Regulation of Caveolin-1 Expression and Secretion by a Protein Kinase Cε Signaling Pathway in Human Prostate Cancer Cells*

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Caveolin-1, androgen receptor, c-Myc, and protein kinase Cε (PKCε) proteins are overrepresented in most advanced prostate cancer tumors. Previously, we demonstrated that PKCε has the capacity to enhance the expression of both caveolin-1 and c-Myc in cultured prostate cancer cells and is sufficient to induce the growth of androgen-independent tumors. In this study, we have uncovered further evidence of a functional interplay among these proteins in the CWR22 model of human prostate cancer. The results demonstrated that PKCε expression was naturally up-regulated in recurrent CWR22 tumors and that this oncoprotein was required to sustain the androgen-independent proliferation of CWR-R1 cells in culture. Gene transfer experiments demonstrated that PKCε had the potential to augment the expression and secretion of a biologically active caveolin-1 protein that supports the growth of the CWR-R1 cell line. Antisense and pharmacological experiments provided additional evidence that the sequential activation of PKCε, mitogen-activated protein kinases, c-Myc, and androgen receptor signaling drove the downstream expression of caveolin-1 in CWR-R1 cells. Finally, we demonstrate that mitogen-activated protein kinases were required downstream of PKCε to derepress the transcriptional elongation of the c-myc gene. Our findings support the hypothesis that PKCε may advance the recurrence of human prostate cancer by promoting the expression of several important downstream effectors of disease progression.

Caveolin-1 is an unusually versatile membrane protein that not only regulates cholesterol trafficking and the assembly of multimeric signaling complexes within the cell but is also able to function as an autocrine/paracrine factor when secreted by human prostate cancer cells (1). For an integral membrane protein that is normally transported to, and retained by, the plasma membrane (2), the latter finding was unexpected. However, there is now good evidence that biologically active caveolin-1 escapes into the medium of cultured human and mouse prostate cancer cells, where this protein functions to suppress apoptosis and regulate their androgen responsiveness (1, 3). Moreover, the finding that caveolin-1 antibodies suppress the growth and metastasis of prostate cancer tumors in mice (1)

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tion of c-Myc might influence the expression of caveolin-1 in prostate cancer cells. In rodent prostate cancer cells, c-Myc has been reported to repress caveolin-1 expression at the transcriptional level (9). At the same time, there is evidence that c-Myc-Max heterodimers interact with a novel exonic region of the androgen receptor (AR) gene to enhance AR autoregulation in the human PC3 prostate cancer cell line (10). The latter finding may be important because testoster-

region of the androgen receptor (AR) gene to enhance AR activity by reducing transcriptional attenuation (pause) at the 3′ end of exon 1 and, thereby, reinitiate transcriptional elongation (13). Studies using the MAP kinase (MEK) inhibitor PD98059 (50 μM, 48 h; Calbiochem) indicate that PKCε stimulates the elongation of c-myc transcripts through a MEK-dependent signaling pathway(s). Finally, we report that AR activity is required downstream of c-Myc to sustain the translation of caveolin-1 in CWR-R1 cells. The cumulative evidence supports the notion that PKCε transduces important mitogenic and survival signals that are propagated to the caveolin-1 gene through the sequential activation of MEK, ERK, c-Myc, and AR in androgen-independent CWR-R1 cells.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—The CWR-R1 cell line was clonally selected for androgen-independent growth from recurrent CWR22 xenograft tumors (12). These cells were cultured in Richter’s improved minimal essential medium (Invitrogen) supplemented with 100 ng/ml epidermal growth factor (Becton Dickinson Labware, Franklin Lakes, NJ), 10 μg/ml insulin/transferrin/selenium (BD Biosciences; Bedford, MA), 10 mM nicotinamide, 900 ng/ml linoleic acid (Sigma), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2% fetal bovine serum. Where specified, CWR-R1 cells were cultured in serum-free medium in the absence or presence of either brefeldin A (10 μM, 24 h; Calbiochem, San Diego, CA) or the MEK inhibitor PD98059 (50 μM, 48 h; Calbiochem). Subconfluent cultures of CWR-R1 cells were also exposed to the anti-androgen flutamide (1 μM, Sigma) for 48 h in complete medium.

**Expression Plasmid and Transfection into CWR-R1 Cells**—CWR-R1 cells were infected with either the empty pLXSN recombinant retrovi-
rus (CWR-R1/v) or pLXSN harboring the gene for p3/PKCε (CWR-R1ε) as described previously (14). Stably expressing cells were sorted and subcloned by limiting dilution in 500 μg/ml G418, as described previously (13). Studies using the MAPK kinase (MEK) inhibitor PD98059 (50 μM, 48 h; Calbiochem) indicate that PKCε stimulates the elongation of c-myc transcripts through a MEK-dependent signaling pathway(s). Finally, we report that AR activity is required downstream of c-Myc to sustain the translation of caveolin-1 in CWR-R1 cells. The cumulative evidence supports the notion that PKCε transduces important mitogenic and survival signals that are propagated to the caveolin-1 gene through the sequential activation of MEK, ERK, c-Myc, and AR in androgen-independent CWR-R1 cells.

**Results**

**PKCε Expression in Human Prostate Cancer CWR22**

**Androgen-independent human prostate cancer cell lines (DU145 and PC3) express elevated levels of endogenous PKCε, relative to the androgen-sensitive LNCaP cell line (3). In this study, we have extended this analysis by performing an immunoblot analysis of PKCε expression during the in vivo progression of prostate cancer using the CWR22 xenograft model. CWR22 tumors grow subcutaneously in intact male mice and undergo complete regression after castration but recur as androgen-independent tumors after several months (17). This reliable pattern of tumor regression and recurrence made it possible to compare the steady-state levels of endogenous PKCε in androgen-dependent and recurrent CWR22 tumor lysates. An analysis of seven pairs of such tumors indicated that castration induced a reproducible and significant increase in the levels of PKCε protein in CWR22 tumor cells (Fig. 1A). This increase may have resulted from the selective outgrowth of preexisting androgen-independent clones or an up-regulation of PKCε transcription/translation following androgen ablation. Results of a quantitative immunohistochemical analysis of PKCε expression in individual cancer cells within benign and recurrent primary tumor spec-
imens indicate that the overall increase in PKCε protein

**Regulation of Caveolin-1 Expression in Prostate Cancer**

**Immunoblot Analyses**—Immunoblot analyses were performed as de-
scribed previously (15). Antibodies purchased from Santa Cruz Biotech-
ology (Santa Cruz, CA) were raised against AR (N-20), caveolin-1 (N-20), ERK1 (K-23), c-Myc (C-19), and PKCε (C-15). Anti-β-actin

**RT-PCR Analyses**—CWR-R1 cells (1 × 10⁶) were collected by trypsinization and centrifugation at 2,000 × g for 5 min. Total RNA was prepared using an RNeasy Mini kit (Qiagen, Valencia, CA). The SuperScript one-step RT-PCR system (Invitrogen) was used to analyze the relative abundance of c-myc exon 1 and/or exon 2 transcripts. RT-PCR primers for human c-myc were derived from the published sequences. The sequences of the primers and their positions in the gene sequence are as follows: c-Myc, 5′- primer, 5′-CGTCCTCGAGAAGAGGAGGTC-TTCT-3′ (48–73); 3′-primer within exon 1, 5′-TCAGAGAAGCGGTC-CTTGGAGGCGGGGGAATG-3′ (453–486); 3′-primer within exon 2, 5′-GTGGGTGAAGCTACGTTGAGGGATCCTGCGCGGA-3′ (549–585). A RT-PCR primer and control set (Invitrogen), containing RT-PCR primers for β-actin, was used to confirm equal loading of all samples. RT-PCR reactions were performed in a PerkinElmer Life Sciences DNA thermal cycler GeneAmp PCR system 2400. The thermal cycling conditions comprised a cycle of CDNA synthesis and pre-denaturation at 45 °C for 15 min and 94 °C for 2 min, followed by 35 cycles of amplification at 94 °C for 15 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. RT-PCR products were analyzed using 5% PAGE. The size of the products for human c-myc exon 1, exon 2, and β-actin were 439, 538, and 350 bp, respectively.

**Preparation of Conditioned Medium**—CWR-R1 cells were serum-starved overnight and cultured in fresh serum-free medium for 3 days before collecting the conditioned medium. Possible contamination of membranous caveolin-1 was minimized by a sequential centrifugation of the conditioned medium; 1,000 × g for 5 min followed by 20,000 × g for 30 min. Trichloroacetic acid and deoxycholic acid were added to the conditioned medium to final concentrations of 10% and 2%, respectively. After 1 h on ice, the mixtures were centrifuged at 10,000 × g for 10 min, and the resultant pellets were dissolved in 3 μl Tris buffer. For in vitro viability assays, caveolin-1 antibody (Santa Cruz Biotechnology) was added to conditioned, or complete, medium and incubated for 16 h at 4 °C. Normal rabbit IgG (R & D Systems Inc., Minneapolis, MN) was used as a control.

**Data Analysis**—Values shown are representative of three or more experiments, unless otherwise specified, and treatment effects were evaluated using a two-sided Student’s t test. Errors are S.E. values of averaged results, and values of p < 0.05 were taken as a significant difference between means.

**Antisense Oligodeoxynucleotide Treatment**—Phosphorothioate oligodeoxynucleotides (ODN) were obtained from Invitrogen. Sequences for the antisense PKCε and c-Myc ODNs, and their corresponding scrambled or sense controls, were exactly as specified (3, 16). CWR-R1 cells were treated with ODNs as described previously (3). Briefly, sub-

confluent (70–80%) cultures were washed with Opti-MEM 1 (Invitro-
gen) before introducing a mixture of ODN and Lipofectin (2 μg/ml; Invitrogen). After 6 h at 37 °C, the cells were washed twice with Opti-
MEM 1 and incubated 18 h in Lipofectin-free medium containing ODN and 2% fetal bovine serum. Medium was replenished, and the cells were incubated for an additional 3 days in complete medium containing ODN before harvesting using trypsinization.

**Cell Lines and Culture Conditions**—The CWR-R1 cell line was clonally selected for androgen-independent growth from recurrent CWR22 xenograft tumors (12). These cells were cultured in Richter’s improved minimal essential medium (Invitrogen) supplemented with 100 ng/ml epidermal growth factor (Becton Dickinson Labware, Franklin Lakes, NJ), 10 μg/ml insulin/transferrin/selenium (BD Bio-

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PKCe protein levels in CWR22 tumors and the anti-proliferative effects of antisense PKCe in CWR-R1 cells. A, immunoblot analysis of endogenous PKCe and β-actin (loading control) in lysates of CWR22 and recurrent tumors. Immunoblots are representative of two experiments using a total of 14 tumor lysates. B, subconfluent cultures of CWR-R1 cells were exposed to Lipofectin alone (C), Lipofectin plus 2 μM scrambled control (SC) PKCe ODN, or 2 μM antisense (AS) PKCe ODN for 3 days. PKCe and ERK1/2 protein levels in CWR-R1 whole cell lysates were analyzed by immunoblotting. The results shown are representative of two independent experiments. C, subconfluent cultures of CWR-R1 cells were treated, as in B, with Lipofectin alone (C), scrambled control (SC) ODN, and either 1 or 2 μM antisense (AS) PKCe ODN, as indicated. After 3 days, viable cells were counted using the trypan blue exclusion assay. Data represent the means of triplicate determinations in three independent experiments. Bars, S.E.

shown in Fig. 1A most likely arose from the clonal selection of PKCe-positive glandular epithelial cells.

Antiproliferative Effects of Antisense PKCe ODNs in CWR-R1 Cells—The CWR-R1 cell line is an androgen-independent derivative of recurrent CWR22 tumors that, unlike DU145 and PC3 cells, expresses a functional mutated AR (H874Y) with diminished ligand specificity (12). This finding suggests that the CWR-R1 model may more accurately recapitulate the genetic composition of recurrent tumors than the immortalized DU145 or PC3 cell lines. We have recently shown that antisense PKCe ODN significantly inhibits the androgen-independent proliferation of DU145 and PC3 cells (3). In this study, we have extended this analysis to include the androgen-independent CWR-R1 cell line. The results shown in Fig. 1C indicated that by specifically interfering with normal PKCe function, even in the presence of many other PKC isozymes, it was possible to significantly compromise the ability of CWR-R1 cells to sustain their normal rate of proliferation. Translation of PKCe mRNA was selectively and effectively down-regulated by exposing CWR-R1 cells to antisense PKCe ODN without altering the steady-state concentration of ERK1/2 (Fig. 1B). Under identical conditions, neither Lipofectin alone nor Lipofectin plus the scrambled PKCe ODN inhibited PKCe or ERK expression in CWR-R1 cells. The decreased expression of PKCe induced by the antisense PKCe ODN was associated with a sequence-specific and dose-dependent inhibition of CWR-R1 growth/survival (Fig. 1C). After a 3-day exposure to 2 μM PKCe AS ODN, there was ~75% reduction in the number of viable CWR-R1 cells, relative to the number of CWR-R1 cells growing in the presence of an equimolar concentration of the scrambled ODN. These and other data suggest that PKCe may be an important factor in the pathways governing growth and proliferation within genetically diverse androgen-independent prostate cancer cells (3).

PKCe Enhances Caveolin-1 Synthesis and Secretion by CWR-R1 Cells—In the LNCaP cell line, PKCe overexpression stimulates the expression and secretion of biologically active caveolin-1 (3). CWR-R1 cells express moderate levels of both endogenous PKCe and caveolin-1 (Fig. 3) but did not accumulate sufficient amounts of caveolin-1 in their culture medium to be detected by immunoblotting. However, if the signaling networks linking PKCe to caveolin-1 have been retained during acquisition of an androgen-independent growth capacity in the CWR-R1 cell line, PKCe overexpression would be expected to both increase caveolin-1 expression and secretion. To test these predictions, we established CWR-R1 variants harboring either an empty pLXSN retroviral vector (CWR-R1/v) or the PKCe cDNA (CWR-R1/ε). An immunoblot analysis of whole cell lysates confirmed that CWR-R1/ε, but not CWR-R1/v, cells maintained high levels of PKCe in subconfluent cultures while also augmenting the expression of caveolin-1 (Fig. 2A). At the same time, PKCe overexpression stimulated the secretion (expulsion) of caveolin-1 from CWR-R1 cells (Fig. 2B). When treated with brefeldin A, to collapse the Golgi apparatus into the ER (18), it was possible to detect small amounts of caveolin-1 in CWR-R1 conditioned medium (Fig. 2C, top left panel) and in the CWR-R1/ε cell line it was evident that this protein escaped via a Golgi-independent route of vesicle/lipid droplet-mediated transport. Following a 24-h exposure to brefeldin A (10 μM), the amounts of caveolin-1 detected in CWR-R1/ε conditioned medium and cell lysates coordinately increased and decreased, respectively (Fig. 2C). In contrast, brefeldin A did not alter the steady-state concentrations of the cytosolic protein ERK1/2 in CWR-R1/ε cells. These results indicated that any genetic aberrations that may be peculiar to the LNCaP cell line do not account for the powerful influence of PKCe-meditated signaling on the synthesis and post-translational sorting of caveolin-1 in human prostate cancer cells.

PKCe-induced Caveolin Secretion Promotes the Proliferation/Survival of CWR-R1 Cells—The proliferation of CWR-R1 cells was inhibited by 25% when the complete growth medium was
replaced with one containing no exogenous growth factors and charcoal-dextran treated fetal bovine serum (Fig. 2D, bars 1 versus 2). Under these conditions, a cell-free medium that had been preconditioned by CWR-R1/H9280 cells partially reversed this suppression of CWR-R1 autocrine growth and an antibody raised against the N terminus of caveolin-1 not only reversed this modest growth promoting effect of CWR-R1/H9280 conditioned medium but titered down the proliferation rate of CWR-R1 cells to as low as 25% of the untreated controls (Fig. 2D). These results confirm recent reports that highly malignant mouse and human prostate cancer cells generally depend on caveolin-1 as an autocrine/paracrine growth factor to sustain their proliferation/survival (1, 3). Equivalent concentrations of IgG were significantly less effective in suppressing the autocrine growth and proliferation of CWR-R1 cells. These results indicate that caveolin-1 secreted by prostate cancer cells may either activate mitogenic signaling pathways and/or neutralize the growth suppressive effects of unknown factors that accumulate in the medium of CWR-R1 cells under autocrine growth conditions. Anti-caveolin-1 antibody also significantly inhibited the proliferation/survival of CWR-R1 cells grown in complete medium (Fig. 2E), suggesting that secreted caveolin-1 makes an important contribution to the optimal growth of these cells in the absence and presence of androgens.

**c-Myc Expression and AR Signaling Are Both Required for PKCe to Enhance Caveolin-1 Expression in CWR-R1 Cells**—The positive influence that PKCe exerts on the expression of c-Myc and caveolin-1 in androgen-sensitive (LNCaP) and androgen-independent (CWR-R1) prostate cancer cells may be of clinical relevance, as increased levels of both c-Myc and caveolin-1 proteins are common in advanced prostate cancer and appear to be independent markers of progressive disease (19). However, these two molecular signatures of advanced prostate cancer had not previously been linked at a functional level. To the contrary, when c-Myc has been overexpressed in various ways and in both rodent and human prostate cancer cells, investiga-
mors have observed a repression of caveolin-1 transcription (9). This implied that at least some of the important downstream responses induced by the enforced expression of c-Myc may not accurately recapitulate the natural progression of those to be found in prostate cancer. We therefore questioned whether PKCε might be capable of transducing signals that either bypassed the transcriptional repression of the caveolin-1 gene by c-Myc or was actually reliant on the physiological influence of this oncogene for the up-regulation of caveolin-1 expression. As discussed below, the latter explanation would be consistent with independent reports that AR is a short-lived protein that is degraded by a ubiquitin-mediated proteolytic pathway that may be inhibited by c-Myc-regulated genes (20, 21).

To determine whether PKCε overexpression was capable of simultaneously augmenting the expression of both endogenous c-Myc and AR proteins, we initially performed an immunoblot analysis of whole cell lysates prepared from subconfluent cultures of CWR-R1, CWR-R1/ε, and CWR-R1/ε cells. Immunoblots shown in Fig. 3A demonstrated that such an expression profile could be induced by PKCε overexpression in the CWR-R1 cell line. Next, we performed a series of antisense experiments to determine whether the expression of endogenous PKCε was an important factor in the regulation of c-Myc, AR, and/or caveolin-1 expression in the CWR-R1 cell line. Artificial down-regulation of PKCε with antisense ODN effectively inhibited the translation of c-Myc, AR, and caveolin-1 mRNAs without altering the expression of either ERK1/2 or β-actin in CWR-R1 cells (Figs. 1B and 2B). Importantly, the levels of these proteins were not different in CWR-R1 cells treated with Lipofectin alone or Lipofectin combined with the scrambled PKCε ODN. As an additional test, we treated subconfluent cultures of CWR-R1 cells with antisense c-Myc ODN. These studies demonstrated that c-Myc translation is required to maintain normal levels of AR and caveolin-1 proteins but not endogenous PKCε or ERK1/2 (Fig. 3C). Controls treated with the sense c-Myc ODN maintained a basal level of AR and caveolin-1 proteins, indicating that the expression profiles observed in these experiments were specifically targeted toward at least two of the putative downstream effectors of endogenous c-Myc in CWR-R1 cells (Fig. 3C). Finally, we used the antiandrogen flutamide to determine whether AR-dependent signaling selectively influences caveolin-1 expression. When subconfluent cultures of CWR-R1 cells were incubated in the presence of complete growth medium plus flutamide (1 μM for 48 h), the levels of caveolin-1 protein were decreased without altering the levels of endogenous c-Myc, AR, or ERK1/2 (Fig. 3D). The data from these studies support the notion that elevated levels of caveolin-1 expression may be maintained in CWR-R1 cells through the activation of a cascade that relies on the basal expression of endogenous PKCε and c-Myc and the ligand-dependent activation of a downstream effector for the AR signaling pathway.

PKCε Relies on a MAPK Signaling Pathway to Promote the Transcriptional Elongation of c-myc Transcripts in CWR-R1 Cells—Exon 1 of the c-myc gene is a relatively large, untranslated, leader sequence that contains two alternative initiation sites. Despite its lack of coding potential, this exon exerts an important influence on expression of the c-myc gene. Following recruitment and activation of a pre-initiation complex, the presence of a paused RNA polymerase restricts the transcriptional elongation of c-myc beyond the 3’ end of exon 1 (22). In erythroid progenitor cells, erythropoietin is able to override this repression of c-myc elongation by activating a PKCε- and MEK-dependent pathway (13, 23). To determine whether downstream effectors of PKCε regulate the transcriptional activity of the c-myc gene through a similar mechanism in CWR-R1 cells, we performed a series of RT-PCR assays to compare the relative steady-state concentrations of c-myc exon 1 versus exons 1 and 2. For c-myc, factors capable of diminishing the ratio of exon 1 (transcriptional initiation) to exon 2 (transcriptional elongation) increase the levels of c-myc transcripts. Using primers targeted for nucleotides 48–73 of exon 1 or nucleotides 453–486 within exon 2 of the human c-myc gene (see “Experimental Procedures”), we consistently detected relatively higher basal levels of the exon 1 transcript in control CWR-R1 cells treated with the scrambled PKCε ODN (Fig. 4, A and B). When the expression of endogenous PKCε was artificially down-regulated using antisense ODN, there was no change in the levels of exon 1 transcripts, whereas transcriptional elongation of c-myc exon 2 was significantly inhibited (Fig. 4A). At the same time, antisense PKCε ODN treatments did not alter transcription of the β-actin gene (Fig. 4A). Finally, an inhibitor of MEK1/2 activity (PD98059) was used to determine whether this mitogenic pathway propagates PKCε signals to the c-myc gene in CWR-R1 cells. The results shown in Fig.
FIG. 4. PKCe promotes the transcriptional elongation of c-myc transcripts via a MAPK signaling pathway. A, RT-PCR analysis of total RNA from subconfluent cultures of CWR-R1 cells incubated with scrambled (SC) PKCe ODN or antisense (AS) PKCe ODN for 3 days. Equal amounts of total RNA (1 μg) were subjected to RT-PCR and products of the predicted size encoding exon 1 (top panel) and exon 1/2 (middle panel) of the human c-myc gene and β-actin (bottom panel) were detected. The results shown are representative of two independent experiments. B, RT-PCR analysis of total RNA from subconfluent cultures of CWR-R1 cells incubated in the absence or presence of PD98059 (50 μM), as indicated, for 48 h. Equal amounts of total RNA (1 μg) were subjected to RT-PCR and products of the predicted sizes, as in A, were detected. The results shown are representative of two experiments.

4B demonstrate that the signal by PKCe to c-myc requires constitutive MEK activity to override the transcriptional repression of c-myc in CWR-R1 cells. In contrast, PD98059 treatments did not alter the initiation of c-myc transcription or the levels of β-actin transcripts in CWR-R1 cells (Fig. 4B). This finding is consistent with our observation that the MEK/ERK pathway is an imminent, if not direct, target of PKCe in LNCaP cells (3) and provides convincing evidence that this signaling cascade has the potential to release paused RNA polymerase molecules downstream of c-myc promoter 2 and promote transcription of its two coding exons in CWR-R1 cells.

DISCUSSION

This study has unraveled the hierarchical organization of at least two signaling pathways that PKCe engages to maintain the basal expression of c-Myc, AR, and caveolin-1 in human CWR-R1 prostate cancer cells. Several independent lines of evidence from gene transfer, antisense, and pharmacological experiments are presented in this paper that support the notion that PKCe is capable of sequentially activating MEK, ERK, c-myc, and AR to promote caveolin-1 expression and the proliferation of CWR-R1 cells in culture. Detailed descriptions of the factors governing the activity of the molecular components featured within this PKCe signaling cascade have been proposed and additional intermediary events should be anticipated (9, 10, 13). There is no clear and direct evidence that explains how caveolin-1 is diverted from the classic exocytotic pathway to reach, and cross, the plasma membrane and then function as an autocrine/paracrine factor in prostate cancer progression. Determining the mechanisms controlling caveolin-1 biogenesis and bioactivity in prostate cancer cells will be a significant prerequisite for understanding the malignant progression of this disease. Although growth of CWR-R1 cells is androgen-independent (12), we have demonstrated that PKCe has the potential to promote the proliferation of prostate cancer cells in the presence and absence of testicular androgens in vitro (3). Therefore, it should be anticipated that androgen status might not be a critical determinant of how PKCe influences the growth of these cells. However, we have now presented evidence of a functional link between PKCe-mediated signal transduction and the androgen-independent proliferation of a variety of prostate cancer cells (Ref. 3 and this paper). Identification of c-myc, AR, and caveolin-1 as important factors in the propagation of growth/survival signals downstream of PKCe provides crucial support for the hypothesis that these statistically independent predictors of disease progression may actually be interdependent components of a significant regulatory pathway that permits some prostate cancer cells to escape the apoptotic penalty that is generally imposed by androgen withdrawal.

Multiple factors contribute to the progression of prostate cancer. Although there is no question that Raf-1 is a downstream target of PKCe, Ras/Raf induction alone is insufficient to promote the androgen-independent proliferation of prostate cancer cells (24). PKCe must therefore signal to additional downstream targets to promote caveolin-1 expression and the androgen-independent growth of LNCaP and CWR-R1 cells (3). This would be consistent with the inability of the MEK inhibitor PD98059 to completely inhibit c-myc transcriptional elongation in CWR-R1 cells (Fig. 4B) and the androgen-independent proliferation of DU145 and PC3 cells. Nuclear factor κB is an attractive alternative candidate because this transcription factor is a known downstream target of PKCe (25) that transcriptionally activates c-myc in a variety of cell types (26). In the case of the MEK/ERK-dependent arm of the PKCs signaling cascade, we have now demonstrated that the PKCe-dependent activation of MEK/ERK permits RNA polymerases to resume elongation of c-myc transcripts beyond exon 2.

Even minor fluctuations in the transcriptional activity of c-Myc can have profound consequences. Decreasing c-Myc levels by one-half prolongs the cell cycle (27), and unscheduled c-Myc activity is tumorigenic in many cell types (28). The essential and strict control of c-Myc activity is provided by a variety of mechanisms, including chromatin remodeling, long distance interactions between regulatory sequences, proximal cis-sequences within the promoter, alternative sites of transcriptional initiation, and repressors of transcriptional elongation (29, 30). c-myc has a three-exon/two-intron structure and contains two major promoters (P1 and P2) and two minor promoters (P0 and P3). Transcriptional initiation at the c-myc P2 promoter is repressed by a ME1a1 site at position −40 bp relative to the P2 start site (30). After initiation at the upstream P1 promoter, RNA polymerase II seems to pause at the ME1a1 site and requires further activation signals for processive transcription and elongation. It has been suggested that

3 D. Wu and D. M. Terrian, unpublished observations.
the P1 promoter may repress P2 activity and that ME1a1 binding factors are required for opening up the DNA strand of the c-myc P2 promoter region (30). However, the identity of functional ME1a1 binding factors and the signals regulating their interaction with the c-myc promoter have not yet been well established. The present study provides the first evidence that PKCε signaling through MEK/ERK may be capable of reinitiating c-myc transcription in CWR-R1 cells. A potential mechanism for this step in the PKCε to caveolin-1 signaling cascade may involve the inhibition of pocket protein (pRb, p107, and p130) recruitment to the E2F site located at position –64 bp relative to the P2 promoter (31). This suggestion is in line with the observation that PKCε overexpression leads to the hyperphosphorylation (inactivation) of Rb in LNCaP cells (3).

A consistent decrease in the steady-state concentration of AR protein but not mRNA (Fig. 3 and data not shown) accompanied antisense neutralization of c-Myc in the CWR-R1 cell line. Previous studies demonstrated that levels of AR transcripts do not differ between CWR22 and recurrent CWR22 tumors, whereas recurrent CWR22 tumors and CWR-R1 cells maintain significantly elevated levels of AR protein and decreased AR degradation rates (12). Androgen binding prolongs the half-life of AR by forestalling ubiquitin-mediated proteosomal degradation (20, 32) and, in the absence of hormone, Hsp90 stabilizes the transactivation/derepression of the caveolin-1 gene have not been defined. The gene encoding caveolin-1 is localized to the q31.1 region of human chromosome 7 and contains a TATA-

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