

# Identity between TRAP and SMCC Complexes Indicates Novel Pathways for the Function of Nuclear Receptors and Diverse Mammalian Activators

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## Summary

The human thyroid hormone receptor-associated protein (TRAP) complex, an earlier described coactivator for nuclear receptors, and an SRB- and MED-containing cofactor complex (SMCC) that mediates activation by Gal4-p53 are shown to be virtually the same with respect to specific polypeptide subunits, coactivator functions, and mechanisms of action (activator interactions). In parallel with ligand-dependent interactions of nuclear receptors with the TRAP220 subunit, p53 and VP16 activation domains interact directly with a newly cloned TRAP80 subunit. These results indicate novel pathways for the function of nuclear receptors and other activators (p53 and VP16) through a common coactivator complex that is likely to target RNA polymerase II. Identification of the TRAP230 subunit as a previously predicted gene product also suggests a coactivator-related transcription defect in certain disease states.

## Introduction

Nuclear hormone receptors bind to and, in a ligand-dependent manner, activate transcription of specific genes that regulate diverse biological processes (reviewed in Mangelsdorf and Evans, 1995). Like other DNA-binding activators, nuclear receptors require various coactivators for effecting the formation of functional preinitiation complexes (containing the general transcriptional machinery) on target genes. One prominent group of nuclear receptor-interacting coactivators includes SRC-1-related factors, p300/CBP, and PCAF (reviewed in Glass et al., 1997; Fondell et al., 1999). These factors contain histone acetyltransferase (HAT) activities and are thought

to work, at least in part, through chromatin template modifications (reviewed in Struhl, 1998).

Our own studies of receptor function have identified a large multisubunit complex of thyroid hormone receptor- (TR-) associated proteins (TRAPs) that, in a ligand-dependent manner, both interact with TR and facilitate TR function on naked DNA templates in conjunction with general initiation factors and USA-derived cofactors (Fondell et al., 1996, 1999). The combined observations have led to a multistep model for thyroid hormone receptor function involving distinct receptor interacting coactivators (Yuan et al., 1998; Fondell et al., 1999). The further demonstration of ligand-dependent interactions of a single subunit (TRAP220) of the TRAP complex with multiple nuclear receptors, through a region containing signature nuclear receptor recognition (LXXLL) motifs, has suggested a broad role for the TRAP complex in nuclear receptor function (Yuan et al., 1998). Thus, the novel TRAP complex (Fondell et al., 1996, 1999; Yuan et al., 1998), as well as the subsequently identified and apparently identical DRIP complex (Rachez et al., 1998), provided new perspectives on the nuclear receptor problem in terms both of target gene activation mechanisms (interfaces with general transcription factors) and the interaction of other signaling pathways with specific ligand-receptor pathways. Nonetheless, the possibility that the TRAP complex or components thereof might have more global functions with other types of transcriptional regulatory factors has not been excluded and, indeed, has been suggested by the identification of several TRAPs within a recently described mammalian SRB- and MED-containing cofactor complex (SMCC) that is distantly related to the yeast Mediator complex (Gu et al., 1999).

The yeast Mediator is a multisubunit complex that interacts reversibly with RNA polymerase II, forming a holoenzyme, and serves both coactivator and (co)repressor functions (reviewed in Hampsey, 1998; Myer and Young, 1998). It is comprised of a group of SRB proteins that were first identified genetically as suppressors of RNA polymerase II CTD mutants (Koleske and Young, 1994), other genetically defined regulatory factors (SIN4, GAL11, RGR1, PDG1, and ROX3), and a group of biochemically identified MED proteins (Kim et al., 1994). Various Mediator components have been implicated in both global and gene-specific transcriptional activation and repression events (reviewed in Myer and Young, 1998; Hampsey, 1998). Mammalian holoenzymes containing homologs of yeast SRBs (SRB7, SRB10, and SRB11) and various variably associated factors have been described and found to mediate activator function (reviewed in Parvin and Young, 1998). However, more recent studies have described RNA polymerase-free mammalian complexes containing homologs of a subset of yeast Mediator components (SRB7, SRB10, SRB11, MED6, MED7, RGR1, and NUT2/MED10), as well as other metazoan-specific components (Jiang et al., 1998; Sun et al., 1998; Gu et al., 1999). The human NAT complex was shown to have a repressive (antiactivation) function (Sun et al., 1998), whereas the human SMCC

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complex was shown to exhibit both a similar repressive function and a novel coactivator function for Gal4-AH and Gal4-p53 (Gu et al., 1999). The identity of three SMCC components with three TRAP components (TRAP220, TRAP100, and TRAP170/RGR1) raised the possibility either of shared components between functionally distinct complexes, as observed for a subset of TAFs that are shared between TFIID and HAT-containing complexes (reviewed in Struhl and Moqtaderi, 1998), or of a more intimate relationship between the TRAP and SMCC complexes (Gu et al., 1999).

The results presented here show virtual identity, both with respect to polypeptide compositions and coactivator functions, for the TRAP and SMCC complexes. These surprising but highly significant studies thus reflect a convergence of diverse coactivator studies and, at the same time, indicate novel activation pathways in mammalian cells both for nuclear hormone receptors and for diverse activators that include p53 and VP16.

## Results

### Cloning of Human cDNAs for TRAP240, TRAP230, TRAP150, TRAP95, and TRAP80

The previously described TR-TRAP complex was immunopurified (on M2-agarose) from ligand-treated HeLa cells that express FLAG-tagged TR $\alpha$ . Human cDNAs encoding the 240 kDa, 230 kDa, 150 kDa, 95 kDa, and 80 kDa subunits (TRAP240, TRAP230, TRAP150, TRAP95, and TRAP80, respectively) of the TR-TRAP complex were isolated on the basis of amino acid sequences derived from cognate polypeptides in the immunopurified complex (Experimental Procedures).

The TRAP240 cDNA encodes a 2174-amino acid protein (Figure 1A) that shows a regional identity of 29% and similarity of 46% with a hypothetical *C. elegans* protein (CEK08F8 and CEF07H5, accession number 3642220). It shows no obvious relationship with known consensus sequences, other than two ligand-dependent nuclear hormone receptor signature recognition motifs (LXXLL sequences; reviewed in Voegel et al., 1998) at positions 1188–1192 and 1279–1283 and a short leucine zipper at position 1331–1352.

The TRAP230 cDNA encodes a 2212-amino acid protein (Figure 1B). This sequence was later found in two other reports of partial sequences: CAG H45 (Margolis et al., 1997; accession number U80742) and an OPA-containing protein (Philibert et al., 1998; accession number AF071309) located on chromosome locus Xq13. TRAP230 also contains two overlapping LXXLL motifs near the amino terminus and a highly glutamine-rich C-terminal region that results from the CAG trinucleotide repeats (Figure 1B). A computer search of the database disclosed that TRAP230 also has significant homology with a hypothetical *C. elegans* protein (CEF47A4, accession number Z49888). This protein has a regional identity of 23% and similarity of 40% with TRAP230 and also possesses a characteristic glutamine-rich sequence near the C terminus.

The TRAP150 cDNA encodes a 955-amino acid protein with no obvious known sequence motifs (Figure 1C). However, a BLAST database search revealed a gene

(KIAA0164; accession number D79986) of unknown function encoding a related human protein (overall identity of 40% and similarity of 55%), thus indicating a putative gene family.

The presumptive full-length TRAP95 cDNA encodes an 877-amino acid protein that has no obvious motifs other than a short leucine zipper near the amino terminus (Figure 1D). The TRAP80 cDNA encodes a 717-amino acid protein that has no obvious motifs other than a short leucine zipper in the middle of the sequence (Figure 1E). Human TRAP95 and TRAP80 appear to be equivalent to the p96b and p78 components, respectively, of the mouse Mediator (Jiang et al., 1998).

Although one of the previously identified TRAP subunits, TRAP170, appears on the basis of weak sequence similarity to be the human homolog of both a *C. elegans* protein and the yeast Mediator component RGR1 (Gu et al., 1999), none of the newly described TRAP sequences or the previously described TRAP220 and TRAP100 sequences (Yuan et al., 1998) show any obvious homology with yeast Mediator components or other yeast proteins. Somewhat surprisingly, in view of the existence of presumptive *C. elegans* homologs to TRAPs 240, 230, and 170, as well as to SRB7, SRB10, SRB11, MED6, and MED7, a database search revealed no *C. elegans* proteins with significant sequence relationships to TRAPs 220, 150, 100, 95, and 80. These results further emphasize the distant relationship between the yeast Mediator and the human TRAP and SMCC complexes, as well as the likelihood of a *C. elegans* cofactor complex that is somewhat more closely related to the TRAP and SMCC complexes than to the yeast Mediator.

Northern blot analyses of multiple human tissues showed that most human TRAPs are expressed ubiquitously and have similar expression patterns in different tissues (Figure 2). However, as reported for TRAP220 and TRAP100 (Yuan et al., 1998), they are variably abundant in skeletal muscle, heart, and placenta.

### Structural Relationship of the TRAP Complex to the Human SRB/MED Cofactor Complex (SMCC)

We recently isolated an SRB and MED protein cofactor complex (SMCC) by immunopurification (on M2-agarose) from HeLa cells expressing FLAG-tagged SRB10. Surprisingly, three of the large SMCC subunits were identified as TRAP220, TRAP170/RGR1, and TRAP100, respectively, by peptide microsequence and immunoblot analyses; and, as mentioned above, TRAP170 has overall homology with the RGR1 component of yeast Mediator (Gu et al., 1999). A further analysis of the comparably sized components of SMCC by mass spectrometry (Ogryzko et al., 1998) revealed multiple polypeptide sequences that showed perfect matches to sequences within TRAP240, TRAP230, TRAP150, TRAP95, and TRAP80, respectively (Figure 1).

The above results further indicated that the TRAP complex (lacking TR $\alpha$ ) might be closely related or even identical to the SMCC complex. To explore these possibilities, TR-TRAP and SMCC complexes that were selected on M2-agarose under comparable buffer conditions (300 mM KCl, 0.1%–0.2% NP40) were compared

**A TRAP240**

MSASFVPGASLEDCNCLFCLADLTGKWKVYVQGPSTAPILFVTEEDPILSSFSRCLKADVLGVWRRDQGRRELWIFWGWGDPVLLTFTMTYQ 100  
KKKMECGRMDFPMAVLCFNSKAVHNLRLCMLNRFVRIKGVFVKPYEKDEKPKINKSEHLSCSFTFFLHGSDNVCTSVIENHQFVYLLSEHITLAQQS 200  
NSSPQVILCFPGNGTITGQAFPMDSATKLGWQKVPYPTSCCLKEMSEBEKQEDMDWEDDLSAAVEVLVAVGVRMTYPAFCVFLVQSDIPTSPVSGSTH 300  
NCSSELGVHQVPASTRDPAMSVTLTPPTSPEEVQTVDPQSVQKWKVFSVSDGFNSDSTSHHGKIPRKLANHVDRVWQECNMNRAQNKKKYASASSG 400  
LCREATAAKVASMVDVFAQRNCSCLRHKNLKRNSRAGQQQAPSLGQQQQLFKPHKNTNEKQKSEBFQKRPLTPFHHRVSVSDVGMADASASQRLVIS 500  
APDSQVRFVSNIRNTDVAKTQMHGTEMANSPPPLSPHPCDVVDVDEGVTKFTSTPQSQHFYQMPPTDPLVPSKPMEDRIDLSQSFPQYQEAPEPTVVV 600  
GTAVNLEDEANIAWKYKFKPKKDEFLPQLPSDKFKDDPVGPFQGESVTSVTELMVQCKPKLVSDLELQQOYQIKNQKLSAASDARQEPKIDPYAF 700  
VEGDEFLFPPDKKDRQNSREAGKHKHVEDGTSVTVLSHEEDAMSLFSPSIKQDAPRPTSHRPPSTSLIYSDSLAVSYTDLNLFNSDEDELTPGSKR 800  
SANGSDKASCKEKSTGNLDPLSCITADLHKMYPTPSLEQHLMGFSPMNNKKEYGMDTPGGTVLEGNSSSIGAQFKIEVDEGFCSPKPSIKDFS 900  
VYVPCENCQILVQSMFAPLKTLPQSYLPLIKLPEECIRYQSWTVGKLELLSSGSPMPFIKEGDSNMDEYGTAYTPQHTSCOMPSSAPPSSGAGI 1000  
LPSFSTPRFPTPTPTPTPRGAGGPAQAQSVKVENSDLYSPASTPSTCRPLNSVEPATVPSIPEAHSYLVNLLISEVMNLFKDCNSDSCCICVCM 1100  
NIRKGAADVGVYIPDPTEAQYRCTCGFSAMNRRKFGNNSGLFLEDELDIRNTDCGKEAEKRFALRATSAEHVNGGLKESEKLSDDLILLQDQCTNLF 1200  
SPFGAADQDFPKSGVINSWVVEERDCNDCYLALEHGRQFMDNMSGGKVDALVKSLSLHPWSKRNVDVMSQSDILRMLLSLQVLDQAIQKRRTVR 1300  
PWGVQPLTQQFHKMAGRSYGTDESPEFLPITPTFLGVDYDYLVSFPFALPYWERLMLPEYGSQDIAYVVLCPENEAALLNGAKSFRDLTAIYESCR 1400  
LQHRVRSLRLLDGMIRVGTASKKLEKVAEWFVSAADGNNEAFSKLKYAQCVRVYDGLPYLASELPLDSSLLSQPNLVAPTQSLSITPPQMTNTGNAN 1500  
TPSATLASAASMTVTSVGTVAISTVATANSTLTASTSSSSSSNLSGVSSNKLPSFPFPGMNSNAAGSMSTQANTVQSGQLGGQTSALQTAGISGE 1600  
SSSLTPQHPDVSSESTMDRDKPDPDGDHAVTYPPAVVYIIDPFTYENTDESNSSSVTLGLLRFCLEMYVQLTPPHIKSTVSQVQIQCQYLLQPVK 1700  
HEDRETYQPHKLSLAFSAFTQRRPLPTSTNVKTLTGFGPLAMETALRSDRPECIRLYAPFLLAPVVKDKQTELGETFGEAGQYVNLVFGVQLSHDQ 1800  
RWLASCSDYLGELLETCIINIDVNPARRKKSARKFQLKLEWECGLVQMSLPCRWHVIGRIGRIGHGELEKDWSCLLSRNQLSKRKLDMCMRCM 1900  
ISAADSPILSACLVMEMEPQGSFVMPDSVSTGSVFRSTLNMQTSQNLTPQDTSRILVFPSTASVQVASTYITENLDLAFNPNDDGADMGFLDL 2000  
LDTGDLDLDFDILNLPASPTGSPVHSPGSHYPHGGDACKQSTDRLLSTPEPHEVPTLQOPLALGYVSTAKAGPLPWFVWASCPAQYQYQCPFLKASL 2100  
LHVSPVQSDDELLHSHSHPLSDNQTSDVLRFLVLEQYANLWLTCDPATQDRRSCLPIHFVVLNQLYFIMNML 2174

**B TRAP230**

MKQSPSLHTKILFCYFHLTNSWCLRRYGLGKMAAFGILSYEHRPLKRPRLGPPDVPYQDPKQKEDELTALNVKQGFNNQPAVSGDEHGSKAVNSFN 100  
PAKISNFSSTIAEKRLCNTLPTDTRRRKQVQNKDNFVLTARSQAINTWFTDLAGTKPLTQAKKVPISFKKEEVFGYLAQYVPMRAAWLIKMTCA 200  
YAAISETVKKRHVDYFMEWTQIIITKYLWEQLQMAEYRPGPAGSGGCTIGPLPHDVEAIRQWDVTEKLMFMPQDMLDRHEFTLWVLECFPEI 300  
RPGDELLKLLPLLLYSGEFVQSAYLRRLAYPCTRRALQLDGVSSHSHSVISAQSTSLTPTTAPQPTTSTPTSPFSDLMLCPQHRPLVFLGSLCT 400  
LQITLCCCPGALVWHYSITDSTRIKTGSPLDHLPIAPSMLPMPGNSAFTQVRAKLRIBQIKERGAQVVRVSPDKQEAATAGFTGRVHLHLEVLDS 500  
HSPERSDFNSNLSLCLNRLFLGLGSPDKGHEISSDDDAVSLCEWAVSCRSRHRAMVAKLEKRAEIEAERCGESEAADEKGSIASGSLASAPAI 600  
FQDVLQPLDTQAFLMLDPRSESRVFFNLVLLFCLELIRHDFVSHNMYTCTLISRGDLAPGAPGRPPSPFPDPADEPHKEAEGSSSKLEDPGLSES 700  
MDLDPSSSVLFDVDMKPDFLSFTSTPCCEGKSPSPPEKPDVEKVKPPPKKIEGTLGLVYDQPRHVQYATHFPIQGEESCSHECNRLLVFLVGVGQRD 800  
DARHAIKIKTDLKPLKNSRKTGABTQDLAIVPLNPGDLTFGGEDGQKRRNRNPEAFPTAEDIKFAKQHLSHYDQHVTAQVSRNVLEQITSFALGSI 900  
HLPLVQHQQIFDLMESYLSISGLIDFATQLNLSLVVAEALLKSSDLVGSYTTSLCLCTIVAVLRHYHACLIINQDQMAQVFEGLCGVVKHGMNRSDD 1000  
SAPRCILAYLDLYTSCSHLKNKFGELSDFCCKVNTIYCNVPEFSNMRWAPFEMDITLENPAHTFTYTGKLSLENPANRYSFVCLNLMHVCGH 1100  
HDPDRVNDIALLCABELGYCKSLSAEWLGLKALCCSNNGTCFNDLCLNVDSLDFHDSLATFVAIILARQCLLEDLIRCAAIPLSLLNAACSQDS 1200  
EPGARLTCRLLHLKPKTQNLNPKGSRGKTVIRSSCDRLHAAQNRIVDGAFAVLKAVFLGDAELKGSFTVTGTEELPEEGEGGGSGGRQGG 1300  
RNLVSTASLDVYAKYVLRISIQEVEWGERCLKSCEDSNDLQDFVLSAAQAQLMQLICYPHRLDNEEDGENPQRQIRKRIQLNLDQWMTMQSSLELQ 1400  
MIKQTPNEMNLENLAKATLIEVFRSAETGSSSGSTAANNPSSSKTKPVLSSLSRSGVWLVAFLAKLPTVQGHVLAAGELEKRGHLSGSSSKR 1500  
DRQKQMSLMSLQDFLVLVLCIKQDQREGLTSLYSQVHQVNNRRDDQVLDLDDCKPKQMHFAIKIRINLVGGMFDTVQRSTQOTTWAMALLLEII 1600  
ISGTVMQSNLELFTVLDMLSVLNGTLAADSSISQSGMEENKRAYMNLAKKQKELGERQSDSLEKVRQLLFLPKQTRDVTCEPQSLIDTKNGKI 1700  
AGFDSIFKKEGLQVSTQKTSIPWDLFEGLKPSALSWGWFVTRVRRVARGEEQRLLYHTHLRPRRAYILEPLPEDEEPPATLLEPEKAPL 1800  
PPTKRGSPKAPPSTREERKKTSMKMRADPKHEEMDDQDKDKAKRKESEFDDDFKMSKIVGANKNQEEKSGKWEGLVYAPPKQKQKTELEEESEFPERSK 1900  
KEDRGRSEGGHGRVPEKPNFVYAYKAVQEKSSPPFRKTESRDKLGAAGDFPTGKSSPITREAGVNVMDSEDEDLARPSGLLAEQKLCRDLVLS 600  
NKKEQEFRSIFQHLTQSAQSQSPSELFAQHI VTIHVHVEKHEHSGSGMTHLHERFTKYLKRGTEQEAANKKSPELHRRIDI SPSTFRKHGLAHDEMKS 700  
EPGYKAEQKVDKVDLRLDIRRKHKHERDLKRGKRSRESDSRSSHSRNSAEKTEKTHGSKKQKHHRRARDRSRSSSSQSSSHYKABEYETE 800  
EREESTGFDKRLGTDFVGPSEGGGRARGTFQFRARGRWGRNYSNNNNNNDFKNRREFEWDEPYTFSKSKKYVLDHDDREGGSDKWSRGRG 900  
RGAFPGRGRFMRKSSSTPKWAHDKFSGEBGELEDEDESTEENREKDIQPTTE 955

**C TRAP150**

MSKTNKSKSGRSRSRASRSRSGRSPFKSRSRSLRSRKRRLSSRSRSYSYSPAHNRERNRPVYQNRDFRGNRGRYRPPYFRGRNRFYVWQVYN 100  
VKGYGNYSRNMNQYRQAYSRRGRSRSRSPKRRSPSPRSRSHSRNSDKSSDRSRSSSSSSSSNHRVSESSKRSKAKKSSSKSDRSPSQAAGNDQDE 200  
RGEOTFSQTSQDASESSKWPDAITYGTGASRASAVSELSPRSPALKSPQSVVVRSSRSPSPVPPKPSPLSSTSQMGSSTLPGSAGYQSGTHQG 300  
QFDHSGSLSPKSKSPVGSPPSTGTVGSSQKEESAASGGAAYTKRYLEEQKTEGKDKKQKQNTDKKIKKQKSFSDTGLGDKMKSDSFAPKTDE 400  
KFFRGSQSPKRYLKRDFEKKMADPKHEEMDDQDKDKAKRKESEFDDDFKMSKIVGANKNQEEKSGKWEGLVYAPPKQKQKTELEEESEFPERSK 500  
KEDRGRSEGGHGRVPEKPNFVYAYKAVQEKSSPPFRKTESRDKLGAAGDFPTGKSSPITREAGVNVMDSEDEDLARPSGLLAEQKLCRDLVLS 600  
NKKEQEFRSIFQHLTQSAQSQSPSELFAQHI VTIHVHVEKHEHSGSGMTHLHERFTKYLKRGTEQEAANKKSPELHRRIDI SPSTFRKHGLAHDEMKS 700  
EPGYKAEQKVDKVDLRLDIRRKHKHERDLKRGKRSRESDSRSSHSRNSAEKTEKTHGSKKQKHHRRARDRSRSSSSQSSSHYKABEYETE 800  
EREESTGFDKRLGTDFVGPSEGGGRARGTFQFRARGRWGRNYSNNNNNNDFKNRREFEWDEPYTFSKSKKYVLDHDDREGGSDKWSRGRG 900  
RGAFPGRGRFMRKSSSTPKWAHDKFSGEBGELEDEDESTEENREKDIQPTTE 955

**D TRAP95**

MCDLRRAAGGMDLAVYCEWKEKSKSTHCPSVLACWASCRNLTFTTMDLRSDDQDLTRMHIIDTEHPWDLHSPSEHHEATITCLEWDQSGSRLLSAD 100  
ADGQIKCVMSADHLASWBSVSGSLVBDGDI VALSWLHNGVKLALHVEKSGASSFGEKFSRVKFSPLTLFGGKPMEGVIAVTVSGLVYSLKLPQSQVL 200  
TSTESLCLRGRVLAADIAFTGGGNTVATADGSSASPVQYKCVSVVSEKRIDTEILFSLFMRCTDNLNRKDFPAITHLKFLARDMSQEVLLCASS 300  
QTSISVLEWALRKEGLPVNNIIQQISPVVGDQKPTLKWRLSANTDLDRVSAVALPKLPI S LNTDLKVASDQFYVGLGLALAFHDSVHIVHRLSLQ 400  
TMAVYFSAAPKFDVDEPAMKRPRTAGPAVHLKAMQLSNTSLALVGDIDSHGKLSVLRLLSPSMGHLEVLGLALRHLFLLEYCMVTVGDWMDLHVVQSPMV 500  
QSLVLEHIEYTRQAAALQQLVSTRFLAMKASLCKLSCTVTRVCDYHTKFLFLIATSTLKSLLRPHLNTPKSPGDRLETEICTKI TDVDIDKVMNLK 600  
TEBFVLDNMTLQALQQLQVWGDVFLVLLASLPNQGSLRRPGHSFLRDGTSGLMLRELMVIRIWLGLKPSCLPVYATSDTQDSMSLFRLLTKLWICC 700  
RDEGPASEPEALVDECCLLPSQLLPSLDLWLPASDGLVSRLOPKQLRQFGRAPTLPGSAATQLDGLARAPGQKIDHRLRHLGACPECKEACTR 800  
CGCVTMLKSNRNTAVKQWQRWKNCLLAVEGRGPDACVTSRASEAPAFVQLGQPSHHSPTPRSLDHLHPEDRP 877

**E TRAP80**

MGCVLKLLGQLCFQSERCVRFPEGLLRHRCGLLSRSLAGKPLPRTSFFGSGVLPPLADAASMGVRAVRISIESACQVHEVGLDGTETYLPLP 100  
SNGNLARLAQRIDFSQSGSSEEEAATQEDGADQWPGAGSADQDEEGVVKFQPSLWFDVSVNRLRSALTEMCVLYDVLSTVDRKFMFLDPSQDA 200  
LFPKQNPOTQLCSKSKSLGAAOITLKGAEERIKSVTENQENKLRDFNSLRLRQHWKLRKVDKILGDLVYRAGSLFPHHGTETVIRKNTDLDBK 300  
KTPEDYCPLDVQIPSDLEGSAYIKVSIQKQAPDIDGLGTVNLFRPLPKSPGSPHWQTKLEAAQNVLLCKEIPAQLSREAVQIKSQVPHVTVKNQIISQ 400  
PPPSLQLSILCHSSNDKSKQFAEKQCPEDHLYVLEHNLHLLIREFHQKTLSSIMMHPASA PFGHKRMLRSGPAQRNEINLSQSEGLELEKIIKQ 500  
AKHTFLRSAAATIDSLASREDEDPQIAHWSNINDVYESVVKLITSGGYEQICKSIQLQNLTVBEQIRVHVRDGRVITLSYQEQELQDFLLSQMSQHQ 600  
HAVOQLAKVMGQVLPFSNHLVGLPESIGNASAITVASPSGDYASVNRNGPESGSKLMVQFPRNQCKDLFPKSDVLQDKWHSRLRGPPEVQVKNMEGRN 700  
FVYKMLLMSALSPLN 717

Figure 1. Amino Acid Sequences of Human TRAPs

- (A) TRAP240.
- (B) TRAP230.
- (C) TRAP150.
- (D) TRAP95.
- (E) TRAP80.

A highly Q-rich region resulting from CAG trinucleotide repeats in TRAP230 and leucine zipper structures in TRAP240, TRAP95, and TRAP80 are boxed. Peptide microsequences found in SMCC components by mass spectrometry are underlined. Peptide sequences found in TRAP components by conventional microsequence analysis are listed in Experimental Procedures, and all are present within the protein sequences shown.

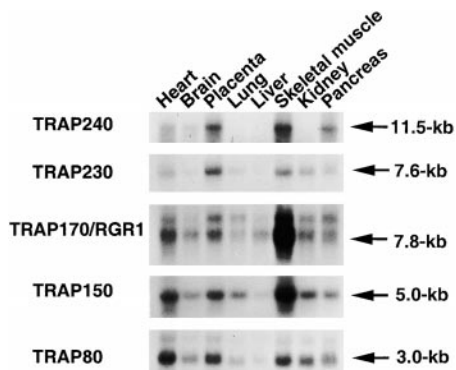


Figure 2. Expression of Novel TRAPs in Human Tissues  
A multiple tissue Northern blot (Clontech) was hybridized with cDNA probes as indicated.

directly by gradient SDS-PAGE followed by silver staining. This analysis (Figure 3A) revealed virtual identity between the complexes with respect to major polypeptides, the main exceptions being the presence of FLAG-TR $\alpha$  in the TR-TRAP complex and FLAG-SRB10 (rather than natural SRB10) in SMCC. Immunoblot analyses (Figure 3B) with available antibodies confirmed the presence of TRAP240, TRAP230, TRAP220, TRAP150, TRAP100, TRAP80, SRB10, MED6, SRB11, and SRB7 in both complexes and of TR $\alpha$  exclusively in the TR-TRAP complex.

The major polypeptides identified here in both the TRAP and SMCC complexes include the ten previously observed TRAPs between 80 and 240 kDa (Fondell et al., 1996), components (SRB7, SRB10, SRB11, MED6,

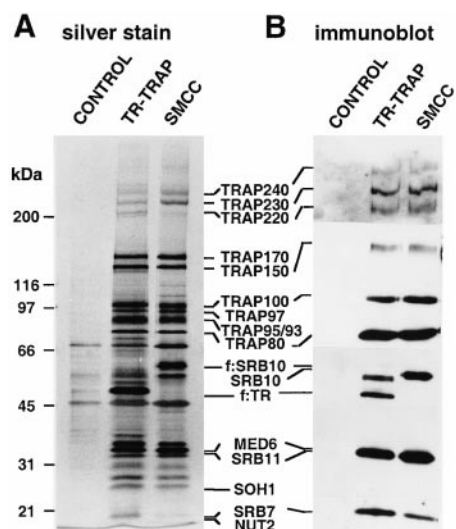


Figure 3. Near Identity in Polypeptide Compositions of TR-TRAP and SMCC Complexes

(A) SDS-PAGE and silver staining. Amounts (5  $\mu$ l) of TR-TRAP (lane 2), SMCC (lane 3), and control eluates from untransfected HeLa extracts (M2/control, lane 1) were analyzed.

(B) Immunoblot analyses. Blots were probed with anti-hTRAP240, anti-hTRAP230, anti-hTRAP220, anti-hTRAP150, anti-hTRAP100, anti-hTRAP80, anti-hSRB11, anti-hSRB10, anti-hSRB7, anti-hMED6, and anti-hTR $\alpha$ .

SOH1, and NUT2) first detected in our own studies in the SMCC complex (Gu et al., 1999), and additional low molecular weight TR-TRAP/SMCC polypeptides (most notably in the circa 25–40 kDa range) that were selectively bound to M2-agarose relative to components in the control HeLa nuclear extract. Of all these, components corresponding to RGR1/TRAP170, SRB7, SRB10, SRB11, and MED6 were identified in the human NAT complex (Sun et al., 1998), and components corresponding to MED6, RGR1/TRAP170, and the currently described TRAP95 and TRAP80 were found in the mouse Mediator complex (Jiang et al., 1998). Based on peptide sequences reported by Jiang et al. (1998) and additional sequence information on TRAP/SMCC components (data not shown), the p34 component of the mouse Mediator corresponds to the circa 36 kDa component of the SMCC/TRAP complex. Immunoblot analyses (data not shown) have also shown the presence of MED7 and a circa 26 kDa human homolog (hTRFP) of *Drosophila* TRF proximal protein in the TR-TRAP and SMCC complexes, consistent with the presence of these components in the murine Mediator complex and in a human holoenzyme preparation (Jiang et al., 1998; Xiao et al., 1999). Hence, the SMCC/TRAP complex appears related, but not identical, to the mouse Mediator and human NAT complexes.

Although our initial report (Fondell et al., 1996) of the TR-TRAP complex failed to note the presence of specific components under 80 kDa, this likely reflected suboptimal electrophoretic resolution and staining procedures, and a reexamination of the original data prompted by the present results has indicated additional low molecular weight polypeptides. The failure to clearly visualize these polypeptides in the earlier analysis may also have reflected variability in complex extraction, binding, and washing conditions. However, while more extensive washes have been found to lower somewhat the fractional content of some smaller polypeptides, the overall composition of the TR-TRAP complex is relatively constant from 180 to 300 mM KCl (data not shown). Hence, the currently described TR-TRAP complex appears to be a rather stable entity and virtually identical to the SMCC complex when isolated under comparable conditions. Nonetheless, and as suggested by a careful inspection of Figure 3A, there might be differences between these complexes with respect to a few minor polypeptides that remain to be characterized.

#### SMCC Mediates TR Function In Vitro

To establish the functional equivalence of SMCC and the TRAP complex, we first asked if SMCC could function as a coactivator for TR in an in vitro coactivator assay that was used previously to uncover the coactivator potential of SMCC (Malik et al., 1998; Gu et al., 1999). This system was reconstituted with core RNA polymerase II, essentially homogeneous general factor preparations (including TFIIA, TFIIB, TFIIID, TFIIE, and TFIIIF, but lacking TFIIH) and the USA-derived cofactor PC4. Control reactions indicated that although our present assay conditions were somewhat different from those in our initial report (Fondell et al., 1996), in which inadequately defined TFIIH-containing and total USA fractions were used, the TR-TRAP complex, but not liganded TR alone,

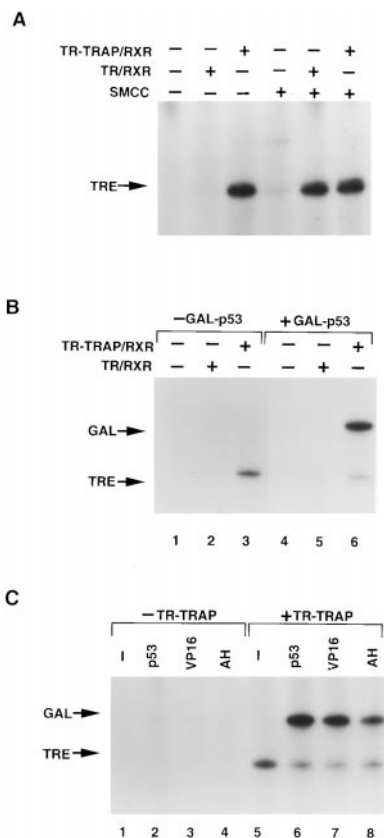


Figure 4. Functional Equivalence of TR-TRAP and SMCC Complexes

(A) SMCC mediates transcription activation by TR. In vitro transcription reactions contained the following general transcription factors: 50 ng TFIIA, 10 ng TFIIB, f:TFIID (10 ng TBP), 10 ng TFIIE $\alpha$ , 5 ng TFIIE $\beta$ , 25 ng TFIIF, 50 ng RNA polymerase II, and 150 ng PC4. TR $\alpha$  (10 ng, lanes 2 and 5), RXR $\alpha$  (20 ng, lanes 2, 3, 5, and 6), SMCC (3  $\mu$ l, lanes 4–6), or TR-TRAP (10 ng TR content by Western blot analysis, lanes 3 and 6) were added as indicated. All reactions had 50 ng each of the test template, TRE $\Delta$ 53 (TRE). (pG5HML was also included to maintain total DNA amount at 100 ng but is not indicated.) TRIAC (10 $^{-6}$  M) was included in all reactions.

(B) TR-TRAP complex mediates transcription activation by Gal4-p53. In vitro transcription reactions were reconstituted as in (A). Gal4-p53 (30 ng) was added to reactions in lanes 4–6. All reactions contained 50 ng each of TRE $\Delta$ 53 (TRE) and pG5HML (GAL) templates.

(C) TR-TRAP is a coactivator for heterologous activators. In vitro transcription reactions were reconstituted as in (A) with the following additions: Gal4-p53 (30 ng), lanes 2 and 6; Gal4-VP16 (30 ng), lanes 3 and 7; Gal4-AH (25 ng), lanes 4 and 8; and TR-TRAP, lanes 5–8. In (B) and (C), reactions were preincubated at 30°C for 5 min to allow any potential activator-TRAP complexes to form prior to addition of RXR $\alpha$  ([B], as indicated; [C], lanes 5–8), template DNA, general transcription factors, and nucleotides. For reactions in (B) only, TRIAC (10 $^{-6}$  M) was also added at this time.

could activate transcription from the TRE-containing template in the presence of RXR (Figure 4A, lane 3 versus lanes 2 and 1). Upon inclusion of SMCC, which alone had little effect on transcription (lane 4 versus lane 1), the level of activated transcription by liganded TR (lane 5) approached that of the TR-TRAP complex (lane 3). Furthermore, activated transcription by TR-TRAP was refractory to further stimulation by SMCC (lane 6). These

results indicate that SMCC can function as a coactivator for liganded TR and are consistent with the postulated identity of SMCC and the TRAP complex.

#### TR-TRAP Is a Coactivator for Heterologous Activators

Given the demonstration that SMCC can mediate TR function (above) and the earlier demonstration that SMCC can function as a coactivator for p53 (Gu et al., 1999), we also asked if, conversely, the TR-TRAP complex could substitute for SMCC in mediating p53 function. We therefore assessed the effect of the TR-TRAP complex on p53 in a coactivator assay reconstituted as above. As shown in Figure 4B, activation by Gal4-p53 (GAL template) could be elicited with the TR-TRAP complex (lane 6 versus lane 3) but not with TR alone (lanes 2 and 5). Thus, and despite the presence of preassociated TR, the TRAP complex can provide a coactivator function for p53. An extension of these studies showed that the activators Gal4-VP16 and Gal4-AH, like Gal4-p53, could function with the TR-TRAP complex (Figure 4C, lanes 6–8), but not alone (lanes 2–4 versus lane 1), to activate a target (GAL) promoter. Altogether, these results establish both the structural similarity, if not identity, and the functional equivalence of SMCC and TRAP complexes.

#### Interaction of SMCC and TR-TRAP Complexes with TR $\alpha$ , VDR, and VP16

To gain further insight into the mechanism of action of TR-TRAP and SMCC, the ability of both natural (intact) complexes and individual subunits to interact with activators was examined. Although several of the TRAP complex components (TRAPs 240, 230, 220, 170, and 100) possess the signature ligand-dependent nuclear receptor recognition motifs (LXXLL), only TRAP220 shows significant ligand-dependent binding to TR (Yuan et al., 1998; data not shown). This suggests that TRAP220 is the main target for the relay of nuclear receptor signals to the TRAP complex. Since SMCC also contains TRAP220, and to further document functional equivalence with the TRAP complex, interactions of SMCC with GST-TR $\alpha$  and GST-VDR fusion proteins were tested. As indicated both by SDS-PAGE silver stain (Figure 5A) and immunoblot (Figure 5B) analyses, SMCC binds to both receptors in a ligand-dependent fashion.

Our previous analysis of SMCC documented an interaction with p53 that was eliminated by two activation domain point mutations that inactivate transcriptional activation by p53 (Gu et al., 1999). To extend this analysis to another activator, and to compare SMCC and TR-TRAP complexes, interactions with the VP16 activation domain were analyzed. As shown in Figure 5C and Figure 5D, respectively, TR-TRAP and SMCC complexes both interacted with VP16 in a ligand-independent manner. Thus, these studies indicate functional equivalence at the level of activator interactions between TR-TRAP and SMCC complexes. Significantly, they also indicate that the presence of one strongly bound activator (TR) does not necessarily preclude the binding of another, consistent with the functional data of Figure 4C. Moreover, and related, the retention of a stoichiometric amount of TR along with the TRAPs on the GST-VP16

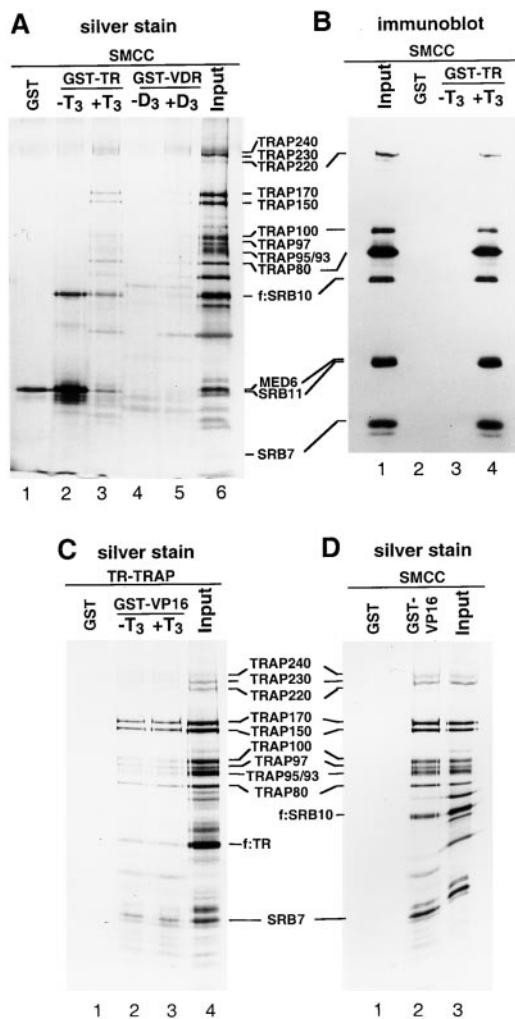


Figure 5. Activator Interactions with TR-TRAP and SMCC Complexes

(A and B) SMCC binds to TR $\alpha$  and VDR in a ligand-dependent fashion. SMCC (5  $\mu$ l) (input, lane 6) was incubated with 1  $\mu$ g each of GST alone (lane 1), GST-TR $\alpha$  (lanes 2 and 3), or GST-VDR (lanes 4 and 5) without (lanes 2 and 4) or with (lanes 3 and 5) its cognate ligand (10<sup>-7</sup> M) in BC180 buffer with 0.1% NP40 and 1 mM  $\beta$ -mercaptoethanol. Bound proteins were eluted, resolved by 5%–15% SDS-PAGE, and either visualized by silver staining (A) or blotted with antibodies as indicated (B).

(C) The TR-TRAP complex binds to VP16 in vitro. GST alone (control, lane 1) or GST-VP16 fusion protein (lanes 2 and 3) (1  $\mu$ g each) immobilized to glutathione-sepharose was incubated with 5  $\mu$ l input (lane 4), and bound proteins were eluted and analyzed as in (A). The reaction in lane 3 also contained T<sub>3</sub>, showing ligand-independent binding of VP16.

(D) SMCC binds to VP16 in vitro. GST alone (control, lane 1) or GST-VP16 fusion protein (lane 2) (1  $\mu$ g each) immobilized to glutathione-sepharose was incubated with 5  $\mu$ l input (lane 3), and bound proteins were visualized in the same condition as in (A).

column (Figure 5C) indicates that two activators (TR and VP16) can simultaneously occupy a single TRAP complex. A similar analysis showed that TR and p53 also can simultaneously bind to the TRAP complex and that activation domain mutations reduce the p53 interaction (data not shown).

To complement previous studies showing that TR and

other nuclear receptors interact with the TRAP220 subunit (Yuan et al., 1998), radiolabeled TRAP subunits were screened for interactions with p53 and VP16 activation domains. The analysis in Figure 6 shows that TRAP80, but not TRAP220 and TRAP100, interacts with GST fusion proteins containing intact p53 (lanes 16–18) and VP16 (lanes 22–24) activation domains, but not with GST alone (lanes 13–15) or with GST fusion proteins containing mutant p53 (lanes 19–21) or mutant VP16 (lanes 25–30) activation domains that are defective for transcription (Cress and Triezenberg, 1991; Lin et al., 1994). In a further analysis of TRAP interactions with full-length wild-type and mutant p53 proteins immobilized on M2-agarose, TRAP80 showed interactions only with wild-type p53 (lane 9 versus lane 12), consistent with the above results with isolated wild-type and similarly mutated activation domains, whereas TRAP220 (but not TRAP100) showed interactions with both wild-type and mutant full-length forms of p53 (lanes 7 and 10). The latter result is consistent with an earlier report of p53 interactions with an independently identified protein (RB18A) equivalent to TRAP220, but for which no transcriptional activation function was demonstrated (Drané et al., 1997). In contrast, the activation domain-dependent interactions of p53 and VP16 with TRAP80 suggest that the latter may be an independent target for p53 and VP16 within the TRAP/SMCC complex.

## Discussion

There are several significant features to the present work. First, we have shown virtual identity, with respect to structure and function, between two independently isolated human coactivator complexes—one (TRAP) that was isolated in a stable ligand-dependent association with a nuclear receptor and one (SMCC) that was isolated on the basis of components originally identified in the yeast holoenzyme-associated Mediator. Second, we have identified novel components (TRAPs) that distinguish the human SMCC/TRAP complex from the distantly related yeast Mediator and, in one case, suggest a link between human disease and a TRAP-related transcriptional defect. Third, we have identified a novel target (TRAP80) for p53 and VP16, distinct from that (TRAP220) of the thyroid hormone receptor, in the TRAP/SMCC complex, indicating distinct pathways of communication to the general transcriptional machinery through a common and potentially global coactivator. These results have important implications for the mechanism(s) of action of TR and the nuclear receptor superfamily, as well as p53, VP16, and other cellular and viral activators. They also suggest a basis for the synergistic functions of different activators, as well as for their regulation through parallel signaling pathways that may affect, positively or negatively, the functions of the cofactor complex.

## Novel TRAP Polypeptides

We describe five novel subunits of the TRAP/SMCC complex. While all of their individual functions are not yet understood, their general relevance as integral components of the TRAP/SMCC complex is underscored by their presence in two complexes isolated by two

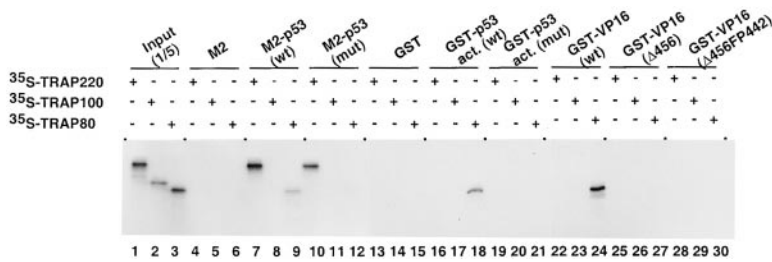


Figure 6. Specific Interactions of TRAP Components with Acidic Activators  
M2-agarose alone (control, lanes 4–6) and 1  $\mu$ g each of wild-type (lanes 7–9) and mutant (lanes 10–12) full-length p53 immobilized to M2-agarose were incubated with  $^{35}$ S-labeled TRAP220, TRAP100, and TRAP80 (inputs, lanes 1–3), and bound radiolabeled proteins were visualized by autoradiography after resolution by SDS-PAGE. Likewise, GST alone (control, lanes 13–15), wild-type (lanes 16–18), and mutant (lanes 19–21) GST-p53 activation domain fusion proteins, and wild-type (lanes 22–24) and mutant ( $\Delta$ 456, lanes 25–27;  $\Delta$ 456FP442, lanes 28–30) GST-VP16 activation domain fusion proteins immobilized on glutathione-Sepharose were incubated with  $^{35}$ S-labeled TRAPs and bound radioactive proteins detected as above. The mutant forms of p53 and p53 activation domains contained two point mutations at residues 22 and 23. VP16 $\Delta$ 456 contains a deletion of the C-terminal part of the complete activation domain (residues 457 to 490) while  $\Delta$ 456FP442 contains an additional point mutation at residue 442.

independent affinity purification methods and by the presence of at least two components (TRAP80 and TRAP95) in a mouse Mediator complex isolated by extensive conventional chromatographic procedures. The absence of any yeast homologs of these components also underscores the fact that the human SMCC/TRAP complex is only distantly related to the yeast Mediator.

Among these new TRAPs, TRAP230 is of special interest in that it contains a long CAG trinucleotide repeat region that encodes a glutamine-rich C-terminal region with demonstrated activation functions in Gal4-fusion protein assays (data not shown). Such CAG repeats have been associated with many hereditary diseases, most notably with neuropsychiatric disorders. Indeed, a mutated TRAP230 gene (a variant allele on the X chromosome containing a dodecamer insertion within the CAG repeat) was most recently correlated with a significant susceptibility to mental retardation in males (Philibert et al., 1998). Since many nuclear receptors play crucial roles in the development and homeostasis of the central nervous system in metazoans, an altered cofactor function of the TRAP complex in the brain as a result of mutant TRAP230 might be a potential cause of male mental retardation. More significantly, Philibert et al. (1998) also reported that those males who carry this mutation have high histories of hypothyroidism and treatment with antidepressants, supporting the idea that TRAP230 is a component of a coactivator complex involved in TR function in human.

Another TRAP, TRAP150, is distinct in that there exists in humans a hypothetical related protein of unknown function in the database, thus indicating a potential gene family for a transcriptional coactivator component (see below).

#### Structural Identity of TRAP and SMCC Complexes and Relationships to Other Complexes

The TRAPs were originally isolated as an associated complex necessary for TR function in vitro (Fondell et al., 1996), whereas SMCC was isolated solely on the basis of resident homologs to yeast SRB proteins and later shown to have both repressive and coactivator functions (Gu et al., 1999). It was therefore surprising to find virtual structural identity between these complexes, but the present studies firmly establish this fact on the basis of comparative electrophoretic, direct sequence, and immunoblot analyses. The currently characterized TRAP

and SMCC complexes contain (1) the ten previously identified TRAP components: TRAP240, TRAP230, TRAP220/RB18A/PBP, TRAP170/RGR1, TRAP150, TRAP100, TRAP97, TRAP95, TRAP93, and TRAP80; (2) homologs of yeast Mediator components SRB7, SRB10, SRB11, MED6, MED7, and MED10/NUT2 (in addition to TRAP170/RGR1) and the yeast regulatory factor SOH1 (Gu et al., 1999); and (3) additional polypeptides, minimally, p26, p28, p36, and p37, which include the human homolog (TRFP) of *Drosophila* TRF proximal protein.

A comparison of TRAP and SMCC complexes with other demonstrated or presumptive cofactor complexes reveals moderate to strong similarities, but not identities. Like the various RNA polymerase II holoenzyme preparations (reviewed in Parvin and Young, 1998), TRAP and SMCC complexes contain SRB7, SRB10, and SRB11, but, unlike these holoenzymes, no general transcription factors, SWI2, CBP/p300, or BRCA1 (Gu et al., 1999; our unpublished data). The overall complexity of TRAP/SMCC resembles that of the human NAT complex, which minimally shares RGR1, MED6, SRB7, SRB10, and SRB11 components with TRAP/SMCC, although NAT appears to lack both high molecular weight components and coactivator functions (Sun et al., 1998). The TRAP/SMCC complex also is related to the structurally less complex mouse Mediator complex, which minimally shares RGR1, TRAP95 (p96b), TRAP80 (p78), MED7, MED6, and TRFP components but appears to lack SRB components (Jiang et al., 1998). Consistent with the ability of TRAP220 to interact with the vitamin D receptor (Yuan et al., 1998), the recently isolated VDR-interacting DRIP complex (Rachez et al., 1998) also appears very similar to TRAP and SMCC complexes; however, the presence of an additional 180 kDa VDR-interacting subunit and a histone acetyltransferase activity may signify some differences and/or heterogeneity. Further, although sequence information has not yet been reported for a recently described SREBP-interacting complex implicated in SREBP and Sp1 synergism on chromatin templates (Naar et al., 1998), the overall composition of this complex (with the exception of associated CBP) looks very similar to that of TRAP/SMCC.

#### Functional Properties of the TRAP/SMCC Complex: A Putative Global Cofactor

Independent studies have implicated the TRAP complex in the function of TR (Fondell et al., 1996) and other

nuclear receptors (Yuan et al., 1998) and SMCC in the function of Gal4-AH and Gal4-p53 (Gu et al., 1999). Consistent with near identity between the structures of TRAP and SMCC complexes, the present studies have further shown that SMCC can mediate activation by TR and, reciprocally, that the TRAP complex can mediate activation by Gal4-AH, Gal4-p53, and Gal4-VP16. Importantly, and consistent with the activator interaction studies (below), the TRAP-mediated coactivation function is unaffected by prior association of TR. Thus, even though we have not conducted a broad survey of activators, the present results suggest a more global role for TRAP/SMCC in activator function. This hypothesis is consistent with the broad functions of the distantly related yeast Mediator. In this regard, it is important to emphasize that some yeast Mediator components are involved mainly in transcriptional activation whereas others are involved in transcriptional repression, and that some components globally affect transcription whereas others are more gene-selective (reviewed in Myer and Young, 1998; Hampsey, 1998). Indicative of potential negative functions for human cofactor complexes as well, both SMCC and NAT complexes have been shown to inhibit activated transcription under certain conditions (Sun et al., 1998; Gu et al., 1999). However, the selective manifestation of repressive functions with NAT and SMCC, but not with the TR-TRAP complex (data not shown), may reflect the specific purification of NAT and SMCC complexes via the SRB10(CDK8) component and a relative excess of the free SRB10-SRB11 kinase-cyclin pair in the preparations.

Given the diversity of mammalian coactivators, especially for nuclear receptors, it will be important to determine whether there is a potential functional redundancy in these factors. Although we and others have shown TAF<sub>II</sub> requirements for the function of many activators in partially purified systems (reviewed in Roeder, 1996; Verrijzer and Tjian, 1996; Oelgeschläger et al., 1998), the apparent lack of a general TFIID-specific TAF<sub>II</sub> requirement not only in yeast (reviewed in Hahn, 1998) but also in HeLa nuclear extracts (Oelgeschläger et al., 1998) supports the idea of redundancy in cofactors acting at the level of the DNA template. Interestingly, the TAF<sub>II</sub>s also appear not to be essential for activation by TR in conjunction with TRAPs on DNA templates in purified systems (Fondell et al., 1999), although this does not preclude their possible function *in vivo* or with chromatin templates. At the same time, TR function with SMCC and TRAP complexes does require other general coactivators (e.g., PC4) derived from the USA fraction (Fondell et al., 1996, 1999). In the case of Gal4-VP16 and Gal4-p53, high level activation from DNA templates in purified systems also requires USA-derived coactivators (e.g., Ge and Roeder, 1994; Gu et al., 1999) as well as the TAF<sub>II</sub> components of TFIID (Klemm et al., 1995; Lu and Levine, 1995; Thut et al., 1995). However, the finding of TRAP/SMCC interactions and coactivator functions with p53 and VP16 (below) raises the possibility that these coactivator complexes may play a dominant role in the function of these activators. This is consistent with our observation that optimal activation by Gal4-VP16 in nuclear extracts requires SRB7 and/or associated proteins but not TFIID-specific TAF<sub>II</sub>s (Oelgeschläger et al., 1998).

#### Novel Mechanisms for the Function of Metazoan Activators

Based on the function of TRAP and SMCC complexes in purified systems with DNA templates, they must act, at least in part, downstream of coactivators that are thought to act through modifications of chromatin templates. Our past (Fondell et al., 1996; Yuan et al., 1998; Gu et al., 1999) and present (Figure 5) studies indicate that the TRAP/SMCC complexes interact directly with the activation domains of the potent activators VP16 and p53 and, in a ligand-dependent manner, with nuclear receptors. They further show that activators may target specific components of the TRAP/SMCC complex—TRAP220 in the case of TR and other receptors and TRAP80 in the case of p53 and VP16—analogous to the situation described for Gal4 interactions with the SRB4 component of yeast Mediator (Koh et al., 1998). Our further demonstration that two distinct activators can bind simultaneously to the SMCC/TRAP complex (Figure 5) also provides a mechanism for the synergistic functions of activators (Ptashne and Gann, 1997).

Although the downstream targets in the basal transcriptional machinery are not well documented for the TRAP/SMCC complex, precedent from yeast Mediator/holoenzyme studies suggests the core RNA polymerase II (Koleske and Young, 1994; Kim et al., 1994; reviewed in Myer and Young, 1998). Consistent with this possibility, several of the TRAP/SMCC components described here have been reported in various partially purified mammalian RNA polymerase II holoenzymes (reviewed in Parvin and Young, 1998; Xiao et al., 1999). In addition, RNA polymerase II can be isolated in association with SMCC at low ionic strength (Gu et al., 1999), as well as with the TR-TRAP complex (data not shown), and interactions of the related NAT and mouse Mediator complexes with core RNA polymerase II and the CTD, respectively, have been reported (Sun et al., 1998; Jiang et al., 1998). Thus, as reported for yeast Mediator, RNA polymerase II recruitment to the promoter may be a major consequence of activator-mediated recruitment of the coactivator complex. In this regard, it is interesting to note that the ability to isolate a stable ligand-induced TR-TRAP complex from the cell suggests that an activator may actually target the coactivator complex before the activator itself is bound to target sites in the promoter—a novel mechanism for activator function.

#### Regulatory Modifications of the TRAP/SMCC/Mediator Complex

A major unanswered question concerns the possibility of natural heterogeneity, as a result of structural modifications, in the TRAP/SMCC/Mediator complex. This possibility is suggested by the finding of a hypothetical human gene product closely related to TRAP150 and by the presence in the mouse Mediator of a component (Ring 3) that is part of a multigene family (Jiang et al., 1998) and evidently not in the human TRAP/SMCC complexes. Such variations may generate gene- or activator-selective cofactor complexes; and in accord with the principle first established by the B cell-specific coactivator OCA-B (Luo et al., 1992), cell-specific modifications of the TRAP/SMCC/Mediator complex may also

play a key role in cell-specific transcription events. Thus, despite the very significant contributions of the yeast Mediator/holoenzyme studies to our understanding of eukaryotic transcription, the rather distantly related mammalian complexes offer the possibility for novel regulatory modifications and interactions unique to mammals.

#### Experimental Procedures

##### Immunopurification of TR-TRAP and SMCC Complexes

The TR-TRAP complex was immunopurified (on M2-agarose) from ligand-treated HeLa  $\alpha$ -2 cells expressing FLAG-tagged TR $\alpha$  as described (Fondell et al., 1996). The SMCC complex was immunopurified (on M2-agarose) from HeLa K-8 cells expressing FLAG-tagged SRB10/CDK8 as described (Gu et al., 1999). On the basis of immunoblot and/or mass spectrometric analysis, the complexes were devoid of detectable amounts of general transcription factors including TFIID.

##### cDNA Cloning and Northern Blot Analysis of TRAPs

Polypeptides in immunopurified TR-TRAP were resolved by SDS-PAGE and subjected to microsequence analysis. Derived sequences included DPYAFVE(G)DEEFLF(P/M)(D/Q)A(K) for TRAP240; AETDQLAPIVPLNPG for TRAP230; LEEESFPERSK for TRAP150; FPAITHL for TRAP95; and EIFAQLSREAVQIK, MRLSQPQAFDK, and IMVQFPRNQCK for TRAP80. Putative full-length cDNAs corresponding to TRAPs 240, 230, 150, 95, and 80 were obtained from a HeLa cDNA library (Gu et al., 1999). TRAP240 cDNA was cloned with PCR products amplified with primers based directly on the cognate peptide sequence, and a subsequent BLAST search revealed identity between sequences encoding the C-terminal half of TRAP240 and a randomly sequenced partial gene of unknown function (KIAA0593, accession number AB011165). TRAP150 and TRAP80 cDNAs were cloned via PCR products amplified with primers based on EST clones (accession numbers 1234764 for TRAP150 and 717840 and AA282298 for TRAP80) that contained cognate peptide sequences. TRAP230 cDNA was cloned via oligonucleotide probes based on a partial nucleotide sequence for a gene (KIAA0192, accession number D83783) of unknown function that encoded TRAP230 peptide sequences. TRAP95 cDNA was cloned with a degenerate oligonucleotide based on the peptide sequence. Oligonucleotide and PCR primer sequences are available upon request.

For Northern blot analyses, a 0.9 kb EcoRI cDNA fragment of TRAP240, a 1.8 kb cDNA fragment encoding residues 252–851 of TRAP230, a 0.6 kb cDNA fragment encoding residues 400–603 of TRAP170/hRGR1, a 1.8 kb cDNA fragment encoding residues 1–601 of TRAP150, and a 1.8 kb BamHI cDNA fragment of TRAP80 were used to probe a human multiple tissue Northern blot (CLONTECH).

##### Antibody Production and Western Blot

6His-TRAP240(706–1000), 6His-TRAP230(587–851), GST-TRAP150(488–573), and 6His-TRAP80(353–717) were expressed in and purified from *Escherichia coli* strain BL21 (pLysS), and 10 mg amounts of each were gel isolated for antibody production in rabbits (Covance, Denver, PA). Polyclonal antibodies against TRAP220 and TRAP100 were described previously (Yuan et al., 1998). Antibody to cyclin C/SRB11 was from Santa Cruz Biotechnology. Western blots were visualized by enhanced chemiluminescence (Amersham).

##### In Vitro Transcription Assays

In vitro transcription assays employed a highly purified system (Malik et al., 1998) and previously described templates and activators (Fondell et al., 1996; Gu et al., 1999), except that baculovirus-expressed FLAG-tagged TR $\alpha$  and RXR $\alpha$  were additionally purified over S-Sepharose after M2-agarose affinity purification.

##### In Vitro Pull-down Assays

Immobilized GST-TR $\alpha$ , GST-VDR, and GST-VP16 (Fondell et al., 1996; Yuan et al., 1998) and either purified SMCC or TR-TRAP complex were used in GST pull-down assays in BC180 with 0.1% NP40

and 1 mM  $\beta$ -mercaptoethanol in the absence or presence of  $10^{-7}$  M T<sub>3</sub> or  $1\alpha,25(\text{OH})_2\text{D}_3$ . After incubation for 1 hr at 4°C, beads were washed extensively with the binding buffer, and bound proteins were eluted with binding buffer plus 0.2% Sarkosyl, separated by gradient SDS-PAGE, and visualized by silver staining.

#### Acknowledgments

We thank Drs. E. A. Nigg, Y. Tao, and Y.-J. Kim for anti-CDK8 (SRB10), anti-SRB7, and anti-MED6 antibodies and members of the Roeder lab for helpful discussions and critical comments on the manuscript. Protein and DNA sequencing analyses for cloning of TRAP cDNAs were provided by the Protein/DNA Technology Center of the Rockefeller University. This work was supported by a Human Frontier Science Program long-term fellowship and a fellowship from the Japan Society for the Promotion of Science to M. I., a postdoctoral fellowship from Life Science Research Foundation (LSRF) for Advanced Cancer Studies to W. G., and grants from the NIH to R. G. R.

Received January 21, 1999; revised February 24, 1999.

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#### GenBank Accession Numbers

The accession numbers for the TRAP240, TRAP230, TRAP150, TRAP95, and TRAP80 cDNA sequences are AF117754, AF117755, AF117756, AF121228, and AF117657, respectively.