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Review

Protein kinase CK2 signal in neoplasia

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Summary. Protein kinase CK2 (previously known as casein kinase II) is a protein serine/threonine kinase that has been implicated in cell growth and proliferation. The focus of this review is on the apparent role of CK2 in cancer. Studies from several laboratories have shown a dysregulated expression of the kinase in tumors. Nuclear matrix and chromatin appear to be key sites for signaling of the CK2 activity in relation to cell growth. Several types of growth stimuli produce a common downstream response in CK2 by enhancing its nuclear shuttling. The neoplastic change is also associated with changes in intracellular localization of the kinase so that a higher nuclear localization is observed in tumor cells compared with normal cells. Experimental studies suggest that dysregulated expression of the α subunit of CK2 imparts an oncogenic potential in the cells such that in cooperation with certain oncogenes it produces a profound enhancement of the tumor phenotype. Recent studies have provided evidence that overexpression of CK2 in tumor cells is not simply a reflection of tumor cell proliferation alone but additionally may reflect the pathobiological characteristics of the tumor. Of considerable interest is the possibility that CK2 dysregulation in tumors may influence the apoptotic activity in those cells. Approaches to interfering with the CK2 signal may provide a useful means for inducing tumor cell death.

Key words: Protein kinase CK2, Prostate, Nuclear matrix, Chromatin, Squamous cell carcinoma, Immunohistochemistry, Signaling, Cell growth, Apoptosis, Cancer biology

Abbreviations: CK2: casein kinase 2 or II; NM: nuclear matrix; BPH: benign prostatic hyperplasia; SCCHN: squamous cell carcinoma of the head and neck; DES: dietylstilbestrol

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Introduction

The protein kinase commonly identified as casein kinase 2, and now generally referred to by the preferred name of CK2, has been known for almost half a century though not by this specific name. Over the past 25 years, CK2 has continued to gain increasing attention for its potential involvement in a variety of cellular processes, and especially for its role in regulation of cell growth and proliferation. Much progress has been made on the analysis of its structure and properties, and several recent reviews provide a broad overview of the general characteristics and functions of CK2 (Tuazon and Traugh, 1991; Ahmed, 1994, 1999; Pinna and Meggio, 1997; Allende and Allende, 1998; Guerra and Issinger, 1999; Guerra et al., 1999a). In the present overview, we aim to focus largely on the potential role of CK2 in the process of neoplasia.

Properties and general features of CK2 activity

Protein kinase CK2 is a highly conserved enzyme having extensive homology across species and almost complete structural identity among the mammalian tissues. The kinase consists of two catalytic subunits (α, α') and the regulatory subunit (ß) existing as $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ configurations, such that the α subunits are linked through the ß subunits. The protein kinase is localized in the cytoplasmic and nuclear compartments. Within the nucleus, chromatin and nuclear matrix appear to be important loci of its signaling in response to various stimuli (Ahmed, 1999). Further, it appears that certain cysteine residues in the ß subunit may play a role in anchoring the kinase to the nuclear structures (Zhang et al., 1998).

A large number of cellular (cytoplasmic as well as nuclear) proteins have been suggested as putative substrates of CK2 which points to its multiple functional activities. CK2 is a protein serine/threonine kinase which is active generally towards acidic protein substrates with the general consensus sequence of S/TXXD/E. The reader is referred to recent detailed compilations of the

currently known protein substrates of CK2 (Allende and Allende, 1998; Pinna and Meggio, 1997; Guerra et al., 1999a; Guerra and Issinger, 1999; Blanquet, 2000); for the reader's convenience, here we have provided a summary of this information in Table 1 based on a functional classification of some of the protein substrates (the original references can be found in these review articles). It is noteworthy that many of the putative substrates are related to growth and proliferation activities of the cell, especially those associated with the nucleus. The critical nature of the role of CK2 in cell biology is further underscored by the observation that it also appears to be essential for cell survival (Padmanabha et al., 1990).

The cellular regulation of CK2 remains poorly defined since it is not clear how its activity is regulated in response to various stimuli. The crystal structure of the α subunit (of maize CK2) has provided an explanation of the possible intrinsic activation of the catalytic activity (Guerra and Issinger, 1999; Guerra et al., 1999a). Other aspects of the CK2 regulation in the cell have also been considered, and may be pertinent to its biological activity. For example, it has been suggested that the catalytic α subunit may not be available because of its being complexed with other entities in the cell (Stigare et al., 1993; Guerra and Issinger, 1998; Guerra et al., 1999b). It has been proposed that interaction of the individual subunits of the kinase with other proteins may serve as a functional regulatory mechanism for its signaling (Allende and Allende, 1998). Likewise, specific modulations in various locales via translocation of the kinase (e.g., in the nuclear components) in response to certain stimuli which regulate growth may represent a mode of regulation of the functional activity of the enzyme (Tawfic et al., 1996; Ahmed, 1999). It is conceivable that all these factors play a role in controlling the functional kinase activity at specific loci in response to various stimuli, as discussed subsequently.

Dysregulation of CK2 in neoplasia

It has been noted in several studies that CK2 is markedly elevated in hematopoietic and solid tumors (Issinger and Boldyreff, 1992; Ahmed, 1994). Among the solid tumors in which increased levels and activity of CK2 have been documented are tumors of the prostate (Rayan et al., 1985; Yenice et al., 1994), kidney (Münstermann et al., 1990; Stalter et al., 1994), colon (Seitz et al., 1989), chemical carcinogenesis in liver (Ahmed, 1974), lung (Daya-Makin et al., 1994), and squamous cell carcinoma of the head and neck (Gapany et al., 1995; Faust et al., 1996). Likewise, increased CK2 has been noted in hematopoietic malignancies such as leukemia and lymphomas (Phan-Dinh-Tuy et al., 1985; Wang et al., 1995; Landesman-Bollag et al., 1998; Roig et al., 1999). Summarized in Table 2 are these observations on dysregulation of CK2 in tumors.

As noted in these various examples, the enhancement in CK2 activity (measured by biochemical analysis on tissue extracts or specific isolated cell

Table 1. A few of the protein substrates of CK2 that are potentially involved in the process of oncogenesis* .

FUNCTIONAL GROUP	SUBSTRATES
Proteins involved in nucleic acid synthesis and repair	DNA ligase, DNA topoisomerases I and II, RNA polymerases I and II, BRCA1 gene product
Transcription factors including oncogenes and tumor suppressor genes	c-myc, N-myc, C-jun, p53, Rb, androgen receptor, serum response factor, mdm2
Factors involved in protein synthesis	eIF1ß, eIF2ß, eIF3, eIF4B, eIF5
Proteins involved in signal transduction	$p34^{cdc-2}$, Protein kinase C, Protein kinase A – regulatory subunit, Insulin receptor, IGF-II receptor, Protein phosphatase 2A
Viral proteins	Human papilloma: E7, SV40: large T, Polyoma: VP1, HIV: Vpu, Herpes simplex: VP22, VP16, protein α22, R1 subunit, EBV: ZEBRA protein, EBNA-2

*: for specific references, see Pinna and Meggio, 1997; Guerra and Issinger, 1999; Guerra et al., 1999a; Blanquet, 2000.

Table 2. Observations on dysregulated expression of CK2 in tumors.

TUMOR TYPE	INCREASE IN CK2 ENZYME ACTIVTY	METHODS OF DETECTION	REFERENCE
Colorectal adenocarcinoma	Up to 8-fold	Protein level by immunoblot and activity assays; immunohistochemistry	Munstermann et al., 1990 Seitz et al., 1989
Breast carcinoma		Immunohistochemistry	Munstermann et al., 1990
Renal cell carcinoma	2-fold	Protein level by immunoblot and activity assays	Stalter et al., 1994
Non-small cell carcinoma of lung	2- to 8-folds	Activity assay	Daya-Makin et al., 1994
Acute myeloid leukemia and chronic myeloid leukemia in acute blast crisis	3- to 5-fold	Activity assay	Phan-Dinh-Tuy et al., 1985
Prostatic adenocarcinoma	3- to 5-fold	Activity assay and immunohistochemistry	Yenice et al., 1994
Squamous cell carcinoma of the head and neck (SCCHN)	2- to 8-fold	Activity assay and immunohistochemistry	Gapany et al., 1995; Faust et al., 1996, 1999a

fractions) was observed to be in the range of 2- to 8-fold compared with the corresponding normal controls. However, it must be emphasized that these values are based on crude cellular materials from whole tissues (which may consist of numerous cell types, etc.) and as such may not reflect the precise nature of the dysregulation in the affected cells. However, when the biochemical and immunohistochemical analyses on the same tumor sample are compared a reasonable correlation of the two is noted. In general, these results can be taken to indicate a general relation of the altered CK2 to the neoplastic state. A careful biochemical analysis of CK2 in the well-defined tumor specimens can lead to additional general conclusion on the pathological status, as discussed subsequently (Faust et al., 1999a). The observations that CK2 is elevated in cancer cells as well as normal proliferating cells may suggest that the kinase is simply a proliferation marker. However, as discussed subsequently, recent immunohistochemical studies using anti-CK2α antibody and the commonly employed proliferation marker antibody Ki-67 have revealed that this conclusion may not be appropriate to fully represent the biological function of CK2 (Faust et al., 1999a). Thus, we suggest that careful immunohistochemical analysis of CK2 expression in tumor cells may yield important information in evaluation of its potential role in the pathological manifestations of the dysregulated CK2.

The change in the CK2 enzyme expression in tumor cells does not appear to be related to an alteration in the level of the CK2 message at the transcriptional level, as noted by us from studies of the RNA expression in prostate cancer and squamous cell carcinoma of the head and neck (Table 3). The mRNA expression levels for the α and ß subunits of CK2 were determined in prostate and SCCHN samples by employing the slot blot technique followed by densitometric analysis of the autoradiograms. The results in Table 3 do not reflect a significant change in the expression of CK2 mRNA in prostate cancer or SCCHN specimens compared with the corresponding normal tissues. This suggests that the dysregulation of CK2 observed in cancer may occur at the post-transcriptional level. That no mutations in CK2α gene in neoplasia have been described supports this notion.

Table 3. CK2 mRNA levels in normal and neoplastic prostate and oropharyngeal tissue specimens*.

	CK2-α	CK2-ß	ACTIN
Human Prostate			
Normal	1.00	1.00	1.00
BPH	1.55	1.21	1.61
Tumor	1.15	1.15	1.13
SCCHN			
Normal	1.00	1.00	1.00
Metastatic lymph node	1.14	1.08	0.90
Primary tumor	1.00	0.72	1.47

*: mRNA expression levels for CK2 subunits are presented as relative values per DNA equivalent in the sample (based on 4-8 samples in each case).

CK2 in tumor pathobiology

The ubiquitous dysregulation of CK2 in tumors would suggest that it reflects diverse pathobiological characteristics of tumor cells. Studies dealing with various aspects of the dysregulated expression in tumors and their implications in tumor cell biology are discussed in the following.

Nature of CK2 expression in tumors

Based on the above discussion, it would be of interest to determine whether the degree of dysregulation in CK2 activity in the tumor cells, compared with the corresponding normal cells, reflects the severity of the disease. There is a paucity of information on this subject. However, it was recently documented that the level of CK2 in the head and neck tumor cells correlated with the tumor grade, stage, and clinical outcome (Gapany et al., 1995). Concordant with these studies was the observation suggesting an association of CK2 in the chromatin of head and neck tumors with malignant transformation (Faust et al., 1996). For prostate cancer also, the CK2 activity in the tumor showed a correlation with Gleason grade (Yenice et al., 1994). Taken together, these studies point to the possible involvement of CK2 in many aspects of tumor biology such as differentiation, invasion, metastasis, and response to therapy.

Immunohistochemical analysis of CK2 in tumors (such as prostatic adenocarcinoma and SCCHN) reveals interesting features of its localization in tumor cells as well as in cells (e.g., tumor infiltrating lymphocytes) invading the tumors (Yenice et al., 1994; Faust et al. 1999a). It appears that the distribution of CK2 within the tumor cells in the tissue is not uniform. In general, the tumor cells at the periphery (or the infiltrating edge) of the tumor show a higher concentration of CK2 than in tumor cells located more centrally. This may be pertinent to the consideration that the infiltrating edge of tumors has the capacity to secrete soluble factors that facilitate the process of local invasion of surrounding stroma and basement membranes. Biochemical and immunohistochemical studies have shown that intracellular elevated levels of CK2 are differentially distributed in various compartments of the cell and may be distinct from that observed for the normal cells. In tumor cells compared with normal, CK2 is localized preferentially in the nuclear compartment (Yenice et al., 1994; Faust et al., 1999a). The results of the CK2 activity measurements by biochemical methods accord with those obtained by employing immunohistochemical analysis of the same tumor specimens although additional features of CK2 differential cellular localization were noted with the use of immunohistochemistry (Faust et al., 1999a). By employing the antibodies to the α subunit of CK2 (anti-CK2-α antibody), we found that the catalytic subunit

was localized predominantly to the nuclei in tumor cells but that not all tumor cells were stained with the same intensity with the anti-CK2-α antibody. Interestingly, the CK2-α staining pattern in benign squamous mucosa of head and neck was distinct from that observed in its malignant counterpart (Faust et al., 1999a). The chiefly nuclear distribution of CK2-α immunostaining found consistently in these tumor cells (and tumor-infiltrating lymphocytes) contrasted with a relatively more predominant cytosolic staining pattern demonstrated by cellular constituents of normal oropharyngeal mucosa. In general, the proliferating front of squamous infiltration was strongly positive for CK2-α staining. This is analogous to the previous observations on prostate and colon cancers (Seitz et al., 1989; Yenice et al., 1994). Further, it was noted that nuclei of the infiltrating lymphocytes in the squamous cell carcinoma specimen also stained intensely for CK2-α; the significance of this observation is not entirely clear but it may be related to increase in secretory/proliferative or anti-apoptotic activities of lymphocytes within the tumor cells. At still higher magnification, it was observed that the nuclear staining of the squamous cell carcinoma of the head and neck cells exhibited a punctate pattern for CK2-α staining. This suggests non-uniform distribution of CK2 in the nucleus with focal collections. This is consistent with the association of CK2 with chromatin and nuclear matrix structures, as documented by us in several studies (Ahmed and Goueli, 1987; Ahmed et al., 1993; Faust et al., 1996; Tawfic et al, 1996; Guo et al., 1998; Ahmed, 1999; Yu et al., 1999). Of note, desmoplastic fibroblasts that were found in the stroma of the tumor sections showed significant staining of the cytoplasm as well as nucleus. This may accord with the proliferative activity of desmoplastic stroma around the tumor. In summary, immunohistochemical studies showed enhanced, mostly nuclear, staining in tumor cells, tumor-infiltrating lymphocytes, and surrounding desmoplastic stroma compared to normal cellular counterparts. In addition, the differential high-intensity staining of tumor cells at the periphery of the tumor may reflect the additional biological activities assigned to tumor cells at this location as mentioned above. In other work, we have provided evidence on the growth stimulus mediated signaling of CK2 to chromatin and nuclear matrix (Ahmed, 1999). The immunohistochemical observations on the tumor specimens (Faust et al., 1999a) further point to the important role of these nuclear structures in dysregulated CK2 signaling.

Other studies on immunoblots of certain tumor derived samples (e.g., from acute myeloid leukemia, HL-60, and mouse Krebs ascites cells) have demonstrated multiple forms of the α subunit of CK2 (Guerra and Issinger, 1999; Roig et al., 1999) which have been suggested to arise from their proteolytic degradation (Roig et al., 1999). Of interest are also the observations which indicate asymmetric expression of the α and ß subunits in certain tumors. For example, in renal clear cell carcinomas it was noted that the ratio of the α subunit in the tumor versus normal specimen was 1.58±0.47 whereas that for the ß subunit was 2.65±1.1 implying that in tumors altered expression of the subunits may have some pathobiological relevance (Stalter et al., 1994). A careful analysis of specific tumors in relation to their pathological characteristics compared with the corresponding normal specimens is warranted to generate additional data along these lines which might indicate the role of this disturbance in the pathogenesis of the tumor.

CK2 reflects not only proliferation but also pathobiological character of the cells

The association of CK2 with rapid proliferation (normal as well as abnormal) has prompted the general notion that it might simply reflect the growth status of the cells. However, a high level of cellular CK2 does not simply correlate with the proliferative activity as indicated, for example, by the high level of CK2 in brain (see e.g., Guerra and Issinger, 1999). Given that CK2 has an apparent role in a rather wide range of cellular activities, including cell growth, it would seem that the actual level of CK2 in a given cell type may be dictated by the functional needs of the cell. Thus, the extent of the CK2 activity alone may not be informative; rather, the *change* from the normal for the particular cell type may be a critical determinant for an alteration in the biological response of the cells. As discussed subsequently, support for this concept comes from the experimental studies which suggest an oncogenic potential of even a small dysregulation of CK2 in the cell (Landesman-Bollag et al., 1998). In this regard, it is pertinent that the effects of overexpression of CK2 on cell proliferation can yield apparently contradictory results. It was observed that overexpression of CK2-α and CK2-α' can enhance proliferation in fibroblasts (Orlandini et al., 1998), whereas another report has suggested that overexpression of CK2 caused a reduction in proliferation especially when enzyme containing the α' subunit was compared with that containing the α subunit (Vilk et al., 1999). The reasons for this discrepancy are not entirely clear but it would appear that the type of cells used for overexpression studies may influence the results on the effects of overexpression of CK2.

To address the question whether CK2 expression is simply a reflection of proliferation activity, we compared the immunohistochemical profiles of the same tissue sections by employing the anti-CK2-α antibody and the antibody for the commonly utilized proliferation-marker Ki-67 (Faust et al., 1999a). Ki-67 is a nuclear antigen whose expression is an absolute requirement for progression through the cell-division cycle (Scholzen and Gerdes, 2000). Our results clearly indicated distinct patterns of CK2-α and Ki-67 immunostaining in the same tissue sections (Fig. 1). As expected, immunostaining for Ki-67 was most intense at the mitotically active areas of the tumor which contrasted with CK2-α

immunoreactivity that was found to be positive to varying extents throughout the same tumor in addition to the enhanced staining at the leading edge of the tumor as mentioned earlier. In general, it appeared that anti-CK2-α immunostaining was particularly remarkable in moderately to poorly differentiated carcinomas, whereas in well-differentiated carcinomas there was a relatively decreased CK2-α immunoreactivity in the central keratinizing zone. These observations clearly indicate that CK2 cannot be regarded strictly as a proliferation marker per se. Rather, it appears that it does associate with proliferation but more importantly its activity may relate to the pathobiological characteristics of the tumor cells such as the state of differentiation, etc. In this regard, it should be noted that our data on the CK2 in squamous cell carcinoma of the head and neck demonstrated a highly significant Kaplan-Meier cumulative survival analysis. It was observed that survival in the high-CK2 activity patient group was greatly reduced suggesting the potential usefulness of CK2 as a prognostic indicator in certain malignancies (Gapany et al., 1995).

Oncogenic potential of CK2 and modes of CK2 function

Transgenic models of CK2 function in tumor growth

Various lines of evidence suggest that dysregulated presence of CK2 may augment the oncogenic potential in the cell by cooperating with other factors (Xu et al., 1999). A more direct evidence in support of the potential oncogenic role of CK2 comes from the studies on the incidence of lymphoma in mice carrying CK2-α transgene (see e.g., Xu et al., 1999). Based on the observation that in cattle infected with *Theileria parva* there was a marked upregulation of CK2 associated with incidence of pseudo-leukemia in infected cells, studies were undertaken to demonstrate that ectopic coexpression of CK2-α along with c-myc in transgenic mouse model resulted in a large increase in the incidence of lymphoma (Seldin and Leder, 1995). Further studies along these lines demonstrated similar responses to transgenic co-expression of CK2-α with Tal-1 or altered p53 expression (Kelliher et al., 1996; Landesman-Bollag et al., 1998). In these studies, ectopic expression of the CK2-α transgene was not extensive such that it was not detectable by simple means. Thus, the results suggest that even a very mild dysregulation in the 'intrinsic' level of CK2 in a given cell could contribute toward significant pathological consequences.

Of considerable interest are also several recent reports implicating phosphorylation of viral proteins by CK2 in pathological processes. Virally transformed cells demonstrate higher levels of CK2 (Brunati et al., 1986). It now appears that many of the viral proteins are phosphorylated by CK2; at least 13 different viruses have been found to contain CK2-mediated phosphorylated proteins, and the number of viral proteins which are candidates for phosphorylation by CK2 seems to continue to increase (for a review, see e.g., Guerra and Issinger, 1999). These viral proteins phosphorylated by CK2 appear to play multiple roles, which strongly suggests a role of CK2 in virally mediated pathologies including cancers.

CK2 and apoptosis

Oncogenic proteins might influence the growth by stimulating proliferation and/or by an effect on the apoptotic activity in the cell. Our studies on the androgenic regulation of the prostate had indicated that CK2 might be involved in both of these activities. For example, androgen deprivation which results in receptor-mediated apoptosis in rat ventral prostate results in a rapid loss of CK2 from chromatin and NM and that this event temporally precedes the appearance of apoptosis (Ahmed et al., 1993; Tawfic and Ahmed, 1994a,b; Tawfic et al., 1994, 1995; Guo et al., 1998; Ahmed, 1999). Of equal importance, the translocation of CK2 from the cytoplasm to the nuclear compartments occurs very rapidly (during the pre-replicative phase) on induction of growth by androgenic stimulus in the animal. Thus, the receptor-mediated loss of CK2 from

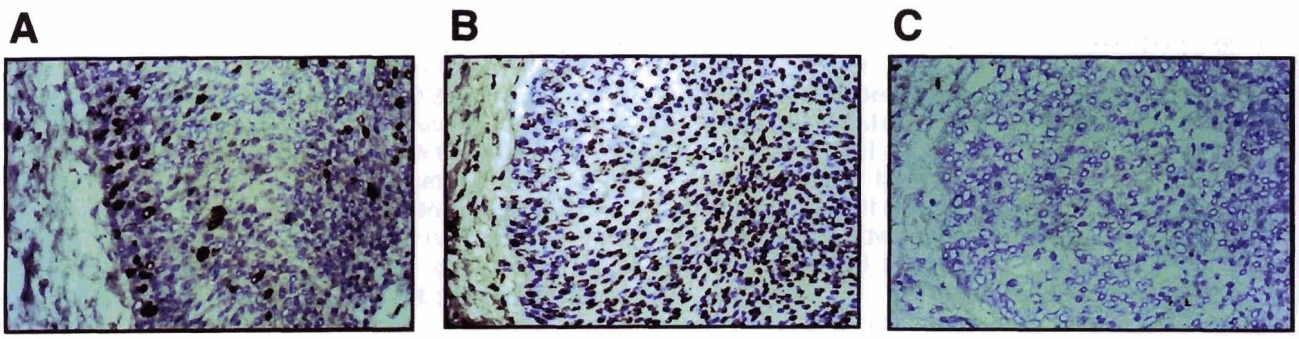

Fig. 1. Immunohistochemical staining for CK2-α and Ki-67 in the same tumor specimen. **A.** Pattern of Ki-67 staining. **B.** Immunostaining pattern of CK2-α. **C.** Negative control. All sections are lightly counter-stained with hematoxylin. For further details, see text. (Reproduced from Faust et al., 1999a, with permission). x 100

the nucleus can be equated with cessation of cell growth activity as well as induction of apoptosis, whereas the reverse would imply involvement of CK2 in stimulation of growth and inhibition of apoptotic activity. To address the role of CK2 in apoptosis more directly, we examined the effects of chemical-mediated apoptosis on nuclear dynamics of CK2. We observed that in several cancer cell lines, treatment with etoposide resulted in induction of apoptosis; however, there was a marked concomitant increase in the NM-associated CK2. This appeared to result in part from its translocation from the cytoplasm suggesting a possible protective effect of CK2 in response to etoposide-mediated cell death. In support of this notion we found that forced transient overexpression of CK2-α and CK2-αß resulted in significant protection of the cells against etoposide mediated apoptosis. The critical role of the α subunit of CK2 was indicated by the observation that overexpression of CK2-ß did not evoke such a response (Guo et al., 2000a,b; Yu et al., 2000). Diethylstilbestrol (DES) another chemical agent which is known to induce apoptosis in prostate cancer cells (Robertson et al., 1996), gave similar results as etoposide (Yu et al., 2000). Thus, our data provide evidence for the first time in support of the view that CK2 has the ability to exert an effect on the apoptotic activity in cells. This observation has strong implications for a role of this kinase in neoplasia. It is noteworthy that treatment of cancer cells with antisense oligonucleotides against the α subunit of CK2 produces a strong inhibition of cell growth which appears to be through induction of apoptosis (Faust et al., 2000). Interestingly, the antisense to CK2-α produced nearly a 100% growth inhibition and cell death at concentrations which reduced the CK2 activity by 40%, implying that even a modest alteration (reduction) in the CK2 level intrinsic to the tumor cells might compromise viability of tumor cells under these conditions. This is the first study which employed antisense to CK2-α as an anticancer modality suggesting a potential of using this approach to anticancer therapies.

Possible mechanisms of CK2 regulation and involvement in tumors

Based on the above discussion, there seems no doubt that CK2 plays a significant role in the process of oncogenesis. Therefore, it would be important to define the mechanism(s) of its involvement in this process. This is a problematic question at the present time since the mechanism(s) by which CK2 is regulated in the cell even under normal growth has not been fully defined. As this field progresses, it should find application in yielding insights into the mechanism(s) that are involved in the normal and cancerous cell growth. Nonetheless, sufficient information on this subject has emerged to enable us to speculate and hypothesize.

The primary issue regarding the regulation of CK2 in vivo remains unresolved. Various studies have indicated that the catalytic subunit of the kinase is intrinsically active. Certainly, recent studies on the crystal structure of CK2-α have provided an explanation why the catalytic subunit of CK2 could remain constitutively active (Guerra and Issinger, 1999; Guerra et al., 1999a), and this may account for why CK2 isolated from cells always appears to be active. However, since CK2 is involved in many cellular functions including control of cell growth and proliferation which are unlikely to be random processes, this raises the problematic issue of the response to various stimuli which affect the functional activity of the kinase in various intracellular locales. In this context, a number of possibilities have emerged from studies in various laboratories which hint at several potential subtle modes of the functional regulation of CK2 in the cell.

Evidence has been provided in support of the notion that the α and ß subunits of CK2 in the cell may not always be fully complexed, and may exist as free subunits or in complex with other proteins (Stigare et al., 1993; Guerra et al., 1999b; Guerra and Issinger, 1998). Among the latter are included key molecules such as nucleolin, B23, topoisomerases, RNA polymerases, p53, MDM2, p21waf1/cip1, p34cdc, Myc/Max, tubulin, etc. Phosphorylation and/or association of these molecules with subunits of CK2 often reveals complex interactions and consequences, including a potential role in neoplasia (see e.g., Bousset et al., 1993; Tawfic et al., 1994, 1995; Filhol et al., 1996; Götz et al., 1996, 1999a,b, 2000; Bosc et al., 1999; Egyhazi et al., 1999; Faust et al., 1999b; Ghavidel et al., 1999; Leroy et al., 1999; McKendrick et al., 1999). Further, the ability to independently interact with a rather large number of proteins (or partners) (Grein et al., 1999; Guerra and Issinger, 1999; Kusk et al., 1999) would suggest that activity of CK2 may be modulated by such interactions in the cell, thus creating a subtle means of regulating functional cellular activity of the kinase under various conditions. It has been proposed that there might be certain "wild-card" proteins which could serve as switches by interacting with CK2 subunits (Allende and Allende, 1998). In this regard, the α subunit of CK2 has been shown to be a target for the Abl and Bcr-Abl tyrosine kinases implying that CK2 may represent a mediator for Bcr-Abl (Hériché and Chambaz, 1998). Similarly, CD5 which is a T-cell marker and is expressed aberrantly on B lymphocytes of chronic lymphocytic leukemia was found to directly interact and enhance CK2 activity (Raman et al., 1998). Another example relates to the BRCA1 gene which encodes a complex protein that appears to be involved in some aspects of DNA repair. Mutations in BRCA1 gene on chromosome 17p account for many cases of familial breast carcinoma and predisposition to ovarian carcinoma. This protein has been found to bind to CK2 which phosphorylates it. The interaction is reduced in vitro when BRCA1 fragment bearing a disease-associated mutation was used implicating CK2 as a potential mediator of BRCA1 activity (O'Brien et al., 1999).

As we have proposed, a mode of cellular regulation of CK2 may relate to the potential of its dynamic

shuttling to different compartments in the cell in response to various stimuli (Ahmed, 1999). This hypothesis is based on our observations that CK2 undergoes very rapid modulations in its association with chromatin and nuclear matrix with altered status of growth stimuli, including hormones and growth factors (Ahmed et al., 1993; Tawfic and Ahmed, 1994a,b; Guo et al., 1998, 1999a,b; Ahmed, 1999; Yu et al., 1999). In addition, it seems that intranuclear activities (such as transcription and DNA synthesis) that are associated with displacement of histone proteins qualitatively and quantitatively affect CK2 activity focally in these locations which may afford yet another subtle means of CK2 regulation and its involvement in these essential activities (Ahmed, 1999; Tawfic et al., 1999). We have recently observed specific modulation of nuclear matrix-associated CK2 during cell cycle progression (Wang, Yu, Davis and Ahmed, unpublished data). These observations accord with previously reported cell cycle related changes in nuclear CK2 (Marshak and Russo, 1994; Pinna and Meggio, 1997; Bosc et al., 1997). Further work is needed to understand the process of spatio-temporal regulation of CK2 in the nucleus in response to growth stimuli and in neoplasia.

Given the present state of knowledge on CK2 regulation in the cell, one can only speculate on the various means by which it is involved in the process of oncogenesis. First, based on the studies employing transgenic expression of CK2 (Xu et al., 1999), it would seem that the absolute amount of CK2 in a given cell under normal conditions would be relatively constant and that even a small deviation from this (e.g., by overexpression) could disturb the system in a way as to make it highly susceptible to the presence of other oncogenic stimuli (Seldin and Leder, 1995; Landesman-Bollag et al., 1998). Likewise, the shuttling of the kinase to distinct sites can be a feature of importance to the cancer phenotype as observed by immunohistochemical analysis which showed a more diffuse presence of CK2 in the cytoplasm of benign or normal specimens while in the cancer specimen an intense localization in the nucleus was the more obvious feature (Yenice et al., 1994; Faust et al., 1999). This may not only influence the activities associated with growth and proliferation but also those associated with regulation of apoptotic activity in the cancer cells (Guo et al., 2000a,b; Yu et al., 2000). The latter feature of CK2 function may be particularly important in regard to the process of neoplasia as it may come into play when the cell encounters dysregulation in the intrinsic level of CK2.

Involvement of CK2 in neoplasia by virtue of its activity in the phosphorylation of certain proteins also remains a major candidate for this role. As stated earlier, the list of the putative substrates for CK2 is quite large including a variety of nuclear proteins and oncogenes which influence cell cycle and proliferation (for general reviews see e.g., Tuazon and Traugh, 1991; Ahmed, 1994, 1999; Pinna and Meggio, 1997; Guerra and Issinger, 1999; Blanquet, 2000). Complex interactions of CK2 and p53 are further highlighted by observations that cell type specific p53 transactivation may produce varying degrees of alteration of the promoters for MDM2, cyclin G, Bax and Fos (Schuster et al., 1999). Also germane to these considerations are our studies which have suggested that several proteins associated with the nuclear matrix and chromatin are phosphorylated by CK2 (see e.g., Ahmed, 1999). The involvement of CK2 in the phosphorylation of chromatin-associated proteins in relation to transcriptional activity in the nucleosomes indicates a number of proteins that are candidates for phosphorylation by CK2 under different conditions. Of interest was the observation that phosphorylation of certain proteins was noted only in the transcriptionally-active nucleosomes and of some others only in the transcriptionally-inactive nucleosomes (Guo et al., 1998, 1999a).

Concluding remarks

Our recent proposal on a role of CK2 at the interface of the nuclear matrix and chromatin structures may be of interest as it suggests a paradigm for involvement of phosphorylation of nuclear matrix and chromatin proteins in a spatio-temporal manner thereby influencing the status of chromatin with respect to its transcriptional activity (Ahmed, 1999). Such proteins may represent transcription factors and other regulatory molecules involved in the control of growth related activity in the cell (Guo et al., 1998, 1999a,b; Ahmed, 1999). The modulations in signaling of CK2 in response to diverse stimuli could reflect its key function in the pathobiological features of cancer cell growth (Ahmed, 1999; Guo et al., 1999b). The involvement of structural constraints in the biological functions such as cell growth and proliferation continues to be recognized (for a detailed review, see e.g., Stein et al., 2000). Given that nuclear structural alterations represent a hallmark of cancerous transformation, it would be important to identify and define the role of the factors relating to the functionality of the structures such as chromatin and nuclear matrix and what factors contribute to their altered state. We propose that CK2 is one such factor which interacts with the chromatin and nuclear matrix, and merits further consideration from these points of view, especially relating to neoplastic transformation.

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