Protein Kinase Cδ Inhibition of S-Phase Transition in Capillary Endothelial Cells Involves the Cyclin-dependent Kinase Inhibitor p27Kip1*

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Distinct protein kinase C (PKC) isoforms differentially regulate cellular proliferation in rat microvascular endothelial cells (EC). Overexpression of PKCδ has little effect on proliferation, whereas PKCδ slows endothelial cell proliferation and induces S-phase arrest. Analyses were performed on EC overexpressing PKCα (PKCαEC) or PKCδ (PKCδEC) to determine the role of specific cyclin-dependent regulatory proteins in the PKCδ-induced cell cycle arrest. Serum-induced stimulation of cyclins D1, E, and A-associated kinase activity was delayed by 12 h in the PKCδEC line in association with S-phase arrest. However, the protein levels for cyclins D1, E, and A were similar. Nuclear accumulation of cyclin D1 protein in response to serum was also delayed in PKCδEC. In the PKCδEC line, serum induced p27Kip1 but not p16Ink4a or p21Cip1. Serum did not affect p27Kip1 levels in the control vascular endothelial cell line. Immunoprecipitation-Western blotting analysis of p27Kip1 showed serum stimulation of the vascular endothelial cell line resulted in increased amounts of cyclin D1 bound to p27Kip1. In the PKCδEC line, serum did not increase the amount of cyclin D1 bound to p27Kip1. Transfection of full-length p27Kip1 antisense into the PKCδEC line reversed the S-phase arrest and resulted in normal cell cycle progression, suggesting a critical role for p27Kip1 in the PKCδ-mediated S-phase arrest.

The vascular endothelium is a dynamic organ controlling hemostasis, vasodilation, and wound healing. The endothelium is influenced by shear stress, hypoxia, and chemotactic/mitogenic gradients that promote migration and division of its cells. Endothelial cell division is an important component of the angiogenic response to many stimuli (2–4). As several different external agents promote or inhibit endothelial cell proliferation, the secondary messengers mediating these responses are being actively investigated. The protein kinase C (PKC) family of Ser-Thr kinases is a common intracellular signaling pathway that coordinates a diverse array of signals that arise in the extracellular environment. Activation of the PKC pathway by phorbol esters, for example, induces endothelial cell proliferation and angiogenesis in vivo (5–7). In contrast, inhibition of the PKC pathway by prolonged treatment with phorbol esters inhibits mitogenesis of endothelial cells (8, 9). The molecular mechanisms regulating endothelial cell proliferation in response to mitogens and PKC activation are poorly understood. However, it is likely that specific components of the cell cycle regulatory apparatus may govern these responses. Recent studies have suggested that individual isozymes modulate specific cell cycle transitions in specific cell types. The G1-S transition is regulated by the PKCδ isoform in NIH3T3 cells (10), whereas in vascular smooth muscle cells PKCα/ε regulate this transition (11). Overexpression of PKCα and PKCδ affect cellular proliferation and cell cycle progression in several different cell types. PKCα promotes cellular proliferation in human breast cancer and other cells (12, 13). In contrast, overexpression of the PKCδ isoform in Chinese hamster ovary fibroblasts in the presence of phorbol ester induces G2/M-phase arrest (14).

The components of the cell cycle regulatory apparatus governing progression through the G1 phase are increasingly well understood (15–17). The cyclin-dependent kinases (CDKs) are serine-threonine holoenzymes, consisting of a regulatory and catalytic subunit that phosphorylate target substrates to promote progression through the G1 phase of the cell cycle. The phosphorylation of the pRB protein is mediated in part by cyclin D1/Cdk4 and cyclin E/Cdk2 (18, 19). The phosphorylation of pRB inactivates its ability to block G1 phase progression. Phosphorylation of pRB is associated with release of E2F/DP proteins from their binding site on the pRB protein and progression through G1 into a phase of DNA synthesis. The activity of the CDKs is inhibited by members of the p21 family (p21Waf1/Cip1 family (p21cip1, p27kip1, p57kip2) and the INK family (p16Ink4a, p15Ink4b, p16Ink4a, and p19Ink4a). These proteins inhibit CDK enzymatic activity in part through binding to the CDK regulatory subunit, thereby inhibiting holoenzyme association. The p21 family proteins are referred to as “universal inhibitors” because of the ability to block the activity of the cyclin D, cyclin E, and cyclin protein kinase A. As the CDK holoenzymatic activity is directed at nuclear substrates, the activity of the

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††† The abbreviations used are: PKC, protein kinase C; CDK, cyclin-dependent kinase; CRI, CDK inhibitor; EC, endothelial cell(s); V-EC, vector EC (cell line); PBS, phosphate-buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GST, glutathione S-transferase; MACS, magnetic activated cell separation system; pRB, retinoblastoma protein; JAB, JAK-binding protein.
CDK inhibitor (CKI) is in part determined by its subcellular distribution. Thus, the CKI is inhibitory in the nuclear but not the cytoplasmic location.

In recent studies we showed that overexpression of PKCδ, but not PKCa, in EC inhibited cellular proliferation through an arrest in S-phase (1). These findings were consistent with several other studies in which the loss of PKCδ expression was associated with increased cellular proliferation or transformation (20–22). Together these studies suggested a role for PKCδ as an inhibitor of cellular proliferation, which may play an important role in slowing cell cycle progression in normal cells. The current studies were performed to understand the molecular mechanisms governing PKCδ-mediated cell cycle arrest. We report here that PKCδ overexpression (PKCδEC) delays serum-induced expression of kinase activity associated with cyclins D1, E, and A. However, the cyclin protein levels induced by serum in the PKCδEC were unchanged compared with the induction seen in control cells. Expression of the INK family members (p16Ink4a and p18Ink4c) and the CKI p21Cip1 was unchanged. PKCδEC contained higher nuclear levels of the cyclin-dependent kinase inhibitor p27Kip1 than vector controls. p27Kip1-antisense reduced p27Kip1 levels and relieved the cell cycle defect induced by PKCδ, strongly suggesting that increased expression of p27Kip1 was responsible for the prolongation of S-phase in PKCδEC.

MATERIALS AND METHODS

Cell Culture—Cells used in this study were those reported by Harrington et al. (1). The parent endothelial cells were derived from the rat epididymal fat pad and were stably transduced with full-length cDNA encoding the PKC isoforms alpha (PKCαEC) and delta (PKCδEC) or vector (pcDNA1) without insert (V-EC). Cells were cultured in medium 199 supplemented with 15% fetal calf serum and 2 mM l-glutamine with 5% (w/v) dry milk to block nonspecific binding sites. For immunoprecipitation, cell cultures were harvested 48 h after transfection using EDTA (5 mg/ml) in PBS. Cells were pelleted by centrifugation and resuspended in PBS containing 5 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1% Tween 20, 0.1 mM sodium orthovanadate (Sigma), at 4°C. Lysates were centrifuged at 10,000 x g for 5 min, and protein concentrations were determined using the Bio-Rad protein assay. Protein expression and changes were confirmed by Western blot analysis using antibodies to cyclins A, E, and D1 and antibodies to the CKI p21Cip1.

Serum Induction of Cyclin-dependent Kinase Activity—In our previous studies a delay in S-phase exit, in response to serum, was observed in a cell line overexpressing PKCδ (PKCδEC) (1). Serum induced extracellular signal-regulated kinase-2 activity equally in both the PKCδEC and parental ECs (1). Serum treatment of fibroblasts and primary myocytes induces the G1 cyclins, cyclin D1 and cyclin E, and their associated kinase activity (18, 19). The induction of these kinases is required for cell cycle progression. To determine whether the proliferative defect observed in the PKCδEC stable line was associated with alterations in cyclin-associated kinase activity, the cell lines PKCδEC, PKCaEC, and V-EC were analyzed. Cells were stimulated with serum, cell extracts were harvested at sequential time points, and immunoprecipitation kinase assays were performed. The experiments were conducted on three separate occasions with similar findings. Cyclin D1 kinase activity, assessed using GST-pRB as substrate, was increased 15-fold at 24 h after serum addition in the V-EC (Fig. LA). In the PKCδEC, cyclin D1 kinase activity was induced only 4-fold at 24 h (Fig. LA), with maximal induction at 36 h in the PKCδEC line (Fig. LA). The cyclin E and cyclin A kinase activities were assessed by electrophoresis, and Western blotting was performed using antibodies for cyclins A, E, and D1 as described above.
Immunoprecipitation kinase assays were performed on cell extracts derived from either a control stable cell line containing the empty expression vector cassette (Vector-EC) or the PKCδEC stable cell line. Analyses were performed after the addition of serum for the time points indicated (0–36 h). Immune complex kinase activity was assessed for cyclin D1 using GST-pkB as the substrate (A). Cyclin A and cyclin E kinase activities were assessed using histone H1 as substrate (B and C). The induction of each of the cyclin-dependent kinases occurred in a delayed manner in the PKCδEC stable cell line.

**Serum Regulation of the Cyclins**—The activity of the cyclin-dependent kinases (CDK) is dependent in part upon the relative abundance of both the cyclin subunit and the presence of CDK inhibitors (CKI) (15–17). To determine whether the altered activity of the cyclin-dependent kinases in the PKCδEC was caused by differences in the abundance of cyclin proteins, Western blotting was performed. We hypothesized that the delayed induction of cyclin-dependent kinase activity in the PKCδEC was the result of delayed induction of the cyclin proteins. In the V-EC line (Fig. 2A), the addition of serum resulted in a modest induction of cyclin D1 at 24 h. The induction of cyclin D1 by serum was also observed in the PKCoEC and PKCδEC lines (Fig. 2, B and C). When the levels of cyclins A and E were compared following serum treatment, there were no significant differences between the V-EC and PKCoEC or PKCδEC lines (Fig. 2, compare A, B, and C). These results suggest that differences in the levels of cyclin D1, cyclin E, and cyclin A were not responsible for the inhibition of serum-induced cell cycle progression consequent upon PKCδ overexpression.

To examine further possible mechanisms for the delayed induction of cyclin kinase activity in serum-treated PKCδEC, we analyzed the nuclear abundance of the cyclins upon serum treatment. The cyclin D1 protein undergoes nuclear-cytoplasmic shuttling (35), which is critical for DNA synthesis in fibroblasts (36). We hypothesized that the S-phase arrest in the PKCδEC line may have been the result of delayed entry of cyclins to the nuclear compartment. To examine this possibility, nuclear fractions of serum-treated cells were examined (Fig. 3). The addition of serum to the V-EC line was associated with an increase in nuclear cyclin D1 levels at 12 h, which subsequently began to decrease at 24 and 36 h (Fig. 3A). The increase in nuclear cyclin D1 levels with serum treatment and subsequent decrease is consistent with previous observations in fibroblasts (35). Cyclin E nuclear abundance was relatively unchanged, and cyclin A levels increased at 12 h, peaking at 24 h. In the PKCδEC line (Fig. 3B), the induction of nuclear cyclin D1 was minor at 12 h and peaked at 24 h. The increase in nuclear cyclin D1 levels in the PKCδEC line was therefore delayed when compared with the V-EC line. The changes in cyclin E and cyclin A with the addition of serum in the PKCδEC were not significantly different from the V-EC line.

**p27Kip1 Levels in the Presence of PKCδ Overexpression**—Cyclin-dependent kinase activity is modulated in vitro by the cyclin-dependent kinase inhibitors, which include the INK4s (p16Ink4a, p15Ink4b, p18Ink4c, and p19Ink4d), specific inhibitors of Cdk4 or Cdk6, and the CIP/KIP family (p21Cip1, p27Kip1, and p57Kip2) of "universal inhibitors" (15, 37, 38). We first hypothesized that increased INK4 protein abundance may contribute to the delayed serum-induced cyclin-dependent kinase activity in the PKCδEC line. Western blotting for p16Ink4a abundance, however, showed no difference between the V-EC and the PKCδEC lines (Fig. 4A). Western blotting was also performed for p18Ink4c and p21Cip1. No difference was observed in the abundance of either p16Ink4a (data not shown) or p21Cip1 (Fig. 4A) in PKCδEC as compared with the control cells or PKCoEC. In starved cells p27Kip1 levels were increased 2-fold in the PKCδEC (Fig. 4C) when compared with the PKCoEC or V-EC lines (Fig. 3, A and B). Furthermore, p27Kip1 levels increased...
was performed with the p27Kip1 antibody, and the precipitates were with serum for the time points indicated in hours. Immunoprecipitation and p27Kip1. The relative amount of cyclin D1 bound to p27 Kip1 in- the immunoprecipitate with antibodies to cyclin D1, cyclin E, cyclin A, and p27Kip1. The relative amount of cyclin D1 bound to p27Kip1 increased in the serum-treated V-EC line at 36 h (A) compared with the relative binding of cyclin E. In the PKC\textsuperscript{A} line at 36 h, the relative amount of cyclin D1 compared with cyclin E bound to p27Kip1 was not significantly changed.

4-fold after 24 h of serum treatment in the PKC\textsuperscript{A} line (Fig. 4C) but were unchanged in the PKCoEC or V-EC (Fig. 4, A and B).

\textit{p27Kip1} Immunoprecipitation Assays—These observations suggested that the p27Kip1 level increased in response to serum in the PKC\textsuperscript{A} line but that this increase was not observed in the PKCoEC or the V-EC line. p27Kip1 is capable of binding cyclin D1/Cdk4 and cyclin E/Cdk2. It is thought that p27Kip1 inhibits cyclin E/Cdk2 activity but may not inhibit phosphorylation of pRB by cyclin D1/Cdk4 under certain circumstances (39). Thus, the relative binding of the CIP (p21/p27Kip1) family proteins to cyclin D1 complexes compared with cyclin E complexes may determine the cytostatic activity of the complex (39). Immunoprecipitations were therefore performed on the V-EC and the PKC\textsuperscript{A} lines using a p27Kip1-specific antibody (Fig. 5). The relative amount of p27Kip1 in each immunoprecipitate was examined by Western blotting (Fig. 5, bottom panel). The relative amount of cyclin protein bound to the p27Kip1 was also assessed by Western blotting and was compared with the amount of p27Kip1 in the immunoprecipitate. The relative amount of cyclin D1 bound to p27Kip1 increased at 36 h in the V-EC line (Fig. 5A). The binding of cyclins E and A to p27Kip1 was not significantly changed after serum addition (Fig. 5A). In the PKC\textsuperscript{A} line (Fig. 5B), the relative increase in cyclin D1 compared with cyclin E binding, found in the V-EC line at 36 h, did not occur. The relative binding of cyclins E and A to p27Kip1 was also unchanged. The lack of increase in binding of p27Kip1 to cyclin D1 may have caused by the relative delay in nuclear entry of cyclin D1 in the PKC\textsuperscript{A} line compared with the V-EC line (Fig. 3). These results suggest that in the V-EC line there is a modest increase in the amount of cyclin D1 bound to p27Kip1 compared with cyclin E after 36 h of serum treatment. This increase in cyclin D1/p27Kip1 is not observed in the PKC\textsuperscript{A} line.

\textit{p27Kip1} Antisense and the Cell Cycle Delay Induced by PKC\textsuperscript{A}—The analysis of the CKI in the PKC\textsuperscript{A} line indicated preferential induction of p27Kip1 but not the other CKI in response to serum. Because p27Kip1 overexpression is capable of inducing cell cycle arrest in fibroblasts (32), these findings raise the possibility that p27Kip1 may be involved in the PKC\textsuperscript{A}-induced S-phase arrest in endothelial cells. To examine the role of p27Kip1 in PKC\textsuperscript{A} cell cycle delay, we used a p27Kip1 antisense construct. Transfection of rat microvascular endothelial cells with the p27Kip1 antisense expression plasmid reduced basal p27Kip1 levels 2-fold (Fig. 6A). The p27Kip1 antisense vector was also used to transfect the PKC\textsuperscript{A} line. The increased p27Kip1 levels were reduced 10-fold by the p27Kip1 antisense expression plasmid. The overexpression of the empty expression vector cassette did not affect p27Kip1 levels. These studies demonstrated that the p27Kip1 antisense expression plasmid can reduce p27Kip1 levels in the PKC\textsuperscript{A} line.

To determine the effect of p27Kip1 antisense on the cell cycle...
arrest induced by PKCδEC, cells were transfected with the p27Kip1 antisense expression plasmid in conjunction with CD4 expression plasmid, and magnetic cell sorting was conducted with cell cycle analysis of the transfected cells. The cell cycle histograms are shown in Fig. 6, B–E. The control V-EC were transfected with the p27Kip1 expression vector. The relative proportion of cells in S-phase was increased from 24 to 40% (Fig. 6F). An increased proportion of the PKCδEC were in S-phase (47%) compared with the V-EC line, as described previously (1). Overexpression of the p27Kip1 antisense in the PKCδEC line reduced the proportion of cells in S-phase by 17% (Fig. 6F), with the resultant histograms similar to the V-EC line. These results suggest that the increase in p27Kip1 found in the PKCδEC line may play an important role in the increased proportion of cells found in S phase. The reversal of the S-phase arrest by p27Kip1 antisense was observed in three additional PKCδEC stable lines (data not shown).

DISCUSSION

The molecular mechanisms by which specific isoforms of the PKC family regulate cellular proliferation are poorly understood. The current studies extend our previous findings that PKCδ delays S-phase progression in rat microvascular endothelial cells (1). The PKCδ-mediated inhibition of S-phase progression was associated with a delay in the induction of the kinase activities associated with cyclins D1, E, and A. An investigation of the abundance of the cyclin-dependent kinase inhibitors associated with the inhibition of the cyclin kinase activity revealed an increase in the abundance of the "universal inhibitor," p27Kip1. The relative abundance of p21Cip1 and the INK4 protein family was unchanged, suggesting that the induction of p27Kip1 was a relatively specific change. In addition, overexpression of an antisense expression plasmid for p27Kip1, which was shown to reduce p27Kip1 protein levels, was also shown to reverse the S-phase arrest observed in the PKCδEC lines. These studies are consistent with a model in which the induction of p27Kip1 may play an important and specific role in PKCδ-mediated S-phase arrest in microvascular endothelial cells.

The pRB protein is a critical regulator of cell cycle progression, and the phosphorylation of pRB during G1 phase coincides with passage of the cell through the restriction point in G1 (15, 16). pRB undergoes continued phosphorylation throughout the S-phase (40), and the cyclin D1-dependent phosphorylation, which is required for cyclin D1 to promote cell cycle progression, occurs on specific phosphorylation sites that can be assessed in immunoprecipitation assays using a pRB fragment containing this site (25–28). Upon phosphorylation by cyclin D1, cyclin E kinase further phosphorylates pRB at distinct sites (41). Cyclin E kinase activity phosphorylates and inactivates additional substrates that contribute to cell cycle progression in a pRB-independent manner (42). In the current studies the induction of cyclin D1-dependent pRB phosphorylation was maximally induced at 24 h in the V-EC and PKCδEC but occurred in a delayed manner in the PKCδEC. The induction of cyclin E kinase activity by serum was also delayed in the PKCδEC. These data are consistent with the role of p27Kip1 as a "universal inhibitor" of both cyclin E and cyclin D1 kinase activity. The phosphorylation of pRB coincides with the loss of the ability of pRB to bind and inhibit E2F/DP complexes. The corresponding induction of "free E2F activity" activates genes involved in DNA synthesis. In the current studies, the delayed induction of cyclin A kinase activity, a marker of S-phase entry, in the PKCδEC line, is consistent with the delayed entry into S-phase. pRB is a poor substrate for cyclin E kinase, and cyclin E overexpression can promote S-phase entry independently of pRB, suggesting that cyclins D1 and E function in parallel pathways to promote S-phase entry (21, 42). The current studies suggest that PKCδ inhibits these parallel pathways in EC lines.

In the current studies, PKCδ induced p27Kip1 in rat microvascular endothelial cells. The induction of p27Kip1 by serum was enhanced in the PKCδEC in association with S-phase arrest. Antisense p27Kip1 expression blocked the PKCδEC-induced S-phase arrest. Overexpression of p27Kip1, initially characterized as a protein homologous to p21Cip1 (32), can delay cell cycle progression in fibroblasts (43, 44). In recent studies p27Kip1 reduced the proliferation of smooth muscle induced by angioplasty and mediated the inhibition of smooth muscle cell proliferation by fibrillar collagen (45, 46). Together these studies suggest that p27Kip1 may be an important inhibitor of vascular remodeling (45). Our finding that p27Kip1 is involved in the cell cycle delay by PKCδ extends the known cytostatic signaling pathways in which p27Kip1 is involved, p27Kip1 also mediates the cytostatic effects of rapamycin and cAMP (32, 47–49).

In the current studies, p27Kip1 immunoprecipitation assays were performed to assess the effect of the PKC isoforms on the multimeric complexes bound to p27Kip1. The cyclin/CDK complex to which p27Kip1 is bound determines its functional activity. p27Kip1 associates with cyclin E in a variety of cell types during quiescence (47, 50). When bound to cyclin D1/Cdk4, p27Kip1 may not be inhibitory (47, 51–53), whereas cyclin E/Cdk2 activity is inhibited by p27Kip1. In the current studies, we compared the relative proportion of p27Kip1 bound to either cyclin D1 or cyclin E after 36 h of serum stimulation. In the PKCδEC line, p27Kip1 was bound to both cyclins D1 and E after 36 h stimulation; however, there was relatively more cyclin D1 bound to p27Kip1 in the V-EC line (Fig. 4). Thus, in the PKCδEC line the serum-induced increase in cyclin D1 binding to p27Kip1 is reduced. It is thought that the removal of p27Kip1 from the cyclin E/CDK complex is an essential step for S-phase entry. Through binding cyclin D1/Cdk4, p27Kip1 is sequestered from cyclin E/Cdk2, reducing its inhibition by p27Kip1 (47, 51–53). Thus, in the PKCδEC line it may be expected that p27Kip1 is incorporated proportionately more in an inhibitory complex with cyclin E than in the case in the V-EC line. The failure of p27Kip1 to bind increasing amounts of cyclin D1 may be the result of the delayed nuclear entry of cyclin D1 in response to serum (Fig. 3). Thus, these studies suggest that PKCδ overexpression both increases the amount of p27Kip1 induced in the cell in response to serum stimulation and also alters the multiprotein complex with which p27Kip1 is associated in the cell.

The mechanisms responsible for the increased p27Kip1 levels in the PKCδEC line remain to be fully evaluated. The abundance of p27Kip1 is regulated primarily at a post-translational level, and p27Kip1 protein levels decrease after mitogenic stimulation in quiescent NIH3T3 cells (50, 54, 55). The degradation of p27Kip1 upon mitogen stimulation is dependent upon prior phosphorylation. Cyclin E/Cdk2-induced phosphorylation of p27Kip1 on T187 in murine fibroblasts (56) and phosphorylation of p27Kip1 by cyclin E/Cdk2 enhanced degradation of p27Kip1. The delayed induction of cyclin E/Cdk2 activity in the PKCδEC line (Fig. 1C) may have delayed phosphorylation of p27Kip1, in turn delaying its phosphorylation-dependent degradation. The binding of the JAB-1 gene product to p27Kip1 causes p27Kip1 degradation (57). An alternate mechanism may be that JAB-1 is a downstream target of PKCδ, with phosphorylation resulting in functional inactivation of JAB-1.

The mechanism by which p27Kip1 inhibits cell cycle progression may vary with the cell type, although our studies are consistent with the model in which p27Kip1 inhibits cell cycle progression.
progression in part by binding to Cdk2 and thereby reducing cyclin E/Cdk2 kinase activity (32). Increased p27Kip1 levels induced by overexpression in VSMCs was associated with reduced Cdk2 activity (45). Consistent with our findings overexpression of p27Kip1 in the nuclear, rather than the cytoplasmic, compartment was required for the cell cycle arrest (58), indicating that the subcellular distribution of p27Kip1 is important in the inhibition of cellular proliferation. In recent studies an alternate mechanism of p27Kip1 action was proposed. In LAP-3 cells, derived from NIH3T3 cells, p27Kip1 overexpression induced a cell cycle arrest associated with a specific E2F pocket protein complex. One consequence of the p27Kip1/Cdk2 association was disruption of the interaction between Cdk2 and both the E2F-130 and the E2F-p107 repressor complexes (59). The p130/p107 complexes that were induced by p27Kip1 were similar to the complexes induced by serum starvation (59). Further studies will be directed at analyzing the effect of p27Kip1 in the presence of the PKCαEC on components of the E2F-130 and E2F-p107 complexes. The present studies indicate, however, that alterations in cyclin E protein abundance do not appear to be important in the cell cycle effects mediated by p27Kip1, arguing against an indirect effect of p27Kip1 on E2F-130/p107 complex activity.

The results presented here are consistent with recent studies in which PKC isoforms were implicated in the inhibition of cellular proliferation and cell cycle progression. PKC has been shown to inhibit cell cycle progression in intestinal epithelial cells (60), IMR-90 fibroblasts (61), melanoma cells (62), and vascular endothelial cells (63, 64). Both the PKCa and PKCβ isoforms have been implicated in the inhibition of cellular proliferation in different cell types. Overexpression of PKCα inhibited cell cycle progression in Chinese hamster ovary cells (65), B16 melanoma cells (66), and F9 teratocarcinoma cells (67). In rat microvascular capillary endothelial cells (EC), PKCa does not inhibit cell cycle progression but rather promotes migration of the endothelial cells in response to growth factors (1). The studies suggest the cell cycle regulatory effect of the PKC isoforms may be cell type specific. PKCβ is the only isoform to undergo tyrosine phosphorylation (68), and PKCβ was inactivated by tyrosine phosphorylation in v-Src (21) and v-Ras (22) transformed cells, raising the possibility that inactivation of PKCβ may promote unregulated cellular proliferation and transformation. In view of the current findings that p27Kip1 is required for the cell cycle inhibitory function of PKCβ and the prior observations that PKCα inactivation may play an important role in oncogene induced transformation, future studies are warranted to examine the role of p27Kip1 in oncogene/PKC-induced transformation.

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