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Histone Acetyltransferase Activity Is Conserved between Yeast and Human GCN5 and Is Required for Complementation of Growth and Transcriptional Activation

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Yeast and human ADA2 and GCN5 (y- and hADA2 and y- and hGCN5, respectively) have been shown to potentiate transcription in vivo and may function as adaptors to bridge physical interactions between DNA-bound activators and the basal transcriptional machinery. Recently it was shown that yGCN5 is a histone acetyltransferase (HAT), suggesting a link between enzymatic modification of nucleosomes and transcriptional activation. In this report, we demonstrate that hGCN5 is also an HAT and has the same substrate specificity as yGCN5. Since hGCN5 does not complement functional defects caused by deletion of yGCN5, we constructed a series of hGCN5-yGCN5 chimeras to identify human regions capable of activity in yeast. Interestingly, only the putative HAT domain of hGCN5, when fused to the remainder of yGCN5, complemented *gcn5*$^-$ cells for growth and transcriptional activation. Moreover, an amino acid substitution mutation within the HAT domain reduced both HAT activity in vitro and transcription in vivo. These findings directly link enzymatic histone acetylation and transcriptional activation and show evolutionary conservation of this potentially crucial pathway in gene regulation.

Transcriptional activation by RNA polymerase II (RNAPII) is highly regulated and requires coordinated function of at least three classes of transcription factors. Activators, composed of separable DNA-binding and activation domains, bind upstream of the transcriptional start site to the enhancer or upstream activation site. The basal transcriptional machinery, comprising RNAPII and accessory factors, binds to the core promoter consisting of the TATA box and initiation site (23, 54). A third class of proteins, termed coactivators, mediators, or adaptors, are also required for transcriptional activation (24). These cofactors may have multiple roles and mechanisms; however, a unifying theme may be that they facilitate productive interaction between activators and the basal machinery.

Coactivators include several types of factors or complexes which have been isolated through associations with basal factors or with activators. TATA-binding protein (TBP)-associated factors (TAFs) assemble with the TBP into the TFIID complex. TFIID, and not TBP alone, is required for transcriptional activation in vitro (18). Certain TAFs directly interact with activators (10), leading to a model in which specific interaction between activators and coactivators recruits the TFIID complex to the promoter during transcriptional activation. Recently, kinase activity has been associated with TAF250 (14); however, the relevance of enzymatic activity to transcriptional activation has not been demonstrated.

Other coactivators were discovered through their interaction with specific activators and later shown to potentiate activation in vivo. CREB-binding protein (CBP) was isolated as a protein that binds directly and specifically to the phosphorylated form of CREB and was subsequently shown to potentiate transcription of a variety of activators (12, 36). OBF-1 (53), or OCAB (39), is a lymphocyte-specific coactivator for Oct1, and TIF1 is a coactivator for nuclear steroid receptors (16).

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Genetic approaches with *Saccharomyces cerevisiae* (yeast) have identified more global transcriptional regulators, including the SWI/SNF complex, which alters chromatin structure (for reviews, see references 9 and 43). SWI/SNF2, as well as related higher eukaryotic homologs, possesses ATPase activity, which is required for normal growth and transcriptional activation in vivo (30, 37) as well as stimulation of GAL4 activator binding to nucleosomal DNA in vitro (13). SRBs are associated with the RNAPII holoenzyme (33, 34) and, as the mediator components of the holoenzyme, are required for transcriptional activation (31, 33). Finally, SPTs are functionally involved with both basal factors and chromatin and include multiple potential mechanisms (15, 19, 45).

Another genetic selection in yeast identified proteins that functionally interact with the acidic activation domain of the herpes simplex virus activator VP16. Several genes, termed *ADA2* (2), *ADA3* (44), *GCN5* (42), and *ADA5* (41), were cloned. Mutations in any of these genes slowed yeast growth and reduced transcriptional activation by some acidic activators, such as VP16 and yeast GCN4 (yGCN4), but had relatively little effect on other activators, such as HAP4 (2, 44). ADA2 physically interacted with activation domains derived from VP16 and GCN4 and also with TBP (1, 51). ADA2, ADA3, and GCN5 interacted with each other in vitro (28, 42) and in vivo (7), which argued strongly for the existence of a physiologically relevant ADA complex. Taken together, these data suggest that the ADA complex functions as an adaptor, to bridge interactions between activation domains and the basal machinery.

Recently, GCN5 was identified as a nuclear histone acetyltransferase (HAT) (6). Histone acetylation has long been correlated with modulation of gene activity (55, 59). Acetylation of lysines in histone amino-terminal tail domains reduces the positive charge to weaken histone-DNA interactions. This alteration in binding may derepress chromatin structure and ultimately activate transcription. There are several correlative

lines of evidence to support a role for histone acetylation in gene regulation. Hyperacetylation of large chromosomal domains, such as the mammalian globin gene cluster (27), has been correlated with gene activity. In contrast, hypoacetylated histones have been localized to domains of gene inactivity (3, 29). Genetic evidence has related acetylation and deacetylation of specific histones to activities of specific genes. For example, deletion and substitution mutations of specific lysines in the amino-terminal tail of histone H3 induced the yeast *GAL1* gene (40), while similar mutations in H4 decreased the expression of *GAL1* and *PHO5* (17). Biochemical approaches have indicated that acetylation influences the ability of transcription factors to bind to promoter elements (38, 56). Thus, the finding that GCN5 possesses HAT activity suggests, but does not establish, a direct relationship between regulation of chromatin structure by GCN5 and its gene activation properties.

Human proteins with strong sequence homology to yADA2 and yGCN5 have been identified (8). These human proteins (hADA2 and hGCN5) show functional similarities to the yeast proteins; for example, hGCN5 interacted with both hADA2 and yADA2 in the yeast two-hybrid assay and hGCN5 and yGCN5 had similar stabilizing effects on yADA2 in vivo. Furthermore, hADA2 and hGCN5 augmented GAL4-VP16 activation in HeLa cells. In spite of these similarities, hGCN5 did not complement deletion of yGCN5 in yeast, either for growth or transcriptional activation (8).

Here, we report additional similarities between hGCN5 and yGCN5. Recombinant hGCN5 and yGCN5 acetylated histones with comparable levels of strength and identical substrate specificities. Chimeric proteins were constructed with yGCN5 and hGCN5 to identify possible functional domains of hGCN5 in yeast. We demonstrate that, whereas the full-length hGCN5 failed to complement yGCN5 function in yeast, the HAT region of hGCN5 functionally substituted for this region of yGCN5 for both growth complementation and transcriptional activation. Moreover, a single amino acid substitution mutation within the HAT region of the chimeric GCN5 that lowered HAT activity also reduced the ability to complement transcriptional activation. These findings directly link HAT activity to transcriptional activation in vivo.

MATERIALS AND METHODS

Construction of GCN5 chimeras. Chimeras are named according to the species of origin of each subregion (in yGCN5, the regions are 1 to 116, 117 to 260, 261 to 348, and 349 to 439, and in hGCN5, the regions are 1 to 110, 111 to 251, 252 to 339, and 340 to 427). All chimeric DNA fragments were generated by PCR.

For the *hhyy* construct, yGCN5(261-439) was cloned as a 5′-*Not*I/3′-*Bgl*II fragment into the pBTMN vector (8) at *Not*I and *Bam*HI sites. hGCN5(1-251) was generated as a 5′-*Not*I/3′-*Eag*I fragment and inserted into pBTMN-yGCN5(261-439) at the *Not*I site.

For the *yyhy* construct, yGCN5(349-439) was cloned as a 5′-*Bam*HI/3′-*Bgl*II fragment and inserted into pBTMN at the *Bam*HI site. The construct was opened at the *Bam*HI site, and the hGCN5(252-339) fragment with 5′-*Bam*HI/3′-*Bgl*II ends was ligated. Finally, the construct was opened at *Not*I and *Bam*HI sites to ligate yGCN5(1-260) as a 5′-*Not*I/3′-*Bgl*II fragment.

For the *yyyh* construct, hGCN5(340-427), with 5′-*Bam*HI/3′-*Bgl*II ends, was inserted into pBTMN at the *Bam*HI site. The construct was opened at the *Not*I and *Bam*HI sites to insert yGCN5(1-348) with 5′-*Not*I/3′-*Bgl*II ends.

For the *hhhy* construct, hGCN5(1-339) was amplified with 5′-*Not*I/3′-*Eco*RI-stop-*Bam*HI primers, and the fragment was inserted into pBTMN at the *Not*I/*Bam*HI site to yield hGCN5ΔBrD. Then the construct was opened at the *Eco*RI and *Bam*HI sites to ligate yGCN5(349-439) as an *Eco*RI/*Bgl*II fragment.

For the *yhhh* construct, hGCN5(111-427), with 5′-*Not*I/3′-*Eco*RI ends, was inserted into pBTMN at *Not*I/*Eco*RI sites; the resulting clone was opened at the *Not*I site to ligate yGCN5(1-116) as an *Eag*I fragment.

The yeast expression vector pPC87 was modified from pPC86 (11) by removing the GAL4-DNA-binding domain and inserting a new linker with *Not*I and *Sal*I sites. The *yyhy*, *hhyy*, *hhhy*, and *yhhh* constructs were subcloned into pPC87 as *Not*I/*Sal*I fragments.

Two-hybrid plasmid construction. yGCN5(1-250) and yGCN5(250-440) were isolated from pSP64 clones (7) as *Eag*I fragments and inserted into BTMN at the *Not*I site. hGCN5(1-224) and hGCN5(225-427) were amplified by PCR as *Eag*I fragments and inserted in BTMN at the *Not*I site.

Yeast strains and assays. The wild-type strain is the *trp1* derivative of PSY316 (*MAT***a** *ura3-52 leu2-3,112 his3*Δ*200 lys2*). The construction of the *gcn5*$^-$ strain, which was used in all complementation experiments, was described previously (8). Yeast transformation was carried out by the lithium acetate protocol. β-Galactosidase assays were performed by breaking cells with glass beads, and β-galactosidase activity was normalized to protein (46). Yeast two-hybrid assays were done with the L40 strain (57).

Protein expression. *hGCN5* containing 5′-*Bgl*II/3′-*Eco*RI ends isolated from the original phage clone (8) was inserted into the pRSET vector (Invitrogen) at *Bam*HI-*Eco*RI sites. The *hhyy* construct from the pPC87 clone was inserted into pRSET at *Not*I-*Hin*dIII sites. GCN5 proteins, expressed with six histidine residues fused to the amino terminus, were purified on Ni^{2+}-nitrilotriacetic acid agarose (Qiagen) from bacteria sonicated under nondenaturing conditions, according to the manufacturer's instructions. The concentrations of recovered GCN5 proteins were estimated by visual comparison to known amounts of bovine serum albumin stained with Coomassie blue (data not shown). Although preliminary experiments suggested these estimates were sufficiently accurate to enable comparisons of enzyme activities, we employed antiserum to hGCN5 in immunoblot assays (see Fig. 7B) for final normalization of the enzyme concentrations employed in HAT determination.

hGCN5 antiserum was prepared as described previously for yGCN5 antiserum (8).

HAT assay and analysis of histone acetylation. Liquid assays for HAT activity were performed essentially as previously described (5). Acid-extracted chicken erythrocyte core histones (10 μg) and enzyme samples were incubated for 10 min at 30°C in a final volume of 50 μl of buffer A (50 mM Tris-HCl [pH 8.0], 10% [vol/vol] glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA). Reactions were initiated by the addition of [^{3}H]acetyl coenzyme A (100 nCi, 6.1 Ci/mmol; ICN) to a final concentration of 0.328 μM. HAT activity was determined by spotting aliquots (typically 20% of the total) of each reaction mixture on P-81 filters (Whatman) which were then processed for liquid scintillation counting as described before (5). Aliquots (typically 80% of the total) of assay reaction mixtures were resolved on 12% polyacrylamide gels, stained with Coomassie blue, and then fluorographed to identify acetylated proteins.

GST binding assays. Glutathione *S*-transferase (GST)-hADA2 was constructed by inserting h*ADA2* as a 5′-*Bam*HI/3′-*Eco*RI fragment into pGEX-3 (Pharmacia). GST-hGCN5 was constructed by inserting h*GCN5* as a 5′-*Bgl*II/3′-*Eco*RI fragment into pGEX-3.

GST fusion protein beads were prepared as described previously (1). The amounts of GST fusions were estimated by visual inspection of Coomassie blue-stained protein following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently, amounts were normalized for each binding assay. In vitro-translated proteins (5 to 20 μl; TNT kit from Promega) were incubated with GST fusion beads in 200 μl of binding buffer (50 mM NaCl, 20 mM HEPES [pH 7.6], 5 mM $MgCl_2$, 0.5 mM EDTA, 15% glycerol, 1 mM dithiothreitol, and proteinase inhibitors) as indicated in the figures. The binding reaction mixtures were rotated at 4°C for 1 h and then at room temperature for 1 h. The beads were washed in binding buffer with 250 mM NaCl and 0.05% Nonidet P-40. Proteins bound to beads were eluted in SDS-gel buffer and boiled for 5 min just before being loaded on SDS-polyacrylamide gels. The amount of input protein was either 33 or 50% of the protein used for reaction, as indicated in the legend to Fig. 2.

Site-directed mutagenesis. A Chameleon double-stranded site-directed mutagenesis kit (Stratagene) was used to make the point mutation in the *hhyy* construct with the oligonucleotide 5′-TTTGACCCGAAGGCCAAGACTCTG GCC-3′ .

RESULTS

Histone H3 acetylation by hGCN5 and yGCN5. We compared recombinant hGCN5 to yGCN5 for HAT activity. GCN5 from various sources was tested for HAT activity in a liquid assay with an equimolar mixture of free chicken core histones H2A, H2B, H3, and H4 as the substrate. Incorporation of [^{3}H]acetyl label into individual histones was then visualized by fluorography of reaction products separated by SDS-PAGE (Fig. 1). In this assay, both yGCN5 and hGCN5 showed similar labeling efficiencies and strong preference for H3, and little, if any, acetyl label was observed in any of the other core histones (Fig. 1). Thus, the yeast and human catalytic HAT subunits had comparable activities when assayed in this fashion.

hGCN5 and yGCN5 interactions with ADA2. We previously reported that hGCN5 interacted with both hADA2 and yADA2 in a yeast two-hybrid assay (8). We wished to deter-

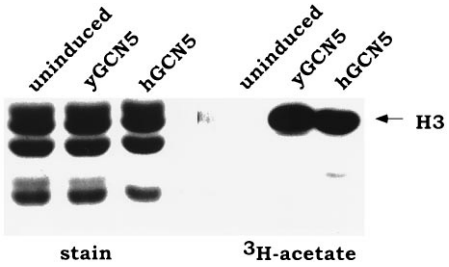

FIG. 1. hGCN5 has HAT activity and acetylates H3 in vitro. HAT liquid assay aliquots with crude extracts from uninduced bacteria, Ni^{2+}-nitrilotriacetic acid-purified yGCN5, and hGCN5-expressing bacteria were analyzed by SDS-PAGE and fluorography. The left panel (stain) demonstrates that all four core histones were present in approximately equimolar amounts. The right panel ([^{3}H]acetate) demonstrates that H3 is the major acetate acceptor for both yGCN5 and hGCN5. Only the core histone region of the gel is shown. Labeling was not observed for proteins migrating elsewhere in the gel.

mine whether the interactions of hGCN5 with the ADA2s were similar in vitro, in the absence of other yeast proteins. Thus we tested pairwise interactions of the human and yeast proteins using GST fusions (52) and standard pull-down assays (Fig. 2A). GST fusion proteins to hADA2 and hGCN5 were prepared and coupled to Sepharose-glutathione beads. Similar amounts of GST fusion proteins were incubated with in vitro-translated h- or yGCN5 or h- or yADA2, and the percent retention of each protein relative to input was determined. GST-hGCN5 retained similar percentages of hADA2 and yADA2 input (21 versus 36%), while GST-hADA2 retained a percentage of hGCN5 input protein (16%) higher than that of yGCN5 (4%) (Fig. 2A). Thus in vitro binding results paralleled previous two-hybrid interaction results (8), indicating similar physical interactions between hGCN5 and either hADA2 or yADA2.

Since hGCN5 interacted with both hADA2 and yADA2 in vivo and in vitro, we wished to determine whether a similar region of hGCN5 interacted with hADA2 or yADA2. We previously found that amino acid residues 250 to 350 of yGCN5 were necessary and sufficient for interaction with yADA2 (7, 8). Therefore, we fused subregions of GCN5 to the LexA DNA-binding domain (LexA) to test interaction of GCN5 with ADA2 in the yeast two-hybrid system.

Three fusion proteins were prepared with hGCN5 and LexA (Fig. 2B). The first contained a deletion of the bromodomain (BrD) (LexA-hGCN5ΔBrD), a conserved motif present in a variety of transcriptional regulatory factors (26). The second fusion protein contained the amino-terminal half of GCN5 (LexA-hGCN5$_N$), and the third contained the carboxyl-terminal half (LexA-hGCN5$_C$). For comparison, similar fusions were made between LexA and portions of yGCN5. All fusions were tested for interaction with h- or yADA2 fused to the VP16 transcriptional activation domain (hADA2-VP16 or yADA2-VP16).

The fusion proteins were cotransformed into yeast, and interaction between them was quantitated at the *lexA* promoter driving the bacterial *lacZ* gene (Fig. 2B). LexA-hGCN5ΔBrD interacted with either hADA2-VP16 or yADA2-VP16 (Fig. 2B), as had been reported previously for LexA-yGCN5ΔBrD (42). As previously reported (8), yGCN5 did not interact with hADA2. The carboxyl-terminal half of hGCN5 (LexA-hGCN5$_C$), but not the amino-terminal half, interacted with hADA2, just as the carboxyl-terminal half of yGCN5 (LexA-yGCN5$_C$) interacted with yADA2 (Fig. 2B). Moreover, LexA-hGCN5$_C$ also interacted with yADA2. Overall, the nature of

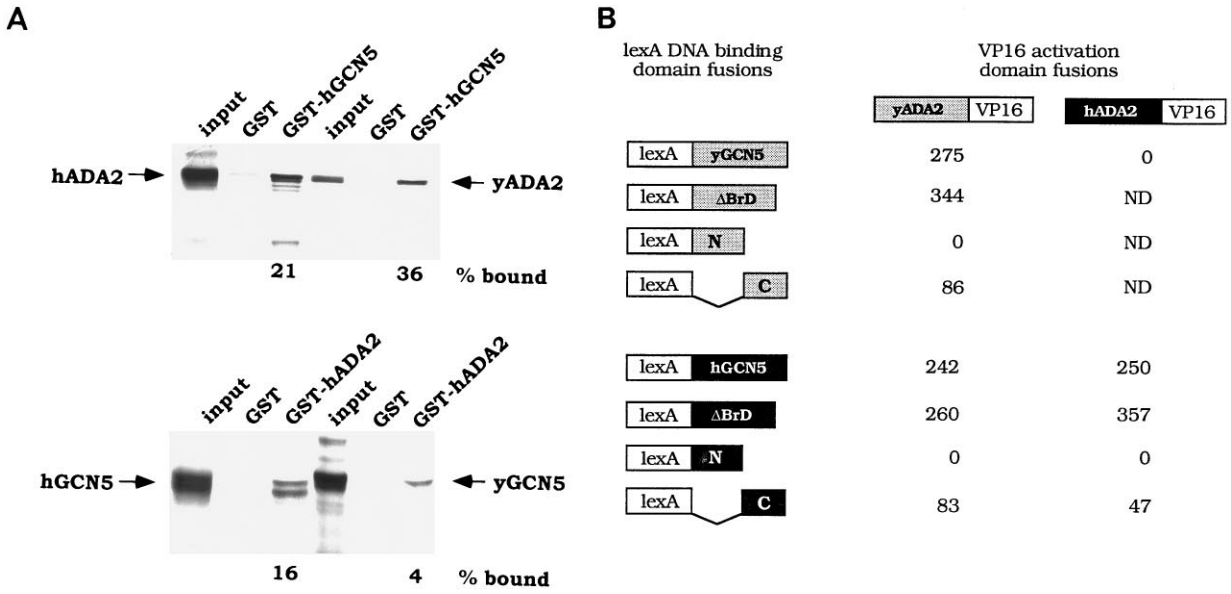

FIG. 2. Interspecies interaction between GCN5 and ADA2 in vitro and in vivo. (A) Interspecies interaction of GCN5 and ADA2 in vitro. (Top) In vitro-translated hADA2 (left) and yADA2 (right) were incubated with GST or GST-hGCN5 coupled to Sepharose beads. ^{35}S-labeled, in vitro-translated proteins bound to GST fusions were analyzed by autoradiography following SDS–8% PAGE. The input amounts were one-third the amounts of proteins used in binding assays. (Bottom) In vitro-translated hGCN5 (left) and yGCN5 (right) were incubated with GST or GST-hADA2-coupled beads. The proteins were analyzed as described above. The input amounts were 50% of the amounts of in vitro-translated proteins used in binding assays. The percentages of bound proteins were determined by phosphorimaging. The data shown is representative of three independent binding assays. (B) Two-hybrid interaction assay between GCN5 and ADA2. y- or h*GCN5* or *GCN5* deletion mutants fused to the bacterial LexA DNA-binding domain were cotransformed into yeast strain L40 with y- or h*ADA2* fused to the VP16 activation domain, as indicated schematically. The chromosomally integrated reporter contained eight LexA binding sites upstream of the bacterial *lacZ* gene. β-Galactosidase activity was determined as units per milligram of protein. The background activity of each construct was less than 1 U. The β-galactosidase activities represent averages of between two and six independent experiments, and the standard error varied between 1 and 12%. ND, not determined; N and C, N and C terminus, respectively.

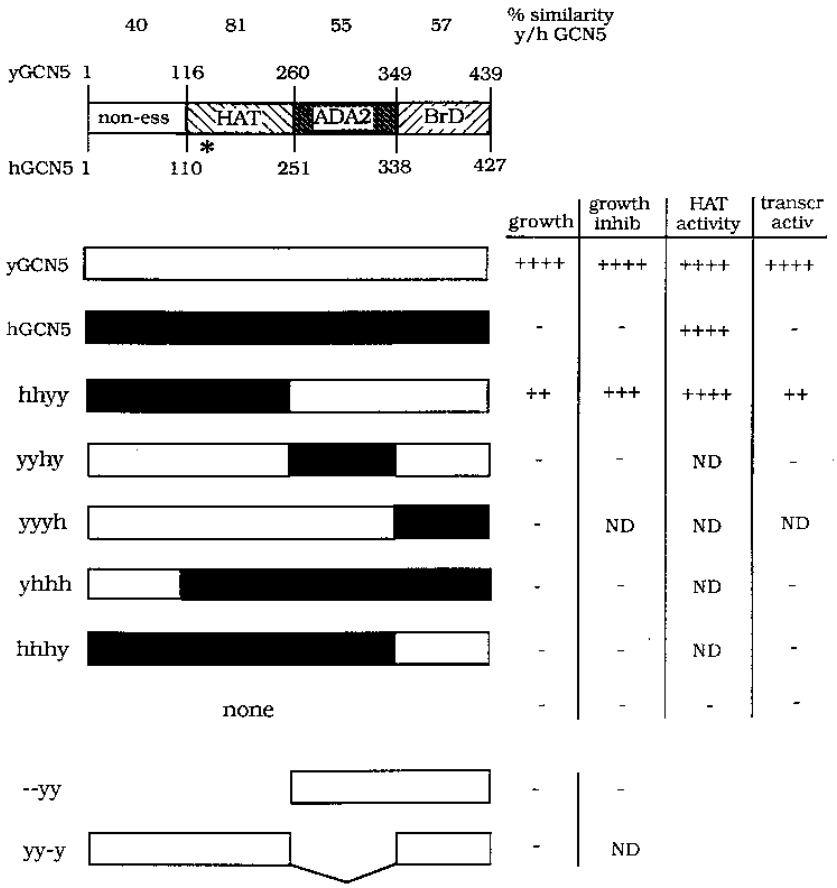

FIG. 3. Structures and phenotypes of yGCN5, hGCN5, and yGCN5-hGCN5 chimeras. In the schematic, y- and hGCN5 were divided into four regions based on deletion analyses of yGCN5: nonessential for growth (non-ess), histone acetylation (HAT), interaction with ADA2 (ADA2), and BrD motif. The percentage of sequence similarity between yGCN5 and hGCN5 is indicated above each region. Histidine 138 in hGCN5 is indicated by an asterisk. In the representations of chimeras, yGCN5 sequences are pictured as open boxes and hGCN5 sequences are pictured as filled boxes. Each chimera is designated by a four-letter code indicating the species origin of each of the four regions comprising the chimeric proteins. The experiments summarized in the columns are (from left to right) growth complementation in the *gcn5*$^-$ strain (Fig. 4), complementation of growth inhibition (inhib) by GAL4-VP16 in the *gcn5*$^-$ strain (Fig. 5), HAT activity in vitro (Fig. 1 and 7A), and complementation of transcriptional activation (transcr activ) by GAL4-VP16 and GAL4-p53 in the *gcn5*$^-$ strain (Fig. 6 and 7C). Activity similar to that of wild-type yGCN5 is indicated by degree with + signs, with ++++ indicating highest similarity; activity similar to deletion of yGCN5 is indicated by −. ND, not determined.

interactions between hGCN5 and both h- and yADA2 was parallel to that of yGCN5-yADA2 interactions.

Engineering of human-yeast chimeric GCN5 proteins. Thus, these assays of h- and yGCN5 indicated considerable similarities in function between the proteins, including comparable levels of strength and specificities of HAT activity, as well as comparable levels of interaction of hGCN5 with either hADA2 or yADA2. It was therefore surprising that these functional similarities were not reflected in growth and transcriptional complementation of hGCN5 in a yeast strain with y*GCN5* deleted (8). To determine whether subregions of hGCN5 are conserved in function in vivo, a series of chimeric proteins were constructed and tested for complementation (summarized in Fig. 3).

The protein was divided into four subregions, based on sequence conservation between yGCN5 and hGCN5 (Fig. 3) and functional analyses. The first region was at the amino terminus (in yGCN5, amino acids 1 to 116; in hGCN5, amino acids 1 to 110); this region has low sequence similarity between yGCN5 and hGCN5 and, in yGCN5, was not required for growth complementation in the *gcn5*$^-$ strain (8a). The second region (in yGCN5, amino acids 117 to 260; in hGCN5, amino acids 111 to 251) has very high sequence similarity between yGCN5 and hGCN5. This region in yGCN5 comprises the enzymatic HAT domain and was critical for growth complementation and transcriptional activation in vivo (8a). The third region (in yGCN5, amino acids 261 to 348; in hGCN5, amino acids 252 to 339) has intermediate sequence similarity and was necessary and sufficient for GCN5 interaction with ADA2 (Fig. 2) (7, 8). The fourth and last region (in yGCN5, amino acids 349 to 439; in hGCN5, amino acids 340 to 427) contains the BrD motif, which was required for full growth complementation of yGCN5 in the *gcn5*$^-$ strain (42).

Based on these subregions, several chimeric proteins were constructed with hGCN5 and yGCN5. Each of the chimeras diagrammed in Fig. 3 is designated by a four-letter code indicating the species of origin (yeast or human) of the four regions described above. The ability of the chimeras to function in yeast was tested in several assays.

The hhyy chimera, containing the human HAT domain, partially complemented the *gcn5*$^-$ growth defect. We tested growth complementation by each of the chimeras in the *gcn5*$^-$ strain. As was previously observed (21, 42), yeast lacking yGCN5 grew extremely poorly on minimal medium (Fig. 4A). As noted before (8), transformation of y*GCN5* completely complemented the growth defect, while transformation of

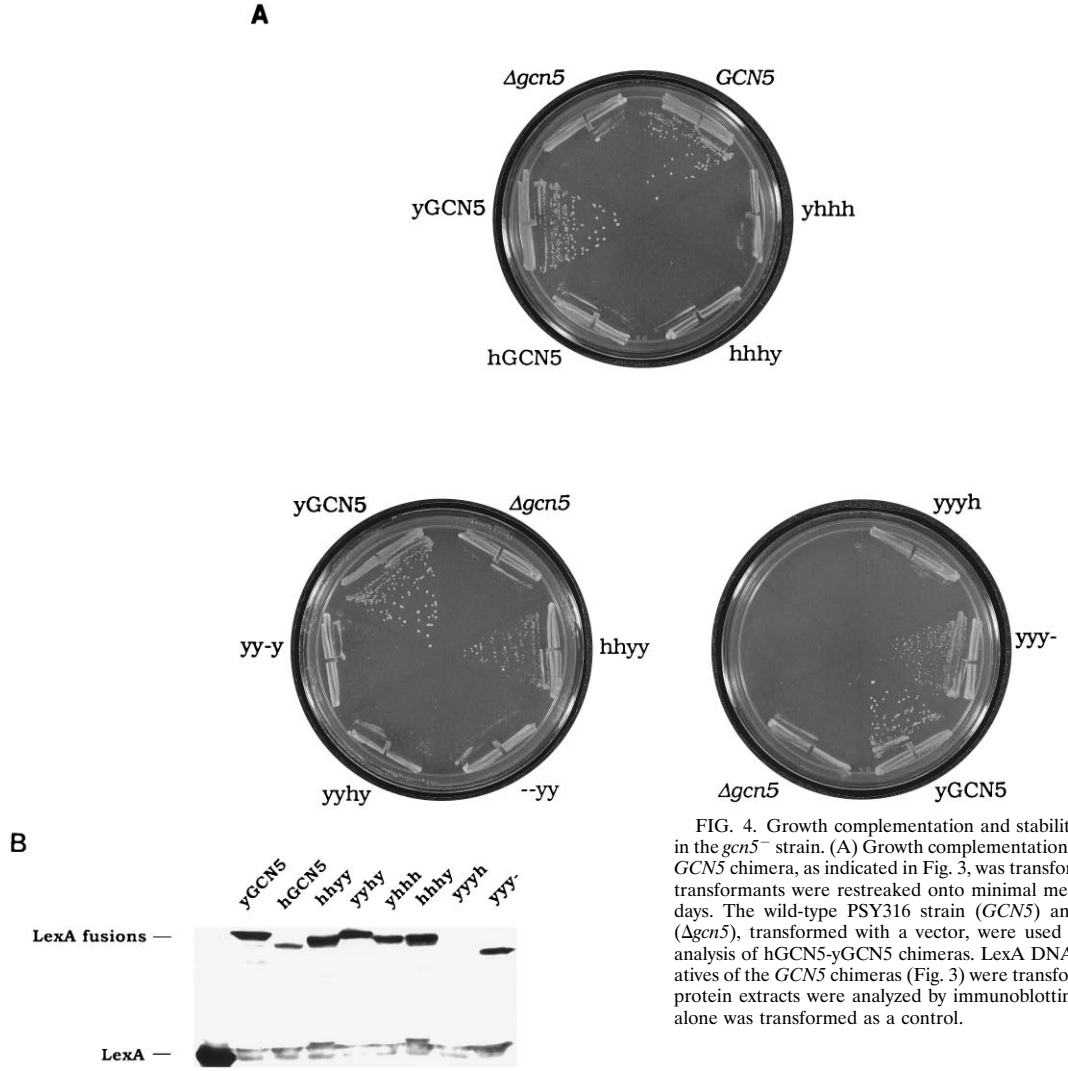

FIG. 4. Growth complementation and stability of hGCN5-yGCN5 chimeras in the *gcn5*$^-$ strain. (A) Growth complementation assay of the *gcn5*$^-$ strain. Each *GCN5* chimera, as indicated in Fig. 3, was transformed into the *gcn5*$^-$ strain, and transformants were restreaked onto minimal medium and grown at 30°C for 2 days. The wild-type PSY316 strain (*GCN5*) and the yGCN5 deletion strain (Δ*gcn5*), transformed with a vector, were used as controls. (B) Western blot analysis of hGCN5-yGCN5 chimeras. LexA DNA-binding domain fusion derivatives of the *GCN5* chimeras (Fig. 3) were transformed into yeast strain L40, and protein extracts were analyzed by immunoblotting with LexA antiserum. LexA alone was transformed as a control.

h*GCN5* had no ameliorative effect (Fig. 4A), even when expressed at levels that far exceeded normal endogenous yGCN5 levels.

Neither the amino nor the carboxyl terminus of yGCN5, separately fused to appropriate deletions of hGCN5 (yhhh and hhhy), complemented the *gcn5*$^-$ strain (Fig. 4A). These findings indicate that the diverged amino and carboxyl (i.e., BrD) termini are incapable of providing critical functions missing in hGCN5.

Next, to identify conserved functions, the chimeras containing human subregions fused to yGCN5 were tested. Complementation was not observed with either replacement of the ADA2-interaction region (yyhy) or the BrD (yyyh) (Fig. 4A). The failure of yyyh to complement may have resulted from intrinsic instability of the protein (see Fig. 4B; discussed below) or from inappropriate contacts of the hGCN5 BrD with still unidentified cellular components. Only the chimera hhyy, comprising the first half of hGCN5 (including the HAT domain) fused to the second half of yGCN5, showed partial complementation (Fig. 4A). In contrast, a deletion mutant of yGCN5, containing only the ADA2-interaction domain and BrD, did not complement growth in the *gcn5*$^-$ strain (Fig. 4A). The chimeric proteins demonstrate that the histone acetylation domain provides a highly conserved function for growth complementation.

The stability of each chimera was determined as a LexA DNA-binding domain fusion, which can be detected in yeast extracts with LexA antiserum (Fig. 4B). The fusion proteins were detected at comparable levels, except yyyh, which was present at very low levels (the protein was detected after extended times of exposure [data not shown]), even though the deletion mutant of the yGCN5 BrD, yyy−, was stable in vivo. The LexA fusion versions of the chimeras exhibited similar patterns of growth complementation compared to those of the normal, unfused proteins, as described above.

The hhyy chimera restored GAL4-VP16 growth inhibition in the *gcn5*$^-$ strain. A second measure of complementation by the human-yeast chimeras was their ability to restore GAL4-VP16 growth inhibition in the *gcn5*$^-$ strain. We have shown previously that overproduction of GAL4-VP16, a chimeric activator composed of the GAL4 DNA-binding domain fused to the VP16 transcriptional activation domain, inhibited wild-type yeast growth. We used this as a selection assay to identify proteins required for transcriptional activation (2), since such inhibition was thought to result from sequestration of essential factors (22). Mutations in components of the ADA complex, including yGCN5, relieved the inhibition (2, 42).

The human-yeast GCN5 chimeras were cotransformed with

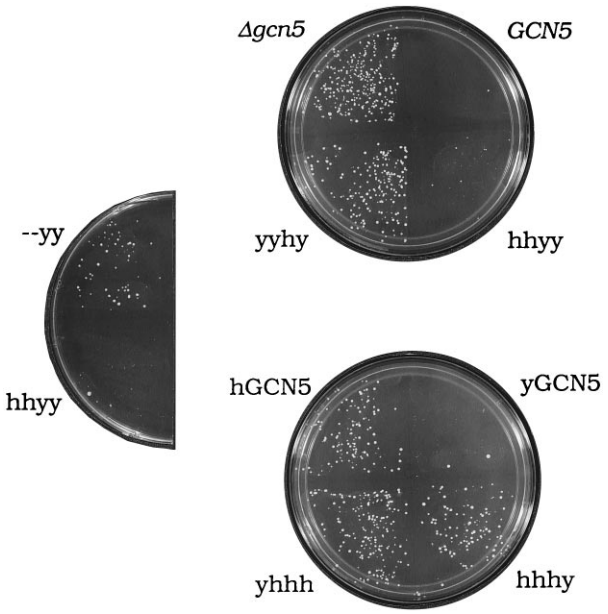

FIG. 5. Growth inhibition of GAL4-VP16 in the presence of hGCN5-yGCN5 chimeras. The ability of the various chimeras to confer the slow growth phenotype in the presence of high-copy-number GAL4-VP16 (2) is shown. The *GAL4-VP16* expression plasmid was cotransformed with the indicated *GCN5* chimeras (Fig. 3) into the *gnc5*$^-$ strain. Transformants were plated directly onto minimal synthetic medium and were grown at 30°C for 4 days. The wild-type PSY316 strain (*GCN5*) and yGCN5 deletion strain (*gnc5*$^-$) transformed with a vector were used as controls.

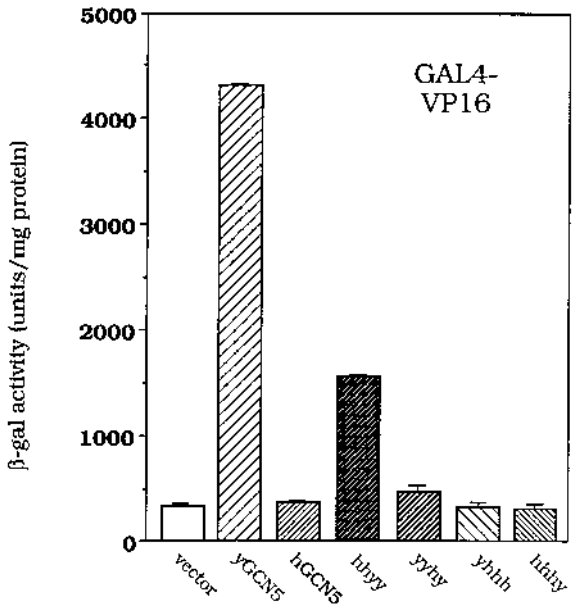

FIG. 6. GAL4-VP16 transcriptional activation in the presence of hGCN5-yGCN5 chimeras. The chimeric *GCN5* plasmids (Fig. 3) were cotransformed into the *gnc5*$^-$ strain, along with plasmids expressing low-copy-number *GAL4-VP16*$_{FA}$ (containing the mutation F442A within the VP16 activation domain to lower toxicity [2]) and reporter pLGSD5 (25), containing bacterial *lacZ* driven by the *GAL1-10* promoter. β-Galactosidase (β-gal) activity was determined as units per milligram of protein. Error bars represent the standard errors about the means from two independent experiments.

GAL4-VP16, cloned onto a high-copy-number yeast expression plasmid, into the *gcn5*$^-$ strain (Fig. 5). As in the previous growth complementation, only the hhyy chimera, and not hGCN5 or other chimeras, restored growth inhibition by GAL4-VP16 (Fig. 5). In contrast, a deletion mutant of yGCN5 containing only the carboxyl-terminal ADA2-interaction domain and BrD was not capable of restoring growth inhibition (Fig. 5). Thus, the HAT domain of hGCN5 fused to yGCN5 restored both growth (Fig. 4A) and growth inhibition by GAL4-VP16 (Fig. 5) in the *gcn5*$^-$ strain.

The hhyy chimera partially complemented transcriptional activation in the *gcn5*$^-$ strain. Since the human HAT domain possesses a conserved and therefore presumably crucial function for growth, we wished to test whether this function was also conserved for transcriptional activation in vivo. Activation by GAL4-VP16 (expressed at low, noninhibitory levels) was tested in the *gcn5*$^-$ strain in the presence of yGCN5, hGCN5, or the chimeric GCN5 proteins. The reporter used was the bacterial *lacZ* gene, whose expression was driven by GAL4 DNA-binding sites (25). GAL4-VP16 transcriptional activation requires components of the adaptor complex for full activity in yeast (2, 41, 42, 44).

GAL4-VP16 activity was reduced in the *GCN5* deletion strain approximately 14-fold (Fig. 6). The hhyy chimera, containing the hGCN5 HAT domain, partially complemented the GCN5 deletion (Fig. 6). In this case, GAL4-VP16 activity was increased fivefold, to one-third of the activation observed in the presence of yGCN5. In contrast, neither hGCN5 nor any chimera other than hhyy was capable of complementation of transcriptional activation (Fig. 6). Therefore, the human HAT domain provided a critical function both for growth complementation (Fig. 4A and 5) and transcriptional activation by GAL4-VP16 (Fig. 6).

A single amino acid substitution in the HAT domain lowered both histone acetylation and activation. Within the HAT domains of GCN5 homologs are four highly conserved sequences (regions I to IV [6]). Region I contains a histidine residue strongly conserved with several previously identified acetyltransferases (Fig. 3), leading to speculation that this histidine may represent an active site residue (6). Therefore, a single amino acid substitution mutation was introduced at the conserved histidine at amino acid 138 in the chimera hhyy. Equivalent amounts (Fig. 7B) of the recombinant mutant protein hhyy(H138A) and the parental hhyy and hGCN5 were compared for HAT activity. The assay was done at several enzyme concentrations within their linear ranges of activity (Fig. 7A). The HAT activity of mutant protein was found to be three- to sixfold lower than that of parental hhyy or hGCN5 (Fig. 7A).

The mutant chimeric gene was then transformed into yeast to determine its ability to complement growth and transcriptional activation in the *gcn5*$^-$ strain. The growth rate of hhyy(H138A) transformants in liquid culture was approximately 40% lower than that of the hhyy parent (data not shown). In addition, transcriptional activation by GAL4-VP16 in the presence of hhyy(H138A) was 40% lower than that in the presence of the parental hhyy (Fig. 7C). The effect of the substitution mutation was also tested on activation mediated by GAL4-p53, a fusion protein of the GAL4 DNA-binding domain and the activation domain derived from the human tumor suppressor p53. (We have recently shown that full transcriptional activation in yeast by GAL4-p53 requires yGCN5 [7a]). Activation by GAL4-p53 in the presence of hhyy(H138A) was 50% lower than that in the presence of hhyy (Fig. 7C). The relative intracellular levels of the hhyy(H138A) mutant were compared to those of the hhyy parent and were found to be similar (Fig. 7D). Thus, a single substitution mutation in the HAT domain of the hhyy chimera that lowered HAT activity caused a comparable reduction in growth

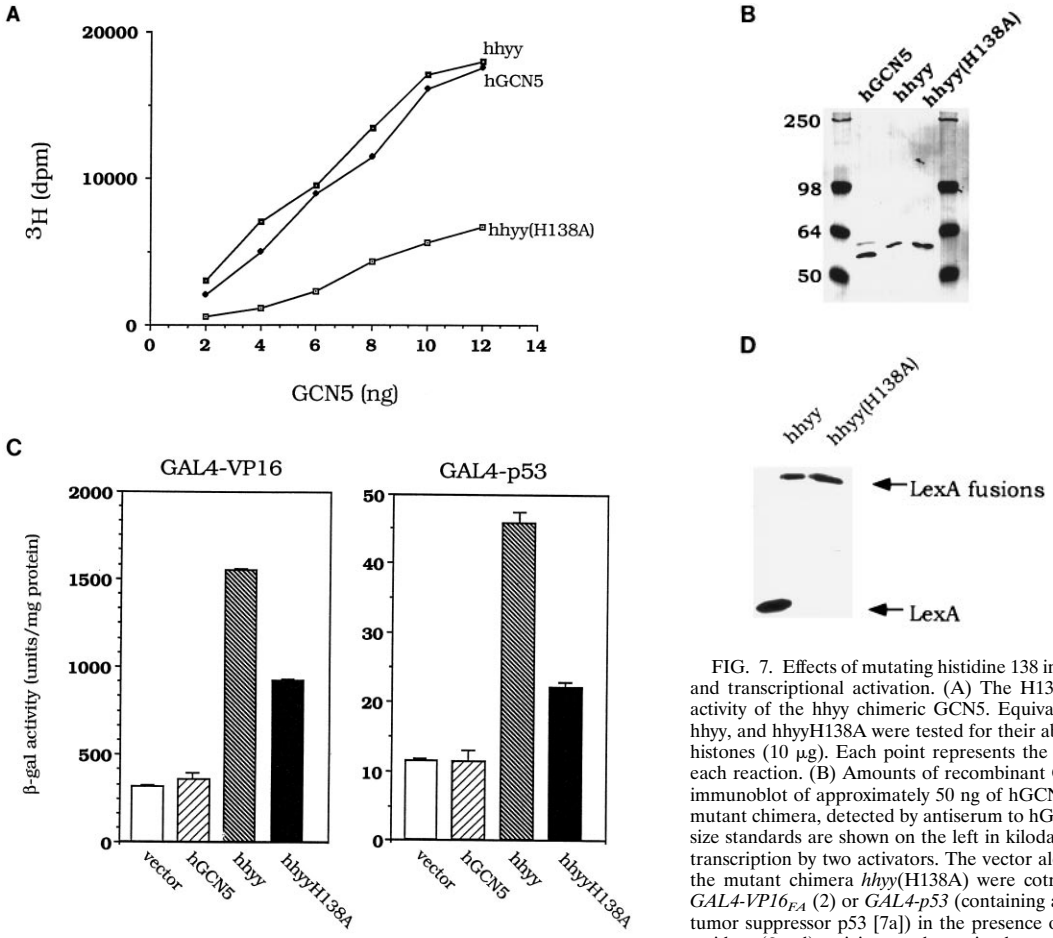

FIG. 7. Effects of mutating histidine 138 in the hhyy chimera on HAT activity and transcriptional activation. (A) The H138A mutation decreased the HAT activity of the hhyy chimeric GCN5. Equivalent amounts of purified hGCN5, hhyy, and hhyyH138A were tested for their ability to acetylate free chicken core histones (10 μg). Each point represents the average of two filters counted for each reaction. (B) Amounts of recombinant GCN5 used in the HAT assay. An immunoblot of approximately 50 ng of hGCN5, hhyy chimera, and hhyyH138A mutant chimera, detected by antiserum to hGCN5 is shown. Positions of protein size standards are shown on the left in kilodaltons. (C) H138A mutation lowers transcription by two activators. The vector alone, *hGCN5*, the chimera *hhyy*, or the mutant chimera *hhyy*(H138A) were cotransformed with low-copy-number *GAL4-VP16*$_{FA}$ (2) or *GAL4-p53* (containing amino acids 40 to 83 of the human tumor suppressor p53 [7a]) in the presence of the reporter pLGSD5. β-Galactosidase (β-gal) activity was determined as units per milligram of protein. Error bars represent the standard errors about the means from two independent experiments. (D) Immunoblot analysis of hhyy compared to hhyy(H138A). LexA fusions of each chimera were transformed into L40 cells. Equal amounts of proteins were immunoblotted with antiserum against LexA. LexA alone was used as a control.

complementation and transcriptional activation by two fusion activators.

DISCUSSION

We identified human proteins that are conserved in sequence with the putative yeast transcriptional adaptors yADA2 and yGCN5 (8). These human proteins, termed hADA2 and hGCN5, showed functional similarities to yADA2 and yGCN5, including mutual interaction (8) (Fig. 2) and potentiation of transactivation in human cells (8). Surprisingly, despite the common structural and functional characteristics, neither of the human genes complemented growth or transcriptional activation when expressed in yeast strains bearing deletions of yADA2 or yGCN5 (8) (Fig. 4A and 6).

Recently, we (6) determined that a *Tetrahymena* protein, p55, which shows strong sequence conservation with both y- and hGCN5, possesses HAT activity. This finding suggested that the adaptor complex may affect transcription, at least in part, by targeting chromatin for specific covalent modification. However, direct experimental evidence linking the HAT activity of GCN5 with gene activation has not been obtained.

Since several activities have been ascribed to yGCN5 (histone acetylation, ADA2 interaction, growth dependence, and transcriptional activation), we assayed hGCN5 for these functions and also engineered human-yeast chimeras to determine which functions are highly conserved evolutionarily. In the course of this work, we obtained data which demonstrate experimentally that enzymatic acetylation, probably of histone substrates, and transcriptional activation are directly linked.

Human GCN5 is an HAT. hGCN5 was identified because of strong sequence and functional conservation with yGCN5 (8). The discovery that yGCN5 was an HAT (6) prompted us to determine whether hGCN5 also possesses histone acetylation activity. Our results demonstrated that hGCN5 has HAT activity with enzymatic properties similar to those of yGCN5 (6) and p55 (35). Moreover, both recombinant yGCN5 and hGCN5 showed strong preference for H3 as a substrate, providing additional evidence that yGCN5 and hGCN5 are functionally conserved proteins. Since both yGCN5 and hGCN5 associate with ADA2, both the yeast and human HATs may fulfill their enzymatic activities in the form of complexes, as discussed below, not as free catalytic subunits. Thus, it will be interesting to determine enzymatic properties of the adaptor complex, for example, whether the other components direct GCN5, and thus the HAT, to chromatin and whether the presence of other components alters or expands the histone substrate specificity of GCN5. As we completed the present study, a novel hGCN5-related protein (P/CAF) was identified and shown to possess HAT activity (60). Recombinant P/CAF, unlike hGCN5, was capable of acetylating nucleosomal histones. This finding suggests that P/CAF may not require asso-

ciation with other ADA proteins for nucleosomal histone acetylation in vivo and thus may represent a distinct activation pathway in human cells.

The HAT domain is functionally conserved. The middle region of yGCN5 (amino acids 116 to 260) is highly conserved among hGCN5 and *Tetrahymena* p55 and shares features with other acetyltransferases (6). This region was predicted to possess HAT activity (4), and indeed, analysis of deletion mutants of yGCN5 has confirmed this prediction. Deletion of this region in yGCN5 resulted in failure to complement growth (Fig. 4), transcriptional activation, and complete loss of HAT activity (8a). When the amino-terminal half of hGCN5 (amino acids 1 to 251), including the HAT region, was fused to the functionally inert carboxyl-terminal half of yGCN5 (amino acids 262 to 439), the resulting chimera (hhyy) partially complemented function in the *gcn5*$^-$ strain in three assays: growth, growth inhibition by GAL4-VP16, and transcriptional activation by both GAL4-VP16 and GAL4-p53 (summarized in Fig. 3). All other chimeras failed to complement these functions. Thus, the interspecies chimeras demonstrated that the HAT domain is functionally conserved and important for both cell growth and transcriptional activation.

The relationship between HAT and transcriptional activation. Histone acetylation has long been associated with transcriptional activation (55, 59). In this study, we altered the HAT activity of GCN5 and observed a reduction in transcription in vivo. Among yGCN5, hGCN5, and *Tetrahymena* p55, as well as HAT B (32), a histidine residue within a putative HAT signature domain is conserved. The histidine was suggested to be important for HAT activity (6) based on analogy to mutagenesis analysis of chloramphenicol acetyltransferase (49). To test this hypothesis directly, this histidine (amino acid 138 in hGCN5) was mutated to alanine in the hhyy chimera, wherein the HAT domain of hGCN5 was fused to the remainder of yGCN5. (A complete mutagenesis of yGCN5 by site-directed alanine scanning is in progress and will be presented in a separate study.) The HAT activity of hhyy(H138A) was reduced three- to sixfold relative to that of the parental hhyy, suggesting that this residue is an important, although not essential, active site residue in GCN5. Interestingly, compared to the hhyy parent, hhyy(H138A) grew more slowly and failed to reach full transcriptional activation by both GAL4-VP16 and GAL4-p53. This correlation between lowered HAT activity and lowered transcriptional activity exhibited by hhyy(H138A) supports the model that the HAT activity of GCN5 is required for its role in transcription.

In light of previous evidence of gene-specific effects of ADA mutations (2, 42, 44), as well as physical association of ADA proteins with activators and basal factors (1), the link established in these experiments between histone acetylation and gene activation properties in GCN5 supports an appealing model: particular promoters may be targeted for histone acetylation through transcription factor recruitment of the ADA complex (58). The consequence would be gene-specific regulation via GCN5-mediated histone acetylation.

The function of the BrD and ADA2 interaction domain in HAT activity. We have seen that a deletion mutant of yGCN5, containing only the HAT domain, acetylated histones as efficiently as full-length yGCN5 in vitro but failed to complement growth and transcription in vivo (8a). Thus, HAT must be targeted to chromatin via other functions of GCN5. Two possibilities are through interactions of the GCN5 BrD or through GCN5 interactions with the ADA complex.

The BrD is present at the carboxyl termini of yGCN5, hGCN5, and *Tetrahymena* p55, and it is also found in other transcriptional regulatory factors (26). Although the BrD of yGCN5 is required for full complementation of growth and transcription (42), its role remains unclear. The BrD of yGCN5 was suggested to be involved in targeting HAT activity to chromatin (6). We observed that the fusion of the yGCN5 BrD to hGCN5 (chimera hhhy) did not complement in yeast. Since our data show that the human HAT domain functions in yeast, we conclude that the BrD is not likely to be the sole mechanism of targeting to chromatin.

The second possible targeting mechanism is through interaction of GCN5 with other components of the ADA complex, such as ADA2. We have shown that interactions between hGCN5 and yADA2 are indistinguishable from interactions between yGCN5 and yADA2. The failure of the yyhy chimera (containing the ADA2-interaction domain from hGCN5) to complement in the *gcn5*$^-$ strain suggests that either subtle differences exist in the hGCN5-yADA2 association that were not obvious in our assays or additional ADA components that did not properly associate with hGCN5 in vivo are required for targeting. In any case, we conclude that, in general, GCN5 interaction with ADA2 is not sufficient for targeting.

In this study, we have shown that hGCN5 is an HAT and that the HAT function is evolutionarily conserved. Importantly, we have linked enzymatic HAT activity within GCN5 to its transcriptional activation properties. Other enzymatic activities have been associated with transcriptional factors. The coactivator TAF250 has kinase activity that targeted the basal transcriptional factor RAP74 (TFIIF [14]). Also, CAK, which is associated with TFIIH, phosphorylated the carboxyl-terminal repeat domain of RNAPII (20, 47, 48, 50). These studies did not show a direct functional role for enzymatic activity in transcription. The ATPase activity of SWI2 has been linked to growth and transcription in vivo (30, 37). Thus, the relationship we have established between acetylation and transcriptional competence in GCN5 is among the first in vivo evidence linking enzymatic activity to transcriptional activation.

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