A Potential Role of Nuclear Matrix-associated Protein Kinase CK2 in Protection against Drug-induced Apoptosis in Cancer Cells*

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Protein kinase CK2 (CK2) has long been implicated in the regulation of cell growth and proliferation. Its activity is generally elevated in rapidly proliferating tissues, and nuclear matrix (NM) is an important subnuclear locale of its functional signaling. In the prostate, nuclear CK2 is rapidly lost concomitantly with induction of receptor-mediated apoptosis after growth stimulus withdrawal. By contrast, chemical-induced apoptosis in prostate cancer and other cells (by etoposide and diethylstilbestrol) evokes an enhancement in CK2 associated with the NM that appears to be because of translocation of CK2 from the cytoplasmic to the nuclear compartment. This shuttling of CK2 to the NM may reflect a protective response to chemical-mediated apoptosis. Supporting evidence for this was obtained by employing cells that were transiently transfected with various expression plasmids of CK2 (thereby expressing additional CK2) prior to treatment with etoposide or diethylstilbestrol. Cells transfected with the CK2α or CK2αβ showed significant resistance to chemical-mediated apoptosis concomitant with the corresponding elevation in CK2 in the NM. Transfection with CK2β did not demonstrate this effect. These results suggest, for the first time, that besides the commonly appreciated function of CK2 in cell growth, it may also have a role in protecting cells against apoptosis.

Protein kinase CK2 (CK2) has long been extensively studied in recent years for its potential role in multiple functional activities including the regulation of cell growth and proliferation. It is a ubiquitous protein Ser/Thr kinase, localized in the cell cytoplasm and nucleus, existing as a heterotetramer consisting of α, α′, and β subunits (~42, 38, and 28 kDa, respectively) with the possible αβββ, αα′ββ, or αα′ββ configuration. A number of putative substrates for CK2 have been identified in both the cytoplasm and the nucleus. Many of these are based on in vitro phosphorylation studies although a number of them have also been shown to be substrates for CK2 in vivo. Among the nuclear substrates are proteins involved in growth including RNA polymerases, topoisomerase II, protein B23, nucleolin, SV40 large T antigen, certain proto-oncogene products, and growth factors, as well as certain nonhistone proteins, which might include transcription factors, etc. (for examples see Refs. 1–10).

In previous work, we have demonstrated that CK2 is dynamically regulated with respect to its nuclear localization, such that chromatin and NM appear to be its preferential sites of association within the nucleus. The association of CK2 in these compartments is profoundly responsive to the status of growth stimuli (1, 9, 11–13). To that end, we have employed androgen action in the prostate epithelial cells that is mediated via the androgen-receptor system as an experimental model. It is well known that withdrawal of androgenic growth signal via castration of adult rats induces rapid apoptosis in the epithelial cells of the gland and that this process is reversed on androgen administration to castrated rats (14, 15). In the same paradigm, androgen withdrawal evoked a rapid loss of nuclear CK2 that was most apparent in the NM fraction. The treatment of castrated rats with androgen, and consequent induction of gene activity and cell growth in the prostatic epithelial cells, concurred with a rapid translocation of the CK2 from cytoplasm to the nuclear compartment such that the enhancement in the NM-associated CK2 was the most prominent (1, 2, 9, 11–13).

The above studies also point to a hitherto unappreciated functional role of CK2, namely that growth stimulus-mediated changes in nuclear CK2 were associated not only with early growth responses but also with changes in apoptotic activity in the cells, depending on the availability of physiological growth factor signals such as androgens or other mitogenic growth factors (1–3, 16). Thus, under conditions of androgenic stimulation in the prostate the growth-related activities would predominate, whereas under conditions of growth stimulus withdrawal the apoptosis would predominate. This would imply that the presence or absence of CK2 in the nucleus may exert a significant effect on apoptotic activity. Apoptosis, or programmed cell death, has been increasingly appreciated to play a significant role in the maintenance of tissue homeostasis (for examples see Refs. 17–20). The process of apoptosis can be initiated by either the receptor-mediated or chemical-mediated pathway. The former relates to withdrawal of growth stimuli (e.g. on androgen deprivation in the prostate), whereas the latter type of apoptosis is induced by physical agents such as γ-radiation or by chemicals such as etoposide. Some of the apoptosis-inducing chemical agents are known to induce DNA damage as an initial step, which is a distinguishing feature from receptor-mediated apoptosis (21–23). Our previous work
emerging receptor-mediated apoptosis in the prostate epithelial cells has shown a distinct response of NM-associated CK2 such that a rapid loss of CK2 from the NM compartment temporally precedes the appearance of apoptosis (1, 9, 11, 14, 15, 24). However, no such studies have been reported on the response of the CK2 signal in relation to chemical-mediated apoptosis in cancer cells. Considering the distinct nature of the receptor-mediated and chemical-mediated apoptosis, we decided to examine the nature of CK2 dynamics in the NM under the latter conditions.

In the present paper we report that etoposide and diethylstilbestrol (DES), employed as models for chemical-induced apoptosis, demonstrate a distinctly different response of CK2 in the NM when compared with the previous observations on receptor-mediated induction of apoptosis. Unlike the latter, which evoked a rapid loss of CK2 from the NM in the prostate epithelial cells preceding apoptosis, the chemical-induced apoptosis mediated by etoposide or DES treatment of cells resulted in a dramatic initial increase in the CK2 association with the NM. A possible explanation of these results is that the presence of CK2 in the nuclear compartment may exert a protective role against apoptosis, in addition to its previously suggested role in activities related to stimulation of growth. A direct support for this notion was obtained from the observation that overexpression of CK2 by transient transfection of cells with expression plasmids of catalytic subunit CK2α or bicistronic holoenzyme CK2αβ resulted in a significant protection against chemical-induced apoptosis. The specificity of this action appears to reside in the catalytic subunit α of CK2, because transfection with the regulatory subunit CK2β did not afford such a protection.2

**EXPERIMENTAL PROCEDURES**

**Materials**

**Chemicals—**Synthetic dodecapeptide substrate (RRADDDDDDDDD) (25) was purchased from Peptide Technologies (Gaithersburg, MD). Etoposide and DES were purchased from Sigma. Cell proliferation reagent WST-1 was supplied by Roche Molecular Biochemicals. The expression plasmids pCI-CK2α, pCI-CK2β, and bicistronic pCI-CK2αβ were prepared as detailed previously (26). Anti-CK2α antibody was purchased from Transduction Laboratories (Lexington, KY). TRIZOL reagent was from Life Technologies, Inc., Grand Island, NY. All other reagents were of the highest purity available.

**Cells—**Human prostate adenocarcinoma cell lines LNCaP and PC-3 were purchased from American Type Culture Collection (Manassas, VA). They were maintained in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 2 mM l-glutamine, 25 mM HEPES, 10% defined FBS (HyClone Laboratories, Logan, UT) in an atmosphere containing 5% CO2. Culture medium for LNCaP cells also included 10−8 M 5α-dihydrotestosterone. Human prostate carcinoma cell line ALVA-41 was obtained from Dr. Richard C. Ostenson (University of Washington, Seattle, WA). These cells were maintained in RPMI 1640 supplemented with 2 mM l-glutamine, 25 mM HEPES, 6% defined FBS in an atmosphere containing 5% CO2. A cell line from squamous carcinoma of head and neck CA-9–22 was a gift from Dr. T. Kuroki, University of Tokyo, Tokyo, Japan. These cells were also maintained in the same medium used for ALVA-41 cells. Shionogi mouse mammary cancer cells were kindly supplied by Dr. John Isaac of Johns Hopkins University, Baltimore, MD. They were maintained in RPMI 1640 supplemented with 2 mM l-glutamine, 25 mM HEPES, 10−8 M 5α-dihydrotestosterone, 10% charcoal/dextran-striped defined FBS in an atmosphere containing 5% CO2. The CHO cell line was purchased from CLONTECH, Palo Alto, CA. They were maintained in Ham's F-12 medium supplemented with 2 mM l-glutamine, 6% defined FBS in an atmosphere containing 5% CO2.

2 An abstract based on aspects of this work was presented at the 18th International Congress of Biochemistry and Molecular Biology. An abstract on parts of this work has also been accepted by the American Society for Cell Biology for presentation at its annual meeting, San Francisco, 2000.

**Methods**

**Cell Proliferation Assay—**Cell viability and proliferation was determined by employing the 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 to 2 × 10⁶ cells) was plated in each well of 96-well plates. Cells were cultured for 24 h to allow reattachment. Following the treatment of cells with etoposide or DES, 10 µl of WST-1 was added to each well, and incubation was carried out at 37°C for 30 min. An automated plate reader was employed to measure A490.

**Cell Transfection with Expression Plasmids of CK2—**The transient transfection with expression plasmids pCI-CK2α, pCI-CK2β, and pCI-CK2αβ was carried out in PC-3 cells as described previously (26). The period of transfection was 24 h, yielding a transfection efficiency of about 50–60%. These cells were treated with etoposide for varying periods of time as described in the text and figure legends. Control cells carrying only the pCI vector were treated in parallel in a similar manner. Similar approaches were followed for transfection of ALVA-41 cells.

**Preparation of Nuclear Matrix—**Subfractionation of cells to isolate NM was carried out as described previously (16, 26, 27). All the procedures were performed at 4°C except where indicated otherwise. Cells were scraped from the flasks after various treatments, washed twice with cold 0.9% saline solution, and then suspended in CSK Buffer (10 mM PIPES, pH 6.8, 100 mM NaCl, 0.3 mM sucrose, 3 mM MgCl₂, 1 mM EGTA, 0.5% Triton X-100, 4 mM vanadyl ribonucleoside complex, 1 mM phenylmethylsulfonyl fluoride, 10 µg/mL leupeptin) for preparation of the cytosolic and NM fractions as described previously (16, 26, 27). The final NM fraction was suspended in a buffer consisting of 0.2 M NaCl, 5

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**Fig. 1. Effect of etoposide on the growth of various prostate cancer cells in culture.** A. Effect of varying concentrations of etoposide on viability of ALVA-41 cells over time. Control (no added etoposide), ○, 10 µM etoposide, ▽, 30 µM etoposide, ■, and 100 µM etoposide, ◇. The time of treatment with etoposide was as shown. B, effect of treatment for 48 h with etoposide at varied concentrations on the viability of LNCaP (○) and PC-3 (◇) cells. Procedures for cell culture and measurement of cell viability were as described under “Methods.” The results are expressed as A490 ± S.E.
mm MgCl₂, 1 mM EDTA, 0.5 mM dithiothreitol, 50 mM Tris- HCl, pH 7.9. Protein content of these preparations was assayed as described previously (28).

**Protein Kinase CK2 Assays**—Synthetic dodecapeptide was used as the substrate to assess CK2 activity associated with various cell fractions (cytosol and NM) as described previously (16, 26). Briefly, the reaction buffer consisted of 30 mM Tris- HCl, pH 7.4, 5.0 mM MgCl₂, 150 mM NaCl, 1.0 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 40 mM β-glycerophosphate, 0.2 mM synthetic dodecapeptide substrate, 0.05 mM γ-32P[ATP (specific radioactivity, 3 x 10⁶ dpm/nmol of ATP). The reaction was started by the addition of the enzyme source, generated from untreated controls and subjected to immunoblot analysis by employing anti-CK2α antibodies. Lane a, controls (no etoposide added); lane b, 10 μM etoposide; lane c, 30 μM etoposide; and lane d, 100 μM etoposide. The relative densitometric values for cytosolic fractions were 1.00, 0.96, 0.25, and 0.19 for lanes a–d, respectively. The relative densitometric values for the NM fractions were 1.00, 1.28, 1.81, and 2.69 for lanes a–d, respectively.

**Western Blot Analysis**—Samples were denatured by heating at 95 °C for 5 min in a sample buffer consisting of 10 mM sodium phosphate buffer, pH 7.0, 4 mM urea, 2.5% SDS, 1% 2-mercaptoethanol and were subjected to SDS/urea/10% polyacrylamide gel electrophoresis as described previously (16, 26). The separated proteins were transferred from the gel to a nitrocellulose sheet. After blocking the sheet with a medium consisting of 10 mM Tris- HCl, pH 7.4, 0.9% NaCl, 3% dry milk, the blot was successively incubated with mouse anti-human CK2α IgM and goat anti-mouse IgM-alkaline phosphatase-conjugated antibody. Immobilized alkaline phosphatase was visualized using 5-bromo-4-chloro-indolyl phosphate and nitro blue tetrazolium as described previously (16, 26).

**RNA Analysis**—Cells subjected to various treatments were collected for total RNA isolation using TRIZOL reagent according to the manufacturer's guidelines. Different amounts (0.2, 1, and 5 μg) of total RNA from each sample were used for hybridization with CK2α and CK2β cDNA probes as described previously (26).

**RESULTS**

To investigate the dynamics of CK2 in response to chemical–induced apoptosis, we have employed LNCaP, PC-3, and ALVA-41, three prostate cancer cell lines of diverse biological properties. Further, to confirm the general nature of these responses, we have also included three diverse nonprostate cell lines, which are Shionogi mouse mammary carcinoma, CA-9–22 (a squamous cell carcinoma of the head and neck), and CHO (a noncancerous cell line). For induction of chemically–induced apoptosis we have utilized two well established agents, namely etoposide (22) and DES (29); these drugs have also been employed as cancer chemotherapeutic agents.

**Effect of Etoposide on Prostate Cancer Cells**—The results represented in Fig. 1 show that etoposide is a potent inducer of cell death in a time- and dose-dependent manner in the three diverse prostate cancer cell lines LNCaP, PC-3, and ALVA-41. For example, the viability was most markedly reduced at concentrations of etoposide between 50 and 100 μM in the culture medium and was apparent within 20 h of treatment with the drug. The nature of the cell death under these conditions was...
confirmed by DNA ladder analysis, and Fig. 2 shows a representative result of the effect of 48-h treatment of ALVA-41 cells with varying concentrations of etoposide (Fig. 2A) and the effect of 30 μM etoposide over a period of 1 to 96 h (Fig. 2B). These results confirmed that etoposide-induced cell death was via apoptosis; similar results were observed with the other two prostate cancer cell lines (result not shown).

Effect of Etoposide on Cytosolic and NM-associated CK2 in Prostate Cancer Cells—Fig. 2C shows that ALVA-41 cells treated with 30 μM etoposide over time evoked a marked change in NM-associated CK2 activity compared with the corresponding controls. This effect was time-dependent and was maximally apparent at about 48 h following the etoposide treatment of cells.

Western blot analysis was undertaken to determine whether the change in the NM-associated CK2 in response to etoposide treatment was explained by translocation of CK2 from the cytoplasmic compartment, as observed previously when prostatic cells were subjected to changes in growth stimuli (1, 9, 11, 16, 24). Fig. 2D shows a representative result employing ALVA-41 cells treated with 30 μM etoposide over time and evoked a marked change in NM-associated CK2 activity compared with the corresponding controls. This effect was time-dependent and was maximally apparent at about 48 h following the etoposide treatment of cells.

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Fig. 3 shows the effect of varying concentrations of etoposide on ALVA-41, LNCaP, and PC-3 cells with respect to CK2 activity in the cytosolic and NM fractions. Cells were treated for 48 h with etoposide at the concentrations shown, after which CK2 activity was determined in the cytosolic and NM fractions isolated from various cells. CK2 activity associated with the NM fractions from control ALVA-41, LNCaP, and PC-3 cells was 33.9 ± 3.1, 9.6 ± 0.7, and 15.2 ± 1.5 nmol of 32P/mg of protein/h, respectively. CK2 activity in the cytosol fractions of control ALVA-41, LNCaP, and PC-3 cells was 21.6 ± 2.0, 17.9 ± 1.2, and 35.8 ± 2.2 nmol of 32P/mg of protein/h, respectively. The CK2 activity is expressed as a percentage of that in the fractions from etoposide-treated cells compared with that in the corresponding fractions from control untreated cells ± S.E.

Fig. 4. Response of various nonprostate cell lines to etoposide. Shionogi mouse mammary carcinoma, human squamous cell carcinoma of head and neck CA-9–22, and CHO cell lines were treated with etoposide for 48 h, after which cell viability and NM-associated CK2 activity were determined as described under “Methods.” A, growth response of the cells after treatment with 10 or 30 μM etoposide, expressed as A450 ± S.E. B, the CK2 activity associated with the NM fraction following treatment with 30 μM etoposide, expressed as a percentage of untreated control ± S.E. Control values of the CK2 activity in the NM fraction from Shionogi, CA-9–22, and CHO cells were 45.3 ± 3.6, 28.4 ± 2.6, and 27.8 ± 0.7 nmol of 32P/mg of protein/h, respectively.
the concentration of etoposide required for similar effects on LNCaP and PC-3 cells was 100 μM. This correlated with the sensitivity of these various cell lines to induction of apoptosis by etoposide shown in Fig. 1.

Response of Various Other Cell Lines to Etoposide—The effects of etoposide on growth of Shionogi, CA-9–22, and CHO cell lines were also studied (Fig. 4A). The results indicated that cell growth was affected in a manner similar to that observed for prostate cancer cell lines (Fig. 1). Measurement of NM-associated CK2 activity in these cell lines showed an enhancement in CK2 in response to etoposide analogous to that observed for the prostate cancer cell lines (Fig. 4B compared with Fig. 3).

Other Considerations of Etoposide Action on CK2—In control experiments we established that treatment of ALVA-41 cells with etoposide at 10, 30, and 100 μM did not evoke a significant change in the expression of CK2α or CK2β mRNA over a time course of 0 to 48 h, as examined by slot blot analysis using CK2-specific cDNA probes. This suggests that the above-described effects were not due to changes at the transcriptional level. Likewise, addition of etoposide at these concentrations to isolated nuclei or nuclear matrix or purified CK2 had no influence on the enzyme activity thus ruling out a direct effect of this agent on the enzyme activity itself (results not shown).

Effect of Diethylstilbestrol on Prostate Cancer Cells—The effect of DES, another agent for induction of chemical-mediated apoptosis (29), on growth of ALVA-41, PC-3, and LNCaP cell lines was examined. The results represented in Fig. 5A show that DES affects the growth of ALVA-41 cells in a dose- and time-dependent manner similar to that observed for the effects of etoposide (Fig. 1A). Further, PC-3 and LNCaP cell lines also respond similarly to DES in a dose-dependent manner (Fig. 5B compared with Fig. 1B). The nature of the cell death induced in these cell lines by DES as being due to apoptosis was confirmed by DNA ladder analysis (Fig. 5C).

Effect of Transient Overexpression of CK2 on Chemical-mediated Apoptosis—The above results, taken together with our previous observations on receptor-mediated apoptosis in prostate cells and the associated changes in CK2 (1, 9, 11, 16, 24), hinted that CK2 may exert a protective effect against apoptosis. To test this more directly, we transfected PC-3 cells (as a representative prostate cancer cell line) with expression plasmids of CK2α (the catalytic subunit), CK2β (the regulatory subunit), and bicistronic CK2αβ for 24 h prior to treatment with etoposide. Measurement of CK2 activity in the cytosolic and NM fractions from transiently transfected cells was carried out to confirm the CK2 overexpression. The relative overexpression of CK2 activity in the cytosolic and NM fractions in response to transient transfection with various plasmids of CK2 was analogous to our previous observations in that despite a modest change in the cytosolic CK2 activity there was a much greater increase in the NM-associated CK2 activity (26). A representative result of the CK2 expression in PC-3 cells in response to transient transfection with bicistronic pCI-CK2αβ followed by etoposide treatment is shown in Fig. 6A. Transfected cells treated with 30 μM etoposide demonstrated modest change in CK2 activity in the cytosolic fraction, but there was a significantly greater enhancement of CK2 activity in the NM, compared with that in the corresponding controls. Fig. 6B shows the corresponding effect of transient transfection with CK2 plasmids on the etoposide-mediated apoptosis of PC-3 cells. Treatment of control or pCI-transfected cells with 30 μM etoposide for 48 h resulted in about 51% loss in cell viability. This was partially reversed in cells transfected with pCI-CK2α, and the effect was even more pronounced in cells transfected with the bicistronic pCI-CK2αβ, such that in the latter case the cell viability as compared with the control cells was about 71%. Under the same conditions, transfection of cells with pCI-CK2β was without effect suggesting that the presence of the catalytic subunit of CK2 is the primary mediator of this protective effect. Considering that the efficiency of transient transfection was no more than about 60%, these results suggest that the extent of protection against apoptosis by CK2αβ is likely to be of a much higher magnitude than that observed in Fig. 6B.

We also confirmed the above observation by employing ALVA-41 cell line treated with DES. ALVA-41 cells were transiently transfected with various expression plasmids of CK2 as described above for PC-3 cells. The profile of CK2 expression in these cells was similar to that observed for PC-3 cells under the same conditions (result not shown). These cells were treated with 30 μM DES for 48 h, which resulted in a loss of about 53% of cell viability in control and pCI-transfected cells (Fig. 7). On the other hand, cells transfected with pCI-CK2α and the bicistronic pCI-CK2αβ resulted in a significant protection (about 72% of the control) against DES-induced apoptosis. No protection against chemical-mediated apoptosis induced by DES was observed when cells were transfected with the pCI-CK2β expression plasmid (Fig. 7).
DISCUSSION

Protein kinase CK2 has long been known as a multifunctional protein Ser/Thr kinase with possible roles in the cytoplasmic and nuclear compartments (1–9, 30). In particular, a considerable amount of evidence has accumulated implicating CK2 in normal and abnormal cell growth and proliferation (1–9, 30). Along these lines, our studies on the functional dynamics of CK2 in relation to growth control have employed the temporal androgenic response of prostatic epithelial cells as a model. In the in vivo model of rat ventral prostate, androgen deprivation results in receptor-mediated apoptosis, which is reversed on androgen administration. Cell culture models employing various prostate cancer cells responsive to androgens and/or growth factors have also demonstrated similar growth responses in vitro (1, 16, 31). By employing the in vivo, as well as in vitro, androgenic regulation of prostate cells, our previous studies have established that CK2 undergoes dynamic modulations in the nuclear compartment in response to altered status of growth signals, and within the nucleus there appears to be distinct loci of the functional association of CK2. For example, removal of the growth signal that controls rat prostatic growth (i.e., by androgen deprivation in the animal) evokes a rapid differential loss of CK2 from the NM, whereas introduction of the growth stimulus for the prostatic epithelial cells (by administration of androgen to the animal) results in a rapid translocation of CK2 from the cytoplasmic compartment to the nuclear compartment where it demonstrates a differential association with the NM (1, 9, 11–13). These responses of CK2 are among the earliest events that occur with altered growth signals. Analogous studies carried out on prostate cancer cells in culture have also demonstrated that NM is a common downstream site of CK2 signaling that is profoundly modulated by altered androgenic or growth factor stimulus in the corresponding responsive cells (1, 16). In other studies, transcriptionally active nucleosomes that are organized on NM were found to harbor a higher level of CK2 compared with that in the transcriptionally inactive nucleosomes (2, 10). Further, it is noteworthy that transient overexpression of CK2 in prostate cells by transfection with expression vectors was found to yield a higher degree of differential enhancement in the NM as compared with that in the cytoplasm (24). Thus, based on several lines of evidence it appears that NM is a key site of CK2 signaling in the nucleus (1, 9).

In the aforementioned rat prostate epithelial cell model, removal of the growth stimulus resulted in an early loss of CK2 associated with the cessation of cell growth and initiation of receptor-mediated apoptosis. It may be recalled that receptor-mediated apoptosis in this case does not involve events such as an initial DNA damage or up-regulation of p53 (14, 15) and is a direct consequence of the removal of the growth stimulus, androgen, or the growth factors, as the case may be. More importantly for the present discussion, the receptor-mediated dynamic loss of CK2 from the NM is initiated prior to a significant appearance of apoptosis in rat prostate epithelial cells (1, 9, 11). Accordingly, it would be pertinent to discuss the present results on CK2 dynamics in chemical-mediated apoptosis in the context of the previous observations on CK2 dynamics in receptor-mediated apoptosis.

To reiterate, CK2 has been proposed to have a major functional role in growth-related activities, especially in stimulation of cell growth (1–4, 6–8). However, based on our previous observations taken together with the present results, an adjunct functional role of CK2 under various growth conditions also merits consideration. We suggest that an early and rapid loss of NM-associated CK2 in the receptor-mediated induction of apoptosis may additionally be interpreted in terms of the removal of a protective role of CK2 against apoptosis under these conditions. Analogous considerations on the role of CK2 should apply to the chemical-mediated apoptosis induced by the commonly employed agents such as etoposide and DES. Unlike the receptor-mediated apoptosis, the chemical-mediated apoptosis initially involves damaging effects at upstream sites prior to the execution phase of apoptosis mediated by caspases (21–23, 29, 32). With respect to the present results on the dynamics of CK2 in response to etoposide and DES, an increase in the NM-associated CK2 was observed as an early response to this mode of induction of apoptosis. Clearly, this is opposite of the previously observed response of CK2 to receptor-mediated apoptosis where a rapid loss of CK2 from the nuclear compartment was demonstrated. However, based on
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The present results we propose that these two apparently disparate responses of NM-associated CK2 to the receptor-mediated apoptosis compared with chemical-mediated apoptosis can be interpreted to reflect a common role of CK2 as a protective agent against apoptosis. If the presence of CK2 would be expected to block receptor-mediated apoptosis, early loss of CK2 from the nucleus following androgen deprivation (1, 9, 14, 15) would be commensurate with the progression of apoptosis. On the other hand, in chemical-mediated induction of apoptosis, a rapid recruitment of CK2 to the NM may be interpreted to serve a primary protective response under these conditions. As discussed subsequently, our results also provide a more direct support for this notion.

A key test of the role of CK2 in protection against apoptosis, as proposed above, would be to demonstrate that cells overexpressing CK2 would exhibit a resistance to apoptosis. Thus, our results provided a direct evidence that etoposide and DES induction of apoptosis was modulated by the overexpression of the CK2 catalytic subunit or holoenzyme. The fact that overexpression of the CK2 subunits for a period of 24 h, as for Fig. 6. They were then treated with 30 μM DES for 48 h, and cell viability was determined. Results are expressed as A_{450} ± S.E. ANOVA was used to test the significance of the differences between the CK2-overexpressing cells and the cells treated only with DES. The p values of 6.3 × 10⁻⁶, 0.17, and 2.9 × 10⁻⁵ were obtained for cells overexpressing CK2α, CK2β, and CK2αβ, respectively.

In summary, we have shown that two different chemical agents that are known to induce apoptosis evoke a qualitatively similar response of CK2 translocation to the NM in diverse type of cells. The distinct nature of the dynamic response of NM-associated CK2 to chemical-mediated or receptor-mediated apoptosis is compatible with its possible role in the process of apoptosis. This is supported by the observation that overexpression of CK2 catalytic subunit or the holoenzyme significantly blocks the chemical-mediated apoptosis. The lack of such an effect of the overexpression of the β subunit of CK2 suggests a specificity of the role of the α subunit in protection against apoptosis. Thus, our results document for the first time a potential role of CK2 in protection of cells against apoptosis, in addition to the previously recognized role of CK2 in cell growth. These observations could have significant pathobiological implications as they may explain a mechanism of the resistance of cancer cells to apoptosis-inducing therapeutic approaches.

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