding proteins and, in general, are localized to interfaces between membranes and cytoskeletal structures [Ron and Mochly-Rosen, 1995]. RICKs do not need to be substrates for PKC. The *Drosophila* InaD is a protein, which fulfills the criteria for RICKs [Jaken and Parker, 2000]. RACKs are proteins that directly interact with the C2 domain of PKC [Ron and Mochly-Rosen, 1995; Ron et al., 1995]. Interestingly, RACK1 is upregulated in angiogenesis and human carcinomas [Berns et al., 2000], while ethanol treatment induces uncoupling of PKC-βII from RACK1 [Ron et al., 2000]. Elucidation of PKC–RACK interactions allowed the synthesis of PKC translocation inhibitors and activators [Johnson et al., 1996, Yedovitzky et al., 1997]. These isozyme-selective translocation inhibitors and activators are of potential therapeutic value [Dorn et al., 1999]. Several PKC isoforms have been demonstrated to be present in the nucleus, either resident there or translocated to this organelle after a variety of stimuli [Olson et al., 1993; Buchner, 1995; Martelli et al., 1999]. Nevertheless, our knowledge about nuclear PKC-binding proteins is almost nonexistent. Overlay assays have frequently been exploited to identify PKC-binding proteins [e.g., Chapline et al., 1996, 1998; Izumi et al., 1997]. In such experiments, cell extracts are separated by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE) and probed for the presence of binding proteins after transfer to nitrocellulose, by incubating the blots with PKC preparations in the presence of lipid activators. In agreement with these previous results, by means of overlay assays performed in the presence of lipid cofactors with recombinant PKC-α on two-dimensional blots, we have identified in extracts from Swiss 3T3 cell nuclei some PKC-binding proteins, namely lamin A, B, and C, and nucleolin [Martelli et al., 2000]. Since all these proteins are recognized substrates for PKC [Martelli et al., 1999], they might be considered STICKs. However, due to the presence of lipids in these overlay assays, it was unclear whether or not these interactions were direct protein–protein interactions or mediated by phospholipids. Moreover, PKC-protein binding could have simply been a consequence of interactions with the substrate-binding site of PKC, which is present in the catalytic domain (C4 region) [Hug and Sarre, 1993]. Therefore, we undertook the present study to elucidate the elements of PKC-α responsible for binding to lamin A, also in the absence of lipid cofactors. To this end, we employed fusion proteins, consisting of GST fused to elements of either the regulatory or the catalytic domain of PKC-α. We reasoned that a full understanding of the amino acidic sequences involved in these binding events may represent a fundamental step in the clarification of how PKC isozymes are activated within the nucleus and may also lead to a better comprehension of the interactions between PKC and its nuclear substrates.

MATERIALS AND METHODS

Materials

Protease inhibitors, detergents, isopropyl-β-thiogalactoside (IPTG), and Lumi-Light^{Plus} enhanced chemiluminescence (ECL) detection kit were from Roche Molecular Biochemicals, Milan, Italy. Mouse monoclonal antibodies to GST (clone B-14) and to lamin A/C (clone 636) were purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Protein Assay kit (detergent compatible) was obtained from Bio-Rad Laboratories (Hercules, CA). The pGEX4T1 expression vector was from Amersham Phar-

macia Biotech, Uppsala, Sweden. *Escherichia coli* (strain B21) was from Novagen, Madison, WI. pQE-30 and the Qiaexpress expression system were obtained from Qiagen, Milan, Italy. ExSite polymerase chain reaction (PCR)-based site directed mutagenesis kit was from Stratagene, La Jolla, CA. Restriction enzymes were purchased from Promega Corporation, Madison, WI. Glutathione-agarose matrix, reduced glutathione, peroxidase-conjugated anti-mouse antibody, PS, fetal calf serum, and tissue culture media were obtained from Sigma Chemical Co., St. Louis, MO.

Protein Assay

This was performed according to the instruction of the manufacturer.

SDS–PAGE and Western Blotting of Proteins

Recombinant lamin A (200 pg) was run on a 10% SDS–PAGE and transferred to nitrocellulose sheets using a semi-dry blotting apparatus.

Expression and Purification of Recombinant Human Lamin A

We used a mammalian expression construct encoding the whole open reading frame of human lamin A to generate the cDNA corresponding to the full length and to the truncated forms 1–377, 1–439, and 1–499. The forward (LAF) and the reverse (LAR) primers for lamin A (see also Fig. 1) were:

LAF1 (5′CGGGGATCCCCGGATGGAGACCC-CGTCCCAGCGG3′),
LAR665 (5′CGGGGTACCCCGTTACATGATG-CTGCAGTTCTGGGG3′),
LAR377 (5′CGGGGTACCCCGGCGGTAGGC-GTGGATCTCCATG)
LAR439 (5′CGGGGTACCCCGGCGCCCGCT-AGTGCGTGCGTG3′),
LAR499 (5′CGGGGTACCCCGAGCCCAGATC-GTCACCACCTG3′).

All the primers contained a KpnI-restriction site and the PCR products were then subcloned in the KpnI-digested pQE-30, producing an in-frame fusion protein with GST. For the expression and purification of the recombinant protein, the QIAexpress Expression System was used following manufacturer's instructions. Briefly, the bacterial culture was grown at 37°C until the OD_{600} was 0.5–0.7, then protein expression was induced by adding 1-mM IPTG for 4–5 h. Cells were harvested by centrifugation at 4,000*g* for 20 min, lysed by gently vortexing in lysis buffer (100 mM NaH_2PO4, 10 mM Tris, 6 M GuHCl), and cell debris was eliminated by centrifugation at 10,000*g* for 20 min. The clear lysate was incubated for 45 min with the 50% Ni-NTA slurry and then the lysate-resin complex was loaded onto an empty column for the purification. During the elution of the recombinant protein, several fractions were collected and analyzed by SDS–PAGE.

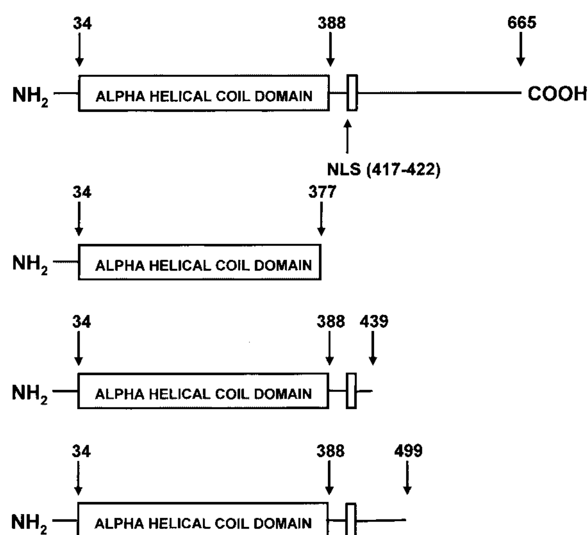

Fig. 1. Molecular schematic of human lamin A (amino acid 1–665) and of its truncated forms (amino acid 1–377; 1–439; 1–499) as employed in the present study. NLS, nuclear localization signal.

PKC-α Elements Expressed as GST Fusion Proteins

The different binding regions of rat PKC-α were expressed in *E. coli* as C-terminal fusion proteins with GST (see Fig. 2). Briefly, the cDNA encoding regions of interest were amplified by PCR from a rat PKC-α cDNA. Both primers contained an EcoRI restriction site. DNA amplification was carried out for 30 cycles, each at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min (Perkin Elmer 2400 thermal cycler). The list reported below shows the primers used to amplify the different regions that are indicated by the first or the last amino acid (single-code letter and number) still present (see also Fig. 2):

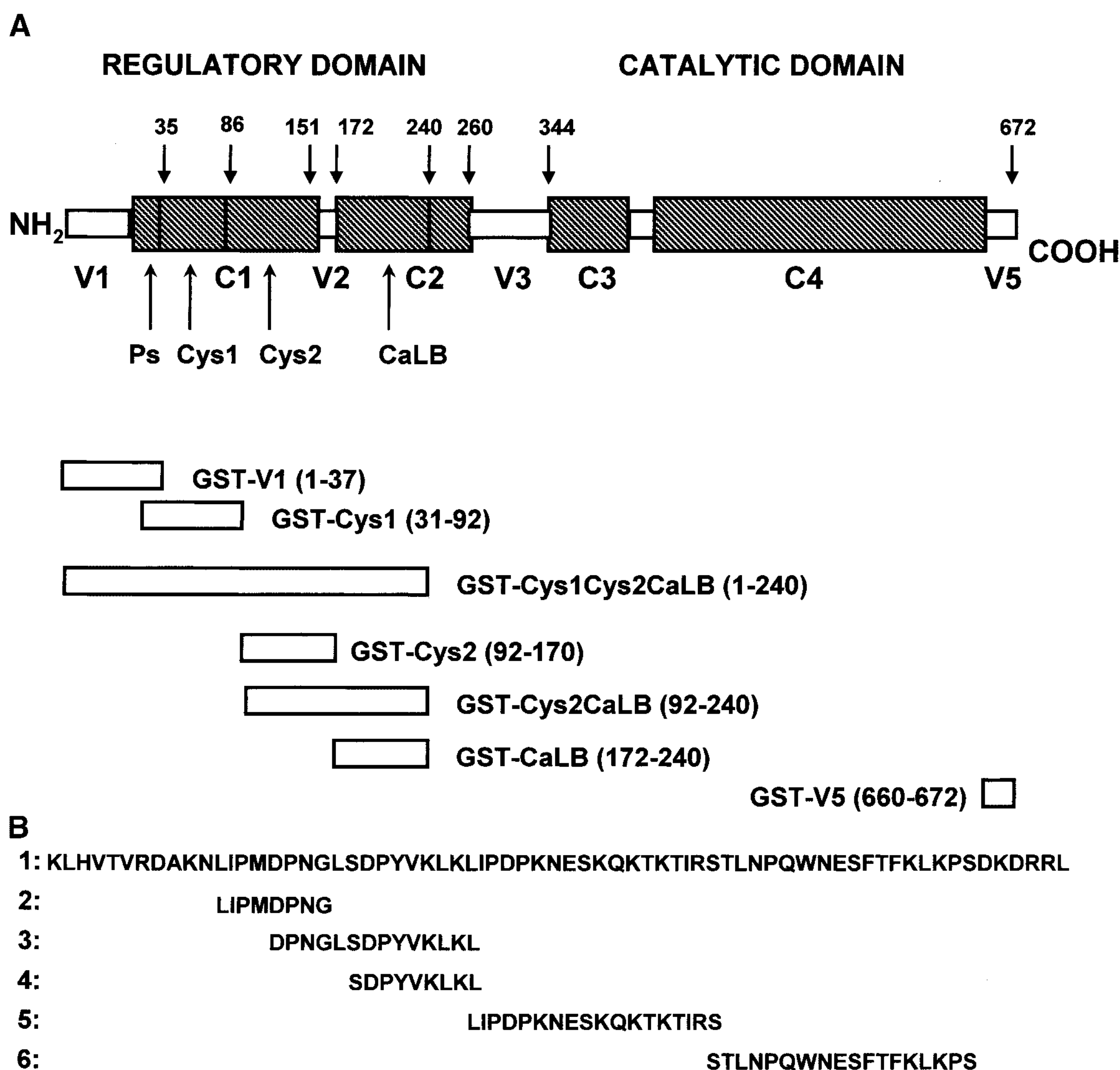

Fig. 2. **A**: Schematic representation of rat PKC-α and fusion proteins employed in this study. The variable (V) and common (C) regions of PKC-α are shown. Ps, pseudosubstrate site (amino acid 19–35) [see Hug and Sarre, 1993]. **B**: Sequence of the CaLB domain (1) as well as amino acids that were deleted in the GST-CaLBΔ fusion proteins, (2) Δ183–190, (3) Δ187–200, (4) Δ192–200, (5) Δ200–217, (6) Δ217–234 (see also Fig. 5).

V1 F(1M): GCGCGGGAATTCATGGCTGACGTTTACCCGG
V1 R(H37): GCGCGCGAATTCGTGGTCTTTCACCTCATGCA
Cys1 F(31V): GCGCGCGAATTCGTGCATGAGGTGAAAGACCA
Cys1 R(92G): GGGCTGGAATTCCCCGGACAAGAGAAAGTAAC
Cys2 F(92G): GCGCGCGAATTCGGGTGCGGATAAGGGACC
Cys2 R(170D): GCGCGCGAATTCATCTGTGACCTCTGCCTT
CaLB F(172K): GCGCGGAATTCAAGCTGCACGTCACCGTAC
CaLB R(240L): GCGCGGAATTCCAGTCGCCGGTCTTTGTC
V5 F (N660): GGGCGGAATTCAACCCCCAGTTTGTGCACC
V5 5 (N672): GGGCGGAATTTCATACTGCACTTTGCAAGAT

The PCR products, analyzed by agarose gel, were subcloned in the EcoRI digested pGEX4T1. Deletions in the CaLB domain (Δ183–190, Δ187–200, Δ192–200, Δ200–217, and Δ217–234) and mutations in the CaLB fragment spanning from amino acid 200 to 217 in which the four different lysines (K205, K209,

K211, and K213) were replaced by isoleucine, were generated by PCR mutagenesis using the ExSite PCR-based site directed mutagenesis kit, following manufacturer's instructions. The resulting cDNA fragments encoding the mutants reported above were subcloned in the same expression vector. All constructs were confirmed by DNA sequencing before use.

The plasmids were transformed into *E. coli* (protease deficient, strain BL21, used to avoid proteolytic degradation of fusion proteins) which was grown in LB medium at 37°C to an OD_{600} of 0.5. Bacteria were then transferred to 20°C until the culture reached an OD_{600} of 0.7. At that density, $ZnCl_2$ was added (final concentration in the medium 1 μM) and the fusion protein was expressed by induction with IPTG to a final concentration of 0.2 mM. After 16 h, the bacteria were harvested by centrifugation and the pellets frozen at −70°C. The pellets were then lysed and extracted (1 ml of extraction buffer for cell pellet derived from 15 ml of *E. coli* culture medium). Extraction buffer was: 50 mM HEPES, pH 8.0, 1 mM dithiothreitol, 0.1% Triton X-100, 10% ethylene glycol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml benzamidine, 2 μg/ml antipain, 1 μg/ml leupeptin. Insoluble cell debris were removed by centrifugation at 15,000*g*. Supernatants were applied three times to an equal volume of glutathione-agarose matrix equilibrated at room temperature with 1 mM sodium phosphate buffer, pH 7.3, 15 mM NaCl. After washing with 10 column volumes of phosphate buffer to remove nonspecifically bound proteins, GST fusion proteins were specifically eluted with 5 mM reduced glutathione in 50 mM HEPES, pH 8.0, 10% ethylene glycol. All fusion proteins were stored at−70°C in elution buffer [Quest et al., 1995].

Overlay Assays

The procedure outlined by Ron et al. [1995] was employed. The blots were incubated for 30 min at room temperature in overlay buffer (50 mM Tris-HCl, pH 7.5, 0.1% bovine serum albumin, 5 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 0.1% polyethylene glycol, 0.2 M NaCl, 0.1 mM $CaCl_2$, and 12 mM β-mercaptoethanol). The fusion proteins (at ≥ 10 μM) were added in the presence or absence of 50 μg/ml of PS and 1 mM $CaCl_2$, and the mixture further incubated for 30 min at room temperature. The blots were then washed three times for 5 min with overlay wash buffer (0.1% polyethylene glycol, 0.2 M NaCl, 0.1 mM $CaCl_2$, 12 mM β-mercaptoethanol, and 50 mM Tris-HCl, pH 7.5). Binding of fusion proteins was detected with an anti-GST monoclonal antibody (diluted 1:5,000), followed by a peroxidase-conjugated anti-mouse IgG (diluted 1:8,000). Bands were visualized by ECL. The synthetic peptides, corresponding to amino acid 200–217 or 217–234 of rat PKC-α, were synthesized at the Microchemical Facility of the University of Trieste. All peptides used in this study were at least 90% pure and were employed at 10 μM in the overlay buffer.

RESULTS

Binding of GST/Elements of PKC-α Fusion Proteins to Lamin A

We employed overlay assays to investigate the binding to lamin A of fusion proteins consisting of PKC-α elements fused to GST. As shown in Figure 3A, GST carrier protein alone did not bind recombinant human lamin A. A fusion protein consisting of GST fused to the V1Cys1Cys2CaLB region of rat PKC-α (amino acid 1–240, it also includes the V2 region) reacted with recombinant human lamin A. Interestingly, the binding occurred both in the absence and in the presence of PS and $CaCl_2$ in the overlay mixture (Fig. 3A). When the fusion protein consisted of GST fused to the Cys1 region of rat PKC-α (amino acid 31–92), we never detected any binding to lamin A, irrespectively of the presence or the absence of cofactors (Fig. 3B). Also if GST was fused to the V1 region of rat PKC-α (amino acid 1–37, it also includes the pseudosubstrate site of the C1 region), the binding to lamin A did not occur (Fig. 3C). In contrast, binding was detected with the GST-V5 fusion protein (Fig. 3D).

CaLB Domain of PKC-α Binds Lamin A and the Carboxyl Terminus of the Lamin Is Essential for the Binding

As shown in Figure 4, the Cys2 domain (amino acid 92–170, it also includes the V2 region) alone did not bind recombinant human lamin A, whereas a fusion protein containing the Cys2 domain plus the CaLB domain (amino acid 92–240, it also includes the V2 region) did. The CaLB domain (amino acid 172–240) fused to GST bound recombinant lamin A. We next tried to identify the region(s) of lamin A

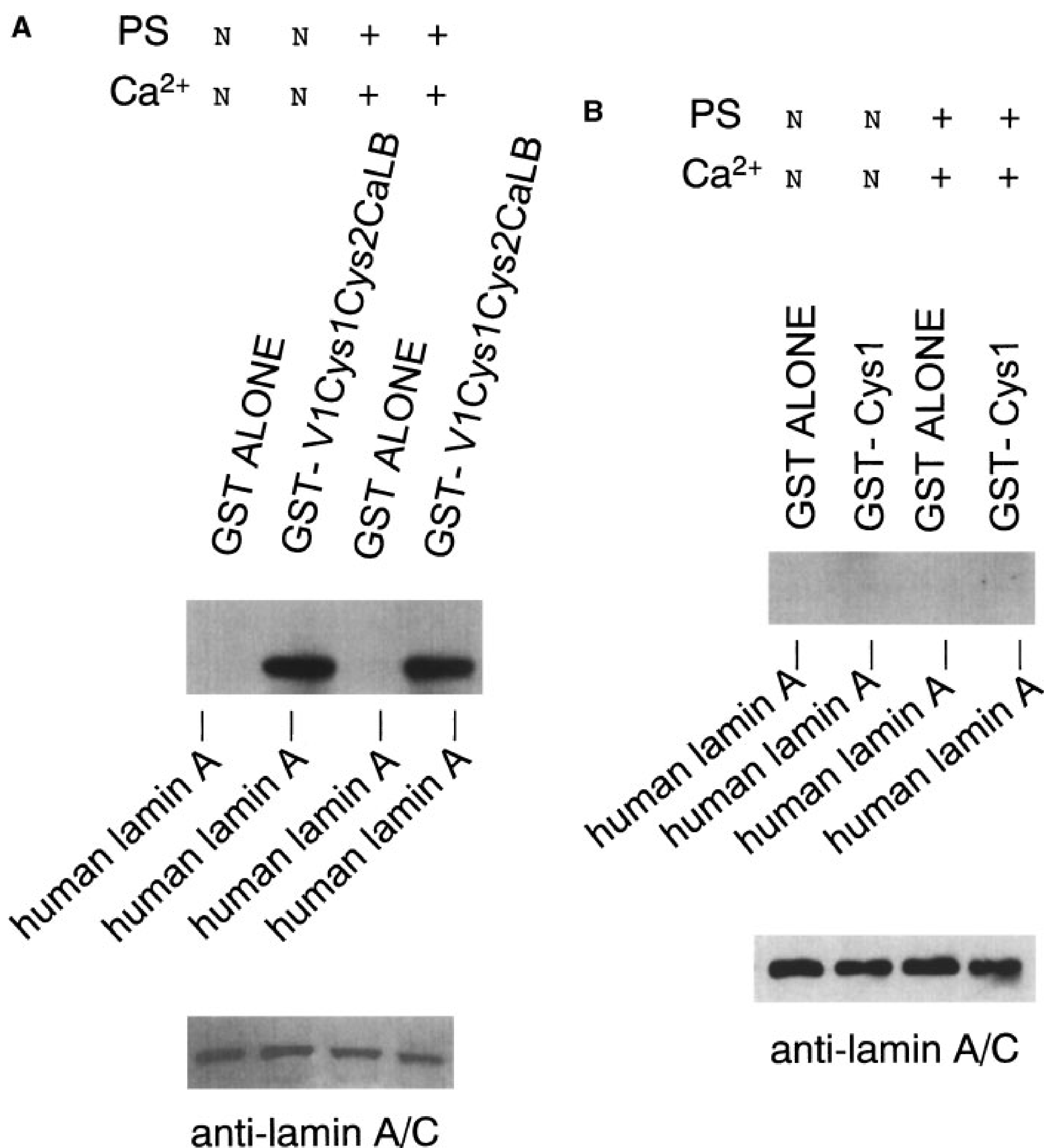

Fig. 3. Lamin A binds elements of rat PKC-α. Recombinant human lamin A separated by SDS–PAGE, was blotted to nitrocellulose sheets. Sheets were then subjected to overlay assays in the presence of the fusion proteins (10 μM). **Panel A**: GST-V1Cys1Cys2CaLB; **panel B**: GST-Cys1; **panel C**: GST-V1; and **panel D**: GST-V5. Binding was revealed by a monoclonal antibody to GST, followed by ECL detection. Overlay assays were performed either in the absence (N) or in the presence (+) of the PKC cofactors PS and Ca^{2+}, as indicated. Duplicate blots were probed with a monoclonal antibody to lamin A/C.

essential for the binding to CaLB domain. Therefore, we made a series of truncated forms of lamin A (see Fig. 1). As presented in Figure 4, all the truncated forms of lamin A we tested did not react with the CaLB, indicating that the binding site resides in the last 166 amino acids of the carboxyl-terminus of lamin A. Also in this case, binding of the entire CaLB domain to full-length lamin A was independent of the presence of cofactors during the overlay assays (Fig. 4).

Identification of the Sequence Present in the CaLB Domain of PKC-α Which Binds Lamin A

We next moved to the identification of the amino acid sequence of the CaLB domain, which is essential for binding lamin A. To this end, we

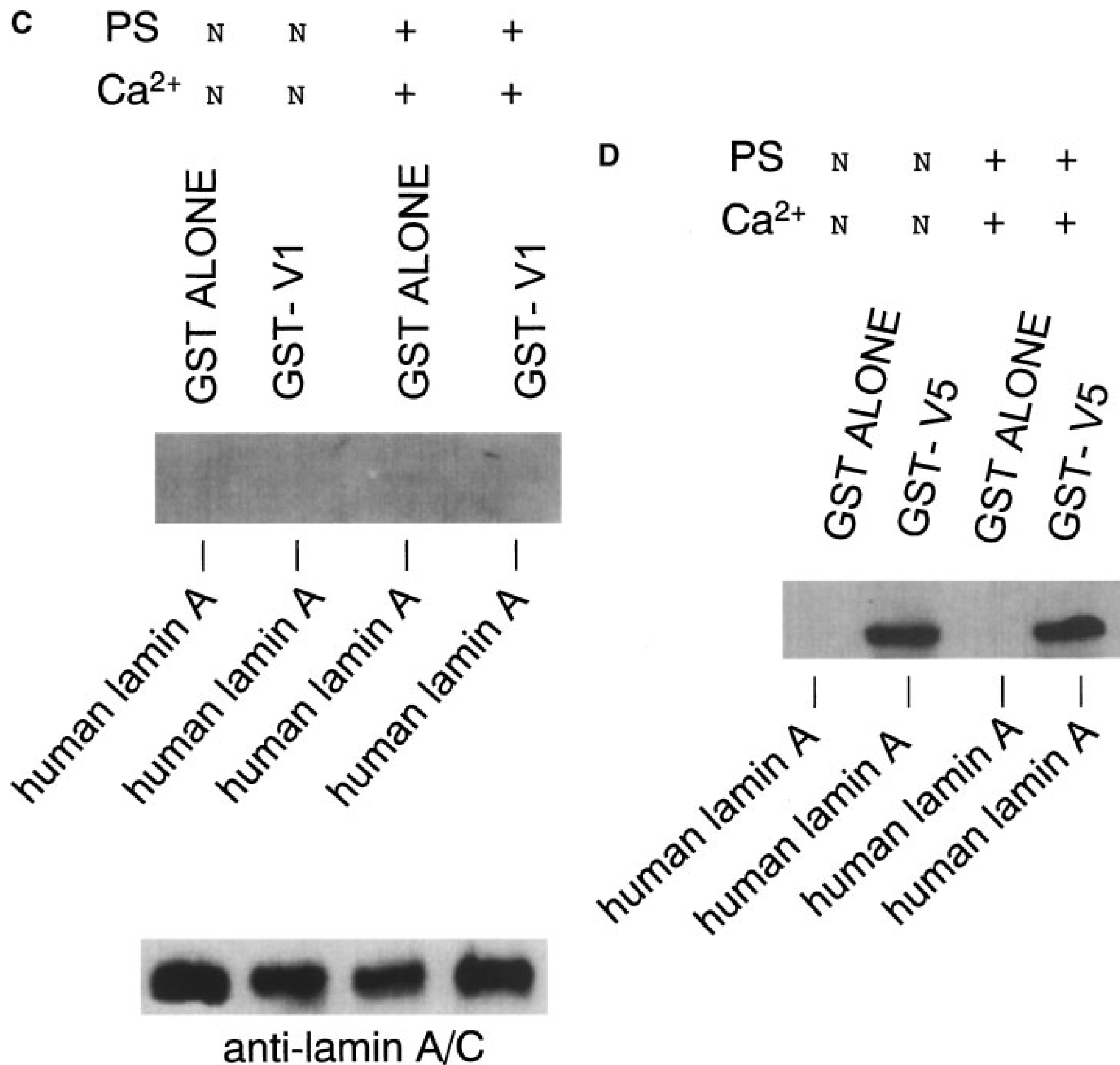

Fig. 3. (*Continued*)

constructed a series of fusion proteins consisting of GST fused to CaLB domain of the kinase in which different stretches of amino acids had been deleted. The results are presented in Figure 5. It is evident that the binding did not occur if the deleted amino acids were 200–217 (LIPDPKNESKQKTKTIRS), but not 183–190 (LIPMDPNG), 187–200 (DPNGLSDPYVKLKL), 192–200 (SDPYVKLKL), or 217–234 (STNLPQWNESFTFKLKPS).

To determine whether the lysine residues present in the 200–217 stretch of amino acids were important for binding, four different lysines (K205, K209, K211, and K213) were replaced by isoleucine. If only one lysine was replaced (GST-CaLB1: K205), the binding was not affected. If two or three lysines were replaced (GST-CaLB2: K-205 and K209; GST-CaLB3: K205, K209, and K211), binding was reduced. Finally, if all the four lysines were replaced with isoleucine (GST-CaLB4), binding was abrogated (Fig. 6).

Synthetic Peptide, Corresponding to Amino Acid 200–217 of Rat PKC-α, Blocks CaLB Binding to Lamin A

Finally, we sought to determine whether or not a synthetic peptide, corresponding to amino acid 200–217 of rat PKC-α, could block the binding to recombinant human lamin A of the GST-CaLB fusion protein. As shown in Figure 7, if the nitrocellulose sheets, prior to be subjected to the overlay assays, were incubated for 30 min at room temperature with the peptide 200–217,

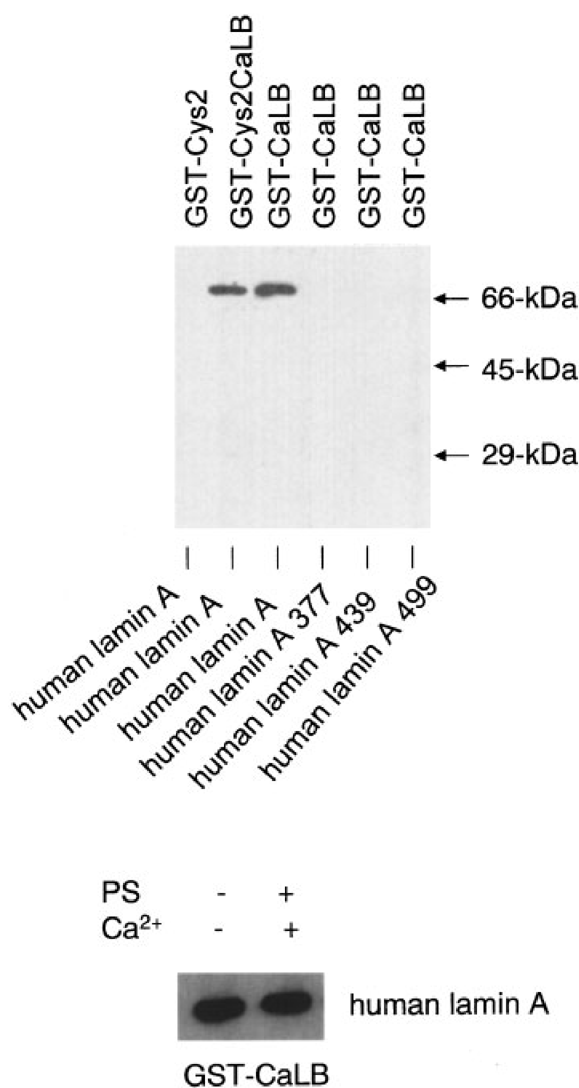

Fig. 4. Lamin A binds the CaLB domain (amino acid 172–240) of rat PKC-α. **Upper panel**: Recombinant human lamin A (either full length or truncated) was blotted to nitrocellulose sheets. Sheets were then subjected to overlay assays in the presence of the fusion proteins (10 μM) without PKC cofactors. **Lower panel**: GST-CaLB fusion protein binds recombinant human lamin A independently from the presence of PKC cofactors.

subsequent binding of GST-CaLB domain fusion protein to lamin A was precluded. As a control, we repeated these experiments with another synthetic peptide, corresponding to amino acid 217–234. In this case, the binding of the fusion protein to lamin A was not blocked at all (Fig. 7).

DISCUSSION

In the present study, we have identified the elements of the PKC-α that are important for

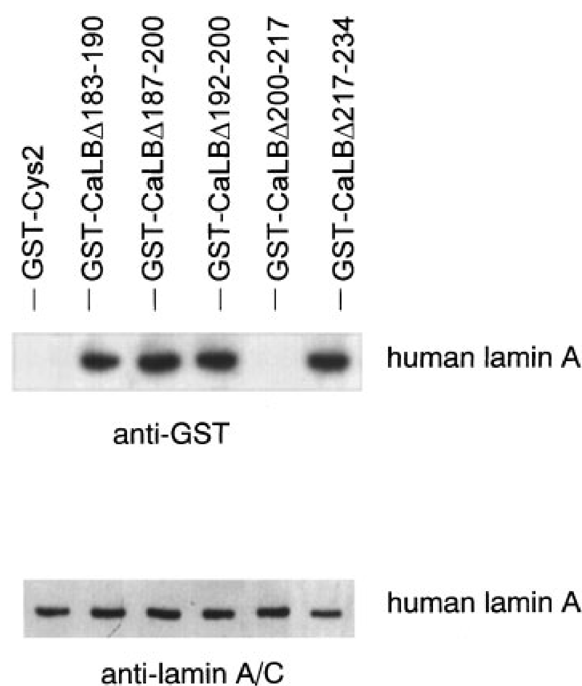

Fig. 5. Identification of the amino acidic sequence in the CaLB domain of rat PKC-α which interacts with lamin A. Recombinant human lamin A was blotted to nitrocellulose sheets that were then subjected to overlay assay in the presence of a series of GST-CaLB domain fusion proteins, in which the indicated amino acid stretches had been deleted.

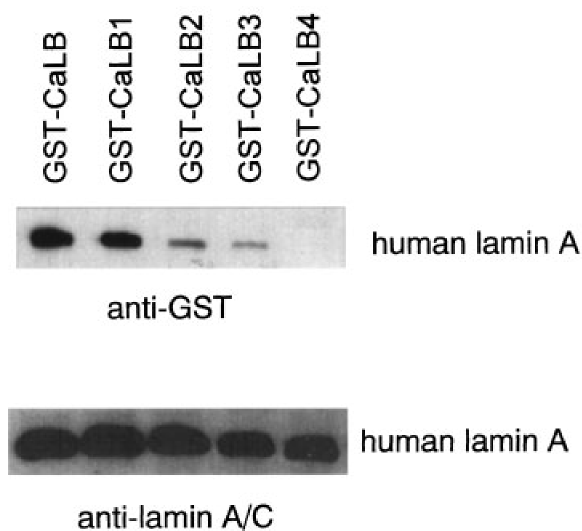

Fig. 6. Lysine residues in the stretch of amino acid 200–217 are fundamental for binding of CaLB to lamin A. Recombinant human lamin A was blotted to nitrocellulose sheets that were then subjected to overlay assay in the presence of a series of GST-CaLB domain fusion proteins, in which lysine residues had been replaced with isoleucine. GST-CaLB1: K205 replaced; GST-CaLB2: K205 and K209 replaced; GST-CaLB3: K205, K209, and K211 replaced; GST-CaLB4: K205, K209, K211, and K213 replaced.

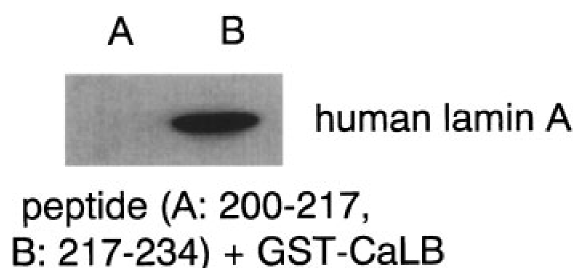

Fig. 7. A synthetic peptide corresponding to amino acid 200–217 prevents the binding of GST-CaLB domain fusion protein to lamin A. Recombinant human lamin A was blotted to nitrocellulose sheets that were then incubated at room temperature for 30 min in the presence of 10 μM of the synthetic peptides LIPDPKNESKQKTKTIRS or STNLPQWNESFTFKLKPS. Then, the overlay assay was carried on with a GST-CaLB domain fusion protein (10 μM), followed by incubation with anti-GST antibody and ECL detection.

binding lamin A. Our findings indicate that both the C2 and the V5 region of PKC-α bind lamin A. As far as the C2 region is concerned, we have identified a particular stretch of amino acids (200–217), present in the CaLB domain, which is essential for the binding. This conclusion is also supported by the fact that a synthetic peptide, corresponding to this stretch of amino acids, blocked the interaction. This particular stretch of amino acids is enriched in lysine residues (four lysines) and also contains an arginine residue. In the other stretches of amino acids we deleted from the CaLB domain, only in that comprised between amino acid 271–234 there are basic residues (two lysines). By deleting these basic residues from stretch 200–217, we have determined that these amino acids are indeed essential for the binding to occur. Indeed, mutation of all four lysine residues to isoleucine abrogated binding. Mutation of only one lysine (K205) did not affect binding, whereas mutation of two or three lysines decreased the binding. It may be that these basic amino acids are very important for binding through electrostatic interactions with lamin A.

In this context, it should be emphasized that our data are in agreement with the results of others who have investigated other PKC-binding proteins. Indeed, the RACK-1 binding site for PKC-βII is located in the C2 region of the kinase, although in this case, there are multiple stretches of amino acids involved in the interactions [Ron et al., 1995]. Fragments of the synaptic vesicle-specific protein p65 synaptotagmin that are homologous to C2 region of PKC bind RACKs [Mochly-Rosen et al., 1992]. Moreover, it has very recently been shown that the C2-like domain of PKC-δ is a binding site for actin in neutrophils, where it enables F-actin redistribution [Lopez-Lluch et al., 2001].

However, we found that also the V5 region of PKC-α binds lamin A. In this connection, it is worth reminding that Stebbins and Mochly-Rosen [2001] have demonstrated—the V5 region of PKC-βII contains part of the binding site for RACK-1. The sequence of PKC-βII, which binds RACK1 corresponds to amino acid 645–650 (QEVIRN). However, the V5 domain of PKC-α does not possess a similar sequence. The V5 region of PKC-α interacts with PICK1, a PKC-binding protein, through PDZ domain-like interaction [Staudinger et al., 1997]. The PDZ-binding domain of PKC-α (QSAV) is located at the extreme carboxyl-terminus of the kinase. We are presently trying to identify the sequence of the V5 domain, which is essential for binding lamin A. Since the C2 region of PKC is a conserved region among the various conventional isoforms, it is conceivable that the existence of an additional binding site located in a variable region of the kinase, such as V5, should confer the lamin A-binding specificity. Indeed, our unpublished results indicate that the V5 regions of either PKC-βI or-βII do not bind lamin A.

The interaction between lamin A and the C2 or V5 region of PKC-α occurred independently of the presence of cofactors, such as PS and Ca^{2+}. In a previous investigation, in which we used recombinant PKC-α to identify nuclear PKC-binding proteins [Martelli et al., 2000], we employed these cofactors during the overlay assays. We have found that PS and Ca^{2+} are indispensable for binding of PKC-α whole molecule to lamin A (G. Tabellini, unpublished results), but they are not necessary when elements of PKC-α are employed in overlay assays. Therefore, our findings agree with those of other investigators [Ron et al., 1995] and suggest that PKC cofactors are only required to expose lamin A binding sites in the intact PKC-α.

Human and mouse lamin A are substrates for PKC [Eggert et al., 1993; Haas and Jost, 1993]. However, three truncated forms of lamin A did not bind the CaLB domain of PKC-α. Thus, the interaction we detected is not in the helical rod-like section of the lamin, but rather in its carboxyl-terminal section. However, this interaction could not be related to the physiologically relevant PKC phosphorylation sites, because

a lamin A truncated at amino acid 499 still did not bind the CaLB domain. In particular, PKC phosphorylates lamin A on two serine residues that are serine 403 and 404 [Leukel and Jost, 1995].

An important issue is that the intranuclear distribution of PKC-α translocated to the nucleus in Swiss 3T3 cells treated with insulin-like growth factor-1, when studied by immunofluorescence staining, is rather diffuse [Neri et al., 1998; Martelli et al., 2000], whereas antibodies to lamins usually identify a ring at the nuclear periphery [e.g., Pugh et al., 1997], even though some intranuclear foci are also evident [Moir et al., 1994; Neri et al., 1999]. Usually, the distribution of PKC-binding proteins correspond to the distribution of the translocated PKC isoform [Ron et al., 1995; Lopez-Lluch et al., 2001]. This may be a further indication that other important PKC-binding proteins exist within the nucleus that are yet to be identified.

As far as we know, this is the first detailed investigation aimed at defining the interactions between PKC-α and lamin A. Lamin phosphorylation by PKC has been linked with both cell proliferation and apoptosis [Thompson and Fields, 1996; Shimizu et al., 1998]. Aberrations in growth factor signaling pathways, regulating cell proliferation and apoptosis, are strongly connected with developmental abnormalities and a variety of chronic diseases, chief of which is cancer [reviewed in Reed, 2000]. At present, PKC isoforms are regarded as very attractive targets for antineoplastic treatment. A serious problem of this therapeutic approach is the lack of PKC inhibitors with selectivity towards specific isozymes.

A body of evidence indicates that PKC isoforms located at the nuclear level may play a role in carcinogenesis [Martelli et al., 1999]. Therefore, a better understanding of the complex protein–protein interactions, involving PKC isoforms at the nuclear level, should allow the full elucidation of isozyme specific-signaling in this peculiar cell compartment and should also help to rationally design isoform-specific pharmacological tools [Hundle et al., 1997] to be employed in the therapy of various types of cancer.

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