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Association of Protein Kinase CK2 With Nuclear Matrix: Influence of Method of Preparation of Nuclear Matrix

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Abstract Nuclear matrix (NM) plays roles of fundamental structural and functional significance as the site of replication, transcription, and RNA processing and transport, acting as an anchor or attachment site for a variety of enzymes and other proteins involved in these activities. We have previously documented that protein kinase CK2 translocates from the cytosol to the nucleus, where it associates preferentially with chromatin and NM, in response to certain growth stimuli. Considering that characteristics of the isolated NM can depend on the procedure employed for its isolation, we compared three standard methods for NM preparation to confirm the association of intrinsic CK2 with this structure. Our data suggest that the method used for isolating the NM can quantitatively influence the measurable NM-associated CK2. However, all three methods employed yielded qualitatively similar results with respect to the stimulus-mediated modulation of NM-associated CK2, thus further supporting the notion that NM is an important site for physiologically relevant functions of CK2. In addition, core filaments and cytoskeleton that were isolated by two of the preparative methods had a small but significant level of associated CK2 activity. J. Cell. Biochem. 64:499–504.

Key words: protein kinase CK2; nuclear matrix; cytoskeleton; chromatin; intermediate filaments; core filaments; carcinoma; prostate

Nuclear matrix (NM) is the filamentous structure that provides the internal scaffold for the nuclear architecture. Much evidence suggests that NM plays a fundamental role in a variety of nuclear activities, especially those related to gene activity and cell growth [Berezney, 1991; Getzenberg et al., 1990; Nickerson et al., 1990; Stein et al., 1996]. Thus, it is reasonable to consider that this structure would serve as the binding site for a variety of signal-transduction molecules to promote functional activities. Indeed, NM serves as a binding site for a wide range of such molecules [Berezney, 1991; Getzenberg et al., 1990; Nickerson et al., 1990; Stein et al., 1996; Pinna, 1990].

Protein kinase CK2 (also known as casein kinase 2 or II) is a multifunctional protein serine/threonine kinase that has been implicated in growth control [Pinna, 1990; Tuazon and Traugh, 1991; Issinger, 1993; Litchfield and Lüscher, 1993; Ahmed, 1994; Allende and Allende, 1995]. Although the functional regulation of CK2 is not fully understood, its spatiotemporal translocation may represent a mode of its cellular regulation in response to various

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signals. Recently, we demonstrated that NM serves as an anchor for CK2 and provided evidence that the level of NM-associated CK2 is dynamically regulated in response to a growth signal [Tawfic and Ahmed, 1994a,b]. It has been suggested that the characteristics of NM preparations can depend on the isolation procedure employed [e.g., Berezney, 1991; Getzenberg et al., 1990; Nickerson et al., 1990]. The NM employed in our previous studies [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996] was prepared by the procedures described by Fey and Penman [1988] and Payrastre et al. [1992]. However, we reasoned that if CK2 association with NM is physiologically important, it should also be found in NM isolated by other procedures. We have employed three methods for NM preparation [He et al., 1990] and have demonstrated that CK2 association is intrinsic to the NM regardless of the isolation procedure employed. Furthermore, these data reinforce the concept of growth stimulus-mediated regulation of CK2 activity associated with the NM, which was apparent in these preparations isolated by three different procedures.

METHODS

Cells and Tissues

We have employed cells in culture and a rat tissue as experimental models. The Ca9-22 cells, established from a human squamous cell carcinoma of the head and neck [Yamamoto et al., 1986], were cultured in RPMI-1640 medium supplemented with 6% fetal bovine serum, as described [Yamamoto et al., 1986]. At confluency, Ca9-22 cells were washed once in phosphate-buffered saline (PBS), and harvested by trypsinization (0.25%, in PBS), followed by two spin-washes in PBS at 4°C. All studies reported here were carried out with Ca9-22 cells between passages 30 and 60. Rat ventral prostate, studied previously with respect to CK2 association with NM of this tissue and its androgenic regulation [Tawfic and Ahmed, 1994a,b; Tawfic et al., 1996]. Glands were procured from normal Sprague-Dawley rats or from rats that had been castrated 4 days earlier, as described previously [Tawfic and Ahmed, 1994b]. The rats were purchased from Harlan-Sprague-Dawley (Indianapolis, IN) and were treated precisely as before [Tawfic and Ahmed, 1994a,b].

Preparative Methods

On the basis of the procedures described by He et al. [1990], we started with 500 mg of the prostate tissue or 2 flasks (75 cm^2) of confluent Ca9–22 cells. Prostatic tissue was minced and suspended at a concentration of about 10% (w/v) 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 complex, 1 mM phenylmethylsulfonyl fluoride (PMSF), and leupeptin 2 µg/ml. The cultured cells were suspended at 5×10^6/ml (final vol. 3 ml) in the CSK buffer. Cells were homogenized for 1 min at 720 rpm, whereas the prostate tissue was homogenized for 10 strokes at 720 rpm in a Teflon-glass Potter-Elvehjem homogenizer. Prostate homogenate was filtered through a single layer of #110 mesh monofilament nylon screen. Both types of homogenates were divided into three equal portions. All subsequent procedures were the same for the materials from both sources.

The samples were centrifuged for 5 min at 600*g* at *4*°C. Supernatant fluids were collected and centrifuged for 1 h at 100,000 × g. The pellets from this high-speed centrifugation were discarded, and the supernatant fluids were collected as the cytosol fraction. This fraction probably also contains soluble nuclear proteins, which might be extracted in the CSK buffer containing Triton X-100. The pellets from the first centrifugation step were used as the starting material for isolation of the various nuclear subfractions by the three preparative procedures [Yamamoto et al., 1986]. The details of the three methods, designated A, B, and C, as employed by us, are outlined in Figure 1.

Method A. The pellet was suspended in 2 ml of "digestion buffer," which consisted of 10 mM PIPES, pH 6.8, 50 mM NaCl, 0.3 M sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 4 mM vanadyl ribonucleoside complex, 1 mM PMSF, leupeptin 2 µg/ml, RNase A 100 µg/ml, and DNase I 100 µg/ml. This suspension was incubated for 30 min at room temperature. (Addition of vanadyl ribonucleoside complex in various media appears to improve the yield of nuclear matrix.) Subsequently, 1 M $(NH_4)_2SO_4$ was added dropwise to a final concentration of 0.25 M. The material was centrifuged for 5 min at 600*g*, and the supernatant fluid was collected as the "chromatin" fraction. The pellet was suspended in TMED buffer consisting of 50 mM Tris-HCl, pH 7.9 at 25°C, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM dithiothreitol, 200 mM NaCl, 0.5 mM PMSF, and leupeptin 1 µg/ml. This suspension was designated as the "NM."

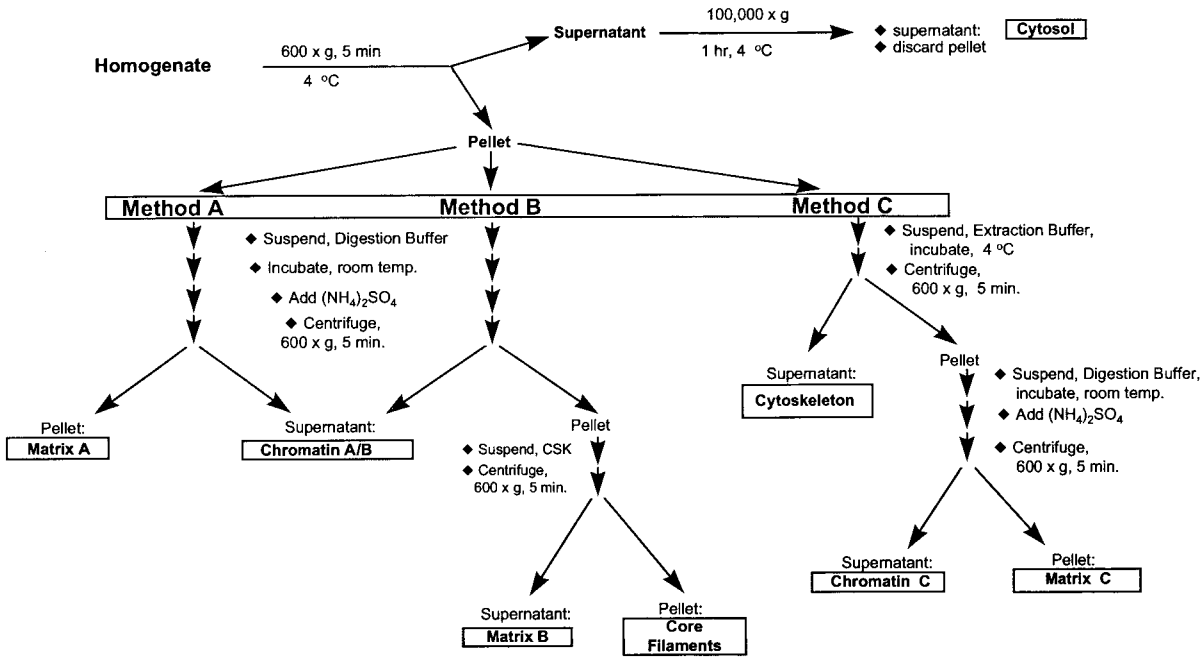

Fig. 1. Outline of the procedures employed for preparation of cellular and nuclear subfractions. The various cellular and nuclear subfractions isolated are shown in the boxes in the diagram. The details of the methods designated as A, B, and C are given in the text. Nuclear matrix prepared by method A represents NM plus intermediate filaments (NM-IF). Nuclear matrix prepared by method B represents a 2.0 M NaCl extract of NM-IF, whereas that isolated by method C represents NM plus intermediate filaments prepared after extraction of cytoskeleton (NM-IFe). The chromatin isolated by method C differs from that from methods A and B in that it was prepared after extraction of cytoskeleton.

Method B. The initial 600*g* pellet was suspended in 2 ml of the digestion buffer as described under Method A. After an incubation of 30 min at 25°C, a solution of 1 M $(NH_4)_2$ SO_4 was added to a final concentration of 0.25 M. The material was centrifuged at 600*g* for 5 min and the supernatant fluid was collected as the "chromatin" fraction. The pellet was suspended in 2 ml of the CSK buffer containing 2 M NaCl and centrifuged at 600*g* for 5 min. The supernatant fluid was collected as the NM fraction. The pellet from this step was suspended in TMED buffer and was designated the "core filaments" fraction.

Method C. The initial 600*g* pellet was suspended in 3 ml of "extraction buffer" consisting of 10 mM Tris-HCl, pH 7.4 at 25°C, 10 mM NaCl, 3 mM $MgCl_2$, 1% Tween 40, 0.5% sodium deoxycholate, 4 mM vanadyl ribonucleoside complex, 1 mM PMSF, and leupeptin 2 µg/ml. The mixture was incubated for 5 min at 4°C followed by centrifugation for 5 min at 600*g*. The supernatant fluid was collected as the "cytoskeleton" fraction. The pellet was suspended in 2 ml of digestion buffer containing RNase A 100 µg/ml and DNase I 100 µg/ml. After incubation for 30 min at 25°C, a solution of 1 M $(NH_4)_2$ SO_4 was added to a final concentration of 0.25 M. The material was centrifuged at 600*g* for 5 min, and the supernatant fluid was collected as the "chromatin" fraction. The pellet was suspended in TMED buffer, and the suspension represented the NM fraction.

Assays

All protein assays were carried out using the Bradford method with the modification described by us to counter interference by the vanadyl ribonucleoside present in the various preparations [Ahmed et al., 1996]. The dye-binding reagent was purchased from BioRad Laboratories (Hercules, CA).

All CK2 assays were carried out in a final reaction volume of 100 µl and consisted of 30 mM Tris-HCl, pH 7.45 at 37°C, 5 mM $MgCl_2$, 150 mM NaCl (except for assay of NM B), 1 mM DTT, 50 µM ATP (containing [γ-^{32}P]ATP to achieve 3×10^6 cpm/nmol of ATP), 40 mM β-glycerophosphate, 0.5 mM PMSF, leupeptin 10 µg/ml with or without 200 µM peptide sub-

strate (RRRADDSDDDDD). The characteristics of the peptide substrate for CK2 assay have been described [Marin et al., 1994]. The amounts of various sources of CK2 activity were as follows: cytosol, 5 µg/reaction; core filaments B, 5 µg/reaction; cytoskeleton C, 20 µl of undiluted sample/reaction (the protein could not be assayed in this sample because of the presence of detergent); chromatin, 2 µg/reaction; and NM, 2 µg/reaction. Under these conditions, the reaction proceeded at a linear rate for at least 20 min of incubation at 37°C. All other details of the assay and the procedure to measure the amount of ^{32}P incorporated into the peptide substrate were the same as described previously [Tawfic and Ahmed, 1994a,b]. Each experiment was repeated at least three times.

RESULTS AND DISCUSSION

The purpose of this study was to evaluate three standard cell fractionation methods for isolation of the NM fraction [He et al., 1990] in the context of our observations [Tawfic and Ahmed, 1994a,b] on the regulation of CK2 in the NM preparation [Fey and Penman, 1988]. Further, to establish the general applicability of our observations, we employed two sources of material. Initially, we examined the differences in the level of CK2 activity localized in the NM isolated from a tumor cell line (Ca9–22); these methods also yielded a few other cell and nuclear fractions that were included in this study. The results of these experiments are provided in Table I. As expected, the activity in the cytosolic fraction isolated by the three methods was identical. In accord with previous results [Tawfic et al., 1996], the presence of CK2 was consistently found in the chromatin fraction isolated by all three methods, although the activity was lower in the preparation isolated by method C. Core filaments, isolated only by method B, were replete with CK2 activity. All of the NM preparations demonstrated the presence of CK2. Methods A and B gave comparable levels of activity while preparations from method C had about half as much activity.

The data presented in Table II show the total units of CK2 recovered in various fractions prepared by the three methods. Again, as expected, the total units of CK2 in cytosol were the same for all three preparations. The activity units in chromatin isolated by methods A and B were approximately twice as great as in the preparation isolated by method C. It may be noted that method C also yielded a cytoskeletal fraction which was found to contain some CK2. The NM isolated by method A appeared to have nearly twice as many units of enzyme activity as that prepared by methods B and C. These observations on Ca9–22 cells in culture suggest that CK2 is consistently associated with chromatin and NM. In addition, they document the presence of CK2 in the cytoskeletal fraction and core filaments, which is of interest as certain proteins in these fractions also have been sug-

TABLE I. Specific Activity of Protein Kinase CK2 in Various Nuclear Fractions and Cytosol Isolated From Ca9–22 Cells Using Three Preparative Procedures*

Cell or nuclear fractions	Protein kinase CK2 activity (nmol ^{32}P/mg of protein/h)		
	Method A	Method B	Method C
Cytosol	30.2 ± 1.2	30.2 ± 1.2	30.2 ± 1.2
Chromatin	42.0 ± 1.5	43.7 ± 2.0	8.6 ± 0.3
Core filaments	[a]	90.0 ± 2.1	[a]
Cytoskeleton	[a]	[a]	[b]
Nuclear matrix	116.5 ± 8.7	144.3 ± 1.2	62.0 ± 3.6

*Values are shown as mean ± SEM. The characteristics of the NM fraction from each method were as follows: the NM from method A represents matrix plus intermediate filaments (NM-IF); that from method B represents a 2M NaCl extract of NM-IF; NM from method C represents NM plus intermediate filaments prepared after extraction of cytoskeleton (NM-IFe). Chromatin from method C differs from that obtained by methods A and B in that it was prepared after extraction of cytoskeleton.

[a]The method does not yield this preparation.

[b]CK2 activity was present in the fraction but could not be quantitated because of the difficulty of measuring protein in the presence of detergent. (However, see Table II for the amount of CK2 present in this fraction).

TABLE II. Total Units of Protein Kinase CK2 Recovered in Various Nuclear Fractions and Cytosol Isolated From Ca9–22 Cells Using Three Preparative Procedures*

Cell or nuclear fraction	Protein kinase CK2 activity (nmol ^{32}P/h in total fraction)		
	Method A	Method B	Method C
Cytosol	86.1 ± 3.4	86.1 ± 3.4	86.1 ± 3.4
Chromatin	18.7 ± 0.7	19.8 ± 0.9	8.7 ± 0.3
Core filaments	[a]	34.4 ± 0.8	[a]
Cytoskeleton	[a]	[a]	12.7 ± 0.5
Nuclear matrix	40.7 ± 3.0	16.4 ± 0.4	20.8 ± 1.2

*Values are shown as mean ± SEM.

[a]The particular fraction is not isolated by the procedure. Total units were calculated based on a sample of 5×10^6 cells. The characteristics of the NM fractions isolated by the three methods and those of chromatin are as described in Table I.

gested as putative substrates for CK2 [Berezney, 1991; Getzenberg et al., 1990; Nickerson et al., 1990; Stein et al., 1996; Pinna, 1990]. It is also noteworthy that the NM preparations isolated by all the three methods employed here [He et al., 1990] contained the intermediary filaments, and in this regard, the NM used in this study differed from those employed by us previously [Fey and Penman, 1988], in that the latter were free of intermediary filaments [Tawfic and Ahmed, 1994a,b]. Taken together, our results clearly show that CK2 is associated with the NM in the presence or absence of intermediate filaments.

We previously reported that NM-associated CK2 is modulated depending on the growth stimulus [Tawfic and Ahmed, 1994b]. Thus, having observed the above-described results using the Ca9–22 cell line, we decided to evaluate changes in CK2 activity associated with NM from rat ventral prostate in response to altered androgenic status (Tables III and IV). The results in Table III document the presence of CK2 activity in various cellular and nuclear fractions from prostatic tissue from normal and 4-day castrated rats. In accord with our previous observations, androgen deprivation resulted in differential loss of CK2 activity in various fractions. However, the loss of activity in chromatin isolated by the procedures followed here was not as extensive as described previously for chromatin prepared by our conventional procedure [e.g., Ahmed et al., 1993]. The loss of activity associated with the core filaments was relatively modest. The activity associated with the NM fraction was similar for the preparations isolated by methods A and B but was somewhat lower in NM prepared by method C. However, the CK2 activity in the NM from method C demonstrated the largest decrease in response to androgen deprivation. The results in Table IV show the total CK2 units in each fraction that is listed in Table III. For chromatin, method C yielded the lowest number of units. The core filaments (method B) had a small number of units, and activity was further reduced in tissue from androgen-deprived animals. Also, the cytoskeleton (method C) had a similar number of CK2 units present, which declined on androgen deprivation. The units in the NM fractions isolated by these procedures showed varied recovery of the associated CK2, and the extent of the response to androgen deprivation was somewhat different in the three preparations. The loss was maximal in NM prepared by method C and comparable to that observed previously [Tawfic and Ahmed, 1994b].

The results presented here document that units of CK2 activity associated with chromatin and NM may vary depending on the preparative method employed. This difference is understandable, as there are significant differences in the various treatments of the tissue (e.g., salt and detergents) that could result in variable removal of associated CK2. For example, treatment with salts can result in gradual removal of nuclear CK2, depending on the concentration of the salt [Goueli and Ahmed, 1983]. Thus, taking into consideration that even though there are quantitative differences in the amount or activity of CK2 in the variously isolated NM preparations, none of the preparations was devoid of kinase activity. Furthermore, the activity of CK2 associated with all these preparations was modulated in response to altered growth stimulus, thus reiterating the possible

TABLE III. Specific Activity of Protein Kinase CK2 in Various Nuclear Fractions and Cytosol Isolated From Ventral Prostate of Normal and 4-Day Castrated Rats Using Three Preparative Procedures*

Cell or nuclear fraction	Protein kinase CK2 activity (nmol ^{32}P/mg of protein/h)					
	Method A		Method B		Method C	
	Normal	Castrate	Normal	Castrate	Normal	Castrate
Cytosol	23.5 ± 1.3	12.7 ± 1.6	23.5 ± 1.3	12.7 ± 1.6	23.5 ± 1.3	12.7 ± 1.6
Chromatin	15.9 ± 1.0	7.5 ± 0.4	13.7 ± 1.0	7.0 ± 0.4	6.2 ± 0.2	2.8 ± 0.4
Core filaments	[a]	[a]	9.2 ± 1.0	7.3 ± 0.4	[a]	[a]
Cytoskeleton	[a]	[a]	[a]	[a]	[b]	[b]
Nuclear matrix	54.0 ± 3.0	26.9 ± 1.0	60.9 ± 5.9	35.9 ± 4.9	32.3 ± 1.6	7.6 ± 1.5

*Values are shown as mean ± SEM. The characteristics of the NM fractions isolated by three methods and those of chromatin are as described in Table I.
[a,b]As in Table I.

TABLE IV. Total Units of Protein Kinase CK2 Recovered in Various Nuclear Fractions and Cytosol Isolated From Ventral Prostate of Normal and 4-Day Castrated Rats Using Three Preparative Procedures*

Cell or nuclear fraction	Protein kinase CK2 activity (nmol ^{32}P/h in total fraction)					
	Method A		Method B		Method C	
	Normal	Castrate	Normal	Castrate	Normal	Castrate
Cytosol	324 ± 18	139 ± 17	324 ± 18	139 ± 17	324 ± 18	139 ± 17
Chromatin	39.4 ± 2.3	8.0 ± 0.4	34.7 ± 2.6	8.9 ± 0.5	9.9 ± 0.2	3.0 ± 0.4
Core filaments	[a]	[a]	5.8 ± 0.7	3.1 ± 0.2	[a]	[a]
Cytoskeleton	[a]	[a]	[a]	[a]	5.8 ± 0.6	2.3 ± 0.4
Nuclear matrix	22.0 ± 1.0	12.0 ± 0.4	8.8 ± 0.8	3.8 ± 0.5	31.3 ± 0.5	5.8 ± 1.2

*Values are shown as mean ± SEM. Total units were calculated based on the prostate sample of 167 mg. The characteristics of the NM fractions isolated by the three methods and those of chromatin are as described in Table I.
[a]As described in Table I.

physiological relevance of NM-associated protein kinase CK2.

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