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Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID

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ABSTRACT **Coactivators previously implicated in ligand-dependent activation functions by thyroid hormone receptor (TR) include p300 and CREB-binding protein (CBP), the steroid receptor coactivator-1 (SRC-1)-related family of proteins, and the multicomponent TR-associated protein (TRAP) complex. Here we show that two positive cofactors (PC2 and PC4) derived from the upstream stimulatory activity (USA) cofactor fraction act synergistically to mediate thyroid hormone (T_3)-dependent activation either by TR or by a TR-TRAP complex in an *in vitro* system reconstituted with purified factors and DNA templates. Significantly, the TRAP-mediated enhancement of activation by TR does not require the TATA box-binding protein-associated factors of TFIID. Furthermore, neither the pleiotropic coactivators CBP and p300 nor members of the SRC-1 family were detected in either the TR-TRAP complex or the other components of the *in vitro* assay system. These results show that activation by TR at the level of naked DNA templates is enhanced by cooperative functions of the TRAP coactivators and the general coactivators PC2 and PC4, and they further indicate a potential functional redundancy between TRAPs and TATA box-binding protein-associated factors in TFIID. In conjunction with earlier studies on other nuclear receptor-interacting cofactors, the present study also suggests a multistep pathway, involving distinct sets of cofactors, for activation of hormone responsive genes.**

In eukaryotes activation of the transcription of specific genes by RNA polymerase II involves the concerted action of gene specific activators bound to cognate regulatory elements, general transcription initiation factors that form the preinitiation complex at common core promoter elements, and a number of transcriptional coactivators (reviewed in refs. 1 and 2). Broadly defined as factors necessary for activator function, but not for transcription from core promoter elements by the minimal basal factors, the coactivators fall into several non-mutually exclusive groups: (*i*) factors intimately associated with the general transcriptional machinery, including TFIIA and the TATA box-binding protein (TBP)-associated factor (TAF_{II}) components of TFIID (reviewed in refs. 1 and 3), and SRB/MED components associated with RNA polymerase II (reviewed in ref. 4); (*ii*) general positive cofactors (PCs) derived from the USA fraction in human cells (reviewed in ref. 5); and (*iii*) factors whose primary interactions are with DNA-bound activators, such as the B cell-specific OCA-B (reviewed in ref. 6), the more pleiotropic CBP and p300 (reviewed in ref. 7) and nuclear hormone receptor-interacting factors (below). This multiplicity of general and gene-specific cofactors raises important questions regarding synergistic interactions and multistep pathways, as well as the possibility of functional redundancies and alternate activation pathways.

In the case of nuclear hormone receptors (reviewed in ref. 8) a number of ligand-dependent receptor-interacting proteins have been identified and shown to be required for optimal receptor function *in vivo* (reviewed in refs. 9–11). The best characterized of these are the SRC-1 family of proteins (including SRC-1/p160, TIF-2/GRIP-1, ACTR/PCIP/RAC3/AIB1/TRAM1; reviewed in refs. 9–11), the pleiotropic coactivator p300/CBP (reviewed in ref. 7), and the interacting PCAF (12). By contrast, a distinct coactivator complex, comprised of 9–10 major thyroid hormone receptor (TR)-associated proteins (TRAPs), was recently identified and shown to markedly enhance activation by thyroid hormone receptor in a cell free system comprised of purified factors and naked DNA templates (13). A broader role for the TRAP complex in nuclear hormone receptor function was indicated by the demonstration of ligand-dependent interactions of one subunit (TRAP220) not only with TR but also with several other receptors (11), by the finding of ligand-dependent receptor interactions of a mouse homologue of TRAP220 (14), and by the isolation of a VDR-interacting group of proteins that are apparently the same as the earlier described TRAP proteins (15). Like the other cofactors (including p300/CBP and SRC family members) that interact with receptors through NR boxes with LXXLL signature motifs (reviewed in ref. 10), TRAP220 interacts with receptors in a ligand-dependent manner through similar domains (11).

The diversity of nuclear receptor-interacting coactivators raises questions regarding their mechanisms of actions and potential synergistic vs. redundant functions of these factors with each other and with general coactivators. The present study addresses these questions by analyzing the effects of TRAPs, PC2 and PC4, and TAF_{II}s on TR-mediated activation.

MATERIALS AND METHODS

Purification of Transcription Factors and Coactivators. Both hRXRα and hTRα were expressed as FLAG-tagged fusion proteins (f:RXRα and f:TRα, respectively) in Sf9 cells from baculovirus vectors and purified as described (13). The f:TRα/TRAP coactivator complex was immunopurified from

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Abbreviations: TR, thyroid hormone receptor; TRAP, thyroid hormone receptor-associated protein; TBP, TATA box-binding protein; SRB, suppressor of RNA polymerase B; MED, mediator protein; PCAF, p300/CBP-associated factor; TAF_{II}, TBP-associated factor within TFIID; PC, positive factor; CBP, CREB-binding protein; USA, upstream stimulatory activity; HAT, histone acetyltransferase; RXRα, retinoid X receptor α; SRC-1, steroid receptor coactivator-1.

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α-2 cells grown in thyroid hormone (T_3)-containing medium essentially as described (13). Natural TFIID containing a FLAG-tagged TBP (f:TFIID) was immunopurified from 3–10 cell nuclear extracts as described (16). RNA polymerase II (Mono Q fraction); TFIIA (Ni^{2+}-NTA fraction); a partially purified mixture of TFIIE, TFIIF, and TFIIH (Mono S fraction); and USA (heparin-Sepharose fraction) were purified from HeLa nuclear extracts as described (16). PC2 (Mono S fraction) was purified exactly as described (17, 18). Recombinant PC4, TFIIB, and TBP were expressed in *Escherichia coli* and purified as described (16).

***In Vitro* Transcription Assays.** *In vitro* transcription assays were carried out in 25-μl reactions containing 20 ng of the adenovirus ML200 template (13), 50 ng of the $TRE_3\Delta53$ template (13), 250 ng of TFIIA, 20 ng of recombinant TFIIB, an amount of f:TFIID containing 4 ng of TBP, 450 ng of TFIIE/F/H, 150 ng of RNA polymerase II, and 200 ng of BSA using previously described (16) buffer conditions and nucleoside triphosphate (including ^{32}P-CTP) concentrations. In specific experiments (see Fig. 2), recombinant TBP (4 ng) was added in place of TFIID. USA, PC2, PC4, f:TRα, f:TRα/TRAP, and TRIAC (2 μM) were added as indicated in the figure legends. f:RXRα (20 ng) was added to all reactions that contained TR. After incubation for 60 min at 30°C, RNA was purified and analyzed (urea/PAGE/autoradiography) as described (16). In some cases data were quantitated by PhosphorImager analysis.

Antibodies. The anti-CBP antibodies were directed against the carboxy-terminal domain (residues 1990–2441) of CBP (19). Anti-p300 mAb was provided by D. Livingston (20). Anti-SRC-1 antibodies (BCM9) were provided by M. Tsai and B. O'Malley (21). Anti-TIF2 antibodies (801-III) were provided by H. Gronemeyer and P. Chambon (22). Western blot analysis was done by standard procedures with an enhanced chemiluminescence detection kit (Amersham).

RESULTS

Synergistic Action of PC2 and PC4 in Mediating TR Function. We recently demonstrated that T_3-dependent transcriptional activation by an RXR/TR heterodimer *in vitro* requires the general cofactor activity USA (13). Originally identified as a crude fraction that substantially enhances transcriptional activation by multiple activators *in vitro* (23), USA has been further fractionated into at least five independent PCs (PC1, PC2, PC3, PC4, and p52/75) (5, 24). Although the exact composition of the ≈500-kDa PC2 remains unresolved (17), PC1 has been identified (all or in part) as poly(ADP-ribose) polymerase (25); PC3 as DNA topoisomerase I (26, 27); and PC4 as a 19-kDa single-stranded DNA-binding protein capable of interactions with both activators and with specific components (TFIIA, RNA polymerase II) of the general transcriptional machinery (28–30). p52/75 represent related polypeptides that, like PC4, appear to have broad specificities and are able to interact both with activators and with the general transcriptional machinery (24).

To determine the specific USA components that mediate activation by liganded-TRα, purified or recombinant PCs were tested for their ability to functionally replace the USA activity in an *in vitro* transcription system comprised of purified RNA polymerase II, purified general initiation factors TFIIA, TFIIB, and TFIID, and a chromatographic fraction containing a mixture of TFIIE, TFIIF, and TFIIH. Two different TR sources were assayed: human TRα expressed in and purified from Sf9 cells as a FLAG-tagged fusion protein (f:TRα) and f:TRα immunopurified from a T_3-treated HeLa cell line (α-2) that stably expresses the f:TRα fusion protein (13). The liganded-f:TR protein purified from α-2 cells is associated with a novel set of coactivators (TRAPs for TR-*a*ssociated *p*roteins) that are immunologically distinct from other nuclear receptor cofactors (see below) and, in conjunction with USA, markedly enhance transcriptional activation by liganded-f:TRα *in vitro* (ref. 13; Fig. 1*A*, lane 4 vs. Fig. 1*B*, lane 4).

When substituted for USA, neither recombinant PC4 nor a highly purified PC2 fraction conferred significant T_3-dependent activation by f:TRα (Fig. 1*A*, lanes 5–8). However, when PC2 and PC4 were added together, much of the stimulatory activity conferred by USA alone was restored (Fig. 1*A*, lanes 4 and 9). We next examined whether TRAPs affect the PC requirements for activation by TR. In contrast to the observations with TR alone, transcription by the liganded-f:TRα/TRAP complex was significantly stimulated both by PC4 alone and, to a lesser extent, by PC2 alone (Fig. 1*B*, lanes 5 and 7 vs. lane 3), albeit not to the level reached by USA (lane 4); the reduced level of transcription at the higher concentration of PC4 (Fig. 1*B*, lane 6), is consistent with previous reports of conditional inhibitory effects of PC4 (28–31). However, and as described for f:TRα/T_3-mediated transcription, the level of f:TRα/TRAP-dependent transcription that was observed with a combination of PC2 and PC4 was greater than that observed with either cofactor alone and comparable to the level conferred by USA (Fig. 1*B*, lane 9 vs. lane 4). Furthermore, and reinforcing the idea that TRAPs serve as coactivators for TR, both USA-mediated and PC2/PC4-mediated transcriptional activation by liganded-f:TRα were markedly higher in the presence of TRAPs (lanes 4 and 9 of Fig. 1*A* vs. lanes 4 and 9 of Fig. 1*B*, respectively). These findings show that two distinct components of the crude USA activity, PC2 and PC4, can act synergistically to mediate T_3-dependent activation by TR *in vitro*. The modest stimulations effected by PC2 or PC4 alone (Fig. 1, lanes 5, 7, and 8) suggest that TRAPs act in concert

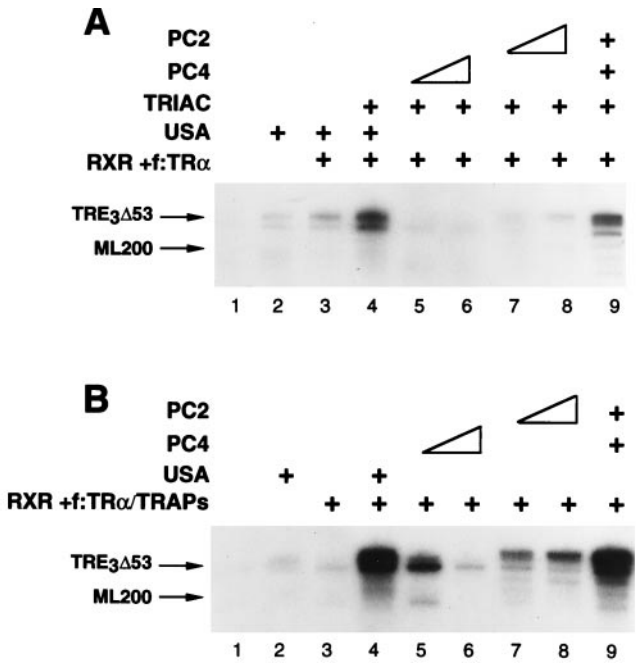

FIG. 1. Effect of PC2 and PC4 on activation by TR and TRα/TRAPs *in vitro*. (*A*) PC2 and PC4 act cooperatively to mediate T_3-dependent activation by f:TRα. In addition to basal transcription factors and RNA polymerase II, reactions contained, as indicated, TRIAC (2 μM) f:TRα (6 ng), f:RXRα (20 ng), USA (250 ng), PC4 (35 ng, lane 5; 100 ng, lane 6; 50 ng, lane 9), and PC2 (1 μl, lanes 7 and 9; 2 μl, lane 8). (*B*) PC2 and PC4 act cooperatively with TRAPs to mediate activation by TR. Reactions and additions were identical with those in *A* except for the replacement of f:TRα with immunopurified f:TRα/TRAP complex from α-2 cells (6 ng f:TRα, 40 ng total protein including TRAPs), the absence of TRIAC, and the addition of USA (250 ng), as indicated.

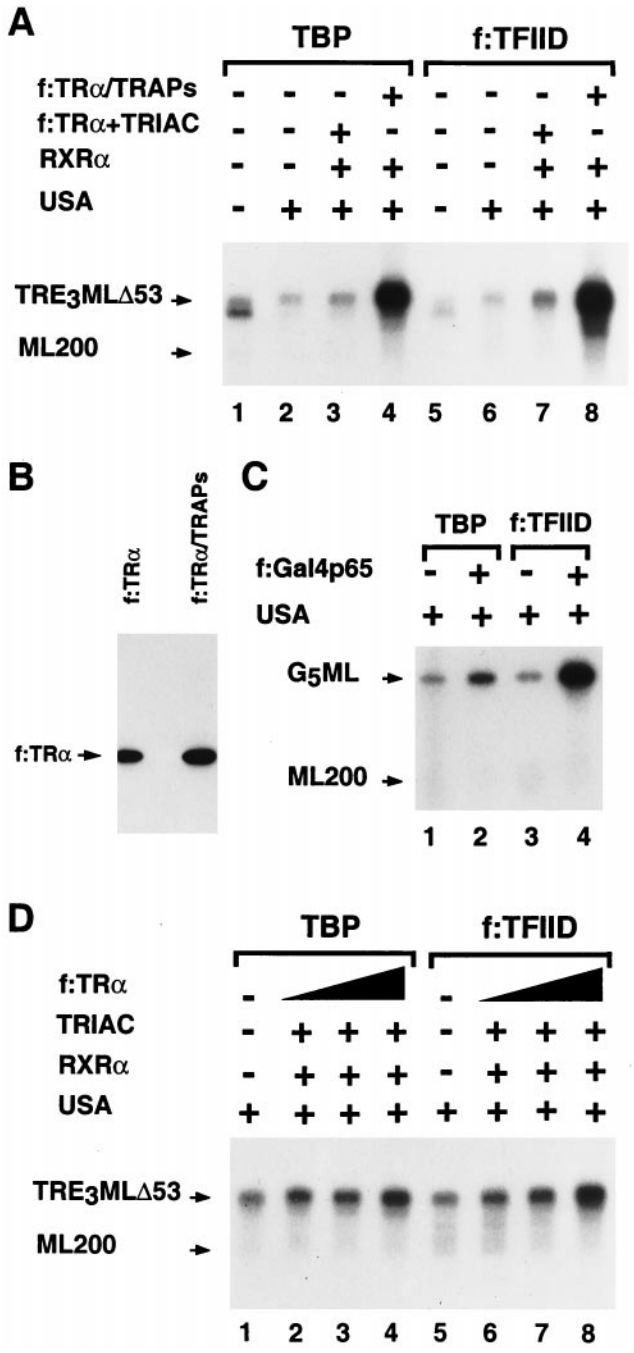

FIG. 2. TRAP-mediated TR activation does not require TAF_{II}s. (*A*) Activation by the f:TRα/TRAP complex in the absence of TAF_{II}s. Reactions contained RNA polymerase II and general initiation factors as in Fig. 1, except that f:TFIID (containing 4 ng TBP) was replaced with TBP (4 ng) in lanes 1–4. Other additions, as indicated, included f:RXRα (20 ng), f:TRα (12 ng) plus TRIAC (2 μM), f:TRα/TRAPs (12 ng f:TRα, 80 ng total protein including TRAPs), and USA (250 ng). Quantitative immunoblot analyses revealed the presence of low amounts of TAF_{II}s in the TFIIE/TFIIF/TFIIH preparation, but the amounts contributed to the reactions were only 5–10% of the amounts contributed by the f:TFIID preparation and were insufficient to mediate normal high-level activation by Gal4-p65 (*C*); furthermore, f:TRα/TRAP-mediated activation was also observed, albeit at a somewhat reduced level, with a transcription complementation system that is TFIID/TAF_{II}-free by both functional and Western blot analyses (data not shown). (*B*) Quantitation of TR in f:TRα and f:TRα/TRAP preparations. Purified f:TRα (12 ng) and immunopurified f:TRα/TRAP complex (12 ng of f:TRα, 80 ng of total protein including TRAPs) were fractionated by SDS/PAGE and assayed by Western blot analysis using mouse monoclonal antisera against the FLAG-epitope. (*C*) Activation by Gal4-p65 requires TAF_{II}s. Reactions contained the same amounts of RNA polymerase II and general transcription factors as in Fig. 1, except that f:TFIID (containing 4 ng of TBP) was replaced by TBP (4 ng) in lanes 1 and 2, in addition to USA (250 ng) and f:Gal4-p65 (25 ng), as indicated. A template containing Gal4 binding sites fused to the adenovirus ML core promoter replaced the TR-responsive promoter (18). (*D*) Dose response of f:TRα-mediated activation in the presence and absence of TRAPs. Variable amounts of f:TRα (6 ng, lanes 2 and 6; 12 ng, lanes 3 and 7; 24 ng, lanes 4 and 8) were added to transcription reactions containing either 4 ng TBP (lanes 1–4) or an amount of highly purified f:TFIID containing 4 ng TBP (lanes 5–8) and other components, as indicated, at the concentrations employed in Figs. 1 and 2*A*.

with these cofactors to enhance their individual activation properties.

TAF_{II}-Independent Activation by the f:TRα/TRAP Complex. The above results indicate that, whereas PC2 and PC4 are minimally required for T_3-dependent activation by TR *in vitro*, the addition of TRAPs significantly enhances the activation level. We next asked whether the general coactivator properties attributed to the TAF_{II}s in TFIID are required for high levels of f:TRα/TRAP-mediated activation. To this end, we compared the ability of TBP and TFIID (at nearly equimolar concentrations of TBP based on quantitative Western blot analysis) to mediate activation *in vitro* by liganded-f:TRα and by the f:TRα/TRAP complex. The f:TRα/TRAP complex activated transcription 20-fold in the presence of TFIID (Fig. 2*A*, lane 6 vs. lane 8), effecting a large net increase in the absolute level of transcription above the basal level, whereas liganded-f:TRα alone activated transcription only 2–3-fold under the same conditions (lane 7 vs. lane 6). Quantitative Western blot analysis verified that the f:TRα and f:TRα/TRAP samples analyzed in these experiments contained nearly equimolar amounts (250 fmol) of TRα (Fig. 2*B*). Significantly, and surprisingly, the f:TRα/TRAP complex activated transcription 11-fold in the presence of TBP (Fig. 2*A*, lanes 2 and 4), also effecting a large net increase in the absolute level of transcription above the basal level, whereas liganded-f:TRα alone activated transcription only 1.3-fold under the same conditions (Fig. 2*A*, lane 3 vs. lane 2). In contrast to these results, and consistent with previous studies (18, 28, 32), control experiments showed a continued TAF_{II} requirement for the optimal function of other activators in this purified system. Thus, the activator Gal4-p65 (18) activated transcription $\approx$14-fold activation in the presence of TFIID but only $\approx$2.5-fold when TBP replaced TFIID (Fig. 2*C*). This latter result, as well as the failure of liganded f:TRα to activate transcription in the absence of TBP-associated TAF_{II}s (Fig. 2*A*), argues against any significant contribution to the TBP-mediated activation from minor TAF_{II} contaminants in the natural GTF fractions (see legend to Fig. 2). These findings with the f:TRα/TRAP complex provide the first demonstration that the TBP-associated TAF_{II}s within TFIID are not essential for the high absolute level of activated transcription that is normally observed with intact TFIID in a human cell-free system reconstituted with purified factors. Moreover, they suggest that TRAPs might fulfill functional roles mechanistically analogous to those performed by the TFIID TAF_{II}s.

We next asked whether the level of activation mediated by f:TRα/TRAPs could be achieved by simply increasing the molar concentration of liganded-f:TRα in the absence of TRAPs. However, even at levels as high as 500 fmol, f:TRα activated transcription only 2.5-fold in the presence of TFIID (Fig. 2*D*, lanes 5–8) and 1.6-fold in the presence of TBP (Fig. 2*D*, lanes 1–4). These data further underscore the importance of TRAPs in mediating optimal transactivation function by liganded-f:TRα.

f:TRα/TRAP-Mediated Activation in the Absence of CBP/p300 and SRC-1-Related Coactivators. Prior studies have

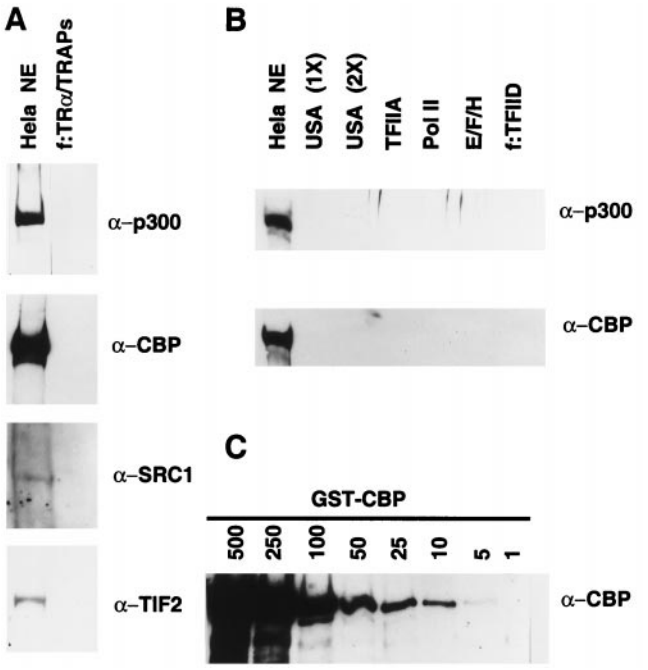

FIG. 3. Absence of CBP/p300 and SRC-1/TIF-2 in the immunopurified f:TRα/TRAP complex and the *in vitro* assay system. (*A*) The f:TRα/TRAP complex. Immunopurified f:TRα/TRAP complex (40 ng of f:TRα, 266 ng of total protein including TRAPs) and HeLa nuclear extract (30 μg) were assayed by Western blot analysis with antibodies to TIF2, SRC-1, CBP, and p300. Only the relevant regions of the blot are shown. (*B*) Transcription assay components. Purified factors TFIIA (500 ng), f:TFIID (100 ng), TFIIE/F/H (900 ng), RNA polymerase II (600 ng), and USA (250 and 500 ng), as well as nuclear extract (30 μg), were assayed by Western blot analysis with antibodies to p300 and CBP. Only the relevant regions of the blot are shown. The amount of each factor loaded on the gel corresponds to 2–4 times the amount used for a single *in vitro* transcription reaction (Fig. 1). The general factors TFIIB and TBP were not included in this assay as they were expressed in *E. coli* and, thus, are devoid of CBP/p300. (*C*) CBP standard. The indicated amounts (in fmoles) of a recombinant glutathione *S*-transferase-CBP fusion protein (containing CBP residues 1990–2441) were determined by Western blot analysis with anti-CBP antibodies under the same conditions employed in *A* and *B*.

documented the involvement in receptor function (*in vivo*) of two groups of coactivators that show ligand-dependent interactions with TR (and other nuclear receptors) and with each other: the SRC-1 related family (SRC-1/p160, TIF2/GRIP-1, and PCIP/ACTR/RAC3/AIB1/TRAM1) of coactivators and CBP/p300 (Introduction). Although peptide microsequencing and Western blot analyses indicated that TRAPs are both structurally and immunologically distinct from p300/CBP and SRC-1-related factors (refs. 11 and 13; M. Ito, C.-Y. Yuan, S.M., W. Gu, J.D.F., S. Yamamura, Z.-Y. Fu, X. Zhang, J. Qin, and R.G.R., unpublished data), substoichiometric amounts of the latter might have copurified (in association with f:TRα) along with the f:TRα/TRAP complex and contributed to the activity. Importantly, however, Western blot analyses of the f:TRα/TRAP preparations (6–7-fold higher amounts than those used in typical transcription reactions such as those in Fig. 1*B*) failed to reveal any SRC-1, TIF-2, CBP, or p300 (Fig. 3*A*). These findings support the idea that TRAPs comprise a novel group of coactivators and that other coactivators do not contribute to the observed activity of the TR-TRAP complex *in vitro*.

Whereas CBP and p300 are clearly not TRAPs, they have been reported to be present in various RNA polymerase II/general initiation factor (holoenzyme) preparations (33, 34) and to contribute to the function of certain activators on DNA templates *in vitro*. Hence, the possible contamination of our purified *in vitro* transcription system with p300/CBP also might have accounted for at least some of the enhanced activation by liganded-f:TRα in the presence or absence of TRAPs (Fig. 1 *A* and *B*). However, as shown by the quantitative Western blot analyses in Fig. 3*B*, neither p300 nor CBP (sensitivity of detection as low as 5 fmol, Fig. 3*C*) were detected in any of the natural purified fractions used for the transcription assay.

DISCUSSION

Various studies have uncovered a surprising array of coactivators that appear either to be generally required for activator function or to be more specifically required for certain activators. These observations raise the possibility either of a multistep activation pathway(s), with different coactivators acting at distinct steps, or of redundant cofactors and alternate activation pathways.

In the case of TR-interacting coactivators, our results with TRAPs contrast with other studies indicating roles for CBP/p300 (35–38), the interacting PCAF (12) and SRC1-related factors (21, 22, 39–44) in nuclear hormone receptor function. However, several observations indicate that the combined results reflect the presence of a multistep activation pathway for nuclear hormone receptors *in vivo*. First, the reported functions of CBP/p300 (35–38), PCAF (12), and SRC1-related factors (21, 22, 39–44) were based mainly on *in vivo* assays involving ectopic expression of coactivators and/or microinjection of cognate antibodies, whereas TRAP functions were observed in cell-free systems containing purified factors and naked DNA templates that were not assembled into chromatin (13). Second, CBP/p300 (45, 46), PCAF (47), and SRC family members (41, 48) have been shown to have intrinsic histone acetyltransferase (HAT) activities; and HAT activities have been linked to activation through modifications of chromatin templates (49–52). Preliminary studies have failed to reveal an intrinsic HAT activity in the f:TRα/TRAP complex (T. Kundu, C.-X. Yuan, M. Ito, and R.G.R., unpublished data). Based on these and other observations of receptor-interacting corepressors (53, 54) and general coactivator requirements (present studies) we propose the following multistep model for thyroid receptor function. (*i*) ligand-independent binding of thyroid receptor (to target sites within chromatin) along with corepressors (SMRT/N-CoR, Sin3, and histone deacetylases) that maintain a repressed state by deacetylation of nucleosomal histones, (*ii*) ligand-mediated dissociation of corepressors with concomitant binding of coactivators (SRC-1-related factors, CBP/p300, and PCAF) that contain, or interact with factors that contain, HAT activities–with resulting acetylation of nucleosomal histones or possibly of other factors (55), (*iii*) binding of TRAPs (or a receptor-TRAP complex), possibly with displacement of other coactivators (or a receptor-coactivator complex); and (*iv*) subsequent or concomitant interactions of the receptor-TRAP complex either with general initiation factors or with general cofactors (see below). The latter step could also involve direct ligand-independent receptor interactions with general initiation factors (reviewed in ref. 10).

An important contribution of the present studies is the demonstration that with the apparently specialized TRAP coactivators, there is a continued requirement for some general coactivators. An analysis of the USA fraction has shown that its potent and essential coactivator function can be recapitulated by the combined action of PC4 and PC2, whereas the individual components show essentially no activity on liganded-TRα and only partial activity with the TRα/TRAP complex. This joint requirement may reflect the apparently different functions of PC4 and PC2 (17, 28–30, 56). However, these results are similar to those of recent studies with Oct-1 and the B cell-specific coactivator OCA-B (6), and suggest that assays with natural activators and templates may be necessary

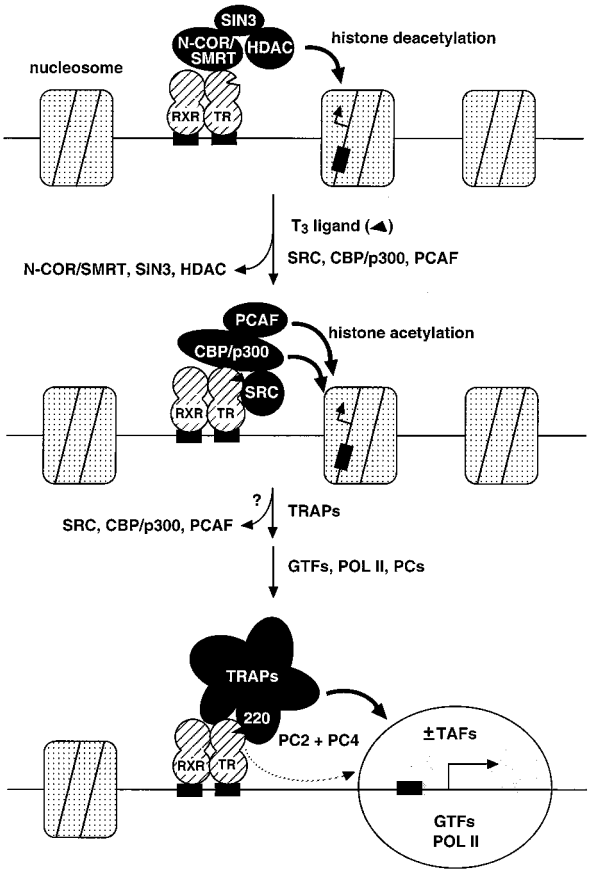

FIG. 4. Model for gene activation by TR. The multistep model is based on past and present studies showing ligand-independent TR-mediated repression through TR-interacting corepressors (SMRT/N-COR, SIN3) and the associated histone deacetylase (HDAC), ligand-dependent dissociation of corepressors and corresponding association of coactivators (CBP/p300, SRC-1-related factors, and PCAF) with HAT activity, and ligand-dependent association of TRAPs that act in conjunction with general coactivators PC2 and PC4 to mediate transcription from DNA templates by RNA polymerase II and general initiation factors. For further details and references see *Discussion*.

to elicit synergism and, presumably, more physiological functions.

The present results also show TAF_{II}-independent functions of the TRα/TRAP complex. This result is surprising in view of previous studies of activator functions in purified metazoan cell-free systems (reviewed in refs. 1, 3, 18, and 57). However, it is consistent with the more recent studies indicating (*i*) that TFIID-specific TAF_{II}s may not be generally required for normal activator functions either in yeast cells (58, 59) or in unfractionated HeLa nuclear extracts containing a more natural complement of nuclear proteins (57), whereas in some cases they may be involved in core promoter specific functions (60, 61 and references therein) and (*ii*) that components of SRB/mediator complexes may be generally or specifically required for activator functions in various situations (reviewed in ref. 62). In the present case, the lack of an apparent TAF_{II} requirement for TR activation of naked DNA templates in the presence of TRAPs suggests the possibility of a functional redundancy, at least at the level of DNA transcription *in vitro*, between TRAPs and certain TAF_{II}s. These results do not exclude the possible involvement of TAF_{II}s in nuclear hormone receptor functions *in vivo* (63, 64), either through effects on more natural (chromatin) templates or through core promoter specific interactions and either as constituents of TFIID or as constituents of HAT complexes (reviewed in ref. 65).

Finally, our recent studies have defined a novel human SRB- and MED-containing cofactor complex (SMCC) that contains homologues of a subset of yeast mediator components and mediates activation by various Gal4-based activators (66). The further identification of several SMCC components as TRAPs (66), along with the identification of a p53-interacting protein (RB18A) identical with TRAP220 (67), raises questions regarding the possible use of coactivators in different complexes, the possibility of a closer relationship between SMCC and the TRAP complex, and, related, the possibility of TRAP complex function with activators other than nuclear receptors.

Note Added in Proof. Recent studies have shown virtual identity between the SMCC and TRAP complexes with respect to subunit composition, coactivator functions (for TR, Gal4-p53, and Gal4-VP16), and activator interactions, indicating that the TRAP/SMCC complex is a potentially global cofactor (M. Ito, C.-Y. Yuan, S.M., W. Gu, J.D.F., S. Yamamura, Z.-Y. Fu, X. Zhang, J. Qin, and R.G.R., unpublished data).

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