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Proteasome Activity Is Required for Androgen Receptor Transcriptional Activity via Regulation of Androgen Receptor Nuclear Translocation and Interaction with Coregulators in Prostate Cancer Cells*

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Upon binding to androgen, the androgen receptor (AR) can translocate into the nucleus and bind to androgen response element(s) to modulate its target genes. Here we have shown that MG132, a 26 S proteasome inhibitor, suppressed AR transactivation in an androgen-dependent manner in prostate cancer LNCaP and PC-3 cells. In contrast, MG132 showed no suppressive effect on glucocorticoid receptor transactivation. Additionally, transfection of PSMA7, a proteasome subunit, enhanced AR transactivation in a dose-dependent manner. The suppression of AR transactivation by MG132 may then result in the suppression of prostate-specific antigen, a well known marker used to monitor the progress of prostate cancer. Further mechanistic studies indicated that MG132 may suppress AR transactivation via inhibition of AR nuclear translocation and/or inhibition of interactions between AR and its coregulators, such as ARA70 or TIF2. Together, our data suggest that the proteasome system plays important roles in the regulation of AR activity in prostate cancer cells and may provide a unique target site for the development of therapeutic drugs to block androgen/AR-mediated prostate tumor growth.

The ubiquitin-proteasome system degrades misfolded or unfolded proteins in order to control a variety of biological functions, including cell proliferation, differentiation, and stress response (1–3). The multicomplex 26 S proteasome contains two 19 S regulatory complexes and a 20 S catalytic core complex that may be responsible for 80–90% of protein degradation in the cell (4). The 19 S complexes are responsible for recognition of the polyubiquitinated protein substrates and work to bridge the substrates to the 20 S core complex for degradation. The barrel-shaped 20 S complex contains four rings, each of which is made up of seven different subunits. The two outer rings contain α-type subunits, whereas the inner rings contain β-type subunits (4, 5). Several proteins involved in cell cycle regulation, like p27 and cyclin, are known to be degraded by the ubiquitin-proteosome pathway (6–8). The protein ubiquitination is initiated by multiple enzyme reactions catalyzed by a single ubiquitin-activating enzyme (E1), a few ubiquitin-conjugating enzymes (E2s),[1] and a large variety of ubiquitin-protein ligases (E3s). The intrinsic E3 ligase activity represents the rate limiting step of ubiquitin modification of proteins. Therefore, the control of the E3 ligase activity may influence proteasome-dependent protein degradation (2, 5).

Proteasomes are known to play an essential role in thymocyte apoptosis and inflammatory responses (9–12). The proteasome inhibitors, such as MG132, suppress the inflammatory response by blocking NF-κB activation or induction of heat shock protein expression, which may allow cells to resist higher temperatures and other toxic agents as well as prevent leukemia cell apoptosis (13–15). In contrast, proteasome inhibitors can also induce cancer cell apoptosis, accompanied by activation of several caspases, such as caspase-3 or caspase-7 (16, 17). Although apoptosis elicited by proteasome inhibitors is universal and not restricted to only one cancer cell type, the molecular mechanisms by which the proteasome inhibitors induce apoptosis remain largely unknown.

The androgen-androgen receptor (AR) complex may cooperate with various coregulators to modulate their target genes for proper or maximal function (18–25). Some of these coregulators contain E3 ligase activity, which may regulate AR activity via the ubiquitin-proteasome pathway (19, 22, 24, 25). Early evidence suggested that the ubiquitin-proteasome system might be involved in the regulation of AR protein turnover (26). For example, UBC9, an E2 enzyme, can bind to AR and enhance AR transactivation (27). A putative PEST sequence located in the hinge region of AR is also proposed to play important roles in ubiquitination-related AR degradation (26). Collectively, these results imply that the ubiquitin-proteasome system may be involved in the regulation of AR activity. Here we have demonstrated that inhibition of the proteasome suppresses AR transactivation, AR nuclear translocation, and interaction between AR and AR coregulators, whereas proteasome subunits enhance AR transactivation in a dose-dependent manner. These results suggest that the proteasome system is required for AR activity.

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[1] The abbreviations used are: E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein ligase; AR, androgen receptor; DHT, 5α-dihydrotestosterone; MMTV-luc, mouse mammary tumor virus-luciferase; PSA, prostate specific antigen; GR, glucocorticoid receptor; Dex, dexamethasone; ARE, androgen response element; DAPI, 4,6-diamidino-2-phenylindole; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling.

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EXPERIMENTAL PROCEDURES

Plasmids and Reagents—Gal4-AR (DBD-LBD), VP16-AR, VP16-ARA70 (amino acids 1–401), Gal4-ARA70 (amino acids 176–401), and VP16-TIF2 have been described previously (20, 21). pFLAG-PSMA7 was kindly provided by Dr. S. Cho (Korean Research Institute of Bioscience and Biotechnology, Yusong, South Korea). MG132, lactacystine, and Z-VAD-fmk were purchased from Calbiochem. 5α-dihydrotestosterone (DHT) was purchased from Sigma, and the anti-AR polyclonal antibody, NH27, was produced as previously described (20, 24). The Texas Red-conjugated secondary anti-rabbit antibody was obtained from ICN Pharmaceuticals, Inc.

Cell Culture and Transfections—The human prostate cancer PC-3 cells and African green monkey kidney COS-1 cells were maintained in Dulbecco's minimum essential medium containing penicillin (25 units/ml), streptomycin (25 μg/ml), and 5% fetal calf serum. The human prostate cancer LNCaP cells were maintained in RPMI 1640 with 10% fetal calf serum. Transfections were performed using SuperFect™ according to standard procedures (Qiagen).

Apoptosis Assay—LNCaP cells were treated with 40 μM Z-VAD-fmk 30 min prior to 5 μM MG132 treatment. After 48 h, the cells were harvested for the TUNEL assay to measure cell apoptosis according to standard procedures (Oncogene Research Products). At least 200 cells were scored for each sample, and the data are means ± S.D. from three independent experiments.

Luciferase Reporter Assays—The cells were transfected with pSG5-AR along with vector or PSMA7 and the mouse mammary tumor virus-luciferase (MMTV-luc) reporter for 16 h and then treated with ethanol or 10 nM DHT for another 16 h. The cells were lysed, and the luciferase activity was detected by the dual luciferase assay using pRL-SV40 as an internal control, according to standard procedures. Each sample was normalized by pRL-SV40, and data are means ± S.D. from three independent experiments.

Cell Fractionation Preparation and Western Blotting—The nuclear and cytosolic fractions were prepared as previously described (28). Briefly, LNCaP cells were treated with dimethyl sulfoxide, Me_2SO, or 5 μM MG132 for 30 min prior to 10 nM DHT treatment. After 8 h of treatment, the cells were dissolved in buffer A (10 mM HEPES-KOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) for 10 min and then centrifuged for 30 s. The supernatant was collected as a cytosolic fraction. The pellets were dissolved in buffer C (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) for 20 min and centrifuged for 2 min. The supernatant was then collected as a nuclear fraction. Western blotting was performed as previously described (29).

Immunofluoresence and Microscopy—COS-1 cells were plated on 12-mm coverslips and incubated overnight. The cells were transfected with pSG5-AR for 16 h, followed by treatment with Me_2SO or 5 μM MG132 for 30 min prior to addition of 10 nM DHT. After 16 h of treatment, the cells were fixed with 4% paraformaldehyde/phosphate-buffered saline for 20 min on ice; the cells were then permeabilized with 100% methanol for 15 min on ice. The following experiments were performed at room temperature. The coverslips were rinsed twice with phosphate-buffered saline and incubated in 5% bovine serum albumin for 30 min. The primary antibody against AR (NH27) was added for 1 h, and cells were then washed four times with phosphate-buffered saline. The secondary antibody was added for 1 h, and cells were then washed four times with phosphate-buffered saline, followed by application of the counting medium containing 4,6-diaminodino-2-phenylinodel (DAPI). A Texas Red anti-rabbit antibody was used as the secondary antibody. Coverslips were examined with a confocal microscope.

RESULTS

The Proteasome Activity Is Required for AR Transactivation—Androgen/AR signaling plays important roles in prostate cancer cell growth and cell apoptosis (29–32). In accordance with previous reports (16, 17), we observed that MG132 (5 μM) could markedly induce apoptosis in prostate cancer LNCaP cells (Fig. 1). The MG132-induced apoptosis in LNCaP cells was dramatically reduced by Z-VAD-fmk, a general caspase inhibitor, suggesting that MG132-induced apoptosis is, at least in part, achieved through a caspase-dependent pathway (Fig. 1). To study the potential linkage between MG132-induced cell apoptosis and androgen/AR signaling, the AR transactivation assay was performed using three AR reporters including

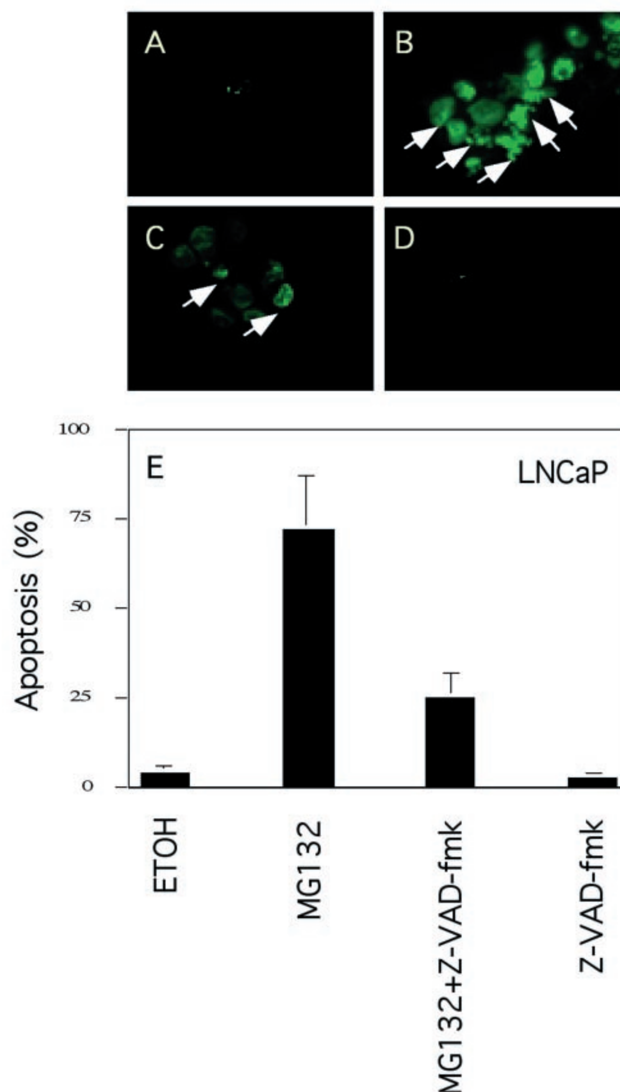

FIG. 1. **MG132-induced apoptosis is via a caspase-dependent pathway.** LNCaP cells were treated with ethanol (ETOH) (*A*), 5 μM MG132 (*B*), 40 μM Z-VAD-fmk plus 5 μM MG132 (*C*), or 40 μM Z-VAD-fmk (*D*) for 48 h. Cells were then harvested for an apoptosis assay using the TUNEL method. *Arrows* indicate apoptotic cells. *E*, representation of statistical results from *A–D*.

MMTV-luc, prostate specific-antigen (PSA)-luc, and four copies of a synthetic androgen response element, (ARE)4-luc, in the presence or absence of MG132. As shown in Fig. 2*A*, 10 nM DHT activated the three AR reporters markedly, and addition of 5 μM MG132 dramatically suppressed the DHT-induced AR transactivation of all three AR reporters in LNCaP cells. Similar results were obtained in PC-3 cells, an androgen-independent prostate cancer cell line transfected with wild-type AR (Fig. 2*B*), as well as in COS-1 cells that were transfected with wild-type AR (data not shown). To determine whether MG132 affects steroid receptor transactivation in general, we used the glucocorticoid receptor (GR) for comparison. As shown in Fig. 2*C*, MG132 did not suppress dexamethasone (Dex)-induced GR transactivation. Suppression of AR transactivation by MG132 seems to occur upstream of its apoptotic activity, because the caspase inhibitor Z-VAD-fmk, which blocked MG132-induced apoptosis (Fig. 1), did not affect the MG132-mediated suppression of AR transactivation (Fig. 2*D*). This result suggests that suppression of AR transactivation by MG132 is not an effect secondary to its induction of apoptosis. To rule out the possibility that the suppressive effect on AR transactivation by

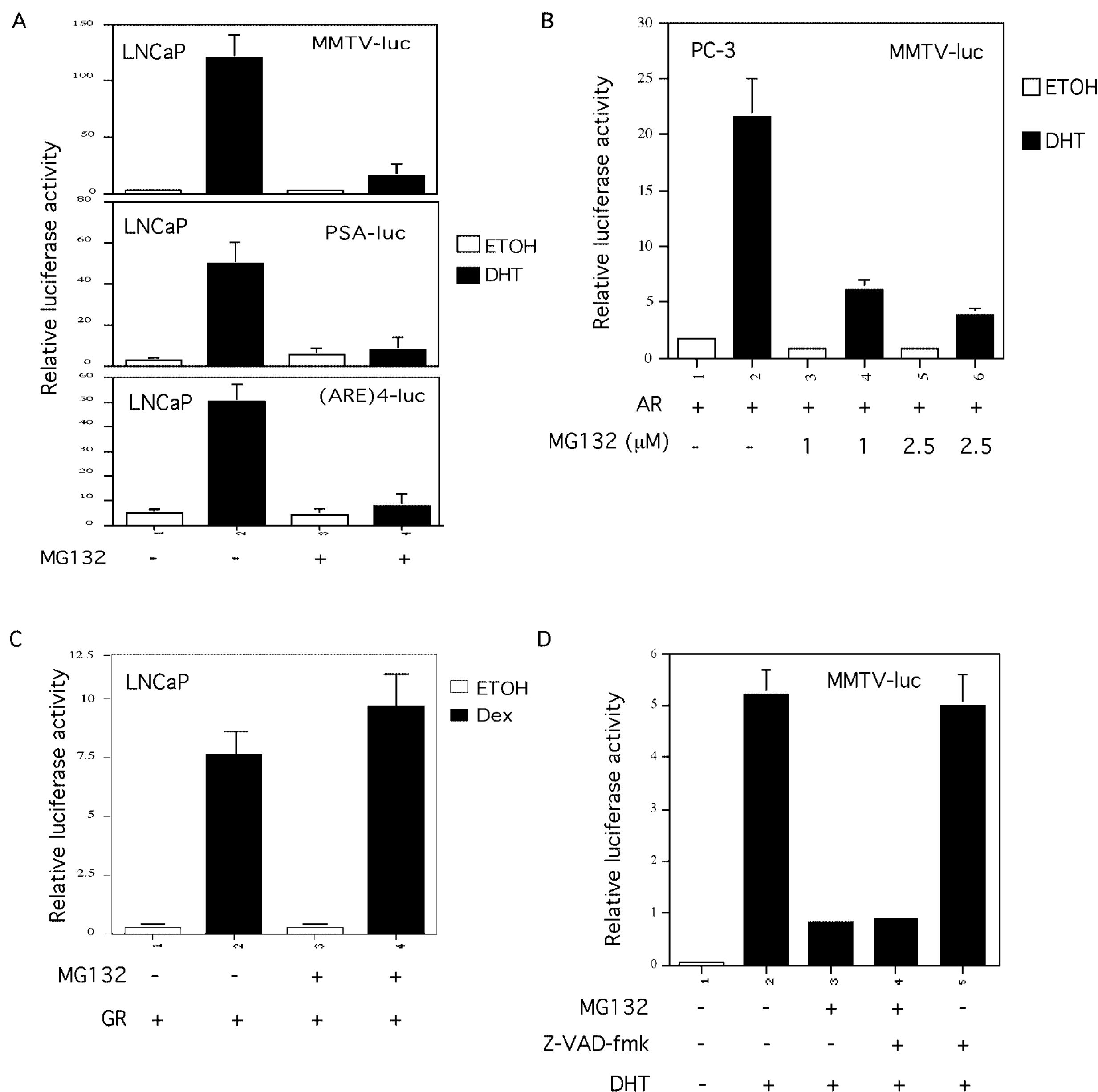

FIG. 2. **The proteasome activity is required for AR transactivation but not for GR transactivation.** *A*, LNCaP cells were transfected with MMTV-luc, PSA-luc, or (ARE)4-luc in combination with PRL-SV40, an internal control, for 24 h. Cells were treated with 5 μM MG132 for 30 min, followed by ETOH or 10 nM DHT treatment for 16 h, harvested, and assayed for luciferase activity. *B*, PC-3 cells were transfected with AR and MMTV-luc for 16 h, treated with MG132 for 30 min followed by 10 nM DHT for 16 h, and then harvested for luciferase activity. *C*, LNCaP cells were transfected with GR and MMTV-luc, as well as PRL-SV40, for 24 h. Cells were treated with 5 μM MG132 for 30 min followed by 10 nM Dex for 16 h and then harvested and assayed for luciferase activity. *D*, LNCaP cells were transfected with MMTV-luc in combination with PRL-SV40 for 24 h. Cells were treated with 40 μM Z-VAD-fmk for 30 min followed by 5 μM MG132 and 10 nM DHT for 16 h and then harvested and assayed for luciferase activity. Data are means ± S.D. from three independent experiments.

proteasome inhibition is mediated only through MG132 activity, we used another proteasome inhibitor, lactacystine, in our reporter study. Lactacystine, like MG132, suppressed AR transactivation of all three AR reporter constructs (Fig. 3*A*) and did not suppress GR transactivation (Fig. 3*B*). Taken together, these results suggest that proteasome activity is important for AR transcriptional activation.

The Proteasome Subunit Enhances Androgen-mediated AR Transactivation—Because blockage of proteasome activity by MG132 could result in the suppression of AR transactivation, it is possible that the induction of proteasome activity may enhance AR transactivation. To test this hypothesis, we transfected PSMA7, an α-type subunit of the 20 S proteasome core complex (33), into PC-3 cells in combination with AR and the MMTV-luc reporter. As shown in Fig. 3*C*, 10 nM DHT induced AR transactivation up to 10-fold, and addition of PSMA7 further enhanced androgen-mediated AR transactivation in a dose-dependent manner. Together, results from Figs. 1 and 2 clearly demonstrate that the proteasome has an important role in modulation of AR transactivation.

Suppression of Androgen Target Gene Expression by Proteasome Inhibition—To reduce the potential artifactual effects of reporter assays, we applied Northern and Western blot analyses of LNCaP cells to assess the MG132 effect on the expression of PSA, an endogenous AR target gene. As shown in Fig. 4, *A* and *B*, 10 nM DHT induced PSA expression at both mRNA and protein levels, and addition of 5 μM MG132 then repressed this effect, suggesting that protea-

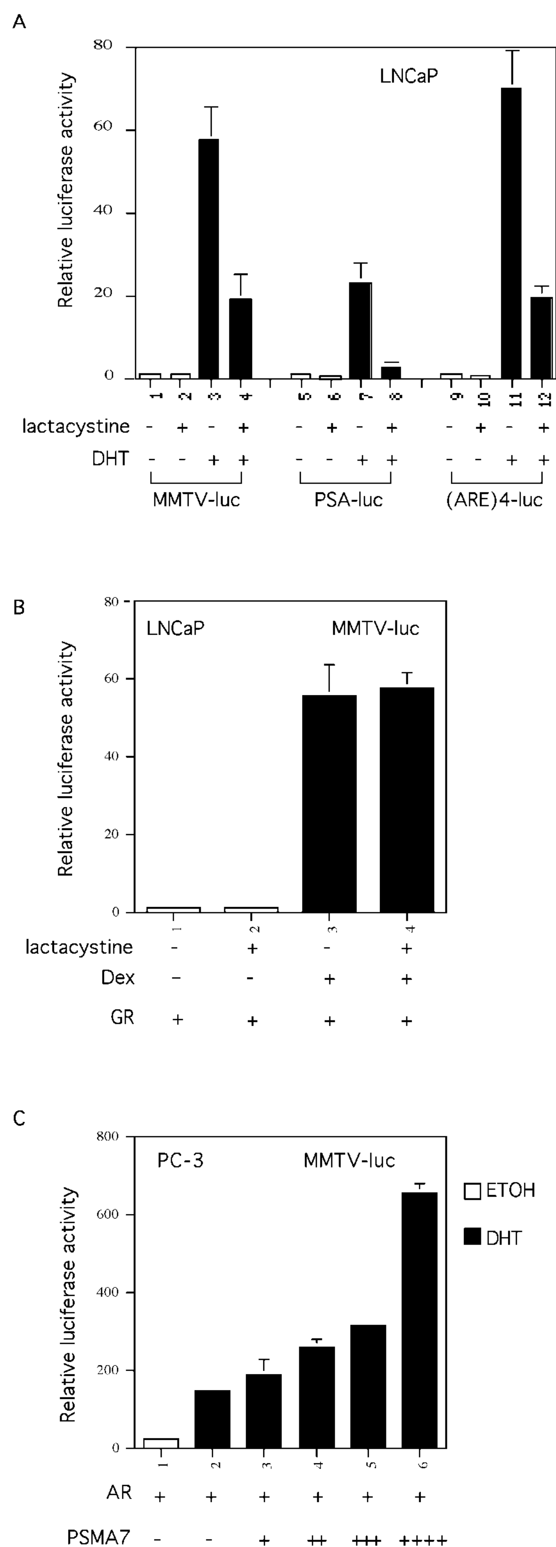

FIG. 3. **Proteasome subunit enhances AR transactivation in a dose-dependent manner.** *A*, LNCaP cells were transfected with MMTV-luc, PSA-luc, or (ARE)4-luc as well as PRL-SV40 for 24 h. The cells were treated with 5 μM lactacystine for 30 min and then with ETOH or 10 nM DHT for 16 h. They were then harvested and assayed for luciferase activity. *B*, LNCaP cells were transfected with GR and MMTV-luc, as well as with PRL-SV40, for 24 h. Cells were treated with 5 μM lactacystine for 30 min, then with 10 nM Dex for 16 h, and harvested and assayed for luciferase activity. *C*, PC-3 cells were transfected with AR and MMTV-luc in combination with different amounts of PSMA7 for 16 h, treated with 10 nM DHT for 16 h, and then harvested and assayed for luciferase activity. Data are means ± S.D. from three independent experiments.

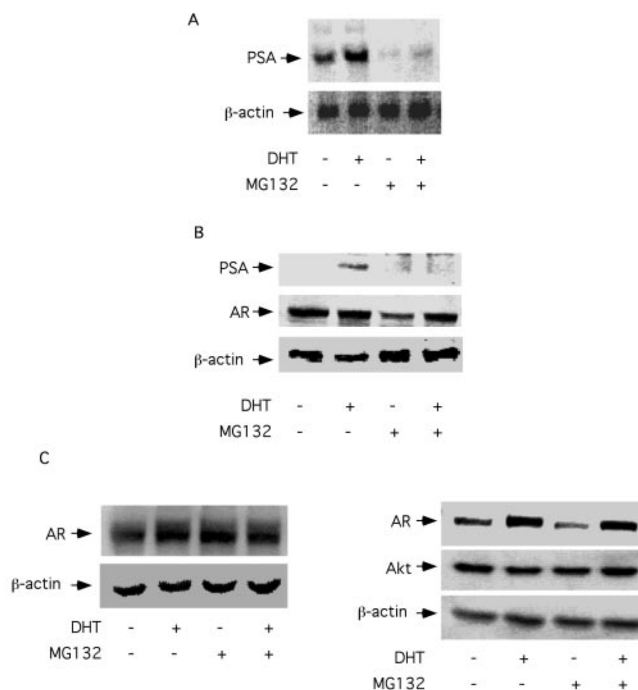

FIG. 4. **Suppression of androgen-induced PSA expression by MG132.** LNCaP cells were treated with Me_2SO or 5 μM MG132 for 30 min, then with 10 nM DHT for 24 h, and harvested for Northern (*A*) and Western blot analysis (*B*). *C*, LNCaP cells were treated with Me_2SO or 5 μM MG132 for 6 h (*left panel*) or 24 h (*right panel*) in the presence or absence of 10 nM DHT and harvested for Western blot analysis.

some activity is important for expression of endogenous AR target genes. It should be noted that MG132 also suppressed the basal PSA expression without androgen treatment (Fig. 4*A*), suggesting that very small amounts of androgen in the medium may contribute to induction of PSA expression, an effect that could be inhibited by MG132. However, we cannot rule out the possibility that MG132 may also suppress PSA expression via an androgen-independent pathway. Interestingly, when we compared AR expression in the presence or absence of MG132, the results (Fig. 4*C*) demonstrated that MG132 treatment for 6 h could increase AR protein expression in the absence of DHT, whereas after MG132 treatment for 24 h AR protein levels were suppressed in the absence of DHT. However, MG132 showed only a marginal effect on AR protein levels in the presence of DHT (Fig. 4*C*), suggesting that reduction of AR protein expression may not play a major role in the MG132-mediated suppression of androgen-induced PSA expression. Although MG132 decreased AR protein levels in the absence of DHT, it did not affect the expression of β-actin (Fig. 4) or Akt, a survival protein (Fig. 4*C*), suggesting that MG132 does not have a general toxic effect on cells.

MG132 Suppresses AR Nuclear Translocation—Because the AR hinge domain contains a putative PEST sequence that overlaps the bipartite nuclear localization signal, it is possible that MG132 may reduce AR transactivation via interruption of AR nuclear translocation. To test this hypothesis, we used immunocytochemistry to monitor AR nuclear translocation in COS-1 cells, a well studied cell model in which androgen-dependent AR nuclear translocation has been demonstrated. As shown in Fig. 5*A*, AR was mainly expressed in the cytosol in the absence of androgen, and the addition of 10 nM DHT resulted in translocation of most of the cytosolic AR into the nucleus (Fig. 5*A*). Interestingly, addition of 5 μM MG132 significantly suppressed (near 50%) AR nuclear translocation (Fig. 5, *A* and *B*).

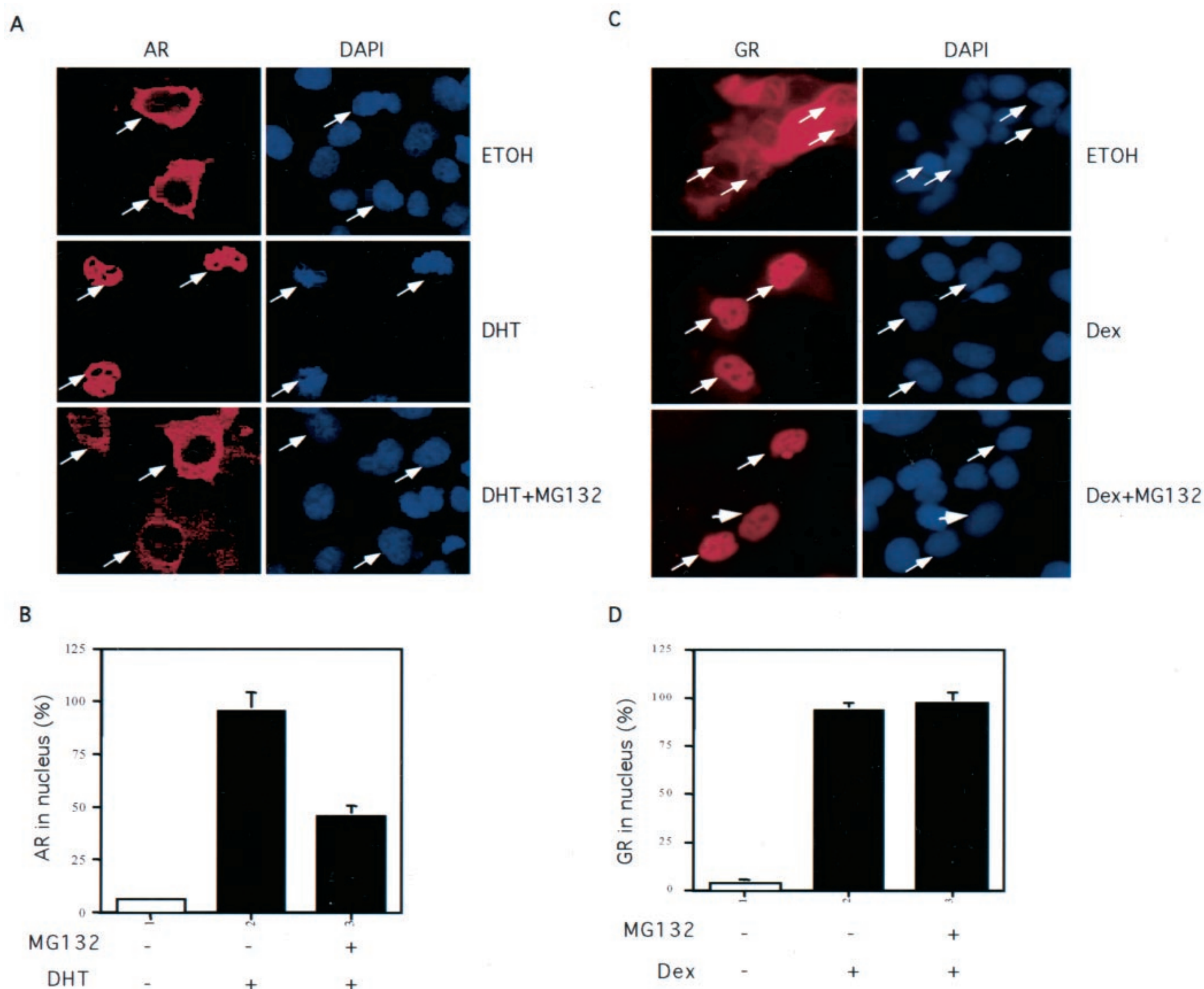

FIG. 5. **Requirement of proteasome activity for AR nuclear translocation.** *A*, COS-1 cells were transfected with AR for 16 h, followed by treatment with 5 μM MG132 30 min prior to ETOH or 10 nM DHT treatment. After 16 h, the cells were fixed, stained with an AR antibody and DAPI, and examined by confocal microscopy. *Red* and *blue* represent AR staining and cell nuclei, respectively. *B*, quantitative representation of the results shown in *A*. *C*, COS-1 cells were transfected with GR for 16 h, followed by treatment with 5 μM MG132 30 min prior to ETOH or 10 nM Dex treatment for 16 h. The cells were fixed, stained with an GR antibody and DAPI, and examined by confocal microscopy. *Red* and *blue* represent GR staining and cell nuclei, respectively. *D*, quantitative representation of the results shown in *C*.

As a control, MG132 showed little influence on GR nuclear translocation (Fig. 5, *C* and *D*). To further confirm the effect of MG132 on AR nuclear translocation, we prepared cytosolic and nuclear fractions for Western blot assay. LNCaP cells were treated with 5 μM MG132 30 min prior to addition of 10 nM DHT. Nuclear and cytosolic fractions were then collected. In Fig. 6, Western blot analysis using the NH27 anti-AR antibody demonstrates that DHT increases AR protein expression in the nucleus and that MG132 significantly reduces AR protein expression in the nucleus. In contrast, MG132 enhances AR protein expression in the cytosol, suggesting that MG132 suppresses androgen-induced AR nuclear translocation. Together, results from Figs. 5 and 6 indicate that MG132 suppresses AR transactivation via interruption of AR nuclear translocation.

MG132 Inhibits the Interaction between AR and AR Coregulators—A recent report suggested that the proteasome system may also have steroid receptor coregulator-like activity and thus modulate steroid receptor transactivation as well as affect the stability of some coregulators (34). It is known that androgen-AR may cooperate with various coregulators to modulate their target genes for proper or maximal function (18–25). It is therefore possible that MG132 suppresses AR transactivation via interruption of AR coregulator function. To test this hypothesis, we applied the mammalian two-hybrid system to monitor the MG132 effect on the interaction between AR and its coregulators. As shown in Fig. 7*A*, 10 nM DHT induced interaction between AR and ARA70, and addition of MG132 significantly blocked this interaction. MG132 did not suppress the basal activity of the reporter gene in the absence of androgen. Similar suppressive effects also occurred when we replaced ARA70 with another AR coregulator, TIF2 (Fig. 7*B*). Results shown in Fig. 7 suggest that MG132 suppresses AR transactivation via interruption of interaction between AR and AR coregulators.

DISCUSSION

Recent advances in the nuclear receptor field have indicated that steroid receptor transactivation is regulated by post-translational modification such as methylation, phosphorylation, and acetylation (21, 29, 35). In the present study, we have demonstrated that the proteasome inhibitor MG132 dramati-

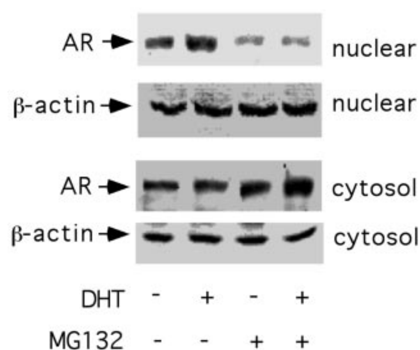

FIG. 6. **MG132 suppresses AR nuclear translocation.** LNCaP cells were treated with 5 μM MG132 30 min prior to 10 nM DHT treatment. After 8 h, the cells were harvested for preparation of cytosolic and nuclear fractions, as described under "Experimental Procedures," and fractions were analyzed by Western blotting.

cally attenuated AR transactivation in prostate cancer PC-3 and LNCaP cells. Northern and Western blot assays further confirmed this result, suggesting that the proteasome is an essential component for AR transcriptional activation and may serve as an AR coregulator. Overexpression of a 20 S proteasome subunit, PSMA7, enhanced AR transactivation in a dose-dependent manner (Fig. 3*C*), providing evidence that the proteasome may act as a coregulator. Thus, the ubiquitin-proteasome system may represent another mechanism through which AR transactivation is regulated.

The PEST sequence within the AR hinge domain, which is conserved throughout many species, may play a role in the ubiquitin-proteasome degradation pathway (36). An early report demonstrated that AR protein expression in LNCaP cells could be increased after adding the proteasome inhibitor MG132 for 4 h (26). In agreement with this finding, we also observed a moderate increase of AR protein expression after short term (4–6 h) treatment with MG132 in LNCaP cells (Fig. 4*C*). However, long term treatment with MG132 (≥ 24 h) caused AR protein expression to decrease (Fig. 4, *B* and *C*) in the absence of androgen, which correlates with the finding that AR mRNA also decreases with long term treatment (data not shown). A potential explanation for this biphasic modulation of AR expression by the proteasome inhibitor MG132 is the combination effect, which entails suppression of AR mRNA expression yet prevention of AR protein degradation.

Accumulating evidence indicates that the proteasome not only plays a proteolytic role in protein degradation but also plays a non-proteolytic role in transcription elongation, nuclear excision repair, and protein trafficking (1, 37–41). Our results demonstrating that inhibition of proteasome function by MG132 attenuates androgen-induced AR nuclear translocation further support the non-proteolytic role of the proteasome in protein trafficking (1, 37, 38). In contrast, proteasome inhibition does not affect Dex-induced GR nuclear translocation, suggesting that the proteasome is not involved in modification of GR cellular localization. How the proteasome is involved in regulation of AR nuclear translocation is currently unknown. Since the PEST sequence in the AR hinge region overlaps the bipartite nuclear translocation region (19, 26), it is possible that ubiquitination of AR in this region may provide the recognition site for proteasome association, resulting in the modulation of AR nuclear translocation. To support the role of the ubiquitin-proteasome pathway in AR nuclear translocation, it has been shown that the Snurf1 coregulator, a RING finger protein, could bind to the AR hinge region to enhance AR transactivation via promotion of AR nuclear translocation (19, 42). However, it remains to be determined whether Snurf1 has

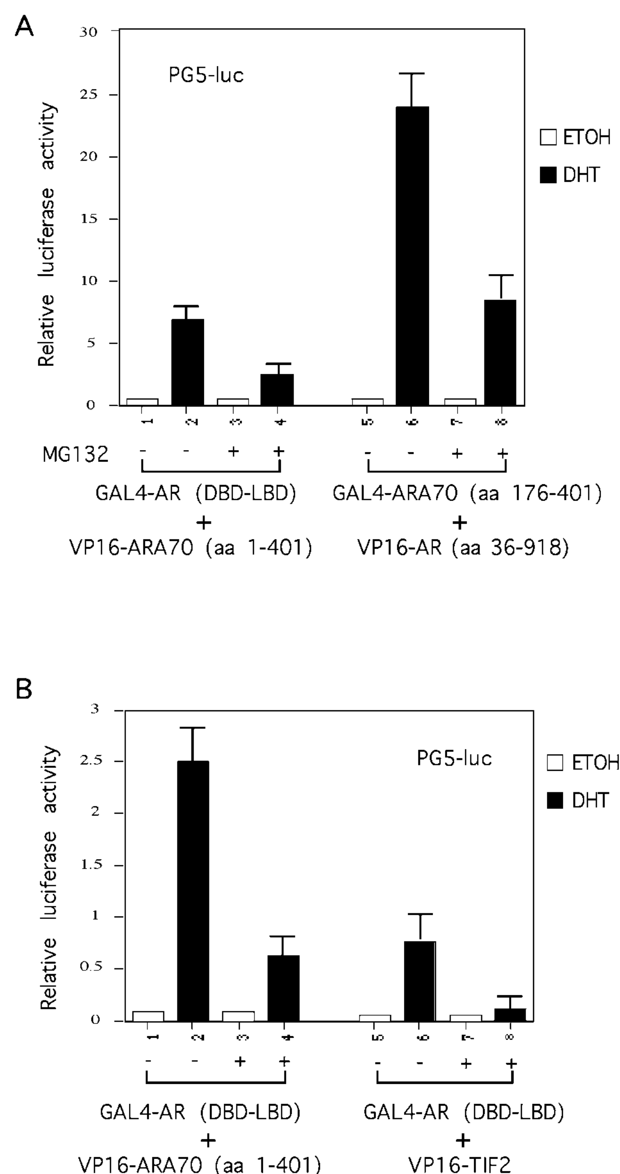

FIG. 7. **Suppression of interaction between AR and its coregulators by MG132.** *A* and *B*, LNCaP cells were transfected with plasmids for 24 h as indicated and then treated with 5 μM MG132 or vehicle 30 min prior to 10 nM DHT treatment. The interaction between AR and its coregulators was determined by luciferase assay using pG5-luc as a reporter. Data are means ± S.D. from three independent experiments.

intrinsic E3 ligase activity to ubiquitinate AR and allow proteasome recognition and thus promotion of AR nuclear translocation.

Upon binding to androgen, AR dissociates from the heat shock protein 70 (hsp70) and translocates into the nucleus. This process may require cooperation with many other coregulators, either in the cytosol or the nucleus (19, 43). Any interruption of the interaction between AR and these coregulators may then alter AR function and result in abnormal androgen action. Our findings that MG132 can interrupt the interaction between AR and these coregulators, such as ARA70 and TIF2 (Fig. 7), further support the concept that both coregulators and the proteasome system are important for the regulation of AR function.

The detailed mechanism of how the proteasome regulates AR activity is currently unclear. Based on our results and those of others, it is possible that the regulation of AR transcriptional activity by the proteasome may involve multiple mechanisms.

First, proteasome inhibition can markedly suppress androgen-induced AR nuclear translocation (Figs. 5 and 6). As a result, AR access to the DNA in the nucleus decreases, leading to a reduction in AR transactivation. Second, proteasome inhibition interrupts the interaction of AR with its coregulators, such as ARA70 and TIF2 (Fig. 7). Recent reports indicate that AR coregulators play a key role in the regulation of AR transcriptional activity (18–25). Supporting evidence has been provided by an additional study (44) showing that overexpression of the dominant negative ARA54, an AR coregulator, suppresses AR transactivation, presumably via interruption of endogenous ARA54 association and function. Furthermore, addition of Pyk2, an ARA55-interacting protein, into PC-3(AR)2 cells could suppress AR transactivation by preventing the interaction between AR and ARA55 (45). Likewise, abrogation of the interaction between AR and its coregulators by proteasome inhibition may result in suppression of AR transactivation. Third, it is possible that AR could be initially ubiquitinated by some unknown E3 ligases, providing the recognition site for proteasome association with AR. This association may then promote recruitment of individual coregulators to the AR complex, allowing proper androgen action. Fourth, it is also possible that the proteasome serves as a bridging factor to recruit transcriptional elongation factors to the AR complex. This assertion is supported by the recent report showing that the 19 S proteasome is required for efficient transcriptional elongation by RNA polymerase II via physical interaction with CDC69, an elongation factor (39). Finally, the proteasome may be recruited to the promoter region of AR target genes, where it could then facilitate AR transcriptional activation. In support of this idea, it has been shown that upon induction with galactose the 19 S proteasome is recruited to the GAL1–10 promoter in yeast via chromatin immunoprecipitation assays (46). Proteasome inhibitors may interrupt proteasome binding to the promoter region of AR target genes, resulting in suppression of AR transactivation. Whether the proteasome is able to bind to the promoter region of AR target genes upon androgen treatment remains for further investigation.

Although the PEST sequence, located in the hinge region of AR, is thought to be involved in ubiquitin proteasome-dependent protein degradation, an AR with a PEST sequence mutation with the lysine at position 638 replaced by arginine shows similar levels of transactivation as the wild-type AR when treated with androgen (data not shown). This result suggests that the PEST sequence may not be involved in AR transcriptional activity or affect protein stability. However, we cannot rule out the possibility that mutations at other positions in the PEST sequence affect AR protein stability and transactivation. Alternatively, the PEST sequence may still be involved in regulation of AR protein stability but not be involved in AR transactivation, because suppression of AR transactivation by MG132 is not via modulation of AR protein levels.

In summary, our data demonstrate for the first time that the proteasome system plays an essential role in modulation of AR transcriptional activity via regulation of AR nuclear translocation and mediation of the interaction of AR with its coregulators. Because androgen/AR signals play essential roles in prostate cancer growth, any new mechanisms successful in blocking this growth could provide new targets for the design of novel therapeutic agents for the treatment of prostate cancer, the second leading cause of cancer-related death in men in the United States. Thus the proteasome system, which is required for optimal AR activity, may serve as such a therapeutic target.

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