Role of nuclear protein kinase C in the mitogenic response to platelet-derived growth factor

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Summary

We have assessed the involvement of nuclear envelope protein phosphorylation in the mitogenic response to platelet-derived growth factor (PDGF) in NIH/3T3 fibroblasts. We find that stimulation of quiescent NIH/3T3 cells with PDGF or with the mitogenic protein kinase C (PKC) activators phorbol 12-myristate 13-acetate (PMA) or bryostatin 1 (bryo) leads to rapid, dose-dependent phosphorylation of several nuclear envelope polypeptides. The predominant nuclear envelope targets for mitogen-induced phosphorylation are immunologically identified as the nuclear envelope lamins. All three lamin species (A, B and C) are phosphorylated in response to PMA or bryo, while lamins A and C are preferentially phosphorylated in response to PDGF. Phosphopeptide mapping and phosphoamino acid analysis indicate that similar serine sites on the lamins are phosphorylated in response to PDGF, PMA and bryo. Both mitogenicity and lamina phosphorylation induced by these mitogens can be inhibited by the selective PKC inhibitor staurosporine at 2 nM. Treatment of quiescent NIH/3T3 cells with PDGF, PMA or bryo leads to rapid translocation of PKC to the nuclear envelope. These data indicate that rapid nuclear events, including translocation of cytosolic PKC to the nuclear membrane and lamina phosphorylation, may play a role in the transduction of the mitogenic signals of PDGF from the cytoplasm to the nucleus in NIH/3T3 fibroblasts.

Key words: nuclear lamina phosphorylation, bryostatin 1, phorbol myristate acetate, NIH/3T3 fibroblasts.

Introduction

The process of cellular proliferation is a highly coordinated series of events that can be initiated by binding of a growth factor such as platelet-derived growth factor (PDGF) to its specific receptor on the cell surface. Growth factor binding often leads to the generation of second messenger molecules that propagate the proliferative signal. Two such second messenger molecules, cyclic AMP and diacylglycerol, work by activating protein kinases (cyclic AMP-dependent kinase and protein kinase C, respectively), suggesting a role for these cellular kinases and for protein phosphorylation in mitogenic stimulation (Nishizuka, 1986; Rozengurt, 1986). The observation that pharmacological activators of protein kinase C (PKC), including the tumor-promoting phorbol esters and synthetic diacylglycerols, can mimic the mitogenic response to PDGF further implicates PKC in the PDGF mitogenic response (Nishizuka, 1986; Fasti et al. 1986; Rozengurt, 1986). In addition, the PDGF receptor, like many other growth factor receptors, possesses tyrosine kinase activity that can be modulated by ligand binding and appears to be involved in mitogenic signalling (Bowen-Pope and Ross, 1982; Eck et al. 1982; Escobedo et al. 1988; Frackleton et al. 1984; Yarden et al. 1986). The mitogenic signal generated by PDGF at the plasma membrane must be propagated rapidly through the cytoplasm to the nucleus, where specific transcriptional events occur (Cochran et al. 1988), including activation of c-fos (Greenberg and Ziff, 1984; Kruijer et al. 1984; McCaffrey et al. 1987), c-myc (Armelin et al. 1984; Fricke et al. 1986; Kaibuchi et al. 1986; Kelly et al. 1983; McCaffrey et al. 1987) and ornithine decarboxylase (Hovis et al. 1986) gene expression. Despite our knowledge of many of the molecular details of the mitogenic response to PDGF, little is known about the mechanisms by which such cell surface signals reach the nucleus. Since several classes of cellular kinase appear to be involved in PDGF mitogenicity (Rozengurt, 1986), it has been suggested that propagation of these signals to the cell nucleus involves the activation of a specific kinase or kinases at the nuclear level. Indirect evidence using the tumor-promoting phorbol esters has suggested that PKC is involved in the nuclear mitogenic events induced by PDGF. For instance, phorbol esters, like PDGF, have been shown to induce activation of c-fos (Greenberg and Ziff, 1984) and c-myc (Kaibuchi et al. 1986) expression. Expression of these genes is under the control of regulatory elements that are responsive to the phorbol esters and PDGF (Muller et al. 1987). Two transcriptional factors that interact with these elements, c-jun and c-fos, are both phosphoproteins whose activity can be modulated by phosphorylation (Muller et al. 1987). However, it is not clear whether PKC acts directly on transcription factors at the nucleus or whether it is involved in a cascade of
proximal events that lead to activation of another kinase at the nucleus.

In the present report, we demonstrate that PDGF, phorbol 12-myristate 13-acetate (PMA) and bryostatin 1 (bryo), all potent mitogens, induce similar patterns of nuclear envelope phosphorylation in quiescent NIH/3T3 fibroblasts. The predominant nuclear envelope substrates for these mitogens are immunologically identified as the lamins A, B and C. Both nuclear envelope phosphorylation and mitogenicity induced by these mitogens can be inhibited by the selective PKC inhibitor staurosporine. Finally, all three mitogens induce rapid translocation of PKC to the nuclear envelope, the site of the observed phosphorylation events. These data support the hypothesis that specific, PDGF-induced translocation and activation of PKC at the nuclear envelope plays an important role in the mitogenic response to PDGF and suggest that lamina phosphorylation may be involved in the early transcriptional events that occur upon mitogenic stimulation.

Materials and methods

Cells and cell culture

NIH/3T3 fibroblasts were obtained from the American Type Culture Collection. Cells were maintained in DME medium supplemented with 10% fetal calf serum (FCS).

Mitogenicity assays

For mitogenicity studies, NIH/3T3 cells were plated into 96-well plates and allowed to grow to confluence. Confluent cells were washed twice with sterile phosphate-buffered saline (PBS) and incubated for 48–72 h in 200 μl of serum-free Dulbecco's modified Eagle's medium (DME) supplemented with 5 μg/ml transferrin and 10 mM Na<sub>2</sub>SO<sub>4</sub>. Mitogens were added to the cells at the indicated concentrations. PDGF (R & D Systems, Inc., Minneapolis, MN) was added from a 1 μg/ml stock in 100 mM acetic acid. PMA and bryo were added from stocks in dimethylsulfoxide (DMSO). The final concentration of DMSO was 0.1% (v/v). Staurosporine was added to cultures 24 h prior to addition of mitogens and remained present throughout the assay period. After 16 h, 1 μCi of [3H]thymidine (Amersham) was added to each well and incubation continued for an additional 6 h. Labelled cells were washed twice with cold PBS followed by two washes with cold 5% trichloroacetic acid (TCA). Cells were solubilized in 0.25 N NaOH and counted after addition of 10 ml of scintillation fluid. Points were counted in duplicate and results are expressed as cts/min of [3H]thymidine incorporated/well.

Metabolic labelling and treatment with mitogens

NIH/3T3 cells were grown to confluence in 100 mm tissue culture dishes in DME supplemented with 10% FCS. Confluent cultures were serum-deprived for 48–72 h by incubation with serum-free DME supplemented with 5 μg/ml transferrin and 10 mM Na<sub>2</sub>SO<sub>4</sub>. Serum-deprived cultures were equilibrated with 100 μCi ml<sup>−1</sup> of [32P]orthophosphate in serum-free DME for 1 h prior to addition of mitogens at the indicated concentrations. For inhibitor studies, staurosporine was added to the cultures 24 h prior to radiolabelling and addition of mitogens. The extent of nuclear lamina phosphorylation was determined by densitometric scanning of 32P autoradiographs of resolved nuclear envelope proteins or by direct counting of excised lamin protein bands from SDS–PAGE gels.

Isolation of nuclear envelopes, phosphopeptide mapping and phosphoamino acid analysis

Nuclear envelopes were isolated as previously described (Fields et al. 1988). All steps were performed at 4°C and all buffers contained 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 μg ml<sup>−1</sup> leupeptin, 0.1 mM sodium vanadate and 20 mM sodium fluoride. The cells were washed twice with cold PBS and scraped into cold lysis buffer (250 mM sucrose, 50 mM Tris–HCl, pH 7.4, 5 mM MgSO<sub>4</sub>, 0.1% β-mercaptoethanol (BME)). Cells were incubated in lysis buffer for 10 min, Nonidet P-40 was added to 0.02% (v/v) and the cells were homogenized in a glass Dounce homogenizer with an “A”-type pestle. All subsequent steps were performed as previously described (Fields et al. 1988). Phosphopeptide mapping and phosphoamino acid analysis were performed on 32P labelled lamins resolved by two-dimensional gel electrophoresis as previously described (Fields et al. 1988).

Gel electrophoresis and immunoblot analysis

SDS–PAGE, IEF/SDS–PAGE and immunoblotting were performed as described (Fields et al. 1988). Rabbit antiserum to the lamins was a gift from Dr L. Gerace. The PKC antiserum was used as previously described (Fields et al. 1989).

Results

PMA, bryo and PDGF induce phosphorylation of the nuclear envelope lamins

Recent studies have shown that the nuclear envelope lamins are targets for phosphorylation when cells are treated with a variety of mitogens including bryo (Fields et al. 1988, 1989), interleukin-3 (Fields et al. 1989) and insulin (Friedman and Ken, 1988). To determine whether the lamins are targets for phosphorylation in response to mitogenic stimuli in NIH/3T3 fibroblasts, quiescent, serum-deprived cells were equilibrated with 32P-orthophosphate and treated for 30 min with either PMA, bryo or PDGF. After treatment, nuclear envelopes were isolated and subjected to SDS–PAGE and autoradiography as described in Materials and methods. Coomassie Blue staining of electrophoretically resolved nuclear envelope preparations reveals the predominance of three polypeptide bands in the 60–70K (K=10<sup>3</sup> Mr) range (data not shown) consistent with the molecular weights of the three lamin species (A, B and C) expressed in this cell type. As seen in Fig. 1, quiescent cells exhibit a low level of nuclear envelope phosphorylation. In contrast, treatment of cells with either PMA, bryo or PDGF leads to increased phosphorylation of several nuclear envelope polypeptides. Prominent phosphorylated bands are observed at 82, 67 and 62K in response to PMA, bryo and PDGF. After treatment, nuclear envelopes were isolated and subjected to SDS–PAGE and autoradiography as described in Materials and methods. Coomassie Blue staining of electrophoretically resolved nuclear envelope preparations reveals the predominance of three polypeptide bands in the 60–70K (K=10<sup>3</sup> Mr) range (data not shown) consistent with the molecular weights of the three lamin species (A, B and C) expressed in this cell type. As seen in Fig. 1, quiescent cells exhibit a low level of nuclear envelope phosphorylation. In contrast, treatment of cells with either PMA, bryo or PDGF leads to increased phosphorylation of several nuclear envelope polypeptides. Prominent phosphorylated bands are observed at 82, 67 and 62K in response to PMA, bryo and a qualitatively similar pattern of phosphoproteins in response to PDGF with the exception of the 67K species.

The three phosphoproteins in the 60–70K range identified in Fig. 1 comigrated in SDS–PAGE gels with the nuclear lamins. In order to determine directly whether the lamins are phosphorylated in response to PMA, bryo and PDGF, nuclear envelopes from treated cells were subjected to two-dimensional isoelectric focusing (IEF)/SDS–PAGE followed by electrophoretic transfer to nitrocellulose. After autoradiography to visualize the resolved phosphoproteins, the same nitrocellulose membranes were subjected to immunoblot analysis using a specific rabbit antilamin antisera as described in Materials and methods. Following IEF/SDS–PAGE, the major 72 and 62K phosphoproteins migrate with apparent pI values in the 6.8–7.2 range (Fig. 2). The 67K phosphoprotein (best seen in response to bryo) migrates with an apparent pI of ~6.0. Immunoblot analysis shows the migration of the nuclear lamins (labelled A, B and C) in the same samples, demonstrating that the phosphoproteins at 72, 67 and 62K correspond to the lamins A, B and C, respectively. All three mitogens induce phosphorylation of lamins A and C;
Fig. 1. PMA, bryo and PDGF induce phosphorylation of nuclear envelope polypeptides. Quiescent, serum-deprived NIH/3T3 cells were radiolabelled with [32P]orthophosphate, treated with either PMA (10 nM), bryo (10 nM), PDGF (10 ng ml⁻¹) or diluent for 30 min at 37°C. Nuclear envelopes were isolated and analysed by SDS-PAGE and autoradiography as described in Materials and methods. A, B and C indicate the positions of the lamins A, B and C on the gels as determined by Coomassie Blue staining.

however, bryo stimulates lamin B phosphorylation to a greater extent than either PDGF or PMA.

Similar serine site(s) on the lamins are phosphorylated in response to PMA, bryo and PDGF
To determine the site(s) of phosphorylation on the lamins in response to PMA, bryo and PDGF, 32P-labelled lamins were excised from two-dimensional IEF/SDS-PAGE gels and subjected to comparative phosphopeptide mapping and phosphoamino acid analysis (Fig. 3). Phosphopeptide mapping of lamins A and C from cells treated with PMA, bryo or PDGF reveal similar patterns, with predominant labelling of the same phosphopeptide (Fig. 3A). The lower level of phosphorylation of lamin B in PMA- and PDGF-treated cells precluded comparative analysis of the phosphopeptides on this lamin species. Phosphoamino acid analysis demonstrates that phosphorylation occurs predominantly on serine residue(s) in all three lamins in response to these mitogens (Fig. 3B).

PDGF-induced lamina phosphorylation correlates with mitogenicity
The finding that three mitogens induce the rapid phosphorylation of the same nuclear envelope lamins suggests that lamina phosphorylation may be involved in the mitogenic response. Previous studies indicated that lamina phosphorylation correlated positively with the mitogenic response of FDC-P1 cells to interleukin-3 and bryo (Fields et al. 1989) and of BHK-21 cells to insulin (Friedman and Ken, 1988). To determine whether lamina phosphorylation correlated with PDGF-induced mitogenicity, cells were treated with various concentrations of PDGF and the extent of lamina phosphorylation and [3H]thymidine incorporation was assessed at each concentration. Fig. 4A demonstrates that a good correlation exists between the level of lamina phosphorylation and the mitogenic response to a given dose of PDGF. The time course of PDGF-induced lamina phosphorylation was assessed by treating quiescent cells with 10 ng ml⁻¹ PDGF. At various times after addition of PDGF, cells were lysed, nuclear envelopes purified and nuclear lamina phosphorylation assessed after SDS-PAGE and autoradiography. Fig. 4B demonstrates that PDGF-induced lamina phosphorylation is rapid, occurring as early as 5 min after exposure to PDGF. The extent of phosphorylation increases over the next hour reaching a maximum at about 60 min.

Mitogenicity and lamina phosphorylation are inhibited by staurosporine
To assess whether mitogenicity and lamina phosphorylation are dependent upon the activation of PKC, we assessed the ability of the selective PKC inhibitor staurosporine to block these events. Quiescent cells were incubated in serum-free medium for 48-72 h prior to addition of mitogens. For inhibition studies, staurosporine was added to the cells for 24 h prior to stimulation as described in Materials and methods. Fig. 5 demonstrates that PDGF, PMA and bryo stimulate a dose-dependent response in these cells as measured by incorporation of [3H]thymidine. The maximal response to either PMA or bryo was seen at approximately 10 nM, while PDGF was maximally stimulatory at concentrations of 5 ng ml⁻¹ and above. The magnitude of the response to either PMA or bryo ranged from 40 to 70% of the maximal response to PDGF. The response to all three of these mitogens could be inhibited by exposure of the cells to 2 nM staurosporine. The inhibitory effect is reversible, since the response was restored in cells that were treated with 2 nM staurosporine for 24 h, followed by removal of the drug for 24 h prior to mitogen stimulation (data not shown).

For phosphorylation studies, quiescent, serum-deprived cells were incubated in the presence of 2 nM staurosporine for 24 h prior to addition of mitogens. After a 1-h incubation, cells were lysed and nuclear envelopes isolated for SDS-PAGE and autoradiography as described in Materials and methods. PMA-, bryo- and PDGF-induced lamina phosphorylation are all inhibited by staurosporine (by 66% for PMA, 82% for bryo and 69% for PDGF).

Our initial experiments indicated that effective inhibition with staurosporine was achieved at lower concentrations when cells were incubated in the presence of
staurosporine for 24 h prior to mitogen stimulation as compared to addition immediately prior to mitogen stimulation. This suggests that staurosporine does not enter intact cells rapidly but that with preincubation the drug can gain access to the interior of the cell. The concentration at which staurosporine can effectively inhibit mitogenicity (2 nM) is approximately 2.5 times the $K_i$ for PKC inhibition (0.7 nM). At this concentration, staurosporine exhibits selective PKC inhibition.

**Fig. 2.** PMA, bryo and PDGF induce phosphorylation of the nuclear lamina. Quiescent, serum-deprived NIH/3T3 cells were treated as described for Fig. 1. Isolated nuclear envelopes from treated cells were resolved by isoelectric focusing (IEF)/SDS–PAGE and transferred to nitrocellulose sheets. The sheets were autoradiographed to reveal the phosphoprotein pattern and then subjected to immunoblot analysis using monospecific anti-lamin antibody. Immunoreactive laminae were detected by horseradish peroxidase-labelled conjugate as described previously (Fields et al. 1988). The positions of lamina A, B and C are shown with arrowheads.

**Fig. 3.** PMA, bryo and PDGF translocate PKC to the nuclear envelope
In a previous report, we demonstrated that treatment of the interleukin-3-dependent cell line FDC-P1 with either bryo or interleukin-3 leads to rapid translocation of protein kinase C to the nuclear membrane (Fields et al. 1989). We therefore assessed whether PMA, bryo and PDGF caused translocation of protein kinase C to the nuclear membrane of quiescent, serum-deprived NIH/3T3 cells.

**A.** Comparative phosphopeptide mapping of lamina A and C isolated from IEF/SDS–PAGE gels of nuclear envelopes from cells treated with either PMA (10 nM), bryo (10 nM) or PDGF (10 ng ml⁻¹) for 30 min. Isolated polyacrylamide spots corresponding to lamin A or C were subjected to limited proteolytic digestion with Staphylococcus V-8 protease as previously described (Fields et al. 1988). B. Comparative phosphoamino acid analysis of laminae A, B and C after treatment with either PMA, bryo or PDGF as described in Materials and methods.
Cells were treated with either PMA (10^{-8} M), bryo (10^{-8} M) or PDGF (5 ng ml^{-1}) for 30 min at 37 °C. The cells were then lysed and nuclear envelopes isolated as described in Materials and methods. Nuclear envelope polypeptides were resolved by SDS-PAGE and transferred to nitrocellulose for immunoblot analysis using a specific rabbit anti-PKC antibody as previously described (Fields et al. 1989).

As can be seen in Fig. 6, nuclear envelopes from quiescent cells contain little detectable PKC. However, treatment of these cells with either PMA, bryo or PDGF leads to rapid association of PKC with the nuclear envelope. In addition to a major staining band at 82K corresponding to intact PKC, a second band is detected at ~52K. This lower band may correspond to proteolytically degraded PKC. It should be noted that the anti-peptide antiserum used in these experiments corresponds to the extreme C-terminal sequences of the regulatory domain; therefore, the 52K form of PKC detected here retains this portion of the regulatory domain. This is consistent with previous observations, indicating that PMA and bryo both stimulate rapid activation of PKC followed by progressive degradation of PKC (Kraft et al. 1987b).

Discussion

Recently, we reported that in the human promyelocytic leukemia cell line, HL60, PKC can be translocated to the nuclear envelope where lamin B is phosphorylated (Fields et al. 1988). Nuclear translocation was observed in response to the PKC activator bryo but not phorbol dibutyrate. The activation of PKC at the nuclear envelope correlated with the growth stimulatory effect of bryo on HL60 cells (Fields et al. 1988). Since our initial observation of nuclear PKC translocation, we have demonstrated the translocation of PKC to the nuclear envelope in the murine interleukin-3-dependent cell line FDC-P1 when these cells are treated with either bryo or interleukin-3, which are both potent mitogens (Fields et al. 1989).

In the present study we have demonstrated that PDGF, PMA and bryo are all cellular mitogens to NIH/3T3 PKC.

Fig. 4. Lamina phosphorylation correlates with PDGF-induced mitogenicity. A. Quiescent, serum-deprived NIH/3T3 cells were stimulated with various concentrations of PDGF and mitogenicity was assayed by ^{3}H^{}thymidine incorporation as described in Materials and methods. Parallel cultures were equilibrated with ^{32}P^{}orthophosphate, treated with various concentrations of PDGF for 30 min and the extent of lamina phosphorylation determined as described in Materials and methods. Results were plotted as cts min^{-1} (mitogenicity) or % control phosphorylation as determined by densitometric scanning of autoradiographs of ^{32}P^{}-labelled lamins (lamina phosphorylation) versus the concentration of PDGF. B. Time course of PDGF-induced lamina phosphorylation. Lamina phosphorylation was assessed as described above after treatment with PDGF (10 ng ml^{-1}) for the indicated times.

Fig. 5. PMA-, bryo- and PDGF-induced mitogenicity is inhibited by staurosporine. Quiescent, serum-deprived NIH/3T3 cells were incubated with the indicated concentrations of PMA, bryo or PDGF and mitogenicity determined by ^{3}H^{}thymidine incorporation as described in Materials and methods. For inhibition studies, staurosporine was added to cells 24 h prior to addition of mitogen and mitogenicity determined as described.
fibroblasts. All three mitogens induce rapid phosphorylation of the nuclear lamins at similar serine sites. The level of lamina phosphorylation induced by PDGF correlates with the mitogenic effect of PDGF. These results are in agreement with our results in the murine interleukin-3-dependent PDC-P1 cell line (Fields et al. 1989) and with those of Friedman and Ken (1988) showing that treatment of quiescent BHK-21 fibroblasts with insulin led to rapid, dose-dependent phosphorylation of lamins A and C, which correlated with the mitogenic effect of insulin. Therefore, accumulating evidence suggests that lamina phosphorylation is an early biochemical event in the mitogenic response in several cell systems. The exact role of PDGF-induced lamina phosphorylation remains unclear; however, inhibition of lamina phosphorylation leads to profound inhibition of PDGF mitogenicity. Our results (Fields et al. 1988, 1989), as well as those of others (Friedman and Ken, 1988; Hornbeck et al. 1988), suggest that mitogen-stimulated lamina phosphorylation does not lead to dissolution of interphase lamina structure. Therefore, it appears that these phosphorylation events do not induce mitotic nuclear envelope breakdown (Ottaviano and Gerace, 1986). There are several possible explanations for this observation. First, in vitro studies indicate that at least three distinguishable events are coordinately involved in nuclear envelope breakdown: lamina phosphorylation, chromosome condensation and nuclear membrane vesicuHzation (Newport and Spann, 1987). Therefore, lamina phosphorylation alone may be insufficient to cause nuclear envelope breakdown. Second, nuclear envelope breakdown in vivo proceeds only after a 30–60 min lag period in which the lamins are progressively phosphorylated (Suprynowicz and Gerace, 1996), suggesting that a critical level of lamina phosphorylation must be achieved to allow lamina dissolution. This level may not have been achieved in our experiments. Third, the sites of mitogen-induced lamina phosphorylation may be distinct from those induced at mitosis. Mitogen-induced lamina phosphorylation may allow for the growth of the nuclear lamina necessary to increase the nuclear volume during S-phase or the release of constraints on nuclear envelope-associated chromatin to allow for initiation of DNA replication or activation of specific gene transcription. Further studies will be required to distinguish between these possibilities.

Both the mitogenic effect and lamina phosphorylation induced by PDGF, PMA and bryo can be inhibited by the selective protein kinase C inhibitor staurosporine. These inhibition studies must be interpreted cautiously, since staurosporine, like many other protein kinase inhibitors, inhibits several classes of protein kinase (Tamaoki et al. 1986). Despite this limitation, staurosporine does show relative selectivity for PKC inhibition ($K_i \sim 0.7 \text{ nM}$) as compared to inhibition of cyclic-nucleotide-dependent kinases ($K_i \sim 7.0 \text{ nM}$; Tamaoki et al. 1986). In addition, staurosporine has been shown specifically to inhibit PMA-induced phosphorylation in platelets at 3 nm (Krisna-murthi and Joseph, 1989), in agreement with our results.

Our immunological studies demonstrate that PKC is translocated to the nuclear envelope of PDGF-, PMA- and bryo-treated cells. Nuclear envelope-associated PKC is detected in two Mr forms of 82 and 52K. The 82K form of the enzyme corresponds to the intact enzyme while the 52K form is consistent with a proteolytically degraded fragment of PKC. These results are similar to those obtained in rat liver (Capitani et al. 1987), antigen-stimulated B-lymphocytes (Cambier et al. 1987) and PMA-treated NIH/3T3 cells (Halsey et al. 1987; Thomas et al. 1988), in which both intact and proteolysed fragments of PKC were detected in the nuclear fraction. In contrast, Leach et al. (1989) found only an 82K form of PKC was associated with the nuclear envelope of PMA-treated NIH/3T3 cells. These results may be due to differences in the antibodies used and/or the time of treatment with PMA prior to isolation of nuclear envelopes. It is interesting to note that both PMA and bryo have been shown to lead to activation followed by progressive degradation of the enzyme (Kraft et al. 1987b), presumably through the action of calpains. It is possible that physiological activation of nuclear PKC by PDGF may not cause PKC degradation to the same extent as pharmacological activation of the enzyme with PMA or bryo.

Accumulating evidence suggests the direct involvement of PKC at the nuclear level in the transduction of external signals to the nucleus (Cambier et al. 1987; Fields et al. 1988, 1989; Hornbeck et al. 1988; Kiss et al. 1988; Kraft et al. 1987a; Misra and Sahyoun, 1987; Thomas et al. 1988). However, many questions remain to be answered with respect to nuclear PKC activation. For instance, the mechanisms by which PKC is specifically translocated and activated at the nuclear are unclear at present. Presumably, PMA and bryo can translocate and activate PKC at the nuclear membrane by direct interaction with PKC and the nuclear membrane. In contrast, growth factors probably stimulate translocation and activation of nuclear PKC through the action of intervening molecules. If translocation of PKC to the nuclear membrane is similar to translocation to the plasma membrane, one might predict involvement of phosphoinositide metabolism with generation of diacylglycerol or similar physiological PKC activators at the nuclear membrane. Indeed, the nuclear membrane is capable of phosphoinositide metabolism (Cocco et al. 1987), suggesting that such activators can be generated at the nuclear membrane. It would be of great interest to determine whether nuclear phosphoinositide metabolism is stimulated by mitogen stimulation, thereby generating a signal for PKC translocation and activation. Interestingly, the mitogenic effects of PDGF can be abol-

Fig. 6. PMA, bryo and PDGF stimulate translocation of PKC to the nuclear envelope. Quiescent, serum-deprived cells were incubated with PMA (10 nM), bryo (10 nM) or PDGF (10 ng ml$^{-1}$) for 30 min at 37$\degree$C. Nuclear envelopes were isolated and subjected to immunoblot analysis using an anti-peptide PKC antibody as previously described (Fields et al. 1989). Antigen was detected with $^{125}$I-labelled protein A.
ished by antibodies to inositol phosphates (Matouka et al. 1989) and PDGF is known to stimulate rapid tyrosine phosphorylation (Wahl et al. 1989) and activation (Berger et al. 1984; Chu et al. 1985; Habenicht et al. 1981; Hasegawa-Sasaki, 1985) of phospholipase C activity. However, the possibility of nuclear membrane-associated phospholipase C activity in response to PDGF activation has not been investigated.

The identities of the relevant nuclear substrates for PKC has only begun to be explored. Several important regulatory nuclear enzymes have been identified as substrates for PKC in vitro, including topoisomerase II (Sahyoun et al. 1986) and RNA polymerase II (Chuang et al. 1989). In addition, the nuclear lamins are phosphorylated in response to a variety of growth stimuli that lead to nuclear PKC activation (Fields et al. 1988, 1989; Hornbeck et al. 1988) and they are excellent substrates for activated PKC, both in whole cells and in vitro (Fields et al. 1988, 1989; Hornbeck et al. 1988). Therefore, it is tempting to speculate that phosphorylation of the lamins and other nuclear proteins by nuclear PKC is involved in the mitogenic signaling process.

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