Mapping of a Molecular Determinant for Protein Kinase C \(\beta_{II}\) Isozyme Function*

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In human erythroleukemia (K562) cells, the highly related protein kinase C (PKC) \(\alpha\) and PKC \(\beta_{II}\) isozymes serve distinct functions in cellular differentiation and proliferation, respectively. Previous studies using two domain switch PKC chimera revealed that the catalytic domains of PKC \(\alpha\) and \(\beta_{II}\) contain molecular determinants important for isozyme-specific function (Walker, S. D., Murray, N. R., Burns, D. J., and Fields, A. P. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 9156–9160). We have now analyzed a panel of PKC chimeras to determine the specific region within the catalytic domain important for PKC \(\beta_{II}\) function. A cellular assay for PKC \(\beta_{II}\) function was devised based on the finding that PKC \(\beta_{II}\) selectively translocates to the nucleus and phosphorylates nuclear lamin B in response to the PKC activator bryostatin. This response is strictly dependent upon expression of PKC \(\beta_{II}\) or a PKC chimera that functions like PKC \(\beta_{II}\). We demonstrate that a PKC \(\alpha/\beta_{II}\) chimera containing only the carboxyl-terminal 13 amino acids from PKC \(\beta_{II}\) (\(\beta_{II}\) V5) is capable of nuclear translocation and lamin B phosphorylation. These results are consistent with our recent observation that the PKC \(\beta_{II}\) V5 region binds to phosphatidylglycerol (PG), a potent and selective PKC \(\beta_{II}\) activator present in the nuclear membrane (Murray, N. R., and Fields, A. P. (1998) J. Biol. Chem. 273, 11514–11520). Soluble \(\beta_{II}\) V5 peptide selectively inhibits PG-stimulated PKC \(\beta_{II}\) activity in a dose-dependent fashion, indicating that PG-mediated activation of PKC \(\beta_{II}\) involves interactions with the \(\beta_{II}\) V5 region of the enzyme. We conclude that \(\beta_{II}\) V5 is a major determinant for PKC \(\beta_{II}\) nuclear function and suggest a model in which binding of PG to the \(\beta_{II}\) V5 region stimulates nuclear PKC \(\beta_{II}\) activity during G2 phase of the cell cycle.

Protein kinase C (PKC) is a family of serine/threonine kinases that play crucial roles in various signaling pathways, including cellular proliferation and differentiation (1–4). Molecular cloning studies have shown that the PKC family has at least 12 distinct members classified into three groups according to their structure, calcium requirement, and cofactor dependence (5, 6). The fact that PKC isotypes exhibit different patterns of tissue expression, subcellular localization, and activation/substrate specificity implies that there is functional diversity among isotypes (6–10). Previous studies demonstrated that PKC \(\alpha\) and \(\beta_{II}\) are involved in the differentiation and proliferation of a variety of cell types (3, 10). In human erythroleukemia (K562) cells, we determined that PKC \(\alpha\) is involved in PMA-induced cytotasis and megakaryocytic differentiation (10). PKC \(\beta_{II}\) on the other hand, is required for K562 cell proliferation (10). PKC \(\beta_{II}\) selectively translocates to the nucleus during the G2/M phase transition of cell cycle and leads to direct phosphorylation of the nuclear envelope poly peptide lamin B at mitosis-specific sites involved in mitotic nuclear lamina disassembly (9, 11–15).

In order to study the molecular basis of PKC \(\alpha\) and \(\beta_{II}\) isozyme function, we expressed two domain switch chimeras between the regulatory and catalytic domains of PKC \(\alpha\) and \(\beta_{II}\) in K562 cells (16). These chimeras demonstrated that the catalytic domains of PKC \(\alpha\) and \(\beta_{II}\) contain determinants that are critical for isozyme-specific function (16). Specifically, a PKC \(\beta_{II}/\alpha\) chimera, consisting of the regulatory domain of PKC \(\beta_{II}\) and the catalytic domain of PKC \(\alpha\), exhibited a phenotype resembling wild type PKC \(\alpha\) (16). Conversely, a PKC \(\alpha/\beta_{II}\) chimera, composed of the regulatory domain of PKC \(\alpha\) and the catalytic domain of PKC \(\beta_{II}\), behaved like wild type PKC \(\beta_{II}\) (16).

Based upon these findings, we wished to define the isozyme-specific determinants within the catalytic domain of PKC \(\beta_{II}\). For this purpose, we constructed and expressed a series of \(\alpha/\beta_{II}\) PKC chimeras in which the variable and constant regions within the catalytic domain of PKC \(\beta_{II}\) were replaced by the corresponding PKC \(\alpha\) regions. Our results demonstrate that the V5 region of PKC \(\beta_{II}\) (\(\beta_{II}\) V5), consisting of the carboxy-terminal 13 amino acids of PKC \(\beta_{II}\), contains the molecular determinant necessary for nuclear translocation and activation of the enzyme. These results are interesting, in light of our recent studies demonstrating that the V5 region of PKC \(\beta_{II}\) binds phosphatidylglycerol (PG), a nuclear activator of the enzyme (17). A soluble peptide corresponding to \(\beta_{II}\) V5 inhibits PG-stimulated PKC \(\beta_{II}\) activity, indicating that interactions between PG and the \(\beta_{II}\) V5 region are important for PG-mediated activation of PKC \(\beta_{II}\). Based on these observations, we propose a model for the cell cycle-regulated activation of nuclear PKC \(\beta_{II}\).

EXPERIMENTAL PROCEDURES

Construction of PKC Chimeras—In previous work, we demonstrated that a chimera consisting of the regulatory domain of PKC \(\alpha\) (\(\alpha\)V1–V3) and the catalytic domain of PKC \(\beta_{II}\) (\(\beta_{II}\) C3–V5) behaved like PKC \(\beta_{II}\) (16). Therefore, we constructed PKC \(\alpha/\beta_{II}\) chimeras in which each of the constant and variable regions within the catalytic domain of this chimera was replaced with the corresponding PKC \(\alpha\) sequence (Fig. 1).
PKC chimeras were constructed by two-step polymerase chain reaction (PCR) as described previously (16). The first step in the chimera construction was to amplify the desired PKC α and PKC β portions of the chimera using the appropriate 5' and 3' end primers, internal chimeric primers, and either PKC α or PKC β cDNA as a template. The internal primers were constructed so that they would anneal in the second PCR step. Products from the first PCR step were gel-purified and combined, along with 5' and 3' end primers, and the complete chimera produced by extension and amplification. The primers used for construction of the chimeras are presented in Table I. The end primers contained restriction sites (5' KpnI and 3' Nhel) to facilitate subsequent cloning. Completed chimeras were gel-purified and cloned into the TA PCR cloning vector pCR 2.1 (Invitrogen). The chimeras were restricted from pCR 2.1 with KpnI and Nhel and ligated into the KpnI and Nhel sites within the multiple cloning site of the episomal expression vector pREP4 (Invitrogen).

**Transfection and Expression of PKC Chimeras in K562 Cells—**Human erythroleukemia K562 cells (ATCC) were maintained in suspension culture as described previously (10). Cells were transfected with the pREP4 plasmids containing the PKC chimera constructs using the manufacturer's protocol. 24 h after transfection, fresh medium containing 100 μg/ml hygromycin B. Expression of PKC chimeras was determined by immunoblot analysis as described previously (10). The chimeric nature of the chimeras was confirmed by immunoblot analysis using isozyme-specific antibodies against the V5 regions of PKC α and β (14). For immunoblotting, K562 cell transfecteds were washed with cold phosphate-buffered saline, sonicated for 30 s in SDS sample buffer (18), and boiled for 5 min. Total cell extracts from 10^5 cells were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and subjected to immunoblot analysis as described previously (10). The chimeric nature of the chimeras was confirmed by immunoblot analysis using isoantibody-specific antibodies against the V5 region of PKC α and β as described previously (16).

**Drug Treatment, Isolation of Nuclear Envelopes, and Lamin B Phosphorylation—**K562 cell transfecteds carrying either control vector (pREP4) or PKC chimera-containing pREP4 vectors were treated with 30 μM PMA (LC Laboratories) or diluent (0.1% MeSO). Previous studies demonstrated that this PMA treatment leads to loss of immunologically detectable PKC βII and loss of bryostatin-mediated lamin B phosphorylation (10, 16). Following the 48-h PMA treatment, total cell lysates were prepared from an aliquot of cells and subjected to immunoblot analysis using isotype-specific PKC α V5 and βII V5 antibodies as described above. The remainder of the cells were incubated in the presence and absence of bryostatin 1 (100 nM, LC Laboratories) for 30 min at 37 °C, followed by nuclear envelope isolation and determination of lamin B phosphorylation as described previously (16).

**Assay of Protein Kinase C βII Activity in Vitro—**In vitro protein kinase C assays were carried out using purified baculovirus-expressed human PKC βII as described previously (15). Briefly, reactions were performed in assay buffer containing 50 mM Tris-HCl, pH 7.5, 10 mM MgSO4, 1 mM diithiothreitol, 100 μM CaCl2, 100 μM ATP, 2 μCi of [γ-32P]ATP (Amersham Pharmacia Biotech), 40 μg/ml (50 μM) phospha-tidylinositol (PS, Avanti Polar Lipids), 20 μg dioctanoylglycerol (DAG, Avanti Polar Lipids), and 10 μg histone H1 for 15 min at 25 °C. In most reactions, 250 μg/ml (315 μM) diethylene glycol (PG) (Avanti Polar Lipids) was added to maximally stimulate PKC βII activity (17). Synthetic peptides corresponding to the carboxyl-terminal regions of PKC V5 (CQKIVSHIQSV) were added at the concentrations indicated in the figure legends. Histone H1 phosphorylation was quantitated using phosphorimaging analysis as described previously (12).

**RESULTS**

**Construction and Expression of PKC Chimeras in K562 Cells—**Based on our previous finding that the catalytic domain of PKC βII is crucial for PKC βII function (16), we generated a series of PKC chimeras in which the variable and constant regions within the catalytic domain of PKC βII were exchanged with the corresponding sequences from PKC α by two-step PCR as depicted in Fig. 1. To investigate the biochemical properties of these chimeras in intact K562 cells, chimeric PKC constructs were subcloned into the pREP4 episomal expression vector and transfected into K562 cells. Expression of the PKC chimeras was confirmed by immunoblot analysis with antibodies against the carboxyl-terminal V5 regions of PKC α and βII (Fig. 2). Expression of each of the chimeras containing the βII V5 region (βII V4, βII C4, βII V5, βII 1504, and βII 1851) was confirmed using the PKC βII V5 antibody (Fig. 2A). Expression of the α βII chimera, which contains the carboxyl-terminal V5 region from PKC α, was confirmed using the PKC α V5 antibody (Fig. 2B). In each case, a band with apparent molecular mass of 85 kDa was detected, corresponding in size to intact PKC α and βII protein. The level of expression for all chimeras was determined by densitometric analysis to be between 2- and 3-fold that of the endogenous levels of PKC α or PKC βII. The chimeric nature of each of the chimeras was confirmed by immunoblot analysis using the appropriate PKC α and βII-specific V3 antibodies as described previously (Ref. 16; data not shown).

**PKC Chimaera Expression Persists after PMA Treatment—** Our previous studies demonstrated that treatment of K562 cells with PMA for 48 h leads to an increase in PKC α expression and a loss of PKC βII expression (10). We therefore wished to assess the fate of PKC chimeras expressed in K562 cells following treatment with PMA. For this purpose, K562 cell transfecteds were treated with PMA (30 nM) for 48 h. Transfectants were harvested and immunoblotted as described under “Experimental Procedures” (Fig. 3). As expected, endogenous PKC βII expression was dramatically reduced as a consequence...
lysates from 1 x 10^6 K562 cells, transfected with either control vector or PKC chimera vectors were treated with 30 nM PMA for 48 h. Following 48 h treatment, total cell lysates from 1 x 10^6 cells were immunoblotted with antibody against the V5 region of PKC βII as described under “Experimental Procedures.” Lane Control (−PMA), pREP4 vector in the absence of PMA; lane Control (+PMA), pREP4 in the presence of PMA; lanes βII V4, βII C4, αβIIα, βII 1504, βII 1851, and βII V5, PKC chimeras after PMA treatment. Panel B, immunoblot analysis as described under “Experimental Procedures” with antibody against the V5 region of PKC α. Lanes: controls are the same as in A; αβIIα, PKC chimera after PMA treatment.

Fig. 2. Expression of PKC chimeras in K562 cells. Total cell lysates from 1 x 10^6 K562 cells, transfected with vector containing the indicated PKC chimera, were subjected to immunoblot analysis as described under “Experimental Procedures.” Panel A, immunoblot detection using isotype-specific antibody against the V5 region of PKC βII. Cell transfectants expressing the following expression vector constructs were subjected to immunoblot analysis: pREP4 control vector (−Control), wild type PKC βII (+Control), βII V4, βII C4, βII V5, αβIIα, βII 1504, and βII 1851 PKC chimeras. Panel B, immunoblot detection using isotype-specific antibody against the V5 region PKC α. Lane −Control, pREP4 vector; lane +Control, wild type PKC α; αβIIα, PKC chimera.

of PMA treatment (Fig. 3A, compare lanes 1 and 2) (10, 16). In contrast, the levels of PKC α were increased as previously reported (10, 16) (Fig. 3B, compare lanes 1 and 2). Interestingly, in contrast to endogenous PKC βII, the expression of each of the transfected PKC chimeras (βII V4, βII C4, βII 1504, βII 1851, βII V5, and αβIIα) persisted after PMA treatment (Fig. 3A and B).

Ability of PKC Chimeras to Reconstitute PKC βII Function—The observation that expression of PKC chimera persists after chronic PMA treatment while endogenous PKC βII levels are dramatically reduced suggested a strategy to specifically determine whether the transfected PKC chimeras could function like PKC βII in intact K562 cells without the contribution of endogenous PKC βII. This strategy is predicated upon several key observations. First, PKC βII is selectively translocated and activated at the cell nucleus in response to bryostatin but not PMA (9, 11, 14). Second, bryostatin-mediated translocation of PKC βII to the nucleus leads to direct phosphorylation of the nuclear envelope polypeptide lamin B (11–15). Third, the ability of bryostatin to stimulate nuclear lamin B phosphorylation is strictly dependent upon the expression of PKC βII (16). Thus, when K562 cells are treated with PMA, both PKC βII expression and bryostatin-mediated lamin B phosphorylation is lost (16). Therefore, we assessed the ability of the transfected PKC chimeras to reconstitute bryostatin-mediated lamin B phosphorylation in PMA-treated K562 cell transfectants as described under “Experimental Procedures” (Fig. 4). Treatment of K562 cells containing an empty control vector with PMA leads to the expected loss in bryostatin-mediated lamin B phosphorylation, whereas cells not treated with PMA exhibited robust lamin B phosphorylation in response to bryostatin (Fig. 4, compare panels labeled Control (−PMA) and Control (+PMA). K562 cells expressing the βII V4, βII C4, βII 1504, βII 1851, and βII V5 chimeras were all capable of reconstituting bryostatin-mediated lamin B phosphorylation after treatment with PMA. In contrast, cells expressing the αβIIα chimera were incapable of reconstituting the bryostatin-mediated response. From these results, we conclude that chimeras containing the functional determinant important for PKC βII function are capable of reconstituting PKC βII-dependent lamin B phosphorylation. Furthermore, our analysis localizes this functional determinant to the extreme carboxyl-terminal 13 amino acids of PKC βII (βII V5), since a chimera containing only these 13 amino acid residues from PKC βII (PKC βII V5) is capable of functioning in this PKC βII-selective pathway.

PG-stimulated PKC βII Activity Involves Interactions with the V5 Region of PKC βII—The results from the PKC chimera studies demonstrate that the carboxyl-terminal V5 region of PKC βII represents a molecular determinant for PKC βII-selective function in intact K562 cells. In recent studies, we identified a component of the nuclear membrane, termed nuclear membrane activation factor (NMAF), which selectively acti-
vates PKC βII (19). More recently, we identified NMAF as PG (17). PG was found to be a potent and selective activator of PKC βII, stimulating the enzyme 3–5-fold above the level of activity seen in the presence of optimal concentrations of PS, DAG, and calcium (19). We also demonstrated that PG binds selectively and saturably to the carboxyl-terminal region of PKC βII (17), suggesting that PG stimulates PKC βII activity through interactions involving βII V5. To specifically test this hypothesis, we assessed the effect of soluble βII V5 peptide on PG-stimulated PKC βII activity in vitro (Fig. 5). As reported previously (17) addition of 315 μM PG stimulates PKC βII more than 4-fold over the level in the absence of PG. This stimulation is specific for PG since it is not observed in the presence of 370 μM PS (data not shown). Inclusion of βII V5 peptide in the kinase assay leads to inhibition of PG-stimulated PKC βII histone kinase activity (Fig. 5A, lane 5). Inhibition is specific for the βII V5 peptide, since no inhibition is observed using the corresponding V5 peptide from PKC α (Fig. 5A, lane 4). Inhibition by βII V5 peptide is dose-dependent with an apparent IC₅₀ of ~100 μM (Fig. 5B), whereas significant inhibition was not observed with the corresponding α V5 peptide. Interestingly, the βII V5 peptide inhibits only the PG-stimulated component of PKC βII activity and not calcium-, DAG-, and PS-stimulated activity. These results are consistent with the conclusion that PG mediates activation through the carboxyl-terminal V5 region of PKC βII, whereas calcium, DAG, and PS mediate activation by binding to the C1 and C2 regions within the regulatory domain of the enzyme.

**DISCUSSION**

The PKC family of serine/threonine kinases serves critical roles in cellular function. Recent studies have begun to elucidate specific roles for individual PKC isoforms (1, 3, 5). We have identified roles for the three major PKC isoforms in human K562 leukemia cells. K562 cells express PKC α, βII, and γ, and each of these isoforms serves distinct functions in these cells. PKC α is important in cellular differentiation (10), PKC βII is required for cellular proliferation (10) and PKC γ plays a critical role in leukemia cell survival and resistance to apoptosis (20). However, despite our knowledge of the importance of these isoforms in cellular physiology, less is known about the specific pathways in which these isoforms function, the relevant cellular targets of their action, and the mechanisms by which they maintain isozyme identity within the intact cell. We have addressed these questions in the case of PKC βII. PKC βII is required for cell cycle progression through the G₂/M phase transition (12, 13). PKC βII is activated at the nucleus during late G₂ phase just prior to mitosis in synchronized cells (12, 13). At the nucleus, PKC βII mediates direct phosphorylation of the nuclear envelope polypeptide lamin B on sites involved in mitotic nuclear lamina disassembly (12, 13, 15). Inhibition of PKC βII activation leads to cell cycle arrest in G₂ phase, demonstrating the importance of nuclear PKC βII activation and lamin B phosphorylation in entry into mitosis (13).

Nuclear PKC βII activation is regulated by several factors. First, a nuclear phosphoinositide-specific phospholipase C activity generates a peak of diacylglycerol at the nuclear membrane during G₂ phase that serves to activate nuclear PKC βII (21). Second, the nuclear membrane contains a potent activator of PKC βII that stimulates its activity above the level observed in the presence of optimal calcium, DAG, and PS (17, 19). This activator, originally termed NMAF, was recently identified as the phospholipid PG (17). The selectivity of PG for PKC βII activation suggested that PG might function through interactions within the carboxyl-terminal V5 region of PKC βII. Indeed, we have demonstrated that the carboxyl-terminal region of PKC βII binds selectively and saturably to PG-containing vesicles, but not to vesicles containing other phospholipids (17).

In the present study, we wished to determine the molecular determinants on PKC βII that allow it to translocate to the nucleus and phosphorylate lamin B in intact cells, a process that we have demonstrated requires PKC βII expression (16). We previously demonstrated that a chimera between PKC α

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**Fig. 4.** PKC chimeras can reconstitute lamin B phosphorylation. K562 cells transfected with either control vector or the indicated PKC chimeras were harvested following 48 h PMA treatment. After washing to remove the PMA, cells were incubated without or with bryo (bryo, 100 nM) for 30 min and assayed for lamin B phosphorylation as described previously (16). The labels are the same as described in Fig. 3. Results are representative of three independent experiments.

**Fig. 5.** PG-stimulated PKC βII activity is inhibited by the βII V5 peptide in a dose-dependent manner. Recombinant human PKC βII was incubated in kinase buffer in the absence or presence of the indicated concentration of either βII V5 or αV5 peptide for 15 min at 25 °C as described under “Experimental Procedures.” Activity was monitored by incorporation of [³²P] into purified histone H1 (10 μg) as described previously (12). **Panel A,** autoradiograph of PKC βII-mediated histone H1 phosphorylation by PKC βII. Reactions were as follows. Lane 1, no PKC βII added; lane 2, PKC βII in reaction buffer without PG; lane 3, PKC βII in reaction buffer with PG; lane 4, PKC βII in reaction buffer containing PG and 1 mM αV5 peptide; lane 5, PKC βII in reaction buffer containing PG and 1 mM βII V5 peptide. Data are from a representative experiment. **Panel B,** dose-dependent inhibition of PG-stimulated PKC βII activity with either βII V5 (open circles) or αV5 (closed circles) peptide. Results are plotted as the percentage of the PG-stimulated PKC βII activity versus peptide concentration (μM). Data represent the mean of five determinations ± S.D. Some error bars are masked by the data point symbols.
and PKC \(\beta_{II}\) containing the catalytic domain of PKC \(\beta_{II}\) was capable of translocating to the nucleus and phosphorylating lamin B (16). In contrast, a chimera containing the catalytic domain of PKC \(\alpha\) was not capable of mediating this response (16). From these data, we concluded that regions within the catalytic domain of PKC \(\beta_{II}\) serve as a molecular determinant of PKC \(\beta_{II}\) function (16). We have now used further PKC \(\alpha/\beta_{II}\) chimeras to map this molecular determinant to the extreme carboxy-terminal 13 amino acids of PKC \(\beta_{II}\). The results of this study are interesting in light of our recent finding that the V5 region of PKC \(\beta_{II}\) mediates binding to PG (17). In fact, we now demonstrate that a \(\beta_{II}\) V5 peptide selectively inhibits PG-stimulated PKC \(\beta_{II}\) activity in a dose-dependent fashion. These results indicate that interactions between PG and \(\beta_{II}\) V5 are important for PG-stimulated PKC \(\beta_{II}\) activity.

The identification of the carboxy-terminal region of PKC \(\beta_{II}\) as an important region regulating isozyme specific function is consistent with recent studies suggesting the importance of this region to PKC function. The V5 region of PKC \(\alpha\) has recently been shown to interact with PICK 1, a PKC-binding protein, through PDZ domain-like interactions (22). PICK1 has been suggested to play a role in targeting PKC \(\alpha\) to appropriate intracellular sites where it can transduce isozyme-specific signals (22). Likewise, we have demonstrated that interaction of the V5 region of PKC \(\beta_{II}\), in this case with the phospholipid PG, is important for the nuclear translocation and activation of the enzyme at the nuclear membrane, where it is required for nuclear lamina disassembly and entry into mitosis (17). Finally, studies investigating the differential regulation of PKC \(\beta_{II}\) and \(\beta_{II}\) activity by calcium have led to the suggestion that the V5 region of PKC \(\beta_{II}\) interacts with the C2 region of the enzyme to influence calcium- and PS-mediated enzyme activation (23).

Given our recent data on nuclear activation of PKC \(\beta_{II}\), the proposed interdomain interactions involving the C2 and V5 regions of PKC \(\beta_{II}\), and related studies on the mechanism of PKC membrane translocation and activation (24), we propose a working model for the cell cycle-regulated, nuclear activation of PKC \(\beta_{II}\) as illustrated in Fig. 6. Soluble PKC \(\beta_{II}\) is stably phosphorylated on at least three known sites (24, 25). First, PKC \(\beta_{II}\) is phosphorylated by a putative protein kinase C kinase at threonine 500 in the activation loop of the enzyme (24, 25). Phosphorylation at threonine 500 makes PKC \(\beta_{II}\) catalytically competent and triggers subsequent autophosphorylation of threonine 641 and serine 660 in the carboxy-terminal region of the enzyme. Phosphorylation of these sites stabilizes the catalytically competent conformation of the enzyme and allows its release into the cytosol (24, 25). In our model, a nuclear PI-PLC isozyme(s), whose activity is linked to cell cycle by an unknown mechanism, is activated during the G2 phase of cell cycle (21). Activation of nuclear PI-PLC leads to generation of inositol trisphosphate (IP3) and DAG. We propose that IP3 binds to nuclear IP3 receptors to mobilize intracellular calcium stores, leading to a rise in intracellular calcium concentrations. It has been demonstrated that nuclei contain functional IP3 receptors (26) and that intracellular calcium concentrations rise in synchronized cells just prior to mitosis (27–29). Furthermore, inhibition of this rise in intracellular calcium, using calcium chelators such as BAPTA, leads to cell cycle arrest in G2 phase, demonstrating the importance of calcium in entry into mitosis (29). We propose that one function of elevated calcium levels is to translocate PKC \(\beta_{II}\) to the inner nuclear membrane. We have not directly determined whether this step involves translocation of PKC \(\beta_{II}\) from cytosolic pools, or alternatively involves translocation of inactive PKC \(\beta_{II}\) from a nucleoplasmic pool. However, based on our fractionation studies (9, 11–15) and the immunofluorescence studies of others in cultured myocytes (30), we favor the hypothesis that this step represents translocation of cytosolic PKC \(\beta_{II}\) to the nucleus. As has been previously demonstrated, binding of calcium to PKC increases its membrane affinity leading to membrane translocation and an increase in affinity of the enzyme for PS (31–33). Membrane bound PKC coordinates multiple PS molecules, possibly within the C2 region of the enzyme (34, 35). It has been demonstrated that PKC induces calcium-dependent clustering of acidic phospholipids into microdomains that may facilitate cooperative binding of phospholipid to the C2 domain (36). We propose that nuclear PG clutches with PS during this phase of nuclear membrane binding. In the presence of DAG, PKC activation occurs, through displacement of the
pseudosubstrate domain from the active site of the enzyme (37). It has recently been proposed that the V5 region of PKC \( \beta_{II} \) interacts with the C2 region of the enzyme when it is in its active conformation (23). We further propose that these V5-C2 interdomain interactions are mediated, at least in part, by binding of the V5 region to PG that is clustered in the same nuclear membrane microdomains containing the PS involved in membrane/C2 domain interactions. Once optimally activated at the inner nuclear membrane, PKC \( \beta_{II} \) directly phosphorylates lamin B at Ser-405 within its carboxyl-terminal activated at the inner nuclear membrane, PKC \( \beta_{II} \) directly phosphorylates lamin B at Ser-405 within its carboxyl-terminal globular domain (12, 15). Lamin B is an ideal substrate for PKC since it is intimately associated with the inner nuclear membrane surface by virtue of its carboxyl-terminal isoprenyl groups (38). Phosphorylation of lamin B by PKC, and p34 cdc2/cyclin B kinase, leads to nuclear lamina disassembly, a process required for entry into mitosis (38). Current studies are focused on determining the identity and mechanism of the cell cycle regulation of nuclear PI-PLC II since it is intimately associated with the inner nuclear membrane surface by virtue of its carboxyl-terminal isoprenyl groups (38). It has recently been proposed that the V5 region of PKC \( \beta_{II} \) participates, along with the C1 and C2 domains, in regulating nuclear PKC \( \beta_{II} \) activity.

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