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The second largest subunit of RNA polymerase II interacts with and enhances transactivation of androgen receptor☆

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Abstract

AR may communicate with the general transcription machinery on the core promoter to exert its function as a transcriptional modulator. Our previous reports demonstrated that AR interacted with TFIIH and positive transcription elongation factor b (P-TEFb), and that phosphorylation of the carboxy-terminal domain in the largest subunit of RNA polymerase II might play important roles in AR-mediated transcription. These results suggest that AR may modulate gene expression by enhancing the efficiency of transcriptional elongation. Here we further demonstrate that co-expression of the second largest subunit of RNA polymerase II (RPB2) enhances AR transactivation. However, co-expression of the other subunits of RNA polymerase II or TFIIB did not show preferential enhancement of AR-mediated transcription. Furthermore, co-transfection of RPB2 with ER showed little effect on enhancement of ER transactivation. Together, AR may be able to interact with TFIIH, P-TEFb, and RPB2 to enhance transcription from AR target genes, such as prostate specific antigen that may play important roles in the prostate cancer progression.

Keywords: AR; PSA; RPB2; RNA polymerase II; Transcription; Transcriptional elongation; TFIIH

The androgen receptor (AR) is a member of the steroid receptor superfamily that is composed of a variable amino-terminal domain, a highly conserved DNA-binding domain, and a ligand-binding domain [1–3]. Ligand-dependent transcriptional activation of steroid receptors is mediated by the carboxy-terminal domain that includes a ligand-binding domain and activation function-2 [4]. Crystallographic studies show that ligand-bound steroid receptors undergo a conformational change in the activation function-2 core motif [5,6]. The ligand-induced conformational change presumably recruits co-regulators as well as the basal transcriptional machinery for the target gene expression [7–11]. Numerous co-regulators of nuclear receptors have recently been cloned and characterized [7–10]. It has been proposed that co-regulators function as a bridge between activators and the basal transcription machinery [7–11]. They may potentiate transactivation of nuclear receptors in transient transfection or in in vitro transcription assays through the modification of nucleosomal structure or the efficient recruitment of basal transcription machinery [7,9,11,12].

The amino-terminal domain of steroid receptors contains a ligand-independent activation function-1, which is under the control of activation function-2 [4]. The amino-terminal domain of steroid receptors has been reported to interact with general transcription factors, as exemplified by AR interaction with transcription factor IIF (TFIIF) [13] and transcription factor IIH (TFIIH) [14]. Transcription factor IIB (TFIIB) has been reported to interact with thyroid receptor [15], vitamin D receptor [16], and hepatocyte nuclear factor 4 [17]. However, the molecular mechanism by which activation function-1 synergistically activates transcription remains unclear.

☆ *Abbreviations:* AR, androgen receptor; ARE, androgen response element; CTD, C-terminal domain of RNA polymerase II largest subunit RPB1; DHT, dihydrotestosterone; E2, 17-β-estradiol; ER, estrogen receptor; GST, glutathione *S*-transferase; PSA, prostate specific antigen; P-TEFb, positive-transcription elongation factor b; RPB1, RNA polymerase II subunit 1; RPB2, RNA polymerase II subunit 2; SDS, sodium dodecyl sulfate; TFIIF and TFIIH, transcription factor IIF and IIH.

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Transcription of eukaryotic genes requires general transcription machinery as well as a variety of activators and co-factors that modulate efficiency of transcription initiation, elongation, and termination [18]. Our previous reports demonstrated that AR interacts with TFIIH and P-TEFb under physiological conditions [14,19]. AR interaction with TFIIH and P-TEFb may enhance the efficiency of transcriptional elongation of androgen-target genes, such as prostate specific antigen (PSA) [19]. Based on intensive molecular and biochemical studies of transcription mechanisms [20], enhancement of transcriptional elongation of androgen-target genes reported in our previous studies [19] may result from efficient phosphorylation of the C-terminal domain (CTD) in the largest subunit of RNA polymerase II (RPB1). This can be supported by our previous report demonstrating that the low concentration of DRB, a CTD kinase inhibitor, inhibited AR transactivation, while it did not inhibit SV40 enhancer-mediated transcription [19].

In an effort to further dissect the mechanisms implicated in AR transactivation, we report here that the second largest subunit of RNA polymerase II (RPB2) interacts with AR. Co-expression of RPB2 resulted in preferential enhancement of AR-mediated transcription, while co-expression of the other subunits of RNA polymerase II as well as TFIIB did not show preferential enhancement of AR-mediated transcription. In addition, co-expression of RPB2 did not enhance estrogen receptor (ER) transactivation. A few RNA polymerase II subunits, such as RPB1, RPB5, and RPB7, have been reported to enhance transcription from specific promoters [21–24]. Recently, RPB2 and RPB10α have been reported to interact with BRCA-1 and enhance BRCA-induced transcription in vitro [25]. In addition, RPB10α, but not RPB2, enhanced Sp1-dependent activation in vitro, indicating that activators may interact with a certain set of RNA polymerase II subunits to enhance transcription [25]. Genetic and biochemical studies demonstrated that mutations in RPB2 increased transcriptional arrest, indicating that RPB2 plays a key role in the elongation stage of transcription [26,27]. AR interaction with RPB2 further supports the hypothesis that AR increases efficiency of transcriptional elongation. Taken together with our previous reports, AR may modulate directly TFIIH, P-TEFb, and RPB2 for the efficient phosphorylation of the CTD of RPB1 in the transcriptional preinitiation complex to enhance transcription of androgen target genes, such as PSA, upon androgen induction.

Materials and methods

Plasmids. The plasmids encoding GST-fused RNA polymerase subunits [25] and the expression plasmid of RPB2 [30] were generous gifts. The complementary DNA fragments of RPB subunits were obtained by polymerase chain reaction and subcloned into the eukaryotic expression vector pSG5 (Stratagene).

Cell culture and transfection assay. COS-1 and PC-3 cells were maintained in DMEM (Gibco/BRL) supplemented with 10% fetal bovine serum (FBS). All media contain 50 U/ml penicillin and 50 μg/ml streptomycin. Cells were seeded to be at a density of 40–60% confluency for transfection. Cells in 12-well plates were refed with fresh medium 2 h before transfection and transfected with 1.5 μg DNA according to the "SuperFect Transfection" instruction (Qiagen). After 2–3 h incubation, cells were treated with medium supplemented with charcoal-dextran treated FBS containing either ethanol or ligands. Cells were further incubated at 37 °C for 24 h, washed with PBS, and harvested. Cell lysates were prepared and used for luciferase assay according to the manufacturer's instructions (Promega). Relative luciferase activities were plotted using the activity of AR in the presence of ligand as 1. The results were obtained from at least three sets of transfection and presented as means ± S.D.

Biochemical binding (GST pull-down) assay. Recombinant proteins of GST-fused RPB subunits were expressed in *Escherichia coli*. Bacterial cells were lysed in 5 ml of binding buffer (20 mM Hepes/pH 7.5, 100 mM NaCl, 5 mM β-mercaptoethanol, and 10% glycerol). Recombinant proteins were purified using glutathione-resin (Pharmacia) affinity chromatography according to the manufacturer's instructions. The amounts of GST-fused proteins were adjusted to an equal molar ratio for the binding assay.

Proteins obtained from 50 ml culture were incubated with 100 μl bed volume of glutathione-resins. The resins were incubated with ^{35}S-labeled TNT expressed AR for 4 h and washed extensively with 20 mM Hepes/pH 7.5, 0.5 mM EDTA, 20% glycerol, and 100 mM NaCl. The bound proteins were eluted by 2× SDS loading buffer (100 mM Tris–HCl/pH 6.8, 4% SDS, 20% glycerol, and 200 mM β-mercaptoethanol), separated on 8% SDS–polyacrylamide gels, and analyzed using Molecular Dynamics PhosphorImager.

Biochemical binding assay with TNT expressed proteins. TNT-expressed ^{35}S-labeled FLAG-tagged RPB2, AR, AR-N terminal domain AR-DNA binding domain, and ligand-binding domain, were prepared according to the manufacturer's instructions (Promega). The M2 affinity resins (Sigma) coupled with anti-FLAG antibody (20 μl bed volume) were incubated with the TNT-expressed proteins for 8 h at 4 °C and then extensively washed with 1× PBS. The bound proteins were eluted with the 2× SDS loading buffer, analyzed on SDS–polyacrylamide gels, and visualized by PhosphorImager.

Immunoprecipitation assay of RNA polymerase II. COS-1 cells in 60 mm culture dish were transfected with pSG5-fRPB2 using SuperFect. Cells were grown in DMEM supplemented with 10% FBS for 24 h and rinsed with PBS. About 200 μl of RIPA buffer was added and total cell lysate was collected. Protein A/G resins (Santa Cruz) coupled with anti-CTD monoclonal antibody (generous gift from Dr. R.G. Roeder) were incubated with 170 μl of total cell lysate for 8 h at 4 °C. Unbound proteins were collected by centrifugation of the resins at 2000 rpm and the resins were extensively washed with 1× PBS. Bound proteins were eluted with SDS loading solution and analyzed on SDS–polyacrylamide (7–16% gradient) gels along with 15% input and 15% unbound proteins. Proteins were transferred to a membrane and incubated with anti-CTD, anti-FLAG (Sigma), and anti-RPB6 (generous gift from Dr. R.G. Roeder) antibodies.

Results

AR interaction with RPB2

Biochemical studies of protein–protein interactions between AR and the general transcription factors indicated that AR may interact with TFIIF, TFIIH, and P-TEFb under physiological conditions [13,14,19]. Based

on biochemical and molecular studies of transcriptional regulation [18,28], AR interaction with TFIIH and P-TEFb implies a possibility that the androgen-AR signaling pathway may enhance the efficiency of transcriptional elongation of AR target genes. This idea was further supported with the observation that AR transactivation was preferentially inhibited at the low concentration of DRB, a CTD kinase inhibitor, which inhibits efficiency of transcriptional elongation [19].

The importance of CTD phosphorylation in RPB1 for efficient transcriptional elongation has been well documented [20,28,29]. In addition to the CTD of RPB1, biochemical and genetic studies indicated that RPB2 may also play important roles in transcriptional elongation [26,27]. Mutations in RPB2 inhibited interaction with the general elongation factor SII and severely increased transcriptional arrest, demonstrating that RPB2 may play important roles in transcriptional elongation [26]. Since our previous studies indicated that the androgen-AR signaling pathway increases the efficiency of transcriptional elongation [19], it is likely that RPB2 may also play a key role in AR transactivation. To test this hypothesis, glutathione *S*-transferase (GST)-fused selective RPBs [25] and TNT-expressed ^{35}S-labeled AR were used to assay the potential interaction between AR and RPB2. Due to the low expression and/or solubility of RPB2 in bacteria, RPB2 was divided into four fragments for GST-fusion proteins [25]. Considering differences of molecular weights, equal molar ratios of GST-fused proteins were used. As shown in Fig. 1A, AR preferentially interacts with the amino-terminal domain (amino acids 1–606) of RPB2. AR was not retained in resins containing GST-RPB6 (lane 6) or GST alone (lane 8), indicating that AR interaction with RPB2 was specific. AR interacts with RPB10α, but to a lesser degree than with RPB2 (lanes 2 and 7 in Fig. 1A).

Since arbitrary division of RPB2 into four fragments used in the GST pull-down assay may alter conformation of RPB2, a co-immunoprecipitation assay of AR and FLAG-tagged RPB2 (fRPB2) was performed (Fig. 1B). Radioisotope labeled AR and fRPB2 were prepared by in vitro transcription/translation and immunoprecipitated using M2 affinity resins coupled with anti-FLAG antibody. AR was detected in the immunoprecipitated samples with anti-FLAG antibody only in the presence of fRPB2 but not in the absence of fRPB2, indicating that AR interacted with the full-length RPB2. Further detailed binding assays were performed to narrow down the domains in AR important for interaction with RPB2. As shown in Fig. 1C, RPB2 interacts mainly with the AR LBD.

Co-expression of RPB2 with AR resulted in preferential enhancement of AR transactivation

Although RNA polymerase II is required for transcription of every gene encoding mRNAs, the roles of each subunit in transcription remain largely unclear. A few RPBs, such as RPB1, RPB5, and RPB7, have been reported to enhance transcription from specific promoters [21–24]. Recently, RPB2 and RPB10α have been reported to interact with BRCA and enhance BRCA-induced transcription in vitro [25]. In addition, RPB10α, but not RPB2, enhanced Sp1-dependent activation in vitro [25]. Thus, activators seem to interact with a certain set of RPBs to enhance transcription.

Our previous reports [14,19] support the idea that AR may interact with TFIIH and P-TEFb for the efficient phosphorylation of CTD in RPB1 to enhance AR transactivation. Genetic and biochemical studies using yeast demonstrated the importance of RPB1 and RPB2 in transcriptional elongation [26,27,29]. Thus, we examined whether AR interacts with RPB2 for the efficient AR transactivation. It is necessary to compare the effect of various RPBs on transcription from the promoters containing androgen responsible elements (AREs) with the effect on transcription from the other promoters containing no AREs. We took advantage of a dual luciferase assay (Promega) using reporter plasmids and internal control plasmid. The luciferase activity from pPSA-luciferase and p(ARE)4-luciferase represents AR-mediated transcription, while the luciferase activity from pRLSV40-luciferase represents SV40 enhancer-mediated transcription. Since the upstream promoter elements may influence transcription efficiency, we used two different reporter plasmids containing ARE in the different context of upstream *cis*-acting elements in order to avoid possible artificial effects due to the difference in promoter structure.

Human prostate cancer PC-3 cells were co-transfected with a reporter plasmid, pRLSV40-luciferase, the AR expression plasmid, and variable amounts of the expression plasmids for various RPBs and TFIIB. When we used p(ARE)4-luciferase as a reporter plasmid, RPB2 enhanced AR-mediated transcription 3–4-fold (lane 2 vs lane 5 in Fig. 2A). In contrast, the other RPBs and TFIIB showed little enhancement on the AR-mediated transcription (lane 2 vs lanes 6–20). None of RBPs and TFIIB enhanced AR transactivation in the absence of androgen (data not shown). When we used another reporter plasmid containing the natural promoter of AR target gene, pPSA-luciferase, similar results were obtained (Fig. 2B). These results suggest that RPB2 is able to enhance AR transactivation in the various contexts of upstream *cis*-acting regulatory elements. The enhancement of AR transactivation by RPB2 was also observed when we replaced human prostate cancer PC-3 cells with COS-1 cells (Fig. 3). Although the biochemical binding assay indicates that AR interacts with RPB10α to some degree (lane 7 in Fig. 1), co-expression of RPB10α showed little influence on the AR-mediated transcription (lanes 12–14 in Figs. 2 and 3). All together, AR interaction with RBP2

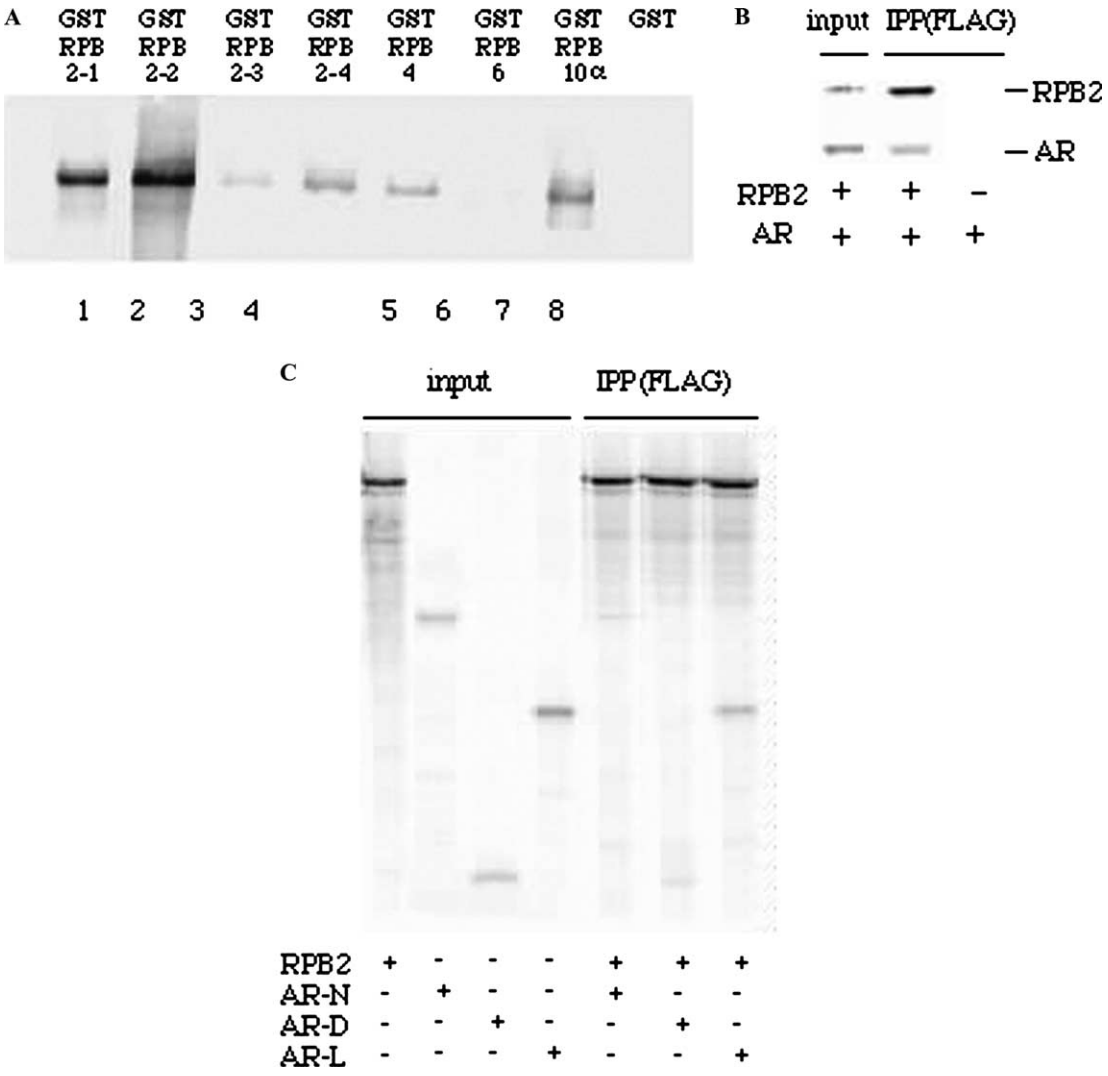

Fig. 1. AR interacts with the N-terminal domain of RPB2. (A) The GST-fused RPB2-1 (amino acids 1–314), RPB2-2 (amino acids 315–606), RPB2-3 (amino acids 607–876), RPB2-4 (amino acids 877–1174), RPB4, RPB6, RPB10α, and GST were expressed in *E. coli* and purified. Equal molar amounts of purified GST-fused proteins were incubated with glutathione-resins and TNT-expressed ^{35}S-labeled AR in the binding buffer as described in Materials and methods. The resins were extensively washed with the binding buffer and the proteins retained on resins were analyzed on SDS–polyacrylamide gels. (B) Radioisotope labeled AR and FLAG-tagged PRB2 were prepared by TNT expression (Promega). The TNT expressed proteins were incubated with anti-FLAG antibody coupled M2 resins (Sigma) for 8 h. The resins were extensively washed with PBS. The bound proteins were eluted, separated on SDS–polyacrylamide gels along with 8% input proteins, and analyzed by PhosphorImager. (C) Radioactively labeled proteins were incubated with M2 resins for 8 h. The resins were extensively washed with PBS. The bound proteins were eluted, separated on SDS–polyacrylamide gels, and analyzed by PhosphorImager. About 10% of each protein, FLAG-tagged RPB2, AR N-terminal domain (AR-N), AR-DNA binding domain (AR-D), and AR-ligand binding domain (AR-L), is shown as input proteins.

increased the efficiency of AR-mediated transcription in a ligand-dependent manner.

Surprisingly, when ER was used instead of AR, RPB2 did not enhance ER transactivation in various conditions of transient transfection (Fig. 4). These results indicate that RPB2-mediated enhancement of AR transactivation may be activator-specific. The contrasting effect of RPB2 on AR and ER transactivation is striking, since AR and ER belong to the sex steroid hormone receptor family, yet they may utilize different mechanisms to communicate with the general transcription machinery on the core promoter.

Effects of various ligands on RPB2-induced AR transactivation

Effects of RPB2 on enhancement of AR transactivation were further analyzed in the presence of various agonist and antagonist ligands, such as dihydrotestosterone (DHT), testosterone (T), 17-β-estradiol (E2), and hydroxyflutamide (HF). RPB2 did not enhance AR activity in the absence of appropriate ligands (lanes 1 and 7 in Fig. 5). As expected, RPB2 enhanced AR transactivation by an average of 3-fold in the presence of DHT at 10^{-9} M and T at a slightly higher concentration of

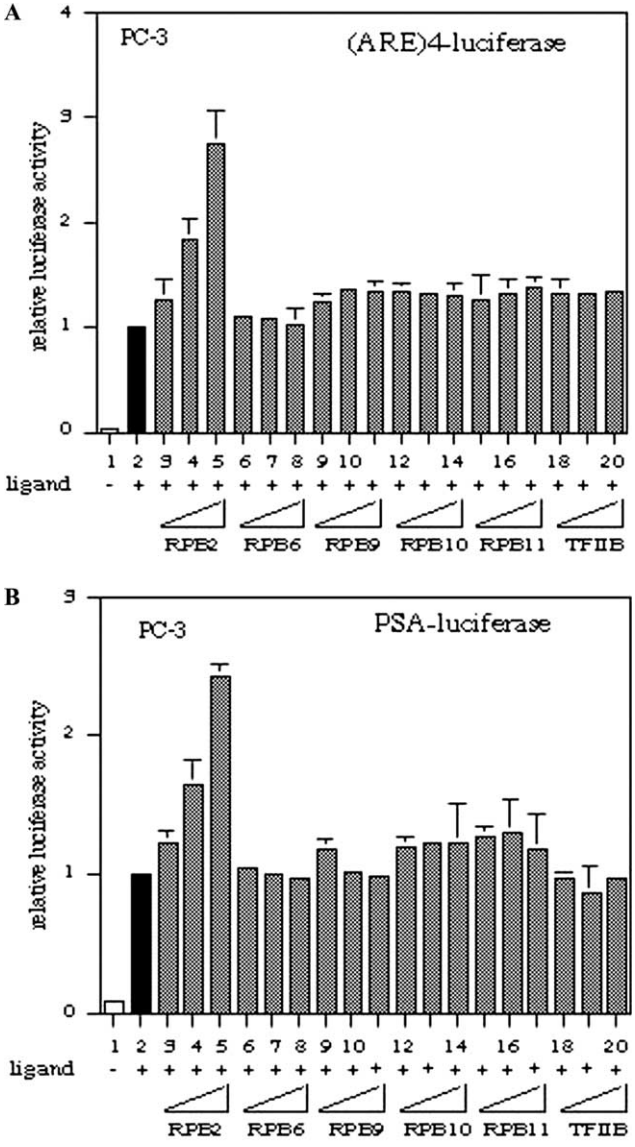

Fig. 2. Co-expression of RPB2, but not the other RPBs, enhanced AR transactivation in PC-3 cells. (A) AR-negative PC-3 cells were transiently transfected using SuperFect transfection reagent (Qiagen) with 350 ng p(ARE)4-luciferase reporter plasmid, 30 ng pRLSV40-luciferase as an internal control, 100 ng AR expression plasmid, and without or with increasing amounts of the pSG5-RPBs or pSG5-TFIIB expression plasmids as indicated. Considering differences in molecular weights of the expression plasmids (8 kb for pSG5-RPB2 and 4.5–5 kb for the other pSG5-RPBs), 300–1000 ng pSG5-RPB2 and 180–720 ng of the other pSG5-RPBs were used. The total amounts of plasmids were adjusted to 1.5 μg with vector plasmid pSG5. The result obtained in the absence and presence of 10 nM DHT is shown as open and closed bars, respectively. The results obtained in the presence of 10 nM DHT and RPBs or TFIIB are shown in shaded bars. Relative luciferase activities were plotted using the activity without RPBs as 1. (B) Experiments were performed and analyzed as described in A using pPSA-luciferase instead of p(ARE)4-luciferase.

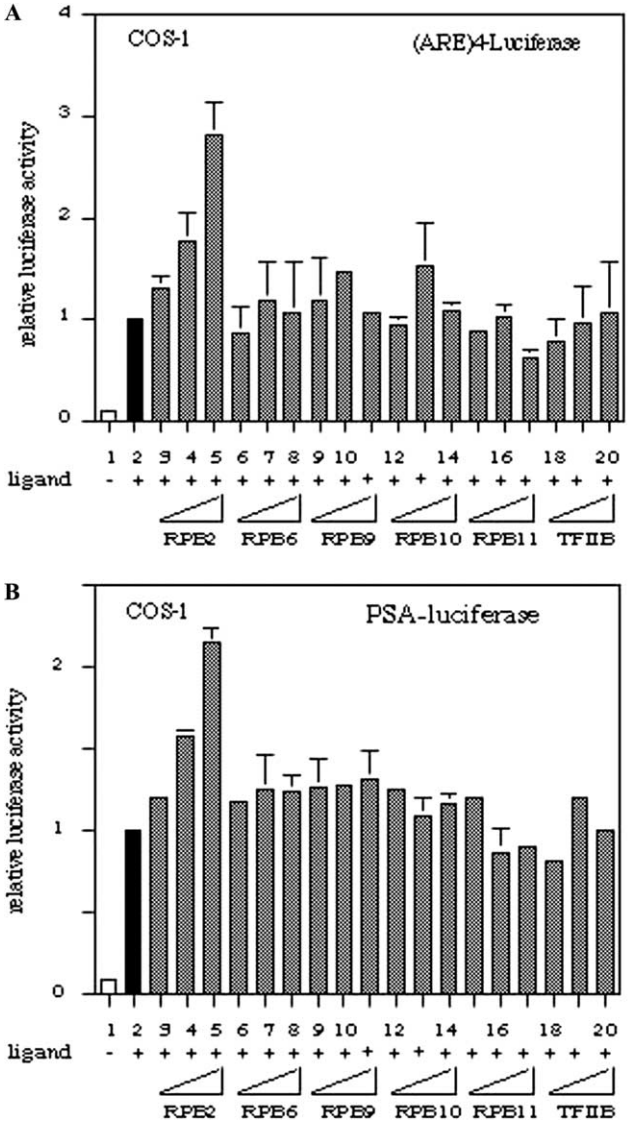

Fig. 3. Co-expression of RPB2, but not the other RPBs, enhanced AR transactivation in COS-1 cells. Experiments were performed and analyzed as described in Fig. 2 using COS-1 cells.

10^{-8} M (lanes 5 and 6 vs 11 and 12). Although the relative transactivation activity of AR in the presence of E2 was much lower than that in the presence of androgens (lanes 2 and 3 vs lanes 5 and 6), co-expression of RPB2 was still able to enhance E2-mediated AR transactivation at a concentration of 10^{-7} or 10^{-6} M (lanes 2 and 3 vs lanes 8 and 9). In contrast, RBP2 showed little effect on AR transactivation in the presence of HF at the pharmacological concentration 10^{-6} M (lane 4 vs 10).

Discussion

AR may communicate with the general transcription machinery on the core promoter to exert its function as a transcriptional modulator. The molecular communication between AR and the general transcription machinery may be achieved either by the direct protein-protein interaction between AR and the general transcription machinery or by the indirect interaction

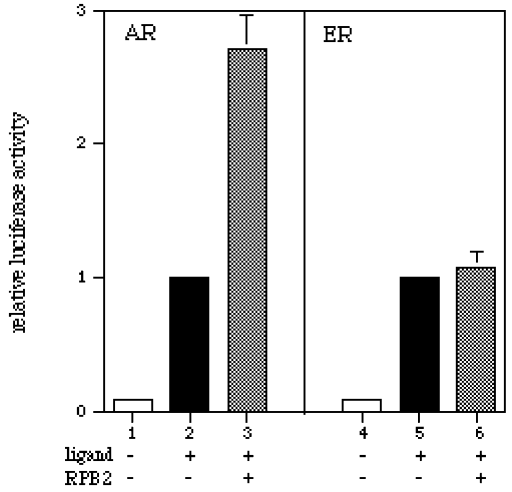

Fig. 4. Co-expression of RPB2 enhanced AR transactivation but not ER transactivation. AR and ER-negative PC-3 cells were transiently transfected using SuperFect transfection reagent (Qiagen) with either 350 ng p(ARE)4-luciferase reporter plasmid or pERE-luciferase, 30 ng pRLSV40-luciferase as an internal control, 100 ng of either AR or ER expression plasmid, and without or with 1 μg pSG5-RPB2. The total amounts of plasmids were adjusted to 1.5 μg with vector plasmid pSG5. The result obtained in the absence and presence of 10 nM DHT or E2 is shown as open and closed bars, respectively. The results obtained in the presence of 10 nM DHT or E2 and RPBs are shown in shaded bars. Relative luciferase activities were plotted using the activity without RPBs as 1.

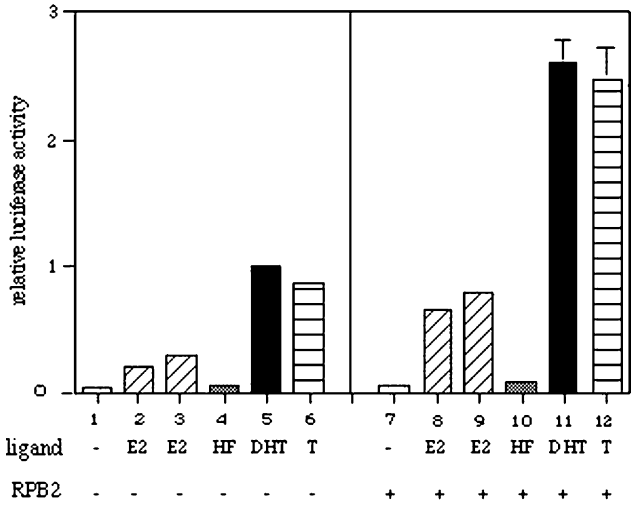

Fig. 5. Effects of various ligands on RPB2-mediated AR transactivation. AR-negative PC-3 cells were transiently transfected using SuperFect transfection reagent (Qiagen) with 350 ng p(ARE)4-luciferase reporter plasmid, 30 ng pRLSV40-luciferase as an internal control, 100 ng AR expression plasmid, and without (lanes 1–6) or with (lanes 7–12) 1 μg pSG5-RPB2. The total amounts of plasmids were adjusted to 1.5 μg with vector plasmid pSG5. The cells were incubated in the presence of 10^{-7} M E2 (lanes 2 and 8), 10^{-6} M E2 (lanes 3 and 9), 10^{-6} M HF, 10^{-9} M DHT, and 10^{-8} M T. The results were analyzed as described in Fig. 2.

mediated by co-regulators. The binding assay shows that AR interacts with RPB2 (Fig. 1). AR interaction with the general transcription machinery on the core

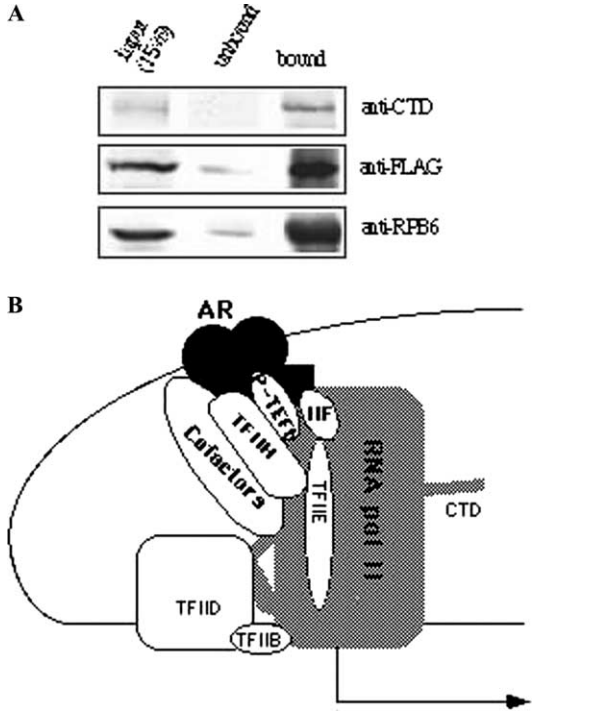

Fig. 6. (A) Immunoprecipitation of RNA polymerase II with anti-CTD antibody. FLAG-tagged RPB2 was expressed in COS-1 cells by transient transfection as described in Fig. 3. Total cell lysate obtained from 60 mm culture dish was incubated with protein A/G (Santa Cruz) resins coupled with anti-CTD antibody for 8 h at 4 °C. The resins were extensively washed with PBS. Bound proteins were analyzed along with 15% input and 15% flow-through on SDS–gradient polyacrylamide gels. Proteins were transferred to a membrane and visualized by Western blot with anti-CTD, andti-FLAG, and anti-RPB6 antibodies. (B) A working model for AR communication with the general transcription machinery. Nuclear specific co-regulators and general co-regulators are omitted in the figure to simplify the relative positions of TFIIH and P-TEFb with AR.

promoter may not only be achieved by AR interaction with RPB2 but also by AR interaction with TFIIH or multiple interactions including co-regulators. Co-expression of RPB2, not the other selective RPBs, enhanced AR transactivation (Figs. 2 and 3), indicating that the effect of RPB2 on enhancement of AR transactivation was selective. Together with our previous reports showing AR can also interact with TFIIH and P-TEFb, we conclude that AR may be able to modulate the expression of androgen target genes by multiple yet selective interactions with the general transcription machinery.

Co-expression of RPB2 enhanced AR transactivation (Figs. 2 and 3) but not ER transactivation (Fig. 4), which indicates that the effect of RPB2 on enhancement of AR transactivation is selective. The possible mechanism by which co-expression of RPB2 enhances AR transactivation might be explained in several ways. In order to understand how co-expression of RPB2 enhances AR transactivation, it is important to know whether the transiently expressed RPB2 is a subunit

incorporated into RNA polymerase II complex or a free form. Total cell lysate obtained from cells after transient transfection of pSG5-fRPB2 was incubated with protein A/G resins and anti-CTD antibody. Either free form of RPB1 or RNA polymerase II complex is retained by the protein A/G resins coupled with anti-CTD antibody. The fRPB2 in the bound fraction by resins coupled with anti-CTD antibody is fRPB2 in RNA polymerase II complex, while the fRPB2 in the unbound fraction is a free form. Due to lack of antibody against RPB2, expression level of endogeneous RPB2 in the transfected cells was not determined. It is not clear at this moment how cells after transient transfection of pSG5-fRPB2 regulate expression levels of fRPB2 and endogeneous RPB2. When we compared the ratio of unbound form over bound form of fRPB2 with that of RPB6 (Fig. 6A), little difference was found between fRPB2 and RPB6. This result indicates that RNA polymerase II subunits are in dynamic equilibrium between a free form and a complexed form in RNA polymerase II. The majority of fRPB2, like RPB6, is incorporated into RNA polymerase II complex.

Co-expression of RPB2 did not enhance SV40-enhancer mediated transcription from the internal control reporter gene pRLSV40. The result showing that co-expression of RPB2 did not enhance ER transactivation (Fig. 4) provides strong evidence, indicating that co-expression of RPB2 may selectively interact with certain types of activators and enhance their activity. From this view, the mechanism by which co-expression of RPB2 enhances AR transactivation may not be related to the total number of RNA polymerase II in cells. The interaction of RPB2 with AR may recruit transcription factors that interact with RPB2 to the transcription machinery and enhance AR transactivation. The transcription factors interacting with RPB2 may be required for the efficient AR-mediated transcription, while they are not required for ER-mediated transcription. RPB2 has been reported to play a key role in transcriptional elongation [26,27], which supports the hypothesis that AR may increase the efficiency of transcriptional elongation based on our previous studies. In addition, the early report suggests that RPB2 interacts with RPB1 [30], therefore, it is possible that interaction between AR and RPB2 may allow AR to influence the function of RPB1. The enhanced phosphorylation of CTD in RPB1 has been reported to be required for efficient transcriptional elongation [28,29]. Interaction of RPB2 with AR may recruit CTD kinases to the transcription machinery and enhance the efficiency of CTD phosphorylation. AR interaction with RPB2 reported here suggests that the orchestrated interaction of AR with TFIIH, P-TEFb, and RPB2 may increase efficiency of the CTD phosphorylation of RPB1 in the transcription machinery to enhance transcription from the androgen target genes, such as PSA. The working model of AR communication with the general transcription machinery based on our results is shown in Fig. 6B.

Androgen-AR signaling pathway not only mediates a wide range of developmental and physiological responses but also promotes prostate cancer progression [31,32]. Thus, interference of AR transactivation provides an essential tool to deter prostate cancer progression. HF, an antiandrogen, has long been used to treat prostate cancer patients. Selective co-regulators, such as ARA70 and ARA55, have been reported to enhance AR transactivation even in the presence of HF [33], which may explain one of the mechanisms leading to the HF withdrawal syndrome. However, RPB2 did not enhance AR transactivation in the presence of HF. Thus, the mechanism by which RPB2 enhances AR transactivation may be different from that of ARA70 or ARA55. Characterization of mechanisms implicated in AR transactivation may facilitate the identification of additional strategies to interfere with AR transactivation to develop potential therapeutic drugs for effective prevention of prostate cancer.

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