Regulation of Aurora-A Kinase Gene Expression via GABP Recruitment of TRAP220/MED1

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The TRAP/Mediator coactivator complex serves as a functional interface between DNA-bound transactivators and the RNA polymerase II-associated basal transcription apparatus. TRAP220/MED1 is a variably associated subunit of the complex that plays a specialized role in selectively targeting TRAP/Mediator to specific genes. Ablation of the Trap220/Med1 gene in mice impairs embryonic cell growth, yet the underlying mechanism is unknown. In this report, we identified distinct cell growth regulatory genes whose expression is affected by the loss of TRAP220/MED1 by RNA interference. Among the down-regulated genes revealed by cDNA microarray analyses, we identified Aurora-A, a centrosome kinase that plays a critical role in regulating M phase events and is frequently amplified in several types of cancer. In general, we found that TRAP220/MED1 expression is required for high basal levels of Aurora-A gene expression and that ectopic overexpression of TRAP220/MED1 coactivates transcription from the Aurora-A gene promoter. Furthermore, chromatin immunoprecipitation assays show that TRAP220/MED1-containing TRAP/Mediator complexes directly bind to the Aurora-A promoter in vivo. Finally, we present evidence suggesting that TRAP/Mediator is recruited to the Aurora-A gene via direct interactions between TRAP220/MED1 and the Ets-related transcription factor GABP. Taken together, these findings suggest that TRAP220/MED1 plays a novel coregulatory role in facilitating the recruitment of TRAP/Mediator to specific target genes involved in growth and cell cycle progression.

The human TRAP/Mediator complex is an evolutionarily conserved multisubunit coactivator that plays a central role in regulating transcription from protein-encoding genes (1). By virtue of its ability to directly interact with both gene-specific transcription factors and RNA polymerase II, TRAP/Mediator acts as an adaptor between promoter-bound activators and the basal transcription machinery. Human complexes similar to TRAP/Mediator have been isolated from several laboratories and include DRIP, ARC, NAT, and human Mediator (reviewed in Refs. 2–5). A smaller, highly related core complex that is apparently a derivative of the larger TRAP/Mediator complex has also been isolated and termed PC2 or CRSP (2, 5). Proteomic analyses of several alternatively purified TRAP/Mediator-like complexes has led to the identification of as many as 32 subunits (6), 22 of which appear to be metazoan homologs of yeast Mediator subunits (7).

TRAP220/MED1 (also termed PBP, ARC/DRIP205 or MED220; reviewed in Ref. 8) is a variably associated subunit of TRAP/Mediator (9, 10) and the human ortholog of the yeast Mediator subunit MED1 (7). TRAP220/MED1 targets TRAP/Mediator to nuclear hormone receptors (NR)2 via two conserved LXXLL motifs (11–13) and thus facilitates an essential activation step during NR-regulated gene expression (8, 14). In fact, TRAP220/MED1 is amplified in estrogen receptor-positive breast cancer cells and may play an oncogenic role in steroid hormone-dependent cancer progression (15, 16). Consistent with an important physiological role in animal development, ablation of the Trap220/Med1 gene in mice is embryonic lethal (17, 18). Interestingly, Trap220/Med1+/− mouse embryos exhibit retarded cellular proliferation, whereas embryonic fibroblasts (isolated prior to embryonic death) likewise display impaired cell cycle progression (18). Furthermore, we recently found that TRAP220/MED1 is a direct target for mitogen-activated protein kinases and becomes phosphorylated and activated in a cell cycle-dependent manner (19). These studies thus suggest that in addition to NR coactivation, TRAP220/MED1 might also facilitate a coregulatory role in mitotic cell growth. In agreement with this view, TRAP220/MED1 has been shown to interact with a number of transcription factors essential for cell growth and development including the GATA family of proteins (20), BRCA-1 (21) and p53 (22). Nonetheless, little is known about the underlying mechanisms by which TRAP220/MED1 facilitates cell growth nor which specific target genes are involved.

Although the importance of TRAP220/MED1 in cell growth and development is clear, its presence within TRAP/Mediator is not critical for the overall functional integrity of the complex. Indeed, TRAP/Mediator subcomplexes lacking TRAP220/MED1 are still capable of facilitating activator-dependent transcription (9, 10) and are similar in subunit composition to complexes containing the subunit (10). Hence, the absence of TRAP220/MED1 does not grossly disrupt the TRAP/Mediator complex nor impair the apparent functional activity of the remaining Mediator subunits. Consistent with this notion, electron micrograph studies of purified human Mediator complexes shows that TRAP220/MED1 assumes a relatively peripheral location on the intact complex (9). Collectively, these findings suggest that TRAP220/MED1 is variably associated with the TRAP/Mediator complex in vivo and likely plays a specialized role in selectively recruiting the complex to specific target genes.

The Aurora family of kinases are evolutionarily conserved and essential for mitotic cell division in eukaryotes because of their key regulatory roles in centrosome cycling, spindle assembly, chromosome segregation, and cytokinesis (reviewed in Ref. 23). In mammals, Aurora-A kinase (also termed AIK, STK6, or STK15) is mainly located at the mitotic spindle where it regulates the centrosome and mitotic microtubules. Aurora-A has attracted significant attention since the discovery

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2 The abbreviations used are: NR, nuclear receptor; RNAi, RNA interference; CHIP, chromatin immunoprecipitation; TF, transcription factor; GST, glutathione S-transferase; Ta, triiodothyronine; HA, hemagglutinin; siRNA, small interfering RNA; PBS, phosphate-buffered saline; RT, reverse transcriptase; TRα, thyroid hormone receptor α; GABP, GA-binding protein.
of its overexpression in several human cancer types, and the demonstration of its ability to induce oncogenic transformation in mammalian cells (reviewed in Ref. 24). Expression of Aurora-A occurs in a cell cycle-dependent manner with peak levels of mRNA and protein occurring at the G1/M phase of the cell cycle (25–28). Amplification of Aurora-A impairs spindle assembly and disrupts the fidelity of centrosome duplication resulting in aneuploidy, genomic instability, and ultimately leads cellular transformation (23, 24).

Here, in an effort to better understand how TRAP220/MED1 selectively targets TRAP/Mediator to physiologically important genes, we used RNA interference (RNai) together with gene microarray to identify specific genes that are affected by the loss of TRAP220/MED1. We report that human Aurora-A was among the subset of cell growth regulatory genes shown to be down-regulated in TRAP220/MED1-depleted HeLa cells. Importantly, chromatin immunoprecipitation (ChIP) and cotransfection assays show that TRAP220/MED1 is directly recruited to, and functionally coregulates transcription from, the Aurora-A gene promoter in vivo. Furthermore, we present evidence suggesting that TRAP220/MED1-containing TRAP/Mediator complexes are recruited to the Aurora-A promoter via the Ets-related transcription factor GABP. In summary, these findings suggest that TRAP220/MED1 plays a novel coregulatory role in facilitating the recruitment of TRAP/Mediator to specific target genes involved in growth and cell cycle progression.

EXPERIMENTAL PROCEDURES

Antibodies—Rabbit polyclonal antibodies against TRAP220/MED1 and TRAP100/MED24 were described previously (29). Antibodies against α-tubulin, Aurora-A kinase, TRAP80/MED17, MED6, GABPα, TFIIB, and the HA-epitope were from Santa Cruz Biotechnology. Mouse monoclonal antibodies against the largest subunit of RNA polymerase II were via the hybridoma 8WG16 provided by Richard Burgess (University of Wisconsin).

Plasmids—The mammalian expression vectors pBK-CMV-FLAG-TRea and pSG5-HA-TRAP220 and the luciferase reporter gene 2xT3REH9251 and pGEX—H9252 were kindly provided by Dr. H. Handa (NCI-Frederick, National Institutes of Health, Fredrick, MD). A class comparison algorithm in Array Tools was used to conduct an univariate test followed by an exact multivariate permutation test (with 1000 permutation) to identify genes showing significant expression value changes between MED1 siRNA samples and control siRNA samples. The cut-off criteria were genes with a permutation p ≤ 0.001.

ChIP—ChIP assays were performed essentially as previously described in detail (31) using antibodies against MED1/TRAP220, MED6, or GABPα. The primers for the Aurora-A promoter were: F1, 5′-GCTTCCAGCATGATAATGGGCCG-3′ (−169), and R1, 5′-TCAGCGCTGTAGAATCAAGTTGTC-3′ (+98); F2, 5′-CGCGGAAATCTGCTGCGCATATCTAC-3′ (−366) and R2, 5′-CCGCGCATTATCGGTTGAAGCGAA-3′ (−145). The primers for the human ISG54 gene promoter were: forward 5′-GAGGAAAAAGAGTCTCTA-3′ and reverse 5′-AGCTGCACTCTTCAGAAAT-3′. In experiments involving the ISG54 promoter, HeLa cells were pre-treated with 1000 units/ml α interferon (ENDOGEN Pharmaceuticals) for 1 h prior to formaldehyde treatment. All ChIP experiments were carried out at least three times. Image processing of the data were performed using Quantity One software (Bio-Rad).

RT-PCR—HeLa cells (1 × 10⁶) were transfected with either TRAP220/MED1 siRNA or control siRNA (100 nM final) for 72 h. 20 μg of total RNA was extracted from each sample using RNeasy (Qiagen) and submitted for microarray analysis at the Core Expression Array Facility (Cancer Institute of New Jersey) using high-density oligonucleotide HG-U133A arrays (Affymetrix). Three independent experiments were performed. Probe level analysis and normalization (using global scaling) were performed using Microarray Analysis Suite version 5.1 (Affymetrix) and Array Tools (developed by Dr. Richard Simon, NCI-Frederick, National Institutes of Health, Fredrick, MD).

RNA Isolation and Microarray Analyses—HeLa cells were transfected with either TRAP220/MED1 siRNA or control siRNA (100 nM final) for 24 h. 20 μg of total RNA was extracted from each sample using RNeasy (Qiagen) and submitted for microarray analysis at the Core Expression Array Facility (Cancer Institute of New Jersey) using high-density oligonucleotide HG-U133A arrays (Affymetrix). Three independent experiments were performed. Probe level analysis and normalization (using global scaling) were performed using Microarray Analysis Suite version 5.1 (Affymetrix) and Array Tools (developed by Dr. Richard Simon, NCI-Frederick, National Institutes of Health, Fredrick, MD). A class comparison algorithm in Array Tools was used to conduct an univariate test followed by an exact multivariate permutation test (with 1000 permutation) to identify genes showing significant expression value changes between MED1 siRNA samples and control siRNA samples. The cut-off criteria were genes with a permutation p ≤ 0.001.

Immunocytochemistry—HeLa cells were seeded on coverslips in 12-well plates (10⁵ cells/well) and either synchronized or transfected with siRNA. Following fixation in 4% formaldehyde/PBS, the cells were permeabilized in 0.2% Triton X-100/PBS, washed in PBS, and subsequently blocked in PBS, 5% goat serum. Primary antibodies were applied to the coverslips for 1 h and then overlaid with Alexa Fluor 568-conjugated goat anti-rabbit secondary antibody (Molecular Probes) for 30 min in 5% goat serum. Following 3 washes in PBS, the coverslips were further incubated in 4’,6’-diamidino-2-phenylindole (1 μg/ml) to stain the nuclei. The coverslips were then mounted using an aqueous mounting medium (Anti-fading Agents; Biomeda Corp.). The images were examined and captured using a Nikon E1000 microscope and analyzed using IMAGEPRO software.

TRAP220/MED1 Coactivation of Aurora-A Expression

21-nucleotide MED1 siRNA is specific for the 3′-untranslated region of the native MED1 mRNA (NCBI accession number NM_004774). A nonspecific (scrambled) siRNA (Dharmacon) was used as a control. HeLa cells were transfected with MED1 siRNA or control siRNA at a final concentration of 100 nM using Lipofectamine in combination with Plus reagent (Invitrogen) according to the manufacturer’s instructions and as described in detail previously (19).
CAAG-3′ and reverse, 5′-CATCTGCTGGAAAGTGGACA-3′) for 25 cycles. The PCR product was analyzed on a 1.5% EtBr-stained agarose gel. To verify that the PCR products were in the linear range of amplification, preliminary experiments were performed varying either the concentration of total RNA or total number of PCR cycles.

**GST Pulldown Assay**—The pGEX-GABPα and pGEX-GABPβ1 constructs, as well as a pGEX-2TK control vector (Amersham Biosciences), were expressed in Escherichia coli strain BL21(DE3) pLysS. The GST and GST fusion proteins were then purified as described (13). Purification of baculovirus HA-TRAP220/MED1 from insect Sf9 cells was carried out exactly as previously described (32). In 250 μl of BC100/Noni
det P-40 (20 mM HEPES, pH 7.9, 100 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.05% Noni
det P-40, 0.5% powdered milk), 0.5 μg of GST-GABPα, GST-GABPβ1, or GST alone, was added together with 40 ng of baculovirally expressed HA-TRAP220/MED1 and then mixed for 1 h at 4 °C on a rotator. Protein complexes were precipitated by gentle centrifugation and washed three times in BC100/Noni
det P-40 followed by resuspension in SDS sample loading buffer. Following 8% SDS-PAGE fractionation, the bound proteins were transferred to nitrocellulose membranes and probed by Western blot with antibodies against TRAP220/MED1, TRAP100/MED24, TRAP80/MED17, TFIIB, and RNA polymerase II.

### RESULTS

**TRAP220/MED1 Knockdown by RNAi**—To better understand the role of TRAP220/MED1 as a signaling mediator of cellular growth, we utilized RNAi to knockdown TRAP220/MED1 expression in human HeLa cells. In these experiments, siRNA duplexes were generated against the 3′-untranslated region of the native TRAP220/MED1 mRNA (see “Experimental Procedures”). To ascertain the efficacy of RNAi as a means of inhibiting TRAP220/MED1 protein expression, HeLa cells were transfected with TRAP220/MED1-specific siRNA and then subjected to immunocyt
chemical staining and immunoblotting with anti-MED1 antibodies. As shown in Fig. 1A, transfection with TRAP220/MED1 siRNA significantly depleted the amount of TRAP220/MED1 in HeLa cell nuclei, whereas transfection with a scrambled siRNA control had no effect. Similarly, immunoblot analyses of HeLa cells transfected with TRAP220/MED1 siRNA revealed a significant (>90%) reduction in TRAP220/MED1 protein expression (Fig. 1B). By contrast, the expression levels of other TRAP/Med
diator subunits (TRAP80/MED17 and TRAP100/MED24), the general transcription factor TFIIB, and largest subunit of RNA polymerase II were all unaffected by TRAP220/MED1 siRNA (Fig. 1B).

**Loss of TRAP220/MED1 Expression Impairs Thyroid Hormone Signaling**—One well established functional role for human TRAP220/
MED1 is as a coactivator for NRs (8, 13, 14). To verify that loss of endogenous TRAP220/MED1 expression impairs NR signaling, HeLa cells were transfected with TRAP220/MED1 siRNA together with a thyroid hormone receptor α (TRα) expression vector and a thyroid hormone (T3)-responsive reporter gene. The cells were then cultured in the presence or absence of T3 and transcription from the reporter gene was measured (Fig. 2). Robust T3-dependent transcription (8–10-fold) was observed in the presence of control siRNA, but as expected, the T3 response was significantly impaired in the presence of TRAP220/MED1 siRNA. Because the TRAP220/MED1 siRNA is specific for the 3′-untranslated region of native TRAP220/MED1 mRNA, we asked whether ectopic TRAP220/MED1 expression (lacking the 3′-untranslated region) could restore the T3 response. Indeed, transient transfection of a recombinant TRAP220/MED1 expression vector restored T3-dependent transacti
vation to near normal levels (Fig. 2). In summary, these data show that RNAi is an effective strategy for inhibiting TRAP220/MED1 expression in human cells.
TRAP220/MED1 Coactivation of Aurora-A Expression

TRAP220/MED1 Coactivates Aurora-A Gene Expression—Tanaka et al. (28) recently isolated the human Aurora-A promoter region and identified an Ets factor binding site (bps 86 to 79) as well as two putative E2F binding sites (bps 307 to 302 and 260 to 254) (Fig. 3A). This study confirmed that the Ets-related factor GABP bound at the Ets site and was essential for activated Aurora-A expression. Using RT-PCR with primers specific for the Aurora-A gene and the microarray data, we found that loss of TRAP220/MED1 expression resulted in a 3.8-fold decrease in Aurora-A mRNA expression as determined by RT-PCR. Importantly, transient cotransfection of recombinant TRAP220/MED1 restored Aurora-A mRNA expression to near normal levels (Fig. 3A). Western blot analyses further showed that the decrease in Aurora-A mRNA expression was associated with a 2.5-fold decrease in Aurora-A protein expression (Fig. 3B). Taken together, these findings strengthen the correlation between TRAP220/MED1 deletion in HeLa cells and loss of Aurora-A expression, and are further suggestive of a potential direct functional role for TRAP220/MED1-containing TRAP/Mediator complexes in regulating Aurora-A gene transcription.

TRAP220/MED1 Depletion Triggers Down-regulation of Aurora-A Gene Expression—Given the essential role of TRAP220/MED1 in cellular growth and animal development (17, 18, 34), we next wanted to identify specific genes that are affected by the loss of TRAP220/MED1. In particular, we were interested in identifying genes that are down-regulated following TRAP220/MED1 depletion, possibly revealing a coactivator role for TRAP220/MED1 in regulating their expression. Toward this end, mRNA was isolated from HeLa cells transfected with TRAP220/MED1 siRNA or the scrambled control and subsequently analyzed using cDNA microarrays representing over 22,000 human genes (U133A, Affymetrix). We selectively identified genes that showed consistent down-regulation following TRAP220/MED1 depletion in three independent experiments. Approximately 150 genes were identified that had a permutation p value less than 0.001 (see “Experimental Procedures”). Interestingly, and consistent with the embryonic growth arrest observed Trap220/Medi1\(^{-/-}\) knockout mice, greater than 10% of the identified genes are involved in cell growth and cell cycle regulation. A representative subset of the genes is shown in Table 1 and includes the human Aurora-A serine/threonine kinase.

In view of the key regulatory role played by Aurora-A kinase in mitotic cell division and oncogenesis, we were interested in determining whether this gene might represent a direct target for TRAP220/MED1 transcriptional regulation. To validate the microarray results linking TRAP220/MED1 loss with down-regulation of Aurora-A expression, RNA was extracted from HeLa cells transfected with TRAP220/MED1 siRNA and subsequently analyzed by RT-PCR using primers specific for the Aurora-A gene. As shown in Fig. 3A, and in agreement with the microarray data, we found that loss of TRAP220/MED1 expression resulted in a 3.8-fold decrease in Aurora-A mRNA expression as determined by RT-PCR. Importantly, transient cotransfection of recombinant TRAP220/MED1 restored Aurora-A mRNA expression to near normal levels (Fig. 3A). Western blot analyses further showed that the decrease in Aurora-A mRNA expression was associated with a 2.5-fold decrease in Aurora-A protein expression (Fig. 3B). Taken together, these findings strengthen the correlation between TRAP220/MED1 deletion in HeLa cells and loss of Aurora-A expression, and are further suggestive of a potential direct functional role for TRAP220/MED1-containing TRAP/Mediator complexes in regulating Aurora-A gene transcription.

FIGURE 2. Loss of TRAP220/MED1 expression impairs TR-dependent transcription. HeLa cells (1 \(\times\) 10\(^{6}\)) were transfected with 50 pmol of either TRAP220/MED1 siRNA or control siRNA (100 nm final) together with pRK-CMV-FLAG-TrxA (1 \(\mu\)g) and 2xTuREtk-luciferase (0.4 \(\mu\)g). The TRAP220/MED1 expression vector pS5S-HA-TRAP220 (0.25 \(\mu\)g) was cotransfected as indicated. 24 h post-transfection, the cells were grown for an additional 24 h in the presence or absence of thyroid hormone (T\(_3\)) (10 \(^{-6}\) M) and then harvested for measurement of luciferase activity. Results are presented as the mean \(\pm\) S.E. of triplicate transfections.

| Gene name | Proposed function | Accession no. | Fold decrease\(^a\) | p value\(^b\) |
|-----------|------------------|--------------|----------------|-------------|
| Cell division cycle 6 homolog (CDC6) | Regulation of cell cycle | NM_001254 | -2.2 | 1.25 \(\times\) 10\(^{-4}\) |
| Mitotic arrest deficient-1 (MAD1L1) | Regulation of cell cycle, mitotic checkpoint | NM_003550 | -2.3 | 7.31 \(\times\) 10\(^{-4}\) |
| Neurofibromin 1 (NF1) | Cell growth and/or maintenance | NM_000267 | -1.7 | 4.89 \(\times\) 10\(^{-4}\) |
| Block of proliferation 1 (Bop1) | rRNA processing | NM_015201 | -3.5 | 6.24 \(\times\) 10\(^{-5}\) |
| Chromatin assembly factor 1, subunit A (CHAF1A) | DNA replication, cell cycle nucleosome assembly | NM_005483 | -2.2 | 6.58 \(\times\) 10\(^{-5}\) |
| Aurora-A serine/threonine kinase 6 (STK6) | Centrosome kinase, regulation of mitosis | NM_003600 | -2.1 | 1.90 \(\times\) 10\(^{-4}\) |
| Anaphase promoting complex subunit 5 (ANAPC5) | G2/M transition, cytokinesis, regulation of cell cycle | NM_016237 | -1.8 | 6.24 \(\times\) 10\(^{-4}\) |
| Lamin B receptor (LBR) | Mitotic dynamics of the nuclear envelope | NM_002296 | -2.4 | 2.30 \(\times\) 10\(^{-4}\) |
| Septin 6 (SEPT6) | Cytokinesis, cell cycle regulation | NM_015129 | -2.5 | 3.73 \(\times\) 10\(^{-5}\) |
| Cyclin J (CCNJ) | Regulation of cell cycle | NM_019084 | -2.0 | 6.69 \(\times\) 10\(^{-4}\) |
| Prothymosin a (PTMA) | Regulation of cell cycle, transcription, development | NM_002823 | -1.6 | 8.23 \(\times\) 10\(^{-4}\) |
| Endothelial differentiation-related factor 1 (EDF1) | Transcriptional activation, cell growth and maintenance | NM_003792 | -3.3 | 1.56 \(\times\) 10\(^{-5}\) |
| Protein phosphatase 3 catalytic subunit B (PPP3CB) | Regulation of cell cycle | NM_204234 | -2.2 | 1.51 \(\times\) 10\(^{-4}\) |
| Zinc finger, MYND domain containing protein (ZMYND11) | Cell cycle regulation, transcriptional regulation | NM_006624 | -2.2 | 1.52 \(\times\) 10\(^{-4}\) |
| Deoxythymidylate kinase (DTYMK) | Nucleotide biosynthesis, cell cycle regulation | NM_012145 | -2.1 | 3.20 \(\times\) 10\(^{-4}\) |

\(^a\) Geometric mean-fold decrease in expression value changes between TRAP220/MED1 siRNA-treated samples and control siRNA samples from three independent experiments.

\(^b\) A class comparison algorithm was used to conduct an univariate \(t\) test followed by an exact multivariate permutation test with a permutation of 1000 (see “Experimental Procedures”). The cut-off criteria were genes with a permutation \(p\) \(\leq\) 0.001.
tional role in regulating Aurora-A gene expression, we measured Aurora-A transcription transiently in HeLa cells from an Aurora-A promoter construct (pGL-370) containing both the E2F and GABP binding sites (Fig. 4, A and B). Importantly, and consistent with a role for TRAP220/MED1 in directly regulating transcription from the Aurora-A promoter, transcription from the reporter gene was inhibited 2.5-fold when cells were cotransfected with TRAP220/MED1 siRNA (Fig. 4B).

To better define the positive regulatory elements in the Aurora-A promoter that are required for TRAP220/MED1 coactivation, we utilized a second Aurora-A promoter deletion construct (pGL-90) that retains the GABP binding site yet lacks the two E2F binding sites (Fig. 4A). Transient overexpression of TRAP220/MED1 markedly enhanced transcription from both the pGL-370 and pGL-90 promoters (3.5–5-fold, respectively) but had no significant effect on the control pGL-basic construct (Fig. 4C). We suspect that the increased basal transcription from the pGL-370 promoter is likely due to the additional presence of the two E2F sites or possibly other potential activator sites upstream of the GABP site. Nonetheless, the ability of ectopic TRAP220/MED1 to
enhance transcription from both the pGL-90 and pGL-370 constructs suggested that the GABP binding site is important for TRAP220/MED1 coactivation. To address this issue more closely, we mutated the GABP site (CTTCCGG to CTTGGAA) within the pGL-370 construct (Fig. 4A, see “Experimental Procedures”). Interestingly, mutation of the GABP site significantly inhibited the ability of ectopic TRAP220/MED1 to coactivate transcription from the Aurora-A promoter. In summary, these findings suggest that TRAP220/MED1 mediates transcription from the Aurora-A gene promoter in a manner that requires the GABP binding site.

Direct Recruitment of TRAP/Mediator to the Aurora-A Promoter—ChIP assays were next performed to investigate whether TRAP220/MED1 is directly recruited to the Aurora-A gene promoter in vivo. Accordingly, HeLa cells were transfected with either TRAP220/MED1 siRNA or control siRNA (100 nM) as indicated. A–D, the chromatin was immunoprecipitated using antibodies specific for TRAP220/MED1, MED6, or GABPα as indicated above the panels. The immunoprecipitates were then subjected to semi-quantitative PCR using two different primer sets (F1/R1 and F2/R2) specific for different regions of the Aurora-A promoter. Primer set F1/R1 spans bp −169 to +98, whereas primer set F2/R2 spans bp −366 to −145. Reactions lacking primary antibodies were included as negative controls. Aliquots of chromatin taken before immunoprecipitation were used as PCR positive controls (denoted as input). E, HeLa cells were stimulated with interferon α (1000 units/ml) for 1 h (see “Experimental Procedures”). ChIP assays were then carried out (per above in A–D) using antibodies specific for MED6 and PCR primers specific for the ISG54 gene promoter.

FIGURE 5. Direct recruitment of TRAP/Mediator to the Aurora-A promoter. Formaldehyde cross-linked chromatin was prepared from HeLa cells transfected with either TRAP220/MED1 siRNA or control siRNA (100 nM) as indicated. A–D, the chromatin was immunoprecipitated using antibodies specific for TRAP220/MED1, MED6, or GABPα as indicated above the panels. The immunoprecipitates were then subjected to semi-quantitative PCR using two different primer sets (F1/R1 and F2/R2) specific for different regions of the Aurora-A promoter. Primer set F1/R1 spans bp −169 to +98, whereas primer set F2/R2 spans bp −366 to −145. Reactions lacking primary antibodies were included as negative controls. Aliquots of chromatin taken before immunoprecipitation were used as PCR positive controls (denoted as input). E, HeLa cells were stimulated with interferon α (1000 units/ml) for 1 h (see “Experimental Procedures”). ChIP assays were then carried out (per above in A–D) using antibodies specific for MED6 and PCR primers specific for the ISG54 gene promoter.
To verify that the observed TRAP220/MED1 occupancy at the Aurora-A promoter is consistent with the recruitment of the intact TRAP/Mediator complex, we repeated the ChIP assay using antibodies against another TRAP/Mediator subunit MED6. Similar to the recruitment of TRAP220/MED1, MED6 was recruited to the Aurora-A promoter between sequences −169 to +98 when HeLa cells were transfected with control siRNA (Fig. 5B). In contrast, no recruitment of MED6 was observed at the upstream promoter sequence containing the E2F sites (data not shown). Importantly, and consistent with a specific role for TRAP220/MED1 in targeting the TRAP/Mediator complex to the Aurora-A promoter, MED6 occupancy was significantly attenuated in cells transfected with TRAP220/MED1 siRNA (Fig. 5B).

GABP is unique among other mammalian members of the Ets family of transcription factors in that it alone is an obligate multimeric protein complex (35). The complex is composed of two different subunits, GABPα and GABPβ, which together form a tetrameric α2β2 structure. To verify that GABP itself binds at the Aurora-A promoter, ChIP was carried out as before using antibodies specific for the GABPα subunit. As expected, GABPα binding was detected at the promoter within the region containing the GABP binding site (−169 to +98) and its occupancy was unaffected by TRAP220/MED1 siRNA treatment (Fig. 5D).

The gene-specific transcription factor signal transducer and activator of transcription 2 (STAT2) can recruit TRAP/Mediator to the interferon-stimulated gene 54 (ISG54) promoter in HeLa cells (Ref. 36; Fig. 5E). In contrast to the results with the Aurora-A promoter, we found that TRAP/Mediator recruitment to the ISG54 gene in HeLa cells was relatively unaffected by TRAP220/MED1 siRNA treatment (Fig. 5E). These findings thus rule out the possibility that TRAP220/MED1 silencing nonspecifically inhibits TRAP/Mediator recruitment at all TRAP/Mediator-regulated promoters. In summary, the results here show that both GABP and TRAP220/MED1-containing TRAP/Mediator complexes are directly recruited to the Aurora-A promoter between sequences −169 and +98, and when taken together with the transient transfection data (Fig. 4), they suggest that the transcription factor GABP may play an important functional role in recruiting the TRAP/Mediator complex to the Aurora-A gene.

TRAP220/MED1 Directly Interacts with GABP—Given our findings showing direct TRAP220/MED1 binding to an Aurora-A promoter region containing a bona fide GABP binding element (Fig. 5), we asked whether GABP might directly interact with TRAP220/MED1 in vitro. Toward this end, GST pulldown assays were carried out using GST-tagged GABPα or GABPβ proteins together with a baculovirus-expressed HA-tagged TRAP220/MED1 full-length protein purified from insect Sf9 cells (Fig. 6A). As shown in Fig. 6B, we found that GST-GABPα was able to directly and efficiently bind TRAP220/MED1 in vitro. By contrast, neither GST-GABPβ nor the GST control protein, exhibited any TRAP220/MED1 binding activity. Reciprocally, we found that HA-TRAP220/MED1 was able to specifically bind GST-GABPα as determined by anti-HA co-immunoprecipitation (Fig. 6C).

To investigate the interaction between GABPα and TRAP220/MED1 under more physiological conditions, we used anti-GABPα antibodies to immunoprecipitate native GABPα-containing protein complexes from HeLa cell nuclear extracts. In agreement with the in vitro GST pulldown results, we found that TRAP220/MED1 coimmunoprecipitated with GABPα, thus indicating that the two proteins associate in vivo (Fig. 6C). As an important negative control, we found that the in vivo GABPα-TRAP220/MED1 association was completely abrogated when the HeLa cells were pretransfected with TRAP220/MED1 siRNA (Fig. 6C). Taken together with the Aurora-A reporter gene findings (Fig. 4), and the ChIP results showing TRAP/Mediator recruitment at the GABP binding element (Fig. 5), the data here suggest that GABP, via direct interactions between GABPα and TRAP220/MED1, can recruit the TRAP/Mediator complex to the Aurora-A gene promoter where it facilitates transcription. Equally important, these data reveal a novel functional role for TRAP220/MED1 in regulating the transcription of specific target genes that are involved in cell growth. Indeed, it is conceivable that other genes summarized in Table 1 might also be direct targets of TRAP220/MED1 regulation that further contribute to regulated cell cycle progression.

DISCUSSION

The Aurora family of kinases play critical roles in chromosome segregation and cell division and are essential for regulating mitosis and meiosis in all eukaryotes (23). Human Aurora-A is a member of this family and is frequently overexpressed in a strikingly high proportion of human cancers thus suggesting that it plays a pivotal role in tumorigenesis (24). In somatic mammalian cells, expression of Aurora-A occurs in a cell cycle-dependent manner with peak levels of mRNA and protein occurring at the G2/M phase of the cell cycle (25–28).

In this study, we identified the human Aurora-A gene as a specific transcriptional regulatory target of the TRAP/Mediator coactivator complex and investigated the underlying molecular mechanism. We found that Aurora-A gene expression is specifically down-regulated in HeLa cells depleted of the TRAP/Mediator subunit TRAP220/MED1. Reciprocally, we found that ectopic TRAP220/MED1 overexpression can specifically enhance Aurora-A gene transcription. We identified a specific regulatory element in the Aurora-A gene promoter containing a GABP binding site that is necessary for TRAP220/MED1 transcriptional coactivation. We further found that TRAP220/MED1-containing TRAP/Mediator complexes are directly recruited to this cis-element in vivo. Finally, we demonstrated that TRAP220/MED1 interacts both in vitro and in vivo with the GABPα subunit of the Ets-related transcription factor GABP.

Tanaka et al. (28) recently reported that the 7-bp GABP-binding sequence CTTCCGG (−85 to −79) in the human Aurora-A promoter was essential for the transcriptional activity of the Aurora-A gene. Indeed, the findings presented here not only confirm this earlier report, they provide a possible molecular mechanism for the critical stimulatory activity facilitated by GABP at the Aurora-A gene. Our transient transfection assays show that TRAP220/MED1 coactivates transcription from Aurora-A promoter fragments containing the GABP binding site (Fig. 4), whereas our ChIP assays show that TRAP220/MED1-containing TRAP/Mediator complexes bind to a promoter element containing the GABP binding site but not binding upstream binding sites for E2F (Fig. 5). Taken together with the TRAP220/MED1-GABPα protein-protein interaction data (Fig. 6), our results suggest that TRAP/Mediator is recruited to the Aurora-A gene via GABP where it then functions interfaces with the basal transcription apparatus. Given that Aurora-A mRNA expression peaks at G2/M, the results here are especially interesting in view of our earlier work showing that TRAP220/MED1 protein expression likewise peaks at G2/M (19). Cell cycle-dependent regulation of Aurora-A transcription additionally requires two tandem repressor elements located downstream of the GABP site at −44 to −35 (28). The tandem repressor elements, termed CDE and CHR (37, 38), presumably bind G2/S-specific repressors that may include submembers of the E2F family of transcription factors (39, 40).

Whereas the findings presented here clearly implicate GABP-mediated recruitment of TRAP/Mediator in positively regulating Aurora-A gene expression, other trans-activation mechanisms are likely involved.
For example, we observed that an Aurora-A promoter construct containing both the GABP site and the two putative upstream E2F sites displayed higher basal activity than a construct containing the GABP site alone (Figs. 4A and 4C). Thus, it appears that “activator” E2F subfamily members function upstream of the GABP site in a constitutive manner, whereas repressor factors likely act downstream of the GABP site in a cell cycle-dependent manner (41). Tanaka et al. (28) also identified a potential Sp1 binding site (bp −129 to −121) upstream of the GABP site. Given that a derivative of the TRAP/Mediator complex termed CRSP (cofactor required for Sp1) is required for Sp1-dependent activation in vitro (42), it is conceivable that Sp1 may play a cooperative role in recruiting TRAP/Mediator to the Aurora-A promoter. Nonetheless, TRAP220/MED1 is capable of coactivating transcription from an Aurora-A promoter lacking the Sp1 site (Fig. 4C) thus suggesting that Sp1 acts in concert with GABP. Important in this regard, Sp1 was recently shown to functionally cooperate with GABP to activate transcription at the CD18 gene promoter in myeloid cells (43).

Because the Aurora-A core promoter lacks a consensus TATA element, potential cooperative binding between TRAP/Mediator and distinct types of TATA-binding protein-associated factors and complexes might also be invoked (Ref. 44 and references therein). Indeed, the possibility exists that distinct TRAP/Mediator complexes specifically containing TRAP220/MED1 might adopt distinct structural conformations that confer promoter-specific recognition (9). Interestingly, estrogen treatment was recently reported to stimulate Aurora-A overexpression in primary breast cancer in female rats (45). Hence, in light of the fact that TRAP220/MED1 itself is amplified in breast cancer cells and acts as an estrogen receptor coactivator (15, 16), the possibility exists that estrogen receptor may play an as of yet ill defined coregulatory role in targeting TRAP/Mediator to the Aurora-A gene.

An important and novel finding to come from the study here is the notion that TRAP220/MED1 specifically targets TRAP/Mediator to distinct genes involved in growth and cell cycle progression. The physiological relevance of such a scenario is supported by the mouse knockout studies indicating that Trap220/Med1−/− embryos are unusually small and exhibit retarded cell growth (17, 18) and that Trap220/Med1−/− embryonic fibroblasts (isolated prior to embryonic death) exhibit impaired cell cycle progression in growth and mitogenicity assays (18). Hence, in this connection, it is plausible that other genes identified in Table 1 may represent direct targets for TRAP/Mediator transcriptional regulation.
In summary, our findings indicate that TRAP220/MED1 plays an important role in regulating Aurora-A gene expression, a centrosome kinase that regulates cell cycle progression and is frequently amplified in several types of cancer. Given that TRAP220/MED1 overexpression itself has been correlated with cancer (15), our findings may have important implications for TRAP220/MED1 involvement in tumorigenesis. Future studies should reveal other growth and cell cycle relevant genes that are targeted by TRAP220/MED1-containing TRAP/Mediator complexes as well as the molecular mechanisms that regulate their expression.

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