Post-transcriptional Destabilization of p21<sup>cip1</sup> by Protein Kinase C in Fibroblasts*<sup>S</sup>

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Janice L. Walker<sup>1,2</sup>, Paola Castagnino<sup>1</sup>, Betty M. Chung, Marcelo G. Kazanietz, and Richard K. Assoian<sup>3</sup>

From the Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084

p21<sup>cip1</sup> inhibits S phase entry by binding to cyclin-cdk2 (cyclin-dependent kinase-2) complexes. The levels of p21<sup>cip1</sup> are rapidly induced after mitogenic stimulation of quiescent fibroblasts and then down-regulate as the cells reach late G<sub>1</sub> phase and activate cyclin E-cdk2. In this study, we have shown that pharmacological inhibition of protein kinase C (PKC), expression of dominant negative PKCα, or knockdown of PKCδ with small interfering RNA elevates p21<sup>cip1</sup> protein levels in mouse embryo fibroblasts. This effect is selective, post-transcriptional, and proteasome-dependent but distinct from previously identified post-transcriptional control mechanisms involving cyclin D1 and Skp2. PKCδ inhibition results in a reduced entry into S phase, and this effect is not detected in p21<sup>cip1</sup>-null cells. Thus, post-transcriptional destabilization of p21<sup>cip1</sup> appears to be a major mitogenic effect of PKCδ in fibroblasts.

p21<sup>cip1</sup> is a dual regulator of the cyclin-dependent kinases (cdks).<sup>4</sup> It was initially described as a cdk inhibitor that could prevent the activation of cyclin-cdk complexes or bind to proliferating cell nuclear antigen and repress DNA synthesis (1–3). Although the cdk inhibitory role of p21<sup>cip1</sup> is now firmly established, subsequent studies have also described a positive role for p21<sup>cip1</sup> as an assembly factor for cyclin D1-cdk4/6 complexes during G<sub>1</sub> phase (4, 5).

One of the best-studied aspects of p21<sup>cip1</sup> is its induction during DNA damage and the activation of its promoter by p53 (6). However, p21<sup>cip1</sup> gene expression is also regulated by many signals that are p53-independent (7, 8). In addition to the control of gene expression, p21<sup>cip1</sup> levels can be regulated post-transcriptionally through the proteasome (9, 10), although the exact mechanism and role of E3 ubiquitin ligases are still under debate. For instance, p21<sup>cip1</sup> can be ubiquitinated, but this modification is not required for the proteosomal degradation of p21<sup>cip1</sup> (11–15).

We and others have previously reported that the levels of p21<sup>cip1</sup> oscillate during cell cycle re-entry of nontransformed quiescent fibroblasts (8, 16, 17). The levels of p21<sup>cip1</sup> mRNA and protein are low in quiescent cells, transiently increase as a consequence of mitogen-stimulated cell cycle reentry, and then decline in late G<sub>1</sub> phase. The early G<sub>1</sub> phase induction of p21<sup>cip1</sup> is thought to play a role in the assembly of cyclin D-cdk4/6 complexes (4, 5), whereas the subsequent decline in p21<sup>cip1</sup> contributes to the activation of cyclin E-cdk2 (16). Extracellular signal-regulated kinase (ERK) activity is required for the growth factor-dependent induction of p21<sup>cip1</sup> mRNA in early G<sub>1</sub> phase but does not affect the late G<sub>1</sub> phase down-regulation associated with S phase entry (16). Rho is thought to have the opposite effect, suppressing p21<sup>cip1</sup> gene expression and destabilizing the protein to allow for efficient S phase entry, at least in transformed cells (18–21).

In addition to ERK and Rho, the PKC family of isozymes has an established role in cell proliferation and as regulators of p21<sup>cip1</sup>. However, the biological outcome of PKC stimulation or inhibition is strongly influenced by the isozyme that is regulated and the cell type in which the effect occurs (22, 23). For example, PKC isozymes can stimulate or inhibit cell proliferation depending on the cell type (24). In epithelial cells, several PKC isozymes have been reported to increase p21<sup>cip1</sup> levels to promote cell cycle arrest and initiate differentiation or apoptosis (25–27). In lung adenocarcinoma cells, the p21<sup>cip1</sup>-dependent G<sub>1</sub> arrest mediated by PKCδ was attributed specifically to PKCδ (28). These effects of PKCδ have been typically accompanied by corresponding changes in the levels of p21<sup>cip1</sup> mRNA.

In this study, we have examined the effect of PKC on p21<sup>cip1</sup> levels and S phase entry in mouse embryonic fibroblasts. Our results show that PKCδ selectively destabilizes p21<sup>cip1</sup> protein levels, and this effect is required for optimal S phase entry. In addition to identifying a novel post-transcriptional effect of PKC, our results emphasize that a single PKC isoform can stimulate or inhibit S phase entry, even when its target is the same cell cycle regulator.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—GF109203X (bisindolylmaleimide I), LY294002, and MG132 were purchased from Calbiochem. PP1 was purchased from Biomol. All stocks were made in Me<sub>2</sub>SO. PMA was obtained from LC Laboratories and dissolved in ethanol. Spontaneously immortalized mouse embryonic fibroblasts (MEFs)

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1. These authors contributed equally to this work.

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3. To whom correspondence should be addressed: Dept. of Pharmacology, University of Pennsylvania School of Medicine, 3620 Hamilton Walk, Philadelphia, PA 19104-6084. Tel.: 215-898-7157; Fax: 215-573-5656; E-mail: rka@pharm.med.upenn.edu.

4. The abbreviations used are: cdk, cyclin-dependent kinase; PKC, protein kinase C; MEF, mouse embryonic fibroblast; FBS, fetal bovine serum; SKF, Src family kinase; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; ERK, extracellular signal-regulated kinase; PMA, phorbol 12-myristate 13-acetate; BrdUrd, bromouridine; DN, dominant negative; PP1, 4-amino-1-tert-butyl-3-(1-hydroxyethyl)pyrazolo[3,4-d]pyrimidine.
PKCα Destabilizes p21cip1 Protein

from wild-type and p21cip1-null mice were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Unless noted otherwise, MEFs were grown to near confluence in 5% FBS-DMEM and then serum-starved in DMEM-1 mg/ml heat-inactivated, fatty acid-free BSA (DMEM-BSA) for 48 h. The quiescent cells were trypsinized, suspended in DMEM-BSA (1–1.5 × 106 cells/10 ml), plated in a 100-mm dish, and stimulated with 10% FBS. In some experiments, the suspended cells were preincubated in suspension with 25 μM LY294002, 10 μM PP1, 10 μM GF109203X, or 10 μM MG132 for 30 min at 37 °C. The cells were collected and lysed as described previously (29).

To monitor entry into S phase, quiescent wild-type and p21cip1-null MEFs were infected with adenoviruses as described below. At the end of the starvation period, the cells were reseeded at 50% confluence in 6-well dishes containing autoclaved glass coverslips and incubated in 2 ml of DMEM-10% FBS with 3 μg/ml bromouridine (BrduRd) (Amersham Biosciences; 1000-fold dilution). Formaldehyde-fixed cells were incubated with anti-BrduRd as described previously (30). Multiple fields of view (usually 50–100 cells in 3–4 fields) were counted per sample to determine the percentage of BrduRd-labeled nuclei.

Adenoviral Infections and Plasmid RNA Interference Transfections—Adenoviruses expressing dominant negative PKCα (DN-PKCα), dominant negative PKCβ (DN-PKCb), LacZ, or green fluorescent protein were generated as described in Refs. 31 and 32 and titered using Adeno-X™ Rapid Titer Kit (BD Biosciences). The dominant negative adenoviruses are kinase-dead due to a mutation from Arg to Lys in the ATP-binding site of the catalytic domain. The Skp2 adenovirus was a generous gift of Keiichi Nakayama. Infections with adenoviruses were performed as follows. Near confluent MEFs in 150-mm dishes were incubated in DMEM-BSA for 8 h. Adenoviruses were then directly added to the culture medium. After overnight infection, the medium was replaced with fresh DMEM-BSA, and the cells were starved for an additional 24 h. MEFs were transiently transfected with cyclin D1 RNA interference in pSUPER (kindly provided by Michael Olson) using 5 μg of plasmid and Lipofectamine Plus (Invitrogen) as described previously (29). The infected/transfected cells were trypsinized, reseeded at subconfluence, and stimulated with FBS as described above.

Western Blotting—Western blotting was performed using 25–75 μg of total cellular protein and the following antibodies: actin (sc-8432, Santa Cruz Biotechnology); cdk4 (AH20202, BIOSOURCE), cyclin D1 (sc-8396, Santa Cruz Biotechnology), p21cip1 (sc-6246, Santa Cruz Biotechnology), PKCα (sc-213, Santa Cruz Biotechnology), PKCβ (K25020, BD Transduction Laboratories), ERK (610031, BD Transduction Laboratories), Skp2 (32-3400, Invitrogen) and PKCα (610107, BD Transduction Laboratories). Proteins were detected using ECL (Amer- sham Biosciences) and film. Autoradiograms were digitized by scanning, and figures were assembled using Photoshop version 6.0. Vertical spaces in Figs. 1C and 4A indicate where extraneous information was removed from a scanned image.

Quantitative Real-time Reverse Transcription-PCR—Isolation of total RNA, cDNA synthesis, and PCR was performed as described previously (33). For mouse p21cip1, the real-time PCR reaction contained 900 nM forward primer TCC ACA GCG ATA TCC AGA CAT T, 900 nM reverse primer CGG ACA TCA CCA GGA TTG G, and 250 nM probe 6FAM-AGA GCC ACA GCC ACC-minor groove binder-nonfluorescent quencher (MGB-NFQ). For 18 S rRNA, we used 150 nM forward primer CCT GGT TGA TCC TGC CAG TAG, 150 nM reverse primer CGG TCG GTA CTT AGA CAT GCA, and 100 nM probe VIC-TTG TCT CAA AGA TTA-MGB-NFQ. p21cip1 mRNA and 18 S rRNA levels were quantified by standard curve using ABI Prism 7000 sequence detection system software. The levels of p21cip1 mRNA were normalized to 18 S rRNA. Duplicate PCR reactions were run for each sample, and results were plotted as mean ± S.D.

RESULTS

Inhibition of PKC Up-regulates p21cip1 Levels as Fibroblasts Progress into Late G1 Phase—Our initial studies used pharmacological inhibitors of several kinases to identify signal transducers other than Rho (see Introduction) that control the down-regulation of p21cip1 during G1 phase progression in fibroblasts. Inhibition of Src family kinases (SFKs) with PP1 (Fig. 1A) reduced the induction of p21cip1, but when we inhibited SFKs after the initial induction of p21cip1 occurred, we found p21cip1 down-regulation was normal in the SFK-inhibited cells (Fig. 1B). Similarly, inhibition of phosphatidylinositol 3-kinase with LY294002 did not affect the down-regulation of p21cip1 during late G1 phase (Fig. 1C). In contrast, inhibition of the PKC family with GF109203X, a pharmacological inhibitor that blocks PKCα, -β, -δ, and -ε (34), prevented the down-regulation of p21cip1 protein seen in late G1 phase (Fig. 1D).

Studies in epithelial cells indicate that phorbol ester-sensitiv PKCs usually act as anti-mitogens and that their anti-mitogenic effects are due to the stimulation of p21cip1 gene expression (Introduction). To determine whether PKCs could stimulate p21cip1 levels in fibroblasts, we compared the levels of p21cip1 after quiescent MEFs were treated with the phorbol ester PMA (an activator of conventional and novel PKCs), serum, or the combination of PMA and serum (Fig. 2A). PMA alone did increase the level of p21cip1 in early G1 phase of MEFs (Fig. 2A, 3 h), but this effect was transient, and p21cip1 levels
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FIGURE 1. PKC inhibition regulates \( p21^{\text{cip1}} \) protein levels. Quiescent MEFs were pretreated with vehicle or 10 \( \mu M \) PP1 (A), 25 \( \mu M \) LY294002 (L; Y; C), or 10 \( \mu M \) GF109203X (G; F; D). The cells were then seeded at subconfluence and stimulated with 10% FBS. \( \beta \), PP1 was added 3 h after serum stimulation to eliminate its effect on \( p21^{\text{cip1}} \) induction. Collected cells were lysed and analyzed by immunoblotting with antibodies to \( p21 \) and cdk4 (loading control). DMSO, Me\(_2\)SO (dimethylsulfoxide).

FIGURE 2. Transient induction of \( p21^{\text{cip1}} \) by PMA in fibroblasts. A, quiescent MEFs were stimulated with 100 nM PMA in DMEM-BSA in the absence or presence of 10% FBS. PMA was removed after 1 h of incubation by washing, and the medium was replaced. Cells were collected, lysed, and analyzed by Western blotting for the expression of \( p21^{\text{cip1}} \) and cdk4 (loading control). \( \beta \), total RNA extracted from the collected cells and analyzed by quantitative real-time reverse transcription-PCR for expression of \( p21^{\text{cip1}} \) mRNA and 18 S rRNA. \( p21^{\text{cip1}} \) mRNA expression is plotted relative to 18 S rRNA. FCS, fetal calf serum.

FIGURE 3. Up-regulation of \( p21^{\text{cip1}} \) protein upon inhibition of PKCδ. A, quiescent MEFs were infected at a multiplicity of infection of 100 with adenoviruses encoding LacZ, DN-PKCα, or DN-PKCδ. Cells were trypsinized, reseeded, and stimulated with 10% FBS for the times indicated. Collected cells were lysed and analyzed by Western blotting for the expression of \( p21^{\text{cip1}} \), PKCα, PKCδ, and cdk4 (loading control). \( \beta \), total RNA extracted from the cells infected with LacZ or DN-PKCα was analyzed by quantitative real-time reverse transcription-PCR for \( p21^{\text{cip1}} \) mRNA and 18 S rRNA. \( p21^{\text{cip1}} \) mRNA expression was plotted relative to 18 S rRNA. C, asynchronous MEFs were incubated with an irrelevant siRNA (Ctrl, control) or two siRNAs (lanes A and B) to PKCδ as described under “Experimental Procedures.” The levels PKCα, PKCδ, \( p21^{\text{cip1}} \), and actin were determined by Western blotting.

were barely detectable by late G1 phase (Fig. 2A, 9–18 h). Serum stimulation also resulted in a transient increase in \( p21^{\text{cip1}} \) levels, and PMA did not enhance the duration of the serum-stimulated signal (Fig. 2A). Although quantitative real-time reverse transcription-PCR did reveal a small stimulatory effect of PMA on \( p21^{\text{cip1}} \) mRNA relative to serum-treated MEFs (Fig. 2B), PMA (alone or in the presence of serum) did not allow for the sustained increase in \( p21^{\text{cip1}} \) gene expression that is characteristic of PMA-stimulated PKC activation in epithelial cells (28). Taken together, the results shown in Figs. 1 and 2 indicate that (i) a pro-mitogenic PKC is effectively activated by serum and inhibited by GF109203X in MEFs, (ii) activation of phospholipase C can stimulate \( p21^{\text{cip1}} \) gene expression in MEFs, but the effect is very short-lived, and (iii) the dominant PKC effect in MEFs is the late G1 phase reduction in \( p21^{\text{cip1}} \).

PKCδ Mediates the Post-transcriptional Down-regulation of \( p21^{\text{cip1}} \). To identify the PKC family member required for the down-regulation of \( p21^{\text{cip1}} \), we infected MEFs with adenoviruses encoding LacZ (control) or DN-PKCα and -PKCδ, two of the major PKC isozymes targeted by GF109203X and expressed in MEFs. Despite similar levels of expression, DN-PKCα did not affect the levels of \( p21^{\text{cip1}} \) during G1 phase, whereas DN-PKCδ strongly inhibited the late G1 phase down-regulation of \( p21^{\text{cip1}} \) (Fig. 3A). Interestingly, the effect of DN-PKCδ on \( p21^{\text{cip1}} \) protein was not accompanied by changes in the level of \( p21^{\text{cip1}} \) mRNA (Fig. 3B). These results indicate that PKCδ is required for the post-transcriptional down-regulation of \( p21^{\text{cip1}} \) in late G1 phase in MEFs. This post-transcriptional regulation of \( p21^{\text{cip1}} \) by PKCδ that we see in fibroblasts is notably distinct from the transcriptional control of \( p21^{\text{cip1}} \) that is commonly seen in response to altered PKCδ activity in epithelial cells (28). Selective knockdown of PKCδ with two different siRNAs increased \( p21^{\text{cip1}} \) levels 2.2–2.6-fold without comparable effect on \( p21^{\text{cip1}} \) mRNA (not shown), confirming that the δ isoform of PKC is responsible for the post-transcriptional down-regulation of \( p21^{\text{cip1}} \) (Fig. 3C).
PKC\(\delta\) Destabilizes p21\(^{cip1}\) Protein

**FIGURE 4.** Inhibition of PKC\(\delta\) regulates p21\(^{cip1}\) protein stability through the proteasome. A, asynchronous MEFs (10^6) were seeded in 100-mm dishes and incubated overnight in DMEM-10% FBS. The cells were then incubated with 10 \(\mu\)g/ml of cycloheximide (CHX) in fresh medium for the times shown. B, near confluent MEFs in 150-mm dishes were infected with DN-PKC\(\delta\) or LacZ adenoviruses at a multiplicity of infection of 100 in DMEM-10% FBS and incubated overnight. Cells were reseeded into four 100-mm dishes and cultured in DMEM-10% FBS for 24 h before the addition of fresh medium containing 10 \(\mu\)g/ml cycloheximide. Samples were collected at the times shown. Light and dark exposures were chosen to normalize the starting levels of p21\(^{cip1}\) in the control and PKC-inhibited cells. C, asynchronous MEFs were transfected with irrelevant siRNA (control) or PKC-\(\delta\) siRNA-A. Cycloheximide was added 48 h after transfection, and the cells were collected at the times shown. D, quiescent MEFs were infected at a multiplicity of infection of 100 with LacZ or DN-PKC\(\delta\) adenoviruses as described under “Experimental Procedures.” Cells (10^6) in 10 ml of DMEM-BSA) were trypsinized and pretreated with Me\(_2\)SO or 10 \(\mu\)M MG132 in suspension for 30 min at 37°C. The cells were seeded and stimulated with 10% FBS for the times indicated. Lysates (A–D) were analyzed by Western blotting for the proteins shown.

PKC\(\delta\) Regulates p21\(^{cip1}\) Protein Stability—Because we found that PKC\(\delta\) regulates p21\(^{cip1}\) post-transcriptionally, we asked whether it was affecting p21\(^{cip1}\) protein stability. The half-life of p21\(^{cip1}\) in MEFs is \(-30\) min (16), and indeed we found that p21\(^{cip1}\) levels were almost undetectable 2 h after exposure of MEFs to cycloheximide (Fig. 4A). We then infected MEFs with an adenovirus expressing LacZ or DN-PKC\(\delta\), blocked protein synthesis with cycloheximide, and determined the stability of p21\(^{cip1}\) during the subsequent 2 h (Fig. 4B). In LacZ-infected MEFs, p21\(^{cip1}\) levels decayed with a halflife of \(-60\) min. In contrast, p21\(^{cip1}\) levels remained nearly constant in cycloheximide-treated MEFs expressing DN-PKC\(\delta\) for at least 120 min. A similar, although somewhat less pronounced, effect was detected after siRNA-mediated knockdown of PKC\(\delta\) (Fig. 4C). Thus, PKC\(\delta\) destabilizes the p21\(^{cip1}\) protein in MEFs. Consistent with this conclusion, we found that the pronounced effect of DN-PKC\(\delta\) on late G\(_1\) phase p21\(^{cip1}\) levels (Fig. 4D, compare lanes 2–4 and 6–8) was lost when the 20 S proteasome was inhibited with MG132 (Fig. 4D; compare lanes 9–11 and 12–14).

**FIGURE 5.** Inhibition of PKC stabilizes p21\(^{cip1}\) independently of Skp2 and cyclin D1—Some studies have implicated the E3 ubiquitin ligase SCF\(^{Skp2}\) in the degradation of p21\(^{cip1}\) (35). We therefore treated MEFs with GF109203X or DN-PKC\(\delta\) to assess the effect of PKC inhibition on Skp2 expression, the rate-limiting component of the SCFSkp2 complex. In some experiments, the expression of Skp2 was partially inhibited by GF109203X (Fig. 5A) or DN-PKC\(\delta\) (not shown), whereas in other experiments, these treatments had no effect on Skp2 levels (not shown). However, even when these treatments caused a reduction in Skp2, the magnitude of the effect was insufficient to prevent the down-regulation of p21\(^{cip1}\) (Fig. 5A), the best established substrate of the SCFSkp2 complex. Moreover, ectopic overexpression of Skp2 did not affect the levels of p21\(^{cip1}\) during G\(_1\) phase progression in either control or GF109302X-treated MEFs (supplemental Fig. 1). We therefore conclude that the effect of PKC on p21\(^{cip1}\) half-life is independent of its effect on the expression of Skp2. Additionally, these data show that the effect of GF109203X and DN-PKC\(\delta\) on p21\(^{cip1}\) levels are not due to a nonspecific inhibition of G\(_1\) phase progression, because p27\(^{kip1}\) down-regulates normally in these PKC-inhibited cells.

Coleman et al. (36) propose that p21\(^{cip1}\) is stabilized by cyclin D1 in Ras-transformed cells, because cyclin D1 competes with the C8\(\alpha\) subunit of the 20 S proteasome for binding to p21\(^{cip1}\). To determine whether cyclin D1 is required for the PKC-dependent destabilization of p21\(^{cip1}\), we transiently transfected MEFs with pSuper vectors encoding cyclin D1 RNA interference. We found that p21\(^{kip1}\) levels were slightly higher after knockdown of cyclin D1, suggesting that cyclin D1 expression...
PKCδ Destabilizes p21cip1 Protein

Effect on S phase entry, DN-PKCδ inhibited the proliferation of MEFs (Fig. 6B), and the magnitude of the proliferative effect was in general agreement with the magnitude of the DN-PKCδ effect on S phase entry.

Discussion

The down-regulation of p21cip1 in late G1 phase is thought to contribute to the activation of cdk2 and entry into S phase. We now report that PKC is required for the post-transcriptional destabilization of p21cip1 protein in MEFs. S phase entry is reduced by inhibiting PKCδ, and this effect is lost in p21cip1-null MEFs. Thus, a major mitogenic effect of PKCδ appears to be its destabilizing effect on p21cip1 protein. Interestingly, similar results have also been reported for Rho (at least in Ras-transformed cells); inhibition of Rho destabilizes p21cip1 protein (18–21) and inhibits S phase entry in wild-type but not p21cip1-null fibroblasts (19). Thus, the p21cip1 protein is the major cell cycle target for both Rho and PKCδ. However, the Rho effect is not selective for p21cip1 protein, as Rho inhibition also results in increased expression of p21cip1 mRNA (19).

The increased level of p21cip1 protein seen upon inhibition of PKCδ was dependent on the proteasome. Although one report linked the degradation of p21cip1 to ubiquitination by the SCFSkp2 complex (35), Sheaff et al. (12) showed that p21cip1 can be degraded by the proteasome, even when all of its lysines are mutated to arginines to prevent its ubiquitination. Additionally, the N terminus of p21cip1 appears to be acetylated in vivo, precluding its use as a site for attachment of ubiquitin (15). Consistent with these findings, we did not find a role for the E3 ubiquitin ligase Skp2 in the regulation of p21cip1 (19). Skp2 levels were slightly reduced by GF109203X or DN-PKCδ, but this effect is not likely to be functionally significant, because it was not accompanied by a change in the level of p21cip1 mRNA, the major SCFSkp2 substrate. Additionally, the elevated levels of p21cip1 seen in PKCδ-inhibited cells were not affected by overexpression of Skp2.

Several studies have identified kinases that phosphorylate p21cip1, although on different sites, and regulate its stability (37–41). For example, Akt-dependent phosphorylation has been linked to the cytoplasmic retention and increased stability of p21cip1 (37–39). Our data indicate that PKC has the opposite effect, decreasing p21cip1 levels and enhancing S phase entry. One study reported that a 3-phosphoinositide-dependent protein kinase-1 (PDK)-dependent activation of PKCζ leads to increased p21cip1 degradation (40), as does PKCδ in our studies. However, PDK is not likely involved in our system, because direct inhibition of phosphatidylinositol 3-kinase (which should inhibit PDK activation) did not up-regulate p21cip1 in MEFs. Moreover, PKCζ is not targeted by GF109203X or the other approaches we used to inhibit PKC.

Our data reveal specificity in the PKC isoform regulating p21cip1 stability; the effect is readily detected by inhibiting PKCδ but not by inhibiting PKCα. One difference between the two isoforms is that PKCδ can be tyrosine-phosphorylated by SFKs (42). However, our data indicate that SFK-dependent phosphorylation is not playing a role in regulating p21cip1 stability, because direct inhibition of SFKs did not mimic the effect of PKCδ inhibition and up-regulate p21cip1. Similarly, the abil-
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ity of PKCδ to bind RasGRP3 and enhance ERK activity (43) is unlikely to underlie its effects in cells, it can increase the expression of p21cip1 because changes in ERK activity regulate p21cip1 mRNA levels (16), and p21cip1 mRNA levels are not strongly affected by inhibiting PKCδ. Another difference between PKCα and PKCδ is that the δ isoform has a much broader subcellular distribution, being detected in mitochondria and in the perinuclear region as well as in the cytosol and plasma membrane (32, 44). We have yet to determine whether redirecting PKCα to these noncanonical compartments would allow it to regulate p21cip1 stability. Unfortunately, detailed analysis of the pathway that mediates PKC-dependent p21cip1 stability is compromised by the fact that the mechanism of p21cip1 degradation is not yet established (see above).

Although the results shown here indicate that PKCδ is responsible for the destabilization of p21cip1 and stimulation of S phase entry in MEFs, we have recently reported that PKC stimulates p21cip1 gene expression and inhibits S phase entry in transformed epithelial cells (28). In that study, we also observed that the PKC effect on p21cip1 was dependent on PKCδ and not PKCα. Thus, it appears that PKCδ can have two opposing effects in cells, it can increase the expression of p21cip1 mRNA, and it can destabilize p21cip1 protein. The final proliferative effect of PKCδ will therefore depend on how cells respond to an activated PKCδ signal. Interestingly, many of the studies showing the anti-mitogenic effect of PKC have been performed with transformed epithelial cell lines (see Introduction). It will be interesting to determine whether the distinct effects of PKCδ on p21cip1 reflect different cell lineages (mesenchymal versus epithelial) or the process of cellular transformation.

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