von Hippel-Lindau Partner Jade-1 Is a Transcriptional Co-activator Associated with Histone Acetyltransferase Activity*

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Jade-1 was identified as a protein partner of the von Hippel-Lindau tumor suppressor pVHL. The interaction of Jade-1 and pVHL correlates with renal cancer risk. We have investigated the molecular function of Jade-1. Jade-1 has two zinc finger motifs called plant homeodomains (PHD). A line of evidence suggests that the PHD finger functions in chromatin remodeling and protein-protein interactions. We determined the cellular localization of Jade-1 and examined whether Jade-1 might have transcriptional and histone acetyltransferase (HAT) functions. Biochemical cell fractionation studies as well as confocal images of cells immunostained with a specific Jade-1 antibody revealed that endogenous Jade-1 is localized predominantly in the cell nucleus. Tethering of Gal4-Jade-1 fusion protein to Gal4-responsive promoters in co-transfection experiments activated transcription 5-6-fold, indicating that Jade-1 is a possible transcriptional activator. It was remarkable that overexpression of Jade-1 in cultured cells specifically increased levels of endogenous acetylated histone H4, but not histone H3, strongly suggesting that Jade-1 associates with HAT activity specific for histone H4. Deletion of the two PHD fingers completely abolished Jade-1 transcriptional and HAT activities, indicating that these domains are indispensable for Jade-1 nuclear functions. In addition, we demonstrated that TIP60, a known HAT with histone H4/H2A specificity, physically associates with Jade-1 and is able to augment Jade-1 HAT function in live cells, strongly suggesting that TIP60 might mediate Jade-1 HAT activity. Thus, Jade-1 is a novel candidate transcriptional co-activator associated with HAT activity and may play a key role in the pathogenesis of renal cancer and von Hippel-Lindau disease.

The von Hippel-Lindau (VHL) protein is the major renal cancer gene in adults. Jade-1 is a novel protein that binds and is stabilized by 30-kDa VHL (1). Jade-1 is highly expressed in kidney and renal proximal tubule cells and may be involved in renal tubular epithelial cell differentiation, growth suppression, and apoptosis (1). Moreover, naturally occurring truncations and mutations of VHL altered its interaction with Jade-1, suggesting a correlation with renal cancer (2). Jade-1 has a strong possible PEST degradation domain (aa 5–28) and numerous possible sites for post-translational modification (1). The 509 amino acid Jade-1 protein contains one canonical plant homeodomain (PHD) finger (aa 203–253) followed by a noncanonical extended PHD (aa 257–371), both of which are zinc-binding motifs. Mutational analysis studies demonstrated that both Jade-1 PHD zinc fingers were required for optimal interaction with wild-type 30-kDa VHL. Jade-1 has also been recently identified as a gene involved in anteroposterior axis development during mouse embryogenesis (3).

More than 300 gene products have been identified so far that contain one or more PHD-type zinc fingers (4–6). Most characterized PHD proteins are found in the nucleus. PHD fingers are protein domains built around two zinc ions coordinated by cysteine residues and a histidine in a Cys$_2$His$_2$z motif. The properties of PHD zinc fingers make them good candidates for intracellular protein scaffolds because they are small, stable, and very diverse in sequence (7, 8). There are several suggested functions for PHD fingers. A line of evidence strongly suggests that the PHD finger functions in chromatin remodeling. Thus, PHD motifs are found in transcriptional co-regulators and proteins of chromatin-modifying complexes, such as p300, CBP (6, 9), ING1 (10), ING3, and TIF1 and Mi-2 family members (4, 6). The extended PHD motif is found in several leukemia-associated proteins, such as AF10, AF17, and the mixed lineage leukemia protein MLL (11). The tandem of a canonical PHD followed by an extended PHD motif characteristic to Jade-1 is found in the closely related E9 (12), AF10, AF17, and BR140 (11). Several reports have provided evidence that PHD fingers may be protein-protein interaction domains. Thus, an extended PHD finger is responsible for oligomerization of the AF10 protein (13). It has recently been reported that the PHD finger of p300 binds isolated nucleosomes in vitro (4). In contrast to the bromodomain, which specifically binds acetylated nucleosomal histones, the PHD finger interacts with nucleosomal histones in an acetylation-independent manner. The PHD finger was also found to be an integral part of the CBP minimal acetyl transferase domain, which represents another function for the PHD finger (9, 14). The PHD fingers of ING2 protein may function as phosphoinositide receptors (15). The PHD fingers in AIRE play a role in subnucleosome targeting of this protein (16). PHD-type zinc fingers are targets of chromosomal translocations and mutations in several diseases, such as acute leukemias (MLL, AF10, AF17), α-thalassemia (ATRX), and autoimmune disease (AIRE-1) (17–19).

The correlation between the acetylation state of histones within chromatin and transcriptional regulation was proposed...
decades ago (20–22). However, only over the last several years have proteins been identified that mediate histone acetylation (23, 24). HAT enzymes acetylate ε-amino groups of specific lysine residues on N-terminal tails of histone proteins that package DNA into chromatin. This packaging is mediated by nucleosome core particles, containing two copies of positively charged histones H2A, H2B, H3, and H4. HATs are recruited to specific promoters by DNA-bound transcriptional activators near specific histones. According to the histone code hypothesis, histone tails acetylated by these HATs serve as docking recognition sites for the binding of transcriptional cofactors and subsequent activation of general transcription (25, 26). Most HATs are remarkably nonrandom and especially in vivo acetylate only certain lysine residues within specific histone tails. An important characteristic of all HATs is that they bind and functionally cooperate with other transcriptional regulators and HATs by assembling into multisubunit complexes, such as SAGA, ADA, PCAF, TIP60, NuA3, and NuA4 (reviewed in Ref. 24).

The most studied role of histone acetyltransferases involves targeted chromatin transcription. However, it has been suggested that global acetylation of histones, specifically histone H4, is required in other types of DNA metabolism, including DNA repair, replication and recombination (27–29). In support of this notion, a novel role for HAT activity has been demonstrated for a member of the MYST family, the known transcriptional regulator and histone acetyltransferase TIP60 (HIV-1 Tat-interactive protein) (27). The first hint suggesting a novel function resulted from purification and identification of proteins composing the TIP60 complex. The TIP60 complex consists of at least 14 distinct subunits, three of which are homologs of known proteins involved in DNA remodeling. Indeed, the purified TIP60 complex possesses ATPase, DNA helicase, and structural DNA binding activities. Most importantly, the ectopic expression of mutated TIP60 lacking HAT activity causes defects in the ability of the cell to repair DNA and to trigger DNA damage-induced apoptosis. It has been suggested that, depending on the level of damage, TIP60, like p53, will initiate DNA repair or an apoptotic response. The mechanism of this dual-role stress response by TIP60 is unclear, but it is hypothesized that the TIP60 complex may interact with cell-cycle checkpoint proteins to activate an apoptotic pathway in response to DNA lesions (27). It is interesting that the TIP60 complex and its yeast homologue Ess1 have unique substrate specificity and, in vivo, specifically acetylate histone H4 and H2A (30).

In this study, we set out to determine the molecular function of Jade-1. Because PHDs are associated with nucleosomal histones and are found in some HATs, we examined the hypothesis that Jade-1 might be a transcription factor associated with HAT activities. We determined Jade-1 nuclear association by Western blotting (lanes 1 and 3) or untagged Jade-1 expression vector (lanes 2 and 4). Cells were extracted with radioimmunoprecipitation assay buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS). Cells lysates (50 μg/lane) were separated by SDS-PAGE and transferred onto nitrocellulose. Single lanes were cut out and probed with either Jade-1 whole serum (lanes 1 and 2) or purified Jade-1 antibody (lanes 3 and 4). Note that three minor bands appearing in Jade-1 overexpressing cells are detected by Jade-1 whole serum and by purified Jade-1 antibody (lanes 2 and 4), bands are indicated by horizontal marks) and are probably related to partial degradation of overexpressed Jade-1. It is noteworthy that the whole Jade-1 serum and Jade-1 purified antibody recognize both endogenous Jade-1 (lanes 1 and 3) and recombinant Jade-1 (lanes 2 and 4), indicating antibody specificity. WB, Western blotting. C, immunohistochemistry of Jade-1 with purified antibody. HeLa cells were processed for conventional fluorescence or scanning confocal microscopy analysis (c and d, magnification 400×; 0.2-μm slice in z-dimension; image shown is taken through the cell nuclei), as described under “Experimental Procedures.” b and d, enlarged selected fields of images a and c, respectively.

BD Pharmingen and Sigma, respectively. Hemagglutinin (HA) monoclonal antibody was from Santa Cruz Biotechnology. Acetylated histones acetyl-H4 and acetyl-H3 polyclonal IgG were from Upstate Biotechnology. Goat anti-mouse and anti-rabbit IgG-HRP conjugate were from Bio-Rad. Protein A/G agarose was from Santa Cruz Biotechnology.

Experimental Procedures

Cell Lines and Transfection—293T17 human embryonic kidney cells and HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, glutamine, and penicillin-streptomycin (Invitrogen). Subconfluent cells grown in 35-, 60-, or 100-mm dishes were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

Antibodies—Jade-1 antiserum has been described previously (1). Human VHL monoclonal and FLAG M5 monoclonal antibodies were from man VHL monoclonal and FLAG M5 monoclonal antibodies were from Bio-Rad. Protein A/G agarose was from Santa Cruz Biotechnology. Goat anti-mouse and anti-rabbit IgG-HRP conjugate were from Bio-Rad. Protein A/G agarose was from Santa Cruz Biotechnology. Goat anti-mouse and anti-rabbit IgG-HRP conjugate were from Bio-Rad. Protein A/G agarose was from Santa Cruz Biotechnology.
amino acids 312–371. FLAG-Jade-1ΔN has a C-terminal deletion of amino acids 418–509. The FLAG-Jade-1ΔN and FLAG-Jade-1ΔC were PCR-amplified from HA-Jade-1ΔN and HA-Jade-1ΔC templates and inserted using HindIII/BglII and BglII/XbaI restriction sites, respectively. FLAG-Jade-1ΔC was PCR-amplified using full-length FLAG-Jade-1 as a template and inserted into pFLAG-CMV2 plasmid using HindIII/XbaI restriction sites. HA-Jade-1 and HA-Jade-1ΔN have been described previously (1).

Reporter Gene Assay—Cells seeded in 60-mm dishes were co-transfected with the indicated amount of either SV40-promoter driven Gal4 DNA binding domain or SV40-promoter driven Gal4-Jade-1ΔN or Gal4-Jade-1ΔC and 2 μg of CAT-reporter plasmid (E1B, SV40, E4, and AdML, described above). After 36 h of transfection, cells were washed in phosphate-buffered saline (PBS), re-suspended in 150 μl of 0.25 mM Tris, pH 8.0, and lysed by freezing and thawing three times (liquid nitrogen/37 °C). Supernatants were clarified by centrifugation (5 min; 12,000 × g). CAT assays were performed as described previously (31, 32), with modifications. In brief, 30 μl of cell lysates were incubated for 1 h at 37 °C in 180 μl of reaction mixture, containing 125 μl of 0.25 mM Tris, pH 8.0, 5 μl of [14C]chloramphenicol (CAT assay grade; Amersham Biosciences) and 20 μl of 4.0 mM acetyl-CoA (American Bioanalytical). Acetylated products of the CAT reaction were excised from thin layer chromatography plates and quantitated on a scintillation counter. Data presented in bar graphs are means of at least three experiments ± S.E.

Endogenous Core Histone Extraction—Cell layers grown in 60-mm dishes were washed with PBS and lysed for 5 min in 0.5 ml of 10 mM Tris buffer, pH 8.0, containing 0.6% Nonidet P-40, 150 mM NaCl, and 1 mM EDTA, supplemented with protease inhibitor mixture (Roche Diagnostics). The nuclear fraction was isolated by centrifuging lysates at 1200 × g for 5 min. Total histones were extracted by suspending nuclei pellets in 100 μl of 0.4 N HSO4 and incubating on ice for 20 min. Histone extracts were cleared by centrifugation (13,000 × g for 10 min) and precipitated by addition of 0.5 ml of ice cold 20% trichloroacetic acid after centrifugation (13,000 × g for 10 min). Precipitated histones were washed twice with acetone and solubilized in 1× SDS sample buffer.

Immunoprecipitation and Histone Acetyltransferase Assay—All procedures were done at 4 °C, unless otherwise noted. Immunoprecipitation and HAT assays were done as described previously (27, 33) with some modifications. Cultured cells grown in 60-mm dishes were lysed in 50 mM Tris, pH 7.8, 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, and 5 mM MgCl2, and protease inhibitor mixture. Lysates were cleared by centrifugation at 12,000 × g for 10 min. Relevant antibodies were added to 1 ml of lysates and incubated overnight. Protein A-agarose/protein G-agarose (1:1 mix, 15 μl total; Santa Cruz Biotechnology) was added and the mixture rotated slowly for 4 h. The immune complexes were pelleted by brief centrifugation and washed three times with 1 ml of lysis buffer. The immune complexes were mixed with 30 μl of HAT reaction mix containing 50 mM Tris pH 7.8, 10% glycerol, 2 mM MgCl2, 0.5 mM EDTA, 15 μM trichostatin A, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.8 mg/ml calf thymus core histones. The HAT reaction was initiated by addition of 1 μl of [3H]Acetyl CoA (7.4 GBq/mmol; PerkinElmer Life and Analytical Sciences). After 30 min of incubation, the reaction was stopped by addition of SDS-sample buffer. One-tenth aliquot of each immunoprecipitation sample was frozen at −20 °C and later used to analyze proteins in immunocomplexes by SDS-PAGE/Western blot with corresponding antibodies. The rest of these samples were used to assess incorporation of [3H]Acetyl-CoA into core histones. Samples were separated on 15% SDS-PAGE, and core histones were visualized by staining with Coomassie Blue. Bands were excised with a razor blade, and histones were extracted from the polyacrylamide gels by incubating in Solvable reagent (PerkinElmer Life and Analytical Sciences) according to the manufacturer instructions. Ultima Gold scintillation mixture was added to the samples, and radioactivity was quantitated with a liquid scintillation analyzer (all from PerkinElmer Life and Analytical Sciences). Data presented in bar graphs are means of at least three experiments ± S.E.

Purification of Monospecific Jade-1 Polyclonal Antibody—A highly specific fraction of Jade-1 polyclonal antibody was affinity-purified from Jade-1 whole rabbit antisemera using the 75-kDa FLAG-Jade-1 antigen and a scaled-up Western blotting approach. FLAG-Jade-1 includes additional 5′ untranslated Jade-1 sequence that was present in the original library clone, increasing its mass to 75 kDa. This size difference was helpful in determining the specificity of the purified Jade-1 antibody (see below andFig. 1B). 293T cells grown in 100-mm dishes were transiently transfected with the FLAG-Jade-1 expression vector. Cells were extracted sequentially with 900 μl of 50, 150, and 420 mM NaCl in ice-cold solution containing 50 mM HEPES, pH 7.0, 5 mM MgCl2, 1 mM EDTA, and 0.2% Triton X-100. The 420 mM fraction of nuclear proteins enriched with FLAG-Jade-1 protein was separated by large scale preparative SDS-PAGE, transferred onto nitrocellulose membrane, and the FLAG-Jade-1 position was localized by Western blot. The membrane region corresponding to 75-kDa FLAG-Jade-1 antigen was cut out and used for affinity purification of antibody. The strip of nitrocellulose was blocked, incubated with Jade-1 antisemera, and washed according to a regular Western blot procedure. Jade-1-specific antibodies were eluted from the strip with 100 mM glycine, pH 2.5. pH was immediately neutralized with 1 M Tris, and antibody was dialyzed and

FIG. 2. Jade-1 activates transcription when tethered to viral promoters. A and B, dose response of promoter activation by Gal4-Jade-1. A-C, 60-mm dishes of 293T17 cells were co-transfected with 1 μg of AdML, E4, SV40, or E1b (data not shown) promoter-reporter constructs (see “Experimental Procedures”) and with the indicated amounts of Gal4-Jade-1 fusion construct. The amount of DNA was adjusted to total 8 μg by addition of empty Gal4 expression vector. Cells were harvested and CAT assays were performed as described under “Experimental Procedures.” Gal4-Jade-1 effects on transcriptional activities of AdML, E4, and SV40 promoters. The amount of product formed after CAT reaction was determined by liquid scintillation analyzer (PerkinElmer Life and Analytical Sciences). For each reporter, the activity derived from Gal4 expression vector was normalized to 1.0, and the activity of Gal4-Jade-1 was expressed relative to this.
was extracted predominantly with buffer containing 420 mM
triton X-100. In both HeLa and 293T cells, Jade-1
Jade-1 with increasing concentrations of NaCl and low concen-
trations of Triton X-100. In both HeLa and 293T cells, Jade-1
expression levels were determined by Western blot with
anti-Jade-1 serum (top).

Highly specific fraction of Jade-1 polyclonal antibody was
affinity-purified from Jade-1 whole rabbit antisera using 75-
kJDa FLAG-Jade-1 antigen and a scaled-up Western blotting
approach (see "Experimental Procedures"). The specificity of
purified Jade-1 antibody was then tested by Western blot.
HeLa cells were transiently transfected with empty vector or
wild-type, untagged Jade-1 expression vector. Total cell lyase
proteins were separated by SDS-PAGE and transferred to a
membrane. Strips of nitrocellulose containing HeLa cell total
lysates were probed with whole Jade-1 serum or with purified
Jade-1 antibody. The whole serum recognized multiple protein
bands in addition to endogenous Jade-1 in untransfected HeLa
cells (Fig. 1B, lane 1). In contrast, the purified antibody
recognized one major band corresponding to 64-kDa endoge-
 nous Jade-1. This demonstrates that Jade-1 purified antibody
is essentially mono-specific and is an appropriate tool for cell
immunostaining. The affinity purified Jade-1 antibody was
used on HeLa cells for conventional immunofluorescence
(Fig. 1C, top) and laser-scanning confocal immunofluorescence
imaging (Fig. 1C, bottom). It was striking that all images revealed
Jade-1 to be predominantly intranuclear, exclusive of nucler.
In addition, sparse cytoplasmic Jade-1 signal was also detect-
able. It has been previously reported that Jade-1 is localized to
cytoskeleton and nuclei in several types of cells (1). The current
study revealed a significantly higher nuclei-to-cytoskeleton ratio
of Jade-1 signal, which is probably attributable to greater spec-
ificity of purified Jade-1 antibody as well as differences in the
method of cell fixation (see "Experimental Procedures").

Jade-1 Tethered to Viral Promoters Activates Transcrip-
tion—The nuclear localization and presence of PHD fingers in
the Jade-1 molecule prompted us to examine Jade-1 transcrip-
To assess Jade-1 transcriptional activity, we used an established promoter-reporter assay that was described previously (35). In this assay, we determined the ability of Jade-1 to activate transcription of specific viral promoters linked to a CAT reporter. We used a set of four vectors with viral promoters (E1B, SV40, E4, and AdML) containing five upstream Gal4 DNA binding sites (gift from Dr T. Kouzarides) and an expression vector that fuses the DNA binding domain of Gal4 transcription factor to the full-length Jade-1 protein. The Gal4 domain provides binding of the Gal4-Jade-1 fusion to the corresponding sites of the promoter and enables Jade-1 to transactivate. Gal4-Jade-1 fusion or control Gal4 expression vectors were transiently co-transfected with each of these specific promoters, and CAT assays were performed. Gal4-Jade-1 up-regulated transcription rates of the AdML and E4 promoters by 5.8- and 3.1-fold, respectively (Fig. 2, A–C). The up-regulation occurred in a dose-dependent manner (Fig. 2, A–C) and correlated with levels of Gal4-Jade-1 protein expression, as assessed by Western blots with Jade-1 antibody (Fig. 2, A and B, bottom). Note that the amounts of recombinant Gal4-Jade-1 protein synthesized by cells are modest and do not greatly exceed the amount of endogenous Jade-1 (Fig. 2, A and B, bottom), indicating that the Gal4-Jade-1 dose response did not reach saturation and is still within its linear range. Thus, Gal4-Jade-1 potently activates transcription of at least two of four tested promoters. Others have shown in similar assays that the minimal acetyl transferase domain of CBP exhibits promoter selectivity, in that it transactivates the AdML and E4 promoters but not the SV40 and E1B promoters (35). It is noteworthy that neither the SV40 (Fig. 2C) nor the E1b (not shown) viral promoters were affected by Gal4-Jade-1, which is in correlation with previously published data for the acetyltransferase domain of CBP (35). Together, these data indicate that nuclear protein Jade-1 might be a transcription factor.

Deletion of the PHD Zinc Fingers Abolishes Transcriptional Activity of Jade-1—PHD fingers participate in protein-protein interactions and are required for transcriptional function of some known transcriptional activators (9, 14). Thus, a series of specific mutations, including a PHD finger deletion, simultaneously inactivated HAT and transcriptional function of CBP. To determine the role of the PHD fingers in Jade-1 transcriptional function, we generated a Gal4-Jade-1dd mutation lacking amino acids 203–253 and 312–371 and examined its transcriptional activity. An AdML promoter-reporter construct was co-transfected with either wild-type Gal4-Jade-1wt or mutated Gal4-Jade-1dd, and CAT assays were performed. As expected, the wild-type Gal4-Jade-1wt reproducibly activated transcription driven by the AdML promoter. However, the mutated Gal4-Jade-1dd lacking PHDs did not activate transcription (Fig. 3). It is noteworthy that both proteins were expressed at comparable levels (Fig. 3, bottom). These data further support the specificity of Jade-1 transcriptional activity and demonstrate that the PHD fingers are essential for Jade-1 transcriptional function.

Jade-1 Promotes Histone H4 Acetylation—PHD zinc fingers are found in nuclear proteins associated with histones, including those with intrinsic HAT activity, such as p300 and CBP. Moreover, it has been shown recently that PHD fingers can bind histones within the nucleosomal context (4). Thus far, we demonstrated that PHD-protein Jade-1 associates with HAT activity. To examine this possibility, we compared the levels of acetylated endogenous histones in cells transiently transfected with wild-type Jade-1, FLAG-Jade-1, or empty vector. Cells were harvested and the total histone fraction was purified from the nuclei by acid extraction (36). Purified total histone samples were analyzed for acetylated histone H3 and

FIG. 5. Jade-1-associated HAT activity requires PHD zinc fingers. A, schematic of Jade-1 mutations. B, 293T17 cells were transfected with 4 μg of each: pFLAG-CMV2 empty vector (lane 1), FLAG-Jade-1wt, FLAG-Jade-1dd, missing aa 203–253 and 312–371, FLAG-Jade-1d, missing aa 312–371, and Jade-1dc, missing aa 418–509. Expression of Jade-1 and Jade-1 deletion mutations (top) was assessed by Western blot (WB) with FLAG antibody, as described in Fig. 4. Histones were extracted (bottom), and levels of histone H4 acetylation were determined (see Fig. 4 and “Experimental Procedures” for details).
Jade-1 Lacking a Single PHD Finger Possesses a Dominant-negative Phenotype—Because PHD fingers are known to participate in protein-protein interactions, we hypothesized that deletion of this domain might result in a dominant-negative form of Jade-1. To investigate this possibility, we examined the effect of the Jade-1d mutation on Jade-1wt-mediated histone H4 hyperacetylation. We reasoned that if Jade-1d could act in a dominant-negative manner, it should functionally compete with its wild-type counterpart and thus prevent Jade-1wt-mediated H4 hyperacetylation. Cells were transfected with increasing amounts of Jade-1dC DNA only or in combination with Jade-1wt, Jade-1wt, and Jade-1d levels of expression were monitored by Western blot. As usual, expression of Jade-1dC protein increased acetyl-H4 in a dose-dependent manner (Fig. 6A, lanes 1–4). In contrast, the presence of Jade-1d significantly diminished histone H4 acetylation by Jade-1wt protein (Fig. 6A, lane 5, compare acetyl-H4 levels in lanes 2–4 with those in lanes 5–7, and B, for quantitation), demonstrating that in a whole cell HAT functional assay Jade-1d acted in a dominant-negative manner.

Histone Acetyltransferase TIP60 Interacts with Jade-1 and Enhances its HAT-Associated Function—Although our data clearly support an association of Jade-1 with HAT activity, the analysis of Jade-1 polypeptide using the Conserved Domain Architecture Retrieval Tool (CDART; www.ncbi.nlm.nih.gov) did not reveal the presence of an acetyltransferase domain. In addition, immunopurified and immobilized wild-type and tagged Jade-1 demonstrated reproducible but rather weak acetyltransferase activities against exogenously added core histones in a cell free HAT assay (Fig. 8A, bar graph, and data not shown) that could not explain the robust effects of Jade-1 on acetylation of endogenous histone H4 in a whole cell HAT assay (Figs. 4–6). This suggested that Jade-1-associated HAT activity might be mediated by another protein with intrinsic acetyltransferase activity interacting with Jade-1. The dominant-negative phenotype of Jade-1d also supported the notion that Jade-1 might physically interact with other proteins mediating Jade-1 HAT activity in vivo and in vitro.

We set out to determine what protein might mediate Jade-1-associated HAT activity and reasoned that a potential Jade-1 partner should be a HAT with histone H4 specificity. Although...
numerous HATs acetylate nucleosomal histones H3 and H4, so far, few known HATs have unique specificity for histone H4 (24). TIP60 has been previously characterized as a HAT with histone H4 specificity and seemed to be a good candidate (27, 30). To examine whether TIP60 might mediate Jade-1 effects, we first determined whether Jade-1 directly interacted with TIP60 using a co-immunoprecipitation approach. Cells were transiently transfected with wild-type Jade-1, HA-Jade-1, or Gal4-Jade-1, and/or FLAG-TIP60. The Jade-1 complex was immunoprecipitated with Jade-1 antisera or corresponding tag antibody, and the presence of FLAG-TIP60 in Jade-1 immunoprecipitates was assessed by Western blot with FLAG antibody (Fig. 7, A). In a reverse experiment, FLAG-TIP60 was immunoprecipitated with Jade-1 antibody, and the presence of Jade-1 was assessed by Western blot with Jade-1 antisera (Fig. 7B). Jade-1, HA-Jade-1, and Gal4-Jade-1 were able to pull down TIP60 (Fig. 7A, top) and TIP60 was able to pull down all three species of Jade-1 (Fig. 7B, top), demonstrating that the two transfected proteins strongly interact. Moreover, endogenous Jade-1 immunoprecipitated with Jade-1 specific polyclonal antisera efficiently pulled down a significant amount of FLAG-TIP60 (Fig. 7A, lane 1), whereas immunoprecipitated FLAG-TIP60 pulled down endogenous Jade-1 (Fig. 7B, lane 1), supporting the specificity of the Jade-1-TIP60 interaction. Therefore, both membranes were stripped and reprobed with Jade-1 and FLAG antibodies (Fig. 7, A and B, bottom). This allowed evaluation of the amount of the interacting partners in the complex and supported the efficiency of Jade-1-TIP60 binding. We then examined whether deletion of the PHD zinc fingers from Jade-1 molecule will affect these interactions. We were surprised to find that full-length Jade-1 and Jade-1Δ8 pulled down TIP60 with similar efficiency (Fig. 8A), indicating that the PHD zinc fingers are not involved in the Jade-1-TIP60 physical interaction. Thus, these results demonstrate that Jade-1 physically interacts with TIP60 and suggest that TIP60 might mediate Jade-1-associated HAT activity.

To further explore this possibility, we examined whether TIP60 co-immunoprecipitated with Jade-1 or Jade-1Δ8 can enhance [3H]acetyl incorporation into core histones in a cell free immunoprecipitation-HAT assay. An aliquot of each immunoprecipitation sample presented in Fig. 8A, top two panels, was subjected to an IP-HAT assay. It is clear that the HAT activities in HA-Jade-1 and HA-Jade-1Δ8 immunoprecipitates were increased up to 3-fold with FLAG-TIP60 (Fig. 8A, bar graph). The increase in HAT activity was proportional to the amount of co-immunoprecipitated FLAG-TIP60; hence, similar amounts of FLAG-TIP60 immunoprecipitated directly without Jade-1 had comparable levels of activity (data not shown). In a reverse experiment, FLAG-TIP60 was immunoprecipitated alone or in the complex with HA-Jade-1, and HAT activities were determined (Fig. 8B, bar graph). As expected, TIP60 alone potently acetylated core histones. TIP60 bound to Jade-1 also acetylated histones with similar efficiency, indicating that the TIP60-Jade-1 complex is capable of acetylating core histones in vitro in a cell free assay. In sum, these data strongly suggest that TIP60 might mediate Jade-1 effects on levels of acetylated endogenous histone H4 in an intact cell.

To examine the functional interactions of Jade-1 and TIP60 in an intact cell, we studied the effects of TIP60 on Jade-1-associated induction of endogenous histone H4 acetylation. Cells were transfected with increasing amounts of Jade-1 without or with TIP60, and levels of endogenous acetylated histone H4 and histone H3 were assessed by Western blot. As expected, Jade-1 alone increased acetylation of histone H4 in a dose-dependent manner (Fig. 9A, lanes 1, 3, 5, and 7, and B), whereas Jade-1Δ8 failed to exert any effects (Fig. 9A, lane 9, and B). Overexpression of TIP60 alone, a potent HAT with histone H4 specificity, failed to modulate levels of endogenous acetylated histone H4.

FIG. 7. Physical interaction of Jade-1 with histone acetyltransferase TIP60. A, endogenous and transfected Jade-1 precipitates recombinant FLAG-TIP60. 293T17 cells grown in 60-mm plates were transfected with FLAG-TIP60 only (lanes 1, 5, and 6) or with FLAG-TIP60 in combination with Jade-1Δ8 (lane 2), HA-Jade-1 (lane 3), or Gal4-Jade-1 (lane 4). The total amount of DNA was adjusted to 8 μg in each transfection sample by filling in with the appropriate empty vector DNA. Thirty-six hours after transfection, cells were lysed in 600 μl of lysis buffer (see “Experimental Procedures”), and endogenous or recombinant Jade-1 was immunoprecipitated (IP) from 300 μl of each lysate with anti-Jade-1 serum (lanes 1 and 2), HA (lane 3), or Gal4 (lane 4) antibody. Lanes 5 and 6 represent negative controls. To visualize FLAG-TIP60 pulled down by Jade-1, equal aliquots of immunoprecipitates were analyzed by Western blot (WB) with FLAG antibody (top). To visualize immunoprecipitated Jade-1, membranes were stripped and re-probed with Jade-1 antiserum (bottom). Note that endogenous Jade-1 (bottom, lane 1) pulled down FLAG-TIP60 (top, lane 1). In addition, note that the amount of Jade-1 in each sample is proportional to the amount of pulled down TIP60, indicating that interactions are efficient and specific. B, FLAG-TIP60 pulls down endogenous and transfected Jade-1. Transfection and immunoprecipitations were done as described above (A), except that FLAG monoclonal antibody was used to precipitate FLAG-TIP60. Co-immunoprecipitated endogenous (top, lane 1) and transfected Jade-1 (top, lanes 2–4) were visualized with Jade-1 antiserum. Lane 5 is a negative control. To visualize immunoprecipitated FLAG-TIP60, membranes were stripped and re-probed with FLAG antibody (bottom).
It is striking that co-expression of TIP60 with Jade-1 further augmented levels of acetyl-H4, demonstrating that Jade-1 co-operates with HAT TIP60 in an intact cell (Fig. 9, A, lanes 4, 6, and 8, and B). In contrast, co-expression of TIP60 with Jade-1dd failed to augment levels of acetyl-H4 (Fig. 9A, lane 10). Note that the levels of acetylated histone H3 were not changed in any samples, supporting the specificity of observed effects (Fig. 9A, middle). These data strongly suggest that TIP60 might mediate Jade-1-associated effects on levels of endogenous acetylated histone H4.

Thus far, we have demonstrated that in vitro TIP60 promotes histone acetylation of core histones whether in the complex with Jade-1 or Jade-1dd. In contrast, in intact cells, TIP60 failed to co-operate with mutated Jade-1dd in promoting hyper-acetylation of endogenous histone H4, indicating a requirement of intact PHD zinc fingers for an in vivo functional interaction of TIP60 and Jade-1. A recent study demonstrated that the PHD zinc finger of CBP binds nucleosomal histones in vitro (4). We speculate that PHD fingers are required for a nuclear Jade-1 complex to bind to the nucleosome, to provide a docking site for TIP60 and thereby promote acetylation of nucleosomal histone H4 (Fig. 10).

DISCUSSION

Jade-1 is a novel PHD zinc finger family protein that interacts with the VHL tumor suppressor (1, 2). Jade-1 is highly
family and their yeast orthologs, the Yng (37) family of proteins. The ING1 candidate tumor suppressor gene expresses a family of PHD proteins that localize to the nucleus and are growth inhibitory. p33ING1b is the most characterized isoform of Ing1 and is involved in apoptosis and cell cycle regulation, presumably via association with p53, PCNA, and HAT complexes (10, 37, 38). Ectopic expression of p33ING1b in human fibroblasts resulted in elevated levels of acetylated histones H4 and H3 (38). We find that overexpression of Jade-1 in 293T cells resulted in a robust increase of endogenous acetylated histone H4, but not histone H3. Yeast protein Yng2 is associated with the yeast homolog of TIP60, Esa1 (37), whereas another ING family member, Ing5 protein, was identified as a specific subunit of the human TIP60 complex (39). Likewise, we find a physical and functional interaction between Jade-1 and TIP60. The most important observation supporting in vivo interaction of TIP60 with Jade-1 is the ability of TIP60 to enhance Jade-1-mediated acetylation of endogenous histone H4. It is interesting that others have shown that TIP60 by itself in vitro can only acetylate individual histones and is unable to acetylate isolated nucleosomal chromatin substrates. Two other proteins were required for the TIP60 to gain affinity toward nucleosomal histones. One of the required proteins of the minimal triple core complex conferring nucleosomal affinity to TIP60 was Ing3 (39). It is conceivable that the whole cell HAT assay in our study is an indirect measurement of Jade-1 or TIP60-mediated effects on acetylation of native nucleosomal histones. We demonstrate that although expression of Jade-1 alone promoted endogenous histone H4 acetylation, expression of TIP60 alone was insufficient to do so. However, co-expression of TIP60 further enhanced H4 acetylation induced by Jade-1, demonstrating the cooperative relationship of these two proteins. We suggest that Jade-1 targets TIP60 to nucleosomal histones and enables TIP60 to acetylate histone H4 lysine residues in vivo. It is possible that, like Ing3, Jade-1 might play a role in conferring TIP60 affinity to nucleosomal histones. More direct examination of Jade-1’s ability to cooperate with TIP60 in acetylating nucleosomal histones in vitro is required to provide evidence for this proposed mechanism.

PHDs are often found in proteins that function in the formation, maintenance, or regulation of chromatin structure and are thought to function as protein interaction domains. According to a recent study, the isolated PHD finger of p300 was capable of binding to nucleosomal histones as determined by an in vitro EMSA assay (4). We show that, in intact cells, deletion of the two PHD fingers abrogated Jade-1-associated HAT activity. It is possible that this mutation impaired Jade-1’s ability to associate with nucleosome and target endogenous HATs, such as TIP60 to their substrate. It is not excluded that other, as-yet unidentified proteins might also be required for this function of Jade-1. Like Yng2 (37), TIP60 physical interaction

expressed in kidney and renal proximal tubule cells (1, 2), which are renal cancer precursors. Jade-1 may be involved in renal tubular epithelial cell differentiation, growth control and apoptosis (1). The molecular function of Jade-1 is undefined and was the specific focus of the current study.

We have made the following major findings: 1) Jade-1 is localized predominantly to the nucleus and is capable of activating transcription when tethered to viral promoters; 2) Jade-1 associates with HAT activity specific for histone H4; 3) Jade-1 is physically and functionally interacts with TIP60, a powerful HAT with histone H4 specificity; and 4) both transcriptional and HAT-associated functions of Jade-1 are fully dependent on its PHD zinc fingers. The presented set of experimental observations indicates that Jade-1 is a candidate transcription factor and is associated with acetyltransferase activity specific for histone H4. Transcriptional and HAT-associated Jade-1 activities require intact PHD zinc fingers within the Jade-1 polypeptide. Our data strongly suggest that TIP60 is at least one of the candidates that might mediate Jade-1-associated in vivo HAT activities.

The association of Jade-1 with HAT activity is, in part, similar to the HAT association of the mammalian ING (10)
Such studies might provide a link between TIP60-in Jade-1 novel nuclear activities, including its interactions with suppressor, an established partner of Jade-1, suggesting a renal cancer relation—demonstrated that naturally occurring mutations of VHL altered processes directly related to cancer development. Previous studies damage stress response, cell cycle and apoptosis, cellular properties, promotes apoptosis, and may be a tumor suppressor. This and other available information suggest that together with TIP60, Jade-1 might be involved in similar biological activities triggered by a cellular stress, such as DNA damage.

In this study, we demonstrated that Jade-1 can activate transcription when tethered to two viral promoters. The magnitude of activation was modest but very reproducible and specific. The specificity of this effect is supported by the fact that, similar to CBP, Jade-1 activated only two of four tested promoters and that the deletion of the two PHD fingers completely inactivated Jade-1 transcriptional function. It is unlikely that the deletion of PHD fingers resulted in impaired nuclear transport and therefore inhibited Jade-1 transcriptional and HAT activities. Thus, genetic manipulations aimed at identifying developmentally regulated genes in the mouse led to Jade-1 gene disruption, resulting in the expression of only a 47-amino acid fragment of Jade-1 (3). This Jade-1 fragment lacking PHD fingers was efficiently targeted to the nucleus in mouse embryo in a developmentally regulated manner. Taking into consideration the current study, as well as data reported by others, we hypothesize that the PHD fingers might be important for Jade-1 association with nucleosomal histone and for targeting other required regulatory proteins to specific promoter regions.

In sum, the current study indicates that VHL-interacting protein Jade-1 is a strong candidate transcription factor and is associated with acetyltransferase activity specific for histone H4. Our data support the idea that TIP60 is at least one candidate that might mediate Jade-1-associated HAT activities in vivo. Jade-1 interaction with TIP60 implies a potential role in DNA damage stress response, cell cycle and apoptosis, cellular processes directly related to cancer development. Previous studies demonstrated that naturally occurring mutations of VHL altered its interactions with Jade-1, suggesting a renal cancer relationship. It would be critical to investigate whether the VHL tumor suppressor, an established partner of Jade-1, might be involved in Jade-1 novel nuclear activities, including its interactions with TIP60. Such studies might provide a link between TIP60-mediated Jade-1 nuclear function and VHL-related renal cancer.

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REFERENCES
1. Zhou, M. I., Wang, H., Ross, J. J., Kuzmin, I., Xu, C., and Cohen, H. T. (2002) J. Biol. Chem. 277, 39887–39898
2. Zhou, M. I., Wang, H., Foy, R. L., Ross, J. J., and Cohen, H. T. (2004) Cancer Res. 64, 1278–1286
3. Tousanou, E., Tweedle, S., and Wilson, V. (2003) Mol. Cell. Biol. 23, 8552–8553
4. Hay, P., and Horikoshi, M. (1997) J. Mol. Biol. 272, 641–643
5. Strahl, B. D., and Allis, C. D. (2000) Nature 403, 442–447
6. Yamamoto, T., and Horikoshi, M. (1997) J. Biol. Chem. 272, 816–826
7. Denu, J. M., and Allis, C. D. (2001) Mol. Cell. Biol. 21, 2886–2893
8. Mege, P. C., Morgan, B. A., and Smith, M. M. (1995) Genes Dev. 9, 1716–1727
9. Yamamoto, T., and Horikoshi, M. (1997) J. Biol. Chem. 272, 30595–30598
10. ashler, A. L., Swaminathan, S., and Sukhatme, V. P. (1993) Mol. Cell. Biol. 13, 4556–4571
11. Cash, K. J., Rich, C. B., Jensen, D. E., Fontanilla, M. R., Bashir, M. M., Rosenblom, J., and Foster, J. A. (1996) J. Biol. Chem. 271, 28853–28860
12. Bannister, A. J., and Kouzarides, T. (1996) Nature 384, 641–643
13. McMillan, S. J., Cerone, S., Panchenko, M. V., Steene, J. P., and Young, D. M. (2000) J. Biol. Chem. 275, 18835–18846
14. Martinez-Balbas, M. A., Bannister, A. J., Martin, K., Haus-Scuffert, P., Meisterernst, M., and Kouzarides, T. (1998) EMBO J. 17, 2886–2893
15. Consalez, G. L., Gallowitz, D., and Alberts, B. M. (1979) J. Biol. Chem. 254, 1716–1723
16. Loewith, R., Meier, M., Lees-Miller, S. P., Raibowol, K., and Young, D. (2000) Mol. Biol. Cell. 11, 3867–3886
17. Vieyra, D., Loewith, R., Scott, M., Bonnin, P., Boisvert, F. M., Cheema, P., Pastyreva, S., Meier, M., Johnson, R. N., and Loewith, R. (2002) J. Biol. Chem. 277, 28963–28973
18. Deyle, Y., Selleck, W., Lane, W. S., and Cote, J. (2004) Mol. Cell. Biol. 24, 1884–1896
19. Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokin, I., Parmely, T., Conaway, R. C., and Conaway, J. W. (2003) J. Biol. Chem. 278, 42733–42736
20. Hebbes, T. R., Thornore, A. W., and Crane-Robinson, C. (1998) EMBO J. 17, 1395–1404
21. Kuo, M. H., and Allis, C. D. (1998) Bioessays 20, 615–626
22. Nakat, G. J., Fan, H. Y., and Kingston, R. E. (1992) Cell 100, 153–167
23. Brownell, J. E., Zhou, J., Ranalli, T., Kubayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) Cell 84, 843–851
24. Blom, S. Y., Deno, J. M., and Allis, C. D. (2001) Annu. Rev. Biochem. 70, 81–120
25. Straub, B. D., and Allis, C. D. (2000) Nature 403, 41–45
26. Turner, B. M. (2000) Bioessays 22, 836–845
27. Ikura, T., Ogryzko, V. V., Grigoriev, M., Grison, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Cell 102, 463–473
28. Barlev, N. A., Polotarovsky, V., Owen-Hughes, T., Ying, C., Liu, W., Workman, J. L., and Berger, S. L. (1998) Mol. Cell. Biol. 18, 1349–1358
29. Mege, P. C., Morgan, B. A., and Smith, M. M. (1995) Genes Dev. 9, 1716–1727
30. Yamamoto, T., and Horikoshi, M. (1997) J. Biol. Chem. 272, 30595–30598
31. Gashler, A. L., Swaminathan, S., and Sukhatme, V. P. (1993) Mol. Cell. Biol. 13, 4556–4571
32. Cash, K. J., Rich, C. B., Jensen, D. E., Fontanilla, M. R., Bashir, M. M., Rosenblom, J., and Foster, J. A. (1996) J. Biol. Chem. 271, 28853–28860
33. Bannister, A. J., and Kouzarides, T. (1996) Nature 384, 641–643
34. D’Camillo, S. J., Carvajal, I., Panchenko, M. V., Stone, E., C. J., and Vagner, M. B. (2003) J. Biol. Chem. 278, 18835–18846
35. Martinez-Balbas, M. A., Bannister, A. J., Martin, K., Haus-Scuffert, P., Meisterernst, M., and Kouzarides, T. (1998) EMBO J. 17, 2886–2893
36. Cuszens, L. S., Gallowitz, D., and Alberts, B. M. (1979) J. Biol. Chem. 254, 1716–1723
37. Loewith, R., Meier, M., Lees-Miller, S. P., Raibowol, K., and Young, D. (2000) Mol. Biol. Cell. 11, 3867–3886
38. Vieyra, D., Loewith, R., Scott, M., Bonnin, P., Boisvert, F. M., Cheema, P., Pastyreva, S., Meier, M., Johnson, R. N., and Loewith, R. (2002) J. Biol. Chem. 277, 28963–28973
39. Deyle, Y., Selleck, W., Lane, W. S., and Cote, J. (2004) Mol. Cell. Biol. 24, 1884–1896
40. Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokin, I., Parmely, T., Conaway, R. C., and Conaway, J. W. (2003) J. Biol. Chem. 278, 42733–42736