MTA1 Interacts with MAT1, a Cyclin-dependent Kinase-activating Kinase Complex Ring Finger Factor, and Regulates Estrogen Receptor Transactivation Functions*

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The transcriptional activity of estrogen receptor-α is controlled by coregulators. MTA1 (metastasis-associated protein 1) represses estrogen receptor-α-driven transcription by recruiting histone deacetylases (HDACs) to the estrogen response element containing target gene chromatin in breast cancer cells. Using a yeast two-hybrid screen with the MTA1 C-terminal domain as bait, we identified MAT1 (ménage à trois 1) as an MTA1-binding protein. MAT1 is an assembly/targeting factor for cyclin-dependent kinase-activating kinase (CAK), which has been shown to functionally interact with general transcriptional factor TFIIH, a known inducer of ER transactivation. We show that estrogen signaling promotes nuclear translocation of MAT1 and that MAT1 interacts with MAT1 both in vitro and in vivo. MAT1 binds to the C-terminal 389–441 amino acids GATA domain and N-terminal 1–164 amino acids bromo-domain of MTA1, whereas MTA1 binds to the N-terminal ring finger domain of the MAT1. In addition, MAT1 interacts with the activation function 2 domain of ER and colocalizes with ER in activated cells. MTA1 deregulation in breast cancer cells led to its interactions with the CAK complex components, ER, and HDAC2. Accordingly, MTA1 inhibited CAK stimulation of ER transactivation that was partially relieved by HDAC inhibitor trichostatin A, suggesting that MAT1 might inhibit CAK-induced transactivation function of ER by recruiting HDAC. Furthermore, MTA1 overexpression inhibited the ability of CAK complex to phosphorylate ER. Together, these findings identified MAT1 as a target of MTA1 and provided new evidence to suggest that the transactivation functions of ER might be influenced by the regulatory interactions between CAK and MAT1 in breast cancer cells.

The eukaryotic genome is compacted with histone and other proteins to form chromatin, which consists of repeating units of nucleosome (1, 2). Formation of nucleosomes and higher order chromatin structures can render the DNA inaccessible to transcription factors and complexes. For transcription factors to access DNA, the repressive chromatin structure needs to be remodeled. Dynamic alterations in the chromatin structure can facilitate or suppress the access of the transcription factors to nucleosomal DNA, leading to transcriptional regulation. One way to achieve this is through alterations in chromatin remodeling factors or in the acetylation state of nucleosomal histones (3–5). Acetylation of core histones occurs at lysine residues on the N-terminal tails of the histones, thus neutralizing the positive charge of the histone tails and decreasing their affinity for DNA. Hyperacetylated chromatin is generally associated with transcription activation, whereas hypoacetylated chromatin is associated with transcription repression (3–6).

A number of recent studies have raised the possibility of a close connection between HDACs and cancer. Because HDAC-mediated deacetylation of nucleosomal histones is known to be associated with transcriptional repression of some genes, it is being proposed that the deregulation of recruitment of HDAC-containing repressor complex to specific target promoters could serve as a potential mechanism by which these enzymes contribute to tumorigenesis. For example, MTA1 (metastasis-associated protein 1) represses estrogen receptor-α (ER)-driven transcription by recruiting HDAC to the ER response element (ERE)-containing target gene chromatin in breast cancer cells (7). The NuRD-70 polypeptide of nucleosome remodeling/histone deacetylase complex is identical to that of the MTA1 (8–11). The MTA1 gene was initially identified by differential expression in rat mammary adenocarcinoma metastatic cells, and its expression has been shown to correlate well with the metastatic potential of several human cell lines and tumors (12).

To better understand the cellular functions of MTA1 in breast cancer cells, we performed a yeast two-hybrid screen to clone MTA1-interacting proteins. One of several isolates was identified as MAT1 (ménage à trois 1). MAT1 was originally discovered as an integral component of the cyclin-dependent kinase (CDK7)-activating kinase (CAK), a complex consisting of catalytic subunit CDK7, regulatory subunit cyclin H, and MAT1 (13). The MAT1 protein consists of three major motifs: the N-terminal RING finger region, the central coiled-coil region, and the C-terminal cyclin-like region (13–16). The functions of MAT1 are mediated by interactions of these motifs with distinct protein-protein interactions. For example, the RING finger domain is linked with general transcription factor TFIIH-mediated transcription (17), the coiled-coil domain in making contact with TFIIH via XBP and XPD helicase subunit.

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The abbreviations used are: HDAC, histone deacetylase; MTA1, metastasis-associated protein 1; ER, estrogen receptor-α; MAT1, ménage à trois 1; CAK, cyclin-dependent kinase-activating kinase; ERE, estrogen response element; E2, 17β-estradiol; AF, activation function; CDK, cyclin-dependent kinase; ChIP, chromatin immunoprecipitation; GST, glutathione S-transferase; TSA, trichostatin A.
units (18), and the cyclin-like region in the formation of trimeric CDK7-cyclin H-MAT1 complex (19). MAT1 facilitates the formation of the ternary CAK complex by assembling and stabilizing the interactions between the CDK7 and cyclin H without involving the activating phosphorylation in the T-loop of human CDK7 (20). MAT1 has been also shown to determine the substrate specificity of CAK, because recruitment of MAT1 to the CDK7/cyclin H as a part of TFIIH preferentially targets the RNA polymerase II large subunit over CDK2 (21). In addition, MAT1 as a part of CDK7-cyclin H-MAT1 complex within the context of TFIIH enhances the phosphorylation of several in vitro substrates, including the POU domain of octamer transcription factor (22), tumor suppressor p53, and pRB proteins (23, 24), retinoic acid receptor-α (25, 26), and estrogen receptor-α (27). In addition, MAT1 also binds to XPB and XPD helicases subunits of TFIIH that recruit CAK complex to the core TFIIH (27). These observations suggest that MAT1 as a part of CAK in conjunction with multi-enzymatic protein complex TFIIH participates in several fundamental aspects of the transcription regulation. Because RING finger and coiled-coil motifs are generally involved in protein-protein interactions, MAT1 could potentially interact with other cellular regulatory proteins.

Here, we show that MTA1 directly binds to the MAT1 and represses CAK-mediated stimulation of ERE transcription and that MAT1 interacts with ER in a ligand-independent manner. In addition, MAT1 is frequently up-regulated in human breast tumors. These findings reveal a novel connection among MTA1, MAT1, and cancer and discovered the existence of regulatory interactions between MTA1 and CAK in breast cancer cells.

**MATERIALS AND METHODS**

**Cell Cultures and Reagents**—MCF-7, MDA-MB-231, T47-D, MDA-MB-453, BT-474, and ZR-75 human breast cancer cells (7, 28) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The following antibodies were used: anti-MAT1, anti-MTA1, anti-cyclin H, anti-CDK7, and anti-HDAC1 (Santa Cruz...
Biotechnology, Santa Cruz, CA), anti-T7 (Novagen, Milwaukee, WI), anti-HER-2 (NeoMarkers, Fremont, CA), anti-vinculin (Sigma), anti-ER (Upstate Biotechnology, Lake Placid, NY), and anti-mouse horseradish peroxidase conjugate (Amersham Biosciences). Alexa 546-labeled goat anti-rabbit and Alexa 488-labeled goat anti-mouse secondary antibodies and the DNA-intercalating fluorescent dye ToPro3 were purchased from Molecular Probes (Eugene, OR).

**Plasmid Construction and Two-hybrid Library Screening**—The full-length MTA1 (1–715 amino acids) (7) was digested with BamHI and XbaI (blunt end) and ligated to the pGBK7 vector that expresses protein fused to amino acids 1–147 of the GAL4-DNA binding domain at BamHI and PstI (blunt end) (Clontech). MTA1 baits were constructed by deleting 1–254 amino acids from the N terminus of MTA1 by cutting and self-ligating with NcoI. The remaining 255–715 amino acids of the C terminus of MTA1 was used as bait. This bait was used to screen a human mammary gland cDNA library fused to the Gal4 activation domain (Clontech) according to the manufacturer’s instructions. A total of 2 × 10^6 clones were screened. The positive clones were isolated and sequenced at the University of Texas M. D. Anderson Cancer Center Core sequencing facility. The positive clones were also verified by one-on-one transformations and selection on agar plates lacking adenine, histidine, tryptophan, and leucine and also by β-galactosidase assay (28).

**Deletion Constructs of MTA1 and MAT1**—Nine MTA1 deletion constructs were generated to map the binding site(s) of MTA1 with MAT1 by PCR-based procedure. Starting from the N-terminal region, the constructs were named N1-MTA1 to N9-MTA1. All of the forward (F) primers including the ATG start codon primer contain an EcoRI site and all of the reverse (R) primers including the stop codon (TAG) primer contain a SalI site. The ATG start codon primer is 5'-GCCGCCGGAATTCACATGGCCGCCAACA-3' and the stop codon (TAG) primer was 5'-AGGTGGGGGTCGACCCTAGTCCTCCCG-3'. The construction of N1-MTA1 through N7-MTA1 used the ATG start codon primer plus N1R, 5'-ACTCGAATGTCGACTTTATCTGCCA-3'; N2R, 5'-AGCTGCTGCCAGTCGACGGCCGTGGAC-3'; N3R, 5'-AGGCCCGGCCGTCGAAGAGGGCTCTGGCCCG-3'; N4R, 5'-ACTGCGGTGTCGACCTGGCCTCTCTCCA-3'; N5R, 5'-ACCGAAGGACGTGGACCGACCAGG-3';...
was performed with a FuGENE 6 kit (Roche Molecular Biochemicals) clean, the PCR products were digested with either RIPA buffer supplemented with 100 mM NaF, 2 mM NaVO₅, 1 protease (29). The sequence of N10-MTA1 deletion construct (amino acids 212–715) has been described previously (28). A nested PCR method was used to generate eight deletion constructs of MAT1. Four of five deletion constructs were made for MAT1 using either start codon primer 5'-GGGAATTCCATGGACGATCAGGGT-3' or stop codon primer 5'-CTTATAGTGACTTAAAGTGAGTCGCGCCA-3'. The following constructs were made by PCR with start codon primer plus 189R (1–189 amino acids), 5'-GATCAGGGT-3'; and using stop codon primer plus 189F (189–309 amino acids), 5'-GATGAAGCTGGGAATTCCATGGACGATCAGGGT-3'. After Greenpeace, the PCR products were digested with either EcoRI and SalI or EcoR1 and NotI and ligated to a pcDNA 3.1A vector at either EcoRI and SalI or EcoR1 and NotI.

Transfection, Cell Extracts, and Immunoprecipitation—Transfection was performed with a FuGENE 6 kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. The cells were lysed with RIPA buffer supplemented with 100 mM NaF, 2 mM NaVO₅, 1 protease mixture (Roche Molecular Biochemicals) on ice for 15 min. Cell lysates containing equal protein were immunoprecipitated with the desired antibody and analyzed by SDS-PAGE (28, 29).

CAK Phosphorylation of ER—The effect of MTA1 on CAK phosphorylation of ER was determined according to Chen et al., (27). Briefly, MCF-7 cells stably expressing either pcDNA or T7-MTA1 were transfected with CDK7 and MAT1 and labeled with [35S]methionine in a reaction volume of 50 μl. The cells were lysed with 1 ml with Nonidet P-40 lysis buffer, and an aliquot (250 μl) containing equal protein was immunoprecipitated with the desired antibody and analyzed by autoradiography.

Chromatin Immunoprecipitation (ChIP) Assay—Approximately 10⁶ cells were treated with cross-linked histones to DNA. The ChIP assay was performed as described (7). After immunoprecipitation with corresponding antibodies, the eluted DNA was amplified by PCR using the primers GAATTAGCTTAGGCCTAGACGGGAATG (forward) and AGGATTGCTGATAGACAGAGACGAC (reverse) for pS2 promoter around the ERE site.

Tissue Samples and Western Blotting—Mouse tissue samples were collected and snap frozen in liquid nitrogen as described previously (7, 30). Human breast tissue samples were obtained from the University of Texas M. D. Anderson Breast Tumor Core Pathology Laboratory maintained by Ayseoglu A. Sahin (31, 32). Thawed tissue samples were homogenized in Triton X-100 lysis buffer (20 mM HEPES, 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate (v/v), 2 mM EDTA, 2 mM NaVO₅, and protease inhibitor mixture), and equal amounts of protein were analyzed by Western blotting. The protein vinculin was used routinely as a loading control.

In Vitro Transcription, Translation, and GST Pull-down Assays—In vitro transcription and translation of the test proteins were performed by using the TNT transcription-translation system (Promega). One microgram of desired DNA in pCDNA 3.1 vector (Invitrogen) was translated in the presence of [35S]methionine in a reaction volume of 50 μl by using the T7-TNT reaction mixture. The reaction mixture was diluted to 1 ml with Nonidet P-40 lysis buffer, and an aliquot (250 μl) was used for one GST pull-down assay. Two pools of the translated reaction mixture were verified by SDS-PAGE and autoradiography. The GST pull-down assays were performed by incubating equal amounts of GST or GST fusion protein immobilized to glutathione-Sepharose beads (Amersham Biosciences) with in vitro translated [35S]-labeled test protein. The mixtures were incubated for 2 h at 4 °C and washed six times with Nonidet P-40 lysis buffer. Two pools of the translated reaction mixture were verified by SDS-PAGE and visualized by fluorography (32).

Immunofluorescence and Confocal Imaging—The cells were plated on glass coverslips in 6-well culture plates. When the cells were ~50% confluent, they were serum-starved for 36 h. Alternatively, 30% confluent cells were maintained in phenol red-free medium supplemented with 5% charcoal-stripped fetal calf serum for 72 h and treated with β-estradiol (10⁻⁹ M) for 30 min with or without pretreatment with the anti-estrogen ICI 182780 (10⁻⁶ M) for 1 h. The cells were rinsed in phosphate-buffered saline, fixed in cold 100% methanol for 10 min, and processed for immunofluorescent localization of MAT1 and Myc-tagged MTA1 or ER. The DNA was visualized by counterstaining with ToPro3. Fluorescent labeling was visualized using a Zeiss LSM 510 microscope and a 40x objective (32).

Immunohistochemistry—For immunohistochemical detection of MAT1, the sections were deparaffinized with xylene and rehydrated using graded ethanol washes. The sections were incubated in 0.3% H₂O₂ and methanol for 30 min to inactivate endogenous peroxidase. The sections were then boiled for 10 min in 0.01 M citrate buffer and cooled for 30 min at room temperature to expose antigenic epitopes. The sections were sequentially biotin- and protein-blocked and incubated
with primary antibody overnight at room temperature followed by biotinylated secondary antibody, streptavidin-biotin complex, amplification reagent, and streptavidin-peroxidase complex (DAKO Corporation, Carpinteria, CA), and then developed with DAB-H2O2 and counterstained with Mayer’s hematoxylin. Anti-MAT1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a dilution of 1:100.

Statistical Analysis—Statistical analysis was done using Student’s t test. The values are considered statistically significant if p < 0.05.

RESULTS

Identification of MAT1 as a MTA1-interacting Protein—To better understand the functions of MTA1 in breast tumor cells, a yeast two-hybrid screening of the mammary gland cDNA expression library was performed using the MTA1 C-terminal amino acids 255–715 as bait. This bait contains several binding motifs (DNA-binding domain, GATA domain, and SH2 and SH3 binding domains). As a negative control, we used a recently identified MTA1s variant that lacks protein-binding motifs as bait (28). Yeast cells expressing the Gal4 fusion protein were transformed with the above bait. Screening of 2 × 10^6 transformants resulted in the isolation of several positive clones. By sequencing of the positive clones, we identified several clones that encoded cDNA of MAT1 (GenBank™ accession number X92669), an integral component of the CAK complex (33). The specificity of the MTA1-MAT1 interactions was verified by one-on-one transformation (Fig. 1A). Interaction was further confirmed by the growth of the transformed colonies in medium lacking adenosine, histidine, tryptophan, and leucine and development of blue coloration in β-galactosidase assay, whereas the cotransfection with the control GBK vector did not do so (Fig. 1A). Subsequent studies were undertaken to understand the significance of MTA1 interactions with MAT1 in breast cancer cells.

MAT1 Interacts with MTA1 in Vitro—To confirm the interaction between MAT1 and MTA1, we next examined the ability of in vitro translated MAT1 protein to bind with GST-MTA1 in vitro. MAT1 interacted efficiently with GST-MTA1 but not with GST alone in GST pull-down assays (Fig. 1B, left panel). Conversely, in vitro translated MTA1 protein also specifically interacted with GST-MAT1 (Fig. 1B, right panel).

MAT1 Interacts with MTA1 in Vivo—To confirm the MAT1 interaction with MTA1 in vivo, MCF-7 cells were cotransfected with the equal amounts of c-Myc-tagged MTA1 and T7-tagged MAT1. Immunoprecipitation of cell lysates with an anti-T7
antibody was followed by immunoblotting with anti-c-Myc antibody. As illustrated in Fig. 1C, cotransfection with T7-MTA1 but not pCDNA coimmunoprecipitated c-Myc-MTA1. To further demonstrate the existence of the noticed interaction between MAT1 and MTA1 in vivo, we used MCF-7 breast cancer cells stably expressing T7-tagged MTA1 or pCDNA (7). Immunoprecipitation of the endogenous MAT1 with an anti-MAT1 monoclonal antibody was followed by immunoblotting with the anti-T7 monoclonal antibody. The results showed specific interaction between the endogenous MAT1 and T7-MTA1 in MCF7/MTA1 cells but not in MCF7/pCDNA cells (Fig. 1D). Next, we used T47D breast cancer cells that express both MAT1 as well as MTA1 to demonstrate the interaction between the endogenous MAT1 and MTA1. Immunoprecipitation of lysate from exponentially growing cells with anti-MTA1 antibody was followed by Western blotting with anti-MAT1 antibody. The results show that endogenous MTA1 and endogenous MAT1 do interact with each other (Fig. 1E).

To explore the spatial relationship between MTA1 and MAT1 and further validate their interactions in vivo, we utilized immunofluorescence and confocal scanning microscopy. MCF-7 cells were transiently transfected with Myc-tagged MTA1 and stained using antibodies against c-Myc antibody to detect c-Myc-MTA1 (green) and estrogen receptor (red). Areas of colocalization of the red and green signals show yellow fluorescence. Control cells, MAT1, and ER were diffusely localized in the cytoplasm. E2-treated cells, MAT1, and ER show strong colocalization at the nuclear periphery and in specific intranuclear regions (×40 magnification).

**Fig. 6. MAT1 interaction with ER.** A. GST pull-down assay involving in vitro translated 35S-MAT1 was incubated with GST-AF1 or GST-AF2 or GST in the presence and absence of E2. B. GST fusion proteins from five functional domains of ER were incubated with in vitro translated 35S-MAT1 and analyzed by SDS-PAGE and autoradiography. C. MAT1 and ER colocalize following estradiol treatment. MCF-7 cells were grown in phenol red-free medium supplemented with 3% charcoal-stripped serum for 72 hours and then treated with E2 for 30 min. The cells were fixed in methanol and immunofluorescently stained for MAT1 (green) and estrogen receptor (red). Areas of colocalization of the red and green signals show yellow fluorescence. Control cells, MAT1, and ER were diffusely localized in the cytoplasm. E2-treated cells, MAT1, and ER show strong colocalization at the nuclear periphery and in specific intranuclear regions (×40 magnification).
el). Together, these observations suggested that MAT1 could interact with Myc-MTA1 under physiological conditions, suggesting the possible influence of MTA1 on the functions of MAT1 in breast cancer cells.

**MAT1 Expression Pattern**—To explore the significance of MAT1 in breast tissues, we examined MAT1 protein expression levels in normal mouse tissues and human breast cancer cell lines. MAT1 protein was easily detectable in both ER-positive (MCF7, ZR75 and T47D) and ER-negative (MDA-MB231, MDA-MB453 and BT474) cell lines (Fig. 2A). As shown in Fig. 2B, various levels of MAT1 protein were present in many mouse tissues, with the highest level in the thyroid and mammary glands of pregnant mice (Fig. 2C). To explore the significance of MAT1 in human breast tumors, we investigated whether MAT1 protein expression was altered in paired normal human breast epithelium and breast carcinoma biopsy samples. As shown in Fig. 3A, MAT1 protein expression was elevated in 8 tumors of 12 as compared with the adjacent normal tissue, which showed either or no expression of MAT1. In 6 of the MAT1-overexpressing tumors, there was also up-regulation of MTA1. The blots were reprobed for vinculin as a loading control. Densitometric scanning of MAT1 and MTA1 protein bands suggested the existence of statistically significant up-regulation of both proteins in breast tumors as compared with adjacent normal appearing tissues (Fig. 3B). To validate these results, we immunostained paraffin-embedded paired tissue specimens with an anti-MAT1 monoclonal antibody. Two representative examples in Fig. 3C demonstrate an intense MAT1 nuclear staining in tumor tissue, and normal tissue showed no positive staining.

**Mapping of MAT1- and MTA1-interacting Domains**—Next, we defined the minimal region of MTA1 required for its interaction with MAT1. MTA1 has several important domains involved in protein-protein interactions, DNA binding, and signaling (Fig. 4A). Several C-terminal MTA1 deletion constructs were generated and expressed as 35S-labeled proteins and then subjected to GST pull-down assays with the GST-MAT1 fusion proteins. The results suggest that amino acids 1–164 of MTA1, which contain the bromo-domain, and 389–441 amino acids representing the GATA domain constitute the binding regions for MAT1 (Fig. 4B). To define the binding region or regions of MAT1 that are important for MTA1 interaction, we generated MAT1 encompassing different regions such as N-terminal RING finger region, the central coiled-coil region, and the C-terminal cyclin-like box (Fig. 5A). The results of the GST pull-down assays indicated that MAT1 uses amino acids 1–66 (representing the ring finger domain) to interact with MTA1 efficiently (Fig. 5B). These findings demonstrated that MAT1...
binds to the C-terminal 389–441 amino acids GATA domain and N-terminal 1–164 amino acids bromo-domain of MTA1, whereas MTA1 binds to the N-terminal ring finger domain of the MAT1.

**MAT1 Interacts with ER**—Because repressed MTA1 has been shown to repress the transactivation functions of ER (7), we hypothesized that MAT1 might physically interact with ER and influence its function. To explore this possibility, we examined the binding ability of in vitro translated MAT1 protein and with the GST-AF1 and AF2 domains of ER in GST pull-down assays. As shown in Fig. 6A, MAT1 protein effectively interacted with the GST-AF2 (ligand-binding domain of ER) but not with GST alone or GST-AF1, and this binding was further increased significantly in the presence of estrogen. To confirm these results, we performed GST pull-down assays using various deletion mutants of ER representing domains of ER (Fig. 6B).

To determine whether MAT1 and ER would colocalize in vivo, we examined the immunolocalization of these proteins by confocal microscopy. MCF-7 cells were grown in estrogen-free medium for 72 h and then were treated with E2 (10^{-9} M) for 30 min. In hormone-depleted cells, both MAT1 (endogenous) and ER were diffusely localized in the cytoplasm and absent from the nucleus (Fig. 6C, top panels). However, after E2 treatment, there was a rapid movement of both proteins to the nuclear periphery, concentration in specific nuclear regions, and also diffuse localization in the nucleus (Fig. 6C, middle panels). Pretreatment of cells with the pure anti-estrogen compound ICI 182780 completely blocked this dramatic estrogen effect on MAT1 and ER intracellular localization (Fig. 6C, bottom panels), confirming the ER-mediated nature of the noticed nuclear colocalization of MAT1 and ER.

**MTA1 Interacts with CAK Complex Components**—The CAK complex is composed of CDK7, cyclin H, and MAT1. To demonstrate the endogenous interaction of MTA1 with the components of CAK complex, cell lysates from exponentially growing MCF-7/T7-MTA1 and MCF-7/pcDNA cells (7) were immunoprecipitated with antibodies against cyclin H, CDK7, and MAT1. As shown in Fig. 7, MTA1 could be detected in complexes consisting of cyclin H, CDK7, and MAT1 only in MCF-7/T7-MTA1 and not in vector-transfected MCF-7 cells. Interestingly, both ER and HDAC2 were also present in the CAK1-MTA1 complex, suggesting a potential role for MTA1 in modifying (i.e. repressing) the effect of CAK on the transactivation functions of ER.

**MTA1 Associates with the ERE-responsive Promoters in Vivo**—To directly demonstrate the potential importance of MTA1-MAT1 interaction in ER transcription, we used the ChiP assay to analyze whether T7-MTA1 or MAT1 associates with the endogenous ERE-containing promoters. MCF-7/pcDNA and MCF-7/T7-MTA1 cells were treated with E2 in the presence or absence of ICI 182780 for 30 min and processed to formaldehyde cross-link and to sonicated chromatin for immunoprecipitation with specific antibodies against T7 or MAT1. T7-MTA1 or MAT1-bound genomic DNA fragments were analyzed by quantitative PCR using primers spanning the ERE elements present in the promoter of the pS2 sequence. The results indicated that E2 treatment triggered a significant increase in the amount of pS2 target gene promoter chromatin associated with T7-MTA1 or MAT1 (Fig. 8). Because both MAT1 and MTA1 interacted effectively with the ER target gene chromatin, these findings raised the possibility that MTA1 might influence the status of ER transactivation by MAT1 as a part of CAK complex. In brief, these findings from Figs. 6 and 8 strongly support the notion that MTA1 might influence the regulation of ER transactivation function by CAK1-associated mechanisms.

**MTA1 Inhibits CAK Stimulation of ER Transactivation**—To assess the potential significance of noticed MTA1-MAT1 interactions, we next examined the effect of MTA1 on the ability of CAK to ER transactivation function in MCF-7 cells. Previous reports have shown that coexpression of all three components but not individual subunits of CAK stimulate transcriptional activity of ER in HeLa cells (27). Consistent with these observations, we observed a significant stimulation of ER-dependent transcription by CAK1 in MCF-7 cells (Fig. 9A). Interestingly, MTA1 expression blocked the ability of CAK1 to ER-mediated ERE transcription. Because MTA1 interacts with HDAC2 (7) and because HDAC2 could be detected in MTA1/CAK1 (Fig. 7) in breast cancer cells, we reasoned that HDAC inhibition by TSA might relieve the inhibitory effect of MTA1 on CAK1. Indeed, TSA treatment of cells was accompanied by a considerable derepression by MTA1. These findings suggested that MTA1 might inhibit CAK-induced transactivation function of ER by recruiting HDAC.

![Fig. 9. MTA1 inhibits CAK-mediated ER transactivation.](image-url)
To further understand the role of MTA1 in the functions of CAK, we next examined the effect of MTA1 deregulation on the CAK activity as determined by its ability to phosphorylate ER in vivo. MCF-7 expressing pcDNA or MTA1 cotransfected with MAT1 and CDK7 were labeled with [32P]orthophosphoric acid. The cell lysates were immunoprecipitated with an anti-ER antibody and analyzed by autoradiography. As expected from the previous work by Chen et al. (27), CAK expression in MCF7/pcDNA cells was accompanied by increased phosphorylation of ER (compare lane 3 with lane 1), which could be further increased by estrogen treatment (Fig. 9B, compare lane 4 with lanes 3 and 2). Interestingly, the CAK failed to phosphorylate ER in MCF7/MTA1 cells in both the absence and the presence of estrogen stimulation (Fig. 9B). These results suggested that MTA1 deregulation could also impair the ability of CAK to phosphorylate ER.

DISCUSSION

In the present study, we have identified MAT1, an essential component of CAK complex with functions in cell cycle control and transcription, as a MTA1-interacting protein using the yeast two-hybrid screening. We show that MAT1 interacts with MAT1 both in vitro and in vivo and that MTA1 could be detected in a complex consisting of CAK components ER and HDAC in breast cancer cells. In an attempt to understand the significance of these biochemical interactions, we followed an earlier finding showing that the CAK complex enhances the ER transactivation function (27) and demonstrated that estrogen stimulation of breast cancer cells promotes rapid nuclear translocation of MAT1 and its association with the endogenous ER. Using recombinant proteins, we further demonstrated that MAT1 directly interacts with the AF2 domain of ER under basal as well as E2-inducible conditions. In the context of MTA1, we show that both MTA1 and MAT1 associate with the ER target gene pS2 promoter chromatin and that MTA1 inhibits its CAK-induced stimulation of transcription function of ER.

The functional significance of these findings is derived from the observations that MAT1 acts as the assembly and targeting factor of CAK, which regulates transcription including transactivation property of ER (27). Because MAT1 expression is deregulated in human breast tumors (28), it is possible that MTA1 up-regulation might suppress CAK regulation of the transactivation activity of ER and, subsequently, leads to loss of ER responses, which is generally associated with the progression of breast cancer to more invasive phenotypes. Additional studies are needed to address these evolving issues.

Inhibition of CAK stimulation of ER transactivation by MTA1 was presumably due in part to the recruitment of HDAC to the MTA1-CAK complex, because inhibition could be partially relieved by the HDAC inhibitor TSA. Also the CAK complex in breast cancer cells with deregulated MTA1 contained HDAC2 (Fig. 7). For the first time, these results show that CAK activity as determined by its ability to phosphorylate ER in vivo.

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