A Coregulatory Role for the TRAP-Mediator Complex in Androgen Receptor-mediated Gene Expression*

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The human thyroid hormone receptor-associated protein (TRAP)-Mediator complex was originally identified as a large multimeric complex that copurifies with the thyroid hormone receptor (TR) from HeLa cells and markedly enhances TR-mediated transcription in vitro. More recent studies have implicated TRAP-Mediator as a coactivator for a broad range of nuclear hormone receptors as well as other classes of transcriptional activators. Here we present evidence that TRAP-Mediator plays a functional role in androgen receptor (AR)-mediated transcription. We show that several subunits of the complex ligand-dependently coimmunoprecipitate with AR from both prostate cancer LNCaP cells and from HeLa cells stably transfected with AR. The 220-kDa subunit of the complex (TRAP220) can contact the ligand-binding domain of AR in vitro, possibly implicating TRAP220 involvement in targeting AR to the holocomplex. Consistent with a TRAP-Mediator coactivator role, transient overexpression of the TRAP220, TRAP170, and TRAP100 subunits enhanced ligand-dependent transactivation by AR in cultured cells. Finally, chromatin immunoprecipitation assays show that TRAP220 is recruited to the androgen-responsive prostate-specific antigen gene promoter in vivo in ligand-stimulated LNCaP cells. Collectively, these data suggest that TRAP-Mediator may play an important coregulatory role in AR-mediated gene expression.

The androgen receptor (AR) is a member of the nuclear hormone receptor (NR) superfamily that mediates the action of lipophilic hormones including steroids, retinoids, thyroid hormone, and vitamin D3 (1, 2). In humans, AR mediates the physiological action of testosterone and dihydrotestosterone, hormones essential for the differentiation, development, and functional maintenance of male reproductive and accessory sex tissues (3). All NRs share a common modular structure consisting of a variable N-terminal domain, a conserved DNA binding domain, a hinge region, and a C-terminal ligand binding domain (LBD) (1, 2). Transcriptional activation by NRs can be mediated by two separable activation functions (AFs): a poorly conserved AF1 in the N-terminal domain (4–7) and a highly conserved, ligand-inducible AF2 in the LBD (8–11).

Transcriptional regulation by NRs involves the binding and recruitment of auxiliary factors (termed coactivators and corepressors) to target gene promoters (12). The p160 family of proteins are among the best characterized NR coactivators (13, 14). By virtue of their ability to associate with potent histone deacetyltransferase enzymes like p300/CREB-binding protein (CBP), the p160 coactivators are thought to play an essential regulatory role in targeted chromatin modification (12–14). The p160 proteins contact NRs through consensus LXXLL motifs (also termed NR boxes), which act as binding surfaces for ligand-activated AF2 domains (15–17). Surprisingly, AR appears to differ from other NRs in that an intramolecular interaction between its ligand-activated AF2 domain and its N-terminal AF1 domain is functionally required for the binding of p160 proteins (18–21).

A second type of NR coactivator complex is the multimeric thyroid hormone receptor (TR)-associated protein (TRAP)-Mediator complex, composed of at least 16 different polypeptides ranging in size from ~15 to 240 kDa (reviewed in Ref. 22). Most (if not all) TRAP-Mediator subunits have been identified in other metazoan holocomplexes including NAT, DRIP, ARC, and CRSP (reviewed in Ref. 23). Cell-free transcription assays show that TRAP-Mediator significantly enhances TR-mediated transcription on nonchromatin DNA templates (24, 25) and in the absence of TATA-binding protein-associated factors (25). Thus, in contrast to the chromatin-modifying activity of p160-CBP/p300 complexes, TRAP-Mediator appears to function by directly influencing the basal transcription machinery, possibly by facilitating direct recruitment of RNA polymerase II. Consistent with this view, several TRAP-Mediator subunits are human homologs of proteins found within yeast Mediator, a large coactivator complex directly associated with both transcriptional activators and the yeast RNA polymerase II holoenzyme (26).

Evidence for a TRAP-Mediator coactivator role in other NR signaling pathways came from the purification of a similar, if not identical, complex of cofactors (termed DRIPs), which associate with the vitamin D receptor (VDR) and stimulate VDR-mediated transcription in vitro (27). A single TRAP-Mediator subunit, TRAP220 (also termed PBP (28) or DRIP205 (27)) directly contacts TR, VDR, and a number of other nonsteroid NRs in a ligand-dependent manner and is thought to anchor TRAP-Mediator to DNA-bound NRs (29–31). Interestingly, and analogous to the p160 proteins, TRAP220 contains two centrally located LXXLL motifs that facilitate ligand-dependent interactions with the AF-2 domain of TR and VDR (29, 30). More recent studies have demonstrated functional TRAP220 interactions with the glucocorticoid receptor (32) and the estradiol-
gen receptor (33–36), thus implicating TRAP-Mediator involvement in steroid hormone signaling pathways. However, the potential physiological role of TRAP-Mediator in androgen signaling pathways remains poorly understood.

In this work, we investigated whether TRAP-Mediator plays a functional role in AR-mediated gene expression. Using human prostatic cancer LNCaP cells and a HeLa cell line stably expressing an epitope-tagged AR gene, we found that several components of the TRAP-Mediator complex coimmunoprecipitate with AR in the presence of ligand. Consistent with a TRAP-Mediator coactivator role, we found that transient overexpression of the TRAP220, TRAP170, and TRAP100 subunits enhanced ligand-dependent transcription by AR. We also found that the AR LBD can bind to TRAP220 in vitro in the presence of ligand, possibly revealing a molecular basis for AR interaction with the holocomplex. We further showed that both TRAP220 and AR are recruited to the prostatic-specific antigen (PSA) promoter in a ligand-dependent manner within intact LNCaP cells. Taken together, these findings are consistent with the idea that TRAP-Mediator plays a coregulatory role in AR-mediated gene expression.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The pSG5-HA-TRAP220 expression vector was described previously (37). The pVL1393-HA-TRAP220 construct was generated by subcloning the Smal/SacI full-length HA-TRAP220 fragment from pGEM-HA-TRAP220 (37) into the Smal site of the baculovirus expression vector pVL1393 vector (Invitrogen). The pBS KS-EXML1 (also known as human TRAP170 expression construct (38)) was kindly provided by Hirohide Yoshikawa (Johns Hopkins University, Baltimore, MD). To construct pSG5-FLAG-TRAP170, a KpnI/NotI fragment from pBS KS-EXML1 was first subcloned into the KpnI/NotI sites of pFLAG-AS (7) generating pFLAG-AS-7-TRAP170. A BamHI/NcoI fragment from pGEX-2TK (Stratagene, La Jolla, CA) was inserted into the BamHI site of pSG5 (Stratagene, La Jolla, CA). The pBS KS-TRAP-100 (KIAA0130) plasmid was a gift from N. Kusuhara (Kazusa DNA Research Institute). To generate pBK-RSV-TRAP170, a SalI/XbaI fragment from KIAA0130 was inserted into the SalI/XbaI sites of pBKS-RSV (Stratagene, La Jolla, CA). The pGEX-2TK-AR-AP2 vector was constructed by PCR, generating BamHI and Smal restriction sites at amino acids 622 and 913, respectively, and subsequently subcloning the corresponding sites of pGEX-2TK (Amersham Biosciences). The pGEX-2TK-AR-AP1 construct was generated by PCR by generating BamHII/AfII sites at AR amino acids 1 and 170 and subcloning the corresponding fragment, together with a AfII/HindIII-blunted fragment from pSV-AR (amino acids 171–564) (39), into BamHII/Smal-digested pGEX-2TK. The androgen-responsive luciferase reporter genes pMMTV-Luc, pAP-1-DS-Luc, and pAP-1–285/–32-Luc were described previously (39). The pGL3-promoter vector was obtained from Promega Corp. The pAP-1-Luc was a gift from D. Kalvakolanu (University of Maryland, Baltimore, MD).

In Vivo Coimmunoprecipitation and Western Blotting—HeLa-derived E19 cells stably expressing a tetracycline-regulated FLAG epitope-tagged AR (39) were routinely maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) (HyClone), 2 mM L-glutamine, 50 units/mL penicillin, 100 μg/mL streptomycin, and 1 mg/mL amphotericin B (Roche Molecular Biochemicals), and 2 μg/mL tetracycline (Sigma). LNCaP cells (obtained from the American Type Culture Collection) were maintained in RPMI 1640 containing 10% heat-inactivated FBS (HyClone), 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 1 mg/mL amphotericin B (Roche Molecular Biochemicals). Cells were plated in 12-well plates (1.5 × 10^5 cells/well) in Dulbecco’s modified Eagle’s medium containing 10% charcoal/dextran-stripped FBS (HyClone) 24 h prior to transfection. A DNA mixture containing 0.4 μg of reporter plasmid (pMMTV-Luc, pARE-DS-Luc, or pAP-1-Luc), 0.2 μg of the internal control plasmid pSV-β-gal, and 0.1 μg of MMTV-Luc was added to 20 μg of pSV-β-gal vector (pSV-β-gal vector contains a lipopectAMINE reagent and added to each well. Cells were incubated at 37 °C in 5% CO_2 for 3 h before replacing the media with fresh RPMI 1640, 10% charcoal/dex-

rotated overnight at 4 °C. For reciprocal anti-TRAP220, -TRAP100, or -TRAP170 coimmunoprecipitation, 5 μl of rabbit polyclonal antibody (anti-TRAP220 and anti-TRAP100 (37)) or 100 μl of goat polyclonal antibody (anti-TRAP230 and anti-TRAP170; Santa Cruz Biotechnology catalog nos. SC-5752 and SC-9420, respectively) were added to 2.5 mg of each bacterial MATCH complex. The lysate was rotated for 1 h at 4 °C and subsequently cleared by centrifugation at 12,000 × g for 10 min at 4 °C. The protein concentration of lysates was determined by Bradford assay. Coimmunoprecipitation of FLAG-AR-cofactor complexes were facilitated by adding anti-FLAG antibodies coupled to agarose beads (M2 Affinity Resin; Sigma) (20 μl packed) to the cellular lysate (2.5 mg of protein). The mixture was
treated with R1881 (10^{-7} M) for 1 h. The cells were treated with the cross-linking reagent formaldehyde (1% final concentration) for 10 min at room temperature; cross-linking was terminated upon the addition of glycine (0.125 M final concentration). Cells were rinsed twice with cold PBS, collected by centrifugation, and washed once in cold PBS plus 0.1 M phenylmethylsulfonyl fluoride. Cells were then sonicated on ice in buffer 1 (5 mM Pipes (pH 8.0), 85 mM NaCl, 0.5% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 100 mM MgCl2, and 100 mM leupeptin) for 20 min. Nuclei were collected by microcentrifugation and resuspended in sonication buffer (50 mM Tris-Cl (pH 8.1), 1% SDS, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, 100 mM NaCl, and 100 mM leupeptin) followed by incubation on ice for 10 min. Samples were then sonicated on ice for 10 min (i.e., until the average length of sheared genomic DNA was 1000–1500 bp), followed by centrifugation for 10 min. The supernatant (i.e., chromatin solution) was then incubated with either 5 μg of mouse monoclonal anti-AR antibodies (Santa Cruz Biotechnology) or 5 μl of rabbit polyclonal anti-TRAP220 antibodies (37) on a rotator at 4°C for 24 h. As a control for total chromatin input (see PCR step below), an aliquot of the chromatin solution from each reaction was saved (prior to the addition of antibodies) and subsequently processed in parallel with the eluted chromatin immunoprecipitates (see cross-link reversal step below). Immunoprecipitated chromatin complexes were isolated by adding 10 μl of packed protein A-agarose beads (Roche Molecular Biochemicals) and rotating the samples for 3 h at 4°C. Precipitates were sequentially washed twice with dialysis buffer (50 mM Tris-Cl (pH 8.0), 2 mM EDTA), followed by four washes in IP wash buffer (100 mM Tris-Cl (pH 8.0), 500 mM LiCl, 1% Nonidet P-40, 1% deoxycholic acid). To elute the immunoprecipitated chromatin complexes from the resin, 150 μl of elution buffer (1% SDS and 50 mM NaHCO3) was added to the beads, and the tubes were vortexed for 15 min. The supernatant was collected, and the elution was repeated with a fresh 150 μl of elution buffer. After combining the eluates in one tube, the protein-DNA cross-linking was reversed by adding 5 μl NaCl to a final concentration of 200 mM. RNA was removed from the samples by adding 10 μg of RNase A (Roche Biochemicals), followed by incubation at 65°C for 4 h. The DNA in each sample was precipitated overnight at −20°C by adding 2 volumes of 100% ethanol. Samples were pelleted and resuspended in 100 μl of Tris-Cl (pH 8.5), 25 μl of 5× proteinase K buffer (50 mM Tris-Cl (pH 8.5), 1.25% SDS, and 25 mM EDTA), and 1.5 μl of proteinase K and subsequently incubated at 42°C for 2 h. Samples were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), and the DNA was precipitated with one-tenth volume of 5 μl NaCl, 3 μl NaAc (pH 5.3), 5 μg of trRNA, and 2 volumes of ethanol at −20°C overnight. The DNA was pelleted by microcentrifugation, resuspended in 25 μl of H2O, and analyzed by PCR. PCRIs contained 5 μl of immunoprecipitate or total input (see above), a 50 μM concentration of each primer, 1.5 mM MgCl2, 2 mM dNTP mixture, 1× thermorphic buffer (Promega), and 1.25 units of Taq DNA polymerase (Promega) in a total volume of 100 μl. The PSA promoter was amplified using the 5′ primer 5′-GAG GTT CAT GTT CAC ATT AGG CAC A-3′ and the 3′ primer 5′-ATT CTG GGT TTT GCA GTC GAG TCC-3′. PCR was performed with a serial dilution of input DNA to determine the linear range of the amplification. Following 30 cycles of amplification, PCR products were run on 1% agarose gel and analyzed by ethidium bromide staining.

RESULTS

AR Interacts with Components of the TRAP-Mediator Complex in Vivo in an Androgen-dependent Manner—We recently generated a HeLa-derived cell line (termed E19) that stably expresses a tetracycline-regulated FLAG-tagged human AR (fAR), thereby rendering the cells responsive to androgens (39). As evidenced by our ability to purify fAR in association with specific p160 proteins2 and the coactivator CBP (39), the E19 line can serve as a useful tool for isolating AR-coregulatory factor complexes that assemble in vivo. In this study, we used E19 cells to begin to investigate whether components of the TRAP-Mediator complex can associate with AR within intact cells. Using anti-FLAG antibodies, fAR was immunoprecipitated from E19 cells grown in the presence or absence of testosterone. Immunocomplexes were then transferred to a membrane and probed by Western blot with antibodies against various TRAP subunits. As shown in Fig. 1A (upper panel), the subunit TRAP220 is clearly associated with fAR in an androgen-dependent manner. To examine whether testosterone affects overall fAR expression, the membrane was stripped and reprobed with antibodies against AR. As shown in Fig. 1A (lower panel), fAR expression levels are roughly the same in the presence or absence of ligand, thus suggesting that the association of TRAP220 with AR is probably regulated by ligand and not by fluctuating AR protein expression.

We next performed reciprocal experiments in which TRAP220 was immunoprecipitated from testosterone-treated or -untreated E19 cells and subsequently probed by Western blot with anti-AR antibodies. In close agreement with the initial findings, AR precipitated with TRAP220 in a ligand-dependent fashion (Fig. 1B, top), and TRAP220 expression was not significantly affected by testosterone (Fig. 1B, bottom). To confirm that these findings were not just restricted to E19 cells, we performed similar immunoprecipitation experiments using the prostate cancer LNCaP line, which expresses AR endogenously. Consistent with the E19 line, AR precipitated together with TRAP220 in a ligand-dependent fashion (Fig. 1C, upper panel), and once again, TRAP220 expression was not significantly affected by testosterone (Fig. 1C, lower panel).

To address whether the association of AR with TRAP220 is reflective of an interaction with the holo-TRAP-Mediator complex, we repeated the experiments using antibodies against other TRAP subunits. As shown in Fig. 1, D and E, fAR and TRAP100 reciprocally coimmunoprecipitated with one another from E19 cells in a ligand-dependent manner, and as before, fAR and TRAP100 expression was not significantly affected by ligand. Furthermore, fAR selectively coprecipitated with TRAP170 (Fig. 1F) and with TRAP220 (Fig. 1G) in the presence of testosterone, and again, neither TRAP170 nor TRAP220 protein levels were significantly affected by testosterone. Collectively, these results show that in the presence of androgen, several subunits of the TRAP-Mediator complex are associated with AR within intact human cells. Given that endogenously expressed TRAP subunits predominantly exist in vivo as components of preassembled, high molecular mass (~2–MDa) multiprotein TRAP-Mediator complexes (41), these findings are consistent with the notion that AR is targeted to a holo-TRAP-Mediator complex in the presence of ligand.

TRAP220 Interacts with the AR-LBD in a Ligand-dependent Manner—The recruitment of TRAP-Mediator-like complexes to specific gene promoters can be facilitated by a broad range of transcriptional activators, including p53, Sp1, and sterol-responsive element-binding protein (SREBP), and presumably involves activator target interactions with distinct TRAP subunits (reviewed in Ref. 23). In the case of NRs, the TRAP220 subunit has been demonstrated to contact the LBD of numerous NRs in a strong, ligand-dependent manner and is thought to target and subsequently anchor the entire TRAP-Mediator complex to DNA-bound NRs (29–31). To determine whether TRAP220 can interact with the LBD of AR, we expressed the AR-AF2 (amino acids 622–919) as a GST fusion protein and tested its ability to interact with baculovirally expressed full-length TRAP220. As shown in Fig. 2A, the AR-AF2 showed a strong AR-TRAP220 interaction possibly indicates that other TRAP-Mediator subunits are involved in targeting the holo-TRAP-Mediator complex to AR (see “Discussion”). Nonetheless, these findings are consistent with the idea that TRAP220, either alone or in concert with other specific TRAP-Mediator

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subunits, serves to help target the holo-TRAP-Mediator complex to AR in the presence of ligand.

Enhancement of Androgen-dependent Transcription by Components of TRAP-Mediator—Our preliminary results revealed a ligand-dependent interaction between AR and components of the TRAP-Mediator complex (Figs. 1 and 2). We thus asked whether TRAP-Mediator might play a functional role in androgen-dependent transcription. Toward this end, E19 cells were transiently transfected with a TRAP220 expression vector, and transcription was measured from androgen-responsive reporter genes in the presence or absence of testosterone. As shown in Fig. 3, transient overexpression of TRAP220 enhanced AR-mediated transactivation greater than 2-fold from both the murine mammary tumor virus (MMTV) promoter and the synthetic ARE2-DS promoter (containing two natural androgen response elements (AREs) cloned into the murine ornithine decarboxylase promoter) (18). These data are consistent with previous studies showing that TRAP220 acts as a potent transcriptional coactivator for other classes of NRs (22, 23).

To demonstrate that the observed TRAP220 enhancement of AR-mediated transcription was not limited to E19 cells, we transiently cotransfected prostate LNCaP cells with TRAP220 and either the MMTV reporter or the PB (−285/+32) reporter (containing nucleotides −285 to +32 of the androgen-responsive rat probasin promoter) (18). As shown in Fig. 3, C and D, TRAP220 once again enhanced transcription in LNCaP cells from both promoters in the presence of ligand. By contrast, and consistent with the notion of a specific TRAP220 coactivation for AR, transient overexpression of TRAP220 in COS cells failed to augment basal transcription from a minimal promoter (Fig. 3E) or activated transcription from an AP-1-dependent promoter in the presence or absence of phorbol esters (Fig. 3F).

Finally, we asked whether other TRAP-Mediator subunits...
could enhance androgen-dependent transcription in vivo. As shown in Fig. 3, G and H, overexpression of either TRAP100 or TRAP170 modestly enhanced AR-mediated transcription from the pPB(−285/+32)-Luc promoter in the presence of testosterone. Taken together, these data are consistent with the idea that TRAP-Mediator plays a transcriptional coactivator role for AR during androgen-dependent gene expression.

**Androgen Induces the Recruitment of AR and TRAP220 to the**

**Fig. 3. Components of the TRAP-Mediator complex enhance androgen-dependent transcription by AR.** A and B, transient overexpression of TRAP220 enhances AR-mediated transcriptional activity in E19 cells. E19 cells were cotransfected with TRAP220 and pMMTV-Luc (A) or pARE2-DS-Luc (B) in the presence or absence of testosterone (T) (100 nM) for 48 h. Transcriptional activity was determined by measuring relative luciferase activity in cellular lysates (see "Experimental Procedures"). C and D, transient overexpression of TRAP220 enhances AR-mediated transactivation in LNCaP cells. LNCaP cells were cotransfected with TRAP220 and pMMTV-Luc (C) or pPB(−285/+32)-Luc (D) in the presence or absence of testosterone (100 nM) for 48 h. E and F, TRAP220 transactivation is activator-specific. COS cells were co-transfected with TRAP220 and the minimal promoter reporter pGL3-Luc (E) in the presence or absence of testosterone (100 nM) or with the AP-1-responsive pAP1-Luc reporter (F) in the presence or absence of phorbol 12-myristate 13-acetate (PMA) (100 nM) for 18 h. G and H, transient overexpression of TRAP100 or TRAP170 enhances AR-mediated transcription activity in LNCaP cells. LNCaP cells were cotransfected with either TRAP100 (G) or TRAP170 (H) and the pPB(−285/+32)-Luc reporter in the presence or absence of testosterone (100 nM) for 48 h. A–H, relative luciferase activities were determined from three independent transfections. Luciferase activity was normalized relative to β-galactosidase activity. Results are presented as the mean ± S.E. of the triplicate transfections.
PSA Gene Promoter in Vivo—To investigate whether TRAP-Mediator is directly recruited to androgen-responsive promoters in vivo, we performed chromatin immunoprecipitation assays. These studies specifically examined AR-cofactor binding at the human PSA gene promoter. The prostate cancer LNCaP cell line was chosen for the chromatin immunoprecipitation studies, since these cells are AR+ and endogenously express PSA in an androgen-dependent fashion (42). LNCaP cells were grown in charcoal-stripped serum for 3 days and then treated with or without the synthetic androgen R1881 (10^{-7} M) for 1 h. Using specific antibodies against either AR or TRAP220, formaldehyde cross-linked chromatin-protein complexes were immunoprecipitated from the ligand-stimulated or -unstimulated LNCaP cells. The immunoprecipitated DNA was subsequently analyzed by PCR using specific primers spanning the most upstream ARE region within the PSA promoter (ARE-III; Fig. 4A). Among the three AREs within the 5.8-kb PSA promoter, ARE-III was identified within a potent core enhancer element (43, 44) and was further shown to exhibit strong AR binding (45). As shown in Fig. 4B, treatment of LNCaP cells with R1881 triggered a marked occupancy of both AR and TRAP220 at the PSA promoter. This finding is consistent with the idea that the TRAP-Mediator complex is concomitantly recruited to the PSA promoter together with AR in a ligand-dependent manner and probably plays a functional role in androgen-responsive transcription in vivo.

The recent cloning and functional characterization of NR-associated coregulatory factors (i.e. coactivators and corepressors) has dramatically increased our understanding of how NRs regulate gene expression (12–14). Indeed, a plethora of AR-associated cofactors have been identified that are believed to regulate AR-mediated transcription (46). Whereas the identification of so many AR cofactors clearly reflects the complexity of AR signaling, the sheer number of putative cofactors further raises concern as to whether many of these proteins actually interact with AR in vivo or physiologically participate in AR-mediated transcription within intact cells. The TRAP-Mediator complex was originally identified as a coactivator that copurifies with liganded-TR from HeLa cells (22, 24) and dramatically stimulates TR-mediated transcription in vitro (24, 25). More recent studies have demonstrated a TRAP-Mediator coactivator role for a broad range of NRs (both steroid and nonsteroid receptors) as well as for a wide variety of non-NR transcriptional activators (22, 23). In contrast to the well characterized NR coactivators that possess chromatin modifying activity (13, 14), the TRAP-Mediator complex is believed to facilitate the expression of target genes by directly influencing the basal transcription machinery (23).

In this study, we investigated whether TRAP-Mediator is involved in androgen-dependent signaling. In support of a functional role for TRAP-Mediator as an androgen-dependent AR coactivator, we found that transient overexpression of several TRAP subunits (TRAP220, TRAP170, and TRAP100) enhanced AR-mediated transcription in vivo. Moreover, we found that AR’s AF2 domain can directly contact TRAP220 in vitro in an androgen-dependent manner. Two additional lines of evidence suggest that the role of TRAP-Mediator in AR signaling is physiologically relevant. First, we found that endogenously expressed AR is associated with endogenously expressed components of the TRAP-Mediator complex in an androgen-dependent manner in both prostate LNCaP cells and HeLa cells stably expressing AR. Second and more importantly, we also found that both AR and TRAP220 are specifically recruited to the androgen-responsive PSA gene in vivo within the genome of androgen-stimulated LNCaP cells.

In view of the numerous other AR coregulatory factors proposed to regulate and enhance AR-mediated transcription (including the p160 family of proteins (46)), the question arises as to the specific molecular role TRAP-Mediator might play in the context of these other distinct AR-cofactors. In the case of TR, the recruitment of TR-TRAP-Mediator complexes to specific TR-target genes appears to occur sequentially after the recruitment of TR-cofactor complexes containing histone acetyltransferase activity (47, 48). These results are thus suggestive of a multistep pathway of NR-mediated gene activation. A similar scenario might be envisaged for AR. For example, AR recruitment of histone acetyltransferase cofactors like p160 proteins (18–21) and/or p300/CBP (49, 50) to specific androgen-responsive genes probably modifies the chromatin structure, thus rendering the promoter accessible to other large multimeric coregulatory complexes. Indeed, the actual recruitment of AR-p160-CBP complexes to the PSA promoter in vivo was recently demonstrated by chromatin immunoprecipitation (51). In a temporally subsequent step, AR presumably recruits TRAP-Mediator, which in turn may more directly interfaces with the basal apparatus, thereby enhancing transcriptional initiation. Alternatively, and as indicated by recent chromatin immunoprecipitation studies with estrogen receptor (52), both TRAP-Mediator and histone acetyltransferase activity might be simultaneously recruited to some androgen-responsive promoters and thus act in concert with one another at the same temporal step.

The findings presented here suggest a functional role for the TRAP220/DRIP205/PBP subunit in targeting TRAP-Mediator to AR in the presence of ligand (Fig. 2). Of note, the ligand-dependent interaction between TRAP220 and the AR-AF2 domain was relatively weak, possibly indicating that other TRAP-Mediator subunits may be involved in binding and subsequently targeting the holo-TRAP-Mediator complex to AR. It is also interesting to note in this regard that DRIP/TRAP-Mediator interactions with the steroid hormone receptor glucocorticoid receptor are thought to involve simultaneous TRAP220/
TRAP-Mediator Coactivation of AR Transcription

DRIP205 interactions with the glucocorticoid receptor AF2 domain and TRAP170/DRIP150 interactions with the glucocorticoid receptor AF1 domain (32). Given that AR undergoes a ligand-induced interaction between its N- and C-terminal domains (18, 19), the resulting intramolecular tertiary structure may provide additional binding surfaces for other TRAP-Mediator subunits. Significantly, and consistent with a TRAP220 role in steroid hormone-dependent neoplasia, TRAP220/DRIP205/PBP was found to be amplified in breast tumors and breast cancer cell lines (33). It will be interesting to examine whether the relative TRAP220 expression levels are likewise elevated in prostate cancer cells, possibly implicating the TRAP-Mediator complex in androgen-dependent carcinogenesis of the prostate.

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