

Retinoblastoma, a Tumor Suppressor, Is a Coactivator for the Androgen Receptor in Human Prostate Cancer DU145 Cells

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The retinoblastoma protein may function as a tumor suppressor by controlling the progression of the normal cell cycle. Inactivation of Rb has been regarded as an important event in prostate carcinogenesis. However, the detailed mechanism of how Rb is linked to androgen-androgen receptor (A-AR), the major factor in promotion of prostate tumor growth, remains unclear. Using GST-Rb pull down assay and mammalian two-hybrid system, we report here that Rb can bind specifically to AR in an androgen-independent manner. Transient transfection assay demonstrates that cotransfection of AR and Rb can further induce AR transcriptional activity 4-fold in the presence of 1 nM dihydrotestosterone in DU145 cells. Interestingly, cotransfection of Rb and ARA70, the first identified AR coactivator, with AR can additively induce AR transcriptional activity 13-fold (from 5-fold to 64-fold). In conclusion, our discovery that Rb can function as a coactivator to induce AR transcriptional activity in prostate cells may represent the first data to link a negative growth regulatory protein function in a positive manner, by inducing the transcriptional activity of AR. © 1998 Academic Press

Prostate cancer has become the most commonly diagnosed cancer in US men (1). Today, most androgen de-

pendent prostate cancers from patients treated with androgen ablation will progress to fatal androgen independent state. While AR mutations may play vital roles in some androgen independent tumors, some androgen insensitive prostate tumors still retain wild-type AR. The alterations in other cellular factors that control the AR function may also play a major role in resistance to androgen ablation therapies (2).

With the cloning of the first AR coactivator, ARA70, and the discovery that ARA70 can enhance AR function in prostate cancer cells (3), the hypothesis that prostate AR may need other cofactors for the proper or maximal androgen activity is being widely accepted. However, many details remain unknown about how the cofactors contribute to the regulation of androgen response in prostate cancer. In addition, the study of retinoblastoma protein (Rb) expression in human prostate tumor also indicated that Rb gene alterations can occur in all grades and stages of prostate cancer, in localized as well as metastatic disease (4). It would be interesting to explore the relevance of Rb to the transactivation of AR and to the androgen-dependent status in prostate tumor progression.

Rb functions in the control of cell proliferation and differentiation (5, 6). In resting cells, hypophosphorylated Rb prevents inappropriate entry of cells into the cell division cycle. Phosphorylation of Rb by cyclin-dependent kinases relieves Rb-mediated growth suppression, and allows for cell proliferation (7, 8). Conversely, dephosphorylation of Rb during G1 progression induces growth arrest or cell differentiation (8, 9). In dividing cells, Rb is dephosphorylated during mitotic exit and G1 entry (10). This dephosphorylation activates Rb for the ensuing G1 phase of the cell cycle, during which Rb exerts its growth suppressive effects. Furthermore, much attention has been given to the functional interaction between Rb and transcription factors. To date, several of these factors have been shown to form com-

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The abbreviations used are: AR, androgen receptor; Rb, retinoblastoma protein; T-ag, SV40 large T-Antigens; CAT, chloramphenicol acetyltransferase; ARE, androgen response element; GR, glucocorticoid receptor; PR, progesterone receptor; ERE, estrogen response element; ER, estrogen receptor; MMTV, mouse mammary tumor virus.

plexes with Rb in cells. Such complex formation and subsequent function studies have revealed that the modulating activity of Rb can take the form of repression of transcription as with E2F (11), or activation as with NF-IL6 (12) and the hBrm/BRG1 complex (13). The hBrm (or SNF2a) and BRG1 (or SNF2b) proteins have not only been shown to associated with Rb, but also to be present in SWI/SNF complexes which are thought to be involved in activating transcription by facilitating the remodelling of chromatin template (14). Very recently, it has also been reported that Rb can associate with histone deacetylase and consequently inhibit the transactivation of E2F target genes (15, 16). Thus, upon association with different cellular proteins, Rb is involved in both positive and negative regulator pathways.

Using immunoblot analysis, Bookstein *et al.* were able to detect a complete loss of Rb expression in one of seven prostate cancers (17). An abnormal short-sized mRNA transcript of Rb exon 21 was found in DU145 cells (18) and transfection of the wild-type Rb gene into the DU145 cells results in suppression of the malignant phenotype (19). Using PCR amplification techniques, Brooks *et al.* found 41 of 46 prostate tumors (89%) were informative and 11 of these (27%) had lost one Rb allele (4). A Rb-dependent apoptotic pathway has also been demonstrated in the prostatic glandular epithelium after castration (20). Together, these data suggested that loss of Rb may play an important role in prostatic tumorigenesis and progression from androgen-dependent to androgen-independent state.

In this report, we present evidence that the Rb protein has a functional and physical interaction with AR. Interestingly, this interaction may be independent of another AR associated protein, ARA70. Together, our data indicate the Rb protein may play an important role for proper A-AR function, in that it can interact with AR and enhances the transcriptional activity of AR in DU145 cells.

MATERIALS AND METHODS

Materials. Dihydrotestosterone (DHT) and 17β -estradiol (E2) were obtained from Sigma and hydroxyflutamide (HF) was from Schering, USA. pSG5-AR and pSG5-ARA70 were constructed as previously described (3). pSG5-Rb is a gift from W. Kaelin Jr. Two mutants of the AR gene (mt AR877 derived from prostate cancer, codon 877 mutation Threonine to Serine; and mt ARe708k derived from a partial androgen insensitive syndrome (PAIS) patient, codon 708 mutation Glutamic acid to Lysine), were provided by S. Balk (Beth Israel Hospital, Boston) and H. Shima (Hyogo Medical College, Japan), respectively. Another AR mutant (mtAR888 derived from an androgen insensitive syndrome patient, codon 888 mutation Valine to Methionine) was constructed as described previously (21). pGAL0-AR (wild type) was provided by D. Chen (University of Massachusetts). pGAL4-VP16 was used to construct fusion of ARA70. pGAL0 contains the GAL4 DNA binding domain (DBD) and pGAL4-VP16 contains the activation domain of VP16. pCMX-Gal-N-RB, pCMX-VP16-AR, and pCMX-VP16-AR-LBD were constructed by inserting fragments, Rb (aa 370-928), AR (aa 36-918), and AR (aa 570-918)

into pCMX-gal-N, and pCMX-VP16 respectively. The sequence of construction junction was verified by sequencing.

Cell culture and transfections. Human prostate cancer DU145 cells were maintained in Dulbecco's Minimum Essential Medium (DMEM) containing penicillin (25U/ml), streptomycin (25 μ g/ml), and 5% fetal calf serum (FCS). Transfections were performed using the calcium phosphate precipitation method, as described previously (3, 22). Briefly, 4×10^5 cells were plated on 60-mm dishes 24 hours before adding the precipitate containing AR expression plasmid (wild type or mutated), chloramphenicol acetyltransferase (CAT) reporter gene, and Rb or ARA70 expression plasmid. A β -galactosidase expression plasmid, pCMV- β -gal, was used as an internal control for transfection efficiency. The total amount of DNA was adjusted to 11 mg with pSG5 in all transcriptional activation assays. The medium was changed to DMEM with 5% charcoal-stripped FCS one hour before transfection. Twenty-four hours after transfection, the medium was changed again and treated with DHT or antiandrogens. After 24 hours, cells were harvested for CAT assay, as described previously (3, 22). The CAT activity was visualized by PhosphorImager (Molecular Dynamics) and quantitated by ImageQuant software (Molecular Dynamics). At least three independent experiments were carried out in each case.

Mammalian two-hybrid assay. Transfections in DU145 cells were performed using the calcium phosphate precipitation method. Briefly, DU145 cells were transiently cotransfected with 3 μ g of a GAL4-hybrid expression plasmid, 3 μ g of a VP16-hybrid expression plasmid, and 3.5 μ g of a reporter plasmid pG5CAT. Transfections and CAT assay were performed as described previously (3, 22). A β -galactosidase expression plasmid, pCMV- β -gal, was used as an internal control for transfection efficiency. The total amount of DNA was adjusted to 10.5 μ g with parent vector in all transcriptional activation assays.

GST and GST-Rb-fusion protein isolation. Induction of GST protein synthesis and isolation by affinity chromatography using glutathione-Sepharose beads (Pharmacia) was performed as described previously (23). Full-length Rb fused to glutathione-S-transferase (GST-Rb₁₋₉₂₈), a gift from S. Mittnacht, was expressed and purified from *E. coli* strain BL21pLys as described recently (24).

AR pull-down experiments. Approximately 2 μ g of His-tag column purified baculovirus AR (purity is near 10%) was mixed with GST-loaded glutathione-Sepharose beads in 1 ml of NET-N (20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5% (v/v) Nonidet P-40) and was then rocked for 3 hr at 4°C. Following low-speed centrifugation to pellet the beads, the clarified supernatant was then mixed with GST-Rb₁₋₉₂₈-loaded glutathione-Sepharose beads and rocked for an additional 3 hr at 4°C. The pelleted beads were then washed 5 times with NET-N, mixed with SDS-sample buffer, boiled, and the proteins separated on a 7.5% polyacrylamide gel (25). After electrophoresis, the proteins were transferred to nitrocellulose paper (26) in buffer containing 25 mM Tris-HCl (pH 8.5), 192 mM glycine, 20% (v/v) methanol, and 0.01% SDS. Blots were blocked in TBST (20 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.1% Tween-20) containing 4% nonfat dry milk. Primary antibodies were incubated overnight in TBST containing 2% nonfat dry milk. Blots were developed using alkaline phosphatase-conjugated secondary antibodies.

Co-immunoprecipitation of AR and Rb. 20 μ l of lysates from *in vitro* translated [³⁵S] labeled full-length of AR and 5mg of Rb (QED Bioscience, Inc) were incubated individually or together with or without 10^{-8} M DHT in the modified RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% NP40, 1 mM PMSF, aprotinin, leupeptin, pepstatin, 0.25% Na-deoxycholate, 0.25% gelatin) and rocked at 4°C for 2 hr. The mixture was incubated with mouse anti-Rb antibodies clone G3-245 (Pharmingen) for another 2 hr and protein A/G PLUS Agarose (Santa Cruz) were added and incubated at 4°C for additional 2 hr. The conjugated beads were washed 4 times with RIPA buffer, boiled in SDS sample buffer and analyzed by 8% SDS/PAGE and visualized by STORM 840 (Molecular Dynamics).

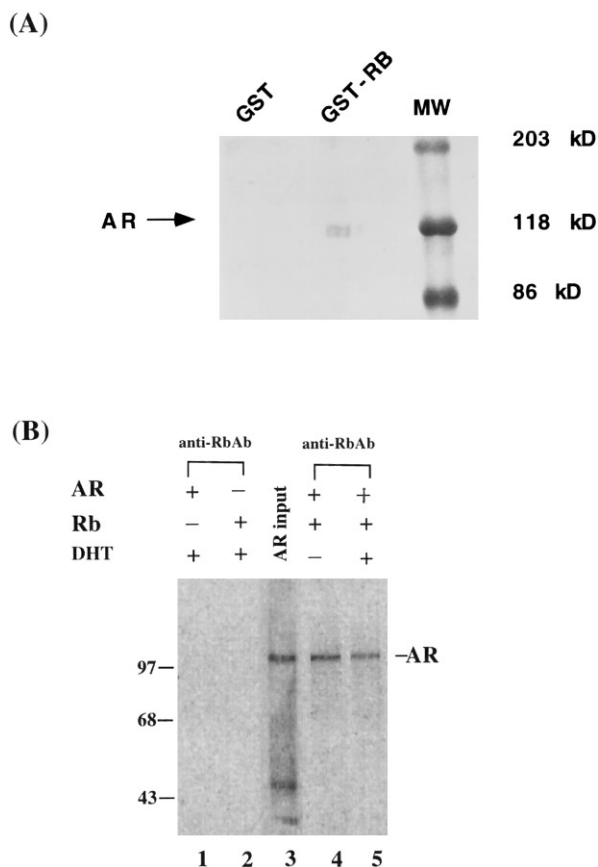


FIG. 1. (A) Detection of AR coprecipitating with GST-Rb. Purified baculovirus expressed AR was allowed to react first with GST-glutathione Sepharose beads (GST), then the clarified supernate allowed to react with GST-Rb₁₋₉₂₈ glutathione Sepharose beads (GST-Rb). The proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with antibody specific for AR. An AR band, with molecular weight closed to 115 kDa, was detected only in GST-Rb lane, but not GST alone. (B) Specific interaction of Rb with AR in DHT-independent manner. The mouse anti-Rb antibody was used here to coprecipitate the *in vitro* translated [³⁵S] labeled AR that interacted with Rb (lane 4, 5). The *in vitro* translated [³⁵S] labeled AR (lane 1) or purified Rb without [³⁵S] labeling (lane 2) was used here as a negative control. The 1/4 amount of input AR was shown in lane 3 to indicate the position of AR. Molecular size markers are shown in kDa.

RESULTS

Interactions between Rb and AR. To demonstrate the potential physical interaction between Rb and AR, we used a GST-Rb₁₋₉₂₈ fusion protein to pull-down AR. The resulting pull-down complexes were subjected to SDS/PAGE-immunoblot analysis using the anti-AR polyclonal antibody NH27 to verify the coprecipitation of AR. As a control, GST-Rb was replaced by GST-alone in the same procedure. As shown in Fig. 1, AR can be found coprecipitating with GST-Rb, but not with GST alone, suggesting that these two proteins can be found in a complex with one another.

In addition to GST-pull down assay, we applied an

immunoprecipitation assay with an anti-Rb antibody (PharMingen) to further confirm the interaction between AR and Rb. This precipitation is specific, as Rb antibody did not precipitate AR in the absence of Rb (Fig. 1B, lanes 1 vs 4 & 5). Interestingly, our data also indicated that this interaction is DHT-independent (Fig. 1B, lanes 4 & 5).

The interaction between Rb and AR was further demonstrated by the mammalian two-hybrid system. For this assay, a Rb fragment (aa 371-928) was generated and fused to the DBD of GAL4. Similarly, near full-length (aa 36-918, missing the first 35 amino acids) AR (nAR) and AR-LBD (aa 570-918) were generated and fused to the transcriptional activator VP16. These fusion plasmids were then coexpressed in DU145 cells with a CAT reporter containing 5 copies of GAL4 DNA binding site. As shown in Fig. 2, transient transfection of either Rb or nAR alone showed no induced-CAT activity (Fig. 2, lanes 1-4). However, the CAT activity can be induced 3-fold by cotransfection of nAR and Rb (Fig. 2, lanes 5 and 6). As a control, we cotransfected nAR and progesterone receptor (PR)-LBD (Fig. 2, lane 7) as well as Rb and ARA70 (Fig. 2, lane 9). Since these results were all negative, these data support our conclusion that the interaction between Rb and nAR is indeed specific. Surprisingly, addition of 10 nM DHT resulted in a negligible difference in the interaction between Rb and nAR (Fig. 2, lanes 5 and 6). The inability of Rb to interact with AR-LBD further suggested the interaction site of AR is located in N-terminal domain and part of DNA-binding domain (aa 36-570) (Fig. 2, lane 8). Taken together, our data suggest that the interaction between Rb and AR is unique in the following ways: first, the interaction is androgen-independent and binding is specific but relatively weak as compared to other AR associated proteins, such as ARA70 (3-fold vs 12-fold induced CAT activity in mammalian two-hybrid assay, data not shown); second, unlike most identified steroid receptor associated proteins that bind to ligand binding domain of steroid receptor, Rb binds to N-terminal domain and part of DNA-binding domain; third, no interaction appears to occur between Rb and ARA70, two AR associated proteins in DU145 cells (Fig 2, lane 9).

Effects of Rb on the transcriptional activity of the wild type and mutant ARs. To address if the interaction between Rb and AR influenced the transcriptional activity of this steroid hormone receptor, we set up a transient transfection system as follows: the DU145 cells, containing a defective Rb with short-sized mRNA transcript (18, 19), were cultured with charcoal-stripped FCS in the absence or presence of 1 nM DHT. When wild type AR and Rb were transiently transfected (at the ratio of 1:3) in DU145 cells without adding androgen, there was no AR transcriptional activity observed. However, AR transcriptional activity could

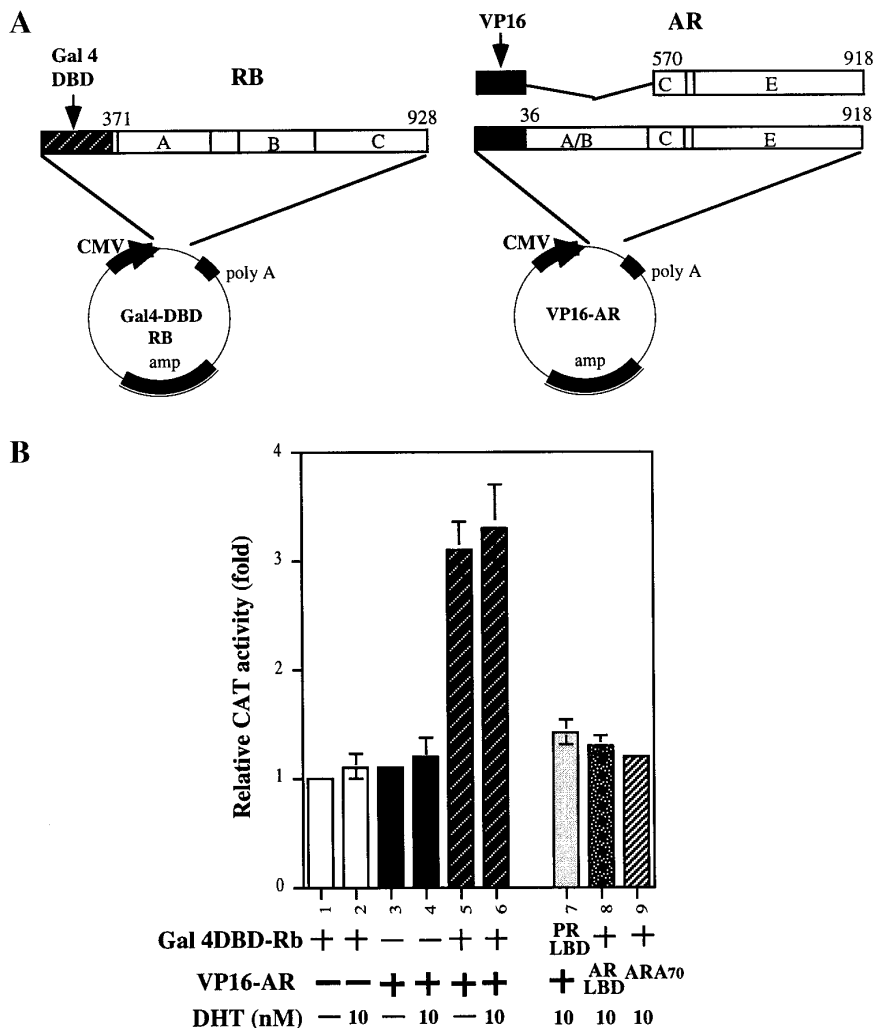


FIG. 2. The interaction of Rb and AR tested in mammalian two-hybrid is DHT-independent. The protein-protein interaction between Rb and AR and other VP16 or GAL4-DBD fusions were tested in mammalian two-hybrid assay. (A) A schematic representation of the constructs of GAL4-DBD Rb and VP16-ARs (B) The protein-protein interaction between Rb and AR and other VP16 or GAL4-DBD fusions were tested in mammalian two-hybrid assay. The fusion genes used include GAL4DBD-Rb, PR LBD, VP16-AR₃₆₋₉₁₈, VP16-AR₅₇₀₋₉₁₈, VP16-ARA70.

be induced to 5-fold when wild type AR was expressed in 1 nM DHT. Cotransfection of Rb with AR can further enhance the AR transcriptional activity from 5-fold to 21-fold in the presence of 1 nM DHT (Fig. 3, lanes 5 & 6). Although our data indicated that the physical association of Rb to AR is ligand independent, the ligand is indeed essential for the functional activation of AR by Rb (Fig. 3, lane 6 vs lane 2). As a control, cotransfection of ARA70 can also enhance the AR transcriptional activity from 5-fold to 36-fold as previously reported (3). Interestingly, when Rb and ARA70 were cotransfected with AR in DU145 cells, the induction of AR transcriptional activity can be additively increased to 13-fold (from 5-fold to 64-fold) (Fig. 3, lane 8).

In our previous report, we have found that ARA70 can confer the partial antagonist activity of hydroxy-

flutamide (HF) to wt AR (27). Our further studies also indicated that cofactors may contribute to the 17 β -estradiol (E2)-mediated AR transactivation (28). We then replaced 1 nM DHT with 10 nM E2 or 1 μ M HF. As shown in Fig. 3, upon transfection of wild type AR without Rb or ARA70, only marginal induction (less than 2-fold) was detected in the presence of 10 nM E2 or 1 μ M HF. Upon E2 and HF treatment, the cotransfection of the wild type AR with Rb can not effectively potentiate the transactivation of AR. In contrast, ARA70 can enhance the AR transcriptional activity to 12-fold (E2) or 3-4-fold (HF), and cotransfection of Rb and ARA70 with AR can further enhance the AR transcriptional activity to 36-fold (E2) or 12-fold (HF), which indicate the cooperative effect between different cofactors.

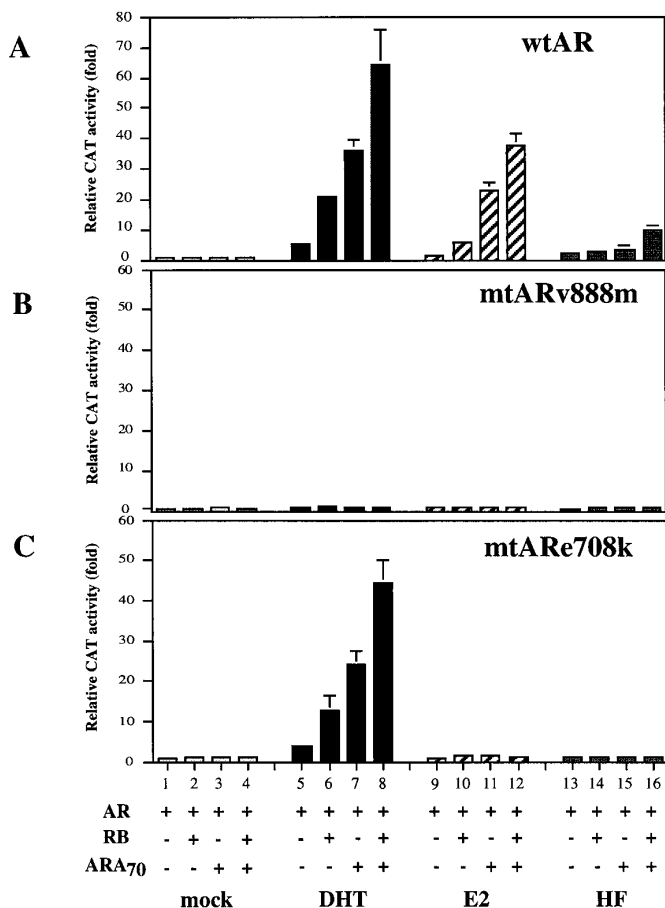


FIG. 3. Effect of Rb on the transcriptional activity of the wild-type AR (A), mt ARv888m (B), and mt ARE708k (C). CAT activity was determined in DU145 cells cotransfected with the expression plasmids of ARs (1.0 μ g) with or without Rb (3.0 μ g) and/or ARA70 (3.0 μ g), and the reporter plasmid MMTV-CAT (3.0 μ g). After transfection, cells were incubated without hormones (mock) or with 1 nM DHT, 10 nM E2, or 1 μ M HF for 24 hours. Values are the mean \pm SD of at least three determinations.

We then extended these findings to include two different AR mutants: mutant AR (mt ARv888m, codon 888 mutation Valine to Methionine) was derived from an androgen insensitive syndrome patient and defective in ligand binding (21); and mt ARE708k with a mutation at amino acid 708 (Glutamic acid to Lysine, named ARE708k) from a partial-androgen-insensitive patient (28). As shown in Fig. 3B, no inductions were obtained when wild type AR was replaced by mt ARv888m. In contrast, while a similar induction was also detected in the presence of 1 nM DHT when we replaced wild type AR with mt ARE708k (Fig. 3C, lanes 5-8), there was almost no induction by cotransfection of mt ARE708k with Rb and/or ARA70 in the presence of 10 nM E2 or 1 μ M HF (Fig. 3C, lanes 9-16). These results indicate that Rb and ARA70 can additively induce the transcriptional activity of wild type AR in the presence of 1 nM DHT, 10 nM E2 or 1 μ M HF. However,

Rb and ARA70 additively induce the transcriptional activity of mt ARE708k only in the presence of 1 nM DHT, but not 10 nM E2, or 1 μ M HF. The inclusion of mutant ARv888m (21), that lost androgen binding, here is to support the fact that, while interaction between Rb and AR is androgen-independent (Fig. 2), the androgen binding remains essential for the transcriptional activity of AR-Rb complex. Also, the differential induction of DHT and E2/HF in mt ARE708k supports the hypothesis that the amino acid 708 in AR may play a vital role for the recognition of androgen vs antiandrogens and for the consequent activation by cofactors, Rb and ARA70.

Rb functions as a relatively specific coactivator for AR in prostate DU145 cells. In our previous report (27), we have found that ARA70 can potentiated the transactivation of wt AR or mutant AR with a mutation on codon 877 (Threonine to Serine and named mtARt877s), which was isolated from androgen-independent prostate cancer (29). It becomes of interest to compare the Rb-mediated transactivation on wt AR and this mtARt877s. We also examined the effect of Rb and ARA70 on the transcriptional activity of other steroid receptors through their cognate DNA response elements [MMTV-CAT for AR, mtARt877s, glucocorticoid receptor (GR), and progesterone receptor (PR); ERE-CAT for estrogen receptor (ER)].

As shown in Fig. 4, while Rb and ARA70 can additively induce AR and mt ARt877s transcriptional activity 13-fold (from 5-fold to 64-fold), Rb and ARA70 only have marginal induction on the transcriptional activity of GR, PR, and ER in DU145 cells. These results sug-

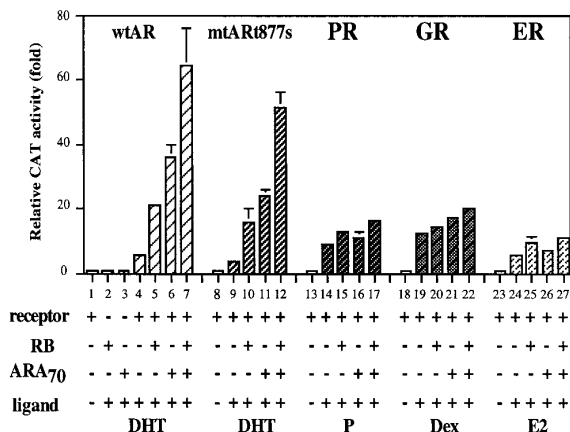


FIG. 4. Effect of Rb on the transcriptional activity of various steroid receptors. CAT activity was determined in DU145 cells cotransfected with the expression plasmid (1.0 μ g of various receptors and reporters, including wild type AR/MMTV-CAT, mt ARt877s/MMTV-CAT, PR/MMTV-CAT, GR/MMTV-CAT, or ER/ERE-CAT. After transfection, cells were incubated without hormones (mock) or with their own ligand (1 nM DHT, 10 nM P, 10 nM Dex, or 10 nM E2) for 24 hours. Values are the mean \pm SD of at least three determinations.

gest that Rb and ARA70 are more specific coactivators for AR in prostate DU145 cells. However, we can not rule out the possibility that our assay conditions for prostate DU145 cells may be a particularly favorable environment for Rb and ARA70 to specifically function as coactivators for AR; perhaps Rb and ARA70 can function as stronger coactivators for GR, PR, and ER in other cell types under different conditions.

Taken together, our results indicate that Rb and ARA70 associate with AR in N-terminus and ligand binding domain, respectively. Both cofactors are more specific coactivators for AR in prostate cancer DU145 cells. In addition, the fact that Rb and ARA70 can induce transcriptional activity of both wild type AR and mutated ARt877s, that occur in prostate tumors, strongly argues for the importance of Rb and ARA70 in normal prostate as well as prostate tumor.

DISCUSSION

The activity of Rb in cell cycle control is related essentially to its ability to bind to several proteins, thus modulating their activity. To date, many cellular proteins have been reported which bind to Rb (5). These include a number of transcription factors, a putative regulator of ras, nuclear structural proteins, a histone deacetylase, a protein phosphatase, and several protein kinases. Whether all of these proteins actually complex, and are regulated by Rb in cells, remains to be elucidated.

Much attention has been given to the functional interaction between Rb and transcription factors. To date, several of these factors have been shown to form complexes with Rb in cells. Such complex formation and subsequent function studies have revealed that the modulating activity of Rb can take the form of repression of transcription as with E2F (11), or activation as with NF-IL6 (12) and the hBrm/BRG1 complex (13). Here, we show that Rb can directly bind to AR and induce the AR transcriptional activity. To our knowledge, this is the first demonstration of a negative growth regulatory protein functioning in a positive manner, by initiating transcription via a signal transduction mechanism involving binding to a nuclear receptor. Meanwhile, Dr. Danielsen's group (Georgetown university, Washington, DC) also found that the AR transactivation promoted by Rb would be inhibited by association of Rb with SV40 large T-antigen, HPV E7 or adenovirus E1a³. When placed in the context of regulating the cell cycle and differentiation, these data indeed suggest a previously undescribed function for Rb which underscores the importance of this protein in regulating transcription by directly binding to transcription factors, but this protein can also regulate

transcription by stimulating at least one type of signal transduction mechanism.

While the study of Rb expression in human breast tumor did suggest a relationship between Rb expression and response to endocrine therapy (30), another study also indicated that Rb gene alterations can occur in all grades and stages of prostate cancer, in localized as well as metastatic disease (4). How to link Rb function to androgen-dependent status in prostate tumor progression, at this time, remains unclear. However, one of the possible explanations is that Rb alteration may be a necessary event in prostate carcinogenesis, but only for a subset of prostatic neoplasms (4). The same may also be true for the AR expression in prostate tumors. The other possibility involves the phosphorylation/dephosphorylation of Rb. Cell proliferation may be the result of phosphorylated Rb (by cyclin dependent kinase) and early study also suggested that hyperphosphorylation of Rb may play some important roles in the E2-induced cell proliferation (31). Therefore, phosphorylated Rb may be able to stimulate cell proliferation by enhancing the AR transcriptional activity in androgen-dependent cells and loss of Rb may lead cells to the androgen-independent state.

In conclusion, our discovery that Rb can function as a coactivator to induce AR transcriptional activity in prostate cells may represent the first data to link a negative growth regulatory protein function in a positive manner, by inducing the transcriptional activity of AR, the major promoter for prostate tumor growth. Further study of Rb and AR may allow us to better understand the roles of these two proteins in prostate cancer progression.

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REFERENCES

1. Parker, S. L., Tong, T., Bolden, S., and Wingo, P. A. (1997) *Cancer Journal for Clinicians* **47**, 5-27.
2. McPhaul, M. J., Marcellis, M., Zoppi, S., Griffin, J. E., and Wilson, J. D. (1993) *J. Clin. Endocrinol. Metab.* **76**, 17-23.
3. Yeh, S., and Chang, C. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 5517-5521.
4. Brooks, J. D., Stevenbora, G., and Isaacs, W. B. (1995) *Prostate* **26**, 35-39.
5. Weinberg, R. A. (1995) *Cell* **81**, 323-330.
6. Kranenburg, O., van der Eb, A. J., and Zantema, A. (1995) *FEBS Lett.* **367**, 103-106.
7. Dowdy, S. F., Hinds, P. W., Louie, K., Reed, S. I., Arnold, A., and Weinberg, R. A. (1993) *Cell* **73**, 499-511.
8. Chen, P. L., Scully, P., Shew, J. Y., Wang, J. Y. J., and Lee, W. H. (1989) *Cell* **58**, 1193-1198.

³ Which was reported in the abstract of 1998 Keystone Symposium.

9. Mihara, K., Cao, X. R., Yen, A., Chandler, S., Driscoll, B., Murphree, A. L., T'Ang, A., and Fung, Y. K. (1989) *Science* **246**, 1300–1303.
10. Ludlow, J. W., Glendening, C. L., Livingston, D. M., and DeCaprio, J. A. (1993) *Mol. Cell. Biol.* **13**, 367–372.
11. Weintraub, S. J., Chow, K. N., Luo, R. X., Zhang, H. S., He, S., and Dean, D. C. (1995) *Nature* **375**, 812–815.
12. Chen, P. L., Riley, D. J., Chen-Kiang, S., and Lee W.-H. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 465–469.
13. Singh, P., Coe, J., and Hong, W. (1995) *Nature* **374**, 562–565.
14. Peterson, C. L., and Tamkun, W. (1995) *Trends Biochem. Sci.* **20**, 143–146.
15. Brem, A. Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998) *Nature* **391**, 579–601.
16. Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorainm, S., Le Villain, J. P., Troalen, F., Trouche, D. and Harel-Bellan, A. (1998) *Nature* **391**, 601–605.
17. Bookstein, R., Shew, J. Y., Chen, P. L., Scully, P., and Lee, U. H. (1990) *Science* **247**, 712–715.
18. Sarkar, F. H., Sakr, W., Li, Y. W., Malorka, J., and Crissman, J. D. (1992) *Prostate* **21**, 145–152.
19. Bookstein, R., Rio, P., Madreperla, S., Hong, F., Allred, C., Grizzle, W. E., and Lee, W. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7762–7766.
20. Day, M. L., Foster, R. G., Day, K. C., Zhao, X., Humphrey, P., Swanson, P., Postigo, A. A., and Zhang, S. H. (1997) *J. Biol. Chem.* **272**, 8125–8128.
21. Mowszowicz, I., Lee H.-J., Portois, M.-C., Kuttenn, F., and Chang, C. (1993) *Endocrine J.* **1**, 203–209.
22. Mizokami, A., and Chang, C. (1994) *J. Biol. Chem.* **269**, 25655–25659.
23. Kaelin, W. G., Jr., Pallas, D. C., DeCaproi, J. aA., Kaye, F. J., and Livingston, D. M. (1991) *Cell* **64**, 521–532.
24. Zarkowska, T. and Mitnacht, S. J. (1997) *J. Biol. Chem.* **272**, 12738–12746.
25. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
26. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
27. Yeh, S., Miyamoto, H., and Chang, C. (1997) *Lancet* **349**, 852–853.
28. Yeh, S., Miyamoto, H., Shima, H. and Chang, C. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 5527–5532.
29. Taplin, M.-E., Bublely, G. J., Shuster, T. D., Frantz, M. E., Spooner, A. E., Ogata, G. K., Keer, H. N., and Balk, S. P. (1995) *N. Engl. J. Med.* **332**, 1393–1398.
30. Anderson, J., Tiniakos, D., Mcintosh, G., Autzen, P., Henry J., Thomas, M., Reed, J., Horne, G., Lennard, T., Angus, B., and Horne, C. (1996) *J. Pathology* **180**, 65–70.
31. Hurd, C., Khattree, N., Dinda, S., Alban, P., and Moudgil, V. K. (1997) *Oncogene* **15**, 991–995.