The metastasis-associated protein MTA1 has been shown to express differentially to high levels in metastatic cells. MTA2, which is homologous to MTA1, is a component of the NuRD ATP-dependent chromatin remodeling and histone deacetylase complex. Here we report evidence that although both human MTA1 and MTA2 repress transcription specifically, are located in the nucleus, and contain associated histone deacetylase activity, they exist in two biochemically distinct protein complexes and may perform different functions pertaining to tumor metastasis. Specifically, both MTA1 and MTA2 complexes exert histone deacetylase activity. However, the MTA1 complex contained HDAC1/2, RbAp46/48, and MBD3, but not Sin3 or Mi2, two important components of the MTA2 complex. Moreover, the MTA1 complex is similar to the HDAC1 complex, suggesting a housekeeping role of the MTA2 complex. The MTA1 complex could be further separated, resulting in a core MTA1-HDAC complex, showing that the histone deacetylase activity and transcriptional repression activity were integral properties of the MTA1 complex. Finally, MTA1, unlike MTA2, did not interact with the pleotropic transcription factor YY1 or the immunophilin FKBP25. We suggest that MTA1 associates with a different set of transcription factors from MTA2 and that this property may contribute to the metastatic potential of cells overexpressing MTA1. We also report the finding of human MTA3, which is highly homologous to both MTA1 and MTA2. However, MTA3 does not repress transcription to a significant level and appears to have a diffused pattern of subcellular localization, suggesting a biological role distinct from that of the other two MTA proteins.

Metastasis represents one of the fundamental differences between benign and malignant tumors and poses a major obstacle in effective cancer treatment. The metastasis-associated gene 1 (MTA1) is closely associated with cancer metastasis. The rat mta1 gene was first identified using differential cDNA library screening techniques in a mammary adenocarcinoma metastatic system (1–3). The expression levels of MTA1 are elevated in human metastatic breast cell lines (2) and metastatic cancer tissues, such as breast, colorectal, gastric, and esophageal carcinomas (2, 4, 5). Overexpression of MTA1 correlates with enhancement of the ability of human breast cancer cells to invade and to grow in an anchorage-independent manner (6). In patients, colorectal and gastric carcinomas overexpressing MTA1 show deeper invasion and higher rates of penetration into lymph nodes (4). In culture, administration of antisense phosphorothioate oligonucleotides specific for MTA1 inhibits the rapid growth of human breast cancer cells that express high levels of MTA1 compared with normal breast epithelial cells (7).

Although evidence shows that MTA1 is closely linked to cancer metastasis, it was unclear how MTA1 is involved in the metastatic process. Genetic studies in Caenorhabditis elegans suggest that MTA1 may function in embryonic patterning, determination of cell polarity, and cell migration (8, 9). Recently, an MTA1 homologue, MTA2, was found to be part of an ATP-dependent chromatin-remodeling complex called NuRD (nucleosome remodeling histone deacetylation) (10). Interestingly, NuRD also has histone deacetylase activity (11). In eukaryotes, DNA is packaged into chromatin (12, 13). Formation of closed chromatin precludes the access of transcription factors to DNA and inevitably leads to transcriptional repression (14, 15). Dynamic changes in the chromatin structure, therefore, lead to either transcriptional activation or transcriptional repression. Alteration of the chromatin structure utilizing energy derived from ATP hydrolysis is called “chromatin remodeling” and is one of the two transcriptional regulatory mechanisms at the chromatin level (reviewed in Refs. 16 and 17). The other mechanism involves covalent modifications of nucleosomes, the basic units of chromatin. Nucleosomes are formed by DNA wrapping around a histone octamer, which composes of two copies of four histone proteins, H2A, H2B, H3, and H4 (12, 18, 19). The N termini of core histones are exposed and unstructured (20); these exposed tails are thought to contact neighboring core histones as well as DNA and are critical in mediating higher order structures of chromatin. As a result, covalent modifications of nucleosomes also lead to changes in the chromatin structure. One of the most well studied nucleosomal modification is acetylation and deacetylation of histone proteins at the unstructured N-terminal tails (reviewed in Ref. 16). The addition of acetyl groups to the N-terminal tails of histones is catalyzed by histone acetyltransferases and the hydrolysis of the acetylated histone tails by HDACs. Acetylation of histone proteins opens up the chromatin structure and leads to transcriptional activation (21). Conversely, deacetylation of histone proteins condenses the chromatin structure and

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is associated with transcriptional repression (reviewed in Ref. 22). The finding that MTA2 is found in a protein complex containing both ATP-dependent chromatin-remodeling activity and histone deacetylase activity suggests that the molecular mechanism of tumor metastasis might involve aberrant regulations of the chromatin structure.

Shortly after the discovery of the NuRD complex, MTA1 was found to be a component of another protein complex with histone deacetylase activity (23). Furthermore, evidence shows that MTA1 may repress estrogen receptor-mediated transcription (23). However, despite the high homology between MTA1 and MTA2, MTA2 has not been shown to involve in metastasis or cancer formation. The level of MTA2 remains constant while MTA1 protein level is up-regulated by heregulin-β1 in breast cancer cells (6). The findings that the homologous proteins MTA1 and MTA2 are both present in HDAC-containing complexes but only MTA1 has been concretely linked to cancer metastasis pose a conundrum.

To further understand the biological functions of MTA1 and MTA2, we used a variety of assays to probe the differences and similarities between the MTA proteins. We also found a third member of the human MTA family, MTA3. We found that both MTA1 and MTA2 mediate transcriptional repression, but MTA3 does not appear to repress transcription to a significant level. In addition, MTA1 and MTA2 form distinct protein complexes, both containing histone deacetylase activity. Our results suggest that although the highly homologous N-terminal regions of the MTA proteins are important in forming protein complexes with histone deacetylases, the divergent C termini are likely to be critical in modulating the histone deacetylase activity associated with the MTA complexes and possibly in the downstream biological events, including the metastatic process.

EXPERIMENTAL PROCEDURES

Plasmids—Gal4-MTA1 was expressed from pm1-MTA1, Gal4-MTA2 from pM2-MTA2, and Gal4-MTA3 from pM2-MTA3. pM1-MTA1, pM2-MTA2, and pM2-MTA3 were constructed by inserting the full-length MTA cDNAs in frame with Gal4 DNA-binding domain in pm1 (24) or pm2 (24). Gal4-MTA1* was made by restriction enzyme digestion and religation of pm1-MTA1. pcDNA3-MTA1, which contained full-length MTA1, was used to express MTA1 without the Gal4 DNA-binding domain. GFP fusion constructs of MTA1, MTA2, and MTA3 were generated by inserting the full-length MTA cDNAs in frame with and C-terminal to the GFP open reading frame in the pEGFP vector (Clontech). GFP-HDAC1, which expressed a N-terminal fusion of GFP to HDAC1, was made by inserting HDAC1 cDNA into pEGFP.

FLAG-tagged MTA1 (FLAG-MTA1) was expressed from pME18S-MTA1, which was generated by inserting the MTA1 cDNA into pME18S (25). FLAG-MTA1* was made by restriction enzyme digestion and religation of pME18S-MTA1. FLAG-MTA2 expression plasmid has been described (10). pGEMZ/SEX-MTA1 and pGEMZ/SEX-MTA2, which were generated in vitro translated MTA1 and MTA2 proteins, were made by subcloning full-length MTA1 and MTA2 into the pGEMZ/SEX vector (26).

Expression plasmids for the following proteins or promoter-reporters have been previously described: GST-p53 (27), GST-FKB2 (28), GST-FKB25 (29), GST-Y1 (28), GST-TKuc and TKloc (29), and HDAC1-Flag (25). GST itself was expressed from pGST (28).

Cell Culture, Transfection, and Luciferase Assay—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and penicillin/streptomycin, 1 × 106 HeLa cells were seeded to 60-mm diameter tissue culture dishes. Sixteen hours later, 0.5 μg of pRL-TK, 5 μg of the MTA construct (with or without Gal4 fusion), and 5 μg of either GST-TKuc or TKloc were transfected into HeLa cells using the calcium phosphate co-precipitation method (31). Forty-eight hours after transfection, cells were harvested and a luciferase assay performed using the dual luciferase assay system (Promega).

Fluorescence Microscopy—HeLa cells were seeded on chamber slides and grown for 18 h. 5 μg of expression plasmids for various GFP fusion proteins were transfected into cells using the calcium phosphate co-precipitation method (31). Forty-eight hours later, cells were washed with ice-cold PBS, fixed with 4% paraformaldehyde, rinsed with cold PBS again, and dried. One drop of anti-fade mounting medium with 4’,6-diamidino-2-phenylindole (Vector) was added to the cells before coverslips were applied. Fluorescence images were observed and analyzed under a fluorescence microscope.

Immunoprecipitation, Western Blot Analysis, and Histone Deacetylase Assay—Immunoprecipitation of FLAG-MTA1, FLAG-MTA1*, and FLAG-MTA2 was carried out using anti-FLAG M2 affinity gel (Sigma) following the manufacturer’s suggestions. Western blot analyses were performed using standard protocols (32).

Histone deacetylase assay was performed using immunoprecipitated MTA proteins or the eluted fractions of the FLAG fusion protein complexes as the source of the enzyme and a labeled peptide corresponding to residues 2–24 of histone H4 as substrate following a previously published procedure except that incubation was performed at room temperature overnight (33). Trichostatin A (400 nm final concentration) (Sigma) was added to the FLAG-MTA1 immunoprecipitate 30 min prior to the addition of the H4 substrate peptide in one of the reactions.

Protein Complex Purification—Anti-FLAG immunoprecipitation columns were prepared using anti-FLAG M2 affinity gel (Sigma) following the manufacturer’s suggestions. Approximately for every 6 × 109 HeLa cells, 5 μg of the plasmids expressing FLAG fusion proteins were transfected using the calcium phosphate co-precipitation method (31). Forty-eight hours after transfection, cells were harvested by scraping. Cells were subsequently lysed by adding PBS plus 0.1% Nonidet P-40 and then briefly sonicating. Cell lysate obtained from about 4.8 × 1010 cells was applied to an equilibrated FLAG column of 1 ml bed volume to allow for adsorption of the protein complex to the column resin. After binding, the column was washed with cold PBS plus 0.1% Nonidet P-40. FLAG peptide (Sigma) was applied to the column as described by the manufacturer to elute the FLAG protein complex. Fractions of 1 bed volume were collected.

For the purification of the MTA1-HDAC complex, anti-HDAC2 antibody (25) was used to pack an immunoprecipitation column following a previously published method (34). Eluted FLAG-MTA1 complex was added to the anti-HDAC2 column and allowed for adsorption. After extensive washing, the MTA1-HDAC complex was eluted with excess HDAC2 peptide that contained the epitope of the anti-HDAC2 antibody (25).

In Vitro Protein-Protein Interaction Assay—35S-Labeled MTA1 was generated from pGEMZ/SEX-MTA1 using T7 RNA polymerase and the TNT Reticulocyte Lysate System (Promega). 35S-labeled MTA2 was generated using the same method from pGEMZ/SEX-MTA2. GST, GST-p53, GST-FKB25, GST-FKB2 and GST-Y1 were expressed in Escherichia coli strain DH5α and captured onto glutathione-agarose beads (Sigma). In vitro translated MTA1 or MTA2 (5 μl) was mixed with 35S-labeled MTA1 or MTA2 (0.1 μg) in the presence of PBS plus 0.2% Nonidet P-40 at 4 °C for 1 h. Beads were washed extensively in PBS plus 0.2% Nonidet P-40. Bound proteins were eluted by boiling in Laemmli sample buffer, separated by SDS-PAGE, and detected by Coomassie Blue staining and autoradiography.

RESULTS

MTA1 and MTA2 Are Highly Homologous in the N Terminus but Divergent in the C Terminus—To further understand the potential functions of MTA1 and MTA2 and to probe the functional differences between them, we began by analyzing the primary structure of the MTA proteins. Human MTA2 is 65% identical to human MTA1, with the highest homology concentrated in the N-terminal half of the proteins. The C-terminal half of the MTA proteins is divergent; data base searches (EMBL) show that in MTA1 there are a GATA zinc finger domain, a bipartite nuclear localization signal embedded in a myb DNA-binding domain, and an Sre homology 3 binding domain (Fig. 1A). MTA2 also contains a GATA zinc finger domain and a nuclear localization signal, but there is no C-terminal myb domain or Sre homology 3 binding domain in MTA2. It has not been shown that MTA1 possesses DNA binding activity; therefore, the consequence of the additional myb DNA-binding domain in MTA1 is unclear. However, the functional differences between MTA1 and MTA2, if any, might relate to the differences in the C-terminal parts of the proteins.
Contrary to the C-terminal half, the N-terminal half of the MTA proteins is highly similar and can be organized into four functional domains. At the extreme N terminus is a BAH (bromo-adjacent homology) domain, followed by an ELM2 (Egl-27 and MTA1 homology 2) domain (InterPro, European Bioinformatics Institute). Next is a conserved leucine zipper. C-terminal to the leucine zipper is a SANT domain related to the myb DNA-binding domain. The BAH domain has been identified in a number of transcriptional regulators including DNA cytosine-5 methyltransferase (35, 36) and the Orc1 (origin recognition complex 1) protein (36). It has been suggested that the BAH domain may serve as a protein-protein interaction module linking DNA methylation, replication, and transcriptional regulation together (36, 37). The ELM2 domain was named after the C. elegans homologue of MTA1, Egl-27, and MTA1. Although many unidentified proteins in C. elegans, Drosophila, Xenopus laevis, Arabidopsis thaliana, and humans also contain the unique ELM2 domain (Fig. 1B), it is unclear what function this domain might have. However, CoREST, a specific co-repressor for REST/NRSF (RE1 silencing transcription factor/neural represser factor) required for the regulation of neuron-specific gene expression (38), also contains the ELM2 domain (Fig. 1B).

Interestingly, CoREST shares another domain, the SANT (SWI3/ADA2/NCoR/TFIIB) domain, with MTA1 and MTA2. SANT domains are present in a variety of transcriptional regulators, including SWI3 of the yeast SWI/SNF transcriptional activation complex; ADA2 of the ADA activation complex; and NCoR and SMRT, two co-repressors mediating inducible repression by steroid hormone receptors (reviewed in Ref. 39). It has been suggested that SANT domains are important in protein complex assembly in a recent study documenting the purification of stable protein complexes containing HDAC1 and HDAC2 (40, 41). The juxtaposition of the SANT domain and the leucine zipper in the MTA proteins suggests the presence of a functional module in the formation of protein complexes.

In summary, domain structures of MTA1 and MTA2 suggest that the MTA proteins are transcriptional regulators capable of forming protein complexes. The unique C-terminal regions of MTA1 and MTA2 might confer additional regulatory roles to the differences in their biological functions.

Human MTA3—Through homology search, we identified a human cDNA clone that is highly homologous to MTA1 and MTA2 (clone KIAA1266; Kazuda DNA Research Institute). This clone was confirmed by dideoxyribonucleotide sequencing to contain a full-length open reading frame, and we named this protein human MTA3 (Fig. 1). Human MTA3 appeared to share the highest homology with MTA1 and MTA2 in the N terminus (Fig. 1C). It is likely that MTA1, MTA2, and MTA3 represent members of the same gene family, and they might possess distinct or overlapping functions.
constructed to test whether they could repress transcription from a promoter containing Gal4-binding sites (Fig. 2A). Gal4-MTA1, Gal4-MTA2, or Gal4-MTA3 was co-transfected into HeLa cells with a reporter plasmid, G5TKLuc, which contained five Gal4-binding sites within the thymidine kinase promoter driving the luciferase reporter gene. As shown in Fig. 2B, whereas Gal4-MTA1 repressed the target promoter more than 10-fold (compare lane 4 with lane 3), it had no significant effect on an identical promoter with no Gal4-binding site (lanes 1 and 2). Similarly, Gal4-MTA2 also repressed transcription from the promoter containing Gal4-binding sites (lane 5). Furthermore, overexpression of MTA1 alone did not repress the thymidine kinase promoter with the Gal4-binding sites, strongly suggesting that the repression is specifically from Gal4-MTA1 (lane 8). Moreover, a carboxyl-terminally truncated form of MTA1 (Gal4-MTA1*, containing amino acids 1–387; lane 6) lost most of the transcriptional repression activity. Most significantly, the transcriptional repression activity of MTA3 appeared to be significantly lower than that of MTA1 or MTA2 (lane 7). These results demonstrate that MTA1 and MTA2 are able to repress transcription when recruited to a target promoter. More importantly, the reduced level of transcriptional repression activity observed for MTA3 and a C-terminally truncated form of MTA1 suggests that the C terminus of the MTA proteins has regulatory roles pertaining to the biological functions of the MTA family.

MTA1 and MTA2, but Not MTA3, Are Located in the Nucleus—Transcriptional regulation takes place in the nucleus. To confirm the functional significance of the MTA proteins with respect to transcriptional repression, the subcellular localization of the MTA proteins was analyzed by direct fluorescence. GFP-MTA1, GFP-MTA2, and GFP-MTA3 fusion constructs were transfected into HeLa cells. After 48 h, transfected cells were fixed, and the localization of fluorescence was observed under a fluorescence microscope. As shown in Fig. 3, GFP-MTA1 and GFP-MTA2 were clearly localized to the nucleus compared with 4',6'-diamino-2-phenylindole (DAPI) counterstaining for DNA. GFP was nonspecifically localized to both the nucleus and the cytoplasm, and GFP-HDAC1 was localized to the nucleus as previously reported (29). GFP-MTA3, however, appeared to have a diffused distribution and failed to localize to a specific subcellular locale (Fig. 3c). This result, together with the finding that MTA3 lacked significant repression activity, suggests that the major function of MTA3 may not involve transcriptional regulation.

MTA1 and MTA2 Contain Histone Deacetylase Activity—Because both MTA1 and MTA2 have been found in protein complexes containing histone deacetylase activities, it is very possible that MTA1 and MTA2 themselves are tightly associated with histone deacetylase activity. To test this hypothesis, a construct that can express FLAG-tagged MTA1 protein was made and transfected into HeLa cells. After transfected cells were harvested, immunoprecipitation experiments were carried out using anti-FLAG antibody on the cell extracts. Precipitated MTA1 was subsequently examined for the histone deacetylase activity with a 3H-labeled H4 peptide as substrate. As shown in Fig. 4A, MTA1 indeed contained a significant level of histone deacetylase activity, but the cells transfected with FLAG vector alone did not. Moreover, MTA1* (containing amino acids 1–387), which lost much of the transcriptional repression activity compared with full-length MTA1 (Fig. 2B), retained only minimal enzyme activity, which further demonstrated that MTA1 indeed specifically contained histone deacetylase activity. Similarly, MTA2 also contained histone deacetylase activity. To avoid the possibility that the FLAG-tagged MTA1 mutant protein was not well expressed, the expression level of each construct was confirmed by Western blot analysis with the anti-FLAG antibody (Fig. 4B). The result from these experiments shows that MTA1 and MTA2 contain associated histone deacetylase activity, and this activity is likely to be instrumental to the transcriptional repression property of these proteins.

MTA1 Forms a Complex Distinct from the MTA2 Complex—We have established that both MTA1 and MTA2 possess transcriptional repression activity, are localized to the nucleus, and contain histone deacetylase activity. To further understand the functional similarities and differences between MTA1 and MTA2, we attempted to isolate MTA1- and MTA2-specific protein complexes by immunopurification.

To purify the MTA1 complex from HeLa cells, an immunopurification column with anti-FLAG antibody was used. The FLAG-MTA1 construct described above was transiently expressed in HeLa cells. Cell extract was obtained and adsorbed on the anti-FLAG column. After extensive washing, bound MTA1 complex was eluted with excess FLAG peptide. Each fraction collected was assayed for its histone deacetylase activity and analyzed by Western blot analysis with the anti-FLAG antibody as shown in Fig. 5A. The amount of MTA1 in each fraction correlates with the histone deacetylase activity. A control experiment with the same amount of extract from HeLa cells transfected with the FLAG vector alone was performed in parallel. As shown in Fig. 5A, no significant level of histone deacetylase activity was observed for the FLAG-alone control. Meanwhile, FLAG-MTA2 and FLAG-HDAC1 complexes were purified with the same method and shown to contain histone deacetylase activity. The peak fraction from each complex, which corresponded to the second elution fraction, was ana-
analyzed by SDS-PAGE, and proteins in the complexes were subsequently visualized by silver staining (Fig. 5B). Most of the proteins appeared to be specific, with a few also present in the FLAG control column and marked as nonspecific. Detailed comparison among the protein complexes revealed that the FLAG-MTA2 complex was remarkably similar to the FLAG-HDAC1 complex. Interestingly, the FLAG-MTA1 complex was distinct from the FLAG-MTA2 complex; several bands around 40–55 kDa were abundant and specific for the MTA1 complex.

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(Fig. 3. Nuclear localization of MTA1 and MTA2. HeLa cells were transfected with GFP-MTA1, GFP-MTA2, GFP-MTA3, or GFP-HDAC1 expression constructs and fixed. Fluorescence microscopy revealed nuclear localization of GFP-MTA1 and GFP-MTA2 similar to that of GFP-HDAC1. GFP-MTA3, on the contrary, had a nonspecific localization pattern similar to that of GFP. DAPI, 4′,6′-diamidino-2-phenylindole.

Fig. 4. MTA1 and MTA2 contain associated HDAC activity. A, FLAG-tagged MTA1, MTA1* (containing amino acids 1–387), and MTA2 were transfected into HeLa cells, immunoprecipitated using anti-FLAG antibody, and assayed for histone deacetylase activity using a labeled H4 peptide. Where indicated, trichostatin A was added to a final concentration of 400 nM prior to the addition of the peptide substrate. B, expression of FLAG-tagged MTA1, MTA1*, and MTA2. Overexpressed FLAG-tagged MTA constructs were immunoprecipitated from HeLa cells, separated by SDS-PAGE, and detected by Western blotting using anti-FLAG antibody.)

The MTA1 Complex Contains HDAC1/2 and RbAp46/48 but Not Sin3 and Mi-2—It has been established that HDAC1/2 and RbAp46/48 form a core complex and exist in most of the histone deacetylase complexes examined (10). To test whether the MTA complexes contain these core factors, we performed Western blot analysis using specific antibodies. As shown in Fig. 6, the HDAC1, MTA2, and MTA1 complexes all contain HDAC1/2 and RbAp46/48. To further examine the difference in the composition between the MTA complexes, we tested for the presence of MBD3 and Mi-2, which were found in the NuRD complex containing MTA2 (10), as well as Sin3, which is found only in the HDAC1 and Sin3 complexes examined to date. As shown in Fig. 6, Sin3 was found in the HDAC1 complex, showing that our immunoaffinity purification result agreed with previous reports. MBD3 was present in both MTA complexes. Moreover, the MTA1 complex did not contain Mi-2. These findings show that the MTA1 complex is indeed different from the MTA2 complex although they share some common factors, such as HDAC1/2, RbAp46/48, and MBD3.
Taken together, our results from protein complex purification strongly suggest that although both MTA1 and MTA2 are involved in histone deacetylation and transcriptional repression, their molecular mechanisms are likely to be different. More significantly, whereas both MTA1 and MTA2 form complexes with the core factors HDAC1/2 and RbAp46/48, their distinct sets of associated proteins might reflect potential differences in their cellular functions, especially with respect to cancer metastasis (see "Discussion").

Purification of the MTA1-HDAC Complex—To confirm the close interaction between MTA1 and the core histone deacetylase factors, the FLAG-MTA1 complex was further purified through an anti-HDAC2 immunoaffinity column. As shown in Fig. 7, MTA1 appeared to tightly associate with the core factors HDAC1/2 and RbAp46/48, as well as with MBD3a/b, and two polypeptides p66 and p68. We called the resulting complex from the sequential immunoaffinity column purification the MTA1-HDAC complex. Although the identity and the functions of p66 and p68 are unknown at this point, the tight association between MTA1 and the core histone deacetylase factors suggests that the cellular functions of MTA1 are intimately linked to histone deacetylation. Moreover, the "MTA-associated proteins" in Fig. 5B were proteins lost upon the second purification step specific for the HDAC core components. These proteins present in the FLAG-MTA1 complex but not in the MTA1-HDAC complex might be involved in the modulation of the histone deacetylation activity of MTA1 or other cellular events.

MTA1, Unlike MTA2, Does Not Interact with YY1 or FKBP25—Although the primary structures of MTA1 and MTA2 are very similar, our protein complex analysis showed that they associated with distinct sets of proteins to form different protein complexes. To pursue the idea that MTA1 and MTA2 might associate with other proteins whose interactions with the MTA proteins are not stable enough to form protein complexes, we performed in vitro binding assays between the MTA proteins and other candidate proteins. MTA1 and MTA2 were expressed by coupled in vitro transcription and translation reactions in the presence of 35S-labeled methionine. Candidate binding proteins p53 and YY1 were expressed in E. coli as GST fusion proteins. The tumor suppressor p53 induces anti-proliferative responses when cells are challenged with stress such as DNA damage (reviewed in Refs. 42 and 43). Recent evidence shows that acetylation and deacetylation of p53, in addition to phosphorylation, are important in p53-dependent growth arrest and apoptosis (reviewed in Ref. 44). YY1 is a transcription factor possessing either activation or repression activity and is absolutely required for development (reviewed in Ref. 45). It has been proposed that YY1 may serve as a specific factor to recruit HDAC1/2 to promoters, and the function of YY1 has been shown to be regulated by acetylation and deacetylation (26). Binding experiments were performed by mixing the labeled MTA proteins with the candidate binding proteins fused to GST. After extensive washing, the GST fusion proteins were retained on glutathione beads together with any bound, labeled proteins. Fig. 8 shows that p53 interacts with both MTA1 and MTA2, confirming a previous report of the interaction between p53 and MTA2 (46) and demonstrating a novel interaction relationship between p53 and MTA1. Surprisingly, YY1 only interacted with MTA2 and not with MTA1. Furthermore, FKBP25, an immunophilin with associated histone deacetylase activity (29), also bound MTA2 but not MTA1. GST alone and GST protein fused to FKBP12, which is an
MTA1, MTA2, and MTA3 are homologous proteins. MTA1 has been linked to tumor metastasis, but experimental data suggest that MTA2 may not be associated with metastasis. From studying the functional domains of the MTA proteins, we realized that the functions of MTA1 and MTA2 are likely to involve histone deacetylation and the formation of protein complexes. The divergent C-terminal regions of the MTA proteins suggest that MTA1 and MTA2 are regulated differently. Indeed, we showed that both MTA1 and MTA2 repress transcription, are located in the nucleus, contain associated histone deacetylase activity, and form multiprotein complexes. However, MTA3 does not appear to repress transcription to a significant level despite sharing a high degree of homology in the N terminus. Interestingly, the MTA1 and MTA2 complexes both exert histone deacetylase activity but are different in composition. Whereas the MTA2 complex is similar to the HDAC1 complex, the MTA1 complex contains specific proteins for the MTA1 complex that are absent in the MTA2 complex. In addition to forming stable complexes, MTA1 and MTA2 are able to make transient association with different transcription factors, reinforcing the idea that MTA1 and MTA2 have different biological functions.

The MTA Model and Cancer Metastasis—Metastasis is a complex series of events. It is believed that for metastasis to occur, cells of the primary tumor must detach, penetrate into the blood stream or the lymphatics, adhere and arrest at the endothelial cells, extravasate and form new blood vessels, and evade host anti-tumor immune response (47, 48). Interestingly, metastasis may not be a late event during the progression of cancer. Emerging evidence shows that many solid tumors, including colorectal, gastric, epithelial, and breast carcinomas, are able to metastasize at an early stage of cancer development, and the bone marrow is the most important site for the detection of micrometastasis (49–51). This finding, contrary to the perception that only late stage tumors form metastasis, suggests that metastasis is an early event where basic biology of the cell has been perturbed. Previously, detailed characterization of MTA2 revealed that MTA2 is involved in chromatin remodeling and histone deacetylation, two key regulatory processes in gene transcription. We now have established that MTA1 also forms a complex with histone deacetylase activity, possibly through the formation of the “core” MTA1-HDAC complex containing HDAC1/2, RbAp48/46, and MBD3. We speculate that these two MTA complexes have different biological functions; in normal cells, MTA2 exists in a complex containing core histone deacetylase factors and Mi-2. This complex is responsible for transcriptional regulation and chromatin remodeling important for maintaining homeostasis of the cell. Targeting of the MTA2 complex may occur through gene-specific DNA-binding repressors such as YY1 or through interactions between mCpG-binding proteins such as MBD2. In this cellular context, the expression level of MTA1 is low, and MTA1 has no effect on the regulatory role of the MTA2 complex. However, in metastatic tumor cells, the expression level of MTA1 is highly elevated, and MTA1 may interfere with the normal functions of the MTA2 complex by forming a specific MTA1 complex and perhaps by interacting with proteins that are also present in the MTA2 protein complex as well. More detailed studies are necessary to prove this hypothesis.

To date, the events downstream of MTA1 overexpression remain poorly understood. Because we have established that the molecular mechanism of the MTA1 complex most likely relies on transcriptional regulation, it is possible that many events of the metastatic process are regulated by MTA1. These events might include decreased cell-cell adhesion, which de-
GST-YY1 and GST-FKBP25 were able to bind in vitro translated MTA2. In vitro translated MTA1 was not able to bind GST-YY1 or GST-FKBP25. Input, one-tenth of the amount of MTA1 and MTA2 used in each binding reaction. Reaction mixtures were separated by SDS-PAGE, and the gels were stained with Coomassie Blue prior to exposure to film to confirm that equal amounts of GST fusion proteins were used in the binding reactions.

**Fig. 8.** p53 interacts with both MTA1 and MTA2, whereas YY1 and FKBP25 interact with only MTA2. A, in vitro translated MTA1 and MTA2 were captured by immobilized GST-p53. B, GST-YY1 and GST-FKBP25 were able to bind in vitro translated MTA2. In vitro translated MTA1 was not able to bind GST-YY1 or GST-FKBP25. Input, one-tenth of the amount of MTA1 and MTA2 used in each binding reaction. Reaction mixtures were separated by SDS-PAGE, and the gels were stained with Coomassie Blue prior to exposure to film to confirm that equal amounts of GST fusion proteins were used in the binding reactions.

HDACs and Cancer—Histone modifying proteins histone acetyltransferases and HDACs affect transcriptional regulation at both transient and sustained states. Therefore, it is not surprising that many human diseases have been found to be associated with mutations or alterations in function of histone acetyltransferases or HDACs, such as hematopoietic disorders (54), Rubinstein Taybi syndrome (55), and some types of colorectal and gastric carcinomas (56, 57). Specifically, aberrant recruitment of HDACs by chromosomal translocations causes acute promyelocytic leukemia and acute myelogenous leukemia (58–60). Therapeutic regimens utilizing HDAC inhibitors have been used in treating these acute leukemias to revert the blockage of differentiation of immature hematopoietic cells that causes these malignancies (61). In addition, HDAC inhibitors are able to stop and revert transformation of some tumor cells (62–67), and clinical trials are under way to evaluate the potential of using HDAC inhibitors to treat cancer patients with solid tumors (68, 69). Although the details of how malfunctions of HDACs subvert the normal transcriptional balance of the cell are currently unclear, our data link MTA1-associated tumor metastasis to HDACs and suggest a potential option using HDAC inhibitors to treat metastasis.

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