The scaffolding protein JADE1 physically links the acetyltransferase subunit HBO1 with its histone H3/H4 substrate

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Running Title: Role of JADE1 in HBO1 Acetyltransferase Activity

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Keywords: HBO1; HAT2 histone acetyltransferase; chromatin regulation, protein complex, epigentics

Abstract

The human enzyme histone acetyltransferase binding to ORC1 (HBO1) regulates DNA replication, cell proliferation, and development. HBO1 is part of a multiprotein histone acetyltransferase (HAT) complex that also contains inhibitor of growth family member (ING) 4/5, MYST/Esa1-associated factor (MEAF) 6, and the scaffolding proteins Jade family PHD finger (JADE) 1/2/3 or bromodomain and PHD finger-containing protein (BRPF) 2/3 to acetylate histone H4 H4K5/8/12 or H3K14, respectively. Within this four-protein complex, JADE1 determines histone H4 substrate specificity of the HBO1–HAT complex. However, the mechanism by which JADE1 controls the H4-specific acetyltransferase activity of HBO1 is unknown. Here, we used recombinant proteins in vitro to dissect the specific regions and activities of HBO1 and JADE1 that mediate...
histone H3/H4 acetylation via the HBO1 HAT domain. We found that JADE1 increases the catalytic efficiency of HBO1 acetylation of an H3/H4 substrate by about 5-fold through an N-terminal, 21-residue HBO1- and histone-binding domain and a nearby second histone core-binding domain. We also demonstrate that HBO1 contains an N-terminal histone-binding domain (HBD) that makes additional contacts with H3/H4 independently of JADE1 interactions with histones and that the HBO1 HBD does not significantly contribute to HBO1’s overall HAT activity. Experiments with JADE1 deletions in vivo recapitulated these in vitro interactions and their roles in HBO1 histone acetylation activity. Together, these results indicate that the N-terminal region of JADE1 functions as a platform that brings together the catalytic HBO1 subunit with its cognate H3/H4 substrate for histone acetylation.

Introduction

Histone acetylation, a covalent modification on lysine residues of histones, predominantly in the N-terminal tail regions, regulates local and global chromatin dynamics—thus playing an important role in modulating DNA accessibility and gene expression in all eukaryotic organisms. Generally, hyperacetylation and hypoacetylation are observed in transcriptionally active and inactive regions of the chromatin, respectively(1-5). Histone acetylation has been shown to promote transcription in at least two ways. First, acetylation neutralizes the lysine positive charge, thus altering its physiochemical properties and making the local chromatin environment more accessible for gene transcription activation(1). Second, lysine acetylation generates a docking recognition sites for various gene-activating proteins containing acetyl-lysine recognition domains such as bromodomains(5-9). Therefore, the tight regulation of acetylation on lysine residues within histones, as well as the enzymes that mediate these modifications, are critical to maintain normal gene expression and regulation. The enzymes that mediate histone acetylation are histone acetyltransferases (HATs), while the enzymes that convert acetyl-lysine residues within histone back to lysine are histone deactylases (HDACs)(10). HDACs are grouped into four classes according to their functional properties and sequence conservation, which fall into two conserved structural folds (classes I, II, IV and the class III sirtuins). HATs show considerably more sequence and structural diversity than HDACs, forming at least five different families, Gcn5/PCAF, MYST, p300/CBP, Rtt109 and HAT1. HATs typically function within multiprotein complexes in vivo where their substrate specificities and activities are modulated by the other subunits of the respective HAT complexes(11,12).

Human HBO1 (also referenced as KAT7 or MYST2), a member of the MYST family, regulates DNA replication(13,14), cell proliferation and development(15). Consistent with its role in gene expression, the HBO1 HAT
complexes are localized at the transcription start sites and coding regions of active genes in the genome (16), and is responsible for the majority of H4(14,17,18) and H3K14(19,20) acetylation. HBO1 has also been shown to play more specific roles in activating the expression of key genes in development and embryonic patterning(19). The aberrant activity of HBO1 has been correlated with several cancers. HBO1 has been shown to be over-expressed in MCF7 and Saos-2 established cancer cell lines and in a subset of human primary cancers such as carcinomas of testis, breast, and ovary(21,22). Finally, a HBO1 catalytic defective mutant inhibits MCM complex loading onto the origin, indicating that HBO1 functions as a DNA replication co-activator in addition to its transcriptional roles(5,13,18).

HBO1 functions in the context of two multi-protein histone acetyltransferase complexes containing ING4/5, MEAF6 and paralogs JADE1/2/3 for targeting the H4 tail or BRPF1/2/3 for H3K14 acetylation(11,20). The activity and substrate selectivity of HBO1 is dependent on the proteins within the HBO1 HAT complex, which also have other functions in various cellular proliferation and tumor suppression pathways. For example, JADE1 was initially identified as a protein partner of the von Hippel-Lindau tumor suppressor to regulate cellular oxygen sensing(23). A recent study demonstrated that an HBO1 complex containing the BRPF1 protein in place of JADE1 has specificity toward H3 tail acetylation, rather than the H4 tail acetylation preferred by the JADE1 containing complex. This suggests that JADE1 contributes specifically toward H4 tail acetylation by the HBO1 complex(5,11,15). Another member of the HBO1 complex, ING, is an inhibitor of growth protein responsible for growth regulation in eukaryotic organisms. Mutation or down-regulation of ING genes leads to tumor developmental pathways(24,25). The HBO1 HAT complex contains three PHD fingers responsible for recognizing various methylated states of H3K4. JADE1 contains two of these motifs (termed PZP), which cooperate to enable acetylation of nucleosome particles and recognition of unmethylated H3K4(11,15,16). ING4 contains the third PHD finger, which recognizes trimethylated H3K4 (H3K4me3). The H3K4me3 modification state has been shown to be correlated with the ability of the HBO1 HAT complex to acetylate H4(5,11,14,16,17). Through its recognition of H3K4me3, ING4 has been proposed to play a lynchpin role in the acetylation process. Thus ING can link its aforementioned tumor suppressor function with chromatin binding, transcriptional activity, and cellular proliferation by nucleating the complex recruitment process(15,24,25). This is further substantiated by the activity of JADE1 spliced variants. Specifically, while the longer JADE1L form harbors tumor suppressor activity, the shorter JADE1 variant that lacks the ING4 binding domain but still binds to HBO1, called JADE1S, does not function as a tumor suppressor(15). Together, JADE1 in the HBO1
HAT complex has been proposed to function as an intermediary to link HBO1 HAT catalytic activity to ING tumor suppressor activity through cross talk between two different epigenetic modifications of methylation and acetylation(16). The absence of any of the HBO1 HAT complex subunits leads to diminished acetylation activity on cognate substrates, implying that these HBO1 HAT complex subunits play important regulatory roles(11,15,16).

JADE1 has been demonstrated to act as a platform to assemble the tetrameric HBO1 HAT complex for histone H4 acetylation, with a region of JADE1 N-terminal to the JADE1 PHD fingers (called region I) shown to recruit HBO1, and a region of JADE1 C-terminal to the JADE1 PHD fingers (called region II) shown to recruit the ING/MEAF6 subcomplex(15). It has also been shown that a modified tetrameric complex, in which BRPF replaces JADE has specificity for H3K14 acetylation(11). These studies highlight the importance of BRPF and JADE subunits in dictating histone substrate specificity. While a recent study has revealed the molecular basis for how BRPF mediates H3K14-specific acetylation by HBO1(26), the molecular basis for how JADE potentiates the H4 specific activity of HBO1 is not known. Given this gap in knowledge, we set out to dissect the specific regions and activities of JADE1 that mediate histone H4 acetylation by the HBO1 HAT complex. We find that JADE1 contains specific HBO1 and histone binding regions that serves to increase the catalytic efficiency of HBO1 acetylation of histone H4. Implications of these studies for the differential regulation of HBO1 by JADE1 and BRPF1 paralogs are discussed.

Results

A 21-residue segment of JADE1 directly contacts the HBO1 HAT domain and histone substrate to facilitate acetylation of free histones

It was previously demonstrated that an N-terminal region of JADE1 between residues 90-199 participated in HBO1 catalytic activity(15). This region of JADE1 contains sequence conservation within residues 115-179 of yeast to human proteins associated with ING/MYST-containing HAT complexes(11,15). We also noted an additional region of sequence conservation among the JADE proteins just N-terminal to this region (Figure 1A). To map the region of the JADE1 N-terminus that directly interacts with the HBO1-HAT domain, we used pull down experiments with the HBO1-HAT and N-terminal truncation constructs of JADE1 (Figure 1B). These experiments revealed that MBP-JADE1(1-188) and MBP-JADE1(60-188) were able to pull down the HBO1-HAT, while MBP-JADE1(80-188) was not. These data revealed the importance of residues 60-80 of JADE1 for the direct interaction with the HBO1-HAT. To further narrow down the key residue(s) within this 21-residue segment critical for the interaction with the HBO1-HAT domain, we introduced triple alanine mutations spanning
residues 60 to 80 within the context of MBP-JADE1(1-188). These studies revealed that each of the mutants resulted in no detectable HBO1-HAT pull down (Figure 1C). The observation that each of the JADE1 mutants disrupted HBO1-HAT interaction suggested that either the entire JADE1(60-80) domain makes important contacts to the HBO1-HAT or that the domain contains a binding surface and tertiary structure that are both important for HBO1-HAT interaction. To more quantitatively assess the binding of the JADE N-terminal region to the HBO1-HAT, we performed Isothermal Titration Calorimetry (ITC) using various constructs of JADE1. The ITC results were consistent with the pull-down data, demonstrating that JADE1(60-188) showed significant binding to the HBO1-HAT with a $K_d$ of $\sim 2.4 \text{uM}$ and a stoichiometry of $\sim 1:1$, while JADE1 (80-188) showed no detectable binding (Figure 1D). Strikingly, a peptide of residues 55-85 of JADE1 showed binding with a comparable dissociation constant ($K_d = 4.18 \text{uM}$) to JADE1(60-188) demonstrating that residues 60-80 of JADE1 are essential for HBO1-HAT binding.

We next asked if residues of 60-80 of JADE1 are also able to interact with histones to potentially bridge the HBO1-HAT acetylation of histones. To do this, we carried out pull-down studies of MBP-JADE1 N- and C-terminal deletion constructs with HBO1-HAT plus H3/H4 (Figure 1B). These studies revealed that only JADE1 constructs harboring residues 60-80 of JADE1 were able to interact with both the HBO1-HAT and H3/H4, confirming that JADE1 residues 60-80 bridge HBO1-HAT/histone interactions. Notably, JADE1 constructs starting at residue 60 and extending to residue 188, showed more robust pull down with HBO1-HAT and histones than JADE1 constructs harboring only residues 60-80, suggesting that additional regions of JADE1 between residues 108 and 188 also contribute to HBO1/histone interactions (Figure 1B). Based on the HBO1-HAT and histone binding properties of residues 60-80 of JADE1, we refer to it as the HBO1 and Histone Binding Domain (JADE1-HHBD).

We then asked if the minimal region of JADE1 that facilitates HBO1 and histone binding is sufficient to potentiate HBO1-HAT activity toward histone tail acetylation. To do this, we titrated JADE1 (55-85) and JADE1(1-188) into the HBO1-HAT and H3-H4 complex and assayed for histone acetyltransferase activity (Figure 2A). This analysis revealed that titration of JADE1(55-85) in vast excess resulted in only marginal effects on HBO1 histone acetyltransferase activity, while titration of JADE1(1-188) showed significantly more potentiation of HBO1 histone acetyltransferase activity, to a level of about 6-fold relative to no added titrant. Taken together with the previous data showing that residues 60-80 of JADE1 bridges HBO-HAT-histone interaction, these data demonstrated that residues 60-80 of JADE1 contribute to but are not
sufficient to mediate HBO1 acetylation of histones.

**JADE1 contains a second histone binding domain that contributes to HBO1 acetylation of histone H4 substrate by anchoring the HBO1/JADE1 complex to the histone core.**

Inspection of the pull-down experiments of Figure 1B reveals that an additional region of JADE1, within residues 108-188, interacts with histones but not the HBO-HAT and the histone acetyltransferase activity assay of Figure 2A further suggests that regions within JADE1 residues 80-188 are also required to fully potentiate HBO-HAT histone acetyltransferase activity. Based on these studies, we carried out further studies to define the regions of JADE1 and histones that mediate this interaction. To specifically probe the requirement for the histone tails for JADE1 interaction, we carried out MBP-JADE1(60-188) pull down with H3/H4 with either full-length or tailless H3 and/or H4 subunits. These studies revealed that JADE1(60-188) pulled down all of the histone complexes, including the completely tailless complexes (H3(TL)-H4(TL)) (Figure 3A). These pull-down experiments demonstrated that the primary JADE1(60-188) binding site exists on the core of the H3/H4 complex.

Previous studies indicate that JADE1 facilitates HBO1 H4-specific tail acetylation(11). For this reason, we asked if JADE1 makes an additional direct or HBO1 dependent contact with the H4 tail. To test this, we prepared GST tagged H4 tail 1 to 19 and performed a GST pull down with JADE1 (1-188) in the presence of the HBO1-HAT. This experiment revealed that HBO1-HAT is unable to interact directly with the isolated H4 histone tail in the absence or presence of JADE1(1-188) (Figure 3B), consistent with the conclusion that JADE1 histone core binding is required for HBO1-HAT acetylation of the histone H4 tail. We refer to the domain of JADE1 that mediates H3/H4 core binding as the Histone Core Binding Domain (JADE1-HCBD).

MBP pull downs with JADE1 deletion constructs and H3/H4 (Figure 1B) suggests that JADE1 contains 2 histone binding sites, JADE1(60-80), JADE1(104-188). To more quantitatively evaluate the histone binding properties of these two regions of JADE1, we performed Isothermal Titration Calorimetry (ITC) with H3/H4 using various constructs of JADE1. The ITC data showed that JADE1(80-188) binds H3/H4 with a dissociation constant of 53 uM (Figure 3C), while JADE1 (60-188), containing both histone binding sites, showed H3/H4 binding with a dissociation constant of about 2-fold higher of 29 uM (Figure 3C). These studies indicate that the HHBD and HCBD regions of JADE1 bind additively to H3/H4.

We also carried out MBP-JADE(1-188) pull down studies with H3/H4 (with or without tails) in the presence or absence of the HBO-HAT, revealing that the histone binding properties of
JADE1(1-188) are independent of HBO-HAT binding (Figure 3D).

**JADE1-HCBD shows selectively for free histone H3/H4 over H2A/H2B and nucleosomes**

To further probe the histone binding specificity of JADE1-HCBD, we performed pull down experiments with MBP-JADE1(1-188) and histones H3/H4 and H2A/H2B. These studies demonstrated that although JADE1(1-188) interacts with histone H3/H4 in the presence or absence of the HBO1-HAT, it does not interact with histone H2A/H2B (Figure 4A). Moreover, if we carry out similar pull-down studies with a mixture of H3/H4 and H2A/H2B complexes, JADE1(1-188) specifically selects for H3/H4 over H2A/H2B, suggesting that JADE1-HCBD does not interact with the H3/H4 core through a nonspecific charge interaction (Figure 4B).

It has been previously demonstrated that the H3 tail is essential for the HBO1-HAT complex to interact with chromatin(16). JADE1 contains 2 PHD finger domains (PHD-Zn knuckle-PHD or PZP domain) C-terminal to the JADE-HCBD that recognize specific methylation states of the H3 tail and allow the HBO1 HAT complex to bind and acetylate nucleosomes(15-17). Given this information, we wanted to further investigate if the JADE-HCBD can bind to H3/H4 when it is assembled into a nucleosome. Pull-down studies with the assembled nucleosome, revealed the absence of an interaction (Figure 4C), suggesting that the octamer or the DNA is excluding the JADE1-HCBD binding surface to H3/H4 (Figure 4C). To further substantiate these findings, we compared the acetylation activity of HBO1-HAT and HBO1-FL with or without JADE1(1-188) against H3/H4 and nucleosomes. These studies revealed that JADE1(1-188) potentiated the activity of HBO1-HAT and HBO1-FL against H3/H4 but not nucleosomes (Figure 4D). This data suggests that other regions of JADE1 are required for HBO1 targeting to nucleosomes. This is consistent with previously reported data that the PZP, PHD1 and PHD2 domains of JADE1, located C-terminal to the JADE1 HHBD and HCBD domains, contribute to HBO1-mediated acetylation of nucleosomes(11,15,16). In contrast, it appears that the HHBD and HCBD domains of JADE1 play an important role in capturing free histones (H3/H4) during transcription (or replication) for further acetylation of the tails.

**JADE1-HHBD and JADE1-HCBD bind independently to their respective partners, but cooperate to potentiate HBO1-HAT acetylation**

After establishing that the HHBD and HCBD regions of JADE1 bind independently to their respective histone and/or HBO1-HAT binding partners, we set out to determine the contribution of these JADE1 regions for HBO1 acetyltransferase activity. We prepared four JADE1 deletion constructs spanning the HHBD and/or HCBD regions: 1-188(HHBD-HCBD), 60-188(HHBD-HCBD), 80-188(HCBD) and 1-80(HHBD). We assayed HBO1 HAT activity in
the presence of 25-fold molar excess of each of these JADE1 constructs. This experiment revealed that only JADE1 construct spanning both the HHBD and HCBD showed potentiating effects of HBO1-HAT activity. (Figure 5A). Moreover, titration with separate fragments of JADE1 1-80(HHBD) and JADE1 80-188(HCBD) did not potentiate HBO1-HAT acetylation activity (Figure 5A). These results demonstrate that the JADE1-HHBD and HCBD regions work cooperatively to potentiate HBO1 HAT activity and that these two regions of JADE1 must be connected to mediate this activity.

To more quantitatively assess the contribution of JADE1 to HBO1-HAT catalytic activity, we carried out bisubstrate kinetics. Due to a significant chemical acetylation that occurs on the core of histones at high concentration, we were not able to carry out a full Michaelis-Menten plot. Instead, we measured the catalytic efficiency ($K_{cat}/K_{m}$) using the slope of the linear range in the Michaelis-Menten plot. We found that JADE1(1-188) increased the catalytic efficiency of HBO1 by ~5 fold (Figure 5B). Together, this data demonstrates the importance of JADE1 in contributing to the overall catalytic activity of HBO1 HAT complex by anchoring the complex to the substrate and by properly orienting the HBO1 HAT domain.

Having established that the HHBD and HCBD regions of JADE1 contact H3/H4, we asked if other regions of HBO1 also contribute to histone binding. To address this, we first carried out pull down studies with MBP-HBO1-HAT, JADE1(80-188) and H3/H4 to demonstrate that the HBO1-HAT is unable to directly pull down H3/H4 (Figure 6A). Although HBO1-HAT does not make stable direct contacts with the histones in the ground state, we asked if the rest of HBO1 has any contribution toward H3/H4 binding independent of JADE1. To address this possibility, we carried out H3/H4 pull down studies with either GST-HBO1-FL or GST alone. This data demonstrated that unlike HBO1-HAT, HBO1-FL is able to pull down H3/H4 indicating that HBO1 also contains an H3/H4 binding site outside of the HAT domain independent of JADE1 (Figure 6B). Revealing this independent histone binding property of HBO1-FL, we asked if JADE1 and HBO1 can interact with H3/H4 in the presence of each other. To do this, we carried out additional MBP-JADE1 pull-down studies with HBO1-FL and H3/H4 in the presence and absence of JADE1-HHBD. As expected, JADE1(1-188) was able to pull down HBO1-FL independent of H3/H4 binding at HCBD. However, JADE1(108-188) was only able to pull down HBO1-FL in the presence of H3/H4 (Figure 6C). This data suggests that unlike HBO1-HAT, HBO1-FL is able to pull down H3/H4 indicating that HBO1 also contains an H3/H4 binding site outside of the HAT domain independent of JADE1 (Figure 6B). While we...
favor an interpretation that H3/H4 is being bound by the full-length HBO1, given the presence of additional bands in the prep we cannot rule out that some of the interactions are through the smaller fragments.

To investigate the role of the additional histone binding domain N-terminal to the HBO1-HAT region (HBO1-HBD), we asked if the full length HBO1 shows higher enzymatic efficiency towards the substrate. When compared, the catalytic efficiencies of HBO-HAT and HBO1-FL are comparable to each other. This analysis demonstrates that despite the additional histone binding property of HBO1-FL, HBO1-HBD does not have a significant contribution to the overall HBO1 catalytic efficiency toward H3/H4 and requires JADE1(1-188) to potentiate its acetyltransferase activity (Figure 5B). The fact that HBO1-HAT and HBO1-FL have comparable catalytic efficiencies suggests that the additional H3/H4 interactions mediated by HBO1-FL relative to HBO-HAT is not productive for catalysis.

**Cellular experiments with JADE1 deletions recapitulate the role of JADE1 in histone and HBO1 interactions and HBO1 acetyltransferase activity**

In order to support the conclusions drawn by the *in vitro* interaction studies, we performed co-transfections in HEK 293T cells with vectors expressing different truncation constructs of JADE1 followed by anti-Flag immunoprecipitation and peptide elution of the complexes. Western blot analysis of the purified fractions with progressive N-terminal truncations of JADE1 indicates that deletion of the first 113 amino acids, which includes the HHBD, does not disrupt the interaction of JADE1 with the HBO1-H3/H4 complex in cells (Figure 7A), while the additional deletion of the JADE1-HCBD abolishes JADE1 interaction with the HBO1-H3/H4 complex, as reported previously(15). The observation that JADE1 constructs that contain residues C-terminal to the HHBD (53-842, 114-842 and 199-842) immunoprecipitate with ING4/EAF6 are also consistent with our earlier studies(15). We performed a similar experiment using progressive C-terminal deletions of JADE1, demonstrating that the region containing the HHBD is also sufficient to interact with HBO1 in cells (Figure 7B).

Consistent with the *in vitro* studies, acetylation assays of cell immunoprecipitated JADE1 deletion constructs demonstrates that acetylation activity requires both the HHBD and HCB1D regions of JADE1 (Figure 7C). These results demonstrate that, like observed *in vitro*, while the JADE1 HCBD is required to stabilize the HBO1 complex, the JADE1 HHBD region is required to enable HBO1 acetylation activity towards histones.

**Discussion**

Histone tail acetylation events have been linked to many important cellular processes through
controlling chromatin dynamics to mediate various DNA transactions including DNA replication and gene transcription\(^2,4\)). While the structure and catalytic activity of HATs have been well delineated, their mode of substrate-specific acetylation has been less rigorously analyzed. GCN5 has been reported to mediate preferential H3K14 acetylation through making extensive direct contact with the residues of the H3 tail flanking the H3K14 target lysine residue using sequence-based recognition\(^27\). On the other hand, numerous HATs function in multi-subunit complexes where the catalytic efficiency and substrate specificities are determined by subunits outside of the catalytic domain\(^11\). A recent structural study on the NuA4 HAT complex revealed a sequence/position double recognition mechanism for substrate specificity, whereby the HAT complex binds to the nucleosome through a subunit outside of the catalytic domain to position the H4 tail proximal to the catalytic domain, which then employs sequence-based recognition for sequence-specific acetylation\(^28\). Additionally, BRFP2 was recently reported to potentiate and mediate H3 specific acetylation by HBO1 through a short N-terminal fragment that bridges interaction between the HAT domain and H3/H4 \(^26\).

In this study, we dissected the role of JADE1 in HBO1 HAT activity toward free histones (H3/H4) based on previous studies suggesting that JADE1 plays a key role in HBO1 complex H4 substrate specific acetylation. The in vitro and cellular data presented here demonstrates that robust histone H3/H4 acetylation by the HBO1 requires the HBO1-HAT domain (residues 311-611), in addition to a 21-residue N-terminal JADE1 region (HHBD, residues 60-80), which bridges HBO1-HAT and H3/H4 contacts and a second N-terminal JADE1 region (HCBD, residues 108-188) that makes additional H3/H4 contacts within the histone core region (Figure 8A). The N-terminal region of JADE1 therefore functions as a platform to bring the catalytic HBO1 subunit and the free histone H3/H4 substrate tail together for catalysis.

The mechanism of JADE1 activation of the HBO1 HAT complex has similarities and differences to that of BRFP2. Both JADE1 and BRFP2 contain short N-terminal fragments (HHBD in the case of JADE1) that bind to both HBO1 and H3/H4, thus bridging appropriate HBO1-histone interaction for substrate-specific acetylation. Unlike BRFP2, JADE1 contains a second N-terminal histone binding domain (HCBD) that also binds to H3/H4 and cooperates with the HHBD to significantly increase the catalytic efficiency of HBO1 for H4 acetylation.

In addition to the role of the N-terminal region of JADE1 in HBO1 acetylation described here, the role of two C-terminal PHD fingers of JADE1 have previously been characterized. These tandem PHD fingers cooperate to recognize the H3 tail and facilitate the targeting of the HBO1 HAT complex to chromatin for nucleosome
Previous studies have suggested that the HBO1 HAT complex is heavily localized to transcriptionally active regions ahead of polymerase to facilitate nucleosome disassembly, consistent with HBO1 complex copurification with the FACT complex(16). In the context of transcription within chromatin, JADE1 PHD finger-mediated recognition of methylated histones may facilitate the subsequent JADE1 mediated HBO1 acetylation of histone H4 to contribute to nucleosome disassembly through increasing chromatin dynamic post nucleosome disassembly and/or provide recognition marks for other proteins involved in transcription elongation. JADE1 has also been linked to HBO1-mediated histone H4 acetylation during DNA replication(18), although the relative roles of the HHBD, HCBD and other C-terminal JADE1 domains in this process remains to be determined.

**Experimental Procedures**

**JADE1/2/3 N-terminal sequence alignment**

Sequence alignment for JADE1/2/3 was carried out using ClustalW2(29) and was further formatted using ESPript (3.0) for more optimal sequence homology of JADE homologs(30),(31)

**Recombinant HBO1 and JADE1 protein production**

HBO1 and JADE1 protein constructs were generated by PCR amplification of the corresponding DNAs, which were cloned into pRSF or pDB.His.MBP vectors using Sacl/NotI sites with a cleavable N-terminal affinity tags (GST,-6XHIS,-MBP-). These constructs were grown and expressed using BL21(DE3) cells in LB. Cells were induced for protein overexpression at an OD ~0.6 with 0.5mM IPTG for 16-18 hours at 18 °C. Cells were harvested by centrifugation at 4000 RPM and lysed by sonicating in lysis buffer (20mM Tris(pH 8) 500mM NaCl, 5mM BME, 0.1mg/ml PMSF). The post lysed sample was spun down at 20,000 RPM for 30 min. and the soluble fraction of the post-sonicated sample was applied to either, Pierce Glutathione Agarose resin(Thermo), Amylose resin (NE Biolabs) or Ni-NTA resin (Biosciences) for batch binding at 4°C for 1 hour. Samples were washed with 20 column volumes (CV) of wash buffer ( 20mM Tris(pH 8), 500mM NaCl, 5mM BME) then eluted with eluant with either 30mM reduced L-glutathione(GST), 20mM maltose monohydrate(MBP), or 300mM Imidazole(6XHIS). Affinity tags were cleaved with TEV protease or were left on for affinity tag pull-downs. Proteins were further purified to homogeneity by either HiTrapQ(GE Healthcare) anion exchange column for JADE1 constructs or HiTrapHP(GE Healthcare) cation exchange for HBO1 constructs. Ion exchange chromatography was performed using a NaCl gradient from 100mM to 1M over 30 CV). To ensure the proper fold of recombinant proteins, they were subjected to size exclusion chromatography(SEC) using a Superdex 200 column in 20mM Tris(pH 8), 500mM NaCl, 5 mM BME. MBP-JADE1(1-188) mutant constructs were generated through site-
directed mutagenesis using Q5 Hot start polymerase (NEB). All of the mutant constructs were verified by DNA sequencing and were expressed and purified using the same protocol mentioned previously for wild-type constructs.

Isothermal Titration Calorimetry
Interaction of HBO1/JADE1 and histone/JADE1 with various JADE1 constructs were quantitatively analyzed using a MicroCal ITC200 (Malvern). All proteins were prepared and dialyzed to 20mM HEPES (pH 7.5), 500mM NaCl, 5mM BME before the analysis. All ITC experiments were conducted by injecting 800uM of JADE1 deletion constructs into a calorimetry cell either containing 40uM of HBO1 HAT of H3/H4. JADE1 deletion constructs were injected in 2uL increments every 2 minutes at 20°C. ITC data were analyzed using MicroCal ITC-ORIGIN (Malvern).

Histone and nucleosome core particle production
Each of the histones (H3, H4, H2A, H2B) were expressed and purified as inclusion bodies. Refolding of H2A/H2B, H3/H4 and their purification was performed as previously described(31,32). Tailless constructs H3(36-135) and H4(20-104) were prepared the same way as the wild-type. These refolded histone components, in addition to the 601 nucleosome positioning sequence, were used to form nucleosome core particles as previously described(32).

Pull down assay (GST-/MBP-)
2uM of tagged protein with 10uM of untagged protein were incubated together with 50uL of resin for an hour at 4°C. The reactions were carried out in sizing buffer (20mM Tris(pH 8), 500mM NaCl, 5mM BME). Resin was then washed extensively with the same buffer and was collected for analysis on SDS-PAGE gel. All of the pull downs were done side by side with the tag alone under the same conditions as controls for background interactions.

Histone acetyltransferase (HAT) activity assay
HBO1 HAT activity was measured using a previously described HAT assay with 14C labeled Ac-CoA (50-60 mCi/mmole; Moravek)(33). A time course for the reaction was initially performed to determine the linear range of enzyme activity at an enzyme concentration of 200nM (except that 2 uM was used in figure 5A) and saturating concentrations of AcCoA (300uM) and H3/H4 (or nucleosome core particles) (100uM). The HAT reaction was done with 20mM Tris (pH 7.5), 500mM NaCl, 0.25mg/ml BSA for 1 hour at 20°C in a volume of 50uL. The reaction was spotted on P81 filter paper. The positively charged and C14-labeled H4 peptide portion of the histone complex(H3/H4) was captured on the filter paper, while free 14C -labeled Ac-CoA was washed away. The paper was analyzed using Liquid Scintillation(34). The amount of 14C -labeled H4 peptide bound to the paper is directly proportional to the amount of acetyl group transferred from the cofactor to.
peptide, thus the data was used to evaluate HBO1 acetyltransferase activity (34). All reactions were done in duplicate. Using the known concentration series of Ac-CoA, a standard curve was generated. The standard curve was used to convert radioactive counts to molar units.

**JADE1/HBO1 complexes cotransfection in 293T cells**

JADE1L, HBO1, hEAF6 and ING4 constructs were generated by PCR amplification of the corresponding DNAs, which were cloned into pcDNA3 vector encoding a N-terminal Flag tag using BglII/XhoI sites or a HA tag. These plasmids were then used to cotransfected 293T cells by the calcium phosphate method for protein complex overexpression with HA-HBO1/HA-ING4/HA-hEAF6 and full length or N-terminal truncated 3xFlag-JADE1L: amino acid 1-842, 53-842, 114-842, 199-842 and 1-113. A second set of cotransfection was done using C-terminal truncated 1x Flag JADE1: the first 113, 198 or 509 amino acids were overexpressed in combination with HA-HBO1.

Cells were harvested 48h post-cotransfection and lysed in high salt buffer (450mM NaCl, 50mM Tris-HCl pH 8.0, 1% TX-100, 2mM MgCl2, 0.1mM ZnCl2, 2mM EDTA, 10% glycerol) supplemented with protease inhibitor mixture. The NaCl concentration was reduced to 225mM and the whole cell extract centrifuged at 14,000 RPM for 30 minutes. The Flag JADE1 was purify from the soluble fraction using anti-Flag M2 affinity beads (Sigma) for 2h at 4°C on rotating wheel. Flag beads were next washed with the 225mM NaCl buffer and eluted with 400ug/ml of 3X or 1X Flag peptide (Sigma) for the N-terminal or the C-terminal truncations, respectively.

**Immunoblotting**

The amount of the different JADE1 construct was normalized empirically following SDS-PAGE and transfer onto nitrocellulose membrane. Anti-Flag M2 conjugated to horse radish peroxidase (HRP; Sigma) was used at a 1:10,000 dilution and the immunoblots were visualized using a Western Lightning plus-ECL reagent (Perkin-Elmer). Anti-HA 3F10 (Roche, rat polyclonal) and anti-HBO1 (Novus, rabbit polyclonal) antibodies were used at a 1:1,000 dilution. HRP conjugated secondary anti-rat and anti-rabbit antibodies (Sigma) were used at a 1:10,000 dilution.

**HAT activity assays on purified JADE1 complexes**

HBO1 activity in the purified JADE1 complexes was measured with 0.125uCi of ³H labeled Ac-CoA (2.1Ci/mmol; PerkinElmer Life Sciences). The amount of the different purified JADE1 complexes used in the HAT assays were normalized based on the immunoblotting data. The HAT reactions were performed in a volume of 15 or 20ul using 0.5ug of human free histones or native short oligonucleosomes as substrate, respectively, in HAT buffer (50mM Tris-HCl (pH 8), 50mM KCl, 10mM sodium butyrate, 5% glycerol, 0.1mM EDTA, 1mM dithiothreitol) for
1 hour at 30°C. The reactions were then captured on P81 filter paper, the free $^3$H-labeled Ac-CoA was washed away and the paper was analyzed using Liquid Scintillation. The $^3$H counts were used to evaluate HBO1 acetyltransferase activity in the complexes containing different JADE1 constructs.

**Acknowledgements:** We thank Rob Magin and Adam Olia for helpful discussions. This work was supported by grants from the National Institutes of Health grants R01 GM060293 and R35 GM118090 and the Ovarian Cancer Research Fund Alliance awarded to R.M., the Canadian Institutes of Health Research (CIHR; FDN-143314) to J.C and National Institutes of Health grant R01 GM082989 to B.E.B. We acknowledge support of the DNA Sequencing Facility at the Perelman School of Medicine, University of Pennsylvania (NIH P30 CA016520). J.C. holds the Canada Research Chair in Chromatin Biology and Molecular Epigenetics.

**Conflict of interest:** The authors declare that they have no conflicts of interest with the contents of this article.

**Author contributions:** JH designed and performed all but the cell-based experiments and prepared all of the figures, excluding Figure 7, and a first draft of the text. Critical reagents and advice for the in vitro experiments were provided by MDR, CEM and MG. CL designed and performed the cell-based experiments shown in Figure 7, prepared Figure 7 and LC and JC wrote a first draft of the associated text. RM designed and supervised the experiments by JH, MDR and CEM. BEB designed and supervised the experiments by MC. JC designed and supervised the experiments by CL. BEB, JC and RM revised the text, and advised regarding figure presentation. All authors read and approved the manuscript.
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Figure Legends

Figure 1. An N-terminal region of JADE1 binds HBO1.
   A. Sequence alignment of the N-terminal region of JADE1/2/3 paralogs with the highest degree of conservation shaded in red with conservative substitutions colored in red. The HBO1 and Histone Binding Domain (HHBD) is highlighted.
   B. MBP-JADE1 pull downs with various N-term and C-term deletion constructs of the HBO1-HAT domain and H3/H4 complex with results resolved on SDS-PAGE.
   C. MBP-JADE1 pull downs of the HBO-HAT with JADE1 triple alanine mutations with results resolved on SDS-PAGE.
   D. Isothermal Titration Calorimetry (ITC) studies of JADE1 constructs titrated into the HBO1-HAT showing the heat profile (top) and the calculated binding isotherm (bottom). Thermodynamic data calculated from this data is shown to the right.

Figure 2. JADE1 requires an additional N-terminal region for H3/H4 binding and HBO1 HAT potentiation.
   A. Titration of JADE1 constructs (55-85, 1-188) from 0- to 16-fold molar excess of HBO1 HAT domain tested for acetyltransferase activity on the H3/H4 complex. Radioactive counts are converted to enzyme rate as described in the methods section.

Figure 3. The additional JADE1 N-terminal Histone Core Binding Domain (HCBD) specifically binds the histone core region.
   A. MBP-JADE1 pull-downs of four different H3/H4 complexes (H3/H4,H3(TL)/H4,H3/H4(TL), H3(TL)/H4(TL)) with results resolved on SDS-PAGE. (TL=tailless)
   B. GST-H4(1-19) pull downs of HBO1-HAT in the presence and absence of JADE1(1-188) with results resolved on SDS-PAGE.
   C. Isothermal Titration Calorimetry (ITC) studies of JADE1 constructs titrated into the H3/H4 complex showing the heat profile (top) and the calculated binding isotherm (bottom). Thermodynamic data calculated from this data is shown to the right.
   D. MBP-JADE1 pull downs of HBO1-HAT and H3/H4 complex in the presence and absence of each other with results resolved on SDS-PAGE.

Figure 4. JADE1 selectively binds to H3/H4 over H2A/H2B and the nucleosome core particle.
   A. MBP-JADE1 pull downs of HBO1 HAT and (H3/H4 or H2A/H2B) in the presence or absence of each other with results resolved on SDS-PAGE.
B. MBP-JADE1 pull downs of HBO1 HAT and H3/H4, H2A/H2B and mixtures of the two with results resolved on SDS-PAGE.

C. MBP-JADE1 pull downs of H3/H4 or nucleosome core particles with results resolved on SDS-PAGE.

D. Activity comparison of HBO1 HAT and HBO1 full length in the presence and absence of saturating concentration of JADE1(1-188) as either H3H4 complex of Nucleosome core particles as the substrate. The activity of these proteins on various substrate was compared using radioactive counts.

**Figure 5. JADE1-HHBD and -HCBD work cooperatively to activate HBO1 HAT activity**

A. Titration of JADE1 constructs (1-188, 60-188, 80-188, 1-80, 1-80/80-188) from 0- to 25-molar excess over HBO1 HAT domain tested for acetyltransferase activity on H3/H4 complex. Radioactive counts are converted to enzyme rate as described in the methods section.

B. Plot of the linear range of the Michaelis-Menten plot of HBO1-HAT, HBO1-FL +/- JADE1 with H3/H4 as the substrate to calculate the catalytic efficiency (slope, $K_{cat}/K_m$). Radioactive counts are converted to enzyme rate as described in the methods section.

**Figure 6. HBO1-FL contains a N-terminal H3/H4 binding domain that binds histones independently of JADE1.**

A. MBP-HBO1-HAT pull down of H3/H4 of H3(TL)/H4(TL) in the presence and absence of JADE1-HCBD with results resolved on SDS-PAGE.

B. GST-HBO-FL and GST control pull down of H3/H4 complex with results resolved on SDS-PAGE. Note that JADE1(80-188) and H3.1 co-migrate on SDS-PAGE.

C. MBP-JADE1(1-188) and (108-188) pull down of HBO1-FL +/- H3/H4 complex with results resolved on SDS-PAGE.

**Figure 7: JADE1 HHBD and HCBD both are required for HBO1 acetyltransferase activity on histones in cells.**

A. Western blot analysis of JADE1 complexes with HBO1, H3, ING4 and EAF6 with different N-terminal JADE1 truncations. Anti-HA immunoblot was done first to visualize the HA-tagged subunits of the Flag-JADE1 complex overexpressed in 293T cells. The membrane was then reprobed with the HBO1 antibody (showing endogenous HBO1 association beside HA-HBO1) and finally with the anti-Flag antibody.
B. HBO1 association with the C-terminal truncated forms of JADE1. Anti-HA was followed by anti-Flag immunoblot on Flag-JADE1 complex overexpressed in 293T cells (* denotes remaining HBO1 HA signal in anti-Flag blot).

C. HAT assay of immunopurified complexes from 7A. HAT assay were performed on free histones (0.5µg) with JADE1 complex using the same ratio as for the immunoblotting. The graphical representation of counts per minutes (CPM) measured by scintillation and a fluorogram of radioactive HAT assay on free histones.

Figure 8. Schematic view of the HBO1, JADE1, H3/H4 complex based on the data obtained in this study.

A. Schematic view of JADE1 and HBO1 domains.

B. Schematic model of JADE1-HBO1 domain interactions.
Fig 1

A

B

C

D

**FIGURE 1**

**A**

- **JADE1** (1-188)
- **MBP** (1-60, 60-104)
- **HBO1-HAT**

**B**

- **JADE1** (1-188)
- **MBP-JADE1** (1-188)
- **HBO1** (60-188)
- **HAT**

**C**

- **JADE1** (1-188)
- **MBP-JADE1** (1-188)
- **HBO1-HAT**

**D**

- **HBO1-HAT** + **JADE1** (60-188)
- **HBO1-HAT1** + **JADE1** (55-85)
- **HBO1-HAT** + **JADE1** (80-188)

**Kd** = 2.4μM

ΔH = -1.479E4

ΔS = -24.8 cal/mol/deg

**Kd** = 4.18μM

ΔH = -1.45E4

ΔS = -24.9 cal/mol/deg
Fig 5

Complex $\left( \frac{K_{cat}}{K_{m}} \right)$ (uM$^{-1}$min$^{-1}$)

HBO1-HAT1 0.01299 ± 0.0004698
HBO1-HAT1/JADE1(1-188) 0.06096 ± 0.001446
HBO1-FL 0.01758 ± 0.0009860
HBO1-FL/JADE1(1-188) 0.06316 ± 0.001065

| Complex                  | $\left( \frac{K_{cat}}{K_{m}} \right)$ (uM$^{-1}$min$^{-1}$) |
|--------------------------|---------------------------------------------------------------|
| HBO1-HAT1                | 0.01299 ± 0.0004698                                           |
| HBO1-HAT1/JADE1(1-188)   | 0.06096 ± 0.001446                                           |
| HBO1-FL                  | 0.01758 ± 0.0009860                                           |
| HBO1-FL/JADE1(1-188)     | 0.06316 ± 0.001065                                           |
Fig. 8

A

JADE1(1-188)

H3/H4 Core

1 60 80 108 160 188

HHBD HCBD

H3/H4 [HAT domain]

1 302 611

HBO1

N-term HBD

HAT domain

H3/H4

B

JADE1

HCBD

H3/H4 Core

HHBD

HAT

HBD

HBO1

N-term HBD
The scaffolding protein JADE1 physically links the acetyltransferase subunit HBO1 with its histone H3/H4 substrate

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*J. Biol. Chem.* published online January 30, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA117.000677

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