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# MED1/TRAP220 Exists Predominantly in a TRAP/Mediator Subpopulation Enriched in RNA Polymerase II and Is Required for ER-Mediated Transcription

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## Summary

**Human TRAP/Mediator is a key coactivator for many transcription factors that act through direct interactions with distinct subunits, and MED1/TRAP220 is the main subunit target for various nuclear receptors. Remarkably, the current study shows that MED1/TRAP220 only exists in a TRAP/Mediator subpopulation (less then 20% of the total) that is greatly enriched in specific TRAP/Mediator subunits and is tightly associated with a near stoichiometeric level of RNA polymerase II. Importantly, this MED1/TRAP220-containing holoenzyme supports both basal- and activator-dependent transcription in an in vitro system lacking additional RNA polymerase II. Furthermore, chromatin immunoprecipitation experiments demonstrate an activator-selective recruitment of MED1/TRAP220-containing versus MED1/TRAP220-deficient TRAP/Mediator complexes to estrogen receptor (ER) and p53 target genes, respectively. Finally, RNAi studies show that MED1/TRAP220 is required for ER-mediated transcription and estrogen-dependent breast cancer cell growth. These observations have significant implications for our current understanding of the composition, heterogeneity, and functional specificity of TRAP/Mediator complexes.**

## Introduction

Nuclear receptors comprise a large family of transcriptional activators that play diverse roles in cell growth, differentiation, and homeostasis in metazoans (Mangelsdorf et al., 1995). Nuclear receptors share a common structure with three functional domains: an N-terminal AF-1 activation domain, a central DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD) with an associated (ligand-induced) AF-2 activation domain. The ultimate action of nuclear receptors on target genes, after site-specific DNA binding, is to enhance the recruitment and/or function of the general transcription machinery (RNA polymerase II and general transcription factors TFII-A, -B, -D, -E, -F, and -H; Roeder, 1996) on cognate core promoter elements. More recent studies have implicated a large multisubunit coactivator complex, TRAP/Mediator, as the main pathway for direct communication between nuclear receptors (as well as other transcriptional activators) and the general transcription machinery (reviewed in Malik and Roeder, 2000). Although capable of mediating the function of transcriptional activators on DNA templates in defined systems, Mediator is thought to act subsequent to other coactivators that modify chromatin structure on more physiological templates (Glass and Rosenfeld, 2000; Malik and Roeder, 2000).

The human TRAP/Mediator complex was first identified through an intracellular ligand-dependent association with thyroid hormone receptor (TR) (Fondell et al., 1996). Identical or closely related complexes (SMCC, DRIP, ARC, CRSP, PC2, NAT, mMediator) were subsequently isolated via other selection methods (reviewed in Malik and Roeder, 2000). TRAP/Mediator is composed of at least 25–30 distinct polypeptides and is related to the earlier-identified yeast Mediator (Boube et al., 2002). Along with earlier reports of yeast Mediator association with RNA polymerase II within a holoenzyme (Kim et al., 1994; Thompson et al., 1993), the demonstration of TRAP/Mediator interactions, through distinct subunits, with various transcription factors (Blazek et al., 2005; Malik and Roeder, 2000) has suggested a model involving activator-based recruitment of TRAP/Mediator followed by the effects of TRAP/Mediator on RNA polymerase II recruitment and/or function.

Whereas TRAP/Mediator has been shown to interact with and/or mediate the function of diverse activators, the most detailed information regarding TRAP/Mediator function has come from studies of nuclear receptor interactions. Thus, TRAP/Mediator has been shown to mediate nuclear receptor functions in purified cell-free systems (Fondell et al., 1996; Ge et al., 2002; Kang et al., 2002), and many nuclear receptors have been shown to interact either with TRAP/Mediator or with the MED1/TRAP220 subunit in a ligand-dependent fashion (Glass and Rosenfeld, 2000; Malik and Roeder, 2000). Further studies with MED1/TRAP220-deficient or mutated TRAP/Mediator complexes (Ge et al., 2002; Kang et al., 2002; Malik et al., 2004) have documented MED1/TRAP220-dependent nuclear receptor interactions with the entire TRAP/Mediator complex. In support of the conclusions of the in vitro analyses, studies of *Med1/Trap220* knockout mice (Ito et al., 2000) and derived *Med1/Trap220*$^{-/-}$ mouse embryonic fibroblasts have shown that although not essential for cell survival (and thus many gene activation events), MED1/TRAP220 is required for the intracellular function of TR and PPARγ on ectopic and endogenous target genes (Ge et al., 2002; Ito et al., 2000). Comparable studies have not yet been reported for estrogen receptor (ER), which is of primary interest in the current study. However, it has been shown that ER interacts with the complete TRAP/Mediator complex, both in vivo and in vitro, through the MED1/TRAP220 subunit (Kang et al., 2002) and that TRAP/Mediator enhances ER-dependent transcription in vitro (Acevedo and Kraus, 2003; Kang et al., 2002).

Consistent with its key role in mediating TRAP/Mediator-dependent nuclear receptor function, MED1/TRAP220

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has generally been considered an integral core subunit of the TRAP/Mediator complex (see Discussion). Consistent with this notion, the presumptive yeast ortholog (Med1) of MED1/TRAP220 has been considered a core subunit of yeast Mediator (Boube et al., 2002). However, both a *MED1* disruption in yeast (Balciunas et al., 1999) and a *Med1/Trap220* deletion in mammalian cells (Ito et al., 2000) display only conditional phenotypes. Moreover, it recently has been shown that the residual TRAP/Mediator complex in *Med1/Trap220*$^{-/-}$ mouse embryonic fibroblasts is essentially intact and retains basal transcription functions and coactivator functions for other activators (Malik et al., 2004), suggesting a tangential association of MED1/TRAP220 with the complex. Therefore, these data raise an important question as to whether MED1/TRAP220 is an integral core subunit of all TRAP/Mediator complexes or whether it exists only in a specialized TRAP/Mediator complex that subserves specific functions.

Toward a better understanding of the role of MED1/TRAP220 and cognate TRAP/Mediator complexes in nuclear receptor function, this study has investigated, through structural and functional studies, heterogeneity within TRAP/Mediator complexes, with a special focus on the MED1/TRAP220-containing TRAP/Mediator. Beyond demonstrating a MED1/TRAP220- and RNA polymerase II-enriched subpopulation of TRAP/Mediator, we have focused functional studies on estrogen receptor (ER)-dependent target-gene activation and cell growth in a breast cancer cell line that is readily amendable to such analyses. Apart from demonstrating a critical role for MED1/TRAP220 in ER-dependent functions, our studies provide evidence for the differential utilization of the newly defined MED1/TRAP220-containing and MED1/TRAP220-deficient TRAP/Mediator complexes in distinct gene activation events.

## Results

### MED1/TRAP220 Exists Only in a Subpopulation of TRAP/Mediator Complexes

Nuclear extracts were repeatedly passed through an anti-MED1/TRAP220 antibody column, and the flow-through fractions were collected and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analyses. A mock-depleted extract treated with preimmune antibodies served as a control. The anti-MED1/TRAP220 antibodies quantitatively removed MED1/TRAP220, whereas the levels of TATA-Binding Protein (TBP) and TFIIH (p65), components of the general transcription machinery, were unaffected (Figure 1). Surprisingly, however, a significant level (at least 80%) of all other TRAP/Mediator subunits tested still remained in the MED1/TRAP220-depleted nuclear extract. This result contrasts with our earlier observation of essentially complete depletion of all TRAP/Mediator subunits by antibodies against the integral TRAP/Mediator subunit MED30/TRAP25 (Baek et al., 2002). These data strongly suggest heterogeneity in cellular TRAP/Mediator complexes and that MED1/TRAP220, unlike core subunits such as MED30/TRAP25, exists only in a subpopulation of TRAP/Mediator complexes.

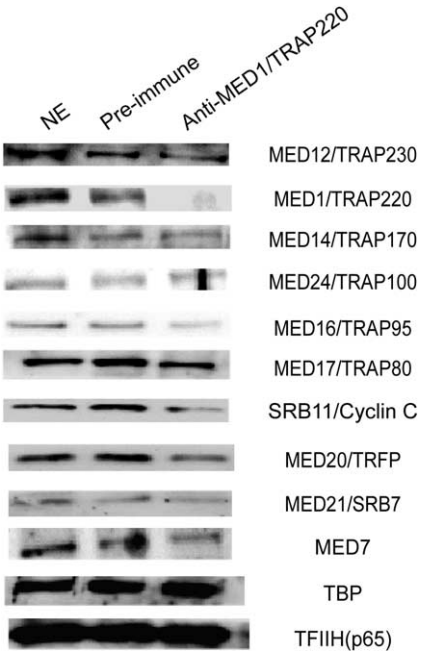


Figure 1. Immunodepletion of MED1/TRAP220 from Nuclear Extract

Immunoblots with indicated antibodies of supernatants after immunodepletion with anti-MED1/TRAP220 antibody are shown. Untreated nuclear extract and derived supernatant after immunodepletion with preimmune serum are also included as controls.

### Isolation and Characterization of MED1/TRAP220-Containing TRAP/Mediator from Nuclear Extracts

To better understand structural and functional similarities and differences between the MED1/TRAP220-containing TRAP/Mediator subpopulation and the total TRAP/Mediator population and to avoid possible disruption of complexes by prior chromatographic steps, we purified MED1/TRAP220-containing TRAP/Mediator directly from nuclear extracts by using anti-MED1/TRAP220 antibodies. Total TRAP/Mediator complex was purified directly from unfractionated nuclear extracts derived from a cell line expressing FLAG-tagged MED10/NUT2 (Malik and Roeder, 2000), a core subunit of the TRAP/Mediator complex, by using M2-agarose under the same conditions. Immunoblot analyses indicated that anti-MED1/TRAP220 antibody and anti-FLAG (M2-agarose) antibody immunoprecipitated all TRAP/Mediator subunits tested, whereas preimmune antibody did not (Figure 2A). A comparison of the subunit levels relative to the amounts of MED17/TRAP80 in these two preparations clearly revealed that MED1/TRAP220 is enriched (about 5-fold) in the anti-MED1/TRAP220 preparation, whereas most other tested subunits showed very similar levels in both preparations. Gel-filtration of nuclear extracts on S300 Sephedex revealed a single MED1/TRAP220 peak (at about 1.6 MDa) that was coincident with the majority of other TRAP/Mediator subunits (data not shown). Along with the fact that antibodies against the MED30/TRAP25 core subunit are able to quantitatively deplete MED1/TRAP220 (Baek et al., 2002), this indicates that MED1/TRAP220 exists mainly as a TRAP/Mediator bound

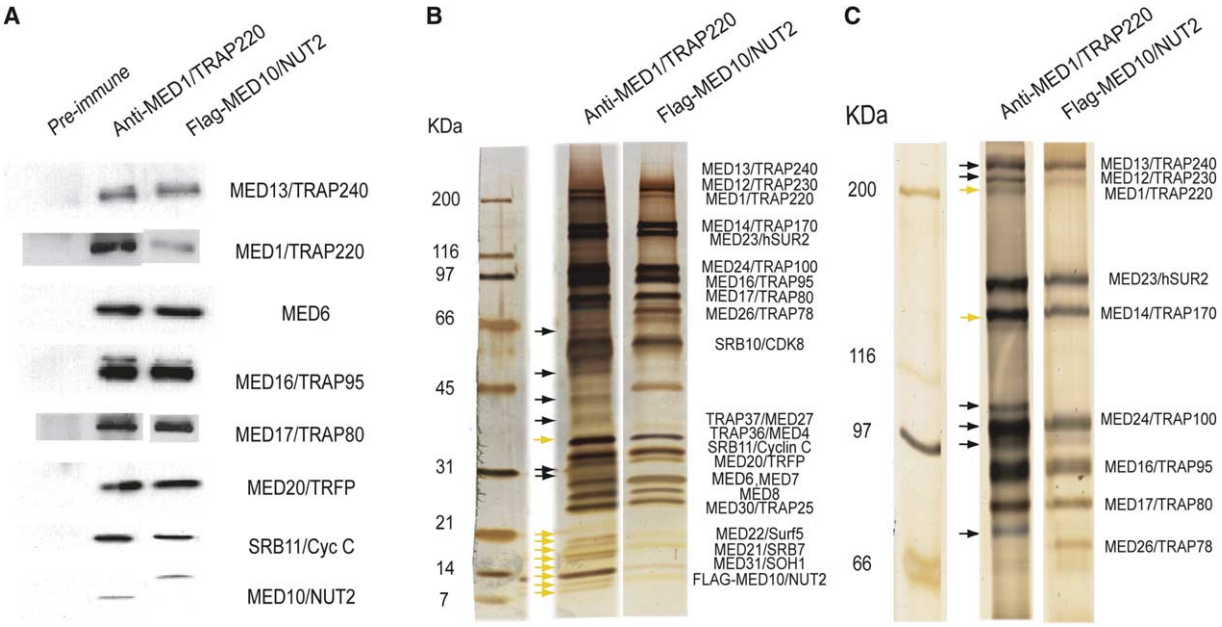


Figure 2. Immunopurification of a MED1/TRAP220-Containing TRAP/Mediator Subpopulation

(A) Immunoblots of preimmune serum immunoprecipitates, anti-MED1/TRAP220 immunoprecipitates, or anti FLAG-MED10/NUT2 purified (total) TRAP/Mediator with indicated antibodies are shown.

(B and C) Complexes were immune isolated with the respective antibodies, resolved by SDS-PAGE, and visualized by silver stain. Two different electrophoretic separations (4%–20% gradient [B] and 6% [C] gels) were run for the same samples. Subunits of the FLAG-MED10/NUT2 complex are listed next to the corresponding bands. New or enriched proteins associated with MED1/TRAP220-containing Mediator are indicated by arrows, with yellow arrows indicating RNA polymerase II subunits.

form. Therefore, we conclude that MED1/TRAP220 is markedly underrepresented (substoichiometeric) in the total TRAP/Mediator population.

We next examined the two TRAP/Mediator preparations by silver stain to assess potential differences in their compositions. Consistent with the immunoblot results, the anti-MED1/TRAP220 and FLAG-MED10/NUT2 preparations showed the expected subunit patterns (Figures 2B and 2C). Interestingly, however, in addition to all known TRAP/Mediator subunits present in the FLAG-MED10/NUT2 preparation, anti-MED1/TRAP220 immunoprecipitates contained a few obvious extra bands (arrows). The most prominent of these were in the sub-21 KDa region, which contains the MED22/Surf5, MED21/SRB7, MED31/SOH1, and MED10/NUT2 subunits in the FLAG-MED10/NUT2 preparation. Importantly, these new protein bands showed silver-stain intensities comparable to those of the MED22/Surf5, MED21/SRB7, MED31/SOH1, and MED10/NUT2 subunits in the same preparation. In addition, this analysis revealed a few other high-molecular weight bands that are either specific to, or enriched in, the anti-MED1/TRAP220 preparation. Because these proteins could potentially be specific to the MED1/TRAP220-containing TRAP/Mediator complex, it seemed that further identification might provide novel insights into the structure and function of the MED1/TRAP220-containing TRAP/Mediator complex.

### Mass Spectrometry Analyses of Purified MED1/TRAP220-Containing TRAP/Mediator

The immunopurified MED1/TRAP220-containing TRAP/Mediator and FLAG-MED10/NUT2 TRAP/Mediator preparations were subjected to Mass Spectrometry (MS) analyses as described in the Experimental Procedures. MS data indicated that both TRAP/Mediator preparations are highly pure, and all TRAP/Mediator subunits detected in these two preparations are listed in Table S1 (see Table S1 in the Supplemental Data available with this article online).

The FLAG-MED10/NUT2 preparation contains, in addition to components previously found in our TRAP/Mediator and PC2 preparations, MED26/CRSP70 (Naar et al., 2002) and several components (MED11/HSPC296, MED18/p28b, MED19/LCMR, and MED15/TIG1) that recently have been reported by others in MudPIT and other analyses of various Mediator preparations (Mittler et al., 2003; Sato et al., 2004). The anti-MED1/TRAP220 preparation was found to contain all of the TRAP/Mediator subunits found in the FLAG-MED10/NUT2 preparation and, in addition, MED13L/KIAA1025, MED25/ARC92/ACID1, MED9/FLJ10193, MED28/FSKG20, and MED29/Hintersex (Mittler et al., 2003; Sato et al., 2004).

We next performed immunoblotting with available antibodies against the newly identified proteins to confirm their association and potential enrichment within the MED1/TRAP220-containing TRAP/Mediator complex. By normalizing the levels of these components to the levels of MED17/TRAP80 in the MED1/TRAP220-containing and FLAG-MED10/NUT2 TRAP/Mediator complexes, we found that whereas MED18/p28b shows similar levels in these two preparations, all of the other tested subunits (MED19/LCMR, MED25/ARC92/ACID1, MED15/TIG1, and MED11/HSPC296) are specifically enriched in the MED1/TRAP220-containing TRAP/Mediator complex (Figure 3A). To further confirm that

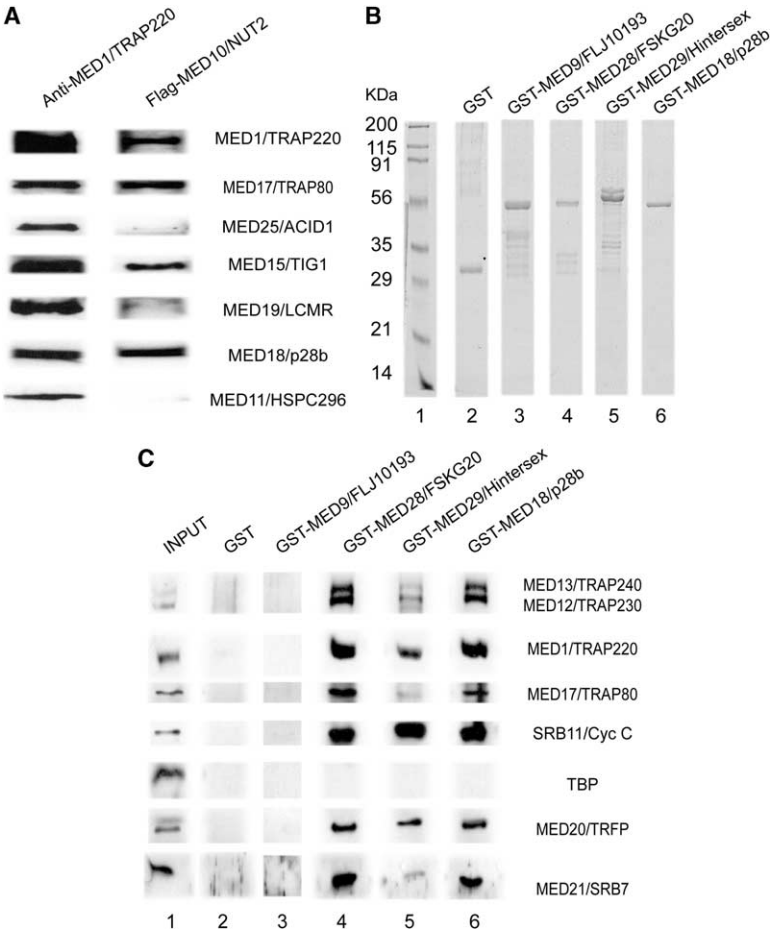


Figure 3. Association of the Newly Identified Proteins with TRAP/Mediator Complex

(A) Immunoblots of anti-MED1/TRAP220 immunoprecipitates or FLAG-MED10/NUT2 TRAP/Mediator preparations with the indicated antibodies are shown.

(B) GST only and indicated GST fusion proteins were expressed in bacteria, purified, and analyzed by SDS-PAGE with Comassie brilliant blue R-250.

(C) Immobilized GST fusion proteins purified in (B) were incubated with Hela nuclear extract, and bound proteins were eluted and analyzed by immunoblot with indicated antibodies.

MED9/FLJ10193, MED28/FSKG20, and MED29/Hintersex (for which appropriate antibodies are not available) associate with the MED1/TRAP220-containing TRAP/Mediator complex, corresponding GST fusion proteins (Figure 3B) were tested for interactions with proteins in Hela nuclear extract (Figure 3C). The MED28/FSKG20, MED29/Hintersex, and MED18/p28b (as a positive control) GST fusion proteins, but not GST alone, were able to pull down MED1/TRAP220 and other TRAP/Mediator subunits. As expected and as an additional control, the TBP component of TFIID was not pulled down by any of the fusion proteins. Although GST-MED9/FLJ10193 failed to pull down any of the Mediator subunits tested, studies with a cell line expressing FLAG-MED9/FLJ10193 have indicated that this protein is mainly associated with the TRAP/Mediator complex (data not shown). These data further confirm an association of all these proteins with the MED1/TRAP220-containing TRAP/Mediator complex but do not establish that the interactions are specific to this complex relative to the MED1/TRAP220-deficient TRAP/Mediator complex. However, in the case of MED28/FSKG20, MED29/Hintersex, and MED18/p28b, the nature of the results (interactions of TRAP/Mediator with extrinsic GST-fusion proteins) suggests either that the extrinsic components readily displace intrinsic subunits or that a fraction of the TRAP/Mediator population is devoid of these components (and thus free to bind).

In addition, MS analysis indicated that the anti-MED1/TRAP220 TRAP/Mediator preparation also contains a few proteins that have no obvious homology to any known TRAP/Mediator subunits. Preliminary data suggest that these proteins are not TRAP/Mediator subunits but rather are proteins that simply associate with MED1/TRAP220-containing TRAP/Mediator complex (data not shown). Indeed, we have found that at least one of these proteins directly interacts with MED1/TRAP220.

Somewhat surprisingly, the cluster of abundant low-molecular weight proteins that appeared (by SDS-PAGE and silver stain) to be greatly enriched in the anti-MED1/TRAP220-purified TRAP/Mediator (Figure 2B) were identified by MS analysis as eight small subunits of RNA polymerase II. Peptides corresponding to other larger RNA polymerase II subunits also were detected by MS analysis in this study, although they were not readily discernable in the gel because of their colocalization with MED4/TRAP36 and MED27/TRAP37 (RPB3) and MED23/MED23/hSUR2 (RPB2) and MED1/TRAP220 (RPB1). Overall, a total of 11 out of 12 RNA polymerase II subunits was identified by MS. The presence of the 12$^{th}$ subunit, RPB5, has been confirmed by immunoblot (data not shown). These results contrast with the results of most other published studies of TRAP/Mediator complexes, in which no RNA polymerase was found, and both confirm and extend more recent studies

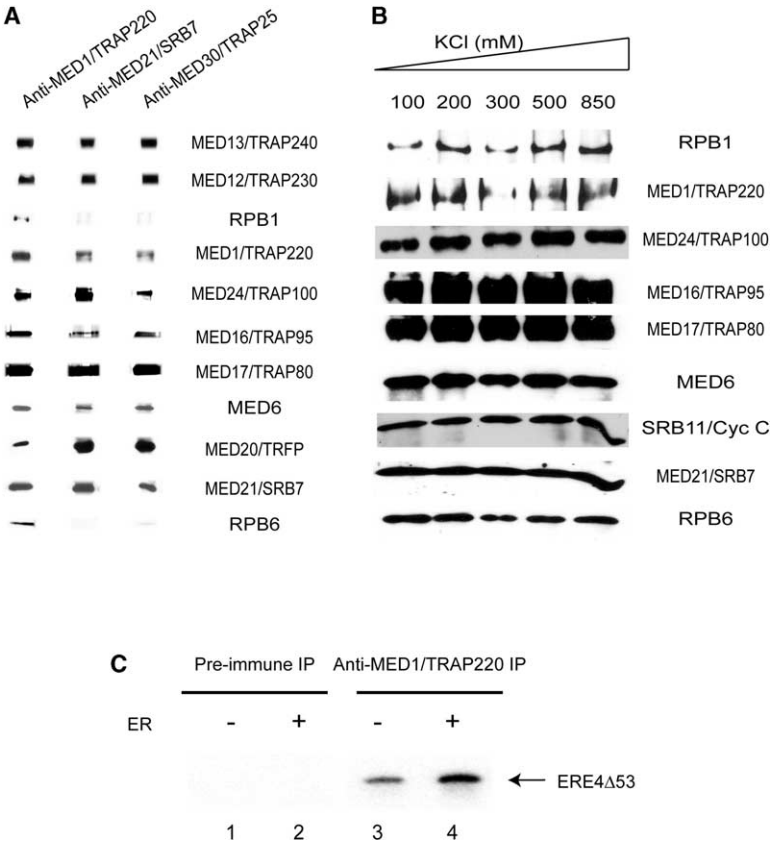


Figure 4. RNA Polymerase II Specifically Associates with MED1/TRAP220-Containing TRAP/Mediator Complex

(A) Immunoblots of purified MED1/TRAP220-containing complex with indicated antibodies. TRAP/Mediator complexes immunoprecipitated through core subunits MED21/SRB7 and MED30/TRAP25 by their respective antibodies were also included as controls.

(B) Immunoblots of MED1/TRAP220-containing complex with the indicated antibodies after extensive washes with BC buffers containing the indicated concentrations of KCl.

(C) Basal and ER-dependent transcription assays on the pSMERE4Δ53 template with preimmune or anti-MED1/TRAP220 immunoprecipitates.

(Malik et al., 2005; Sato et al., 2004), indicating the presence of a low level of RNA polymerase II in human Mediator preparations.

### MED1/TRAP220-Containing TRAP/Mediator Selectively and Tightly Associates with a Near-Stoichiometeric Level of RNA Polymerase II and Supports In Vitro Transcription in the Absence of Additional RNA Polymerase II

To confirm the above data, indicating that RNA polymerase II is specifically associated with the MED1/TRAP220-containing TRAP/Mediator complex, further immunoprecipitations were carried out with antibodies against two other core Mediator subunits (MED21/SRB7 and MED30/TRAP25). MED1/TRAP220 and, especially, RPB1 and RPB2 were present at low levels in both of these immunoprecipitates in comparison to the anti-MED1/TRAP220 immunoprecipitates, whereas most other TRAP/Mediator subunits were present at very similar levels (Figure 4A). These results are consistent with data presented above and further confirm the existence of MED1/TRAP220 in only a subpopulation of the TRAP/Mediator and the preferential association of RNA polymerase II with MED1/TRAP220-containing TRAP/Mediator.

To examine the strength of the RNA polymerase II association, the MED1/TRAP220-containing TRAP/Mediator isolated by immobilized anti-MED1/TRAP220 antibody was divided into equal aliquots that were washed separately with solutions containing different concentrations of salt (KCl), ranging from 100 mM to 850 mM. Remarkably, and indicative of a very tight association, TRAP/Mediator and RNA polymerase remained quantitatively associated under all salt conditions tested (Figure 4B).

The ability of the MED1/TRAP220-containing TRAP/Mediator-RNA polymerase II holoenzyme complex to mediate transcription was tested in an in vitro assay containing all of the highly purified general transcription factors except RNA polymerase II (Fukuda et al., 2001). As expected, preimmune immunoprecipitates failed to support any basal or estrogen receptor (ER)-dependent transcription (Figure 4C, lanes 1 and 2). In contrast, anti-MED1/TRAP220 immunoprecipitates effected both basal (activator-independent) transcription (Figure 4C, lane 3) and ER-dependent transcription (Figure 4C, lane 4). These data indicate that the MED1/TRAP220-containing holoenzyme complex contains a fully functional RNA polymerase II.

### MED1/TRAP220-Containing TRAP/Mediator Is Selectively Recruited to Endogenous Target Gene Promoters through an Activator-Dependent Mechanism

Results presented above indicate that MED1/TRAP220 exists only in a subpopulation of TRAP/Mediator, whereas previous results have shown that MED1/TRAP220 is required only for selected transcription activators (Ge et al., 2002; Malik et al., 2004; Taatjes and Tjian, 2004). This raises the possibility of mechanistic differences in

the activation of target genes by MED1/TRAP220-containing and MED1/TRAP220-deficient TRAP/Mediator complexes. An intriguing model consistent with the above observations is that different TRAP/Mediator subpopulations are recruited to distinct target gene promoters and that the MED1/TRAP220-containing TRAP/Mediator is selectively recruited to target gene promoters through its interacting activators. It is known that MED1/TRAP220 directly interacts with estrogen receptor (ER) and, through TRAP/Mediator, serves as an ER coactivator in vitro (Kang et al., 2002), whereas it neither interacts with nor is required for transcriptional activation by p53 (Ito et al., 2000; Malik and Roeder, 2000). Reciprocally, MED17/TRAP80 directly interacts with p53, but not nuclear receptors, and is thought to mediate TRAP/Mediator-dependent transcription by p53 (Ito et al., 2000; S.Y. and R.G.R., unpublished data). The above model was tested by performing chromatin immunoprecipitation (IP) experiments to examine the recruitment of the MED1/TRAP220-containing TRAP/Mediator subpopulation versus the MED1/TRAP220-deficient TRAP/Mediator population to ER and p53 target gene promoters upon activation by estrogen and UV damage, respectively. Antibodies against MED1/TRAP220 and the MED21/SRB7 core subunit of TRAP/Mediator were employed to distinguish these two populations.

These analyses utilized an estrogen-dependent breast cancer cell line, MCF-7, that shows a strong and relatively rapid induction of several well-characterized genes in response to estrogen. Chromatin IP experiments revealed little or no p53, MED21/SRB7, or MED1/TRAP220 on the estrogen-responsive pS2 promoter before estrogen treatment, whereas high levels of MED21/SRB7 and MED1/TRAP220, but not p53, were recruited to the pS2 promoter after estrogen treatment (Figure 5A, top). These observations are consistent with previous reports of MED1/TRAP220/PBP and ER recruitment to the pS2 gene in response to estrogen (Shang et al., 2000). As controls, occupancy of the p53-responsive p21 promoter by these factors was examined. A low but significant (basal) level of all three proteins was observed on the p21 promoter prior to estrogen treatment, consistent with the basal level of p21 expression in U2OS cells (Espinosa et al., 2003). Importantly, however, their levels did not change after estrogen induction (Figure 5A, bottom), indicating the promoter specificity of ER function and MED1/TRAP220 recruitment.

In contrast to the results with estrogen treatment, UV irradiation resulted in a significant and selective increase of p53 recruitment to the p21 promoter (Figure 5B, bottom). UV irradiation also resulted in an increased occupancy of MED21/SRB7 on the p21 promoter, indicating UV-dependent recruitment of TRAP/Mediator to this promoter. Interestingly, however, in contrast to the increased recruitment of p53 and MED21/SRB7, no significant increase in MED1/TRAP220 recruitment to the p21 promoter was observed after UV treatment. As controls, no p53, MED21/SRB7, or MED1/TRAP220 was detected on the pS2 promoter in response to UV treatment (Figure 5B, top). These data clearly indicate a selective recruitment of two different TRAP/Mediator populations by p53 and ER upon target gene activation by UV and estrogen, respectively. Comparable results were obtained with U2OS cells, which are commonly used to study p53 functions (Figure 5C). These data lend support to the hypothesis that distinct subpopulations of the TRAP/Mediator complex can be differentially recruited to target gene promoters by activator-dependent mechanisms and further indicate a molecular basis for the specificity of TRAP/Mediator complexes. In further support of these conclusions and consistent with their preferential association with the MED1/TRAP220-containing TRAP/Mediator complex, MED19/LCMR, MED25/ACID1, and MED11/HSPC296 also were found to be recruited to the pS2 promoter in response to estrogen (Figure S1).

### MED1/TRAP220 Is Required for ER-Mediated Transcription of Both Ectopic (Reporter) and Endogenous Target Genes

The chromatin IP experiments described above indicate a specific recruitment of MED1/TRAP220 to an ER target gene promoter in ER-positive MCF-7 cells. Recent studies also showed estrogen-dependent interactions of ER with TRAP/Mediator complex through the MED1/TRAP220 subunit and TRAP/Mediator-dependent ER function in vitro (Acevedo and Kraus, 2003; Kang et al., 2002). In an extension of the above studies and to further examine the physiological roles of MED1/TRAP220, the requirement of MED1/TRAP220 for ER-mediated transcription was examined by using RNA interference to reduce intracellular MED1/TRAP220 levels.

MED1/TRAP220 siRNAs selectively reduced MED1/TRAP220 protein to about 10% of the wild-type level (Figure 6A) and significantly impaired estrogen-dependent activation of the ERE-TK-Luc reporter gene but, importantly, showed minimal effects on expression of the control TK-Luc reporter gene (Figure 6B). An additional control with GFP siRNA showed no effect on ERE-TK-LUC activity, indicating the specificity of the MED1/TRAP220 siRNA (Figure 6C).

To examine MED1/TRAP220 siRNA effects on endogenous ER target gene expression, reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out on the cathepsin-D gene (to which MED1/TRAP220 is also recruited in response to estrogen stimulation, but not UV irradiation; Figure S2). Consistent with the above observations, estrogen-dependent activation of cathepsin-D mRNA expression also was blocked by MED1/TRAP220 siRNA (Figure 6D). Because MED1/TRAP220 does not interact with p53 and is neither required for p53-mediated transcriptional activation nor recruited to a p53 target gene promoter in response to p53 activation by UV treatment, another control experiment was designed to examine the effect of MED1/TRAP220 siRNA on p53 target-gene expression. Importantly, and as expected, MED1/TRAP220 siRNA did not affect activation of the MDM2-Luc reporter by p53 (Figure 6E). These data further confirm the specificity of MED1/TRAP220 siRNA treatment. In summary, all these data indicate that MED1/TRAP220 is selectively required for ER-mediated target-gene expression.

### MED1/TRAP220 Plays an Essential Role in Mediating Estrogen-Dependent Growth of MCF-7 Cells

It is well known that estrogen-mediated functions in regulating mammary gland development and in pro-

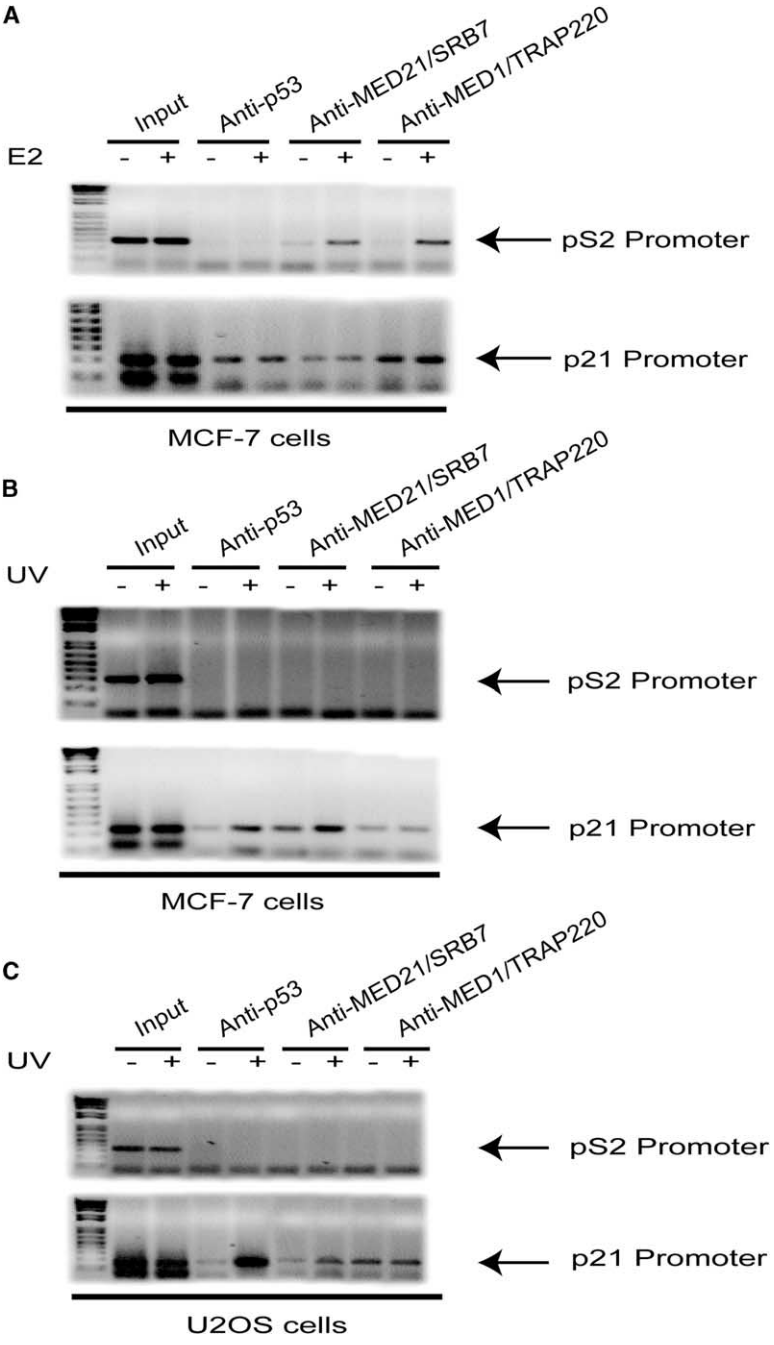


Figure 5. Activator-Selective Recruitment of MED1/TRAP220-Containing and MED1/TRAP220-Deficient Complexes to Target Gene Promoters

(A) Chromatin IP experiments were performed on MCF-7 cells by using anti-MED21/SRB7, anti-MED1/TRAP220, and anti-p53 antibodies before and after estrogen treatment.

(B) MCF-7 cells were processed for chromatin IP after UV treatment by using the same set of antibodies as in (A).

(C) Chromatin IP experiments were performed as in (B) except that U2OS cells were used.

moting growth of breast tumor cells are carried out through estrogen receptors (Nilsson et al., 2001). Because MED1/TRAP220 siRNA was effectively used to document a MED1/TRAP220 requirement for ER-mediated transcription in MCF-7 cells, it was also used to determine whether estrogen-dependent growth of MCF-7 cells is affected by depletion of MED1/TRAP220. As expected, estrogen markedly stimulated the growth of MCF-7 cells (Figure 7A). However, and significantly, MED1/TRAP220 siRNA treatment nearly abolished the estrogen-dependent growth of MCF-7 cells. As a control, the same experiment with GFP siRNA showed no effect on estrogen-dependent growth of MCF-7 cells (Figure 7B). These data demonstrate a MED1/TRAP220 requirement for estrogen-dependent growth of breast cancer cells and raise the possibility of MED1/TRAP220 as a potential therapeutic target in the treatment of breast cancer.

## Discussion

With a principal objective of understanding the role of MED1/TRAP220 through TRAP/Mediator in ER functions, this study has emphasized a structural and functional analysis of MED1/TRAP220-containing TRAP/Mediator. The main conclusions are as follows: (1) that

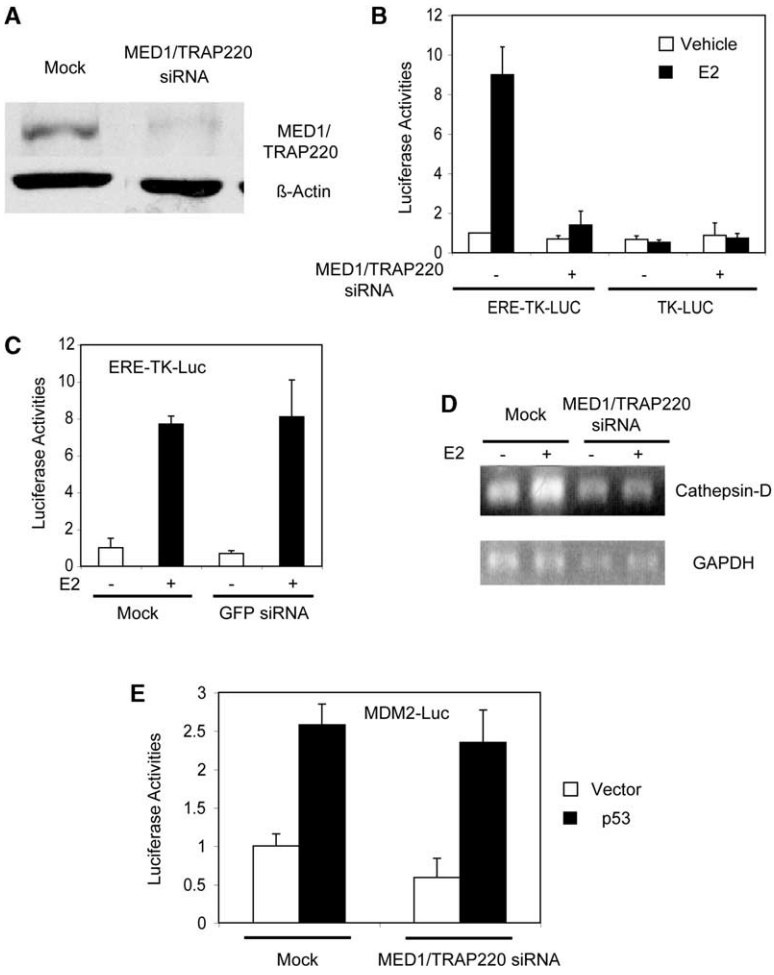


Figure 6. Effects of MED1/TRAP220 siRNA on the MED1/TRAP220 Protein Level and Estrogen-Induced Gene Expression in MCF-7 Cells

(A) Immunoblot of MED1/TRAP220 and β-actin (control) levels in mock and MED1/TRAP220 siRNA-treated MCF-7 cells.

(B) MCF-7 cells were treated with mock (–) or MED1/TRAP220 siRNA (+) for 24 hr, transfected with plasmids containing ERE-TK-LUC and TK-LUC (control) in the presence of vehicle (white bar) or estrogen (black bar) for 30 hr, and harvested for luciferase assays. All luciferase activities reflect an average value ± standard deviation (SD).

(C) Estrogen-dependent ERE-TK-LUC expression (±SD) in MCF-7 cells was examined under the same conditions as described in (B), except that control GFP siRNAs were used.

(D) Estrogen-dependent expression of the endogenous cathepsin-D gene in MCF-7 cells was determined by RT-PCR analysis after mock or MED1/TRAP220 RNAi treatment as described above.

(E) p53-dependent MDM2-LUC expression (±SD) examined in MCF-7 cells after mock or MED1/TRAP220 siRNA treatment is shown.

MED1/TRAP220 only exists in a subpopulation of TRAP/Mediator complexes; (2) that the MED1/TRAP220-containing TRAP/Mediator population is enriched in specific Mediator subunits (or associated proteins) relative to the predominant MED1/TRAP220-deficient population; (3) that the MED1/TRAP220-containing population shows a preferential association, relative to the predominant (MED1/TRAP220-deficient) TRAP/Mediator population, with RNA polymerase II and is able to mediate both basal and activator-dependent transcription in the absence of additional RNA polymerase II in a highly purified in vitro transcription system; (4) that MED1/TRAP220-containing and MED1/TRAP220-deficient TRAP/Mediator complexes are selectively recruited to estrogen-activated and ER-dependent and UV-activated and p53-dependent promoters, respectively; and (5) that MED1/TRAP220 is required both for ER-dependent transactivation and for estrogen-dependent growth of breast cancer cells. The implications of these findings for our current understanding of TRAP/Mediator structure and function are discussed.

## Heterogeneity and Specificity of TRAP/Mediator Complexes

TRAP/Mediator consists of 25–30 subunits that are thought to assemble in relatively discrete modules (Malik and Roeder, 2005; Blazek et al., 2005). It is believed that PC2-, CRSP-, or B-Med-like complexes (minimally defective in MED13/TRAP240, MED12/TRAP230, cyclin C, and CDK8) represent a Mediator core with which other modules loosely (and dynamically) associate. These modules include a subcomplex composed of MED23/hSUR2, MED24/TRAP100, and MED16/TRAP95 polypeptides (Ito et al., 2002; Stevens et al., 2002) and the MED13/TRAP240, MED12/TRAP230, cyclin C, and CDK8 subcomplex (Malik and Roeder, 2005; Samuelsen et al., 2003). MED1/TRAP220 has generally been considered a core subunit despite variations in MED1/TRAP220 levels among different human Mediator preparations (Malik et al., 2004; Taatjes and Tjian, 2004). Because most Mediator complexes documented to date have been isolated through methods that include ion exchange chromatography, variable levels of MED1/TRAP220 in some of these Mediator preparations were thought to reflect disassociation of certain subunits, including MED1/TRAP220, from the complex during chromatography.

Importantly, and contradictory to some of the earlier ideas, this study has provided strong evidence that MED1/TRAP220 naturally exists only in a subpopulation of TRAP/Mediator complexes. This is evident from immunodepletion studies and subsequent immuno-

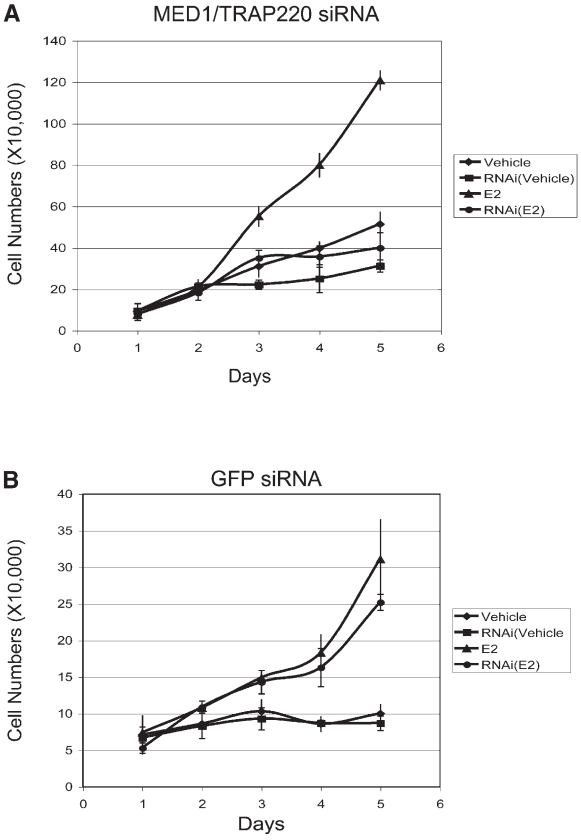


Figure 7. Effect of MED1/TRAP220 RNAi on Estrogen-Dependent Growth of MCF-7 Cells

(A) MCF-7 cells were transfected with mock (–) or MED1/TRAP220 siRNA (+) and grown in the presence of vehicle or estrogen. Cell growth (±SD) measured by counting cell numbers every 24 hr for 6 days under these conditions is shown.

(B) Experiments were performed as described in (A) except that control (GFP) siRNAs were employed.

precipitation experiments with unfractionated nuclear extract and antibodies against MED1/TRAP220 and several TRAP/Mediator core subunits. We also have confirmed the marked enrichment of several Mediator subunits (including MED25/ARC92/ACID1, MED15/TIG1, MED19/LCMR, and MED11/HSPC296) in the MED1/TRAP220-containing complex relative to the predominant MED1/TRAP220-deficient population of TRAP/Mediator; and several of these enriched subunits have also been found to be recruited along with MED1/TRAP220 to the pS2 promoter in response to estrogen stimulation (Figure S1). Other more recently identified subunits for which reliable antibodies necessary for quantitation are unavailable, including MED13L/PROSIT240, MED9/FLJ10193, MED29/Hintersex, and MED28/FSKG20, remain to be scored for selective distribution in MED1/TRAP220-containing versus MED1/TRAP220-deficient complexes. While this manuscript was in preparation, others have identified most of these proteins as TRAP/Mediator subunits (or associated proteins) by other means (Mittler et al., 2003; Sato et al., 2004). However, these analyses failed to show any selective distribution of these, or other Mediator components, among any specific Mediator complexes.

The finding that MED1/TRAP220 exists only in a subpopulation of TRAP/Mediator complexes extends our understanding of TRAP/Mediator heterogeneity beyond the above-mentioned modular model. Moreover, our results indicate an enrichment of certain TRAP/Mediator subunits in the MED1/TRAP220-containing TRAP/Mediator complex. The finding of additional heterogeneity and structural complexity within TRAP/Mediator complexes suggests more diverse functional and regulatory roles through various structurally distinct TRAP/Mediator subpopulations. Related, and most significantly, our results have shown that different TRAP/Mediator subpopulations can be specifically recruited to different target gene promoters. Specifically, the MED1/TRAP220-containing TRAP/Mediator complex is selectively recruited to an ER-dependent target-gene (pS2) promoter in response to estrogen, whereas a MED1/TRAP220-deficient TRAP/Mediator complex is recruited to the p53-dependent p21 gene promoter upon UV treatment.

### TRAP/Mediator and RNA Polymerase II

Mediator was initially isolated from yeast in association with RNA polymerase II in the form of a holoenzyme either with (Thompson et al., 1993) or without (Kim et al., 1994) other associated general transcription factors. However, because only extremely low (Malik et al., 2005; Sato et al., 2004) or nondetectable levels of RNA polymerase II have been reported for the bulk of TRAP/Mediator complexes thus far isolated from mammalian cells, it has been uncertain as to whether any significant level of mammalian Mediator exists as a holoenzyme. This study has documented a high level of tightly associated RNA polymerase II in the MED1/TRAP220-containing TRAP/Mediator preparation, thus providing strong evidence that a mammalian TRAP/Mediator complex can also exist as a holoenzyme. More recently, one study (Sato et al., 2004) detected a low level of RNA polymerase II in bulk Mediator preparations, albeit with no functions demonstrated, whereas a study from our lab (Malik et al., 2005) has documented a functional association of RNA polymerase II with the PC2/Mediator complex. These and other studies (Naar et al., 2002; Samuelsen et al., 2003) also suggest that RNA polymerase II and the MED13/TRAP240, MED12/TRAP230, cyclin C, and CDK8 modules exhibit mutually exclusive, stable interactions with TRAP/Mediator complex. Although our MED1/TRAP220-containing TRAP/Mediator preparation contains MED13/TRAP240, MED12/TRAP230, cyclin C, and CDK8 as well as RNA polymerase II, our results do not necessarily contradict the conclusions of these studies. Thus, it is possible that our MED1/TRAP220-containing preparation is heterogeneous and contains at least two Mediator complexes: one that contains a core Mediator in association with RNA polymerase II and another that contains a core Mediator in association with MED13/TRAP240, MED12/TRAP230, cyclin C, and CDK8. Structural studies of yeast holoenzyme have shown extensive interactions between the "head" domain of Mediator and RNA polymerase II (Chadick and Asturias, 2005), whereas structural studies of human Mediator complexes have assigned

MED1/TRAP220 to the head domain (Taatjes et al., 2004). Therefore, it is highly possible that MED1/TRAP220 itself and/or proteins that are specific to the MED1/TRAP220-containing complex, alone or together with other TRAP/Mediator subunits, may somehow stabilize the interaction of RNA polymerase II with TRAP/Mediator. The underlying mechanisms are currently under investigation through structural and functional analyses.

The isolation of MED1/TRAP220-containing TRAP/Mediator as a holoenzyme with RNA polymerase II raises the question as to whether it can be recruited to the promoter as a holoenzyme. Although this was initially suggested for the yeast holoenzyme, recent structural studies have suggested that threading of template DNA on RNA polymerase II may require dissociation of Mediator and RNA polymerase II (Chadick and Asturias, 2005) and, therefore, favors a two-step recruitment of Mediator and RNA polymerase II. Furthermore, it has been shown both in yeast (Cosma et al., 2001; Kuras et al., 2003) and in *Drosophila* (Park et al., 2001) that Mediator and RNA polymerase II are recruited independently to specific activated promoters. However, these studies do not exclude the possibility of holoenzyme recruitment to select promoters with subsequent rearrangement to accommodate the RNA polymerase II-DNA interactions. It has been reported that binding of Mediator to RNA polymerase II causes significant conformational changes in both Mediator and RNA polymerase II (Chadick and Asturias, 2005) and that binding of activator to Mediator also causes conformational changes in the Mediator (Taatjes et al., 2004). Furthermore, we have found that instead of dissociating RNA polymerase II, estrogen receptor and thyroid hormone receptor both form complexes with MED1/TRAP220-containing holoenzyme in vitro (X.Z. and R.G.R., unpublished data). Related to this notion, a high-resolution kinetic analysis has reported that MED1/TRAP220 and RNA polymerase II are recruited concomitantly to the pS2 promoter upon estrogen induction (Metivier et al., 2003), consistent with a corecruitment model for MED1/TRAP220-containing TRAP/Mediator and RNA polymerase II. Importantly, the MED1/TRAP220-containing holoenzyme complex described here is active in mediating basal and activator-dependent transcription in lieu of ectopic core RNA polymerase II in a purified, cell-free transcription system. Therefore, more detailed studies of the structure of this MED1/TRAP220-containing holoenzyme and how activator interactions elicit conformational changes likely will shed light on our understanding of the assembly and function of holoenzyme complexes on target gene promoters.

## MED1/TRAP220 and Estrogen Receptor Function

Recent studies have shown estrogen-dependent interactions of MED1/TRAP220 (and TRAP/Mediator) with ER as well as TRAP/Mediator-dependent ER functions in vitro (Acevedo and Kraus, 2003; Kang et al., 2002). In a major extension of this work, studies with MED1/TRAP220 siRNA have provided strong evidence that MED1/TRAP220 is indeed required for ER function on both ectopic and endogenous estrogen-stimulated genes in ER-expressing MCF-7 cells. This also has allowed further validation of the studies (described above) showing activator-selective recruitment of MED1/TRAP220-containing TRAP/Mediator (by estrogen-activated ER) versus MED1/TRAP220-defective TRAP/Mediator (by UV-activated p53).

Given that estrogen plays a prominent role in promoting breast cancer, inhibitors that target the production of estrogen and selective estrogen receptor modulators (SERMs) that show tissue-specific effects on ER function have been widely used in the treatment of ER-positive breast cancers (Johnston and Dowsett, 2003; Powles, 2002). In relation to the action of SERMs, coactivator levels can be a critical factor in regulating the activity of their interacting nuclear receptors. Thus, it has been reported that the coactivator SRC-1 is expressed at a higher level in endometrial cells than in mammary epithelial cells and that this represents the molecular basis for the activation versus repression functions of tamoxifen in ER-mediated transcription in the respective tissues (Shang and Brown, 2002). In this study, we have found that MED1/TRAP220 is required for the ER-dependent transcription and estrogen-dependent growth of breast cancer cells and that it exists only in a subpopulation of TRAP/Mediator complexes. Interestingly, it has been reported that MED1/TRAP220 is amplified and overexpressed in a high percentage of breast cancer cells (Zhu et al., 1999). Therefore, it is conceivable that this elevated expression of MED1/TRAP220 could result in a higher concentration of MED1/TRAP220-containing TRAP/Mediator complexes in these cells, which then would enhance the ability of ERs to activate the expression of target genes.

## Experimental Procedures

### Generation of Anti-MED1/TRAP220 Antibody

MED1/TRAP220 fragments corresponding to the NR box region (1516–2259) and to a C-terminal region (4006–4663) were expressed in *Escherichia coli* as $his_6$-tagged fusion proteins and purified for antibody production (Covance) as described (Baek et al., 2002).

### Immunopurification and Depletion of MED1/TRAP220-Containing TRAP/Mediator

Anti-MED1/TRAP220 antibodies were antigen-purified and cross-linked to protein-A Sepharose as described (Baek et al., 2002) for the purification and depletion of MED1/TRAP220-containing TRAP/Mediator from nuclear extract. Proteins were visualized either by immunoblot with an enhanced chemiluminescence SuperSignal West Pico kit (Pierce) or by silver staining with a Rapid-Ag-Stain Kit (ICN).

### Protein Identification by Mass Spectrometry

Tryptic-digested peptides from purified MED1/TRAP220-containing and FLAG-MED10/NUT2 complexes were analyzed with MALDIQq-TOF and MALDI-ion trap mass spectrometers (Krutchinsky et al., 2001). Masses of the tryptic peptides and derived fragments were used to identify proteins by searching the National Center for Biotechnology Information (NCBI) database through Xproteo (http://www.xproteo.com).

### GST Pull-Down Assays

One μg of each immobilized GST fusion protein was incubated with 2.5 mg Hela nuclear extract for 2 hr at 4°C. The beads then were washed extensively with BC300 containing 0.1% NP-40, 1 mM DTT, and 0.25 mM PMSF. Associated proteins were eluted with binding buffer plus 0.2% Sarkosyl and analyzed by immunoblot.

### In Vitro Transcription

Transcription reactions employed recombinant TFIIA, IIB, IIE, IIF, IIH, and PC4 (purified from bacteria or sf9 cells) and affinity-purified f:IID (Fukuda et al., 2001), FLAG-tagged ER (expressed and purified from sf9 cells), immunoprecipitated MED1/TRAP220-containing TRAP/Mediator complex (above), and the DNA template pSMERE3Δ53 (Kang et al., 2002).

### Cell Culture, RNA Interference, Transient Transfection, and RT-PCR Assay

MCF-7 cells were maintained as described (Shang et al., 2000). siRNA duplexes (Dharmacon) were introduced into cells by using Oligofectmine (Invitrogen). Transient transfection assays were carried out with Fugene 6 (Roche), and luciferase activities were assayed by a Dual-Luciferase Reporter Assay System (Promega). For RT-PCR, total RNA was purified with an RNAeasy Mini Kit (Qiagen). Reverse transcription reactions were carried out with the Superscript First-Strand Synthesis System (Invitrogen), and the resulting products were subjected to PCR amplification with previously described primers (Steinfeld et al., 2000).

### Chromatin IP Experiments

Chromatin immunoprecipitation (ChIP) assays were performed essentially as described (Espinosa et al., 2003; Shang et al., 2000). Antibodies against MED1/TRAP220 (this study), MED21/SRB7 (Gu et al., 1999), and p53 (Santa Cruz) were used. Cells were either treated with estrogen (or vehicle) for 40 min or exposed to UV (50 J/m2) and then incubated for 4 hr at 37°C prior to fixation. For PCR, amplifications with a serial dilution of input and various cycles (25–35 cycles) were performed to ensure that all were in the linear range.

### Supplemental Data

Supplemental data including three figures and one table are available online with this article at http://www.molecule.org/cgi/content/full/19/1/89/DC1/.

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### References

Acevedo, M.L., and Kraus, W.L. (2003). Mediator and p300/CBP-steroid receptor coactivator complexes have distinct roles, but function synergistically, during estrogen receptor alpha-dependent transcription with chromatin templates. Mol. Cell. Biol. *23*, 335–348.

Baek, H.J., Malik, S., Qin, J., and Roeder, R.G. (2002). Requirement of TRAP/mediator for both activator-independent and activator-dependent transcription in conjunction with TFIID-associated TAF (II)s. Mol. Cell. Biol. *22*, 2842–2852.

Balciunas, D., Galman, C., Ronne, H., and Bjorklund, S. (1999). The Med1 subunit of the yeast mediator complex is involved in both transcriptional activation and repression. Proc. Natl. Acad. Sci. USA *96*, 376–381.

Blazek, E., Mittler, G., and Meisterernst, M. (2005). The mediator of RNA polymerase II. Chromosoma *113*, 399–408.

Boube, M., Joulia, L., Cribbs, D.L., and Bourbon, H.M. (2002). Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell *110*, 143–151.

Chadick, J.Z., and Asturias, F.J. (2005). Structure of eukaryotic Mediator complexes. Trends Biochem. Sci. *30*, 264–271.

Cosma, M.P., Panizza, S., and Nasmyth, K. (2001). Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. Mol. Cell *7*, 1213–1220.

Espinosa, J.M., Verdun, R.E., and Emerson, B.M. (2003). p53 functions through stress- and promoter-specific recruitment of transcription initiation components before and after DNA damage. Mol. Cell *12*, 1015–1027.

Fondell, J.D., Ge, H., and Roeder, R.G. (1996). Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc. Natl. Acad. Sci. USA *93*, 8329–8333.

Fukuda, A., Yamauchi, J., Wu, S.Y., Chiang, C.M., Muramatsu, M., and Hisatake, K. (2001). Reconstitution of recombinant TFIIH that can mediate activator-dependent transcription. Genes Cells *6*, 707–719.

Ge, K., Guermah, M., Yuan, C.X., Ito, M., Wallberg, A.E., Spiegelman, B.M., and Roeder, R.G. (2002). Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis. Nature *417*, 563–567.

Glass, C.K., and Rosenfeld, M.G. (2000). The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. *14*, 121–141.

Gu, W., Malik, S., Ito, M., Yuan, C.X., Fondell, J.D., Zhang, X., Martinez, E., Qin, J., and Roeder, R.G. (1999). A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol. Cell *3*, 97–108.

Ito, M., Yuan, C.X., Okano, H.J., Darnell, R.B., and Roeder, R.G. (2000). Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. Mol. Cell *5*, 683–693.

Ito, M., Okano, H.J., Darnell, R.B., and Roeder, R.G. (2002). The TRAP100 component of the TRAP/Mediator complex is essential in broad transcriptional events and development. EMBO J. *21*, 3464–3475.

Johnston, S.R., and Dowsett, M. (2003). Aromatase inhibitors for breast cancer: lessons from the laboratory. Nat. Rev. Cancer *3*, 821–831.

Kang, Y.K., Guermah, M., Yuan, C.X., and Roeder, R.G. (2002). The TRAP/Mediator coactivator complex interacts directly with estrogen receptors alpha and beta through the TRAP220 subunit and directly enhances estrogen receptor function in vitro. Proc. Natl. Acad. Sci. USA *99*, 2642–2647.

Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994). A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell *77*, 599–608.

Krutchinsky, A.N., Kalkum, M., and Chait, B.T. (2001). Automatic identification of proteins with a MALDI-quadrupole ion trap mass spectrometer. Anal. Chem. *73*, 5066–5077.

Kuras, L., Borggrefe, T., and Kornberg, R.D. (2003). Association of the Mediator complex with enhancers of active genes. Proc. Natl. Acad. Sci. USA *100*, 13887–13891.

Malik, S., and Roeder, R.G. (2000). Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. Trends Biochem. Sci. *25*, 277–283.

Malik, S., and Roeder, R.G. (2005). The mammalian Mediator complex and its role in transcriptional regulation. Trends Biochem. Sci. *30*, 250–255.

Malik, S., Guermah, M., Yuan, C.X., Wu, W., Yamamura, S., and Roeder, R.G. (2004). Structural and functional organization of

TRAP220, the TRAP/mediator subunit that is targeted by nuclear receptors. Mol. Cell. Biol. *24*, 8244–8254.

Malik, S., Baek, H., Wu, W., and Roeder, R.G. (2005). Structural and functional characterization of PC2 and RNA polymerase II-associated subpopulations of metazon mediator. Mol. Cell. Biol. *25*, 2117–2129.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., et al. (1995). The nuclear receptor superfamily: the second decade. Cell *83*, 835–839.

Metivier, R., Penot, G., Hubner, M.R., Reid, G., Brand, H., Kos, M., and Gannon, F. (2003). Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell *115*, 751–763.

Mittler, G., Stuhler, T., Santolin, L., Uhlmann, T., Kremmer, E., Lottspeich, F., Berti, L., and Meisterernst, M. (2003). A novel docking site on Mediator is critical for activation by VP16 in mammalian cells. EMBO J. *22*, 6494–6504.

Naar, A.M., Taatjes, D.J., Zhai, W., Nogales, E., and Tjian, R. (2002). Human CRSP interacts with RNA polymerase II CTD and adopts a specific CTD-bound conformation. Genes Dev. *16*, 1339–1344.

Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M., and Gustafsson, J.A. (2001). Mechanisms of estrogen action. Physiol. Rev. *81*, 1535–1565.

Park, J.M., Werner, J., Kim, J.M., Lis, J.T., and Kim, Y.J. (2001). Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. Mol. Cell *8*, 9–19.

Powles, T.J. (2002). Anti-oestrogenic prevention of breast cancer–the make or break point. Nat. Rev. Cancer *2*, 787–794.

Roeder, R.G. (1996). The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. *21*, 327–335.

Samuelsen, C.O., Baraznenok, V., Khorosjutina, O., Spahr, H., Kieselbach, T., Holmberg, S., and Gustafsson, C.M. (2003). TRAP230/ARC240 and TRAP240/ARC250 Mediator subunits are functionally conserved through evolution. Proc. Natl. Acad. Sci. USA *100*, 6422–6427.

Sato, S., Tomomori-Sato, C., Parmely, T.J., Florens, L., Zybailov, B., Swanson, S.K., Banks, C.A., Jin, J., Cai, Y., Washburn, M.P., et al. (2004). A set of consensus mammalian mediator subunits identified by multidimensional protein identification technology. Mol. Cell *14*, 685–691.

Shang, Y., and Brown, M. (2002). Molecular determinants for the tissue specificity of SERMs. Science *295*, 2465–2468.

Shang, Y., Hu, X., DiRenzo, J., Lazar, M.A., and Brown, M. (2000). Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell *103*, 843–852.

Steinfeld, S., Maho, A., Chaboteaux, C., Daelemans, P., Pochet, R., Appelboom, T., and Kiss, R. (2000). Prolactin up-regulates cathepsin B and D expression in minor salivary glands of patients with Sjogren's syndrome. Lab. Invest. *80*, 1711–1720.

Stevens, J.L., Cantin, G.T., Wang, G., Shevchenko, A., and Berk, A.J. (2002). Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. Science *296*, 755–758.

Taatjes, D.J., and Tjian, R. (2004). Structure and function of CRSP/Med2; a promoter-selective transcriptional coactivator complex. Mol. Cell *14*, 675–683.

Taatjes, D.J., Schneider-Poetsch, T., and Tjian, R. (2004). Distinct conformational states of nuclear receptor-bound CRSP-Med complexes. Nat. Struct. Mol. Biol. *11*, 664–671.

Thompson, C.M., Koleske, A.J., Chao, D.M., and Young, R.A. (1993). A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell *73*, 1361–1375.

Zhu, Y., Qi, C., Jain, S., Le Beau, M.M., Espinosa, R., 3rd, Atkins, G.B., Lazar, M.A., Yeldandi, A.V., Rao, M.S., and Reddy, J.K. (1999). Amplification and overexpression of peroxisome proliferator-activated receptor binding protein (PBP/PPARBP) gene in breast cancer. Proc. Natl. Acad. Sci. USA *96*, 10848–10853.