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Nuclear Matrix Targeting of the Protein Kinase CK2 Signal as a Common Downstream Response to Androgen or Growth Factor Stimulation of Prostate Cancer Cells[1]

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ABSTRACT

Protein kinase CK2, a messenger-independent serine/threonine kinase, has been implicated in cell growth. Androgenic stimulus in rat prostate modulates its association with nuclear matrix (NM) and chromatin. Because the growth of human prostate carcinoma cells is influenced by androgens and/or growth factors, we determined the nature of CK2 signaling in the NM in response to androgen and growth factor stimuli. Androgen-sensitive LNCaP and androgen–insensitive PC-3 cells were cultured in media to regulate their growth in the presence of 5α-dihydrotestosterone (5α-DHT) or growth factors (epidermal growth factor, keratinocyte growth factor, and transforming growth factor α). The activity of CK2 was measured in the cytosolic and NM fractions isolated from these cells after treatment with growth stimuli. The changes in CK2 in various fractions were also confirmed by immunoblotting with a specific antibody. LNCaP cells responded to both 5α-DHT and growth factors for growth. The presence of these agents in the culture medium evoked a translocation of CK2 to the NM from the cytosol. The PC-3 cells did not respond to 5α-DHT for growth but did respond to growth factors. Under these conditions, there was also a translocation of CK2 to the NM concomitant with a decrease in the cytosolic fraction. These results suggest that CK2 translocation to the NM occurs in response to various growth stimuli in cells in culture. Thus, CK2 is a common downstream signal transducer in response to diverse growth stimuli that may relate to the pathobiology of prostate cancer cells.

INTRODUCTION

Prostatic growth and development are known to be under strict androgenic control mediated via the androgen receptor system (1–3). Androgens are also implicated in prostatic neoplasia (benign prostatic hyperplasia and prostate cancer), although the mechanisms remain poorly understood (4–7). An important development in the progression of prostate cancer is that these cells become androgen insensitive and dependent on paracrine or autocrine growth factors (8–11). Thus, studies of the downstream mechanisms that may be involved in cell regulation in response to these diverse stimuli in prostate cancer cells are of intense interest. Experimental models such as the androgen-sensitive prostate cancer cell line LNCaP as well as a number of androgen-insensitive cell lines such as PC-3 and DU-145 have been used extensively to study the *in vitro* mechanisms of growth regulation in these cells. The LNCaP cell line shows androgen sensitivity and responds to growth factors such as EGF, whereas the PC-3 cell line does not respond to androgen but is stimulated by various growth factors.

We have been interested in the mechanism of growth regulation in the prostate, with a focus on the role of protein kinase CK2 in this function (12, 13). For this, we have used the rat ventral prostate model that has been used extensively for studies of the *in vivo* action of androgen on prostatic growth control (14, 15). Protein kinase CK2 (formerly known as casein kinase 2 or II) is a highly conserved, ubiquitous messenger-independent serine/threonine kinase localized in cell nucleus and cytoplasm. A large body of observations have suggested that CK2 plays a major role in cell growth and proliferation (12, 16–19). Concordant with such functions is its involvement in the phosphorylation of several growth-related nuclear protein substrates including, *e.g.*, topoisomerase II, RNA polymerases, protein B23, certain proto-oncogene products, and growth factors. CK2 also plays a role in cell cycle progression, although the underlying mechanism is unclear (20). Our studies have indicated that CK2 is dynamically regulated with respect to its nuclear localization and preferential association with chromatin and NM[3] in response to androgenic stimulus in the prostate, which is of considerable significance because both NM and chromatin are fundamental to cell growth and proliferation (21–25). In the rat prostate, androgens exert a profound effect on CK2 signaling to NM and chromatin (21, 23–27). CK2 is also dysregulated in human prostatic neoplasia and demonstrates enhanced nuclear localization possibly related to Gleason's tumor grade (28). In view of these various observations, we decided to investigate the effect of androgens and growth factors on CK2 signaling in human prostate cancer cells in culture by using the androgen-sensitive LNCaP and androgen-insensitive PC-3 cells as the model. Our results demonstrate that CK2 association with NM occurs in LNCaP cells stimulated by 5α-DHT and/or growth factors such as EGF, whereas the nuclear signaling of CK2 in PC-3 cells was insensitive to androgens but responded to growth factors. These results indicate that CK2 signaling to NM is a common downstream response to androgen and growth factor stimulation of prostate cancer cells that may be pertinent to the pathobiology of androgen-dependent as well as androgen-independent prostate cancer.

MATERIALS AND METHODS

Chemicals. Synthetic dodecapeptide substrate (Arg-Arg-Arg-Ala-Asp-Asp-Ser-Asp-Asp-Asp-Asp-Asp) for assay of CK2 activity was purchased from Peptide Technologies Corp. (Gaithersburg, MD). Cell proliferation assay reagent WST-1 was purchased from Boehringer Mannheim (Indianapolis, IN). Growth factors human EGF and KGF were purchased from R&D Systems (Minneapolis, MN), and TGF-α was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest purity available.

Cell Culture. Human prostate adenocarcinoma cell lines LNCaP and PC-3 were obtained from American Type Culture Collection (Rockville, MD). They were maintained separately in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 2 mM L-glutamine, 25 mM HEPES buffer, and 10% FBS (Hyclone Laboratories, Logan, UT) in an atmosphere containing 5% CO_2.

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[3] The abbreviations used are: NM, nuclear matrix; 5α-DHT, 5α-dihydrotestosterone; EGF, epidermal growth factor; KGF, keratinocyte growth factor; TGF, transforming growth factor; PMSF, phenylmethylsulfonyl fluoride; IF, intermediary filament.

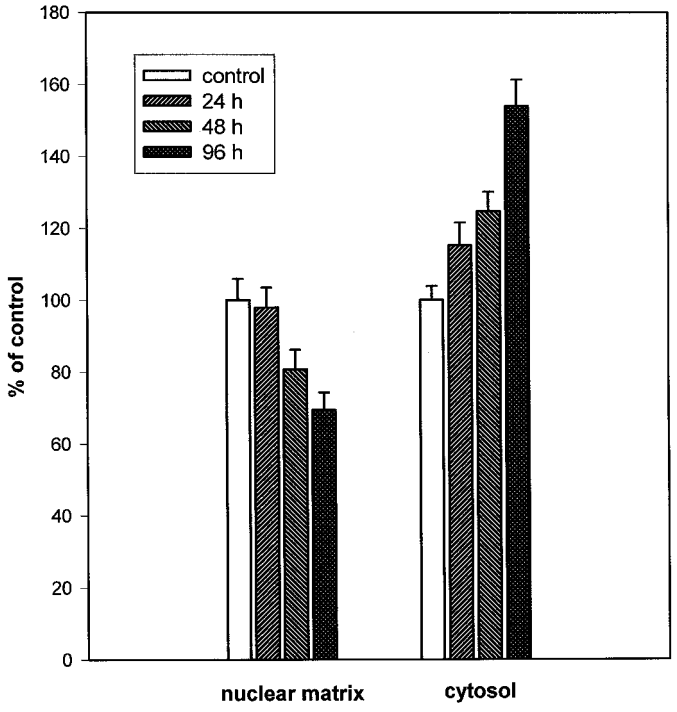

Fig. 1. The effect of androgen withdrawal on CK2 activity in the cytosolic and NM fraction of LNCaP cells. Conditions of cell culture were as described in "Materials and Methods." Androgen withdrawal was achieved by replacing the media with androgen-depleted medium as described. At the times indicated, CK2 activity was measured in the various subcellular fractions. The results are presented as a percentage of the control (*i.e.*, normal system) ± SE.

Treatment of Cells. The cells were cultured in RPMI-1640 containing 10% FBS with 10^{-10} M 5α-DHT (LNCaP cells) or without 5α-DHT (PC-3 cells). To test the effect of 5α-DHT or growth factors, the cells were grown under the conditions described above until a 40–60% confluence was reached. At 24 h before the addition of the test substance (5α-DHT and/or growth factors), the medium was replaced with RPMI-1640 containing 5% heat-inactivated charcoal/dextran-stripped FBS or 0.1% BSA. Treatment of the cells was initiated by replacing the medium with RPMI-1640 containing 0.1% BSA and the desired amount of 5α-DHT and/or growth factors (EGF, KGF, and TGF-α).

Cell Proliferation Assay. The cell viability and proliferation was determined by using cell proliferation reagent WST-1, a tetrazolium salt that is cleaved by mitochondrial dehydrogenases in viable cells. Briefly, 100 μl of cell suspension (containing 0.5–2 × 10^4 cells) were plated in each well of 96-well plates. Cells were cultured for 24 h to allow reattachment. Cell proliferation reagent WST-1 (10 μl) was added to each well. Incubation was continued for 30 min at 37°C. $A_{450\ nm}$ was measured using an automatic ELISA plate reader.

Preparation of Subcellular Fractions. Cell cytosol and NM were prepared as described previously (24). All procedures were performed at 4°C, except when indicated otherwise. Cells were scraped from the flasks after treatment. The cell pellets were washed twice with cold saline solution and suspended in CSK buffer [10 mM PIPES (pH 6.8), 100 mM NaCl, 0.3 M sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 4 mM vanadyl ribonucleoside, 1 mM PMSF, and 10 μg/ml leupeptin]. The cell suspension was homogenized in a Potter-Elvehjem homogenizer, using 12 strokes at 720 rpm. The homogenate was centrifuged at 600 × *g* for 5 min. The supernatant fraction was collected and centrifuged at 100,000 × *g* for 1 h at 4°C. The supernatant fraction was collected as the cytosol fraction. The pellet from the 600 × *g* centrifugation was suspended in extraction buffer [10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 1% Tween 40, 0.5% sodium deoxycholate, 4 mM vanadyl ribonucleoside, 1 mM PMSF, and 10 μg/ml leupeptin] and left on ice for 5 min. The sample was centrifuged at 600 × *g* for 5 min; the supernatant was discarded, and the pellet was resuspended in digestion buffer [10 mM PIPES (pH 6.8), 0.3 M sucrose, 50 mM NaCl, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 4 mM vanadyl ribonucleoside, 1 mM PMSF, 10 μg/ml leupeptin, 100 μg/ml RNase A, and 100 μg/ml DNase I]. After the sample was incubated at room temperature for 60 min, a sufficient amount of 1 M $(NH_4)_2SO_4$ was added to a final concentration of 0.25 M. The sample was centrifuged at 600 × *g* for 5 min, and the supernatant was discarded. The final pellet was suspended in TMED buffer [50 mM Tris-HCl (pH 7.9), 200 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, and 2 μg/ml leupeptin] as the NM fraction. This preparation also contains IFs; hence, the NM studied here represents the NM-IF fraction. We have previously established that CK2 is associated with the NM fraction regardless of the presence or absence of IFs (24).

Assay of CK2 Activity. CK2 activity in various fractions was assayed by using the synthetic CK2-specific dodecapeptide substrate, as describe previously (24). The reaction medium consisted of 30 mM Tris-HCl (pH 7.4), 5.0 mM $MgCl_2$, 150 mM NaCl, 1.0 mM DTT, 0.5 mM PMSF, 10 μg/ml leupeptin, 40 mM β-glycerophosphate, 200 μM synthetic dodecapeptide substrate, and 0.05 mM [γ-^{32}P]ATP (specific radioactivity, 3 × 10^6 dpm/nmol ATP) in a final volume of 0.1 ml. The reaction was started by the addition of the enzyme source such as the cell cytosol or NM (generally a 20-μl sample containing 5–20 μg of protein) to the CK2 assay medium and was carried out for 30 min at 37°C. The ^{32}P incorporated into the peptide substrate was determined by the paper binding method as described previously (24). Blank controls included all components of the reaction except for the peptide substrate. Each experiment was repeated at least three times, and all assays of CK2 activity in various experiments were carried out in triplicate.

Immunoblot of CK2. Samples were denatured by boiling for 5 min in a sample buffer consisting of 10 mM sodium phosphate buffer (pH 7.0), 4 M urea, 2.5% SDS, and 1% 2-mercaptoethanol. The sample was subjected to SDS-urea-10% PAGE. The separated proteins were transferred from the gel to a nitrocellulose sheet using the Hoefer Transphor unit (Western blot). The nitrocellulose sheet was blocked with a medium containing 10 mM Tris-HCl (pH 7.4), 0.9% NaCl, and 3% dry milk. The blot was then successively incubated with mouse antihuman CK2-α IgM (Transduction Laboratories, Lexington, KY) and goat antimouse IgM-alkaline phosphatase-conjugated antibody. Immobilized alkaline phosphatase was visualized using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

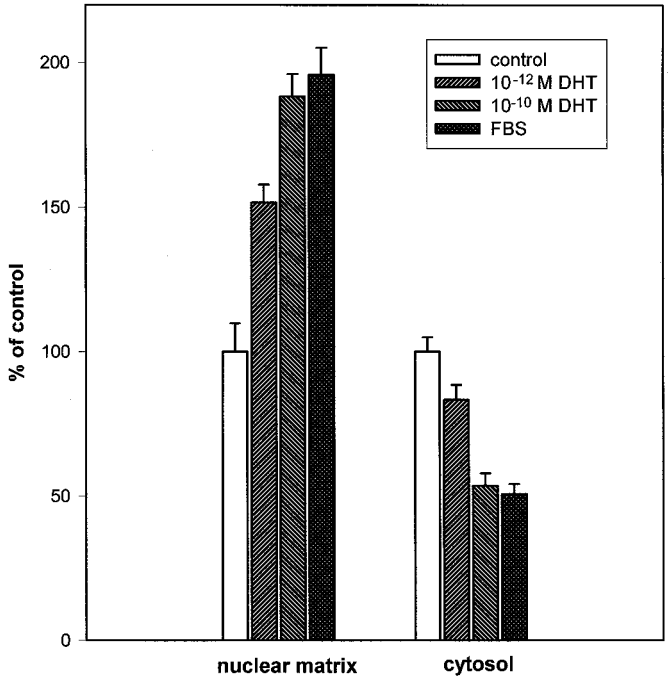

Fig. 2. The effect of varying concentrations of 5α-DHT on CK2 activity in the NM and cytosol of LNCaP cells. LNCaP cells were treated with 5α-DHT at the indicated concentrations or with the normal FBS as described in "Materials and Methods." After 72 h of treatment, cells were harvested, and CK2 activity was determined in the cytosolic and NM fractions. The results are expressed as a percentage of the control (*i.e.*, no 5α-DHT or FBS) ± SE.

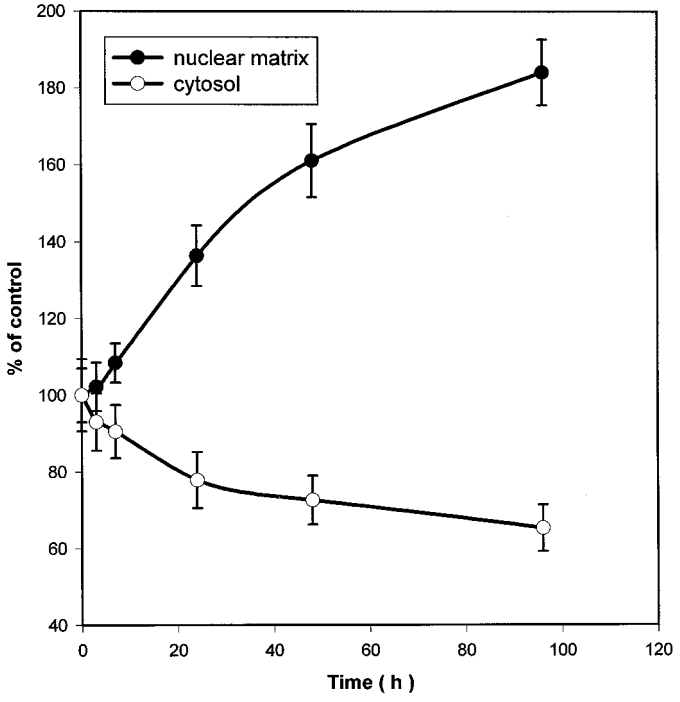

Fig. 3. The time course of the effect of 10^{-10} M 5α-DHT on CK2 activity associated with the NM and cytosolic fractions of LNCaP cells. Cells were treated with 5α-DHT at the indicated concentration for the periods of time shown. The results were calculated as a percentage of the controls (*i.e.*, no 5α-DHT at each time point) ± SE.

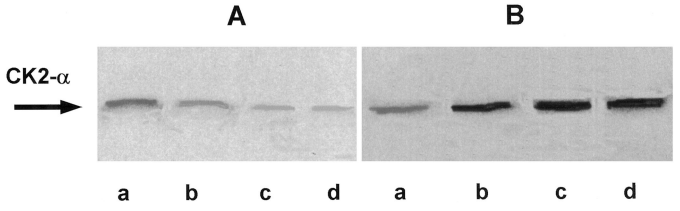

Fig. 4. Immunoblot of CK2-α protein in the NM and cytosol of LNCaP cells treated with 10^{-10} M 5α-DHT over time. Immunoblots of CK2 in the cytosolic fraction (*A*) and NM fraction (*B*) are shown. Each lane was loaded with 50 μg of protein. *Lanes a*, *b*, *c*, and *d* in each panel correspond to control, 24 h, 48 h, and 96 h, respectively. The relative densitometric values corresponding to *Lanes a–d* in *A* were 1.0, 0.65, 0.4, and 0.36, respectively, and for *Lanes a–d* in *B*, they were 1.0, 1.5, 1.7, and 1.85, respectively.

RESULTS

Effect of Androgen Deprivation on NM and Cytosolic CK2 Activity in LNCaP Cells. LNCaP cells were cultured as described in "Materials and Methods," and a dose-response for the effect of 5α-DHT on the growth of these cells was determined; the optimal concentration for promoting cell growth was found to be 10^{-10} M. Because we had observed that androgen deprivation resulted in a loss of nuclear CK2 in the rat prostate, we examined the effect of androgen deprivation from the culture medium on CK2 activity in the cytosolic and NM fractions of LNCaP cells over a period of 96 h. In analyzing the equivalence of changes in cytosolic and NM-associated CK2, a number of points need to be considered. A comparison of the CK2 activity between these two fractions should take into account the fact that these values are based on the initial basal activity in the respective fractions. CK2 appears to be distributed in multiple structures within the cytoplasm and nucleus (16, 19, 25, 26, 29); in the present work, we have not examined the association/dissociation of the kinase with other cellular components. Certain changes in these compartments may also contribute to the level of CK2 detected in the cytosolic fraction. The results in Fig. 1 show a progressive decline in NM-associated CK2 activity with a corresponding increase in CK2 activity in the cytosolic fraction. This suggested that CK2 was translocated from the nucleus to the cytosolic compartment on removal of the androgenic growth signal. These changes in CK2 activity of androgen-deprived LNCaP cells were rapidly reversed by the addition of 5α-DHT.

Effects of 5α-DHT on CK2 Activity in the NM and Cytosol of LNCaP Cells. The results in Fig. 2 show the effect of treating LNCaP cell cultures with different concentrations of 5α-DHT on CK2 activity in the cytosolic and NM fractions. Compared with controls (*i.e.*, in the absence of 5α-DHT), there was a progressive concentration-dependent increase in CK2 associated with the NM in the presence of 5α-DHT. The stimulation was maximal at 10^{-10} M 5α-DHT and was equivalent to that observed in the presence of normal FBS. There was a corresponding decrease in the cytosolic CK2 activity, suggesting that the translocation of CK2 from the cytosol to the nucleus, analogous to the observed androgen effects on CK2 activity in rat prostate (23).

Next we determined the changes in CK2 activity over time in LNCaP cells treated with 10^{-10} M 5α-DHT. As shown in Fig. 3, there was a progressive decline in the CK2 activity in the cytosolic fraction over the 96-h period with a concomitant increase in the NM-associated CK2 activity. Immunoblot analysis of the same samples confirmed that these changes were indeed due to the translocation of the kinase in the presence of the 5α-DHT stimulus, as shown in Fig. 4.

Growth Response of LNCaP and PC-3 Cells to 5α-DHT and Growth Factors. Because prostate tumor cells can become androgen insensitive, it is expected that they are regulated by the paracrine and/or autocrine growth factors. LNCaP cells, although sensitive to androgens, are not strictly androgen dependent, whereas the PC-3 cells are insensitive to androgens and are thus responsive to growth factors alone. The results in Fig. 5 show the growth response in LNCaP and PC-3 cells on treatment with 5α-DHT and human EGF. LNCaP cell growth was equally affected by 10^{-10} M 5α-DHT, 0.2 ng/ml EGF, or both added together. On the other hand, PC-3 cell

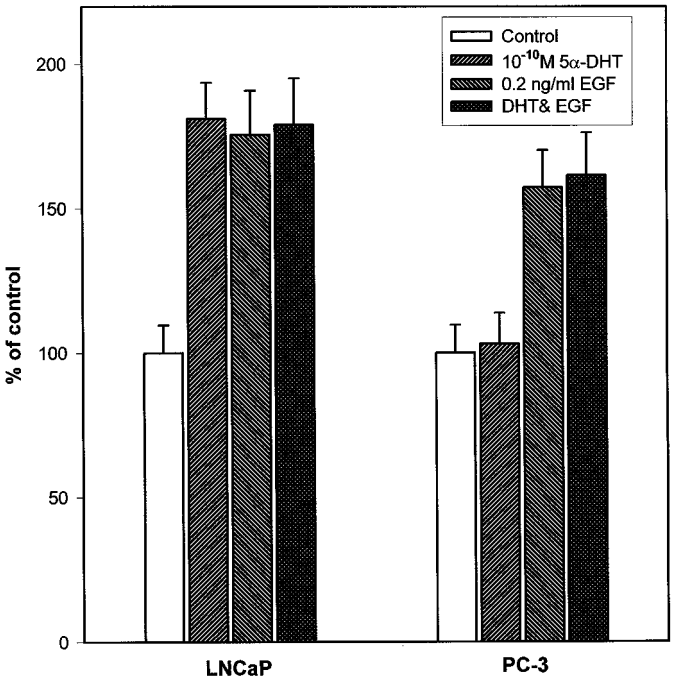

Fig. 5. Growth response of LNCaP and PC-3 cells to 10^{-10} M 5α-DHT and human EGF (0.2 ng/ml). The results are shown as a percentage of the control (in the absence of added 5α-DHT or EGF) ± SE.

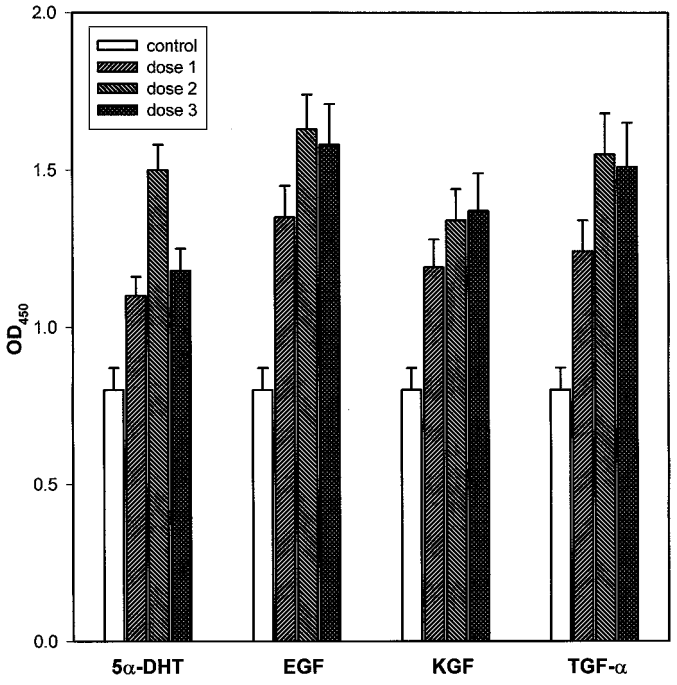

Fig. 6. Growth response of LNCaP cells to varied concentrations of 5α-DHT, EGF, KGF, and TGF-α. The treatment with growth factors was for a period of 72 h. The concentrations of 5α-DHT were 10^{-12} M (*dose 1*), 10^{-10} M (*dose 2*), and 10^{-8} M (*dose 3*). The concentrations for EGF were 0.1 (*dose 1*), 0.2 (*dose 2*), and 0.4 ng/ml (*dose 3*). The concentrations for KGF were 5 (*dose 1*), 10 (*dose 2*), and 20 ng/ml (*dose 3*). The concentrations for TGF-α were 0.1 (*dose 1*), 0.2 (*dose 2*), and 0.4 ng/ml (*dose 3*).

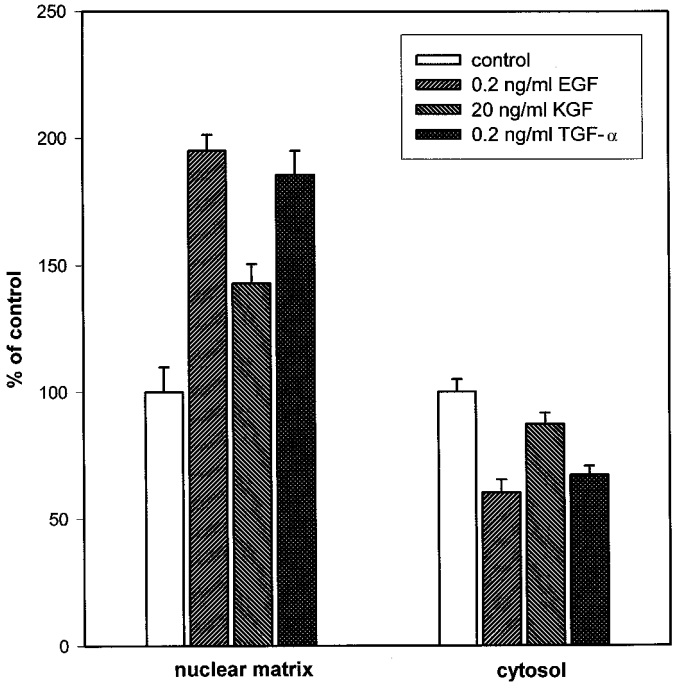

Fig. 7. CK2 activity in NM and cytosol in response to growth factor stimulation of LNCaP cells. The cells were treated with growth factors at the concentrations indicated for a period of 72 h. CK2 activity was determined in the various fractions and is expressed as a percentage of that in the fractions from control cells (no treatment) ± SE.

growth was unresponsive to 5α-DHT but was affected by 0.2 ng/ml EGF. The addition of 5α-DHT with EGF did not enhance the growth response compared with that seen with EGF alone (Fig. 5). Because LNCaP cells are not strictly androgen dependent, we further examined the effects of various growth factors on these cells. LNCaP cells responded to the growth effects of EGF, KGF, and TGF-α in a similar manner (Fig. 6).

Effect of Growth Factors on CK2 Activity in the NM and Cytosol of LNCaP Cells. Considering the effects of various growth factors on the androgen-sensitive LNCaP cells, we decided to examine the CK2 activity in various fractions after treating cells with growth factors. The results in Fig. 7 show that all three growth factors (EGF, KGF, and TGF-α) stimulated a CK2 association with the NM fraction that was accompanied by a corresponding decrease in the cytosolic CK2 activity. The effect of EGF and TGF-α was more marked than that of KGF.

The translocation of CK2 to the NM compartment in this experiment was confirmed by immunoblot analysis of the various fractions isolated from LNCaP cells treated with various growth factors. It is apparent from Fig. 8 that the changes in the NM compartment evoked by treatment with growth factors parallel the activity changes shown in Fig. 7.

Comparison of the Effects of EGF and 5α-DHT on CK2 in LNCaP and PC-3 Cells. When PC-3 cells were treated with EGF, they also demonstrated dynamic changes in CK2 in the NM compartment, analogous to the results described above for LNCaP cells treated with growth factors. The results shown in Table 1 demonstrate the comparison of CK2 dynamics in LNCaP and PC-3 cells treated with 5α-DHT and/or EGF. It is clear that CK2 in LNCaP cells undergoes dynamic changes in the NM and cytosolic fractions in response to either 5α-DHT or EGF. On the other hand, PC-3 cells, which are unresponsive to androgens, show no change in the CK2 present in the NM and cytosol. However, PC-3 cells treated with EGF demonstrate a dramatic response of CK2 in the NM and cytosol fractions, analogous to the aforementioned observations on LNCaP cells.

The CK2 activity data shown in Table 1 were further confirmed by immunoblot analysis, which showed that the activity changes accorded with the alterations in CK2 protein in the NM fraction (Fig. 9). These results also suggest that changes in CK2 association with the NM might follow a common pathway in response to androgenic growth signal or growth factor signal in the prostate cancer cells studied here.

DISCUSSION

The results described in the previous section clearly demonstrate that dynamic changes occur in the intracellular regulation of CK2 activity in prostate cancer cells in culture evoked by different growth stimuli. These studies also indicate that the androgenic responses of CK2 in androgen-sensitive prostate cancer cells in culture are analogous to those observed previously in the rat prostate *in vivo* (21). In each case, it appears that CK2 signaling in the NM is profoundly modulated by altered growth stimulus. These observations hint that the CK2 signal in the NM plays a pivotal role in response to growth stimuli of diverse nature because both androgens and growth factors

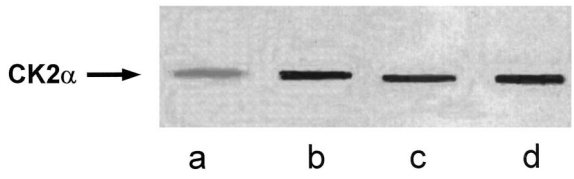

Fig. 8. Immunoblot of CK2-α in the NM of LNCaP cells in response to various growth factors. All of the experimental details were as described in the Fig. 7 legend. Each lane was loaded with 50 μg of protein. *Lane a*, control; *Lane b*, 0.2 ng/ml EGF; *Lane c*, 20 ng/ml KGF; *Lane d*, 0.2 ng/ml TGF-α. The relative densitometric values for *Lanes a–d* were 1.0, 1.8, 1.4, and 1.6, respectively.

Table 1 *The effect of 5α-DHT (10^{-10} M) and human EGF (0.2 ng/ml) on protein kinase CK2 activity in LNCaP and PC-3 cells*

Androgen and EGF treatment lasted 72 h; the control cells were maintained in the absence of these factors as described in "Materials and Methods."

Cells	Cell fraction	CK2 activity (nmol ^{32}P/mg protein/h; mean ± SD)			
		Control	5α-DHT	h-EGF[a]	5α-DHT + h-EGF
LNCaP	Cytosol	9.7 ± 0.9	5.2 ± 0.4	6.9 ± 0.5	7.2 ± 0.6
	NM	6.0 ± 0.5	9.8 ± 0.8	9.2 ± 0.8	9.4 ± 0.9
PC-3	Cytosol	11.5 ± 0.8	12.3 ± 1.0	8.1 ± 0.7	7.8 ± 0.6
	NM	7.3 ± 0.5	7.0 ± 0.7	10.2 ± 0.9	10.9 ± 0.8

[a] h-EGF, human EGF.

evoked a similar response with respect to CK2 dynamics in the NM of the responsive cells. The immunoblot analyses of CK2 in the NM and cytosol fractions indicate that the observed changes in CK2 activity are most likely the result of the translocation of CK2 rather than an activation/inactivation of the enzyme. The effects on the translocation of CK2 to the nucleus appear to relate to the relative effectiveness of the agents in promoting cell growth. For example, KGF, compared with EGF and TGF-α, was less effective in promoting growth in LNCaP cells, as shown in Fig. 6. This corresponds to the relative effects on CK2 dynamics in the nucleus, as shown in Fig. 7. Analogous to this consideration is the apparent lack of androgenic effects on growth as well as CK2 translocation to the nucleus in PC-3 cells. It would appear from the present work that diverse pathways of cell growth stimulation evoke this common downstream signal in the nucleus as well.

A potential significance of these observations relates to the considerable evidence that the NM, the fibrillar proteinaceous substructure of the nucleus, plays a fundamental role in the organization of chromatin and the process of cellular proliferation (30–33). Because both CK2 and NM are implicated in the regulation of cell growth and proliferation, the dynamic changes in NM-associated CK2 in response to the various growth stimuli are therefore of considerable interest. Indeed, growth signal-mediated association of CK2 with NM could conceivably influence the functional activity of this structure via modulations in the phosphorylation of substrates associated with transcriptionally active and inactive nucleosomes as well as with the NM (25).

Much evidence suggests that protein kinase CK2 may be involved in the phosphorylation of a variety of growth-related substrates, leading to proposals of its involvement in normal and neoplastic cell growth. Evidence has been presented that it plays a role in cell cycle progression and seems to be essential for cell survival (34–36). Furthermore, it has been shown to be dysregulated in a number of tumors studied (19, 28, 37–39). Importantly, recent work has documented that moderate overexpression of the kinase in a transgene model enhanced the oncogenic potential (40, 41). It was further demonstrated that p53 deficiency coupled with misexpression of CK2-α acted synergistically in the development of thymic lymphomas in mice (42). In this regard, we have observed that on moderate transient overexpression of CK2, there is a differential enhancement of the kinase in the NM fraction (43). Because a modest dysregulation of CK2 appears to be associated with the imparting of oncogenic potential, it would be reasonable to assume that the dysregulated expression of CK2 may be apparent at a much higher level at the loci involved in gene regulation and control of proliferation, such as the NM. Thus, the observation that a common downstream signaling mechanism via CK2 in the nuclear compartment (such as in the NM) exists in response to diverse stimuli could be of considerable significance in regard to the pathobiology of androgen-sensitive and androgen-insensitive prostate tumor cells.

The primary goal of this study was to determine CK2 dynamics in the NM of LNCaP and PC-3 prostate cancer cells in response to agents that evoke growth stimulation in these cells. Our results clearly show that androgen- or growth factor-stimulated growth in LNCaP cells causes an increase in the NM association of CK2. On the other hand, PC-3 cells that do not respond to the androgenic stimulus show no change in CK2 activity; however, in response to growth factors, CK2 is translocated to the NM, analogous to that observed for LNCaP in response to androgen and/or growth factors. These data suggest that CK2 is a common downstream nuclear signal in response to diverse growth stimuli.

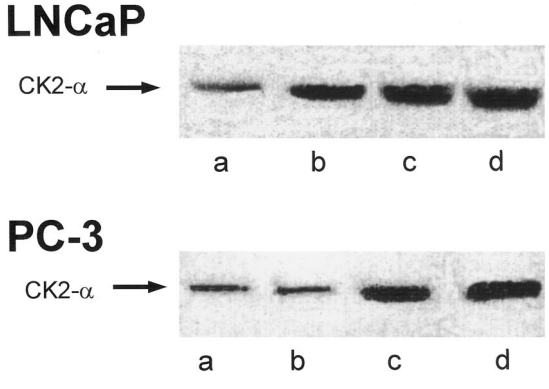

Fig. 9. Comparison of the CK2-α level in the NM of LNCaP and PC-3 cells in response to 10^{-10} M 5α-DHT and 0.2 ng/ml EGF. Cells were treated with the indicated agents for a period of 72 h. Each lane was loaded with 50 μg of protein. *Lanes a*, control; *Lanes b*, 10^{-10} M 5α-DHT; *Lanes c*, 0.2 ng/ml EGF; *Lanes d*, 10^{-10} M 5α-DHT plus 0.2 ng/ml EGF. The relative densitometric values for LNCaP *Lanes a–d* were 1.0, 1.8, 2.0, and 2.1, respectively, and for PC-3 *Lanes a–d* were 1.0, 1.0, 2.2, and 2.2, respectively.

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