THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 27, Issue of July 5, pp. 24648–24652, 2002

Printed in U.S.A.

Gal80 Confers Specificity on HAT Complex Interactions with Activators*

Received for publication, February 27, 2002, and in revised form, April 29, 2002
Published, JBC Papers in Press, May 1, 2002, DOI 10.1074/jbc.M201965200

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Several yeast transcription activators have been shown to interact with and recruit histone acetyltransferase complexes to promoters in chromatin. The promiscuity of activator/HAT interactions suggests that additional factors temporally regulate these interactions in response to signaling pathways. In this study, we demonstrate that the negative regulator, Gal80, blocks interactions between the SAGA and NuA4 HAT complexes and the Gal4 activator. By contrast, Gal80 did not inhibit SAGA and NuA4 interaction with another activator Gcn4. The function of Gal80 prevented Gal4 targeting of SAGA and displaced SAGA targeted by Gal4 to a promoter within a nucleosome array. In the same set of experiments, targeting of SAGA by Gcn4 was unaffected by Gal80. These studies demonstrate that the specificity of HAT/activator interactions can be dictated by cofactors that modulate activation domain function in response to cellular signals.

Regulating promoter activity is a key step in transcription. In eukaryotes, regulation of transcription preinitiation occurs at multiple levels (1). At the very least, this regulation involves the modification of chromatin (2, 3) and modulating the assembly of preinitiation complexes (PICs)[1] (4–6). Regulation at this level is primarily the direct result of activator and repressor proteins that bind DNA and target cellular factors to promoters. It now appears that many of these targeted factors participate in both the modification of chromatin and regulating the establishment of PICs (7).

One of these factors, the yeast SAGA complex, functions as a histone acetyltransferase (HAT) (8) and plays a role in the recruitment of the TATA-binding protein to promoters (9–12). Biochemical and genetic analyses have demonstrated that SAGA is recruited to promoters by sequence-specific DNA-binding transcription activators, where it stimulates transcription in both HAT-dependent (13–18) and -independent manners (10–12). Several components of SAGA have been shown to interact with activators, including Ada2 (19, 20), several TAFs (21), and Tra1 (22). Interestingly, the yeast NuA4 HAT complex also contains Tra1 (23) and is targeted by activators (18). Biochemical and genetic characterization of Tra1 has found that this factor when present within SAGA and NuA4 is contacted by activators and appears to play a crucial role in the activity of these complexes (22). Thus, Tra1 appears to be a mutual interface for activator interaction with SAGA and NuA4.

These observations have made it evident that both SAGA and NuA4 can be targeted to promoters by activators. However, if the interactions between these HATs and activators are related to the regulation of transcription, we would expect them to be modulated by signals controlling gene-specific transcription. To address this possibility, we turned to one of the most widely studied pathways for gene-specific transcription regulation in eukaryotes, the yeast Gal4-Gal80-Gal3 transcription switch, which regulates the expression of galactose-responsive (*GAL*) genes (24–26). The Gal4 activator is responsible for the activation of genes encoding for enzymes necessary for utilizing galactose as an alternative carbon source. Typical of many activators, Gal4 recognizes DNA in a sequence-specific manner through its DNA-binding domain located within amino acids 1–65 (27). Once bound to promoters, the Gal4 activation domains, located within amino acids 148–238 and 768–881 (28), contact and target general transcription components to the promoter (29, 30). Under growth conditions containing glucose, the Gal4 activator binds promoters at very low levels. When cells are grown in other carbon sources, Gal4 binds constitutively to *GAL* promoters. Although bound to promoters, Gal4 only activates transcription in the presence of galactose (24, 26). A combination of biochemical and genetic approaches have established that in the absence of galactose the repressor protein Gal80 is targeted to *GAL* promoters through direct interaction within amino acids 850–871 of the Gal4 C-terminal activation domain (31–38). Through this interaction, Gal80 is believed to block the ability of Gal4 to contact other transcription components (39, 40). In the presence of galactose, the block imposed on activation by Gal80 is alleviated by a third factor, Gal3 (25). The mechanism underlying Gal3 alleviation of Gal80 inhibition remains unclear. In one case, *in vitro* transcription and DNA-binding experiments have demonstrated that Gal3 alleviates this inhibition by directly associating with a Gal4-Gal80 promoter complex (40). However, another study using a Gal3-green fluorescent protein fusion protein found that Gal3 was primarily localized within the cytoplasm (41). Thus, it is unclear how Gal3, if localized in the cytoplasm, would be able to associate with a Gal4-Gal80 promoter within the nucleus.

* This work was supported by NIGMS, National Institutes of Health, Grants GM027925 (to J. E. H.) and GM047867 (to J. L. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PIC, preinitiation complex; HAT, histone acetyltransferase; HA, hemagglutinin; aa, amino acids; GST, glutathione *S*-transferase.

Recent genetic studies have identified a role for SAGA in the expression of *GAL* genes. These studies have shown that the expression of *GAL1* is significantly reduced in cells with defects in the SAGA components Spt3 or Spt20 (10, 11). However, in cells defective in the Gcn5 catalytic HAT subunit of SAGA, *GAL1* expression was less affected. These results indicate that SAGA is required for the transcription of *GAL* genes and provides functions beyond histone acetyltransferase activity. In this study, we demonstrate direct interactions between the Gal4 activator and SAGA. Moreover, the Gal80 protein specifically regulates these interactions with the Gal4 activation domain. These results demonstrate that cofactors that regulate the activity of activation domains in yeast can do so by bringing specificity to activator/HAT interactions.

EXPERIMENTAL PROCEDURES

Plasmids and Strains—SAGA and ADA HAT complexes with a C-terminal HA-tagged Ada2 subunit were isolated from the yeast strain CY947 bearing the plasmid Ycp88-*HA-ADA2* (42). SAGA was also isolated from the yeast strain YJW526, which is a Δ*tra1* strain with an N-terminal FLAG-tagged *TRA1* centromeric plasmid and the plasmid Ycp88-*HA-ADA2*. NuA4 complex was isolated from strain YJW207, which contains three tandem HA tags at the C terminus of the endogenous Esa1. The Gal4-Gcn4AD protein was expressed from pG4Gcn4 that contains the Gcn4 sequences encoding amino acids 9–172 fused to the C-terminal end of the Gal4 DNA binding region (aa 1–93) in pRJR1 (43).

Purification of Proteins—SAGA, ADA, NuA4 HAT complexes were isolated as described previously (44). SAGA was also purified by fractionating whole cell extract containing FLAG-tagged Tra1 over Ni^{2+}-nitrilotriacetic acid-agarose (Qiagen) and MonoQ (AP Biotech) as described by Eberharter *et al.* (44), followed by batch immunoaffinity purification on anti-FLAG M2-agarose (Sigma). Peak SAGA MonoQ fractions were bound to anti-FLAG beads in 0.35 M NaCl buffer B (50 mM Tris-HCl, pH 8.0, 10% glycerol, 0.1% Tween 20, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 2 μg/ml pepstatin A). Beads were washed sequentially in 350 mM NaCl buffer B and 150 mM NaCl buffer B. SAGA was eluted from beads with 1 mg/ml FLAG peptide (Sigma) in 150 mM NaCl buffer B. Fractions from all columns were monitored by assaying for HAT activity (44).

Cross-linked GST-Gal4AD (45) and GST-Gcn4AD (20) Sepharose beads were prepared as described previously (46). Gal4(1–93 + 768–881) (43) and Gal4-Gcn4 were purified as described by Reece *et al.* (43). Gal80 was overexpressed in yeast w303–1*a* bearing the plasmid pAP77 and purified on Ni^{2+}-nitrilotriacetic acid-agarose as described by Platt and Reece (40).

GST Pull-down Assays—HAT complexes were precleared on glutathione-Sepharose (AP Biotech) in 150 mM NaCl buffer PD (50 mM HEPES, pH 7.5, 10% glycerol, 1 mM EDTA, 0.1% Tween 20, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 2 μg/ml pepstatin A). Approximately 0.3 pmol of precleared HAT was added to 0.5 nmol of GST, GST-Gal4AD, or GST-Gcn4-Sepharose preequilibrated in 150 mM NaCl PD buffer and mixed overnight at 4 °C. After several washes in 150 mM NaCl PD buffer, portions of input, supernatants, and equivalent volumes of beads were analyzed for complex subunits by Western blot and HAT activity. For Western blot, anti-HA was used to detect the Ada2-HA subunit in SAGA and ADA and the Esa1-HA subunit in NuA4. For the analysis of HAT activity, samples were mixed into standard HAT reactions (44), run on 15% SDS gels, and subjected to fluorography.

GST-Gal4AD and GST-Gcn4AD complexes were treated with Gal80 by mixing 2.5 nmol of Gal80 with 0.5 nmol of GST-Gal4AD or GST-Gcn4-Sepharose in 150 mM NaCl buffer PD for 2 h at 4 °C, followed by several washes in 150 mM NaCl PD buffer. Pull-down experiments with HATs were then carried out as described above.

Nucleosome Array Recruitment Assays—Nucleosome arrays were reconstituted *in vitro* by salt step dilution (47) and immobilized on magnetic beads (Dynal) (48, 49). The template consisted of a DNA fragment containing five sea urchin 5 S rRNA gene nucleosome-positioning sequences on each side of an adenovirus E4 core promoter with five upstream Gal4 DNA-binding sites (16).

0.5 pmol of Gal4(1–93 + 771–881) or Gal4-Gcn4 were mixed with 2.5 pmol of Gal80 and template beads containing 0.3 pmol of Gal4 binding sites in binding buffer (10 mM HEPES-KOH, pH 7.8, 5% glycerol, 50 mM KCl, 0.25 mg/ml bovine serum albumin, 5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) and incubated for 20 min at 30 °C with occasional agitation. Beads were collected on a magnetic concentrator (Dynal) and washed in binding buffer. Next, 1 μg of competitor HeLa oligonucleosomes (50) and 0.2 pmol of SAGA were added to the binding reactions and incubated for 1 h at 30 °C with occasional agitation. Following another series of washes in binding buffer, reactions were run on 10% SDS gels and analyzed for SAGA by anti-HA Western blot for Ada2-HA.

In the experiment testing for Gal80 displacement of targeted SAGA, Gal4 or Gcn4 was mixed with template beads as described above. Competitor chromatin and SAGA were then added and incubated with the template beads as described. Next, 1 pmol of Gal80 was added to the template beads and incubated for 1h at 30 °C. Samples were then washed in binding buffer and analyzed for SAGA by anti-HA Western blot for Ada2-HA.

RESULTS

The Gal4 Activation Domain Interacts with the SAGA and NuA4 HAT Complexes—To establish the Gal4-Gal80 regulatory switch as a model for studying the specificity of HAT interaction with activators, SAGA and NuA4 were tested for their ability to interact with the Gal4 activation domain (AD) (aa 768–881) in GST pull-down experiments. The yeast ADA HAT complex was included as a negative control, since this complex does not interact with activators (17).

GST and GST-Gal4AD fusion proteins cross-linked to glutathione-Sepharose were incubated with highly fractionated SAGA, NuA4, and ADA. Western analysis in Fig. 1*A* of pull-down samples for SAGA, detected through its subunit Ada2, and NuA4, detected through its catalytic subunit Esa1, shows that both SAGA (*top panel*) and NuA4 (*middle panel*) bind the Gal4AD (compare *lanes 4* and *5*). As expected, the bulk of the Ada2 signal from the ADA complex remained unbound (Fig. 1*A*, *bottom panel*, compare *lanes 4* and *5*), indicating that this complex did not interact with the Gal4AD.

Further demonstration of binding to the Gal4AD was obtained from the HAT activity present in the pull-down samples. Fluorograms of HAT reactions with pull-down samples in Fig. 1*B* show that the bulk of SAGA histone H3 acetylation (*top panel*, *lanes 4* and *5*) and NuA4 histone H4 acetylation (*middle panel*, *lanes 4* and *5*) binds GST-Gal4AD, whereas the histone H3 acetylation carried out by the ADA complex (*bottom panel*, *lanes 4* and *5*) remains unbound. Based on these results, we concluded that that the Gal4AD has high affinity for the SAGA and NuA4 HAT complexes.

Specific Inhibition of Activator Interaction with HATs by Gal80—We next determined whether Gal80 affected the interaction of SAGA or NuA4 with the Gal4AD. This was tested by preincubating GST-Gal4AD beads with Gal80, followed by several washes. The Coomassie stain gel in Fig. 2*A*, *lane 3*, demonstrates that Gal80 binds GST-Gal4AD beads. Pull-down experiments were performed by incubating SAGA and NuA4 with normalized amounts of GST, GST-Gal4AD, or GST-Gal4AD beads pretreated with Gal80. In the absence of Gal80, SAGA bound the Gal4AD (Fig. 2*B*, *top panel*, *lane 5*). By contrast, when SAGA binding was tested using the Gal4AD beads treated with Gal80, SAGA binding was reduced to background levels (Fig. 2*B*, *top panel*, *lane 7*), indicating that Gal80 blocks SAGA interaction with the Gal4AD. The presence of Gal80 in these samples is indicated on the Coomassie-stained gel containing a portion of each pull-down sample (Fig. 2*B*, *bottom panel*, *lane 7*). Gal80 also blocked the binding of NuA4 to the Gal4AD as indicated by the elimination of Esa1 in sample treated with Gal80 (Fig. 2*C*, compare *lanes 5* and *7*).

The specificity of Gal80 inhibition was determined by testing the effect of Gal80 on HAT interaction with the Gcn4AD. This activator is responsible for activation of amino acid biosynthetic genes in yeast and is known to target SAGA to these genes (9, 51). However, Gcn4 is regulated at the translation

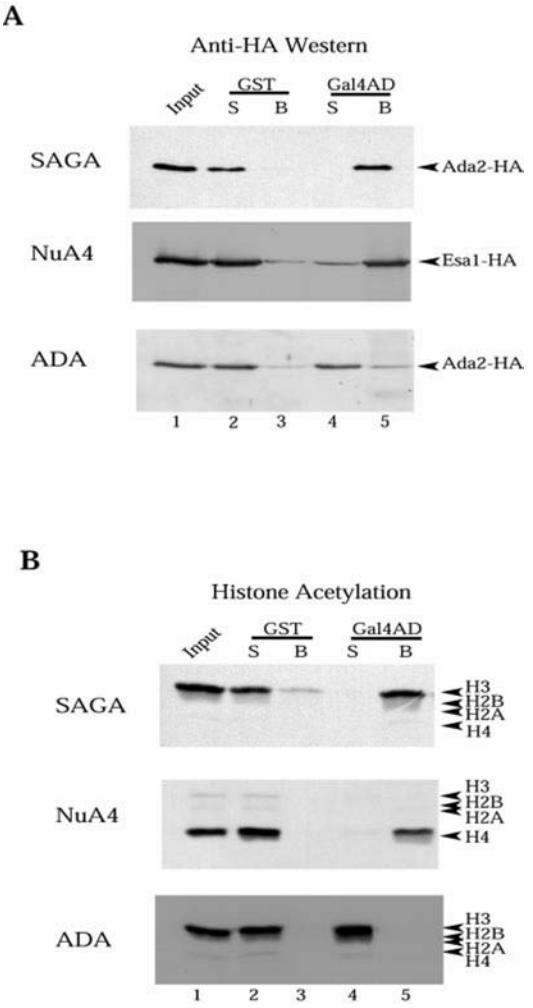

FIG. 1. **SAGA and NuA4 HAT complexes interact with Gal4AD.** SAGA, NuA4, and ADA HAT complexes were tested for binding to GST-Gal4AD or GST protein alone immobilized on glutathione-Sepharose. Input material and sample portions in the unbound supernatant (*S*) and binding to beads (*B*) are indicated at the *tops of panels*. *A*, GST pull-down samples run on 10% SDS-gels and subjected to anti-HA Western blot of SAGA and ADA subunit Ada2-HA and NuA4 subunit Esa1-HA. Migration of Ada2-HA and Esa1-HA is indicated on the *sides of panels*. *B*, HAT activity of pull-down samples run on 15% SDS-gels and subjected to fluorography. Migration of histones H2A, H2B, H3, and H4 is indicated for each *panel*.

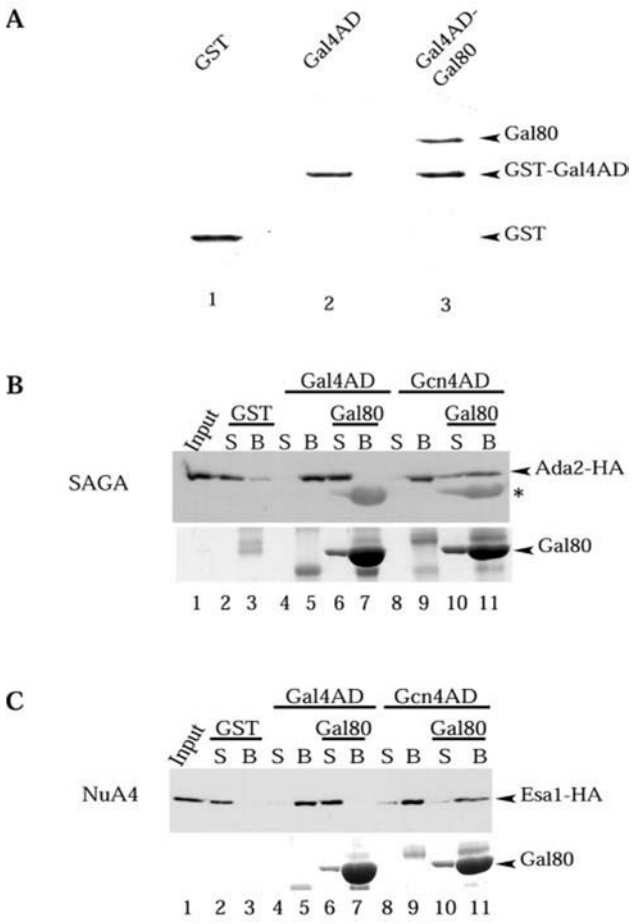

FIG. 2. **Gal80 specific inhibition of activator interaction with HATs.** SAGA and NuA4 complexes were subjected to GST pull-down analysis with GST-Gal4AD and GST-Gcn4AD untreated or treated with Gal80. *A*, Coomassie-stained 10% SDS gel of GST, GST-Gal4AD, and GST-Gal4AD treated with Gal80. Migration of GST, GST-Gal4AD, and Gal80 are indicated by the *arrows*. *B* and *C*, effect of Gal80 on Gal4AD and Gcn4AD interaction with SAGA and NuA4. Input material and sample portions in the unbound supernatant (*S*) and binding to beads (*B*) are indicated *above* the *panels*. *Top panels*, pull-down samples were run on 10% SDS-gels and subjected to anti-HA Western blot for the SAGA subunit Ada2-HA (*B*) and the NuA4 subunit Esa1-HA (*C*). Migration of Ada2-HA and Esa1-HA is indicated at the *side* of each *panel*. *, antibody cross-reactivity with Gal80. *Bottom panels*, Coomassie-stained 10% SDS-gel of pull-down samples. Migration of Gal80 is indicated at the *sides of panels*.

level (52) and should not be affected by Gal80. Both SAGA and NuA4 exhibit efficient binding to the Gcn4AD (Fig. 2, *A* and *B*, *top panels*, *lane 9*). Interestingly, Gal80 also bound the Gcn4AD beads (*bottom panels*, *lanes 11*) but was unable to block SAGA and NuA4 binding to the Gcn4AD (*top panels*, *lane 11*). The presence of Gal80 in the Gal4AD and Gcn4AD pull-downs appears to be a function of the activation domains, since GST alone did not pull down Gal80 (data not shown). Therefore, it appears that the Gal80 confers a specific inhibition of the Gal4 activator interaction with SAGA and NuA4.

Gal80 Regulates Recruitment of SAGA to a Promoter within a Nucleosome Array—To further examine the functional relevance of Gal80 to SAGA function, we tested whether Gal80 would inhibit Gal4-dependent targeting of SAGA to a chromatin promoter *in vitro*. This provides a more physiological context, since potential interactions between the HAT complex and the template chromatin might affect the ability of Gal80 to block HAT complex recruitment to promoter nucleosomes. In addition, we focused on SAGA for these experiments, since it appears to be the most important HAT complex for activation of Gal4-driven genes *in vivo*. Activation of *GAL* genes *in vivo* is dependent on SAGA (10, 11) and chromatin immunoprecipitation experiments have demonstrated that SAGA is recruited to *GAL* promoters as a function of galactose induction (12). However, Esa1, the catalytic subunit of NuA4, has not been observed to cross-link on *GAL* promoters *in vivo* (53).

The nucleosome template used in these experiments consists of five sea urchin 5 S rRNA gene nucleosome-positioning sequences on each side of an adenovirus E4 core promoter with five upstream Gal4 DNA-binding sites (Fig. 3*A*) (16). The DNA template was reconstituted into nucleosomes *in vitro* using salt step dilution with purified histones (47). Once reconstituted, the array was immobilized on streptavidin magnetic beads through a biotinylated nucleotide at the 3′-end (48, 49).

Targeting experiments were performed by first prebinding the template with Gal4(1–93 + 768–881) (40) and Gal80 and then washing on a magnetic concentrator to remove unbound protein (Fig. 3*A*). This was followed by a targeting step with SAGA and competitor chromatin (49, 50) and another series of washes. Initial experiments determined that SAGA exhibited a

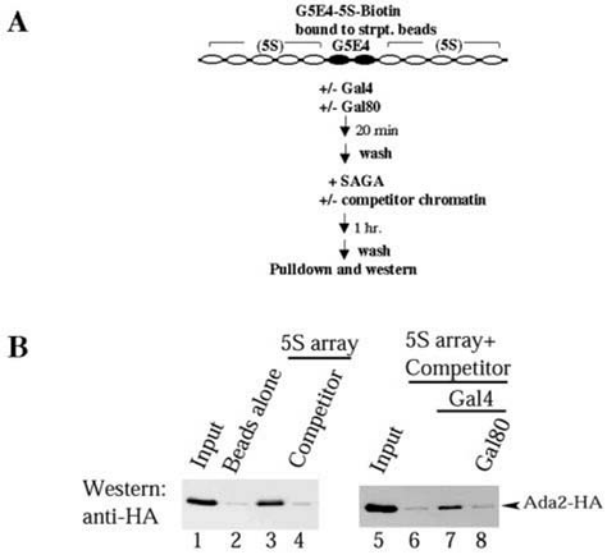

FIG. 3. **Gal80 inhibits Gal4 targeting of SAGA to a nucleosomal promoter.** *A*, schematic depiction of targeting assay. The 5SG5E4 template contains five 5 S nucleosome positioning sequences flanking each end of an adenovirus E4 core promoter with five upstream Gal4 binding sites. Template was reconstituted into nucleosomes *in vitro* and immobilized on magnetic beads. Gal4 contains the Gal4 DNA-binding domain (aa 1–93) fused to the Gal4AD (aa 768–881). *B* (*left panel*), the effect of competitor chromatin on binding of SAGA to the nucleosome template. *Right panel*, effect of Gal80 on targeting of SAGA by Gal4. Binding of SAGA to nucleosome template was determined by anti-HA Western blot for Ada2-HA as indicated at the *sides of panels*.

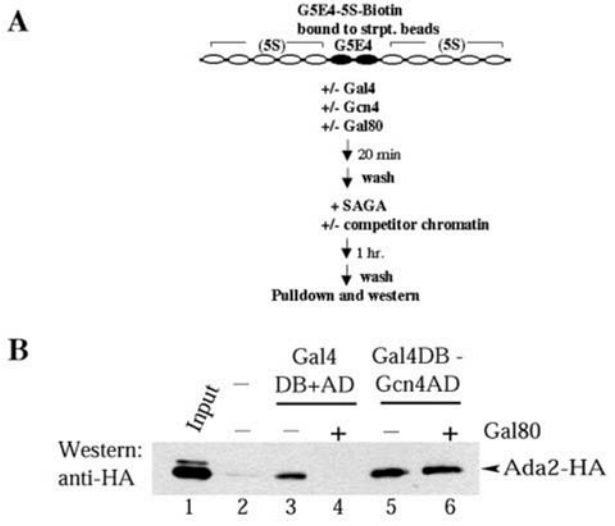

FIG. 4. **Gal80 specifies activator targeting of SAGA.** *A*, schematic depiction of targeting assay. Note that Gal80 is bound to activator prior to the addition of SAGA. *B*, binding of SAGA to nucleosome template was determined by anti-HA Western blot for Ada2-HA as indicated at the *side of the panel*. Gal4DB-Gcn4AD contains the Gal4 DNA-binding domain fused to the Gcn4 activation domain.

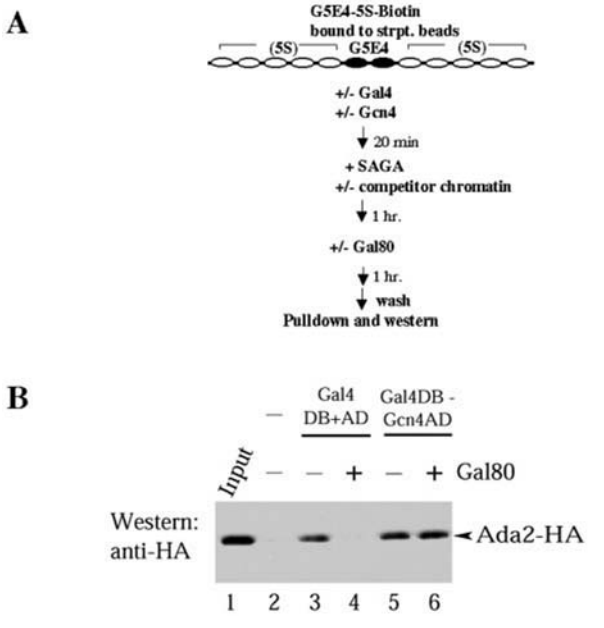

FIG. 5. **Gal80 specifically displaces Gal4-targeted SAGA.** *A*, schematic depiction of activator targeting assay. Note that Gal80 is added after activator targeting of SAGA. *B*, the degree of SAGA retained by Gal4 and Gcn4 following the addition of Gal80 was determined by anti-HA Western blot for Ada2-HA as indicated at the *side of the panel*.

high degree of binding to the immobilized nucleosome array by itself (Fig. 3*B*, *lane 3*). However, with the addition of competitor chromatin, SAGA interaction with the template was lost (compare *lanes 3* and *4*). Under these competitive conditions, the presence of Gal4 resulted in specific targeting of SAGA to the template (Fig. 3*B*, compare *lanes 6* and *7*). This result along with the direct interaction data in earlier figures indicates that the Gal4AD directly targets the SAGA to promoter chromatin *in vitro*. The addition of Gal80 reduced Gal4 targeting of SAGA to near background levels (Fig. 3, *lane 8*). Thus, Gal80 inhibition of SAGA interaction with Gal4 appears to be one mechanism for modulating activator targeting of SAGA.

The specificity of Gal80 inhibition of SAGA targeting was also tested using the Gcn4AD (Fig. 4*A*). To specify Gcn4 for this promoter, the Gal4 DNA-binding domain (aa 1–93) was fused to the Gcn4AD (aa 9–172). Again in Fig. 4*B*, we demonstrate that Gal4 targets SAGA to a nucleosomal promoter (compare *lanes 2* and *3*), and Gal80 effectively reduces this targeting to background levels (*lane 4*). Gcn4 was also able to target SAGA to this promoter (compare *lanes 2* and *5*), but in this case Gal80 was unable to inhibit Gcn4 targeting of SAGA (*lane 6*). Thus, it appears that although both of these activators efficiently target SAGA, Gal80 inhibition of this targeting is specific to the Gal4AD.

In vivo Gal80 is known to bind Gal4 and shut down *GAL* genes following the addition of glucose to cells grown in galactose (54), suggesting that coactivators targeted by Gal4 are displaced following this switch in carbon sources. Therefore, we next determined whether Gal80 was able to specifically displace Gal4-targeted SAGA from a nucleosome template (Fig. 5*A*). To address this possibility, SAGA was first targeted to the immobilized nucleosome with either Gal4 or Gcn4. Following this targeting step, Gal80 was added to activator targeted SAGA templates. Both Gal4 and Gcn4 exhibited efficient targeting of SAGA in the absence of Gal80 (Fig. 5*B*, *lanes 3* and *5*). However, when Gal80 is added following activator targeting, the Gal4-targeted SAGA is no longer bound to the template (*lane 4*), whereas the Gcn4-targeted SAGA remains bound (*lane 6*). Thus, reflective of Gal80-mediated glucose repression *in vivo*, Gal80 specifically displaces SAGA from Gal4-targeted templates.

DISCUSSION

The results presented here illustrate a mechanism whereby HAT targeting by activators can be regulated by additional factors, such as Gal80, that restrict activation domain function. This mechanism is supported by genetic studies using cells with mutations in SAGA components. For instance, deletion of either the *SPT20* or *SPT3* gene, both known components of SAGA, has debilitating effects on *GAL* expression (10, 11).

Furthermore, chromatin immunoprecipitation experiments have demonstrated that SAGA is recruited to *GAL* promoters as a function of galactose induction (12) and the Gal4AD (10). Our results provide biochemical evidence that these observations are the result of Gal4 targeting of the SAGA complex through direct interaction.

Genetic support for a Gal80 role in regulating Gal4 targeting of SAGA is garnered from an experiment testing the effect of an *SPT20* disruption in the background of a *GAL80* disruption (10). In this experiment, the deletion of *GAL80* resulted in the expression of *GAL* genes under growth conditions where these genes are normally inactive. When *SPT20* was disrupted in this *gal80* mutant, *GAL* expression was suppressed. The results of this experiment suggest a relationship between components of SAGA and Gal80. Based on our findings, this relationship is probably the result of Gal80 inhibiting SAGA targeting by Gal4.

Our results also provide important insight into the mechanisms that specify HAT complex interaction with activators. Both SAGA and NuA4 have been shown through biochemical approaches to contact a variety of yeast activator proteins (22). However, the cell must provide mechanisms that specify the appropriate interaction under a particular growth condition. Here we show that coregulatory proteins, such as Gal80, can restrict the interaction between these HATs and Gal4, whereas under the same conditions, these complexes are still able to function with other yeast activators as shown with Gcn4 in this study. This scenario would allow other regulatory pathways, such as activation of amino acid biosynthetic genes by Gcn4, to continue unimpeded by Gal80.

While it would be intellectually pleasing if yeast activation domains were found to interact specifically with distinct targets, the data argue that activation domains do not have the capacity to make these distinctions. Several activators have been shown to interact with the TFIID (21), Mediator (55, 56), SAGA, NuA4 (17, 22), and SWI/SNF (46) complexes. The fact that activation domains appear to have the ability to recruit several different complexes may allow alternative mechanisms to activate many genes in yeast and may account for synthetic phenotypes between components of these complexes (15, 57). Other genes may activate under more stringent circumstances requiring a precise pathway of activation (13, 58). Since it is clear that many yeast activators occupy their target promoters prior to induction of the gene (13, 24, 26, 59), their activation domain function must be regulated. In this report, we provide one example of this by demonstrating that cofactors like Gal80 can regulate the function of activators in recruiting HAT complexes.

Acknowledgments—We thank Richard Reece for the Gal80 and Gal4(1–93 + 771–881) expression plasmids, Kristen Neely for the Gal4-Gcn4 expression plasmid, Shigihiro Osada for the *ESA1-HA* cells, Christine Brown for the Δ*tra1* cells and FLAG-Tra1 plasmid, and Chris Brandl for *Ycp88-HA-ADA2*.

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