Role of Protein Phosphorylation in Post-translational Regulation of Protein B23 during Programmed Cell Death in the Prostate Gland*

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Sherif Tawfic‡, Mark O. J. Olson§, and Khalil Ahmed§

From the Cellular and Molecular Biochemistry Research Laboratory (151) and the Department of Laboratory Medicine and Pathology, Department of Veterans Affairs Medical Center, University of Minnesota, Minneapolis, Minnesota 55417 and the §Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi 34276

Protein B23 is a nucleolar and nuclear matrix-associated phosphoprotein that is involved in ribosome synthesis. Its expression and phosphorylation in rat ventral prostate, an androgen target organ, are profoundly influenced by androgens. Induction of programmed cell death (apoptosis) in the prostatic epithelium by androgen deprivation in the animal induces an early decline in protein B23 in the absence of a corresponding loss of protein B23 mRNA. We have now demonstrated that prostatic nuclei retain the ability to transcribe the B23 mRNA and that a significant amount of this mRNA persists even after 7 days of androgen deprivation when >80% of the prostatic epithelial cells have undergone apoptosis. The B23 mRNA from these nuclei is also translatable in vitro. However, the majority of the B23 mRNA is associated with free and short-stretch polyribosomes, which may account for the castration-induced decline in synthesis of protein B23 in vivo. In addition, the mechanism of down-regulation of protein B23 in apoptotic prostatic cells appears to relate to two coordinate signals, which include loss of phosphorylation of the protein as well as the expression of a protease active toward dephosphorylated protein B23, under these conditions.

Programmed cell death or apoptosis has been described in diverse biological systems mediated by a variety of signals (1–3), and the response of the prostatic glandular epithelium to androgen withdrawal is one of the frequently studied models (3, 4). Androgen withdrawal via orchietomy in the rat induces an energy-dependent cascade of biochemical and morphological changes that lead to the death of 80% of the secretory epithelium between days 2 and 6 (4). Morphologically, the nucleus dissolves (5), and the chromatin is condensed and fragmented to form the membrane-bound apoptotic bodies that appear in appreciable numbers after 2 days of androgen deprivation (6). Biochemically, there is an increase in intracellular calcium and enhancement of calcium/magnesium-dependent endonuclease activity that reaches its maximum 4–5 days after androgen withdrawal (7). Apoptosis in the prostate is associated with modulation of gene expression so that the expression of some genes is repressed and that of others is enhanced. Among the latter are c-fos, c-myc, heat shock proteins (8), glutathione S-transferase (9), and TRPM-2/sulfated glycoproteins (10). On the other hand, the synthesis of ribosomes, especially their assembly into polyribosomes, markedly declines after androgen deprivation (11, 12). Also, prostatic ribosomes from animals treated with 5α-dihydrotestosterone support a significantly higher incorporation of radiolabeled amino acids into proteins than do ribosomes isolated from castrated animal controls (11).

We have been interested in the mechanisms underlying the decline in prostatic rRNA synthesis and assembly after androgen deprivation. Protein B23, a conserved phosphoprotein that is localized to the granular and fibrillar regions of the nucleolus where rRNA synthesis and assembly take place (13, 14), seems to have different functions at different stages of the cell cycle. It is capable of binding nucleic acids and exhibits both helix-stabilizing (15) and ribonuclease activities that implicate the protein in preribosomal RNA processing and transport (16, 17). Various observations suggest that protein B23 plays a role in DNA synthesis (18, 19) and might also have a structural role as one of the components of the perichromosomal layer that is involved in chromosome organization in mitosis (20) and as one of the nuclear matrix-associated proteins (21). Protein B23 is phosphorylated by protein kinase CK2 in interphase (22) and by p34<sup>cdc2</sup> kinase during mitosis (23). The expression and phosphorylation of the protein are enhanced in different cell types in response to mitogens, growth factors, and hormones, including androgen in the prostate, suggesting that protein B23 constitutes a common signal required for cell proliferation (23–28).

In a previous report, we documented that expression and phosphorylation of protein B23 started to decline at a modest rate in the first 24 h after androgen withdrawal (26) and that by 48 h post-castration, B23 was undetectable despite the presence of B23 mRNA up to 7 days of androgen withdrawal (26). These changes in expression coincided with the decline in ribosome synthesis and the morphological alterations associated with apoptosis, suggesting that protein B23 might be involved in these processes (26). In the present work, we have explored the mechanisms by which the prostatic glandular epithelium controls the expression of protein B23 during apoptosis. Our data indicate that regulation of protein B23 expression after androgen withdrawal is not at the transcriptional level. There appears to be no new cis-acting element that would hinder B23 mRNA translation in vitro. This mRNA shows a differential pattern of association with prostatic polyribosomes in response to androgen deprivation, but this effect alone might not explain the specific and abrupt decline in protein B23 expression observed after 48 h of androgen deprivation. It appears that the primary means of regulation of protein B23 expression after
androgen withdrawal is its proteolytic degradation and that the decline in protein B23 phosphorylation as well as the expression (or release of inhibition) of a specific protease(s) after androgen withdrawal may be the prerequisites for protein B23 degradation.

**EXPERIMENTAL PROCEDURES**

**Materials**

Animals—Male Sprague-Dawley rats weighing 295–325 g (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used as the source of ventral prostate tissue. The animals were maintained under standard conditions and were orchietomized via the scrotal route under Metofane anesthesia as described previously (26).

Chemicals—Heparin, phenylmethylsulfon fluoride, poly(γ-glutamyl Tyr) (41), potato acid phosphatase, a-garase-bound alkaline phosphatase, and actin were purchased from Sigma. Rabbit anti-B23 antibody was the same as described previously (15). DNase I (RNase-free) and RNase A were purchased from Boehringer Mannheim. The ribonuclease inhibitor RNasin and the rabbit reticulocyte lysate (nuclease-free) were purchased from Promega. Proteinase K was purchased from International Biotechnologies, Inc. (New Haven, CT). Microcystin-LR was obtained from Promega. All other common reagents and chemicals were of the highest purity available.

**Methods**

Nuclear Run-on Assay—Prostatic nuclei were isolated from rat ventral prostate glands excised either from intact animals or at different times after castration as described earlier (26). Equal amounts of nuclei, as assessed by DNA assay (29), were utilized for each time point examined. Nuclei were suspended in 40% glycerol, 5 mM MgCl₂, 0.1 mM EDTA, and 50 mM Tris-HCl, pH 8; shock-frozen; and stored at –80 °C. The run-on transcription assay was performed as described previously (30). Before hybridization, transcripts (100 μl) were partially hydrolyzed with 4 μl of 10 N NaOH for 10 min on ice, followed by neutralization with 20 μl of 2 M NaH₂PO₄. The alkaline treatment reduces the length of transcripts to ~50–600 nucleotides, the majority of chains being 200–300 nucleotides in length, and leads to more uniformly labeled molecules by removing unlabeled 5'-regions from transcripts that had already been initiated in vivo and then elongated in vitro (31). Excess amounts of denatured B23 DNA (5 μg) as well β-actin cDNA were immobilized on nylon filters with a dot-blot apparatus. Filters were prehybridized for 30 min at 60 °C in prehybridization buffer, followed by hybridization in hybridization buffer containing 1.5 × 10⁶ cpm run-on transcripts for 2 days at 60 °C as described previously (26). The density of different signals was assessed using a two-dimensional Bio-Rad densitometer.

**Northern Analysis**—Total RNA from 3 g of rat ventral prostates was prepared as described previously (26). Total RNA (10 μg) was separated on a formaldehyde-agarose gel and transferred to nylon filters (30). The B23 probe was isolated as described previously (26) and labeled using a random primed DNA labeling kit (Boehringer Mannheim). Prehybridization, hybridization, and autoradiography were as described above (26). The autoradiograms were quantitated by using the two-dimensional Bio-Rad densitometer.

In vitro translation—Prostatic poly(A) RNA was isolated from total RNA using the PolyATtract mRNA isolation system from Promega. Translation was carried out using the rabbit reticulocyte lysate system at 30 °C for 60 min. The reaction containing between 5 and 50 μCi of [35S]methionine (~1000 Ci/mmole) was stopped by adding RNase A (50 μg/ml) in buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 50 mM β-glycerophosphate. The mixture was incubated with B23-specific antibody bound to activated Sepharose 4B beads using cyanogen bromide as described previously (26, 32). The bound protein was washed four times with 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 50 mM β-glycerophosphate and then eluted with buffer containing 10 mM Tris-HCl, pH 7.4, 4 mM MgCl₂, and 50 mM β-glycerophosphate, followed by two washes with the same solution. Bovine serum albumin (500 μg) was added to the eluted material as a carrier, and the mixture was precipitated with 15% trichloroacetic acid, washed with absolute ethanol, and dried. The dried sample was dissolved in β-mercaptoethanol/SDS buffer, boiled for 5 min, and subjected to SDS-PAGE. The gel was dried and exposed to Kodak X-Omat AR film for 4 days at ~80 °C. The density of the bands was quantitated using the two-dimensional Bio-Rad densitometer.

Polysome Gradient—Excised prostate glands were immediately sliced and lysed in ice-cold extraction buffer containing 10 mM HEPES, pH 7.5, 3 mM MgCl₂, 40 mM KCl, 5% glycerol, 20 mM dithiothreitol, and 0.2% Nonidet P-40 supplemented with 1000 unit/ml RNasin, 150 μg/ml cycloheximide, 1 mM phenylmethylsulfonyl fluoride, and 5 mM p-halothan. Mitochondria and nuclei were removed by centrifugation at 10,000 × g for 10 min. Separation of polysomes from the post-mitochondrial supernatant was carried out on linear 15–40% sucrose gradients in extraction buffer centrifuged at 4 °C for 2 h at 38,000 × g as described previously (33). After centrifugation, gradients were separated into fractions and extracted with phenol/chloroform, and the integrity of the transcript was determined by separated RNA in each fraction was assessed by ethidium bromide staining. Subsequently, serial dilutions of each fraction were applied to nylon filters using a slot-blot apparatus, immobilized by heating at 80 °C for 2 h, and then hybridized with randomly labeled B23.1 DNA probe, β-actin probe, or 28 S probe as described above. As a control, an aliquot of each fraction was treated with RNase A before application to the nylon filter to ensure that any observed signal was attributable to RNA in the polysome fraction.

Radiolabeling of Protein B23—Prostatic nuclei were prepared from the isolated nuclei as described previously (34). All preparative solutions contained 50 mM β-glycerophosphate as phosphatase inhibitor. Protein B23 was purified from nuclei using anti-B23 antibody coupled to Sepharose 4B as described previously (32, 33). Induction of purified protein B23 (from tissue or recombinant source) was performed using DOO-BEADS (Pierce) and Na[32P] (36). Radiolabeled protein B23 was dialyzed in a 2000-fold volume of 10 mM Tris-HCl, pH 7.4, to get rid of phenylmethylsulfonyl fluoride and leupeptin prior to the degradation assay. The homogeneity of the protein was assessed using a silver stain of SDS-PAGE and autoradiography.

B23 proteolytic degradation assay—Rat prostate glands were excised either from intact animals or at different times after castration and were immediately homogenized in ice-cold buffer containing 10 mM HEPES, pH 7.5, 3 mM MgCl₂, 40 mM KCl, 20 mM diethiothreitol, and 0.2% Nonidet P-40. The homogenate was subjected within 15 min of excision of the gland. Dialyzed radiolabeled protein B23 was incubated for different times with homogenates obtained from intact and castrated animals. The reaction was stopped by adding sample buffer containing 2% SDS and 5% β-mercaptoethanol, and the mixture was boiled for 5 min prior to SDS-PAGE. The gel was dried and exposed to Kodak X-Omat film overnight. Protease inhibitors such as phenylmethylsulfonyl fluoride (1 mM), leupeptin (20 μg/ml), aprotinin (25 μg/ml), and iodoacetamide (10 mM) were added as a control. When indicated, purified protein B23 was treated with potato acid phosphatase bound to Sepharose 4B for 2 h at 37°C in buffer containing 0.18 M (NH₄)₂SO₄, 1 mM MgCl₂, pH 5.5, followed by dialysis in 3 mM triethanolamine, 0.3 mM NaCl, 0.1 mM MgCl₂, 0.01 mM ZnCl₂, pH 7.6, and incubation with agarose-bound alkaline phosphatase for 2 h at 37 °C prior to the degradation assay (37).

**RESULTS**

Effect of Androgen Deprivation on B23 mRNA Transcription—To determine if the continued high level of B23 mRNA after androgen deprivation was attributable to ongoing transcription or to enhanced stability of the mRNA, the prostatic nuclei obtained from intact and castrated rats were examined for their ability to transcribe B23 mRNA in vitro. As determined by the nuclear run-on assay, the nuclei retained the ability to transcribe B23 mRNA even at 7 days after androgen withdrawal (Fig. 1). The decline in the ability to transcribe B23 mRNA observed after 7 days was comparable to the decline observed in the cellular steady-state level of B23 mRNA examined either by Northern blot analysis (Fig. 2) or by slot-blot analysis (26). The rate of decline in the ability of prostatic nuclei to transcribe β-actin after androgen withdrawal was comparable to that observed for protein B23 (Fig. 1).

Effect of Androgen Deprivation on B23 mRNA Transcription in Prostatic Cytosol—Using a 15–40% sucrose gradient, a different distribution of cytosolic B23 mRNA was observed in prostate glands obtained from castrated animals compared to those obtained from intact animals (Fig. 3A). After androgen withdrawal, the majority of the B23 mRNA existed in association either with...
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Fig. 1. In vitro transcription of B23 mRNA in prostatic nuclei. The nuclear run-on assay was carried out on prostatic nuclei isolated from normal control and castrated rats as described under "Methods." Lane a, normal control rats; lane b, 4-day castrated rats; lane c, 7-day castrated rats. Relative densitometric values for B23 were 1.0 (lane a), 0.8 (lane b), and 0.5 (lane c), and those for β-actin were 1.0 (lane a), 0.7 (lane b), and 0.4 (lane c).

Fig. 2. Effect of androgen deprivation on steady-state level of B23 mRNA using Northern blot analysis. Total prostatic RNA was electrophoresed, transferred to nylon membrane, and probed with randomly labeled B23 cDNA as described under "Methods." Lanes a-c correspond to prostatic total RNA from intact normal and 4- and 7-day castrated rats, respectively. Relative densitometric values were 1, 0.8, and 0.4, respectively.

free ribosomes or with short stretches of polysomes (Fig. 3, A and B). The β-actin mRNA displayed a pattern similar to that of B23 mRNA (Fig. 3C), and this differential distribution of both mRNAs was secondary to the general decline in 28S RNA-containing ribosomal subunits, as evidenced by the decrease in the area under the curve after androgen withdrawal in Fig. 3D. To confirm that the signal obtained by the radiolabeled probe is due to the interaction with RNA in the polysome fractions, a sample of the various polysome fractions was preincubated with RNase A. This treatment abolished the signals, indicating that the probes are indeed recognizing the B23-specific mRNA in the polysome fraction (Fig. 3A).

Effect of Androgen Deprivation on in Vitro Translation of B23 mRNA—Steroid hormones can alter the mRNA initiation sites of many genes (38). To examine the possibility of the development of cis-acting elements in B23 mRNA that would hinder its translation upon androgen deprivation, an in vitro translation assay was performed. Even at 6 days of androgen withdrawal, B23 mRNA was capable of being translated in vitro (Fig. 4).

Effect of Androgen Deprivation on Protein B23 Degradation—Incubation of purified radiolabeled protein B23 with prostatic cellular homogenates from normal and castrated rats did not indicate a significant differential degradation of the protein (Fig. 5, compare top panel with bottom panel). However, we have previously shown that protein B23 phosphorylation in vivo declines dramatically after 2 days of androgen deprivation, suggesting that the extent of phosphorylation may influence B23 degradation (26). Therefore, purified radiolabeled protein B23 was incubated first with acid phosphatase followed by alkaline phosphatase and then with prostatic homogenate in the presence of 20 μg/ml heparin and 2 mg/ml poly(Glu,Tyr) as protein kinase CK2 inhibitors. Under these conditions, a significant appearance of degradation products was observed only when dephosphorylated protein B23 was incubated with prostatic homogenate obtained from 4-day castrated rats (Fig. 6A, compare bottom panel with top and middle panels). The appearance of the degradation products could be minimized by the addition of the protease inhibitors described under "Methods." A Ca++-dependent protease is known to be associated with the nuclear matrix, where it might be involved in the degradation of certain nuclear matrix proteins (39, 40). In accord with this, an addition of 5 mM CaCl2 to the medium markedly enhanced the appearance of degradation products (data not shown).

The nature of the role of phosphorylation of protein B23 in its proteolytic degradation in prostate tissue from castrated rats was further examined. The result in Fig. 6B (lane b), employing recombinant protein B23.1, shows that dephosphorylated protein B23 under conditions that favor dephosphorylation (i.e. presence of excess P1, CK2 inhibitors, phosphatases) was almost completely degraded after 4 h of incubation with prostatic homogenate from 4-day castrated rats. On the other hand, prior incubation of protein B23 with CK2 under conditions that favor phosphorylation (presence of excess ATP, phosphatase inhibitors) protected a 22-kDa segment of protein B23 from being degraded under the same conditions (Fig. 6B, lane c). Since these results suggested that phosphorylation may influence the rate or pattern of proteolytic degradation of protein B23, further experiments were undertaken in which proteolytic degradation of protein B23 was examined at 30 min of reaction time. The results in Fig. 6C (lane b) show that when protein B23 was treated with CK2 (i.e. phosphorylation conditions), some undegraded protein B23 was apparent in addition to the resistant 22-kDa segment that was also observed after 4 h of incubation (e.g. Fig. 6B, lane c). It is noteworthy that intact protein B23 was not detected when nonphosphorylated protein B23 was subjected to the same treatment (Fig. 6C, lane a). Also, it appears that the 22-kDa band has undergone further degradation under these conditions. However, it is possible that the rate and pattern of degradation of protein B23 in vivo may also be influenced by stoichiometry of phosphorylation and/or the presence of other intrinsic kinases. The present observations on the effects of the phosphorylation status of protein B23 on its proteolytic degradation appear to be relatively specific since pretreatment of actin with protein phosphatases under conditions that favor dephosphorylation did not demonstrate a corresponding degradation of actin (or any of the contaminating proteins in the sample) (Fig. 6B, lanes d and e). Thus, the observed degradative changes in protein B23 are not due to the treatment conditions, and furthermore, it appears that protein B23 degradation may be catalyzed by specific proteases that may act on a relatively specific set of proteins.

DISCUSSION

Protein B23 plays an integral role in ribosome synthesis. Changes in rRNA synthesis and assembly are among the earliest responses to androgen action in the prostate. Employing this paradigm, we previously examined the changes in prostatic protein B23 phosphorylation and level in response to androgen withdrawal (i.e. during induction of programmed cell death) and administration (i.e. during prostatic epithelial regeneration) (26). These studies established that although protein B23 expression declines to undetectable levels by 48 h after androgen deprivation, the steady-state level of its mRNA does not change as rapidly, being ~60% of normal even after 7 days of castration (26) when >80% of the prostatic cells have...
The present results demonstrate that persistence of the B23 mRNA after androgen deprivation is the result of ongoing transcription rather than enhanced stability. The decline in the rate of in vitro transcription of protein B23 mRNA was comparable to that of β-actin, which suggests that these changes are general effects of androgen withdrawal and cannot explain the observed specific rapid decline in protein B23 expression.
with either free ribosomes or short-stretch polysomes. How-

after androgen withdrawal, B23 mRNA is mainly associated
probably being translated. We have now demonstrated that
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associated with free or short-stretch polysomes. Furthermore,
it is likely that this differential pattern of association with
polysomes might still be compatible with some protein expres-
sion that is translated from the mRNA associated with long-
stretch polysomes. It is noteworthy that the expression of many
androgen-repressed genes is enhanced after androgen with-
drawal, which again suggests that protein expression can occur
decline in the formation of long-stretch polysomes (8–10). Competition between different mRNAs to associate
with polysomes has been suggested as a mechanism for trans-
lational control (41). In the case of protein B23 mRNA, there
are no cis-acting elements that would arise after androgen
depression and hinder its translation, at least in vitro.

The possibility of proteolytic degradation of prostatic protein
B23 as a result of androgen deprivation was examined. The
initial incubation of radioiodinated protein B23 with prostatic
homogenate obtained after androgen withdrawal did not yield
significant degradation products. However, prior incubation of
protein B23 with phosphatases led to the appearance of degra-
dation products when incubated with prostatic homogenate
obtained from castrated animals, but not when incubated with
homogenates from intact animals. This finding strongly indi-
cates that dephosphorylation of protein B23 and the expres-
sion (or release of inhibition) of protease(s) after androgen
withdrawal are both necessary for the observed decline in protein
B23. This mechanism might be restricted to a specific group of
proteins that includes B23 since, on androgen withdrawal, the
expression of certain proteins is enhanced and actin protein
persists. This accords with our observation that actin is not
degraded under these conditions. Our data also suggest that
phosphorylation of protein B23 enhances its stability and resis-
tance to proteolytic degradation.

The level of phosphorylation of B23 correlates with cellular
proliferative activities and is enhanced at mitosis (17–22). Pro-
tein B23 is phosphorylated by protein kinase CK2 (22), and
CK2 is the rate-limiting factor for B23 phosphorylation in the
prostate gland after androgen deprivation (26). Furthermore,
both protein B23 (21) and protein kinase CK2 (42) are associ-
ated with the nuclear matrix, and phosphorylation of nuclear
matrix-associated protein B23 is directly affected by changes in
nuclear matrix-associated CK2 activity (43). Upon androgen
depression, nuclear matrix-associated CK2 declines rapidly,
which affects the rate of phosphorylation of proteins intrinsic to
that fraction. On the other hand, after androgen administra-
tion to castrated rats, nuclear matrix-associated CK2 is in-
creased within 1 h (43). The physiological significance of these
dynamic changes in the association of CK2 with the nuclear
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in phosphorylation would enhance its susceptibility to proteo-
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B23, which starts to appear within 4 h after androgen ad-

Fig. 4. Effect of androgen deprivation on translation of pro-
tein B23 in vitro. Prostatic poly(A) mRNA was isolated from animals
at the indicated times (days (d)) after androgen withdrawal and trans-
lated in vitro, followed by immunoprecipitation of protein B23 using the
specific antibody as described under “Methods.”

Fig. 5. Effect of androgen on proteolytic degradation of puri-
ﬁed protein B23 added to prostatic homogenate in vitro. Purified
radiiodinated protein B23 was incubated, in a final reaction volume of
100 µl, with 20 µl of prostatic cellular homogenate (20%, w/v) from
intact or from 4-day castrated rats. After incubation for the indicated
periods of time, the reaction was stopped by the addition of gel electro-
phoresis sample buffer. The material was subjected to SDS-PAGE as
described under “Methods.” The dried gel was exposed to Kodak X-
Omat film overnight at −70°C. The top panel shows protein B23
incubated with prostatic homogenate obtained from intact rats. The
bottom panel depicts protein B23 incubated with prostatic homogenate
prepared from 4-day castrated rats. The control lane in both panels
included the addition of protease inhibitors as described under
“Methods.”

B23 mRNA in the prostate gland of the intact animal exists
mainly associated with long-stretch polysomes, where it is
probably being translated. We have now demonstrated that
after androgen withdrawal, B23 mRNA is mainly associated
with either free ribosomes or short-stretch polysomes. How-
ever, this effect is also observed for β-actin mRNA. This similar
association might be attributed to the general reduction in
rRNA synthesis, as evidenced by the decline in 28 S RNA-
containing ribosomal particles, and agrees with the decline in
rRNA synthesis and assembly which is one of the most dra-
matic effects of androgen withdrawal in the prostate gland (11,
12). The observed pattern of association of B23 mRNA or actin
mRNA with ribosomes after androgen withdrawal might con-
tribute to the decline in protein expression in both cases. How-
ever, it cannot explain the aforementioned specific abrupt de-
cline in protein B23 expression (26) in that actin protein is
detected despite the fact that the majority of its mRNA is
associated with free or short-stretch polysomes. Furthermore,
it is likely that this differential pattern of association with
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B23, which starts to appear within 4 h after androgen ad-

ministration in the cascade of events leading to epithelial regeneration (26).

The role of certain proteases in the induction of apoptosis has been documented in several systems (44, 45). Apoptosis induced in thymocytes by staurosporine (a kinase inhibitor) could be inhibited by a protease inhibitor, which suggests a role for both kinases and proteases in this process (46). Nuclear matrix-associated proteins seem to be a preferential target for proteolysis during apoptosis. Lamin A, lamin B, poly(ADP-ribose) polymerase, and topoisomerases are all targets of proteolytic degradation during apoptosis in many systems (39, 47, 48). As is the case for protein B23, nucleolin is another nuclear matrix protein that is localized to the nucleolus and involved in rRNA synthesis and has been shown to be a preferential target of serine proteases during programmed death induced in target cells by cytotoxic lymphocytes (49). Although the nature of the proteases responsible for degradation of these nuclear matrix proteins, including protein B23, is still unknown, a Ca\(^{2+}\)-dependent protease exists in the nuclear lamina (39) and may be involved in this process (40). It is noteworthy that the addition of Ca\(^{2+}\) to the incubation medium markedly enhances the degradation of protein B23. The relation between the decline in protein B23 or its phosphorylation may have certain implications for programmed cell death. An obvious effect of a decline in protein B23 would be on ribosome assembly. Also, protein B23 has been suggested to have a structural role; the decline in protein B23 and its charge might affect the overall tensional integrity of the tissue matrix (50), leading to altered gene

**Protein B23 Degradation during Apoptosis**

![Diagram of protein B23 degradation](image)

**Fig. 6.** Effects of protein B23 phosphorylation status on its susceptibility to proteolytic degradation. A, effect of dephosphorylation of protein B23 on its proteolytic degradation. Purified radiiodinated protein B23 was treated with potato acid phosphatase followed by alkaline phosphatase prior to incubation with prostatic cellular homogenate prepared from intact or 4-day castrated rats as described for Fig. 5. In all panels, heparin and poly(Glu,Tyr) (poly GT) were added as inhibitors of protein kinase CK2. After incubation for the indicated periods of time, the reaction was stopped by the addition of gel electrophoresis sample buffer. The sample was subjected to SDS-PAGE, and the dried gel was exposed to Kodak X-Omat film overnight at -70°C. The top panel represents dephosphorylated protein B23 incubated with prostatic homogenate obtained from intact rats. The middle panel shows phosphorylated protein B23 incubated with prostatic homogenate prepared from 4-day castrated rats. The bottom panel depicts dephosphorylated protein B23 incubated with prostatic homogenate prepared from 4-day castrated rats. The control lane in all panels included the addition of the protease inhibitors listed under “Methods.” B, effects of various conditions on proteolytic degradation of protein B23 and actin by prostatic homogenate from castrated rats. Lane a, \(^{125}\)I-labeled protein B23 (recombinant, nonphosphorylated; 5 \(\mu\)g) was incubated for 60 min at room temperature in medium consisting of 30 mM Tris-HCl, pH 7.45, 5 mM MgCl\(_2\), 1 mM dithiothreitol, 0.1 mM ATP, 40 mM \(\beta\)-glycerophosphate, 2 mM Microcystin-LR, and 350 ng of CK2 in a final volume of 50 \(\mu\)l (phosphorylation medium). Subsequently, 50 \(\mu\)l of control buffer (0.32 M sucrose, 3 mM MgCl\(_2\), 5 mM \(\beta\)-mercaptoethanol) were added, and incubation was continued for 4 h at room temperature (control). Lane b, the conditions were the same as described for lane a, except that phosphatase inhibitors (ATP, \(\beta\)-glycerophosphate, and Microcystin-LR) were omitted, and 0.1 mM Na\(^2\)HPO\(_4\) was included to provide nonphosphorylation conditions. Instead of the control buffer, 50 \(\mu\)l of 50% prostatic homogenate (from 4-day castrated rats) prepared in the control buffer were added, along with inhibitors of CK2 (50 \(\mu\)g/ml heparin, 1 mg/ml poly(Glu,Tyr)), to prevent any phosphorylation of protein B23 catalyzed by CK2 during incubation with the homogenate. Lane c, the conditions were the same as described for lane a, except that prostatic homogenate was added as described for lane b, but without inhibitors of CK2 to promote phosphorylation conditions for CK2. Lane d, 4.5 \(\mu\)g of \(^{125}\)I-labeled actin were incubated under nonphosphorylating conditions as described above for 60 min in medium consisting of 50 mM imidazole HCl, pH 6.3, 2 mM MgCl\(_2\), 2.5 units of alkaline phosphatase, and 2.5 units of acid phosphatase, followed by the addition of control buffer plus CK2 inhibitors and 2.5 mM CaCl\(_2\), and incubation was continued for 4 h as described above. Lane e, the conditions were the same as described for lane d, except that the second incubation (4 h) was carried out following the addition of prostatic homogenate as described above. B, effect of treatment with CK2 on degradation of protein B23 at 30 min by prostatic homogenate from 4-day castrated rats. Lane a, 5 \(\mu\)g of \(^{125}\)I-labeled B23 were incubated under nonphosphorylating conditions as described for B (lane b), except that the second phase of incubation was reduced from 4 h to 30 min. Lane b, the conditions were the same as described for B (lane c), except that the second incubation was for 30 min only as described above. In all panels, arrowheads indicate the position of protein B23.
expression associated with programmed cell death.

In summary, we have documented that cells undergoing apoptosis retain the ability to transcribe B23 mRNA. The existing mRNA for this protein declines very slowly on induction of apoptosis. It appears that the long-lived mRNA is available for translation when the cells are stimulated to grow. Disappearance of protein B23 from the cell appears to relate to its proteolytic degradation, and phosphorylation of the protein (by protein kinase CK2) plays a role in preventing its degradation.

To our knowledge, this is the first report to document the kinetics and mechanism of protein B23 disposition in cells undergoing programmed cell death.

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