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Differential Targeting of Protein Kinase CK2 to the Nuclear Matrix Upon Transient Overexpression of its Subunits

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Abstract Modest dysregulation of CK2 has been shown to enhance the oncogenic potential in transgenic models of cancer. Since nuclear matrix serves as an anchor for CK2 and plays a key role in growth-related activities, we examined the effects of CK2 overexpression on its signaling to the nuclear matrix. Expression plasmids pCI-CK2α, pCI-CK2β, and the bicistronic pCI-CK2αβ containing full length cDNAs encoding the various subunits were employed to transiently transfect two cell lines, BPH-1 and COS-1. Cytosol from transfected BPH-1 cells containing α or β or α + β or αβ showed a modest increase in CK2 activity by 26%, 1%, 20%, and 17%, respectively, over that in the controls transfected with pCI vector. However, the corresponding increase in CK2 activity in the NM fraction was 156%, 8%, 147%, and 152%, respectively. Immunoblot analysis of the CK2 in the NM accorded with these data. Similar results were obtained with COS-1 cells or other expression vectors. The results suggest that moderate overexpression of CK2 in the cells evokes a differential several-fold enhancement in NM associated CK2 relative to that in the cytosol. This process may have a bearing on the functional signaling of this kinase in relation to its possible role in oncogenesis. J. Cell. Biochem. 74:127–134, 1999. © 1999 Wiley-Liss, Inc.

Key words: protein kinase CK2; nuclear matrix; overexpression; prostate; translocation

Nuclear matrix (NM), the fibrillar network that provides the internal scaffold for the nuclear architecture, plays a fundamental role in the nuclear processes related to transcription, cell growth, and proliferation [Berezney, 1991; Getzenberg et al., 1990; Nickerson and Penman, 1992; Stein et al., 1996]. In view of these functions, it is likely that NM is a locus for the association of many signaling molecules. We have previously documented that NM represents a subnuclear site for preferential association of CK2 (protein kinase CK2, formerly casein kinase 2 or II) in response to a growth stimulus, and that the NM-associated CK2 is physiologically relevant [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1995, 1996, 1997]. Further, we have presented evidence that anchoring of CK2 to the NM involves disulfide bond formation [Zhang et al., 1998].

CK2 is a ubiquitous messenger-independent protein serine/threonine kinase consisting of α, α′, and β subunits of M_r 41, 38, and 28 kDa, respectively. The kinase exists as a tetrameric form comprising of $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$ in different tissues and is localized in both the cytoplasmic and nuclear compartments of the cell. Several of the putative substrates of CK2 that are critical to the process of cell growth and prolifera-

Abbreviations used: CK2, formerly known as casein kinase 2 or II; CK2-α or CK2α, protein or cDNA for the catalytic subunit of CK2; CK2-β or CK2β, protein or cDNA for the β subunit of CK2; CMV, cytomegalovirus; DMEM, Dulbecco's modified Eagle's medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NM, nuclear matrix; PIPES, 1,4-piperazinediethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride.

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tion have been identified in the nuclear compartment. CK2 has been implicated in many cellular functions, but most importantly, in the regulation of cell growth and normal and neoplastic proliferation [for reviews see, e.g., Tawfic et al., 1996; Pinna, 1990; Tuazon and Traugh, 1991; Issinger, 1993; Litchfield and Lüscher, 1993; Ahmed, 1994; Allende and Allende, 1995]. A particularly intriguing feature of CK2 function has emerged in observations showing that modest overexpression of CK2-α in transgenic models of cancer causes a significant enhancement of the oncogenic potential in the animal [Seldin and Leder, 1995; Kelliher et al., 1996; Landesman-Bollag et al., 1998].

Considering that NM, a nuclear structure that is intimately involved in regulation of cell growth and proliferation, appears to serve as an anchor for CK2 nuclear signaling, we decided to examine the nature of CK2 association with this structure in response to transient moderate overexpression of various subunits of CK2 in the cell. For these studies, we have employed two different cell lines and two different expression vectors for transient transfection. Our results suggest that upon overexpression of the α or α plus β subunits of the kinase but not the β subunit, a considerably enhanced association of CK2 with the NM occurs relative to the change in the cytosol.

MATERIALS AND METHODS

Expression Vectors

Expression vector pCI and Zero Blunt™ PCR Cloning Kit were purchased from Invitrogen Corp. (Carlsbad, CA). The vector pBluescript II KS (±) was obtained from Stratagene (La Jolla, CA).

Chemicals

Restriction enzymes, calf intestinal alkaline phosphatase, Klenow fragment of DNA polymerase I, Taq DNA polymerase, and T4 DNA ligase were all purchased from Promega Corporation (Madison, WI). Oligonucleotides were synthesized by Oligos, Etc. (Wilsonville, OR). Trizol was purchased from Life Technologies, Inc. (Grand Island, NY). Transfection reagent (DOTAP) and Random Primed DNA Labeling Kit were purchased from Boehringer Mannheim (Indianapolis, IN). [α-^{32}P]dATP was purchased from Amersham Corp. (Arlington Heights, IL). [γ-^{32}P]ATP was purchased from ICN Pharmaceuticals (Costa Mesa, CA). Slot blot apparatus and nitrocellulose, were from Schleicher and Schuell (Keene, NH). The specific peptide substrate (RRRADDSDDDDD) for assaying CK2 was from Peptide Technologies (Gaithersburg, MD). All common reagents and chemicals were of the highest purity available. Monoclonal antibodies against the α and β subunits of CK2 were obtained from Transduction Laboratories (Lexington, KY). Full length cDNA plasmids for human CK2-α and CK2-β (plasmids pBB-3 and pBB-4) were a generous gift from Dr. O.-G. Issinger and Dr. B. Boldyreff (Odense University, Odense, Denmark).

Cells

BPH-1 cell line was supplied by Dr. Simon W. Hayward, Department of Anatomy, University of California School of Medicine, San Francisco, CA. COS-1 cells were purchased from ATCC (Rockville, MD).

Design of Expression Constructs

The full length cDNA of human CK2α was amplified using suitable extension primers containing EcoR I site at 5′ end and Xba I site at 3′ end of CK2α. The CK2α PCR product was digested with EcoR I and Xba I and inserted into the EcoR I and Xba I-digested pCI vector containing the human cytomegalovirus(CMV) major immediate-early gene enhancer/promoter region. The resulting expression construct was designated pCI-CK2α. For the construction of pCI-CK2β, a blunt end PCR product containing full length CK2β cDNA was cloned into the pCR-Blunt vector. Then the full length CK2β cDNA was cut out from this construct with Kpn I and Xho I, and inserted into Kpn I and Xho I-digested pCI vector. The resulting expression vector was designated pCI-CK2β. For the construction of a plasmid which can simultaneously express both CK2-α and CK2-β subunits in mammalian cells, we started from the constructs pCI-CK2α and pCI-CK2β. The pCI-CK2α was digested with BamH I and sticky ends were filled in using the Klenow fragment DNA polymerase. The pCI-CK2β was digested with Bgl II and BamH I, and a restriction fragment containing the CMV enhancer/promoter and the CK2β-coding region was isolated. The sticky ends of the isolated fragment were filled in and the fragment was ligated with the filled pCI-CK2α. A plasmid containing a head to tail orientation of the CK2 α and β genes was selected and was designated pCI-

CK2αβ. PCR analysis, restriction mapping, and DNA sequencing were employed for confirmation of the resulting expression constructs.

Similar strategies were followed to design the pKS-Pro-CK2α and pKS-C3(1)-CK2α which employ the probasin and rat prostate steroid binding protein C3(1) promoters respectively for driving the expression of CK2-α. Briefly, the human CK2-α cDNA was cloned into the vector pPro-SVK_3 (from Dr. J. Green) to yield pPro-CK2α-SVK_3. The DNA fragment probasin-CK2α-SV40 poly (A) from this vector was excised with restriction endonucleases Cla I and Not I, and inserted into plasmid pBluescript KS. The resulting plasmid was named pKS-Pro-CK2α in which the expression of CK2-α is directed by the probasin promoter. Expression plasmid pKS-C3(1)-CK2α was generated by replacing the 0.5 kb probasin promoter with the 4.3 kb C3(1) promoter in the pKS-Pro-CK2α plasmid. In this vector, the expression of CK2-α is driven by the C3(1) promoter.

Transfection of Cells

Transient transfection of expression plasmids into BPH-1 or COS-1 cells was accomplished by using the cationic liposome transfection reagent DOTAP. Briefly, cells were grown under an atmosphere of 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 8% fetal bovine serum (FBS). Twenty hours prior to DNA treatment, the cells were plated at a density of 10^6 cells per 75-cm^2 flask. For each flask 30 μg of plasmid DNA was diluted in 20 mM HEPES buffer, pH 7.4, to a volume of 150 μl and 90 μl of DOTAP was diluted in 20 mM HEPES, pH 7.4, to a volume of 300 μl. These two solutions were combined, mixed well, and left at room temperature for 15 min. This was then added to 15 ml of DMEM plus 10% FBS (previously warmed to 37°C) and gently mixed. The medium in the culture flask was then totally replaced with this DOTAP/DNA medium. After 6 h incubation with the DOTAP/DNA mixture, the media was replaced with fresh DMEM with 8% FBS and the cells were incubated for approximately 72 h prior to harvest. All transfection experiments were repeated at least three times.

Preparative Methods

RNA preparation and analysis of CK2 subunits. The transfected BPH-1 or COS-1 cells were harvested by scraping at 72 h after the DOTAP/DNA treatments. The cells of each sample were divided into two portions. One portion was used for the preparation of cell cytosol and NM described below. The other portion was used for the preparation of total RNA using Trizol. Slot blot analysis of RNA was done using the standard procedure [Ausubel et al., 1993]. Each total RNA sample was applied to a set of at least three slots using quantities of 0.5 to 5.0 μg per slot. Hybridization with labeled CK2α, CK2β, or actin cDNA probes was carried out according to the procedures detailed previously [Lozeman et al., 1990]. The specificity of the CK2-α and CK2-β cDNA probes employed by us was as established previously by Northern blot and S1 nuclease mapping analyses [Ahmed et al., 1993].

Preparation of cell cytosol and nuclear matrix. The preparation of cell cytosol and NM from transfected BPH-1 and COS-1 cells was carried out as described previously [Tawfic et al., 1997]. All the procedures were performed at 4°C except when indicated otherwise. The cell pellets were washed with cold saline solution twice, and then suspended in CSK buffer (consisting of 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, 10 μg/ml leupeptin). The cell suspension was homogenized in a Potter-Elvehjem homogenizer, using 12 strokes (approximately 1 min) at 720 rpm. The homogenate was centrifuged at 600*g* for 5 min. The supernatant fraction was collected and centrifuged at 100,000*g*, 4°C for 1 h. The supernatant fraction was collected as the cytosol fraction. The pellet from the 600*g* centrifugation was suspended in extraction buffer (consisting of 10 mM Tris-HCl pH7.4, 10 mM NaCl, 3 mM $MgCl_2$, 1% Tween 40, 0.5% sodium deoxycholate, 4 mM vanadyl ribonucleoside, 1 mM PMSF, 10 μg/ml leupeptin) and left on ice for 5 min. The sample was centrifuged at 600*g* for 5 min and the supernatant was discarded. The pellet was resuspended in digestion buffer (consisting of 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, 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 (consisting of 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, 2 μg/ml leupeptin) as the NM fraction. The characteristics of this NM preparation were the same as described previously [Tawfic et al., 1997].

Assays

Measurement of CK2 activity. CK2 activity in the cytosolic and NM fractions was determined by using a synthetic dodecapeptide substrate specific for measuring the CK2 activity, as described previously [Tawfic et al., 1997]. The reaction buffer consisted of 30 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 150 mM NaCl, 1 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 of 3×10^6 dpm/nmol of ATP). The reaction was started by the addition of 20 μl of the enzyme source such as the cytosol and nuclear matrix (5–20 μg protein), 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 [Tawfic et al., 1997]. Blank controls included all the components of the reaction except for the peptide substrate. Each assay for CK2 activity was carried out in triplicate.

Immunoblot analysis of CK2 subunits. Immunoblot analysis of the CK2 α and β subunits was carried out by probing the gel electrophoretically separated and blotted NM proteins with monoclonal antibodies against the α and β subunits of human CK2. Appropriate alkaline phosphatase conjugated anti-mouse immunoglobulin antibodies were employed as secondary antibodies. Antibody binding was visualized using Nitro-Blue Tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as described previously [Tawfic and Ahmed, 1994a,b].

RESULTS

RNA Expression Levels of CK2 Subunits in Cells Transiently Transfected with CK2-α and CK2-β cDNAs

The transient overexpression of CK2-α, CK2-β alone or in combination or in a bicistronic construct CK2αβ after transfection of BPH-1 cells

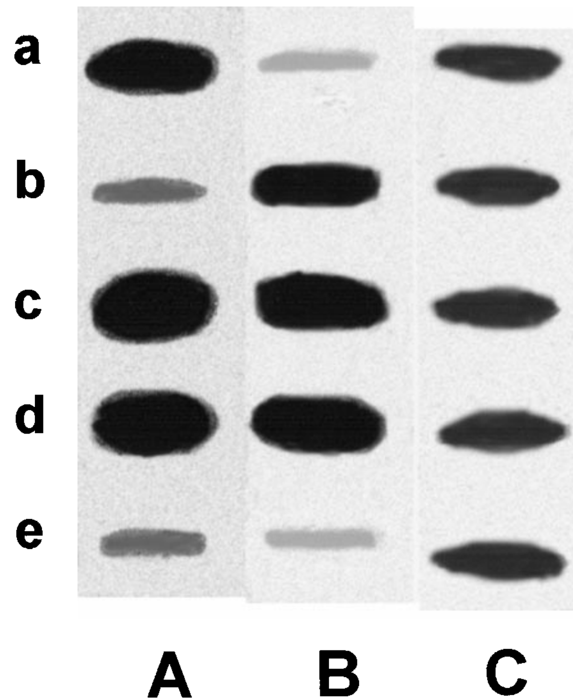

Fig. 1. Slot blot analysis of total RNA from BPH-1 cells transfected with different CK2 subunit expression plasmids and probed with the CK2-α and CK2-β cDNAs. BPH-1 cells were transfected with various overexpression plasmids and blotted RNA was hybridized with probes for CK2-α cDNA **(A)**, CK2-β cDNA **(B)**, or actin **(C)**. Cells were transfected with pCI-CK2α **(lane a)**; pCI-CK2β **(lane b)**; both pCI-CK2α and pCI-CK2β **(lane c)**; bicistronic pCI-CK2αβ **(lane d)**, and pCI vector **(lane e)**.

was monitored by analyzing the RNA in a slot blot. The results in Figure 1A show that when the blot was probed with a CK2-α cDNA probe, there was a strong expression corresponding to transfection by the pCI-CK2α, both pCI-CK2α and pCI-CK2β and the bicistronic pCI-CK2αβ, whereas no enhanced signal was detected for pCI-CK2β as compared with the cells transfected with the pCI vector alone. Figure 1B shows that when slot blot of total RNA was probed with the CK2-β cDNA probe, there was no increase in the signal for the pCI-CK2α but enhancement of the signal was apparent for cells transfected with the other constructs. There was no change in the β-actin signal level in any of the cells overexpressing the CK2 subunits when compared with the control cells (Fig. 1C). These results suggested that successful transient transfection was achieved in the BPH-1 cells. Similar results were observed when expression was monitored in the COS-1 cells; further, transfection of BPH-1 cells with pKS-Pro-CK2α and pKS-C3(1)-CK2α yielded similar

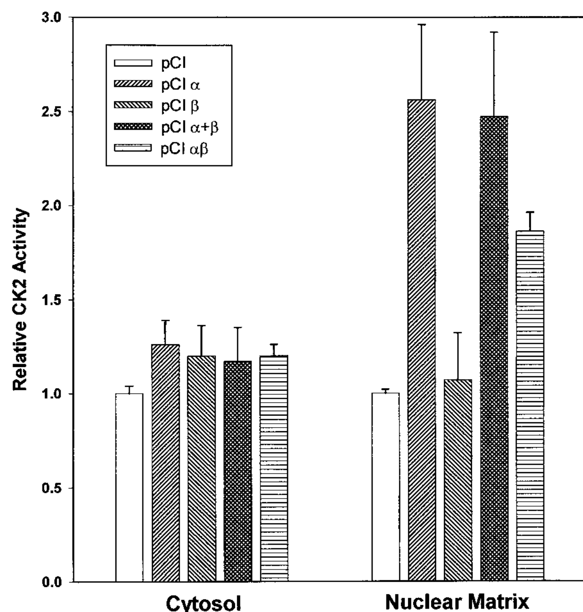

Fig. 2. Relative activity of CK2 in cytosol and nuclear matrix of BPH-1 cells transfected with various subunits of CK2. The results of CK2 activity for various transfection protocols are expressed with the value for the control set at 1.0; the actual CK2 activity values (nmol ^{32}P/mg of protein/h ± S.E.M.) for the pCI control of the BPH-1 cell were 43.9 ± 3.9 for cytosolic activity, and 21.7 ± 1.6 for NM-associated activity.

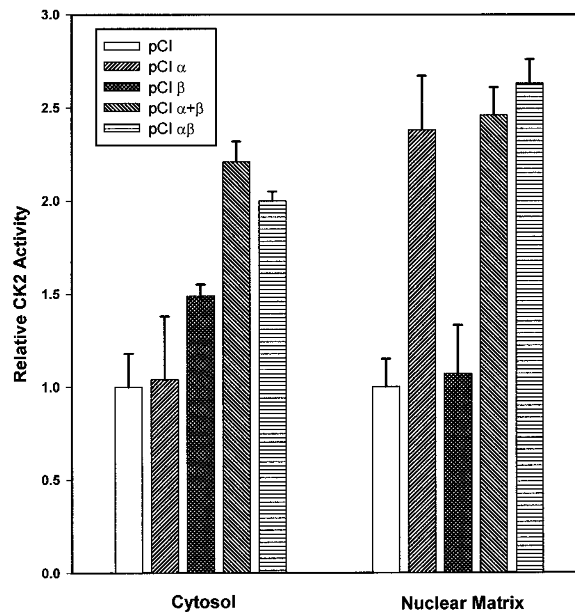

Fig. 3. Relative activity of CK2 in the cytosol and NM fractions of COS-1 cells transfected with various subunits of CK2. The results of CK2 activity for various transfection protocols are expressed with the value for the control set at 1.0; the actual CK2 activity values (nmol ^{32}P/mg of protein/h ± S.E.M.) for the pCI control of the COS-1 cells were 11.7 ± 2.1 for cytosolic activity, and 27.5 ± 2.4 for NM-associated activity.

results suggesting a general response of these various cells to different transfection vectors for overexpression of CK2 (results not shown).

Effect of Transient Overexpression of CK2 Subunits on CK2 Activity in the Cytosolic and NM Fractions

The nature of the response of CK2 activity in the cytosol and NM fractions isolated from BPH-1 and COS-1 cells in response to transient overexpression following transfection with pCI-CK2α, pCI-CK2β, pCI-CK2α plus pCI-CK2β, and bicistronic pCI-CK2αβ was determined. The relative CK2 activity (compared with the activity in the presence of the pCI vector alone) is shown in Figure 2 for the BPH-1 cells and in Figure 3 for the COS-1 cells. The results in Figure 2 show that the cytosolic CK2 activity was increased only modestly (~10–20%) in the presence of the various transfectants. However, there was two- to three-fold increase in the CK2 in the NM fraction when the cells were transfected with the α, α + β or bicistronic αβ expression plasmids, although the latter was somewhat less effective than the other two. Figure 3 shows the results under similar conditions for overexpression of CK2 subunits in COS-1 cells. In this case, the change in the cytosolic activity was somewhat more marked than that observed for the BPH-1 cells and the response to various transfectants was also somewhat different. For example, there was little change in the cytosol from α transfectants, about 40% increase for the β transfectants, whereas there was 80–120% for the bicistronic and α + β transfectants, respectively. However, distinct results were noted for the CK2 activity in the NM fraction. There was a 2.5-fold increase in the NM associated CK2 activity in cells transfected with the α alone, which was the same for the α + β and the αβ bicistronic transfectants. There was no change in the NM-associated CK2 when the transfection was with the β alone. These results suggest that the modest overexpression of the α subunit alone can evoke a much greater preferential increase in the NM-associated CK2 activity. These results also suggest that the level of overexpression in different cell types may vary as determined by CK2 activity in the cytosolic fraction; however, the response of the NM-associated CK2 to overexpression appears to be more consistent in the two cell types studied by us.

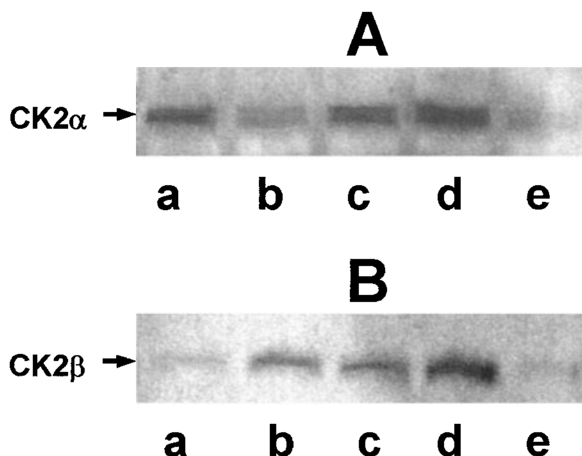

Fig. 4. Immunoblot analysis of NM-associated CK2 from BPH-1 cells transfected with expression plasmids for the α and β subunits of CK2. The NM was prepared from the BPH-1 cells 72 h after treatment with the DOTAP/DNA mixture as described under the Materials and Methods. Each lane of a 10% polyacrylamide-SDS-urea gel contained 50 µg of NM protein which was transferred to nitrocellulose after electrophoresis. The immunoassays used monoclonal antibodies against the CK2-α and CK2-β subunits. **A,B**: The immunoblot of CK2-α and CK2-β subunit, respectively. Each lane represents transfection with different plasmids as follows: pCI-CK2α **(lane a)**; pCI-CK2β **(lane b)**; pCI-CK2α plus pCI-CK2β **(lane c)**; pCI-CK2αβ **(lane d)**, and pCI vector control **(lane e)**.

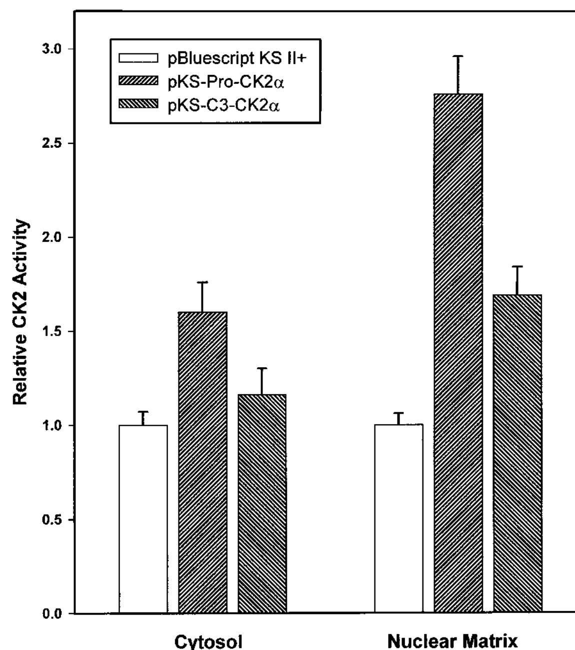

Fig. 5. Relative activity of CK2 in cytosol and nuclear matrix of BPH-1 cells transfected with CK2α expression plasmids driven by probasin or C3(1) promoter. BPH-1 cells were transfected with pKS-Pro-CK2α or pKS-C3(1)-CK2α plasmids and CK2 activity was determined in the cytosolic and NM fractions. All other experimental details were the same as for Figure 3.

Immunoblot Analysis of CK2 Subunits in Cytosolic and NM Fractions of Cells Transiently Overexpressing CK2

The data on the NM association of CK2 under various conditions of overexpression were further confirmed by immunoblot analysis of the expressed CK2 when probed with monoclonal antibodies directed against the CK2-α and CK2-β subunits. As shown in Figure 4, the NM associated CK2-α and CK2-β were elevated and corresponded to the CK2 activity data (shown in Fig. 2) upon transfection of BPH-1 cells with various expression plasmids pCI-CK2α, pCI-CK2αβ, or pCI-CK2α plus pCI-CK2β, but not pCI.

Effect of Probasin and C3(1) Promoters on Transient Overexpression of CK2-α Subunit in BPH-1 Cells

We also examined the effect of transient transfection of the prostatic BPH-1 cells with pKS-Pro-CK2α and pKS-C3(1)-CK2α vectors in which the expression of the CK2-α is driven by the prostate-specific promoters probasin and the rat prostate steroid binding protein C3(1). Again, the results shown in Figure 5 demonstrate that the CK2 activity in the cytosolic fraction of the transfected cells was elevated by about 55% and 15% in the presence of probasin and C3(1) promoters, respectively. However, the corresponding increase in the NM-associated CK2 activity under these conditions was 176% and 60%, respectively. The relatively weaker response in the case of the C3(1) promoter-driven expression may reflect the nature of the promoter. However, it appears that regardless of the type of expression vector employed, there was a significant differential enhancement in the NM-associated CK2 relative to a modest overexpression in the cytoplasm.

DISCUSSION

In the present work, we have achieved transient overexpression of CK2 in different cells by employing different promoters to drive expression of various subunits of CK2. The results suggest that significant enhanced expression of the CK2 subunits occurs in the BPH-1 and COS-1 cells studied. This in general accords with previous studies in COS-1 and COS-7 cells [Heller-Harrison and Czech, 1991; Penner et al., 1997]; however, the present work has also

compared the relative expression of CK2 under the influence of different promoters and CK2 targeting in the cytosolic versus the NM compartments of different cells. These results demonstrate that modest overexpression of CK2 in the cells leads to a disproportionate enhancement of its association with the NM. The significance of this observation relates to the evidence suggesting that both the NM [Berezney, 1991; Getzenberg et al., 1990; Nickerson and Penman, 1992; Stein et al., 1996] and protein kinase CK2 [Tawfic et al., 1996; Pinna, 1990; Tuazon and Traugh, 1991; Issinger, 1993; Litchfield and Lüscher, 1993; Ahmed, 1994; Allende and Allende, 1995] play a role in the processes involved in regulation of normal and abnormal cell growth and proliferation. We have previously shown that NM serves as an anchor for CK2 in response to various mitogenic signals [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996]. It has also been documented that CK2 appears to be dysregulated in neoplastic cells [Issinger, 1993; Yenice et al., 1993; Gapany et al., 1995], and modest overexpression in a transgene has been shown to greatly enhance the oncogenic potential in these models of cancer [Seldin and Leder, 1995; Kelliher et al., 1996; Landesman-Bollag et al., 1998]. However, in the latter experimental models an analysis of the CK2 activity in different compartments of the cell was not undertaken. In an ongoing study of the relative activity of CK2 in the cytosolic and NM compartments of human prostate cancer specimens we have noted a several-fold increase in the CK2 activity associated with the NM relative to that in the cytosol (unpublished observations). Therefore, our observations on the enhanced differential association of CK2 with the NM evoked by modest overexpression of the kinase as determined by its activity in the cytoplasm may be potentially important to the process of oncogenesis.

Our results on the CK2 overexpression as determined by measurement of activity in the cytosolic fraction in the COS-1 cells are in general accord with those reported previously [Heller-Harrison and Czech, 1991] showing that overexpression of the catalytic subunit of CK2 alone was sufficient to increase the CK2 activity suggesting the availability of the β subunit. However, we have observed a relatively lower CK2 activity in the cytosolic fraction compared with that in the previous report [Heller-Harrison and Czech, 1991]. This may relate to differences in the methods of analysis since, unlike the previous work, the present work employed the high speed supernatant fraction for measurement of the CK2 in the cytosolic fraction. Further, the results with BPH-1 cells are somewhat different from those obtained by employing COS-1 cells with respect to overexpression of CK2 in response to the catalytic subunit alone. This suggests that following overexpression the level of CK2 in the cytosolic fraction may depend on the cell type although the data on the NM association of CK2 in response to transfection with the catalytic α subunit or the combined subunits α and β transfection were remarkably similar in the two cell types studied. In this regard, our results accord with the observations showing that the β subunit is produced in excess in the cells [Lüscher and Litchfield, 1994].

An important function of the NM is in the organization of chromatin so that the transcriptionally active nucleosomes are positioned on the NM at the time of transcription. We have recently reported that CK2 in the transcriptionally active and inactive nucleosomes is dynamically regulated in relation to transcriptional activity [Guo et al., 1998]. Thus, the enhanced association of CK2 with the NM in response to overexpression may also impact on the nucleosomal function under the same conditions, i.e., a possibly altered NM function as a consequence of enhanced association of CK2.

In summary, the present results show that NM is a preferential site of CK2 signaling in response to an apparently modest overexpression of the kinase in the cell. This emphasizes the significance of the differential spatial dynamics of CK2 in the nucleus, as suggested for various gene regulators [Stenoien et al., 1998]. To our knowledge, the present results on the overexpression of CK2 in cells leading to its differential enhanced association with the NM are the first to be reported. Such a marked response of the CK2 signal in the NM to modest overexpression of the kinase may be important in its functional signaling in the nucleus especially in the context of its possible role in oncogenesis.

REFERENCES

Ahmed K. 1994. Significance of casein kinase system in cell growth and proliferation with emphasis on studies of the androgenic regulation of the prostate. Cell Mol Biol Res 40:1–11.

Ahmed K, Davis A, Hanten J, Lambert D, McIvor RS, Goueli SA. 1993. Cloning of cDNAs encoding the α and β subunits of rat casein kinase 2 (CK-2): investigation of molecular regulation of CK-2 by androgens in rat ventral prostate. Cell Mol Biol Res 39:451–462.

Allende JE, Allende CC. 1995. Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation. FASEB J 9:313–323.

Ausubel FA, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Strul K. 1993. Current protocols in molecular biology. New York: Greene Publishing and Wiley-Interscience.

Berezney R. 1991. The nuclear matrix: a heuristic model for investigating genomic organization and function in the cell nucleus. J Cell Biochem 47:109–123.

Gapany M, Faust RA, Tawfic S, Davis A, Adams GL, Ahmed K. 1995. Association of elevated protein kinase CK2 activity with aggressive behavior of squamous cell carcinoma of the head and neck. Mol Med 1:659–666.

Getzenberg RH, Pienta KJ, Ward WS, Coffey DS. 1991. Nuclear structure and the three-dimensional organization of DNA. J Cell Biochem 47:289–299.

Guo C, Davis AT, Ahmed K. 1998. Dynamics of protein kinase CK2 association with nucleosomes in relation to transcriptional activity. J Biol Chem 273:13675–13680.

Heller-Harrison RA, Czech MP. 1991. Enhanced casein kinase II activity in COS-1 cells upon overexpression of its catalytic or noncatalytic subunit. J Biol Chem 266: 14435–14439.

Issinger O-G. 1993. Casein kinases: pleiotropic mediators of cellular regulation. Pharmac Therap 59:1–30.

Kelliher MA, Seldin DC, Leder P. 1996. Tal-1 induces T cell acute lymphoblastic leukemia accelerated by casein kinase IIα. EMBO J 15:5160–5166.

Landesman-Bollag E, Channavajhala PL, Cardiff RD, Seldin D. 1998. P^{53} deficiency and misexpression of protein kinase CK2α collaborate in the development of thymic lymphomas in mice. Oncogene 16:2965–2974.

Litchfield DW, Lüscher B. 1993. Casein kinase II in signal transduction and cell cycle regulation. Mol Cell Biochem 127/128:187–199.

Lozeman FJ, Litchfield DW, Piening C, Takio K, Walsh KA, Krebs EG. 1990. Isolation and characterization of humancDNA clones encoding the α and the α′ subunits of casein kinase II. Biochemistry 29:8436–8447.

Lüscher B, Litchfield D. 1994. Biosynthesis of casein kinase II in lymphoid cell lines. Eur J Biochem 220:521–526.

Nickerson JA, Penman S. 1992. The nuclear matrix: Structure and involvement in gene expression. In: Stein GS, Lian JB, editors. Molecular and cellular approaches to the control of proliferation and differentiation. San Diego, CA: Academic Press. p 343–380.

Penner CG, Wang L, Litchfield DW. 1997. Expression and localization of epitope-tagged protein kinase CK2. J Cell Biochem 64:525–537.

Pinna LA. 1990. Casein kinase 2: an “eminence grise” in cellular regulation? Biochim Biophys Acta 1054:267–284.

Seldin DC, Leder P. 1995. Casein kinase IIα transgene-induced murine lymphoma: relation to Theileriosis in cattle. Science 267:894–897.

Stein GS, Stein JL, van Wijnen AJ, Lian JB. 1996. The maturation of a cell: three-dimensional structure of the nucleic acids in the timely expression of genes. Am Sci 84:28–37.

Stenoien D, Sharp ZD, Smith CL, Mancini MA. 1998. Functional subnuclear partitioning of transcription factors. J Cell Biochem 70:213–221.

Tawfic S, Ahmed K. 1994a. Association of casein kinase 2 (CK2) with nuclear matrix: possible role in nuclear matrix protein phosphorylation. J Biol Chem 269:7489–7493.

Tawfic S, Ahmed K. 1994b. Growth stimulus-mediated differential translocation of casein kinase 2 to the nuclear matrix. J Biol Chem 269:24615–24620.

Tawfic S, Olson MOJ, Ahmed K. 1995. Role of protein phosphorylation in post-translational regulation of protein B23 during programmed cell death in the prostate gland. J Biol Chem 270:21009–21015.

Tawfic S, Faust RA, Gapany M, Ahmed K. 1996. Nuclear matrix as an anchor for protein kinase CK2 nuclear signalling. J Cell Biochem 61:165–171.

Tawfic S, Davis AT, Faust RA, Gapany M, Ahmed K. 1997. Association of protein kinase CK2 with nuclear matrix: influence of method of preparation of nuclear matrix. J Cell Biochem 64:499–504.

Tuazon PT, Traugh JA. 1991. Casein kinase I and II. Multipotential serine protein kinases: structure, function, and regulation. Adv Second Messenger Phosphoprotein Res 23:123–164.

Yenice S, Davis AT, Goueli SA, Akdas A, Limas E, Ahmed K. 1993. Nuclear casein kinase 2 (CK-2) activity in human normal, benign hyperplastic, and cancerous prostate. Prostate 24:11–16.

Zhang P, Davis AT, Ahmed K. 1998. Mechanism of protein kinase CK2 association with nuclear matrix: role of disulfide bond formation. J Cell Biochem 69:211–220.