Protein kinase CK2 has been implicated in control of cell growth and proliferation. Since growth stimuli evoke its preferential association with chromatin and nuclear matrix, we examined the dynamics of CK2 in nucleosomes fractionated on the basis of their transcriptional activity in the rat prostate. In this model, androgens induce expression of androgen-dependent genes but inhibit the androgen-repressed genes, whereas absence of androgens has the reverse effect. The level of CK2 was higher in the active than in inactive nucleosomes from normal prostate. Differential alterations in the levels of CK2 activity in the transcriptionally active versus inactive nucleosomes were evoked by androgen deprivation or administration. Comparison of the distribution of CK2 in active and inactive nucleosomes under varying androgenic conditions showed that 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 but increased rapidly on androgen administration (reflecting expression of multiple androgen-dependent genes). We suggest a role for CK2 in promoting the conformational transition of inactive nucleosomes to the active form and in the function of transcriptionally active nucleosomes.

CK2 (formerly known as casein kinase 2 or II) is a ubiquitous protein serine/threonine kinase that has been implicated in multiple cellular functions, including growth control and proliferation. The enzyme is a tetramer consisting of α, α', and β subunits (42, 38, and 28 kDa, respectively) with the possible α2β2, αα'β2, or αα'β2 configuration. There appear to be several substrates for CK2 in both the cytoplasm and the nucleus. Among the putative nuclear substrates are RNA polymerases, topoisomerase II, protein B23, nucleolin, SV40 large T antigen, certain protooncogene products, and growth factors, as well as certain chromosomal nonhistone proteins (1–6). The involvement of CK2 in phosphorylation of these growth-related proteins has provided additional support for its role in growth control.

CK2 appears to be dynamically regulated with respect to its nuclear localization (7, 8) which is manifested by its preferential association with chromatin and nuclear matrix (7, 9, 10). The association of CK2 with these compartments is modulated in response to growth stimuli (7, 9–12) which is of interest since both chromatin and nuclear matrix play fundamental roles in genomic activity and cell proliferation. Nuclear matrix, the structural framework that represents residual components of the nuclear lamina-pore complex, nucleoli, and fibrogranular internal matrix, is believed to play a fundamental role in chromatin organization, regulation of gene activity, and cell proliferation (13–17). The transcriptional machinery resides in chromatin, and the nucleosome is its repeating subunit structure. As might be expected, nucleosomes appear to undergo dynamic conformational transition from inactive to active forms under specific conditions (for reviews see Refs. 18 and 19).

Recent studies have shown that transcriptionally active and inactive nucleosomes can be fractionated on the basis of their conformation, as reflected by the accessibility of thiol groups in the histone H3 (20, 21). This procedure has been successfully employed to fractionate nucleosomes from yeast, 3T3, HeLa S3, COLO 320, and rat liver cells (20, 21). Considering the dynamic association of CK2 with the nuclear matrix and chromatin (9, 11), we decided to examine the role CK2 plays in the nucleosomes in relation to their transcriptional activity by employing the experimental model of androgenic regulation of the prostate. Withdrawal of androgen from adult rats results in apoptosis in the prostate epithelial cells, whereas androgen administration to castrated rats evokes a regrowth of the gland with activation of genomic activity within 1–4 h (22–24). An interesting feature of this model is that during regression of the gland (and overall cessation of the androgen-dependent gene activity), certain genes called androgen-repressed genes (e.g. clusterin) are activated. On the other hand, androgenic stimulation evokes early expression of genes such as C3 and, over time, of a large number of prostatic androgen-dependent genes, whereas the androgen-repressed genes are suppressed (25–27). These markers can thus be used to differentiate the type of nucleosomes isolated from prostatic tissue under varied androgenic conditions. We have used this paradigm previously to demonstrate that CK2 is preferentially associated with the chromatin and nuclear matrix fractions isolated from purified nuclei and that this association is dynamically regulated in a spatiotemporal manner in response to altered androgenic status (7, 9, 11, 12). In the present work, we have documented that CK2 is associated to a greater extent with the transcriptionally active than the inactive nucleosomes. However, androgenic alterations in the transcriptional activity of the prostate evoke a differential regulation of CK2 associated with the active and inactive nucleosomes. The envelope of CK2 in phosphorylation of these growth-related proteins has provided additional support for its role in growth control.
inactive nucleosomes suggesting a possible role for CK2 in nucleosome organization and function.

**EXPERIMENTAL PROCEDURES**

**Materials**

*Animals—Male Sprague-Dawley rats weighing 295–325 g (Harlan Sprague-Dawley, Indianapolis, IN) were used as the source of ventral prostate or liver. The animals were maintained under standard conditions and were orchietomized via the scrotal route under Metofane anesthesia as described previously (9). For restoration of androgens, animals were injected with 5α-DHT in sesame oil (1 mg/100 g of body weight); control animals received an appropriate volume of the vehicle alone.*

*Chemicals—Synthetic dodecapeptide substrate (Arg-Arg-Arg-Ala-Asp-Asp-Ser-Asp-Asp-Asp-Asp) for assay of CK2 activity was purchased from Peptide Technologies Corp. (Gaithersburg, MD). Affi-Gel 501 was purchased from Bio-Rad. Micrococcal nuclease was supplied by Worthington. Mouse monoclonal antibody raised against the last 262 amino acids of the C-terminal end of the CK2-α was from Transduction Laboratories (Lexington, KY). All reagents were of the highest purity available.*

**Methods**

**Preparation of Nucleosomes—**Nuclei were isolated from pooled ven- tral prostate tissue (15 prostate glands from normal, 15 from 2-day castrated, or 45 from 6-day castrated rats) or from liver as described previously (9–11) except that 5 mM sodium butyrate, 0.1 mM EPNP, and 0.5 mM PMSF were included throughout the preparative procedure. DNA content was assayed by measuring the A₄₅₀ or by the Burton method (28), as appropriate. Nucleosomes were isolated from purified nuclei by adapting previously described protocols (9–11). Briefly, purified nuclei were suspended at a concentration of 1 mg of DNA/ml in buffer A (containing of 10 mM Tris-HCl, pH 7.4, 25 mM KCl, 25 mM NaCl, 0.5 mM CaCl₂, 5 mM MgCl₂, 5 mM sodium butyrate, 0.1 mM EPNP, and 0.5 mM PMSF). This suspension was warmed at 37 °C for 2 min followed by addition of micrococcal nuclease at a final concentration of 10 units/ml. The nuclease digestion was carried out for 5 min at 37 °C and was terminated by the addition of 0.2 M EGTA, pH 7.0, to a final concentration of 2 mM. These conditions were established so as to strictly ensure that 10–11% of the DNA was digested. After the tubes stood on ice for 10 min, the samples were centrifuged at 10,000 × g for 20 min. The supernatant phase containing the released nucleosomes was collected.

**Fractionation of Active and Inactive Nucleosomes—**The supernatant fraction was treated with 0.2 M EDTA to a final concentration of 5 mM. The sample was then loaded onto an organismcommercial agarose column (Affi-Gel 501) equilibrated with buffer B (10 mM Tris-HCl, pH 7.4, 25 mM KCl, 25 mM NaCl, 5 mM EDTA, 5 mM sodium butyrate, 0.1 mM EPNP, and 0.5 mM PMSF). To remove the unbound nucleosomes, the column was eluted at a flow rate of 20 ml/h with buffer B until A₄₅₀ reached the baseline fraction. This fraction represented the transcriptionally inactive nucleosomes. The organismcommercial-bound nucleosomes were then eluted with buffer C (buffer B with 0.5 mM NaCl). Column fractions were monitored for A₄₅₀ to identify the peak. Fractions corresponding to the peak were pooled. Finally, elution with buffer C containing 10 mM DTT was used to isolate the bulk of transcriptionally active nucleosomes. The pooled fractions from various peaks were concentrated with the aid of a Centricon 10 (Amicon, Inc., Beverly, MA) and then washed twice with 10 volumes of TMED buffer (50 mM Tris-HCl, 5 mM MgCl₂, 1 mM EDTA, and 0.5 mM DTT, pH 7.9) containing 0.5 mM NaCl. The final protein and DNA concentration were determined as described previously (28, 29).

Sepharcl S-300 Superfine (Amersham Pharmacia Biotech) column chromatography was carried out to determine if there was a contaminant of free CK2 in the nucleosomal preparations. The resin was equilibrated in buffer B in a 0.9 × 12 cm column, and the nucleosomal sample was loaded in a volume of 200 μl in buffer B. The elution was carried out with buffer B at a flow rate of 8 ml/h, and 2-ml fractions were collected and analyzed for A₄₂₀ and CK2 activity.

**Assay of CK2 Activity in Nucleosomal Preparations—**Assay of CK2 activity in various nucleosomal preparations was carried out by employing a synthetic dodecapeptide (30). The reaction mixture consisted of 30 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 150 mM NaCl, 1 mM EDTA, 0.5 mM PMSF, 50 mM 2-glycerophosphate, 0.2 mM dodecapeptide substrate, 0.05 mM [γ-²²P]ATP (specific radioactivity 3 × 10⁷ dpm/nmol of ATP). The reaction was started by the addition of the enzyme source (e.g. various nucleosome preparations). A paper-binding method was used to assay the [²²P] incorporated into the dodecapeptide substrate (9–11, 31).

All assays of CK2 were carried out in triplicate over a time course to ensure the linearity of the reaction. Each experiment was repeated at least three times.

Immunoblot for the CK2-α were carried out by standard procedures. In brief, nucleosomal protein (20 μg) was subjected to electrophoretic separation on a 10% polyacrylamide gel containing SDS and urea. The proteins were transferred to a nitrocellulose sheet and treated with the mouse monoclonal IgM anti-CK2-α antibody. The secondary antibody was alkaline phosphatase-conjugated goat anti-mouse IgM polyclonal from Boehringer Mannheim. Antibody detection was carried out using the standard nitro blue tetrazolium and 5-bromo-4-chloro-indolyl phosphate method (32). A paper-binding method was used to determine protein kinase CK2 activity.

**RESULTS**

**Characteristics of Nucleosomes from Rat Ventral Prostatic Tissue—**Fig. 1 shows the chromatographic profile of the active and inactive nucleosomes isolated from rat ventral prostate nuclei by utilizing the Affi-Gel 501 column fractionation. The first peak (flow-through) represents the transcriptionally inactive nucleosomes, whereas the second large peak represents the transcriptionally active nucleosomes eluted with 10 mM DTT (Fig. 1). Unlike the previous experience (20, 21), elution with 0.5 M NaCl yielded only a negligible peak and was not investigated further. A somewhat larger peak was observed with NaCl elution for liver nucleosomes (result not shown). The inset shows analysis of the DNA pattern of the fractionated nucleosomes as determined by electrophoretic separation of DNA on a 1.6% agarose gel followed by ethidium bromide staining. As expected from previous observations (20, 21), the inactive and active nucleosomes yielded a similar profile. Because our studies involved analyses of the prostatic tissue from rats subjected to altered androgenic status, we also established that isolation profiles of the inactive and active nucleosomes were similar from animals castrated for 48 or 144 h, or 144 h and then treated with 5α-DHT for 4 h (not shown).

The transcriptionally inactive and active nucleosomes from normal and 48-h castrated rats were characterized by probing for sequence abundance of C3 (Fig. 2A) and clusterin (Fig. 2B) genes. As shown, clusterin gene sequences were detected on the inactive nucleosomes from normal rats and on the active nucleosomes from castrated rats. The C3 gene sequences were detected only on the active nucleosomes of normal rat prostate and on the inactive nucleosomes from castrated rats. Thus, the nucleosome preparations from prostatic tissue of rats under
Pooled nucleosomes corresponding to the peaks designated as electrophoretic analysis of DNA (stained with ethidium bromide) of lane e in total nucleosomes, and tral prostate nuclei.

Typically inactive and active nucleosomes isolated from rat ven-
coccal nuclease to allow 11% DNA digestion. The released
ucleosomes were fractionated on an organomercurial-agarose column (Affi-Gel 501) as described under “Experimental Procedures.” The un-
bound fraction (first large peak designated a) represents the transcription-
ally inactive nucleosomes. The nucleosomes bound to the column were eluted in two steps as follows: a minor peak (b) was eluted with NaCl, and the second large peak (c) eluted with DTT represents the transcriptionally active nucleosomes. Peaks were identified by monitor-
ing the A_{260}. The lanes a, b, and c in the inset show the agarose gel electrophoretic analysis of DNA (stained with ethidium bromide) of pooled nucleosomes corresponding to the peaks designated as a, b, and c. Lane d in the inset represents the gel electrophoretic pattern of DNA in total nucleosomes, and lane e shows the DNA standards.

**FIG. 1. A representative chromatographic profile of transcriptionally inactive and active nucleosomes isolated from rat ventral prostate nuclei.** Purified prostate nuclei were digested with micrococcal nuclease to allow 11% DNA digestion. The released nucleosomes were fractionated on an organomercurial-agarose column (Affi-Gel 501) as described under “Experimental Procedures.” The un-
bound fraction (first large peak designated a) represents the transcription-
ally inactive nucleosomes. The nucleosomes bound to the column were eluted in two steps as follows: a minor peak (b) was eluted with NaCl, and the second large peak (c) eluted with DTT represents the transcriptionally active nucleosomes. Peaks were identified by monitor-
ing the A_{260}. The lanes a, b, and c in the inset show the agarose gel electrophoretic analysis of DNA (stained with ethidium bromide) of pooled nucleosomes corresponding to the peaks designated as a, b, and c. Lane d in the inset represents the gel electrophoretic pattern of DNA in total nucleosomes, and lane e shows the DNA standards.

**FIG. 2. Identification of transcriptionally active and inactive nucleosomes by analysis of the androgen-regulated tissue-specific expression of the C3 and clusterin sequences.** DNA (20 μg) from each fraction was slot-blotted on nylon membrane and hybridized with 32P-labeled cDNA probes for C3 (A) and clusterin (B). Lane a, nucleosome DNA from normal rat prostate; lane b, nucleosomes from 48-h castrated rat prostate. Lane 1, genomic DNA; lane 2, DNA from inactive nucleosomes; lane 3, DNA from active nucleosomes. In normal rat prostate, C3 sequences were detected abundantly on active nucleo-
somes, whereas most clusterin sequences were detected on inactive nucleosomes. In castrated rat prostates, C3 gene expression is sup-
pressed by androgen withdrawal resulting in the presence of many more C3 sequences in inactive nucleosomes; clusterin gene expression is activated by androgen withdrawal, and therefore most clusterin sequences were detected in active nucleosomes.

different androgenic conditions (i.e. with varied gene activities) reflect tissue-specific characteristics of transcriptional activity.

**Association of Protein Kinase CK2 with the Nucleosomes—**In previous work, we have shown the translocation of CK2 to the

chromatin of rat prostate in response to androgenic stimulus (9). We therefore decided to examine the nature of CK2 assoc-
iation with the transcriptionally inactive and active nucleo-
somes isolated from rat prostate. As expected, the amount of DNA in the inactive nucleosome fraction was 2–3 times greater than that in the active nucleosomes. Associated CK2 activity was detected in nucleosomes (inactive as well as active) iso-
lated from normal prostate and liver (Table I), but a severalfold greater amount of CK2 was present in the transcriptionally active than in the inactive nucleosomes. The results are similar when calculated per unit of the DNA or protein in each nucleo-
some preparation.

Control experiments were undertaken to establish that CK2 activity measured in the inactive and active nucleosomes did not reflect artifacts due to the presence of free CK2 (i.e. that which may not be intrinsic to the inactive and active nucleo-
somes). First, a purified sample of CK2 was subjected to col-
umn chromatography identical to that for nucleosome fraction-
ation. The results in Fig. 3a show that part of this CK2 was eluted in the flow-through fraction (peak a) and that the re-
maining activity was eluted by 0.5 M NaCl (peak b); the activity corresponding to the peak eluted by DTT (peak c) was negligi-
able. This result would suggest that if free CK2 were present in the unfractinated nucleosomal preparation, it would not be eluted with the active nucleosome fraction (eluted by DTT). However, CK2 measured in the inactive nucleosome fraction would be unreliable if free CK2 were present in the sample prior to fractionation.

To address the above issue, we subjected the unfractinated nucleosomal preparation to Sephacryl S-300 column chroma-
tography. Any free CK2 released during the deoxyribonucleos tone treatment of nuclei (maximal 11% digestion of DNA) would be se-
parated from the nucleosomes by the method. As shown in Fig. 3b, the elution profile indicates a single sharp peak of CK2 activity which corresponds precisely to the nucleosomal peak. The total CK2 activity associated with these nucleosomes prior to chromatography was 13.2 ± 0.3 units and that recovered in the nucleosomal peak was 13.6 ± 0.3 units. This indicates an absence of free CK2 in the nucleosomal preparations that were employed to isolate inactive and active nucleosomes. A com-
parison of the recovery of protein, DNA, and CK2 activity in the inactive and active nucleosomes on Affi-Gel 501 column chroma-
tography of representative samples, with and without prior Sephacryl S-300 chromatography, gave identical results (not shown). Thus, the CK2 activity measured in the isolated active and inactive nucleosome fractions represents the activity in-
trinsic to these fractions.

**Protein Kinase CK2 in Nucleosomes from Prostatic Tissue of**

| Tissue      | Nucleosome fraction | DNA (μg) | CK2 activity (units/mg DNA) | CK2 activity (units/mg DNA) |
|-------------|---------------------|----------|----------------------------|----------------------------|
| Liver       | Inactive            | 150 ± 6  | 2.10 ± 0.26                | 13.9 ± 1.7                 |
|             | Active              | 70 ± 7   | 5.68 ± 0.46                | 81.3 ± 6.6                 |
| Prostate    | Inactive            | 197 ± 25 | 2.41 ± 0.25                | 12.3 ± 1.3                 |
|             | Active              | 75 ± 8   | 3.22 ± 0.23                | 42.8 ± 3.0                 |

**TABLE I** Distribution of DNA and protein kinase CK2 activity in transcriptionally active and inactive nucleosomes isolated from rat liver and ventral prostate.

Transcriptionally active and inactive nucleosomes were isolated from rat liver nuclei (equivalent to 2.4 μg of DNA) and ventral prostate nuclei (equivalent to 2.9 μg of DNA) as described under “Experimental Procedures.” The activity of CK2 in each fraction was determined using the specific peptide substrate. CK2 activity units are defined as micro-
moles of 32P/h. The CK2 activity (total units) refers to the total units in

![Image](https://via.placeholder.com/150)
such as clusterin. This response is followed within 8 h by the expression of bulk mRNA in a target tissue-dependent manner. We, therefore, examined the changes in CK2 associated with the inactive and active nucleosomes in response to early changes in transcriptional activity evoked by androgen within 4 h. The results in Table II show that there was a significant increase in CK2 association with the active and inactive nucleosomes within 4 h after testosterone administration to 144-h castrated rats. Interestingly, there was a hint of a greater rate of increase in CK2 associated with the inactive nucleosomes (Table II).

Immunoblot analysis of CK2 associated with inactive and active nucleosomes corresponding to the data shown in Table II is presented in Fig. 4. The immunoreactive CK2-α in the inactive nucleosomes is significantly lower than in the active nucleosomes (Fig. 4, lanes a and b). At 48 h post-castration, the amount of CK2-α in the inactive and active nucleosomes is reduced, but it is relatively higher in the active nucleosomes (Fig. 4, lanes c and d). At 144 h post-castration, CK2-α is essentially undetectable in the inactive nucleosomes, whereas it persists in the active nucleosome fraction (Fig. 4, lanes e and f). Administration of 5α-DHT to 144-h castrated rats results in a significant increase in the inactive and active nucleosomes (Fig. 4, lanes g and h), although the rate of increase in the inactive nucleosomes would appear to be much greater (lane e compared with lane g and lane f compared with lane h). These results suggest that changes in the CK2 activity in the inactive and active nucleosomes with altered transcriptional activity reflect the changes in the immunoreactive enzyme protein.

A comparison of the distribution of CK2 present in the active and inactive nucleosome fractions as a percent of that in the total nucleosomal fraction corresponding to each treatment is shown in Table III. A higher percentage of CK2 is consistently localized in the active nucleosomes and appears to be relatively stable whatever the androgenic status. This accords with the fact that distinct transcriptional activity continues in the active nucleosomes of prostate epithelial cells in the absence as well as the presence of androgen. Under the same conditions, the percentage of CK2 in the inactive nucleosomes undergoes dramatic modulations in an androgen-dependent manner corresponding to altered transcriptional activity. Upon androgen withdrawal, the proportional amount of CK2 in the inactive nucleosomes declined precipitously but increased rapidly in response to a single injection of testosterone administered to 144-h castrated rats.

**DISCUSSION**

Chromatin is the site of transcriptional activity in the nucleus. Expression of genes in a cell is a dynamic and highly regulated process that is constantly modulated by the influence of external and internal signals. Thus, it is reasonable that the subunit structure of chromatin called the nucleosome would be subject to dynamic conformational changes in accord with the transcriptional activity of the genome (e.g., see Refs. 17–19). Investigation of the mechanisms underlying the conformational changes that define the differences in the functional activities of the inactive and active nucleosomes is therefore of intense current interest. To this end, methods have recently been developed to fractionate the nucleosomes on the basis of their transcriptional activity (20, 21). Because CK2 has been implicated in the regulation of cell growth and proliferation, we have employed these methods to investigate the dynamics of the CK2 signal in the transcriptionally active and inactive nucleosomes from rat ventral prostate in which genomic activity can be modulated by altering the androgenic status of the animal. In our previous studies employing this experimental model, we documented that CK2 rapidly associates with chro-
CK2 activity intrinsic to the fractionated inactive and active nucleosomes isolated from the ventral prostate of animals following androgen deprivation for 48 h (C48) or for 144 h (C144) or 4 h after a single dose of androgens to 144-h castrated animals (C144 T4). The details of androgen deprivation and administration are the same as described under “Experimental Procedures.” Total DNA and protein intrinsic to the inactive and active nucleosomes are also given for each experimental condition. The values are expressed as mean ± S.E.

| Treatment | Nucleosome fraction | DNA (μg) | Protein (μg) | CK2 activity (nmol 32P/mg DNA/h) | CK2 activity (nmol 32P/mg protein/h) |
|-----------|---------------------|----------|-------------|----------------------------------|-------------------------------------|
| Control   | Inactive            | 196.6 ± 14.2 | 324.8 ± 4.2 | 12.3 ± 0.7                      | 7.4 ± 0.4                           |
|           | Active              | 75.3 ± 4.8    | 205.9 ± 0.6 | 42.8 ± 2.0                      | 15.7 ± 0.7                          |
| C48       | Inactive            | 148.7 ± 9.1   | 249.7 ± 3.0 | 0.9 ± 0.2                       | 0.5 ± 0.1                           |
|           | Active              | 66.0 ± 4.2    | 157.3 ± 8.3 | 9.8 ± 0.7                       | 4.1 ± 0.3                           |
| C144      | Inactive            | 187.9 ± 11.1  | 303.5 ± 9.3 | 0.2 ± 0.1                       | 0.1 ± 0.1                           |
|           | Active              | 70.1 ± 3.8    | 196.9 ± 6.6 | 5.4 ± 0.2                       | 1.9 ± 0.1                           |
| C144 T4   | Inactive            | 166.9 ± 11.1  | 285.4 ± 4.0 | 3.1 ± 0.2                       | 1.8 ± 0.1                           |
|           | Active              | 63.9 ± 4.2    | 160.3 ± 1.0 | 15.3 ± 0.8                      | 6.1 ± 0.3                           |

Fig. 4. Immunoblot analysis of catalytic subunit of CK2 (CK2-α) intrinsic to transcriptionally active and inactive nucleosomes from prostatic chromatin of rats with varied androgenic status. Equal amount (20 μg) of nucleosomal protein was subjected to gel electrophoresis, and the proteins were transferred to nitrocellulose sheet and immunostained with anti-CK2-α antibody as described under “Experimental Procedures.” Lanes a, c, e, and g represent inactive nucleosomes. Lanes b, d, f, and h represent active nucleosomes. Lanes a and b, nucleosomes from normal prostate; lanes c and d, nucleosomes from prostates of 48-h castrated rats; lanes e and f, nucleosomes from prostates of 144-h castrated rats; lanes g and h, nucleosomes from prostates of 144-h castrated rats treated with a single dose of 5α-DHT for 4 h. The arrow indicates the location of the CK2-α subunit of kinase.

TABLE III
Percent distribution of nucleosomal associated CK2 in the transcriptionally active and inactive nucleosome fractions

CK2 activity intrinsic to the total nucleosome fraction prior to fractionation was assayed as described under “Experimental Procedures.” The activity recovered in the inactive and active nucleosomes was also determined and expressed as percent of that in the total nucleosomal fraction. All other details were the same as described in the legend to Table II. The values are expressed as mean ± S.E.

| Treatment | Percent of total nucleosomal CK2 activity in | Inactive nucleosomes | Active nucleosomes |
|-----------|---------------------------------------------|----------------------|--------------------|
| Control   | 27.2 ± 1.6 | 72.8 ± 3.4 |
| C48       | 9.8 ± 1.8  | 90.2 ± 6.8 |
| C144      | 5.0 ± 2.7  | 95.0 ± 3.8 |
| C144 T4   | 19.8 ± 1.1 | 80.2 ± 4.0 |

matin and nuclear matrix in response to the androgenic growth stimulus (9–12). We have now demonstrated that differential association of CK2 occurs in the nucleosomes in normal cells such that it is present to a much greater extent in the transcriptionally active than the inactive nucleosomes. However, dynamic changes occur in the CK2 associated with these nucleosomes when transcriptional activity in the chromatin is altered, such as by changing androgen levels (i.e., by castration or administration of androgen to castrated rats). The observed dynamics of CK2 alterations in this experimental model must be considered in the context of the temporal androgenic response of prostatic epithelial cells, as discussed subsequently.

Androgen deprivation in the adult rat leads to a cascade of effects culminating in programmed death (apoptosis) of more than 80% of the prostatic epithelial cells within 5–7 days (27). However, the earliest response of the cells is the cessation of expression of a large variety of the androgen-dependent genes. Concomitantly, there is a specific rapid expression of androgen-repressed genes such as the clusterin gene (25–27). Thus, even during the tissue regression phase, it is possible to isolate both the transcriptionally active and inactive nucleosomes from chromatin. On the other hand, administration of androgen to 6-day castrated animals evokes regrowth of the prostate. The earliest events are an expression of androgen-dependent messages, including the C3 gene (within 4 h after androgen administration), followed by expression of bulk mRNA (i.e., expression of many genes) and protein synthesis. Under these conditions, the androgen-repressed genes are not expressed. In this case, the active nucleosomes would represent the fraction rich in sequences not being expressed (such as clusterin), whereas the active nucleosomes would represent the fraction expressing androgen-dependent genes such as C3.

A number of observations emerge from this study which suggest distinct roles for CK2 in the active and inactive nucleosomes. It appears that CK2 activity is high in the transcriptionally active nucleosomes compared with the inactive nucleosomes under the various tissue-specific changes in the transcriptional activity in the prostate. The proportionally high level of CK2 activity in transcriptionally active nucleosomes (compared with that in inactive nucleosomes) accords with a role in the control of gene activity. On androgen deprivation, CK2 associated with the active nucleosomes declines relatively slowly and remains proportionally high compared with that in the inactive nucleosomes. This finding suggests that CK2 association is required for the function of active nucleosomes.

Equally remarkable is the significant amount of CK2 activity in the transcriptionally inactive nucleosomes in normal prostate, and its dramatic modulation with altered growth conditions which suggests a distinct role for CK2 in this fraction. During the induction of changes in the prostate by androgen withdrawal, the level of CK2 in the inactive nucleosomes declines rapidly so that at 144 h, only a minimal amount is detectable in these nucleosomes. This loss of CK2 associated with inactive nucleosomes is commensurate with the cessation of androgen-dependent gene activity. However, on administration of androgen to castrated rats which initiates extensive transcriptional activity, the level of CK2 localized to the inactive nucleosomes increases at a rapid rate. An interpretation of this observation is that rapid association of CK2 with the inactive nucleosomes may be needed to evoke their transition from the inactive to active conformation. This is supported by the fact that during androgen deprivation most of the gene activity is shut down, precluding the need for a transition of inactive nucleosomes to the transcriptionally active conforma-
tion. On the other hand, androgenic stimulus evokes the expression of numerous genes in the prostate over a sustained period (e.g. see Ref. 32), thus necessitating the generation of transcriptionally active nucleosomes. The increasing association of CK2 observed in this experimental model thus hints at a possible role for CK2 in the conformational transition of the nucleosomes from transcriptionally inactive to transcriptionally active state.

Future work will determine the nature of protein substrates for CK2 in the active and inactive nucleosomes. However, CK2-mediated phosphorylation of proteins such as B23 and nucleolin which are known to be involved in the rRNA synthesis has been documented (33, 34). Also of interest are the observations on the association of CK2 with several growth-related nuclear proteins and transcription factors (35–41) which may participate in mediating the CK2 signal in the nucleus. The present work provides further evidence that translocation of CK2 to its sites of action may serve as a mechanism of its intracellular regulation.

In summary, these studies have demonstrated that CK2, a nuclear protein kinase signal that has been implicated in growth control, is differentially associated with transcriptionally active and inactive nucleosomes and that this association is dynamically regulated in response to altered transcriptional activity. Furthermore, it appears that association of CK2 with the transcriptionally inactive nucleosomes may participate in promoting their transition to the active conformation.

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