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Interdependent Recruitment of SAGA and Srb Mediator by Transcriptional Activator Gcn4p

Hongfang Qiu, Cuihua Hu, Fan Zhang, Gwo Jiunn Hwang,† Mark J. Swanson,‡ Cheunchit Boonchird,§ and Alan G. Hinnebusch*

Laboratory of Gene Regulation and Development, National Institute of Child Health and Human Development, Bethesda, Maryland 20892

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Transcriptional activation by Gcn4p is enhanced by the coactivators SWI/SNF, SAGA, and Srb mediator, which stimulate recruitment of TATA binding protein (TBP) and polymerase II to target promoters. We show that wild-type recruitment of SAGA by Gcn4p is dependent on mediator but independent of SWI/SNF function at three different promoters. Recruitment of mediator is also independent of SWI/SNF but is enhanced by SAGA at a subset of Gcn4p target genes. Recruitment of all three coactivators to *ARG1* is independent of the TATA element and preinitiation complex formation, whereas efficient recruitment of the general transcription factors requires the TATA box. We propose an activation pathway involving interdependent recruitment of SAGA and Srb mediator to the upstream activation sequence, enabling SWI/SNF recruitment and the binding of TBP and other general factors to the promoter. We also found that high-level recruitment of Tra1p and other SAGA subunits is independent of the Ada2p/Ada3p/Gcn5p histone acetyltransferase module but requires Spt3p in addition to subunits required for SAGA integrity. Thus, while Tra1p can bind directly to Gcn4p in vitro, it requires other SAGA subunits for efficient recruitment in vivo.

Transcription initiation by RNA polymerase II (Pol II) is dependent on a set of general transcription factors (GTFs), including the TATA binding protein (TBP), which recognize the core promoter and facilitate initiation from the correct start site (13). Eukaryotic transcriptional activators bind to upstream activation sequences (UASs) and stimulate preinitiation complex (PIC) assembly by disrupting repressive nucleosome structures and recruiting TBP, other GTFs, and Pol II to the promoter. Typically, activators execute these functions indirectly by recruiting cofactors or coactivators to the UAS region (20, 41). The SWI/SNF complex of *Saccharomyces cerevisiae* is a coactivator that uses ATP hydrolysis to displace or destabilize nucleosomes (42, 62). The coactivator SAGA contains a histone acetyltransferase (HAT) subunit, Gcn5p, that acetylates the amino-terminal tail of histone H3 (24, 33). Histone acetylation destabilizes higher-order chromatin structure (60) and may stimulate binding of coactivators harboring a bromodomain (15, 27, 29, 47). SAGA also binds to TBP in vitro (4, 58, 61) and enhances TBP recruitment by activators in vivo (6, 18, 36, 53), most likely functioning as an adaptor between the activators and TBP. The Srb mediator is a coactivator that can interact directly with Pol II to form a holoenzyme complex. In vitro, mediator stimulates basal and activated transcription and enhances phosphorylation of the C-terminal domain of the largest Pol II subunit by TFIIH (40). The mediator is absent from the C-terminal-domain-phosphorylated, elongating form of Pol II (51, 63) and interacts exclusively with nonphosphorylated Pol II at the promoter. Mediator can also interact with various GTFs (9, 40, 55, 56), possibly including TBP (30, 66), and it promotes the recruitment of TBP as well as Pol II to promoters in vivo (35, 38, 39, 53).

Gcn4p is a transcriptional activator of amino acid biosynthetic genes in yeast (45) that is induced at the translational level by starvation for any amino acid (26). Gcn4p activation function is dependent on clusters of hydrophobic residues in its activation domain (16, 28) that contribute to its binding to SAGA, SWI/SNF, and mediator in vitro (17, 23, 43, 68) and its ability to recruit SWI/SNF (72) and Gcn5p HAT activity (33) to target promoters in vivo. Mutations have been identified in multiple subunits of SAGA, SWI/SNF, and Srb mediator that diminish transcriptional activation by Gcn4p (5, 21, 44, 48, 50, 53, 64) and decrease the recruitment of TBP and Pol II by Gcn4p to target promoters in vivo (53).

The molecular mechanisms of coactivator recruitment by Gcn4p are not well understood. Three subunits of SWI/SNF, Swi2p, Snf5p, and Swi1p, can bind directly to Gcn4p in vitro (46). However, we found that Gcn4p cannot recruit Snf2p and Snf5p to target promoters in vivo when the SWI/SNF complex is disrupted, suggesting that SWI/SNF recruitment depends on multiple contacts between Gcn4p and SWI/SNF subunits (72). Indeed, it was shown that particular segments of Snf5p and Swi1p make additive contributions to the binding of SWI/SNF by Gcn4p in vitro (52). Similarly, we found that optimal recruitment of mediator by Gcn4p requires subunits from the head and tail domains of mediator, although the Gal11p/Med2p/Pgd1p triad from the tail domain is efficiently recruited by Gcn4p when separated from the rest of mediator by deleting

* Corresponding author. Mailing address: NIH, Building 6A, Room B1A-13, Bethesda, MD 20892. Phone: (301) 496-4480. Fax: (301) 496-6828. E-mail: ahinnebusch@nih.gov.

† Present address: Life Science Institute, Nanchang University, 235 Nanjing E. Road, Nanchang, Jiangxi, 330047, People's Republic of China.

‡ Present address: Carson Taylor Hall, Room 121, School of Biological Sciences, Louisiana Tech University, 1 Arizona, POB 3179, MC 37, Ruston, LA 71272-3045.

§ Present address: Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6 Rd., Bangkok 10400, Thailand.

the Sin4p subunit (73). It has been proposed that Gcn4p recruits SAGA through a direct interaction with the Ada2p (3) or Tra1p (10) subunit, but these hypotheses have not been tested directly in vivo. Here, we present evidence that optimal Gcn4p recruitment of Tra1p and other SAGA subunits is dependent on both the integrity of SAGA and the Spt3p subunit but occurs independently of the Ada2p/Ada3p/Gcn5p HAT module.

We also investigated whether recruitment of one coactivator enhances the ability of Gcn4p to recruit other coactivators. Genetic evidence suggests that H3 acetylation by Gcn5p (SAGA) enhances recruitment of SWI/SNF via the bromodomain in Swi2p (25). However, we and others found that substantial recruitment of SWI/SNF by Gcn4p occurs independently of both Swi2p (72) and the Gcn5p subunit of SAGA (65, 72). On the other hand, mutations that disrupt SAGA greatly reduced SWI/SNF recruitment, indicating that a non-HAT function of SAGA is important for SWI/SNF recruitment by Gcn4p (72). Here we show that a non-HAT SAGA function also stimulates recruitment of Srb mediator at a subset of Gcn4p target genes. In contrast to recent findings on Gal4p (7, 11, 37), we find that mediator is required for recruitment of SAGA and that SWI/SNF recruitment is independent of PIC formation. These and other new findings allow us to propose an activation pathway for Gcn4p involving interdependent recruitment of SAGA, mediator, and SWI/SNF to the UAS, which enables subsequent recruitment of TBP, other GTFs, and Pol II to the downstream promoter for PIC assembly.

MATERIALS AND METHODS

Yeast strains and plasmids. All strains and plasmids are listed in Tables 1 and 2, respectively. The wild-type (WT) parent strain BY4741 and deletion derivatives thereof were described previously (69) and purchased from Research Genetics. The presence of all reported deletion alleles was confirmed by PCR amplification or complementation of mutant phenotypes by plasmid-borne wild-type genes (64). Strains carrying *gcn4*Δ::*hisG* were created by transformation with plasmid pHQ1240 (72). myc-tagged strains were constructed as previously described (64). To construct *tra1*Δ::*HIS3* strains harboring episomal *TRA1-FL*, the parent strains were first transformed with *URA3* plasmid p4122 carrying *TRA1-FL*. The resulting strains were transformed with plasmid pHQ1376 containing *tra1*Δ::*HIS3* digested with SspI and selected on SC-His. Deletion of chromosomal *TRA1* in the resulting transformants was indicated by their sensitivity to 5-fluoroorotic acid and confirmed by PCR analysis. Strains carrying the *arg1*-Δ*TATA* allele in the chromosome were constructed as described previously (53).

Plasmids pHQ1239, pHQ1303, and pHQ1304 were described previously (64, 72), as was pHQ1344 (53). To construct *tra1*Δ::*HIS3*, 5′ noncoding and 3′ noncoding regions of *TRA1* were amplified by PCR and inserted into pUC18. For the resulting construct, the Eco47III-SspI fragment of *HIS3* from pRS403 (22) was inserted at the EcoRV site (at position −35 upstream of *TRA1*) to produce plasmid pHQ1376. Plasmid p4122 containing the *TRA1-FL* allele was created in several steps. A fragment encoding the 3× Flag epitope ($FLAG_3$) and containing a 5′ XbaI site and 3′ blunt end was created by PCR with Turbo PFU *Taq* polymerase using plasmid p3xFLAG-CMV-7.1 (Sigma) as template, 5′ primer GGGGTCTAGAATGGACTACAAAGACCATGACGGTGAT, and 3′ primer CTTGTCATCGTCATCCTTGTAGTC. (Underlined sequences in PCR primers are the relevant restriction sites used for cloning [in this case, XbaI].) In parallel, a *TRA1* fragment containing nucleotides +1 to +1018 of the open reading frame (ORF) and harboring a 5′ blunt end and 3′ PstI site was PCR amplified from genomic DNA using 5′ primer ATGTCACTCACTGAGCAGATCGAG and 3′ primer GGGGCTGCAGGTTCAGAAGGGCAGTCTTGTAAAA. These two PCR products were ligated together and cloned into pBluescript SK(−) digested with XbaI and PstI. The resulting plasmid was used as template to PCR amplify the *TRA1* ORF from +1 to +1018 fused in-frame to the coding sequences for $FLAG_3$ using 5′ primer ATGGACTACAAAGACCATGACGGTGAT and 3′ primer GGGGCTGCAGGTTCAGAAGGGCAGTCTTGTAAAA, generating a fragment with a blunt 5′ end and a PstI site at the 3′ end. Independently, a fragment spanning nucleotides −875 to −1 upstream of the *TRA1* ORF, containing a SacI site at the 5′ end and a blunt 3′ end, was PCR amplified from genomic DNA using 5′ primer GGGGGAGCTCCAAGAGAGAGCGCTGAA ACACTA and 3′ primer CGGCAAAATGCGGTATTCTTTGTAA. These last two fragments were ligated together and cloned between the SacI and PstI sites of pBluescript SK(−). The sequence of the resulting plasmid was verified by DNA sequencing and then digested with SacI and SnaBI (a unique site in the *TRA1* coding region). The resulting SacI-SnaBI fragment was isolated and used to replace the corresponding segment in the full-length *TRA1* gene cloned into YCplac33, producing p4122.

Biochemical methods. The chromatin immunoprecipitation (ChIP) experiments were conducted as described previously using the same primers described there (53, 64, 72). For Western analysis, whole-cell extracts (WCEs) were prepared as described previously (54) and analyzed using monoclonal anti-myc (Roche) and anti-Flag M2 antibodies (Sigma) and polyclonal anti-Gcd6p antibodies (12). Coimmunoprecipitation assays were conducted essentially as described previously (73), except that EZview Red Anti-FLAG M2 Affinity Gel (Sigma) was used to immunoprecipitate FL-Tra1p.

RESULTS

Optimal recruitment of SAGA subunits by Gcn4p is independent of Gcn5p but requires SAGA integrity and Spt3p. Using the ChIP assay and yeast strains containing functional myc-tagged forms of SAGA subunit Spt7p or Ada2p, we first established that Gcn4p recruits SAGA to the UAS_{GCRE} elements at *ARG1*, *ARG4*, and *SNZ1*. As shown in Fig. 1A, the binding of myc-Spt7p and myc-Ada2p to UAS elements at all three genes occurred at higher levels in cells containing *GCN4* on a high-copy-number plasmid compared to *gcn4*Δ cells when synthesis of Gcn4p was induced by starvation for isoleucine and valine. (We have shown that expression of a myc-tagged form of Gcn4p from a high-copy-number plasmid increases binding of myc-Gcn4p at the *ARG1* UAS by approximately twofold compared to that seen with myc-Gcn4p expressed from a single-copy plasmid [data not shown]. The elevated UAS occupancy afforded by this modest overexpression enhances our ability to quantify recruitment of SAGA and other coactivators by Gcn4p. We showed recently that overexpression of Gcn4p did not alter the subunit requirements for recruitment of Srb mediator to *ARG1* compared to that seen with Gcn4p produced at native levels [73]; hence, we believe that the higher promoter occupancy obtained with Gcn4p overexpression does not qualitatively alter the requirements for coactivator recruitment.) The recruitment of both SAGA subunits to *ARG1* requires the hydrophobic residues in the Gcn4p activation domain, as a strain containing 14 Ala substitutions in these residues (encoded by *gcn4-14Ala*) showed low levels of myc-Spt7p and myc-Ada2p binding, similar to that seen in *gcn4*Δ cells (Fig. 1B). The *14-Ala* substitutions do not reduce binding of myc-tagged Gcn4p to the *ARG1* UAS (72). High-level recruitment of two myc-tagged subunits of Srb mediator, Srb6p and Gal11p, also requires the hydrophobic residues in the activation domain (Fig. 1B).

Ada2p is a subunit of the ADA complex in addition to SAGA (19, 23). To demonstrate that both Spt7p and Ada2p are recruited by Gcn4p as subunits of SAGA, we created *SPT7*-myc and *ADA2*-myc strains harboring deletions of the genes encoding SAGA subunit Ada1p, Ada5p, or Spt7p, which is required for SAGA integrity in vitro (23, 61, 71). Likewise, we analyzed an *ADA2*-myc strain lacking Ahc1p, required for integrity of the ADA complex (19). These strains were subjected

TABLE 1. Yeast strains used in this study

Name	Parent	Relevant genotype[c]	Reference
SPT7-myc			
HQY453	BY4741[a]	*SPT7-myc$_{13}$::HIS3**	This work
HQY457	249[a]	*SPT7-myc$_{13}$::HIS3*gcn4*Δ*::kanMX4*	64
HQY508	1799[a]	*SPT7-myc$_{13}$::HIS3*ahc1*Δ*::kanMX4*	This work
HQY579	4282[a]	*SPT7-myc$_{13}$::HIS3*ada2*Δ*::kanMX4*	This work
HQY580	3534[a]	*SPT7-myc$_{13}$::HIS3*ada3*Δ*::kanMX4*	This work
HQY484	7285[a]	*SPT7-myc$_{13}$::HIS3*gcn5*Δ*::kanMX4*	This work
HQY497	1038[a]	*SPT7-myc$_{13}$::HIS3*ada1*Δ*::kanMX4*	This work
HQY496	7309[a]	*SPT7-myc$_{13}$::HIS3*ada5*Δ*::kanMX4*	This work
HQY581	4228[a]	*SPT7-myc$_{13}$::HIS3*spt3*Δ*::kanMX4*	This work
HQY582	2666[a]	*SPT7-myc$_{13}$::HIS3*spt8*Δ*::kanMX4*	This work
HQY498	LSO2[b]	*SPT7-myc$_{13}$::HIS3*med2*Δ*::kanMX4*	This work
HQY664	6611[a]	*SPT7-myc$_{13}$::HIS3*srb2*Δ*::kanMX4*	This work
HQY499	4734[a]	*SPT7-myc$_{13}$::HIS3*srb5*Δ*::kanMX4*	This work
HQY536	3119[a]	*SPT7-myc$_{13}$::HIS3*rox3*Δ*::kanMX4*	This work
HQY472	1586[a]	*SPT7-myc$_{13}$::HIS3*swi2*Δ*::kanMX4*	This work
HQY706	HQY457[b]	*SPT7-myc$_{13}$::HIS3*gcn4*Δ*::kanMX4 arg1-ΔTATA*	This work
ADA2-myc			
HQY392	BY4741[a]	*ADA2-myc$_{13}$::HIS3**	This work
HQY503	HQY392[b]	*ADA2-myc$_{13}$::HIS3*gcn4*Δ*::hisG*	This work
HQY546	1799[a]	*ADA2-myc$_{13}$::HIS3*ahc1*Δ*::kanMX4*	This work
HQY668	3534[a]	*ADA2-myc$_{13}$::HIS3*ada3*Δ*::kanMX4*	This work
HQY420	7285[a]	*ADA2-myc$_{13}$::HIS3*gcn5*Δ*::kanMX4*	This work
HQY418	1038[a]	*ADA2-myc$_{13}$::HIS3*ada1*Δ*::kanMX4*	This work
HQY520	7309[a]	*ADA2-myc$_{13}$::HIS3*ada5*Δ*::kanMX4*	This work
HQY551	3218[a]	*ADA2-myc$_{13}$::HIS3*spt7*Δ*::kanMX4*	This work
HQY419	4228[a]	*ADA2-myc$_{13}$::HIS3*spt3*Δ*::kanMX4*	This work
HQY669	2666[a]	*ADA2-myc$_{13}$::HIS3*spt8*Δ*::kanMX4*	This work
HQY500	LSO2[b]	*ADA2-myc$_{13}$::HIS3*med2*Δ*::kanMX4*	This work
HQY667	6611[a]	*ADA2-myc$_{13}$::HIS3*srb2*Δ*::kanMX4*	This work
HQY477	4734[a]	*ADA2-myc$_{13}$::HIS3*srb5*Δ*::kanMX4*	This work
HQY573	3119[a]	*ADA2-myc$_{13}$::HIS3*rox3*Δ*::kanMX4*	This work
HQY666	1586[a]	*ADA2-myc$_{13}$::HIS3*swi2*Δ*::kanMX4*	This work
SRB6-myc			
HQY464	BY4741[a]	*SRB6-myc$_{13}$::HIS3**	This work
HQY470	HQY464[b]	*SRB6-myc$_{13}$::HIS3*gcn4*Δ*::hisG*	64
HQY563	7285[a]	*SRB6-myc$_{13}$::HIS3*gcn5*Δ*::kanMX4*	This work
HQY567	1038[a]	*SRB6-myc$_{13}$::HIS3*ada1*Δ*::kanMX4*	This work
HQY568	7309[a]	*SRB6-myc$_{13}$::HIS3*ada5*Δ*::kanMX4*	This work
HQY564	3218[a]	*SRB6-myc$_{13}$::HIS3*spt7*Δ*::kanMX4*	This work
HQY562	1586[a]	*SRB6-myc$_{13}$::HIS3*swi2*Δ*::kanMX4*	This work
HQY705	HQY470[b]	*SRB6-myc$_{13}$::HIS3*gcn4*Δ*::hisG arg1-ΔTATA*	This work
GAL11-myc			
HQY438	BY4741[a]	*GAL11-myc$_{13}$::HIS3**	This work
HQY439	249[a]	*GAL11-myc$_{13}$::HIS3*gcn4*Δ*::kanMX4*	64
HQY552	7285[a]	*GAL11-myc$_{13}$::HIS3*gcn5*Δ*::kanMX4*	This work
HQY549	1038[a]	*GAL11-myc$_{13}$::HIS3*ada1*Δ*::kanMX4*	This work
HQY550	7309[a]	*GAL11-myc$_{13}$::HIS3*ada5*Δ*::kanMX4*	This work
HQY544	3218[a]	*GAL11-myc$_{13}$::HIS3*spt7*Δ*::kanMX4*	This work
HQY662	1586[a]	*GAL11-myc$_{13}$::HIS3*swi2*Δ*::kanMX4*	This work
TRA1-FL			
HQY825	249[a]	*tra1*Δ*::HIS3[TRA1-FL]gcn4*Δ*::kanMX4*	This work
HQY830	BY4741[a]	*tra1*Δ*::HIS3[TRA1-FL]*	This work
HQY836	4282[a]	*tra1*Δ*::HIS3[TRA1-FL] ada2*Δ*::kanMX4*	This work
HQY837	3534[a]	*tra1*Δ*::HIS3[TRA1-FL]ada3*Δ*::kanMX4*	This work
HQY835	7285[a]	*tra1*Δ*::HIS3[TRA1-FL]gcn5*Δ*::kanMX4*	This work
HQY826	1038[a]	*tra1*Δ*::HIS3[TRA1-FL]ada1*Δ*::kanMX4*	This work
HQY827	7309[a]	*tra1*Δ*::HIS3[TRA1-FL]ada5*Δ*::kanMX4*	This work
HQY838	3218[a]	*tra1*Δ*::HIS3[TRA1-FL]spt7*Δ*::kanMX4*	This work
HQY833	4228[a]	*tra1*Δ*::HIS3[TRA1-FL]spt3*Δ*::kanMX4*	This work
HQY834	2666[a]	*tra1*Δ*::HIS3[TRA1-FL]spt8*Δ*::kanMX4*	This work

Continued on following page

TABLE 1—*Continued*

Name	Parent	Relevant genotype[c]	Reference
SWI2-myc			
HQY383	HQY470[b]	*SWI2-myc$_{13}$::HIS3*gcn4Δ::hisG*	This work
HQY707	HQY383[b]	*SWI2-myc$_{13}$::HIS3*gcn4Δ::hisG arg1-ΔTATA*	This work
Strains with myc-tagged GTFs			
HQY382	HQY366[b]	*TBP1-myc$_{13}$::HIS3*gcn4Δ::hisG*	53
HQY366	BY4741[a]	*TBP1-myc$_{13}$::HIS3**	53
HQY692	HQY366[b]	*TBP1-myc$_{13}$::HIS3*arg1-ΔTATA*	53
HQY422	HQY403[b]	*RPB3-myc$_{13}$::HIS3*gcn4Δ::hisG*	53
HQY403	BY4741[a]	*RPB3-myc$_{13}$::HIS3**	53
HQY693	HQY403[b]	*RPB3-myc$_{13}$::HIS3*arg1-ΔTATA*	53
HQY727	249[a]	*TOA1-myc$_{13}$::HIS3*gcn4Δ::kanMX4*	This work
HQY728	BY4741[a]	*TOA1-myc$_{13}$::HIS3**	This work
HQY704	HQY728[b]	*TOA1-myc$_{13}$::HIS3*arg1-ΔTATA*	This work
HQY690	249[a]	*SUA7-myc$_{13}$::HIS3*gcn4Δ::kanMX4*	This work
HQY691	BY4741[a]	*SUA7-myc$_{13}$::HIS3**	This work
HQY698	HQY691[b]	*SUA7-myc$_{13}$::HIS3*arg1-ΔTATA*	This work
HQY777	249[a]	*TFA1-myc$_{13}$::HIS3*gcn4Δ::kanMX4*	This work
HQY787	BY4741[a]	*TFA1-myc$_{13}$::HIS3**	This work
HQY788	HQY787[b]	*TFA1-myc$_{13}$::HIS3*arg1-ΔTATA*	This work
HQY778	249[a]	*TFG2-myc$_{13}$::HIS3*gcn4Δ::kanMX4*	This work
HQY779	BY4741[a]	*TFG2-myc$_{13}$::HIS3**	This work
HQY780	HQY779[b]	*TFG2-myc$_{13}$::HIS3*arg1-ΔTATA*	This work
HQY785	249[a]	*KIN28-myc$_{13}$::HIS3*gcn4Δ::kanMX4*	This work
HQY786	BY4741[a]	*KIN28-myc$_{13}$::HIS3**	This work
HQY776	HQY786[b]	*KIN28-myc$_{13}$::HIS3*arg1-ΔTATA*	This work

[a] Strain purchased from Research Genetics.
[b] Strain isogenic to Research Genetics strain.
[c] *HIS3** designates the *HIS3* allele from *Saccharomyces kluyveri.*

to ChIP analysis to measure the effects of disrupting SAGA or ADA on Gcn4p-dependent recruitment of the myc-tagged proteins to the *ARG1*, *ARG4*, and *SNZ1* UAS elements. The results of multiple, replicate ChIP assays were quantified and summarized in Fig. 2A and B. The values beneath the histograms in these figures give the Gcn4p-dependent component of myc-Spt7p or myc-Ada2p binding in the mutant strains as a percentage of that seen in the wild-type strain. (They correspond to the difference in heights of the histogram bars in the mutants and that measured in the *gcn4*Δ strain as a percentage of the corresponding difference calculated for the wild-type strain.)

The results shown in Fig. 2A indicate that recruitment of myc-Spt7p by Gcn4p is strongly dependent on *ADA1* and *ADA5*, as deletions of these genes reduced binding of myc-Spt7p to the UAS elements at all three genes to nearly the same low levels observed in *gcn4*Δ cells. Deletion of *SPT3* also led to significant reductions in binding of myc-Spt7p at all three genes, but not as severe as those given by *ada1*Δ or *ada5*Δ. By contrast, the *ada2*Δ, *ada3*Δ, *gcn5*Δ, and *spt8*Δ deletions produced little or no reduction in myc-Spt7p recruitment by Gcn4p (Fig. 2A). These last results indicate that the function of the Gcn5p/Ada2p/Ada3p module of SAGA in histone H3 acetylation is not required for high-level binding of SAGA at the UAS_{GCRE}. In fact, it appeared that recruitment of Spt7p to *ARG1* was even higher than WT in the *ada2*Δ, *ada3*Δ, and *gcn5*Δ mutants.

Similar to our findings on myc-Spt7p, recruitment of myc-Ada2p was greatly reduced by deletions of *ADA1*, *ADA5*, and *SPT7*; somewhat less impaired by deletion of *SPT3*; and relatively unaffected by deletions of *SPT8* or *GCN5* (Fig. 2B). The *ada3*Δ mutation reduced the recruitment of myc-Ada2p at all three promoters; however, this probably results from a reduced steady-state level of myc-Ada2p in *ada3*Δ cells (see below) (57). Deletion of *AHC1* had no effect on recruitment of myc-Spt7p and myc-Ada2p by Gcn4p (Fig. 2A and B). We showed previously that none of the SAGA subunit deletions reduced binding of myc-tagged Gcn4p to these target genes (53). We also conducted Western analysis on whole-cell extracts of the *SPT7-myc* and *ADA2-myc* strains to determine whether the reduced levels of myc-Spt7p and myc-Ada2p recruitment in SAGA mutants might result from their reduced expression. The results in Fig. 3A (lanes 1 to 9) eliminate this possibility for myc-Spt7p, which is expressed at wild-type levels in all relevant SAGA mutants. The same was true for myc-Ada2p, except that its expression was reduced in the *ada3*Δ strain (Fig. 3B, lanes 1 to 9).

The results thus far indicate that Ada1p, Ada5p, Spt7p, and Spt3p are required, but that the Gcn5p/Ada2p/Ada3p HAT

TABLE 2. Plasmids used in this study

Plasmid	Description	Reference
YEplac195	Vector	22
pHQ1239	*GCN4-HA$_3$* in YEplac195	64
pHQ1303	*GCN4* in YEplac195	72
pHQ1304	*gcn4-14Ala* in YEplac195	72
pHQ1344	*arg1-ΔTATA* in YIplac211	53
pHQ1376	*tra1::HIS3::tra1* in pUC18	This work
p4122	*TRA1-FL$_3$* in YCplac 33	This work

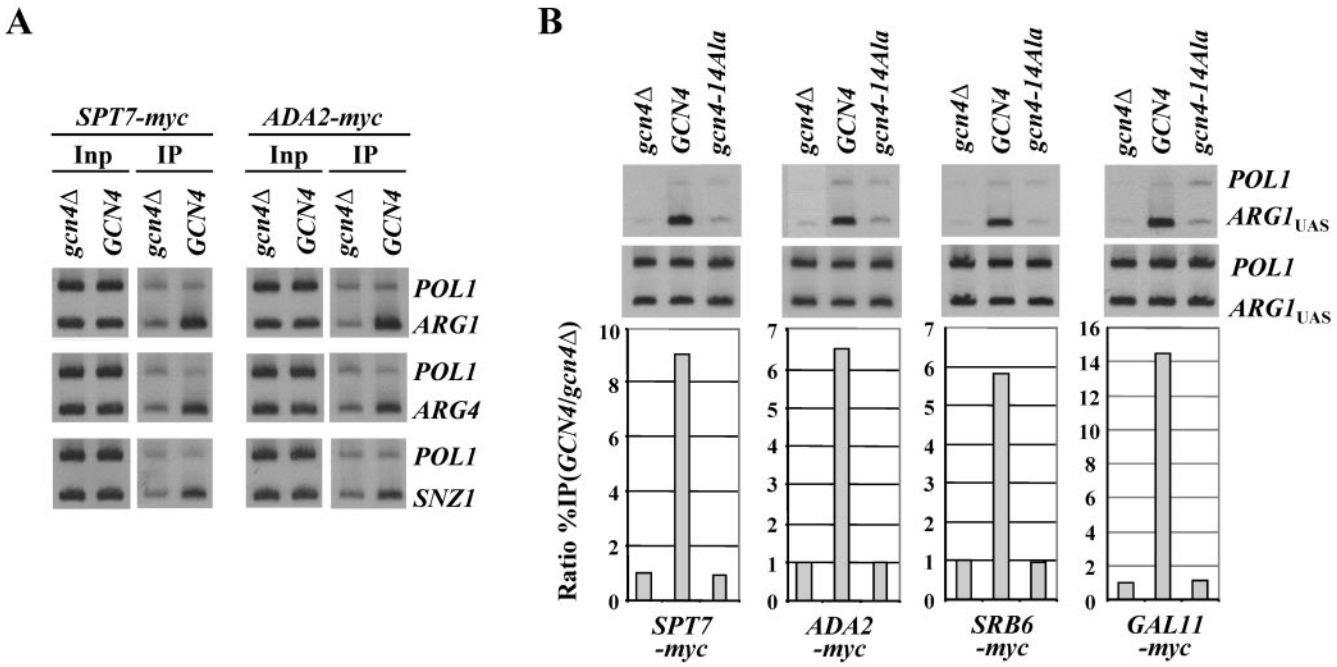

FIG. 1. Gcn4p recruits myc-Spt7p, myc-Ada2p, myc-Srb6p, and myc-Gal11p, dependent on hydrophobic residues in the activation domain. (A) *SPT7-myc gcn4*Δ and *ADA2-myc gcn4*Δ strains bearing empty vector (*gcn4*Δ) and *SPT7-myc GCN4* and *ADA2-myc GCN4* strains carrying high-copy-number *GCN4-HA* plasmid pHQ1239 were cultured in SC medium lacking Ile and Val and treated with sulfometuron for 2 h to induce Gcn4p synthesis by starvation for Ile/Val and then subjected to ChIP analysis with myc antibodies. DNA was extracted from the immunoprecipitates (IP), and 5% of the input (Inp) samples and a 1,000-fold dilution of the Inp and the undiluted IP DNA samples were PCR amplified using primers specific for the $POL1_{ORF}$, $ARG1_{UAS}$, $SNZ1_{UAS}$, or *ARG4* promoter (UAS and TATA sequence), in the presence of [^{33}P]dATP. The PCR products were resolved by polyacrylamide gel electrophoresis and visualized by autoradiography. (B) The *SPT7-myc gcn4*Δ and *ADA2-myc gcn4*Δ strains described above, along with *SRB6-myc gcn4*Δ and *GAL11-myc gcn4*Δ strains, bearing empty vector (*gcn4*Δ), high-copy-number *GCN4* plasmid pHQ1303 (*GCN4*), or high-copy-number *gcn4-14Ala* plasmid pHQ1304 (*gcn4-14Ala*), were subjected to ChIP analysis as above, and the results were quantified with a phosphorimager. The ratios of the $ARG1_{UAS}$ signals to the *POL1* signals in the IP samples were normalized for the corresponding ratios for the Inp samples, and the resulting values measured for the *GCN4* strain were normalized to the corresponding values obtained for the *gcn4*Δ strain to produce the "ratio %IP(*GCN4*/*gcn4*Δ)" values plotted in the histograms for each protein.

module is dispensable, for high-level recruitment of SAGA subunits by Gcn4p. Thus, if Ada2p is a direct target of the Gcn4p activation domain, as suggested previously (3), it cannot be recruited efficiently as an isolated subunit, or as a component of the ADA complex, outside of the intact SAGA complex.

Ada1p, Ada5p, and Spt7p are required to purify an intact SAGA complex from yeast cells (23, 61, 71). To confirm that myc-Spt7p and myc-Ada2p are dissociated from other SAGA subunits in the *ada1*Δ, *ada5*Δ, and *spt7*Δ mutants in vivo, we immunoprecipitated these proteins with anti-myc antibodies and probed the immune complexes for other SAGA subunits. As expected, SAGA subunits Taf12p, Ada3p, Gcn5p, and Tra1p were largely or completely dissociated from myc-Spt7p in *ada1*Δ and *ada5*Δ strains, although Taf9p remained strongly associated with myc-Spt7p in the *ada1*Δ mutant. Ada1p was associated with myc-Spt7p at a reduced level in *ada5*Δ cells, although the total level of Ada1p was reduced in this mutant extract (Fig. 4A). Similarly, myc-Ada2p was dissociated from Taf12p and Taf9p in *ada1*Δ, *ada5*Δ, and *spt7*Δ mutants, and its interaction with Spt7p was greatly reduced in the *ada1*Δ and *ada5*Δ cells (Fig. 4B). By contrast, Gcn5p remained fully associated with myc-Ada2p in the *ada1*Δ, *ada5*Δ, and *spt7*Δ mutants, presumably reflecting an intact Ada2p/Ada3p/Gcn5p subcomplex in addition to the ADA complex in such mutants with disrupted SAGA (2). As expected, deletion of *GCN5* or *SPT3* had little effect on association of other SAGA subunits with myc-Spt7p or myc-Ada2p (Fig. 4A and B) (71). Thus, in agreement with the previous findings cited above, we conclude that Ada1p, Ada5p, and Spt7p are required for SAGA integrity in vivo.

The simplest way to explain the fact that recruitment of myc-Ada2p and myc-Spt7p is reduced in all three mutants where SAGA integrity is disrupted (*ada1*Δ, *ada5*Δ, and *spt7*Δ) is to propose that Gcn4p interacts with only one or two SAGA subunits and that all other subunits must be connected to these targeted proteins to be efficiently recruited by Gcn4p in vivo. Previous evidence indicated that Tra1p is a direct target of the activators Hap4p (10) and Gal4p (7), and it was shown that Gcn4p can interact directly with purified Tra1p in vitro in the absence of other SAGA subunits (10). Assuming that Tra1p is a target of Gcn4p, we wished to determine whether Tra1p can be recruited by Gcn4p in mutant cells where SAGA is disrupted.

To answer this question, we deleted chromosomal *TRA1* in the panel of SAGA mutants described above and replaced it with a functional Flag-tagged allele of *TRA1* expressed from its own promoter on a single-copy plasmid (*TRA1-FL*). ChIP analysis of the resulting strains showed that recruitment of FL-Tra1p by Gcn4p was significantly reduced in the mutants

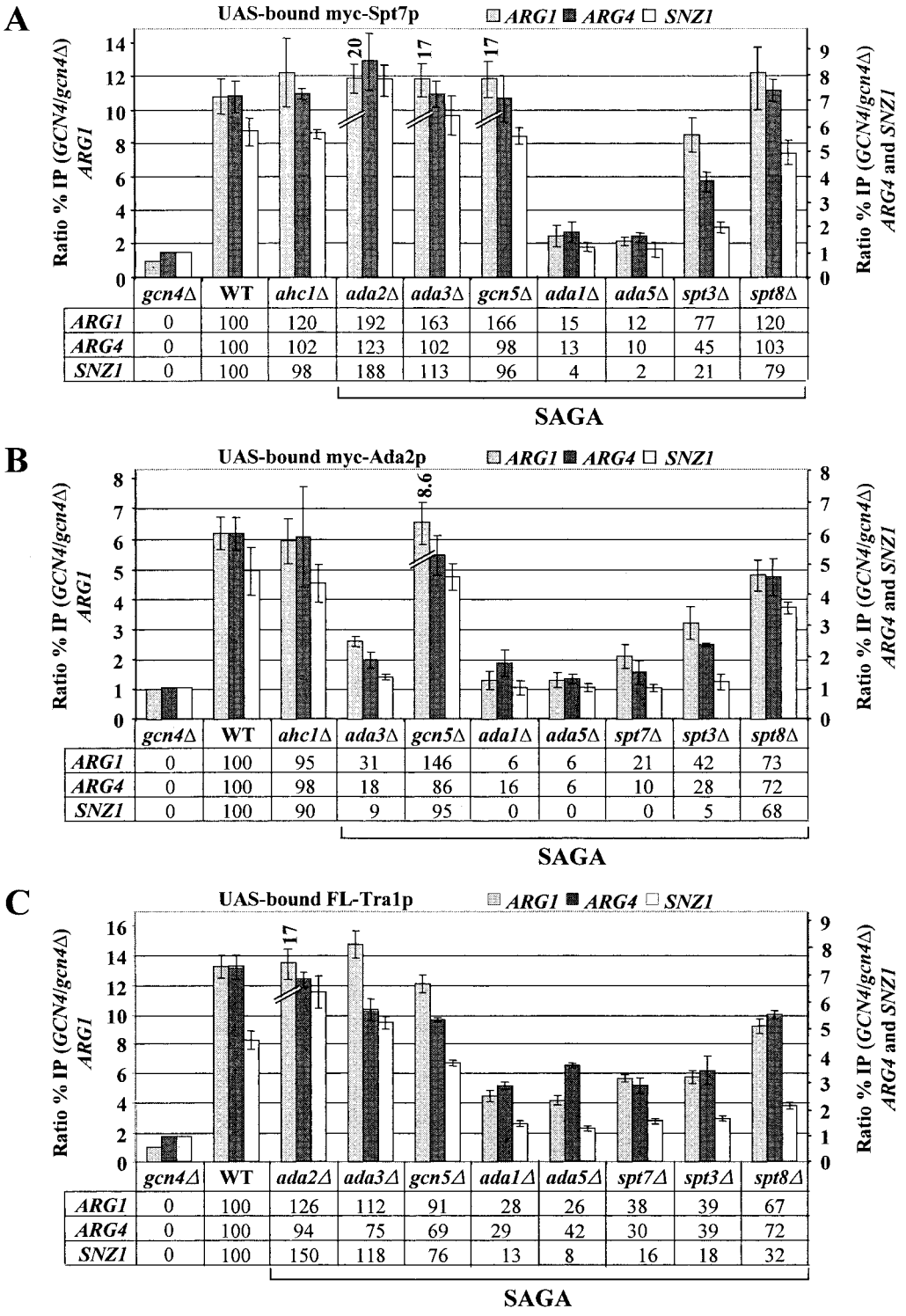

FIG. 2. Deletion of Spt3p or subunits required for SAGA integrity, but not Gcn5p, impairs recruitment of SAGA by Gcn4p. (A) ChIP analysis of a *gcn4*Δ *SPT7-myc* strain (*gcn4*Δ) and *GCN4 SPT7-myc* strains containing WT SAGA subunits or the indicated SAGA subunit deletions and harboring high-copy-number *GCN4-HA* plasmid pHQ1239 was carried out as described in Fig. 1 using anti-myc antibodies. (B) Same as panel A except that *ADA2-myc* strains were analyzed. (C) Same as panel A except that *TRA1-FL* strains were analyzed and anti-Flag M2 antibodies were used. The ratio %IP (*GCN4*/*gcn4*Δ) values as defined in Fig. 1 were calculated for the $ARG1_{UAS}$, $SNZ1_{UAS}$, and *ARG4* probes, and the average results obtained from two or more independent cultures and two or more PCR amplifications for each culture were plotted in the histograms with standard errors shown as error bars. The numbers under the histograms, corresponding to percentages of the WT Gcn4p-dependent binding of myc-Spt7p, myc-Ada2p, or FL-Tra1p, were calculated by subtracting unity from all ratio %IP (*GCN4*/*gcn4*Δ) values for each mutant and expressing the result as a percentage of the corresponding WT value.

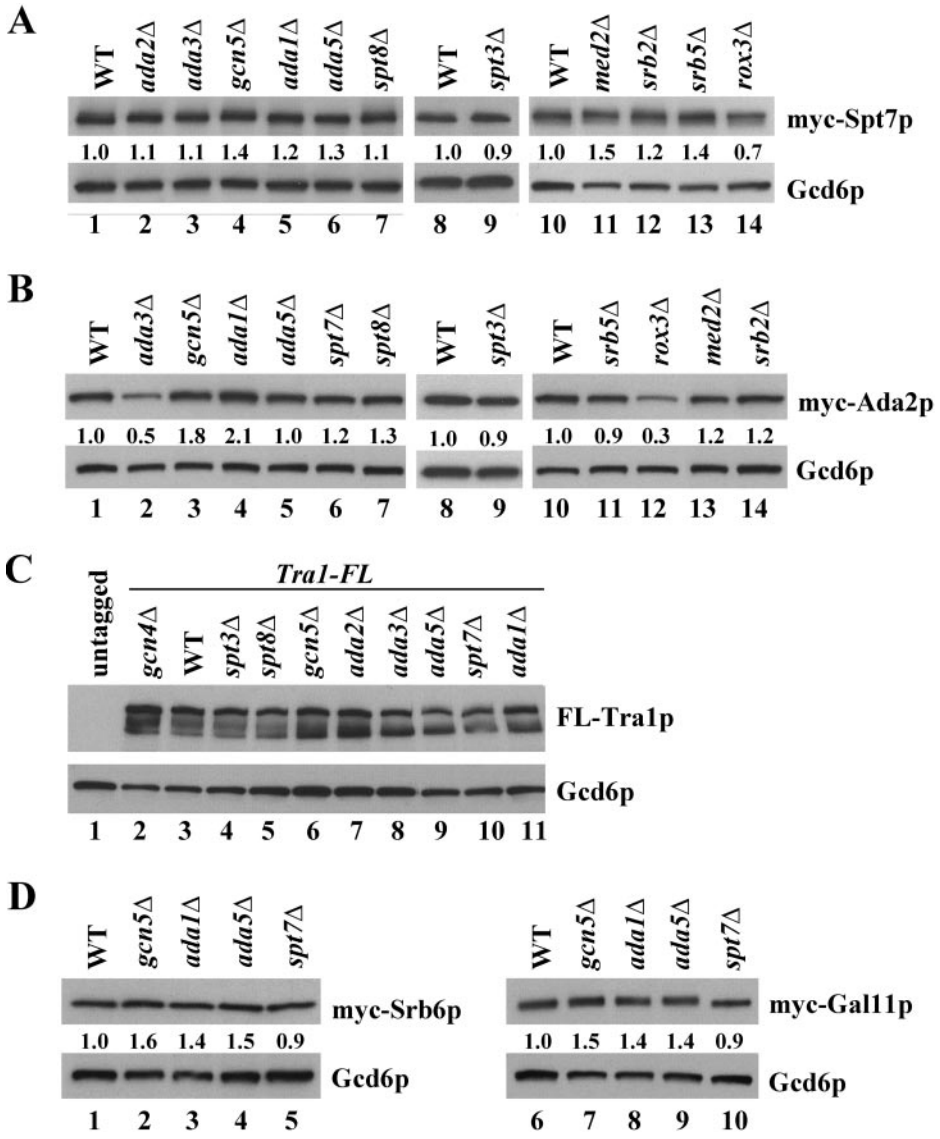

FIG. 3. Western analysis of tagged SAGA or mediator subunits in coactivator mutants. (A-D) myc- or Flag-tagged strains used in Fig. 2 or 5 or the untagged parent strain BY4741 (panel C) was grown under the same conditions used for ChIP analysis, and WCEs were prepared and subjected to Western blot analysis using anti-myc, anti-Flag M2, or anti-Gcd6p antibodies, the last serving as loading control. The Western signals obtained using the ECL chemiluminescence kit (Amersham) were quantified by video densitometry using NIH image software, and the ratios of myc-Spt7p, myc-Ada2p, myc-Srb6p, or myc-Gal11p to Gcd6p signals are listed for each mutant relative to the WT strain between the two blots.

lacking Ada1p, Ada5p, Spt7p, and Spt3p but occurred at essentially wild-type levels in the mutants lacking Ada2p, Ada3p, or Gcn5p (Fig. 2C). Western analysis showed that expression of FL-Tra1p was essentially unaffected by all of the deletions under consideration (Fig. 3C), and the coimmunoprecipitation experiments in Fig. 4A and C confirmed that Tra1p was dissociated from other SAGA subunits in the *ada1*Δ, *ada5*Δ, and *spt7*Δ strains but not in *spt3*Δ cells or in other SAGA mutants. Thus, we conclude that dissociation of FL-Tra1p from other SAGA subunits reduces the efficiency of FL-Tra1p recruitment by Gcn4p in vivo.

Wild-type recruitment of SAGA does not require the ATPase subunit of SWI/SNF but is dependent on multiple Srb mediator subunits. Since Gcn4p can interact specifically with SAGA in vitro, it was possible that efficient SAGA recruitment in vivo would be independent of other coactivators. To explore this possibility, we asked whether recruitment of SAGA is dependent on SWI/SNF and Srb mediator by constructing *SPT7-myc* and *ADA2-myc* alleles in deletion mutants lacking Swi2p; the ATPase subunit of SWI/SNF; or the Med2p, Srb2p, Srb5p, or Rox3p subunit of Srb mediator. We have shown (53) that all four mediator mutants are defective in transcriptional activation by Gcn4p, with *rox3*Δ cells exhibiting the largest reductions in mRNA levels at all three target genes under study. The *swi2*Δ mutant displayed little defect in *ARG1* and *ARG4* mRNA induction but a marked decrease in Gcn4p-dependent induction of *SNZ1* mRNA. Furthermore, we found that none of these mutations reduced binding of myc-Gcn4p to the target genes in vivo (53).

All four mediator subunit deletions impaired the recruit-

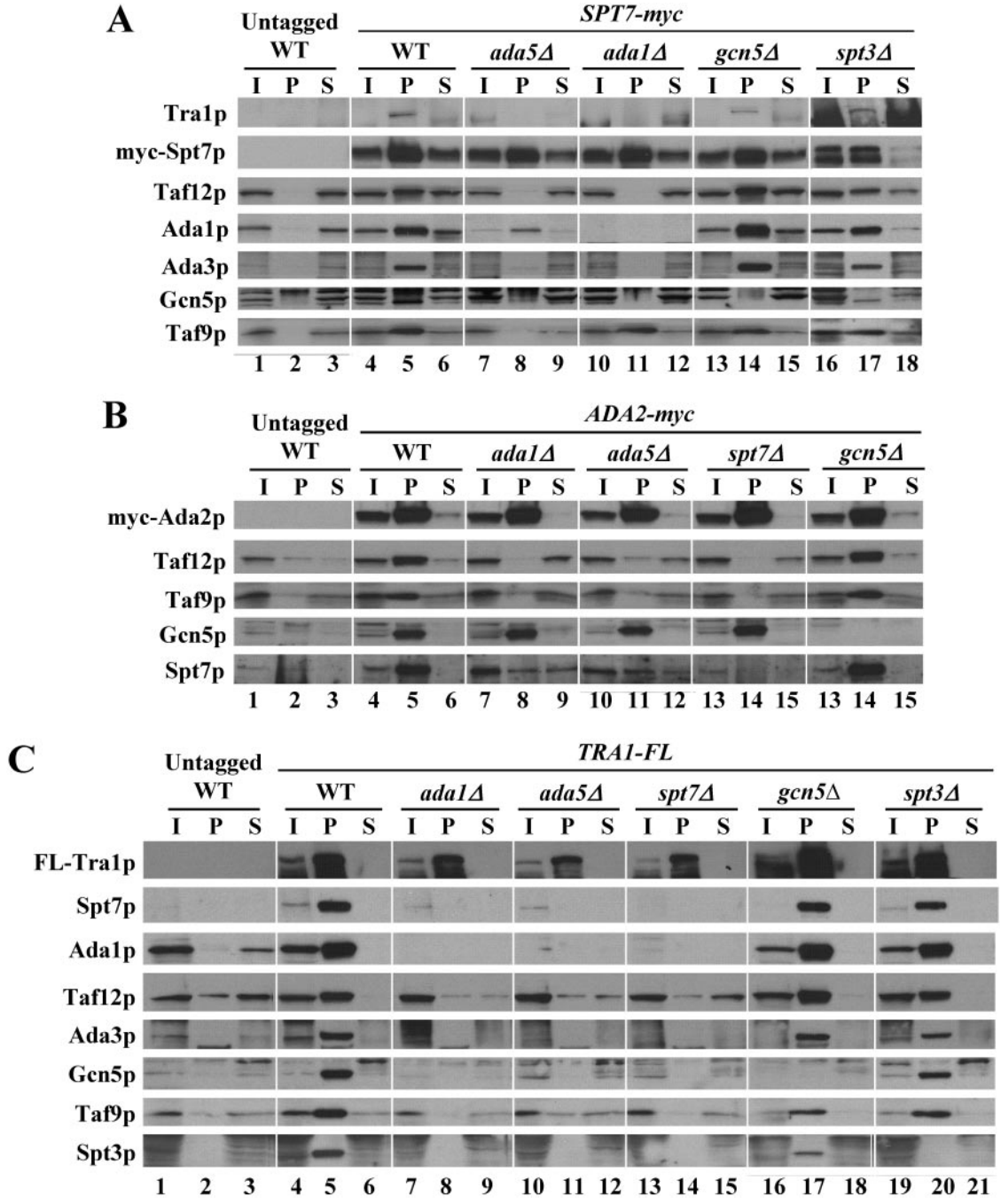

FIG. 4. Coimmunoprecipitation analysis of SAGA integrity in coactivator mutants. WCEs from the appropriate yeast strains were immunoprecipitated with monoclonal c-*myc* or Flag M2 antibodies. The immune complexes were collected, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subjected to Western analysis to detect the proteins listed on the left of each panel or with anti-myc or anti-Flag M2 antibodies to detect the myc- or Flag-tagged proteins. I, 10% of the input WCEs; P, 50% of the pellet fraction from the immunoprecipitates; S, 10% of the supernatant fractions.

ment of SAGA subunits in the following order of increasing severity: *med2*Δ, *srb2*Δ, *srb5*Δ, and *rox3*Δ (Fig. 5A and B). Western analysis shows that these reductions do not arise from decreased steady-state levels of myc-Spt7p or myc-Ada2p in the mediator mutants, with the possible exception of the *rox3*Δ strain (Fig. 3A and B, lanes 10 to 14). Even in this instance, however, the 75 to 87% reductions in myc-Spt7p recruitment (Fig. 5A) significantly exceed the ~30% reduction in myc-Spt7p expression in *rox3*Δ cells (Fig. 3A).

In contrast to the effects of mediator mutations, the *swi2*Δ mutant exhibits wild-type or higher levels of myc-Spt7p recruitment at all three target genes and wild-type or higher levels of myc-Ada2p binding at the *ARG1* and *ARG4* promoters. The only recruitment deficit observed in *swi2*Δ cells was a strong

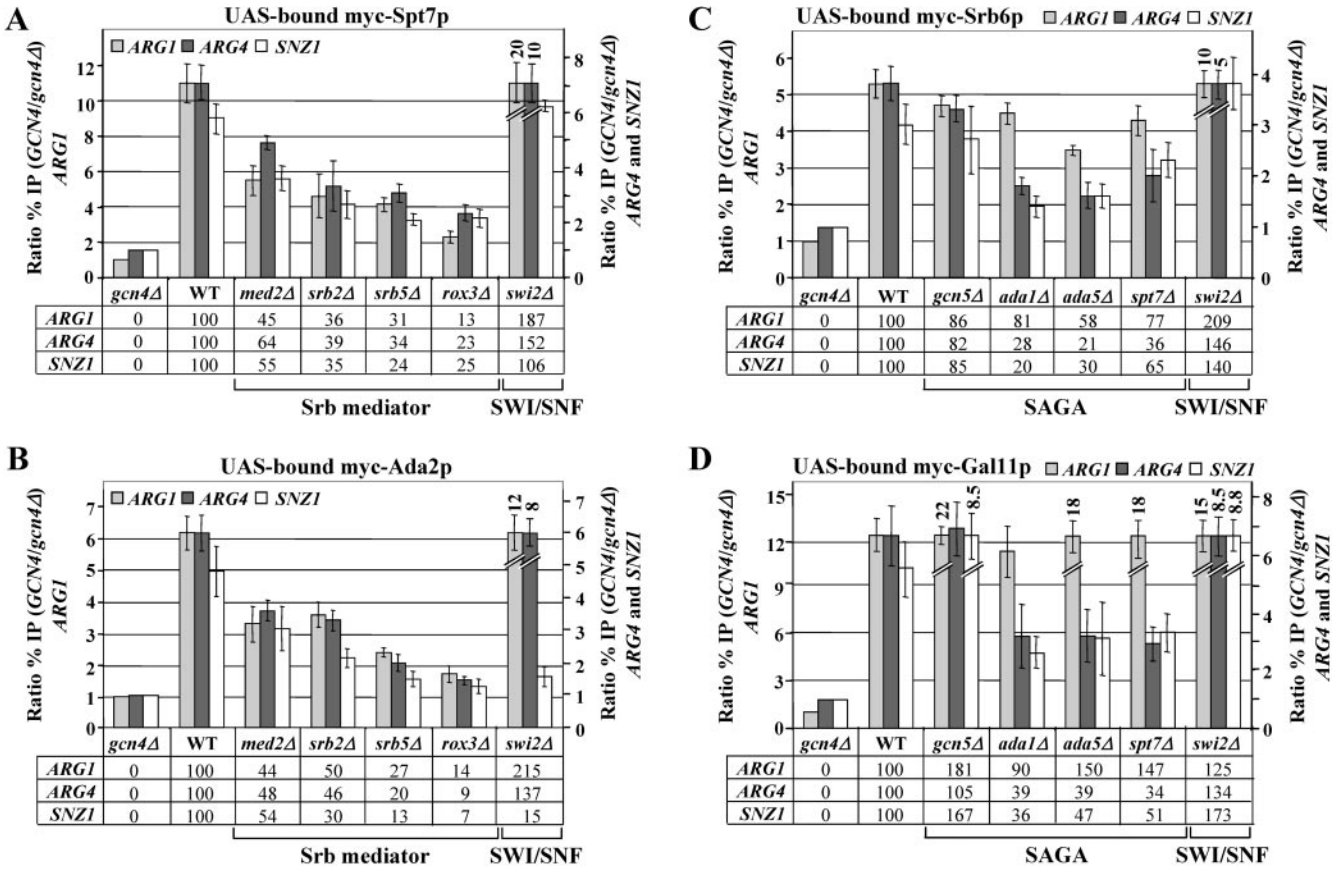

FIG. 5. Interdependent recruitment of SAGA and Srb mediator by Gcn4p. (A) ChIP analysis of a *gcn4*Δ *SPT7-myc* strain carrying empty vector (*gcn4*Δ) or *GCN4 SPT7-myc* strains containing no coactivator mutations (WT) or the indicated deletions of Srb mediator subunits, or *swi2*Δ, and harboring high-copy-number *GCN4-HA* plasmid pHQ1239, conducted as described in Fig. 1 and 2. (B) Same as panel A except that *ADA2-myc* strains were employed. (C-D) Same as panels A and B, except that *SRB6-myc* and *GAL11-myc* strains were employed, containing no coactivator mutations (WT) or the indicated deletions of SAGA subunits or *swi2*Δ.

reduction in myc-Ada2p binding at *SNZ1* (Fig. 5B). We conclude that recruitment of SAGA by Gcn4p is critically dependent on mediator but largely independent of SWI/SNF ATPase function. The fact that *swi2*Δ reduces the recruitment of Ada2p but not Spt7p at *SNZ1* may indicate more stringent requirements for retention of the ADA subcomplex compared to the rest of SAGA at this promoter. Indeed, we previously observed more stringent requirements for Gcn4p recruitment of SWI/SNF (72) and Srb mediator (73) at *SNZ1* versus *ARG1*. Our finding that SAGA recruitment is significantly elevated at *ARG1* and *ARG4* in *swi2*Δ cells (Fig. 5A and B) may indicate that nucleosome remodeling by SWI/SNF somehow limits the recruitment of SAGA by Gcn4p.

Recruitment of Srb mediator requires SAGA complex but not SWI/SNF function. We asked next whether recruitment of mediator is dependent on SAGA or SWI/SNF activity by introducing functional *SRB6-myc* or *GAL11-myc* alleles (64) into the *ada1*Δ, *ada5*Δ, *spt7*Δ, *gcn5*Δ, and *swi2*Δ mutants and conducting ChIP analysis. The results in Fig. 5C and D indicate that wild-type (or higher) levels of myc-Srb6p and myc-Gal11p recruitment occurred in *swi2*Δ cells, showing that SWI/SNF ATPase activity is dispensable for recruitment of Srb mediator by Gcn4p. Inactivating the HAT activity of SAGA by deletion of *GCN5* also had little effect on Srb mediator recruitment by Gcn4p. However, recruitment of myc-Srb6p and myc-Gal11p at the *ARG4* and *SNZ1* promoters was substantially reduced in the *ada1*Δ, *ada5*Δ, and *spt7*Δ mutants that disrupt SAGA integrity and impair recruitment of SAGA itself by Gcn4p (Fig. 5C and D, *ARG4* and *SNZ1*). The reduction in recruitment of myc-Srb6p and myc-Gal11p in these SAGA mutants does not arise from reduced expression of mediator subunits (Fig. 3D). Thus, a non-HAT function dependent on the integrity of SAGA complex is needed for optimal recruitment of Srb mediator by Gcn4p to *ARG4* and *SNZ1*. Deletion of *SPT3* also impaired recruitment of myc-Gal11p to *ARG4* and *SNZ1* by 60 to 70% (data not shown), in accordance with the reduction in recruitment of SAGA itself conferred by *spt3*Δ (Fig. 2). Because efficient recruitment of SAGA at these genes requires mediator subunits, it appears that recruitment of SAGA and mediator is highly interdependent at *ARG4* and *SNZ1*.

Surprisingly, SAGA is much less important for recruitment of Srb mediator at *ARG1* compared to *ARG4* and *SNZ1*. The *ada1*Δ, *ada5*Δ, and *spt7*Δ mutations reduced the recruitment of myc-Srb6p to *ARG1* by only 20 to 40% and had little effect on recruitment of myc-Gal11p by Gcn4p to this gene (Fig. 5C and D). The *spt3*Δ mutation likewise had a small effect on myc-Gal11p recruitment at *ARG1*, reducing it by only ~25% (data not shown). The difference between the results obtained for

myc-Srb6p and myc-Gal11p may be related to our recent finding that the Gal11p/Med2p/Pgd1p triad from the tail domain of mediator is an in vivo target of Gcn4p that can be recruited to *ARG1* independently of the rest of mediator (73). Thus, perhaps binding of the mediator tail domain at *ARG1* can be maintained independently of SAGA, whereas the mediator head domain (to which Srb6p belongs) requires SAGA function for maximal recruitment by Gcn4p. It is currently unclear why recruitment of both mediator head and tail subunits is less dependent on SAGA at *ARG1* than at *ARG4* and *SNZ1*.

Deletion of the TATA element at *ARG1* does not reduce recruitment of SAGA, Srb mediator, or SWI/SNF to the UAS_{GCRE} by Gcn4p. We previously reported that deletion of the TATA element at *ARG1* (Δ*TATA* mutation) greatly reduced recruitment of TBP and Pol II to the promoter by Gcn4p and impaired *ARG1* expression, producing arginine auxotrophy (53). These findings indicated that TBP recruitment is a prerequisite for high-level Pol II binding at *ARG1*. It was reported recently that recruitment of SWI/SNF and Srb mediator at *RNR3* was impaired by mutations in Rpb1p and a TFIID subunit, suggesting a requirement for PIC assembly for retention of SWI/SNF and mediator at this gene (59). Hence, to determine whether recruitment of SAGA, Srb mediator, and SWI/SNF by Gcn4p is dependent on stable TBP and Pol II binding to the promoter, we asked whether the Δ*TATA* mutation at *ARG1* would reduce recruitment of myc-tagged subunits of SAGA, Srb mediator, and SWI/SNF to the *ARG1* UAS. As shown in Fig. 6, subunits of SAGA, Srb mediator, and SWI/SNF were recruited by Gcn4p at the same level, or even higher levels, to the UAS of TATA-less *ARG1* compared to wild-type *ARG1*. For comparison, we included in Fig. 6 the quantification of our previous results indicating that recruitment of TBP and Pol II to the promoter was greatly impaired by the Δ*TATA* mutation (53).

We next investigated whether recruitment of various GTFs by Gcn4p can occur independently of TBP and Pol II binding to the promoter by examining whether the Δ*TATA* mutation at *ARG1* reduces recruitment of functional myc-tagged versions of TFIIB (myc-Sua7) or subunits of TFIIA (myc-Toa1p), TFIIE (myc-Tfa1p), TFIIF (myc-Tfg2p), or TFIIH (myc-Kin28p). These experiments were stimulated by reports of direct binding of activators to GTFs (reviewed in reference 17) and of association of GTFs with mediator (31, 40, 55, 56, 66). As shown in Fig. 7, Gcn4p recruits all of these GTFs to the *ARG1* promoter in a manner dependent on the TATA element. The strong reduction in myc-Toa1p, myc-Tfg2p, and myc-Kin28p recruitment produced by the Δ*TATA* mutation is comparable to that observed for myc-TBP itself, suggesting that TFIIA, TFIIF, and TFIIH recruitment is wholly dependent on TBP recruitment by Gcn4p. Recruitment of TFIIB and TFIIE may be partly independent of TBP, however, as their recruitment was impaired less than that of TBP and Pol II by the Δ*TATA* mutation (Fig. 7).

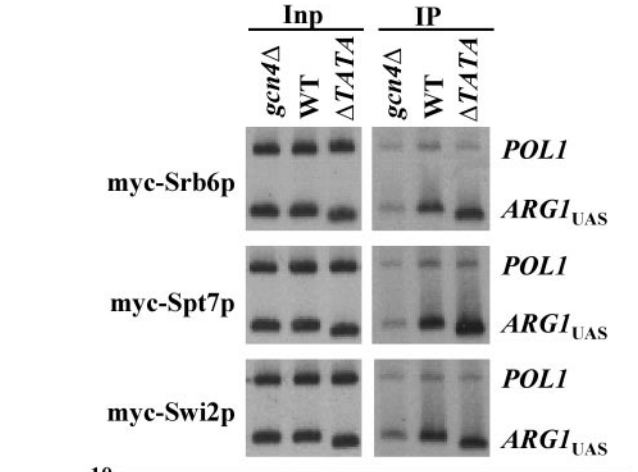

FIG. 6. Deletion of the *ARG1* TATA does not affect recruitment of SAGA, Srb mediator, and SWI/SNF by Gcn4p. Transformants of *gcn4*Δ strains carrying empty vector (*gcn4*Δ) or high-copy-number *GCN4-HA* plasmid pHQ1239 (WT) and of *gcn4*Δ *arg1*-Δ*TATA* strains carrying pHQ1239 (Δ*TATA*), harboring *SRB6-myc*, *SPT7-myc*, or *SWI2-myc*, were subjected to ChIP analysis as described in Fig. 1 and 2. (Data for *TBP-myc* and *RPB3-myc* shown in the histogram were published previously [53] and are provided here only for comparison.)

DISCUSSION

In this report, we have addressed four issues regarding the mechanism of transcriptional activation by Gcn4p in vivo. Regarding the subunit requirements for SAGA recruitment, we showed that recruitment of SAGA to the UAS_{GCRE} elements at three Gcn4p target genes is strongly dependent on Ada1p, Ada5p, and Spt7p; moderately dependent on Spt3p; and largely independent of Spt8p and the Gcn5p/Ada2p/Ada3p HAT module. Evidence was presented previously that Tra1p is a direct target of Gcn4p (10); however, we observed that recruitment of FL-Tra1p by Gcn4p was significantly reduced by deletion of *ADA1*, *ADA5*, *SPT7*, or *SPT3*. Because the SAGA complex is disrupted by the *ada1*Δ, *ada5*Δ, and *spt7*Δ mutations, it appears that optimal recruitment of Tra1p in vivo is dependent on its presence in the intact SAGA complex. Thus, either Gcn4p must interact with one or more SAGA subunits besides Tra1p for efficient recruitment of the entire complex or Tra1p must interact with other SAGA subunits to assume the proper conformation needed for a robust interaction with Gcn4p. Tra1p is a subunit of the NuA4 HAT complex in addition to SAGA (1), and Gcn4p was shown to interact specifically with NuA4 in vitro (68). Thus, much of the residual FL-Tra1p recruited by Gcn4p in the *ada1*Δ, *ada5*Δ, and *spt7*Δ strains may be in the form of NuA4.

Our finding that recruitment of SAGA subunits was reduced

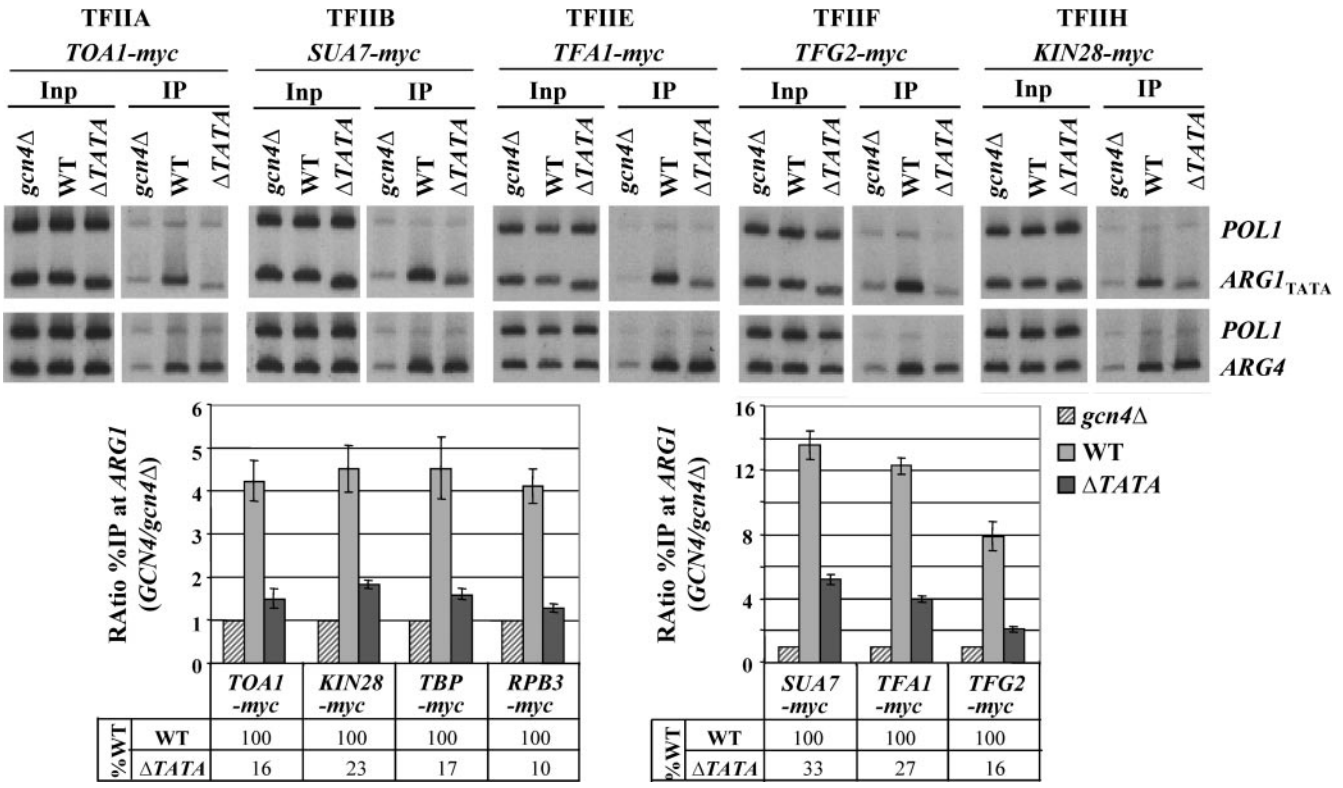

FIG. 7. TBP binding to TATA box is a prerequisite for recruitment of GTFs by Gcn4p. *gcn4*Δ, WT, and *arg1*-Δ*TATA* strains containing chromosomal *TOA1-myc*, *SUA7-myc*, *TFA1-myc*, *TFG2-myc*, or *KIN28-myc* alleles were subjected to ChIP analysis as described in Fig. 1 and 2. (Data for *TBP-myc* and *RPB3-myc* in the histogram were published previously [53].)

by *spt3*Δ, even though the SAGA complex is fully intact in this mutant (71), might indicate that Spt3p provides a second contact for Gcn4p in SAGA besides Tra1p. An alternative possibility, that deletion of Spt3p alters the conformation of Tra1p in a manner that reduces its interaction with Gcn4p, may be unlikely considering a recent structural model of SAGA in which Spt3p resides in a flexible domain distantly located from the bulk of Tra1p in the extended complex (70). We recently investigated whether Spt3p can interact directly with recombinant glutathione *S*-transferase (GST)–Gcn4p in a "GST pull-down" assay employed previously to measure binding of SAGA to Gcn4p in WCEs (17, 43). Whereas Spt3p bound specifically to GST-Gcn4p in a WT extract, this did not occur in an *ada1*Δ extract. By contrast, FL-Tra1p bound to GST-Gcn4p in both WT and *ada1*Δ extracts (data not shown), in accordance with previous results (10). While these data might indicate that Gcn4p does not contact Spt3p directly, it is also possible that Spt3p is not folded properly outside of SAGA or that the dissociation rate of a Gcn4p-Spt3p complex is too high to be detected with this binding assay. Because high-level SAGA recruitment by Gcn4p is dependent on Srb mediator, another intriguing possibility is that Spt3p is required for the stimulatory effect of mediator on SAGA recruitment.

It was shown recently that Spt20p/Ada5p is required for Gal4p-Tra1p interaction and for the recruitment of Tra1p to the *GAL1* UAS by Gal4p in yeast cells (7). Thus, it seems that Gal4p cannot efficiently recruit Tra1p to the *GAL1* promoter outside of the context of SAGA, just as we observed for Gcn4p. Deletion of *SPT3* had a smaller effect on recruitment of Spt20p by Gal4p than was generally observed here for Gcn4p, and it was concluded that Spt3p is not required for SAGA recruitment by Gal4p (36).

We found that *ada2*Δ, *ada3*Δ, and *gcn5*Δ led to higher-than-WT levels of myc-Spt7p recruitment (Fig. 2A), that *gcn5*Δ led to elevated recruitment of myc-Ada2p (Fig. 2B), and that *ada2*Δ increased the recruitment of FL-Tra1p (Fig. 2C) to the *ARG1* promoter. These findings suggest that histone acetylation by Gcn5p may antagonize SAGA recruitment to *ARG1*. The fact that *ada3*Δ did not elevate recruitment of myc-Ada2p at *ARG1* can be explained by the reduced expression of myc-Ada2p in *ada3*Δ cells (Fig. 3B); however, it is more difficult to explain why *ada3*Δ and *gcn5*Δ did not elevate FL-Tra1p recruitment to *ARG1*. Thus, further study is required before drawing any firm conclusion about the impact of Gcn5p HAT activity on SAGA recruitment to *ARG1*. It is also intriguing that *spt8*Δ significantly reduced SAGA recruitment only at *SNZ1*. As noted above, there are more stringent requirements for coactivator recruitment by Gcn4p at *SNZ1* versus *ARG1* (72, 73), although the molecular basis for this difference is unknown. Presumably, the elimination of Spt8p reduces the binding of SAGA to Gcn4p to a small extent that can be compensated for by other interactions at *ARG1* and *ARG4* but not at *SNZ1*.

The second major question addressed in this report is whether the recruitment of one coactivator by Gcn4p enhances the recruitment of others. We showed previously (72) that

TABLE 3. Comparison of requirements for recruitment of mediator, SAGA, and SWI/SNF by three different yeast activators[a]

Requirement	Coactivator recruited and activator								
	Mediator			SAGA			SWI/SNF		
	Gal4p	Gcn4p	Swi5p	Gal4p	Gcn4p	Swi5p	Gal4p	Gcn4p	Swi5p
Mediator				−	+	ND	+	+	−
SAGA	?	+(−)	−				−	+	−
SWI/SNF	ND	−	+	ND	−	+			
PIC assembly	−	−	−	−	−	−	+	−	−

[a] This table summarizes results published previously for coactivator recruitment by Gal4p to the *GAL1* UAS during growth on galactose (6, 7, 11, 37); for Swi5p at URS1 or URS2 at *HO* in early G_1 phase of the cell cycle (8, 14); and for Gcn4p at *ARG1, ARG4*, and *SNZ1* during sulfometuron-induced starvation described here and previously (72). +, strong requirement; −, little or no requirement; ?, conflicting results concerning the requirement for SAGA in mediator recruitment by Gal4p; +(−), mediator recruitment by Gcn4p is substantially dependent on SAGA at *ARG4* and *SNZ1* but largely independent of SAGA at *ARG1;* ND, not determined.

recruitment of SWI/SNF by Gcn4p is impaired by deletions of Srb mediator subunits, including Gal11p, Med2p, and Rox3p, and that Gal11p and Med2p are required for efficient recruitment of mediator itself to *ARG1* (73). Thus, high-level recruitment of SWI/SNF is dependent on recruitment of Srb mediator by Gcn4p. We also reported previously that recruitment of SWI/SNF was dependent on SAGA integrity but independent of the SAGA HAT Gcn5p (72). In contrast to the requirement for mediator and SAGA in SWI/SNF recruitment, we showed here that recruitment of SAGA and Srb mediator was not reduced by inactivating the nucleosome-remodeling function of SWI/SNF by deleting *SWI2*. In fact, recruitment of SAGA and mediator appeared to be increased considerably at *ARG1*, and also slightly at *ARG4*, in the *swi2*Δ mutant (Fig. 5). The latter findings may indicate that remodeling of the nucleosomal array at these promoters by SWI/SNF decreases retention of SAGA and mediator.

We further demonstrated here that efficient recruitment of SAGA is dependent on Srb mediator subunits Rox3p, Srb5p, Srb2p and Med2p and, likewise, that high-level recruitment of Srb mediator at *ARG4* and *SNZ1* is dependent on SAGA integrity but not on Gcn5p (Fig. 5). Thus, even though Gcn4p can interact directly with SAGA, mediator, and SWI/SNF in vitro, these interactions do not suffice for high-level recruitment of these coactivators by Gcn4p to target promoters in vivo. Additional work will be required to understand how recruitment of Srb mediator escapes the dependence on SAGA for wild-type recruitment to the *ARG1* UAS_{GCRE}. In fact, recruitment of the tail domain of mediator (containing Gal11p) seems to be significantly elevated at *ARG1* in SAGA mutants (Fig. 5D). Of even greater importance will be to determine how SAGA and Srb mediator can stimulate SWI/SNF recruitment and also mutually enhance their own recruitment by Gcn4p.

Our findings on Swi2p-independent recruitment of SAGA by Gcn4p are in agreement with a previous analysis of Gcn5p recruitment by Gcn4p to a synthetic *PHO5* promoter harboring a UAS_{GCRE} (65). However, our results contrast with those of Topalidou and Thireos, who observed high-level recruitment of SAGA independent of mediator to various UAS_{GCRE} elements that are separated from core promoter sequences, such as in open reading frames (67). It is unclear at present why mediator is required for efficient SAGA recruitment by Gcn4p to intact bona fide promoters, such as *ARG1* or *ARG4* (Fig. 5A and B), but not to UAS_{GCRE} elements unconnected to core promoter sequences.

While Gal4p and Gcn4p are often regarded as acidic activators of a similar nature, they differ substantially with respect to their mechanisms of coactivator recruitment. Neither Bhaumik et al. nor Bryant and Ptashne observed any reduction in SAGA recruitment by Gal4p in response to mutations in mediator subunits, including *srb4-ts* (7) and *gal11*Δ (11), even though *srb4-ts* abolishes PIC formation at *GAL1* (39). Hence, the marked dependency on Srb mediator for SAGA recruitment observed here for Gcn4p is not shared by Gal4p (Table 3). In addition, it appears that mediator, but not SAGA, is required for high-level recruitment of SWI/SNF by Gal4p (37), whereas both SAGA and mediator contribute substantially to recruitment of SWI/SNF by Gcn4p (72). Furthermore, SWI/SNF recruitment by Gal4p requires Pol II binding to the promoter (37), whereas we showed here that Gcn4p can recruit SWI/SNF independently of PIC formation. There is conflicting evidence concerning the requirement for SAGA in mediator recruitment by Gal4p (7, 11, 37), making it difficult to determine whether the situation is more similar to our findings for Gcn4p at *ARG1*, where mediator recruitment is largely independent of SAGA, or to our findings at *ARG4* and *SNZ1*, where SAGA makes an important contribution to mediator recruitment by Gcn4p (Table 3).

A completely different pattern of coactivator interdependency has been described for the *HO* gene, at which SWI/SNF recruitment by the activator Swi5p is a prerequisite for recruitment of both SAGA (14) and Srb mediator (8), and mediator is not required for SWI/SNF recruitment (8). Note, however, that the requirement for SWI/SNF in SAGA recruitment at *HO* appears to be restricted to late mitosis and applies even to Gal4p- and Gcn4p-regulated promoters in this phase of the cell cycle, most likely involving a highly condensed state of promoter chromatin (32). Thus, the degree of coactivator interdependency can vary for the same activator depending on the chromatin structure of the UAS.

The third question regarding the Gcn4p recruitment program addressed here is whether PIC formation is required for high-level recruitment or retention of coactivators at the UAS_{GCRE} by Gcn4p. Previous studies of Gal4p showed that SAGA and Srb mediator can be recruited by Gal4p to the *GAL1* UAS in the absence of a downstream promoter element and that Ts^- mutations in TBP, TFIIB, or Pol II do not reduce

recruitment of these coactivators to the *GAL1* UAS even though they destroy PIC formation (6, 7, 34). Consistent with this, recruitment of SAGA and mediator precedes that of TBP, GTFs, and Pol II at *GAL1* following induction by galactose (11). Thus, efficient recruitment of SAGA and mediator by Gal4p is independent of PIC formation at the *GAL1* promoter. By contrast, as noted above, recruitment of SWI/SNF by Gal4p seems to require Pol II recruitment to the *GAL1* promoter (37). Similarly, TBP and Pol II binding at the *RNR3* promoter were shown to be required for optimal recruitment of SWI/SNF and mediator at this gene (59). By contrast, we found that recruitment of SWI/SNF, as well as SAGA and mediator, by Gcn4p was unaffected by deletion of the TATA box at *ARG1*, a mutation that impairs recruitment of TBP, GTFs, and Pol II. Thus, Gcn4p recruits all three coactivators to the *ARG1* UAS independently of PIC assembly at the promoter. A similar conclusion was reached for mediator and SAGA using engineered *PHO5* promoters with a UAS_{GCRE} either containing or lacking a TATA box (67). The fact that recruitment of Pol II, but not Srb mediator, is impaired by the Δ*TATA* mutation also indicates that mediator can be recruited by Gcn4p independent of its association with Pol II in the holoenzyme, as concluded previously for other activators (8, 11, 34, 49).

Finally, we found that recruitment of TFIIA, TFIIF, and TFIIH to *ARG1* is completely dependent on TBP binding to the promoter, as deletion of the TATA element impaired recruitment of these GTFs to the same degree that it reduced TBP binding at *ARG1*. Although the TATA deletion also produced a marked reduction in TFIIB and TFIIE recruitment, there appeared to be significant residual binding of these factors to the TATA-less *ARG1* promoter. Thus, TBP-independent binding of TFIIB and TFIIE to the promoter may be enhanced by their interactions with mediator or another coactivator recruited by Gcn4p to the UAS element.

Based on our findings, we can now propose a pathway for the stimulation of PIC formation by Gcn4p. Because Gcn4p can directly interact with SAGA, mediator, and SWI/SNF in vitro, and it recruits all three coactivators to the *ARG1* UAS in the absence of the TATA element, we propose that Gcn4p directly recruits SAGA, Srb mediator (free of Pol II), and SWI/SNF to the UAS_{GCRE}. SAGA and mediator facilitate the recruitment of one another and also enhance SWI/SNF recruitment or retention by Gcn4p. All three coactivators function directly or indirectly to stimulate TBP binding to the TATA element, which, in turn, permits recruitment of the remaining GTFs and Pol II to the promoter to complete the assembly of a preinitiation complex.

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