Significance of Protein Kinase CK2 Nuclear Signaling in Neoplasia

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Abstract   Many stimuli play a role in influencing the structure and function of chromatin and nuclear matrix through post-translational modifications of the component proteins in these dynamic structures. We propose that the protein serine/threonine kinase CK2 (formerly casein kinase II) is one such agent that is involved in signal transduction in the nuclear matrix and chromatin in response to a variety of stimuli. Protein kinase CK2 appears to undergo rapid modulations in its association with nuclear matrix and nucleosomes in response to mitogenic signals and is involved in the phosphorylation of a variety of intrinsic proteins in these structures depending on the state of genomic activity. In addition, its association or loss from the nuclear matrix may also influence the apoptotic activity in the cell. CK2 has been found to be dysregulated in virtually all the neoplasias examined and nuclear association appears to be an important facet of its expression in tumor cells. We hypothesize that CK2 provides a functional paradigm linking the nuclear matrix and chromatin structures. Identification of precise loci of action of CK2 in these structures and how they influence the morphological appearance of the nucleus under normal and abnormal growth conditions would be an important future direction of investigation. J. Cell. Biochem. Suppl. 35:130–135, 2000. Published 2001 Wiley-Liss, Inc.[†]

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It is well recognized that altered nuclear morphology is a reflection of the downstream consequences of biochemical and/or physical stimuli that may have affected the cell. Biochemical changes preceding the morphological changes in the nucleus may be due to the presence of altered or new structural entities such as damaged DNA, oncogenic mutations, etc. [see e.g., Tsatsanis and Spandidos, 2000]. However, often in neoplasia, it is not so much that actual qualitative changes in the chemical make-up of the cell are detected but rather that the activity of one or more of these molecules is "dysregulated" from the normal. This dysregulation may occur at the post-transcriptional as well as at the transcriptional level. Transduction of the biochemical signals is often through post-translational modifications of the proteins in various nuclear compartments. If the signaling is dysregulated from that in the normal cell it presumably translates into altered morphology. The observation that changes in the nuclear structure become rapidly apparent in response to biochemical and physical mediators [e.g., Nicolini et al., 1986; Maniotis et al., 1997] emphasizes the significance of post-transcriptional events leading to altered nuclear morphology.

A major means of achieving altered activity of cellular proteins is via their post-translational modifications, such as by acetylation, phosphorylation, etc. This has provided a general paradigm that can be considered as a key link between the molecular and morphological structure of the nucleus and eventually the cell such that they may appear different from the normal when these signals are dysregulated.

Much evidence has been provided to support the role of protein kinases (and phosphatases) in mediating cellular functions in relation to normal and abnormal growth [e.g., Hunter, 1995; Tsatsanis and Spandidos, 2000]. The protein kinase known as CK2 (formerly, casein kinase II) is one such factor that has been found to be dysregulated in all the neoplasias examined [see e.g., Guerra and Issinger, 1999; Tawfic et al., 2001]. This kinase has been the focus of our investigations for its role in nuclear signaling in response to growth stimuli in normal and cancer cells [Ahmed, 1999]. Here we discuss the evidence that implicates a role of CK2 signaling in the nuclear matrix and chromatin in neoplasia. For these studies we have employed several experimental models. Rat ventral prostate regulation by androgens has provided an excellent physiological model in which removal of androgen (by castration) results in cessation of androgen-dependent gene activity and induction of apoptosis. Administration of androgen to previously androgen-deprived animals results in rapid induction of androgen-dependent genome activation followed by cell proliferation. In addition, we have employed in vitro models of cell growth and proliferation using prostate cancer and head and neck squamous cell carcinoma cell lines [Ahmed, 1999; Guo et al., 1999a; Tawfic et al., 2001].

GENERAL PROPERTIES OF THE CK2 SIGNAL

Protein kinase CK2 is a protein serine/threonine kinase which is a heterotetrameric molecule consisting of catalytic subunits (α, α') and regulatory subunit (β) existing in the $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ configuration. CK2 is localized in both the cytoplasmic and nuclear compartments. It has a special affinity for acidic protein substrates, recognizing the consensus sequence S/TXXD/E. Indeed, there is a fairly large list of putative substrates; importantly, many of these are associated with growth-related activities in the nucleus [see e.g., Ahmed, 1999; Guerra and Issinger, 1999; Blanquet, 2000; Tawfic et al., 2001]. Studies discussed subsequently have emphasized that within the nucleus, chromatin and nuclear matrix are key signaling sites for CK2. The fact that this protein kinase is among the most highly conserved enzymes and appears to be essential for cell viability emphasizes its significance in cell biology [see e.g., Guerra and Issinger, 1999]. A consideration of these various characteristics of CK2 would suggest the likelihood of significant potential consequences of its dysregulation in the cell.

DYNAMICS OF CK2 SIGNALING IN THE NUCLEUS

Chromatin and nuclear matrix represent the two key loci involved in the regulation of normal and abnormal cell growth and proliferation [see e.g., Stein et al., 2000]. Nucleosomes, the subunit structure of chromatin, undergo post-translational modifications (such as acetylation/deacetylation) in their component proteins (histones) which influence their state of activation [for a review see e.g., Stein et al., 2000]. Nuclear matrix is the nuclear substructure that maintains distinct functional and morphologic domains within the nucleus [Ma et al., 1999; Stein et al., 2000]. Among the functions of this dynamic structure is the organization of chromatin in response to its altered activation state [see e.g., Getzenberg et al., 1991; Ahmed, 1999; Barrett and Spelsberg, 1999; Stein et al., 2000]. The nuclear matrix and chromatin appear to be functionally linked through various activities, such as histone acetylase/deacetylase [see e.g., Stein et al., 2000], and, as discussed here, possibly through the involvement of CK2 [Ahmed, 1999].

Original studies undertaken by us employing androgenic regulation of prostate demonstrated the rapid phosphorylation of nuclear non-histone proteins in response to androgen-mediated genome activation [Ahmed and Ishida, 1971]. Several correlative studies emphasized the importance of phosphorylation of these proteins in relation to normal and neoplastic growth [e.g., Stein et al., 1983]. The so-called non-histone phosphoprotein component of the nucleus is now known to be a mixture of a wide variety of proteins including structural proteins, transcription factors, tumor suppressor proteins, various enzymes (including kinases and phosphatases), antigens, receptor proteins, products of oncogenes/proto-oncogenes, etc. Thus, the observation that a preponderant amount of phosphorylation of these proteins (as a group) appeared to be mediated by nuclear-associated CK2 [Goueli and Ahmed, 1991] prompted us to study its spatio-temporal dynamics in chromatin and nuclear matrix in response to altered growth status. Using the aforementioned model of androgenic regulation

of prostate, we demonstrated that CK2 was rapidly and differentially lost from the chromatin compartment compared with that in the nucleoplasm on removal of growth stimulus. On administration of the androgenic stimulus there was a rapid and differential association of the kinase with chromatin compared with that in the nucleoplasm [Ahmed et al., 1993]. We then decided to determine whether or not CK2 signaling occurred to the nuclear matrix also. We demonstrated the presence of CK2 in the isolated nuclear matrix where it appeared to have a role in phosphorylation of intrinsic proteins. Further, in the experimental model of androgenic stimulation of the prostate rapid changes in its loss from the nuclear matrix on androgen withdrawal and rapid translocation to the nuclear matrix on androgen administration were observed, thus demonstrating dynamic changes in its level in response to altered growth stimuli [Tawfic and Ahmed, 1994; Tawfic et al., 1996; Ahmed, 1999]. Evidence supporting the physiological relevance of CK2 signaling to the nuclear matrix related to its involvement in the phosphorylation of protein B23 (nucleophosmin) and nucleolin which are known to be involved in rRNA synthesis. Significantly, the CK2-mediated phosphorylation of these proteins was found to be essential for their stability in the nucleus [Tawfic et al., 1994, 1995]. The loss of CK2 from the nuclear matrix and consequent change in the phosphorylation status of protein B23 was shown to specifically correlate with its proteolytic degradation [Tawfic et al., 1995]. Besides these studies in which androgen was the mitogenic stimulus, we demonstrated CK2 signaling in the nuclear matrix in response to a variety of other mitogenic stimuli [Guo et al., 1999a]. Of note in these observations is that nuclear matrix targeting of CK2 signal appears to be a common downstream response to different types of mitogenic signals in the corresponding responsive cells. For example, it was noted that prostate cancer cells that were sensitive to androgens or growth factors (e.g., LNCaP cells) demonstrated a translocation of CK2 to the nuclear matrix in response to either type of stimulus. Other cell lines that did not respond to androgens but did so to growth factors (e.g., PC-3 cells) responded by translocation of CK2 to the nuclear matrix only in the presence of growth factors and not androgens [Guo et al., 1999a]. That CK2 can serve as a common downstream signal transducer in response to diverse growth factor stimuli has intriguing implications for tumor cell growth.

Analogous studies to define the dynamics of CK2 in chromatin have revealed that the level of CK2 is higher in transcriptionally active than inactive nucleosomes [Guo et al., 1998]. By employing the androgenic regulation of prostate growth as a model, it was found that on androgen deprivation, there were significant differential changes in CK2 associated with the nucleosomes depending on their transcriptional activity. Interestingly, the relative CK2 activity intrinsic to the transcriptionally active nucleosomes remained fairly stable concordant with gene activity specific to the androgenic status. However, CK2 associated with inactive nucleosomes declined to a minimal level on androgen deprivation. As expected, there was an increase in the CK2 associated with active nucleosomes on institution of androgenic growth stimulus; however, even more remarkably there was rapid shuttling of CK2 to the inactive nucleosome fraction. This hinted that such an association of CK2 with the inactive nucleosomes was a means of promoting the conformational transition of inactive nucleosomes to the active form, in addition to its function in transcriptionally active nucleosomes. Study of the phosphorylation of nucleosome-associated proteins revealed that several proteins were potential substrates for CK2, although protein kinase activity other than CK2 was also indicated. Interestingly, there was a distinct difference in the proteins that were phosphorylated in the active nucleosomes compared with those in the inactive nucleosomes [Guo et al., 1999b]. Thus, it is likely that specific structural changes in the nuclear compartments are mediated through these post-translational modifications under varying growth conditions.

DYSREGULATION OF CK2 SIGNALING IN NEOPLASIA

Protein kinase CK2 has been found to be dysregulated in virtually every tumor examined. Based on biochemical studies, a significant increase in its level of activity in tumor cells has been documented in a number of studies [see e.g., Guerra and Issinger, 1999; Tawfic et al., 2001]. The mechanism or consequences of the increase in CK2 in tumor cells has not been fully elucidated. However, it appears that the

changes in the CK2 activity under these conditions may not relate to a change at the transcriptional level which is in accord with the absence of any reported mutations in the CK2 gene [Tawfic et al., 2001]. Of note are the observations that the gene for protein B23 (a substrate for CK2, as discussed above) is involved in two chromosomal translocations that are seen in anaplastic large cell lymphoma, childhood acute myeloid leukemia, and acute promyelocytic leukemia [for review see, e.g., Drexler et al., 2000]. Similar considerations pertain to the interaction of CK2 with the tumor suppressor gene product p53 for its functional activity in the nucleus under various conditions [Schuster et al., 1999].

Important evidence of the role of CK2 in neoplasia has been provided by studies of transgenic mice in which CK2-α was over-expressed in a targeted manner along with Tal-1 gene. In these cases, the dysregulation of CK2 was relatively modest, yet the incidence of neoplasia was greatly enhanced by the presence of CK2-α in the double transgene [Kelliher et al., 1996]. This implies that the actual level of expression of CK2 in a given cell type may be very specific, and that even a small deviation from that would produce an altered or dysregulated biological response in the cell. This notion is supported by the first demonstration that an antisense oligonucleotide based on CK2-α was capable of potently inducing apoptosis while partially reducing CK2 activity in the tumor cells. Thus, it would appear that even a modest alteration (lowering) in the intrinsic level of CK2 in these tumor cells evoked a damaging effect [Faust et al., 2000]. In other words, it may be speculated that dysregulation in the intrinsic CK2 (by increase or decrease) in normal as well as neoplastic cells manifests altered consequences in the biological response of cells. It is important to note that in the case of tumors, elevation in CK2 does not reflect their proliferation status alone, but rather the nuclear CK2 signal may additionally relate to the pathobiological status of the tumor cells [Faust et al., 1999; Tawfic et al., 2001]. The dynamics of CK2 distribution in the cytoplasm and nucleus also appears to be distinctly dependent on the nature of the cell. For example, its localization in the nucleus compared with that in the cytoplasm is much more prominent in neoplasia than in the normal cells further emphasizing its potential involvement in the nuclear function during the

neoplastic process [Yenice et al., 1994; Faust et al., 1999; Tawfic et al., 2001]. The punctate appearance of the distribution of CK2 in the immunostain and its association with protein B23 and nucleolin, which are the basis for silver staining of the nucleolar organizer region for pathological evaluation [e.g., Derenzini, 2000] can be viewed to further accord with nuclear organization which may change with altered kinase and substrate activity.

Another facet of the biological role of CK2 nuclear signaling described by us relates to its potential role in affecting the apoptotic activity in the cell which accords with its proposed role in influencing the oncogenic potential. We have demonstrated that drug-induced apoptosis evokes a rapid translocation of CK2 to the nuclear matrix. This can be interpreted in terms of the effect of CK2 in affording protection against apoptosis. Though not apparent at first, this observation indeed accords with the rapid loss of CK2 from the nuclear matrix and chromatin associated with receptor-mediated induction of apoptosis [Ahmed, 1999]; the difference in these two modes of induction of apoptosis relates to the absence or involvement of initial DNA damage. In support of this, we found that transient transfection of cells with CK2α or CK2$\alpha\beta$ expression plasmids (which produced differential enhancement of CK2 in the nuclear matrix) [Yu et al., 1998] resulted in the protection of cells against etoposide- or diethylstilbestrol-mediated apoptosis. Under these conditions, the transfection with CK2β expression plasmid did not afford such protection suggesting the specificity of this action to reside in the α subunit of CK2 [Guo et al., 2001]. Identification of this novel functional activity of CK2 has important implications for its role in the process of neoplasia, e.g., for its potential role as a prognostic marker [see e.g., Tawfic et al., 2001].

MODES OF CK2 SIGNAL TRANSDUCTION IN THE NUCLEUS

As discussed above, CK2 signaling in the nuclear matrix and chromatin is a dynamic process that responds to various stimuli. Modulations in this signal would be expected to result in an altered level of post-translational modification of various target substrate proteins in these structures, which in turn may produce structural changes in the nuclear

components. Immunohistochemical analysis of CK2 nuclear localization has indicated a punctate appearance of the immunostain with anti-CK2 antibodies [Faust et al., 1999]. This further emphasizes the presence of distinct nuclear functional domains linked to CK2 and its many substrates in the nuclear matrix and chromatin. A few of the various putative substrates for CK2 [see e.g., Guerra and Issinger, 1999; Blanquet, 2000; Tawfic et al., 2001], may be considered as the candidates for these roles. We have already mentioned phosphorylation of proteins B23 and nucleolin which is essential for their stability [Tawfic et al., 1994,1995]. This represented one of the first examples of CK2-mediated phosphorylation serving such a function. Recently, similar observations have been reported with respect to the phosphorylation of β-catenin by CK2 resulting in the stabilization of this protein, which has been implicated in Wnt signaling [Song et al., 2000]. The observations on B23 and its phosphorylation by CK2 are also germane to the role of CK2 in cell-cycle progression as it has been noted that B23 is important for cells to enter mitosis [Jian and Yung, 1999]. Other examples are the enhancement of the activity of topoisomerase II and RNA polymerases mediated by CK2-dependent phosphorylation [for references see, e.g., Guerra and Issinger, 1999; Blanquet, 2000; Tawfic et al., 2001]. Thus, it would appear that nuclear matrix and chromatin have numerous potential molecular sites where CK2 action may play a role through different mechanisms, an area which remains open for much further work.

CONCLUDING REMARKS

In the foregoing discussion we have discussed that CK2, a serine/threonine protein kinase, that is distributed in the cytoplasm and nucleus, is preferentially associated with distinct nuclear domains, especially chromatin and nuclear matrix. We propose that CK2 in the nucleus serves as a link or coordinator of the functional activity of the nuclear matrix and chromatin involved in cell growth as well as apoptosis. Further, we speculate that a dysregulation in the CK2 may produce distinct structural changes through its diverse range of substrates localized in these structures. Another potential mode for providing a link between the nucleosome and nuclear matrix

may be indicated by the observation that certain histones modulate the activity of nuclear matrix-associated CK2 activity [Tawfic et al., 1999]. These various observations lead to the possibility that these factors would produce altered nuclear structural/functional activities in neoplasia. Considerable progress has been made but further work is needed on the precise mechanism(s) of CK2 involvement in these activities along with the identification of specific molecules whose altered phosphorylation influences the structure of nuclear matrix and/or chromatin.

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