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Recruitment of SWI/SNF by Gcn4p Does Not Require Snf2p or Gcn5p but Depends Strongly on SWI/SNF Integrity, SRB Mediator, and SAGA

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The nucleosome remodeling complex SWI/SNF is a coactivator for yeast transcriptional activator Gcn4p. We provide strong evidence that Gcn4p recruits the entire SWI/SNF complex to its target genes *ARG1* and *SNZ1* but that SWI/SNF is dispensable for Gcn4p binding to these promoters. It was shown previously that Snf2p/Swi2p, Snf5p, and Swi1p interact directly with Gcn4p in vitro. However, we found that Snf2p is not required for recruitment of SWI/SNF by Gcn4p nor can Snf2p be recruited independently of other SWI/SNF subunits in vivo. Snf5p was not recruited as an isolated subunit but was required with Snf6p and Swi3p for optimal recruitment of other SWI/SNF subunits. The results suggest that Snf2p, Snf5p, and Swi1p are recruited only as subunits of intact SWI/SNF, a model consistent with the idea that Gcn4p makes multiple contacts with SWI/SNF in vivo. Interestingly, Swp73p is necessary for efficient SWI/SNF recruitment at *SNZ1* but not at *ARG1*, indicating distinct subunit requirements for SWI/SNF recruitment at different genes. Optimal recruitment of SWI/SNF by Gcn4p also requires specific subunits of SRB mediator (Gal11p, Med2p, and Rox3p) and SAGA (Ada1p and Ada5p) but is independent of the histone acetyltransferase in SAGA, Gcn5p. We suggest that SWI/SNF recruitment is enhanced by cooperative interactions with subunits of SRB mediator and SAGA recruited by Gcn4p to the same promoter but is insensitive to histone H3 acetylation by Gcn5p.

Eukaryotic activator proteins depend on coactivator complexes to eliminate repressive chromatin structures at their target promoters. One class of nucleosome remodeling complexes uses the energy of ATP hydrolysis to disrupt histone-DNA contacts and alter the accessibility of protein binding sites in the promoter. The best-studied coactivator of this type in *Saccharomyces cerevisiae* is the SWI/SNF complex (37, 41, 51). Deletion of Snf2p/Swi2p, the ATPase subunit of SWI/SNF, is not lethal but leads to altered transcription of a subset (1 to 2%) of genes in nutrient-rich medium (20, 52). SWI/SNF interacts directly with yeast activators (35, 58) and can be recruited to promoters for nucleosome remodeling and transcriptional activation in vitro (16, 38, 58). Recruitment of SWI/SNF by yeast activators has also been demonstrated in vivo by using chromatin immunoprecipitation (ChIP) assays (8, 13, 53, 54).

The yeast SWI/SNF complex contains 11 different subunits, and genetic studies suggest that many of the subunits are required for the chromatin remodeling function of the entire complex (52). Six subunits have homologs in human SWI/SNF, and four of the latter (related to yeast Snf2p/Swi2p, Snf5p, and Swi3p) form a core complex capable of nucleosome remodeling in vitro (42). However, Swp73p is not necessary for transcriptional stimulation by certain activators that require Snf2p (6), and deletion of Snf11p has little effect on SWI/SNF-dependent genes even though it seems to interact directly with Snf2p (55). Interestingly, Snf2p can mediate repression of *SER3* without the cooperation of certain other SWI/SNF subunits (33, 52).

SWI/SNF remodeling can stimulate various steps in gene activation. It enhances binding of TATA-binding protein (TBP) and RNA polymerase II (PolII) to the yeast *RNR3* promoter (46). Recruitment of SWI/SNF to the *HO* gene stimulates binding of the coactivator complexes SAGA and SRB mediator (4, 8, 26). SAGA is a multifunctional coactivator containing the histone H3 acetyltransferase (HAT) Gcn5p and multiple TBP-interacting proteins (49). SRB mediator is a multifunctional complex associated with PolII and certain general transcription factors (34). The requirement for SWI/SNF function in the recruitment of SAGA to *HO* may be a special case restricted to mitosis involving a hypercondensed state of chromatin (25). Indeed, recruitment of Gcn5p occurred independently of Snf2p at a synthetic *PHO5* promoter regulated by Gcn4p (54). Recruitment of SWI/SNF by Gcn4p to a plasmid-borne copy of *HIS3* leads to a highly labile chromatin domain that extends beyond the promoter region and includes the coding sequences (24). There is also evidence that SWI/SNF can stimulate transcription elongation (9).

The mechanism of SWI/SNF recruitment by activators is not well understood. Gcn4p can bind in vitro to SWI/SNF (35, 38), dependent on bulky hydrophobic residues in the Gcn4p activation domain that are required for transcriptional stimulation in vivo (10, 22). The Snf2p, Snf5p, and Swi1p subunits were photo-cross-linked to Gcn4p and to activator Hap4p in the context of native SWI/SNF, and the corresponding recombinant subunits can bind individually to both activator proteins (37). It was unknown, however, whether these interactions are important for SWI/SNF recruitment in living cells. Recruit-

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TABLE 1. *S. cerevisiae* strains

Strain	Parent strain	Relevant genotype	Reference or source
BY4741	NA	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
BY4742	NA	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Research Genetics
SY1	BY4741	*SNF2-myc$_{13}$::HIS3*[a]*	53
SY162	BY4741	*SNF5-myc$_{13}$::HIS3**	This study
SY163	BY4741	*SNF6-myc$_{13}$::HIS3**	This study
SY160	BY4741	*SWI1-myc$_{13}$::HIS3**	This study
SY161	BY4741	*SWI3-myc$_{13}$::HIS3**	This study
SY175	BY4741	*SWP73-myc$_{13}$::HIS3**	This study
SY127	BY4741	*SNF2-myc$_{13}$::HIS3* gcn4Δ*	This study
249	BY4741	*gcn4Δ::kanMX4*	Research Genetics
SY166	249	*SNF5-myc$_{13}$::HIS3* gcn4Δ::kanMX4*	This study
SY167	249	*SNF6-myc$_{13}$::HIS3* gcn4Δ::kanMX4*	This study
SY164	249	*SWI1-myc$_{13}$::HIS3* gcn4Δ::kanMX4*	This study
SY165	249	*SWI3-myc$_{13}$::HIS3* gcn4Δ::kanMX4*	This study
SY176	249	*SWP73-myc$_{13}$::HIS3* gcn4Δ::kanMX4*	This study
1586	BY4741	*swi2Δ::kanMX4*	Research Genetics
SY169	1586	*swi2Δ::kanMX4 gcn4Δ*	This study
SY206	1586	*swi2Δ::kanMX4 SNF5-myc$_{13}$::HIS3**	This study
SY207	1586	*swi2Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
SY210	1586	*swi2Δ::kanMX4 SNF6-myc$_{13}$::HIS3**	This study
SY228	1586	*swi2Δ::kanMX4 SWI3-myc$_{13}$::HIS3**	This study
SY282	1586	*swi2Δ::kanMX4 SWP73-myc$_{13}$::HIS3**	This study
1250	BY4741	*swi3Δ::kanMX4*	Research Genetics
SY294	1250	*swi3Δ::kanMX4 gcn4Δ*	This study
SY2	1250	*swi3Δ::kanMX4 SNF2-myc$_{13}$::HIS3**	This study
SY222	1250	*swi3Δ::kanMX4 SWP73-myc$_{13}$::HIS3**	This study
SY284	1250	*swi3Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
SY286	1250	*swi3Δ::kanMX4 SNF5-myc$_{13}$::HIS3**	This study
SY324	1250	*swi3Δ::kanMX4 SNF6-myc$_{13}$::HIS3**	This study
7175	BY4741	*snf5Δ::kanMX4*	Research Genetics
SY327	7175	*snf5Δ::kanMX4 gcn4Δ*	This study
SY5	7175	*snf5Δ::kanMX4 SNF2-myc$_{13}$::HIS3**	This study
SY209	7175	*snf5Δ::kanMX4 SNF6-myc$_{13}$::HIS3**	This study
SY219	7175	*snf5Δ::kanMX4 SWP73-myc$_{13}$::HIS3**	This study
SY225	7175	*snf5Δ::kanMX4 SWI3-myc$_{13}$::HIS3**	This study
SY229	7175	*snf5Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
6409	BY4741	*snf6Δ::kanMX4*	Research Genetics
SY295	6409	*snf6Δ::kanMX4 gcn4Δ*	This study
SY3	6409	*snf6Δ::kanMX4 SNF2-myc$_{13}$::HIS3**	This study
SY221	6409	*snf6Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
SY223	6409	*snf6Δ::kanMX4 SNF5-myc$_{13}$::HIS3**	This study
SY300	6409	*snf6Δ::kanMX4 SWI3-myc$_{13}$::HIS3**	This study
SY299	6409	*snf6Δ::kanMX4 SWP73-myc$_{13}$::HIS3**	This study
4008	BY4741	*snf11Δ::kanMX4*	Research Genetics
SY296	4008	*snf11Δ::kanMX4 gcn4Δ*	This study
SY4	4008	*snf11Δ::kanMX4 SNF2-myc$_{13}$::HIS3**	This study
SY214	4008	*snf11Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
SY215	4008	*snf11Δ::kanMX4 SNF5-myc$_{13}$::HIS3**	This study
SY216	4008	*snf11Δ::kanMX4 SWI3-myc$_{13}$::HIS3**	This study
SY218	4008	*snf11Δ::kanMX4 SWP73-myc$_{13}$::HIS3**	This study
SY278	4008	*snf11Δ::kanMX4 SNF6-myc$_{13}$::HIS3**	This study
15398	BY4742	*swp73Δ::kanMX4*	Research Genetics
SY298	15398	*swp73Δ::kanMX4 gcn4Δ*	This study
SY6	15398	*swp73Δ::kanMX4 SNF2-myc$_{13}$::HIS3**	This study
SY217	15398	*swp73Δ::kanMX4 SNF5-myc$_{13}$::HIS3**	This study
SY226	15398	*swp73Δ::kanMX4 SNF6-myc$_{13}$::HIS3**	This study
SY283	15398	*swp73Δ::kanMX4 SWI1-myc$_{13}$::HIS3**	This study
SY290	15398	*swp73Δ::kanMX4 SWI3-myc$_{13}$::HIS3**	This study

Continued on following page

TABLE 1—*Continued*

Strain	Parent strain	Relevant genotype	Reference or source
7285	BY4741	*gcn5*Δ::*kanMX4*	Research Genetics
SY202	7285	*gcn5*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY203	7285	*gcn5*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
SY204	7285	*gcn5*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
1038	BY4741	*ada1*Δ::*kanMX4*	Research Genetics
SY199	1038	*ada1*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY211	1038	*ada1*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY212	1038	*ada1*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
7309	BY4741	*ada5*Δ::*kanMX4*	Research Genetics
SY205	7309	*ada5*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY213	7309	*ada5*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
3218	BY4741	*spt7*Δ::*kanMX4*	Research Genetics
SY273	3218	*spt7*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY276	3218	*spt7*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
2786	BY4741	*srb10*Δ::*kanMX4*	Research Genetics
SY270	2786	*srb10*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY274	2786	*srb10*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY285	2786	*srb10*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
4734	BY4741	*srb5*Δ::*kanMX4*	Research Genetics
SY287	4734	*srb5*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY288	4734	*srb5*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY293	4734	*srb5*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
4393	BY4741	*pgd1*Δ::*kanMX4*	Research Genetics
SY271	4393	*pgd1*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY343	4393	*pgd1*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
SY346	4393	*pgd1*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
3119	BY4741	*rox3*Δ::*kanMX4*	Research Genetics
SY289	3119	*rox3*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY360	3119	*rox3*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY361	3119	*rox3*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study
1742	BY4741	*gal11*Δ::*kanMX4*	Research Genetics
SY197	1742	*gal11*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY198	1742	*gal11*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
LSO2		*MAT***a** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 med2*Δ::*kanMX4*	53
SY292	LSO2	*med2*Δ::*kanMX4 SNF5-myc*$_{13}$::*HIS3**	This study
SY305	LSO2	*med2*Δ::*kanMX4 SNF2-myc*$_{13}$::*HIS3**	This study
SY341	LSO2	*med2*Δ::*kanMX4 SNF6-myc*$_{13}$::*HIS3**	This study

[a] *HIS3** designates the *HIS3* allele from *S. kluyveri* (31).

ment of SWI/SNF to *SUC2* requires both Snf5p and Snf2p (13, 17). At *SER3*, by contrast, Snf5p binding requires Snf2p, while Snf2p binding is independent of Snf5p (33). Experiments with purified components indicate that histone acetylation by SAGA can increase the retention of SWI/SNF on promoter nucleosomes (16) dependent on the Snf2p bromodomain and that the bromodomain is required for activator-induced SWI/SNF recruitment to *SUC2* in vivo (17). However, H3 acetylation by Gcn5p is not required for SWI/SNF recruitment, even though it promotes nucleosome remodeling by SWI/SNF at the synthetic *PHO5* promoter mentioned above (54). Similar conclusions were reached for *RNR3* (46) and *PHO8* (15, 44). Interestingly, SWI/SNF binding at *RNR3* requires the functions of TFIID and the SRB mediator/PolII holoenzyme (46).

Previously, we showed that multiple SWI/SNF subunits are required for wild-type (WT) transcriptional activation of a subset of genes regulated by Gcn4p. Expression of *GCN4* is induced at the translational level by starvation for any amino acid (18), and the induced Gcn4p stimulates transcription of hundreds of genes, including those involved in amino acid, vitamin, and purine biosynthesis (36). Transposon insertions or deletions of SWI/SNF subunits Snf2p, Swi1p, Swp73p, Snf5p, Snf6p, and Swi3p impaired Gcn4p activation of a *lacZ* reporter and conferred sensitivity to amino acid analogs that inhibit biosynthetic enzymes induced by Gcn4p. Mutations in certain SWI/SNF subunits also impaired induction by Gcn4p of a *HIS3-GUS* reporter and the authentic target genes *SNZ1* and *HIS4*; however, other Gcn4p-dependent genes can be induced independently of Snf2p (35, 53). The SWI/SNF-independence of a subset of Gcn4p target genes may reflect the absence of

repressive chromatin in their promoter regions; alternatively, SWI/SNF may have overlapping functions at these genes with SAGA, SRB mediator (45), or RSC complexes—all employed as coactivators by Gcn4p in vivo (27, 35, 53).

We wished to determine the roles of different subunits of SWI/SNF and the coactivators SAGA and SRB mediator in recruitment of SWI/SNF by Gcn4p in vivo. Toward this end, we performed ChIP analysis on deletion mutants lacking different subunits of these coactivators and bearing epitope tags on six different SWI/SNF subunits. We show that Gcn4p recruits all of these SWI/SNF subunits to the *ARG1* and *SNZ1* promoters and that SWI/SNF recruitment is dependent on hydrophobic clusters in the Gcn4p activation domain. Binding of Gcn4p itself to the *ARG1* promoter occurs independently of the activation domain and SWI/SNF function. In contrast to the situation described at *SER3* (33) and *SUC2* (13), we find that recruitment of SWI/SNF by Gcn4p is independent of Snf2p but is strongly dependent on Snf5p, Snf6p, and Swi3p. Since deletions of these latter subunits disrupt the SWI/SNF complex, efficient recruitment by Gcn4p most likely depends on interactions of the activation domain with multiple subunits of the complex. Consistent with this model, overexpressing Snf2p or Snf5p alone does not increase their recruitment by Gcn4p. SWI/SNF recruitment by Gcn4p is independent of Gcn5p but, interestingly, requires the Ada1p and Ada5p/Spt20p subunits of SAGA and multiple subunits of SRB mediator. The latter findings suggest that SWI/SNF recruitment is enhanced by cooperative interactions with SRB mediator and SAGA recruited by Gcn4p to the same promoters.

MATERIALS AND METHODS

Yeast strains and plasmids. All strains listed in Table 1 are derived from BY4741 (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) or BY4742 (*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) or deletion derivatives thereof generated by the *Saccharomyces* Genome Deletion Project and purchased from Research Genetics. The presence of all reported deletion alleles was confirmed by PCR amplification with lysed cells as the source of template chromosomal DNA and commercially available primers. In each case, the upstream primer derives from sequences 5′ of the deleted sequences and the downstream primer corresponds to sequences in the *KanMX4* cassette that replaces the deleted sequences. Except for the *snf11*Δ strain, the presence of the deletion alleles was also verified by showing that sensitivity of the strains to sulfometuron methyl (SM) was complemented by the relevant WT plasmid-borne alleles (53). The absence of Snf2p in the *snf2*Δ strain was additionally confirmed by Western blot analysis using anti-Snf2p antiserum. Insertion of the coding sequences for 13 tandem *myc* epitopes at the C termini of *SWI*/*SNF* genes using the *myc*$_{13}$-*HIS3* cassette in pFA6a-13Myc-HIS3 (31) was conducted as described previously (53). The presence of the *myc*-tagged alleles was verified by colony PCR and by Western blot analysis using anti-Myc antibodies. The deletion of *GCN4* (in all strains except 249) was conducted by transforming with plasmid pHQ1240, containing *gcn4*Δ::*hisG*::*URA3*::*hisG* and digested with *Ssp*I, and selecting for Ura$^+$ colonies. The *URA3* gene was subsequently evicted by selecting for growth on medium containing 5-fluoroorotic acid (2).

Plasmids p2382, pHQ1239, and pSK1 were described previously (53). The empty vector employed throughout was the *URA3 CEN4* plasmid YCp50 (39). Plasmid pHQ1240 was constructed by inserting the *Eco*RI-*Sal*I fragment from p2382 between the corresponding sites in pUC19 and replacing the *Bam*HI-*Bgl*II fragment of the resulting plasmid with a 3.8-kb *Bam*HI fragment containing the *hisG*::*URA3*::*hisG* cassette from pNKY51 (2). Single-copy plasmids pSY284 and pSY285, harboring *gcn4-14Ala-myc* or *gcn4-14Ala-HA*, respectively, were constructed by ligating the larger *Xba*I-*Mlu*I fragment of p2240 (22), a single-copy plasmid harboring *gcn4-14Ala*, with the smaller *Xba*I-*Mlu*I fragments of pSK1 or p2382. The constructs were verified by sequencing and by Western blot analysis of whole-cell extracts (WCEs) of the appropriate yeast transformants by using anti-Myc or -HA antibodies. The high-copy-number plasmids pHQ1303 and pHQ1304, harboring *GCN4* or *gcn4-14Ala*, respectively (see Fig. 3), were produced as follows. The *Eco*RI-*Sal*I fragment of pCD35 (10) was inserted into YEplac195 (14) between the *Eco*RI and *Sal*I sites to produce pHQ1303. The *Sal*I to *Xba*I fragment from p2240 and the *Xba*I to *Eco*RI fragment from pCD35 were inserted into YEplac195 between the *Eco*RI and *Sal*I sites to produce pHQ1304. The constructs were verified by sequencing.

The high-copy-number plasmids pSY286 and pSY287, harboring *SNF2-myc* or *SNF5-myc*, respectively, were generated by introducing the coding sequences for 13 *myc* epitopes at the C termini of the corresponding genes in plasmids pLN138-4 and pAC153 (1). Briefly, yeast strains 1586 and 7175 were transformed with plasmids pLN138-4 and pAC153, respectively, along with the relevant PCR product used for tagging the C termini of chromosomal *SNF2* or *SNF5* with 13 tandem *myc* epitopes (see above). The transformants were screened by Western blot analysis with anti-Myc antibodies for the presence of the relevant *myc*-tagged protein. The plasmids were recovered from cells (19) and reintroduced into the *S. cerevisiae* strains described above to verify that they encode the relevant *myc*-tagged protein, as indicated by Western blot analysis, and to demonstrate their ability to complement the SM sensitivity of the resident *snf2*Δ or *snf5*Δ mutations (53) indistinguishably from that of the parental plasmids.

ChIPs. The experiments were conducted as previously described (53), with the following modifications. Cells cross-linked with formaldehyde were sonicated for 12 cycles of 30 s at 4°C with at least 30-s cooling on ice per cycle. The primer set 5′ TGAAATGCCTGGTGTCAACT 3′ (sense) and 5′ TCTATGCAATCTTGC CAAAG 3′) (antisense) was used to amplify sequences −4724 to −4557 relative to the *SER3* ATG codon (*SER3*$_{-4.5kb}$). The primers used to amplify nucleotides −198 to −43 at the *SNZ1* promoter were 5′ TAGCGCCGCCATTTCTTCAT 3′ (sense) and 5′ TCGTTCCTAAAGGTTTCTCC 3′ (antisense). The primers used to amplify the *ARG1* upstream activation sequence (UAS) and *POL1* open reading frame (ORF) were described previously (53). The PCR conditions were as follows: initial denaturation at 94°C for 4 min, followed by 27 cycles of 94°C for 30 s, 52°C for 30 s, 65°C for 1 min, and a final extension for 5 min at 65°C.

Western blot analysis and coimmunoprecipitation assays. For Western blot analysis, yeast cultures were grown to an optical density at 600 nm (OD_{600}) of 1.0 to 2.0 in synthetic complete medium lacking isoleucine and valine (SC-ILV) (47) at 30°C and treated with 0.6 μg of SM/ml for 2 h. Total proteins were extracted by the trichloroacetic acid method described previously (43) and analyzed using rabbit polyclonal anti-Snf5p antibodies (5), anti-Swp73p antibodies (6), and anti-Gcd6p antibodies (7). The WCEs for coimmunoprecipitation analysis were prepared by a method described previously for glutathione *S*-transferase pull-down assays (11), with the following modifications. The cells were grown in 100 ml of YPD medium (47) to an OD_{600} of 1.0 to 2.0 and resuspended in 200 μl of ice-cold lysis buffer (50 mM Tris-HCl [pH 7.5], 20% glycerol, 50 mM NaCl, 0.1% Triton X-100, 10 μg of leupeptin/ml, 10 μg of pepstatin/ml, 10 μg of aprotinin/ml, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and protease inhibitor cocktail tablet [Boehringer]). The cell suspension was lysed by vortexing with glass beads for five cycles of 30 s, and the supernatant was recovered after centrifugation at 12,000 rpm in an Eppendorf Microfuge for 15 min at 4°C. Coimmunoprecipitation analysis was performed using mouse monoclonal anti-Myc antibodies (Roche). Briefly, the WCEs were incubated for 1 h at 4°C with 1 μg of anti-Myc antibody, 100 μg of bovine serum albumin dissolved in phosphate-buffered saline, and MT buffer (11). The immune complexes were washed three times with 1 ml of MT buffer, dissolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (Invitrogen), and subjected to Western blot analysis with the anti-Myc and anti-Snf5p antibodies described above.

RESULTS

Gcn4p recruits multiple subunits of the SWI/SNF complex to the *ARG1* and *SNZ1* promoters in vivo. Previously, we showed that Gcn4p recruits *myc*-tagged Snf2p to the UAS of the *ARG1* promoter when Gcn4p expression is induced from a high-copy-number plasmid by starvation for isoleucine and valine with the drug SM (53). We wished to determine whether Snf2p and other subunits of SWI/SNF would be recruited to *ARG1* when Gcn4p is being expressed from a single-copy gene. Accordingly, we constructed six pairs of yeast strains, each harboring a different subunit of SWI/SNF (Snf2p, Snf5p, Snf6p, Swi1p, Swi3p, or Swp73p) tagged at the C terminus with 13 *myc* epitopes. One member of each pair bears the WT chromosomal *GCN4* allele, and the other has a deletion of

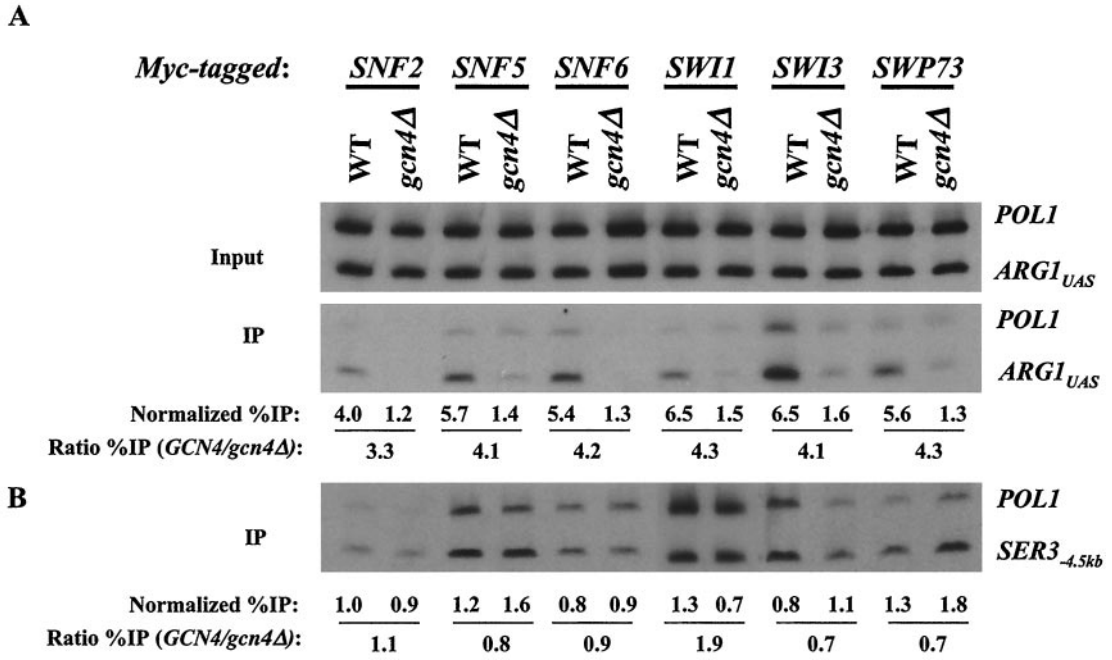

FIG. 1. Gcn4p at native levels recruits the SWI/SNF complex to the *ARG1* promoter. (A) WT (BY4741) and *gcn4*Δ (249) strains containing 13-myc tagged alleles of *SNF2* (SY1 and SY127), *SNF5* (SY162 and SY166), *SNF6* (SY163 and SY167), *SWI1* (SY160 and SY164), *SWI3* (SY161 and SY165), and *SWP73* (SY175 and SY176) were grown to an OD_{600} of 0.8 to 1.0 in SC-ILV medium at 30°C and treated with 0.6 μg of SM/ml for 2 h. Cells were harvested, treated with formaldehyde, and broken by vortexing with glass beads, and the extracts were sonicated to produce chromatin fragments with an average length of ~500 bp. Aliquots (5% of the total) were immunoprecipitated with anti-myc antibodies, and DNA was extracted from the immunoprecipitate (IP) after reversing the cross-links. DNA was extracted directly from another aliquot of the chromatin preparation (5% of the total) to serve as the input control. (A) A 600-fold dilution of the input control and the undiluted IP DNA were amplified by PCR by using primers specific for the *ARG1* UAS or *POL1* ORF in the presence of [^{33}P]dATP. The PCR products were resolved by PAGE and quantified by phosphorimaging analysis, and the ratio of the *ARG1* UAS signals in the IP-to-input samples was calculated and normalized for the corresponding ratio calculated for the *POL1* signals to yield the normalized percentage of IP (Normalized %IP). The ratio of the normalized percentage of IP values for the *GCN4* to *gcn4*Δ strains was calculated to yield the ratio percentage of IP (Ratio %IP) (*GCN4*/*gcn4*Δ). (B) As a negative control, the same IP and input samples described in panel A were analyzed using primers to amplify a sequence located 4.5-kb upstream of the *SER3* gene.

GCN4. All strains were grown in the presence of SM for 2 h to induce Gcn4p synthesis in the *GCN4* strains (Fig. 1A). The results showed that the *ARG1* UAS was immunoprecipitated with each *myc*-tagged SWI/SNF subunit at a level 3.3- to 4.3-fold higher in the WT strain than in the *gcn4*Δ strain (Fig. 1A). As a negative control, we monitored immunoprecipitation of a sequence located 4.5-kb upstream of the *SER3* gene. As shown in Fig. 1B, we observed low-level immunoprecipitation of this $SER3_{-4.5kb}$ sequence relative to *POL1* in WT and *gcn4*Δ strains alike. The comparable levels of *GCN4*-dependent immunoprecipitation of the *ARG1* UAS observed for all six *myc*-tagged strains suggest that Gcn4p recruits the entire SWI/SNF complex to the *ARG1* UAS. We consistently observed a lower level of *ARG1* immunoprecipitation in the strain with *myc*-tagged Snf2p than in strains with other *myc*-tagged SWI/SNF subunits. We assume that this finding reflects a relatively low efficiency of cross-linking by *myc*-tagged Snf2p to the promoter.

A similar analysis was carried out after transforming each of the *myc*-tagged *GCN4* strains with a high-copy-number plasmid bearing *GCN4-HA*, a fully functional allele tagged at the C terminus with the triple-HA tag (53). As shown in Fig. 2A, the *ARG1* UAS was immunoprecipitated with each *myc*-tagged subunit at a level 8- to 15-fold higher in the WT than in the *gcn4*Δ strains. The relatively higher level of *ARG1* UAS immunoprecipitation shown in Fig. 2A than in Fig. 1A presumably reflects increased Gcn4p binding at *ARG1* when Gcn4p is overexpressed. Indeed, other ChIP experiments show that overexpression of *myc*-tagged Gcn4p from a high-copy-number plasmid produces an approximately twofold increase in binding to *ARG1* than that seen with single-copy *GCN4-myc* (H. Qiu and A. G. Hinnebusch, unpublished data). We also analyzed the *GCN4*-dependent recruitment of all six SWI/SNF subunits to the 5′ noncoding region of *SNZ1*, another Gcn4p target gene (36), in the same strains that contain high-copy-number *GCN4-HA*. The percentages of the *SNZ1* promoter that immunoprecipitated with *myc*-tagged SWI/SNF subunits from the WT strains (Fig. 2B) were uniformly lower than those observed for the *ARG1* UAS (Fig. 2A); nevertheless, in all cases they were significantly greater than those seen in the *gcn4*Δ strains (Fig. 2B). Hence, it is likely that the entire SWI/SNF complex is recruited by Gcn4p to both *SNZ1* and *ARG1*.

In most of the ChIP experiments described below, we measured SWI/SNF recruitment to *SNZ1* and *ARG1* in strains overexpressing Gcn4p from a high-copy-number plasmid. This was done because the level of SWI/SNF recruitment in strains expressing the native amount of Gcn4p is too low to distinguish

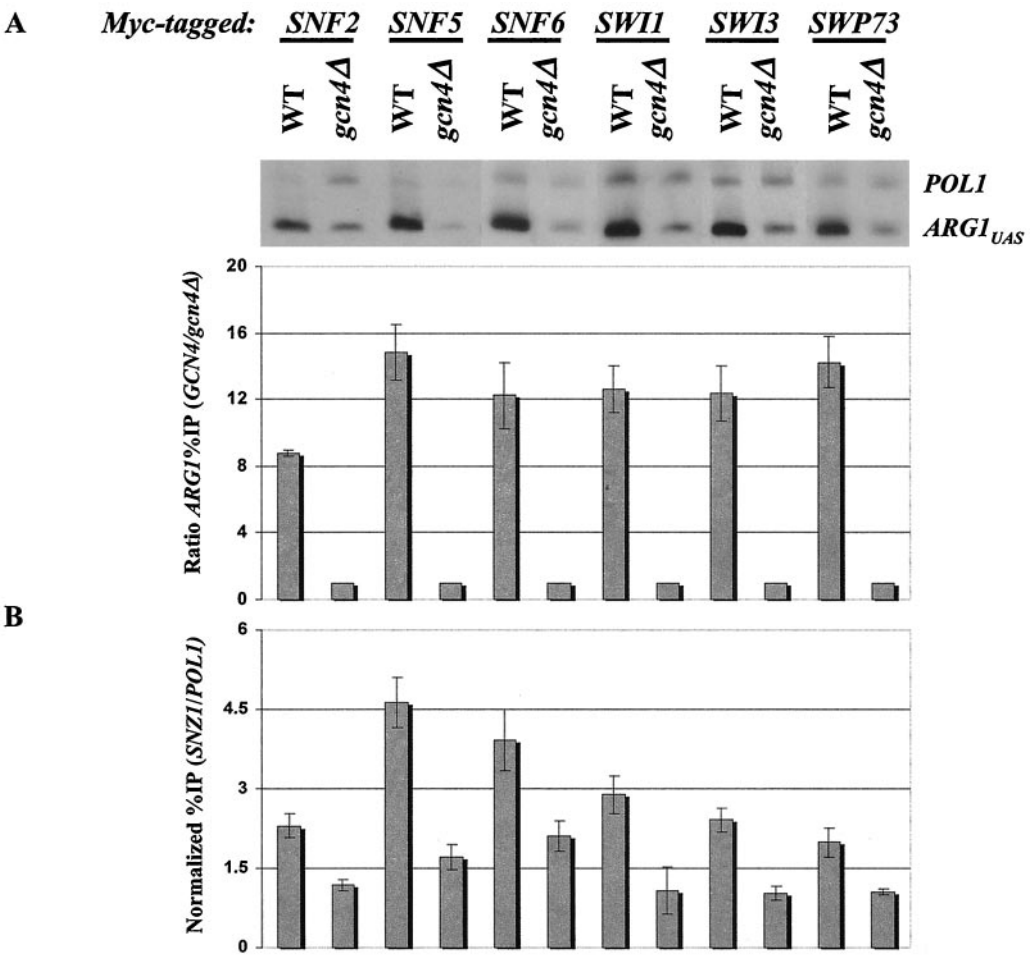

FIG. 2. Overexpression of Gcn4p increases the recruitment of SWI/SNF at *ARG1* and allows detection of SWI/SNF recruitment at *SNZ1*. (A) High-copy-number 2μm plasmid pHQ1239 harboring the *GCN4-HA* allele was introduced into the *GCN4* strains containing myc-tagged SWI/SNF subunits, and empty vector YCp50 was introduced into the *gcn4*Δ myc-tagged strains that were described in the legend to Fig. 1. The strains were cultured in SC-ILV-Ura (SC-ILV also lacking uracil) and induced with SM and then subjected to ChIP analysis as described in the legend to Fig. 1. The results of a typical experiment are depicted at the top, while the histograms below summarize the averages and standard errors of the ratio percent IP (*GCN4*/*gcn4*Δ) (as defined in the legend to Fig. 1) that were derived from multiple determinations for each strain. Two independent cultures and two to four independent immunoprecipitations were analyzed for each strain to provide the data used to calculate the mean values and standard errors shown in the histogram. (B) The same process was used as for panel A except that the SWI/SNF subunit binding to the *SNZ1* promoter was analyzed.

statistically significant differences in SWI/SNF binding between mutant and WT strains at *SNZ1.* As indicated above, promoter occupancy by Gcn4p increases approximately twofold when it is overexpressed from a high-copy-number plasmid. This level of promoter binding by Gcn4p may exist under conditions of severe amino acid limitation in which cell division is blocked, whereas cells continue to grow and divide at the SM concentration employed in our experiments (data not shown). In addition, cells overexpressing Gcn4p exhibit approximately twofold-greater transcriptional induction of *SNZ1* than occurs in WT cells (S. Yoon, S. Kim, and A. G. Hinnebusch, unpublished data), showing that the increased promoter occupancy by Gcn4p is functionally significant. In any event, the experiments described below allowed us to define the requirements for efficient SWI/SNF recruitment at maximal promoter occupancy by the activator Gcn4p.

Recruitment of SWI/SNF is dependent on the Gcn4p activation domain, but promoter binding by Gcn4p is independent of SWI/SNF. In vitro interaction between Gcn4p and the SWI/SNF complex is strongly dependent on bulky hydrophobic clusters in the Gcn4p activation domain, and multiple clusters must be inactivated to observe a strong defect in SWI/SNF binding (35). To determine whether the hydrophobic clusters are required for recruitment of SWI/SNF by Gcn4p in vivo, we conducted ChIP experiments using the *SNF2-myc gcn4*Δ and *SNF5-myc gcn4*Δ strains described above harboring plasmids containing *GCN4* or *gcn4-14Ala* (or the corresponding HA-tagged versions of these alleles). The *gcn4-14Ala* allele contains 14 alanine substitutions in the 7 hydrophobic clusters in the Gcn4p activation domain that contribute to transcriptional activation in vivo (22). As shown in Fig. 3A and B, we consistently observed greater binding of myc-Snf2p and myc-Snf5p to

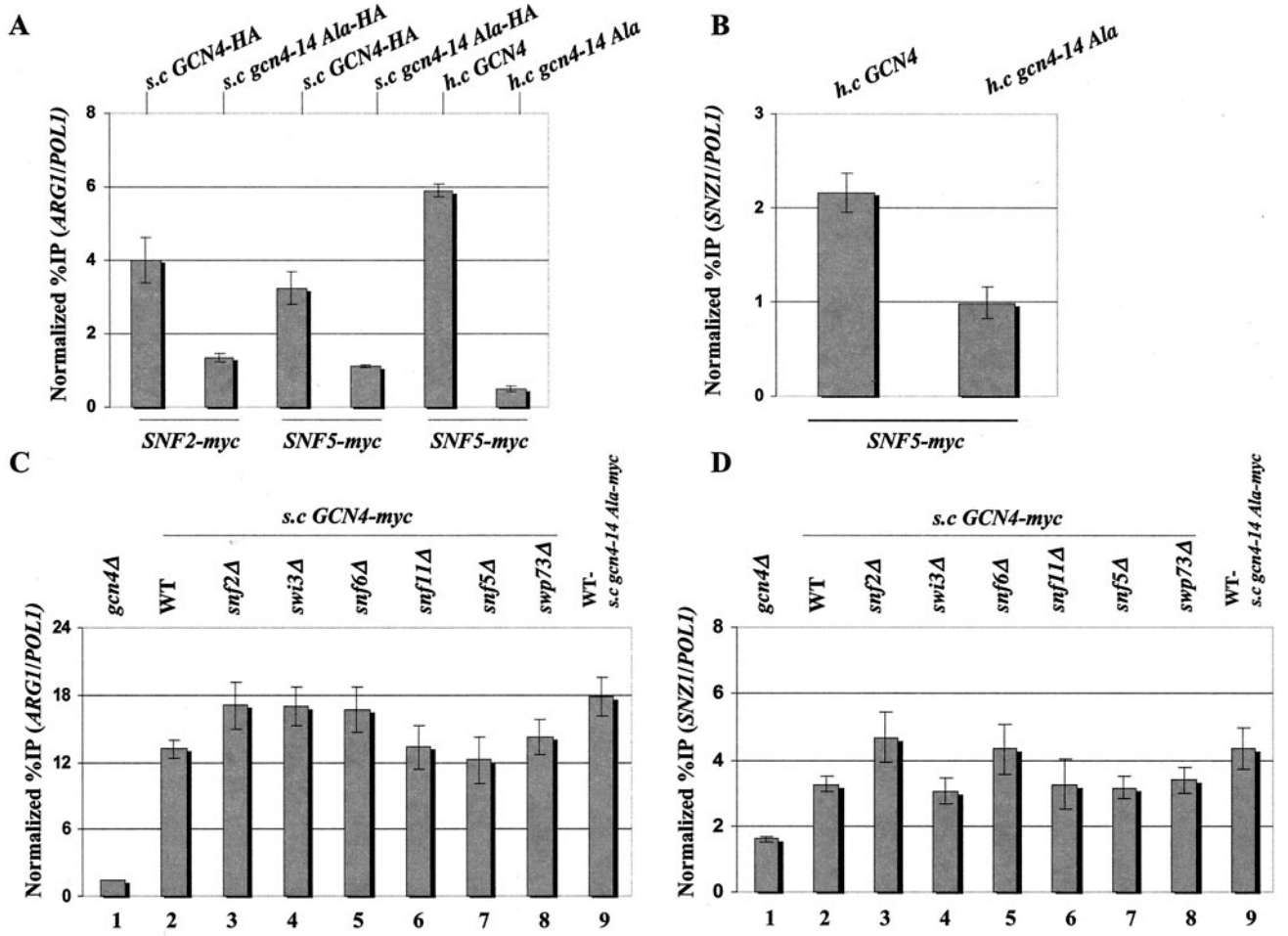

FIG. 3. Hydrophobic clusters in the Gcn4p activation domain are required for recruitment of Snf2p and Snf5p to *ARG1* and *SNZ1*, but Gcn4p binding at these promoters is independent of SWI/SNF. (A and B) The *gcn4*Δ *SNF2-myc* (SY127) and *gcn4*Δ *SNF5-myc* (SY166) strains described in the legend to Fig. 1 were transformed with single-copy (s.c) plasmid p2382 or pSY285, harboring *GCN4-HA* or *gcn4-14Ala-HA*, respectively, or high-copy-number (h.c.) plasmid pHQ1303 or pHQ1304, harboring *GCN4* or *gcn4-14Ala*, respectively, and subjected to ChIP analysis as described in the legend to Fig. 1 to measure binding of myc-tagged Snf2p or Snf5p to *ARG1* (A) or *SNZ1* (B). (C and D) ChIP analysis was conducted as described above to measure binding of myc-Gcn4p to *ARG1* (C) or *SNZ1* (D) in the following strains: transformants of the *gcn4*Δ strains containing either all WT SWI/SNF genes (249), *snf2*Δ (SY169), *swi3*Δ (SY294), *snf6*Δ (SY295), *snf11*Δ (SY296), *snf5*Δ (SY327), or *swp73*Δ (SY298), all bearing single-copy plasmid pSK1 containing *GCN4-myc*, lanes 2 through 8; *gcn4*Δ strain (249) transformed with empty vector YCp50 or single-copy plasmid pSY284 containing *gcn4-14Ala-myc*, respectively, lanes 1 and 9.

the *ARG1* and *SNZ1* promoters and greater binding of myc-Snf2p at *ARG1* in the strains harboring *GCN4* than in those with *gcn4-14Ala*.

It was conceivable that the 14 Ala substitutions in the activation domain reduce SWI/SNF recruitment by impairing the binding of Gcn4p to the *ARG1* and *SNZ1* promoters. As shown in Fig. 3C and D, similar or even greater amounts of *ARG1* and *SNZ1* promoter sequences were immunoprecipitated with myc-gcn4p-14Ala than with myc-Gcn4p when these proteins were expressed from single-copy plasmids (Fig. 3C, compare lanes 2 and 9; Fig. 3D, compare lanes 2 and 9). The somewhat higher level of promoter binding observed for the 14Ala mutant protein can be attributed to its higher steady-state level, as determined by Western blot analysis (data not shown). Higher steady-state levels are frequently observed for Gcn4 proteins with mutations in the activation domain (22).

The results shown in Figs. 3C to D also reveal that deletions of SWI/SNF subunits have little or no effect on binding of *myc*-tagged Gcn4p to the *ARG1* and *SNZ1* promoters. This finding is especially important for the *snf2*Δ mutant, which lacks the ATPase subunit of the complex. Hence, WT binding of Gcn4p at these promoters does not depend on prior nucleosome remodeling by SWI/SNF, nor does Gcn4p binding require prior interaction of any cofactors with the activation domain in a manner that depends on the hydrophobic clusters.

Recruitment of SWI/SNF by Gcn4p is independent of Snf2p but requires Snf5p, Snf6p, and Swi3p. It was shown previously that Swi2p/Snf2p, Snf5p, and Swi1p were photo-cross-linked to Gcn4p in the context of intact SWI/SNF and that each recombinant subunit can interact directly with recombinant Gcn4p in vitro (37). We wished to determine whether these interactions are necessary or sufficient for recruitment of the SWI/SNF complex to the *ARG1* and *SNZ1* promoters in vivo. Towards this end, we constructed a panel of six strains harboring dele-

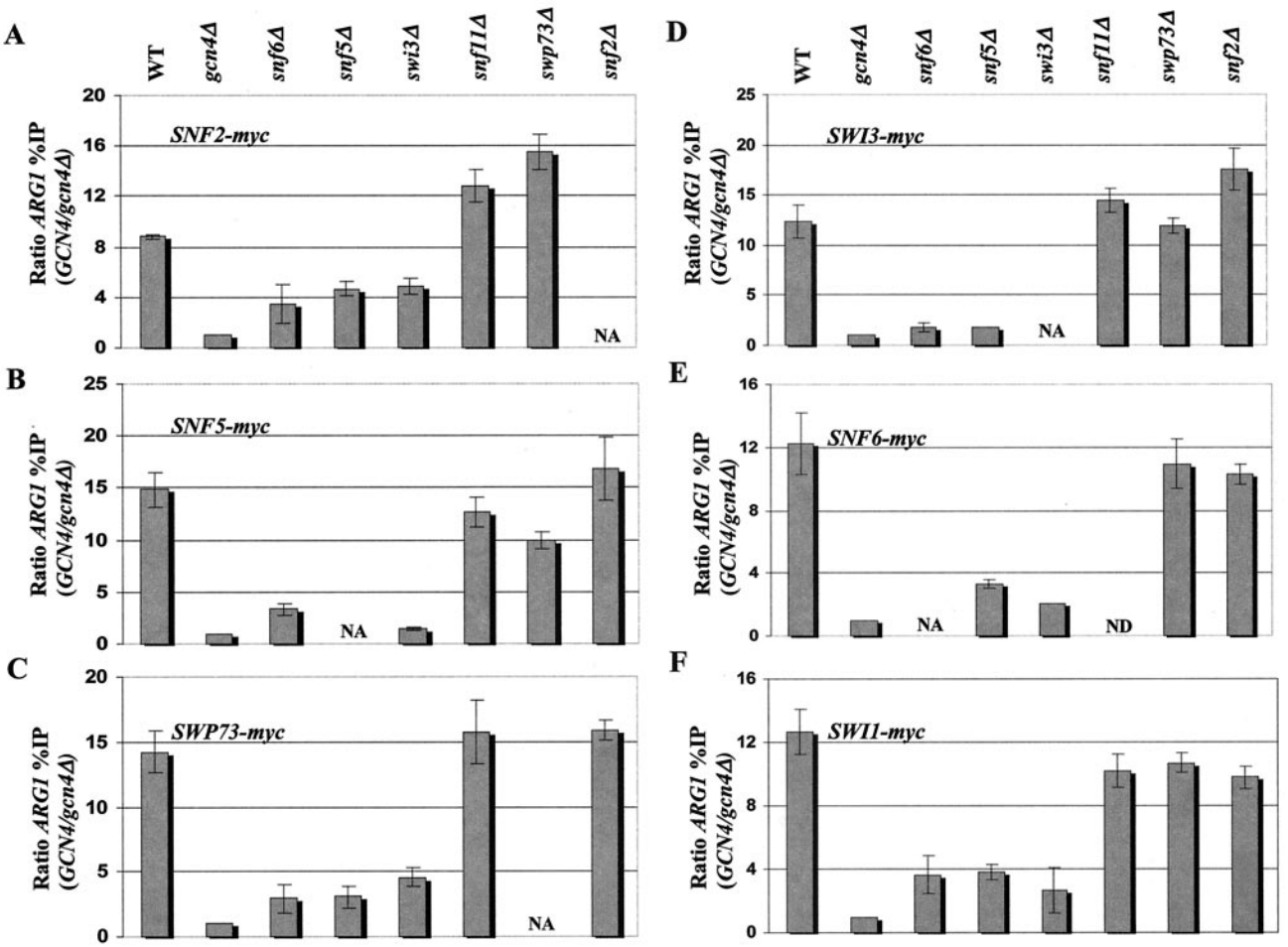

FIG. 4. Recruitment of SWI/SNF subunits by Gcn4p to *ARG1* is impaired in *snf6*Δ, *snf5*Δ, and *swi3*Δ mutants. (A through F) We generated a panel of strains harboring the chromosomal myc-tagged *SWI/SNF* alleles designated in the upper left of each histogram from *GCN4* strains containing all WT SWI/SNF subunits or the indicated *swi/snf* deletion alleles (shown at the top) and from the *gcn4*Δ strain containing all WT SWI/SNF subunits (*gcn4*Δ). (See Table 1 for a list of all strains employed.) High-copy-number plasmid pHQ1239 was introduced into the *GCN4* strains, and empty vector YCp50 was introduced into the *gcn4*Δ strains. The resulting transformants were subjected to ChIP analysis as described in the legend to Fig. 1. NA, not applicable; ND, not determined.

tions of *SNF6*, *SNF5*, *SWI3*, *SNF11*, *SWP73* or *SNF2* for each of the six strains containing a different *myc*-tagged subunit (myc-Snf2p, myc-Snf5p, myc-Swp73, myc-Swi3p, myc-Snf6p, and myc-Swi1p). The resulting 31 strains, along with the 12 tagged strains containing all WT SWI/SNF subunits (WT and *gcn4*), were subjected to ChIP analysis as described above.

The results shown in Fig. 4A through F indicate that the *snf6*Δ, *snf5*Δ, and *swi3*Δ strains consistently showed marked reductions in the Gcn4p-dependent recruitment of all tagged SWI/SNF subunits to the *ARG1* UAS. Recruitment was not completely eliminated by any of these mutations, as the residual binding was consistently higher than that seen in the *gcn4*Δ strain lacking the activator. By contrast, the *snf11*Δ, *swp73*Δ, and *snf2*Δ strains showed little or no reduction in binding of all SWI/SNF subunits to *ARG1*. Similar results were obtained when we analyzed binding to *SNZ1* except that the *snf6*Δ and *swi3*Δ mutations greatly reduced myc-Snf5p binding to the same low level observed in the *gcn4*Δ strain. Moreover, the binding of all tagged SWI/SNF subunits to this promoter was impaired by *swp73*Δ (Fig. 5A and B), even though this mutation had little or no effect on SWI/SNF recruitment to *ARG1* (Fig. 4). Even though Snf2p, Snf5p, and Swi1p are capable of direct binding to Gcn4p in vitro (37), the data in Fig. 4 and 5 suggest that high-level recruitment of these proteins is dependent on SWI/SNF subunits Snf6p, Snf5p, and Swi3p. Interestingly, it appears that interactions between Gcn4p and Snf2p are dispensable for recruitment of other SWI/SNF subunits in vivo.

Impaired recruitment of SWI/SNF in *snf6*Δ, *snf5*Δ, *swi3*Δ, and *swp73*Δ mutants likely results from complex disruption. It was shown previously that deletions of Snf6p, Snf5p, and Swi3p affect the integrity of the SWI/SNF complex (40). Thus, the reduced recruitment of SWI/SNF subunits to *ARG1* and *SNZ1* we observed in *snf6*Δ, *snf5*Δ, and *swi3*Δ mutants and to *SNZ1* in *swp73*Δ cells might result simply from reduced steady-state levels of these subunits in the mutant strains. To address this possibility, we conducted Western blot analysis of WCEs from *swi/snf* mutant and WT strains harboring *SNF2-myc* by using rapid extraction with trichloroacetic acid to minimize protein degradation during preparation of the extracts. The blots were

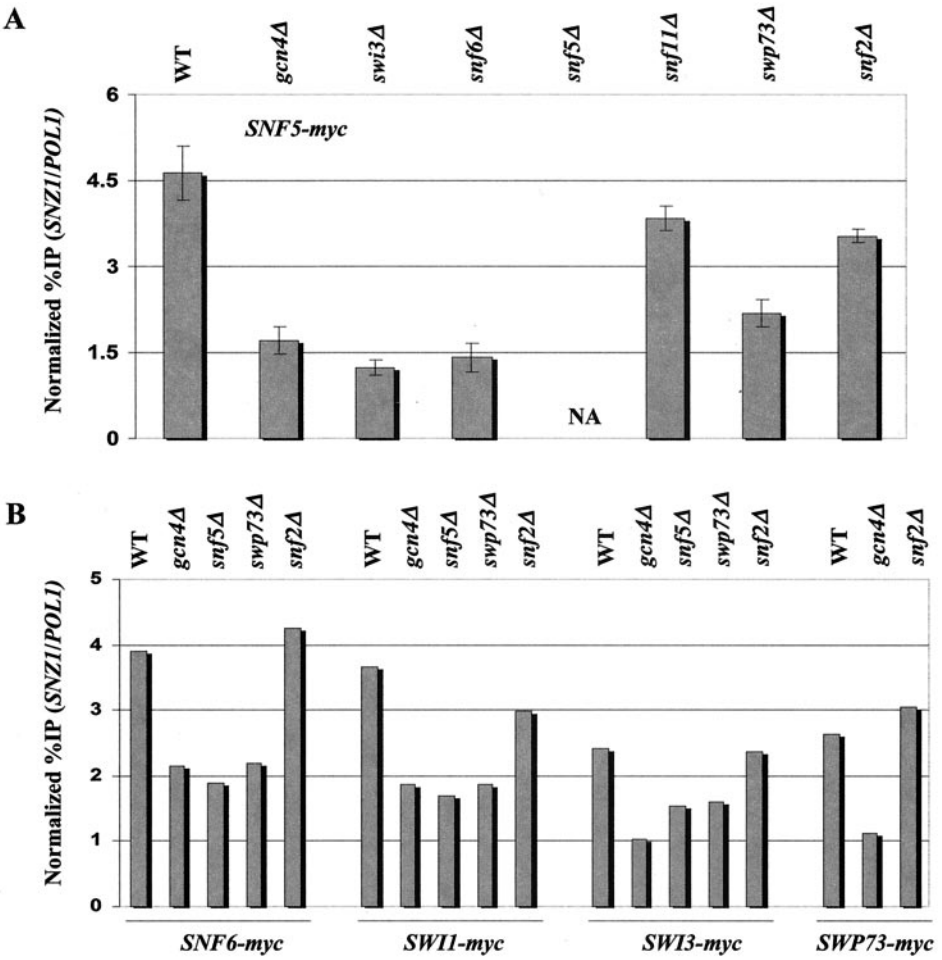

FIG. 5. Recruitment of SWI/SNF subunits by Gcn4p to *SNZ1* is impaired in *snf6*Δ, *snf5*Δ, *swi3*Δ, *and swp73*Δ mutants. The selected transformants described in the legend to Fig. 4 were subjected to ChIP analysis to measure binding of the indicated myc-tagged SWI/SNF subunits to *SNZ1* in *GCN4* or *gcn4*Δ strains containing all WT SWI/SNF subunits (WT and *gcn4*Δ, respectively) and in *GCN4* strains containing the indicated *swi*/*snf* deletion alleles.

probed with Myc antibodies to visualize myc-Snf2p, with antibodies against SWI/SNF subunits Snf5p and Swp73p, and with antibodies against Gcd6p to control for protein loading. Quantification of the results shown in Fig. 6A and B indicated that the steady-state levels of myc-Snf2p, Snf5p, and Swp73p (normalized for Gcd6p) were reduced by one-third or less in *snf6*Δ and *snf5*Δ cells. Greater reductions, of between 40 to 75%, were observed in *swi3*Δ and *swp73*Δ cells. (Owing to the relatively weak Western blotting signals for Swp73p, we could not estimate its reduction in *swi3*Δ cells.)

As shown above, the Gcn4p-dependent recruitment of myc-Snf5p and myc-Swp73p to *ARG1* was reduced by ~80% in *snf6*Δ and *snf5*Δ mutants (Fig. 4B and C) and recruitment of myc-Snf5p to *SNZ1* was completely eliminated in the *snf6*Δ mutant (Fig. 5A). These reductions in recruitment are considerably larger than the <33% decreases in expression of SWI/SNF subunits observed in *snf6*Δ and *snf5*Δ mutants (Fig. 6A). A similar but less pronounced disparity holds for myc-Snf2p, whose recruitment to *ARG1* was decreased by 50 to 65%, versus the <33% reduction in myc-Snf2p expression seen in *snf6*Δ and *snf5*Δ cells. Hence, it seemed unlikely that reduced expression alone could account for the defective recruitment of SWI/SNF subunits in *snf6*Δ and *snf5*Δ cells. The same conclusion cannot be drawn for the *swi3*Δ mutant, because the reductions in recruitment to *ARG1* were comparable to the reductions in subunit expression for myc-Snf2p, Snf5p, and Swp73p in *swi3*Δ cells (compare Fig. 4A through C and 6A and B). It is noteworthy, however, that we observed comparable, strong reductions in SWI/SNF subunit expression in *swi3*Δ and *swp73*Δ cells (Fig. 6B), yet recruitment of myc-Snf2p and myc-Snf5p to *ARG1* occurred at nearly WT levels in *swp73*Δ cells (Fig. 4). Thus, the extensive decreases in myc-Snf2p, Snf5p, and Swp73p expression in *swi3*Δ cells probably cannot fully explain the dramatic reduction in recruitment of these proteins to *ARG1* in the *swi3*Δ mutant. It appears that high-level recruitment of Snf2p and Snf5p by Gcn4p requires contributions from other SWI/SNF subunits that go beyond their roles in maintaining WT levels of Snf2p and Snf5p.

As mentioned above, previous gel filtration analysis suggested that the SWI/SNF complex is partially disrupted in *snf2*Δ, *swi3*Δ, *snf5*Δ, and *snf6*Δ strains, with a particularly strong defect in *swi3*Δ cells (40). We confirmed by coimmuno-

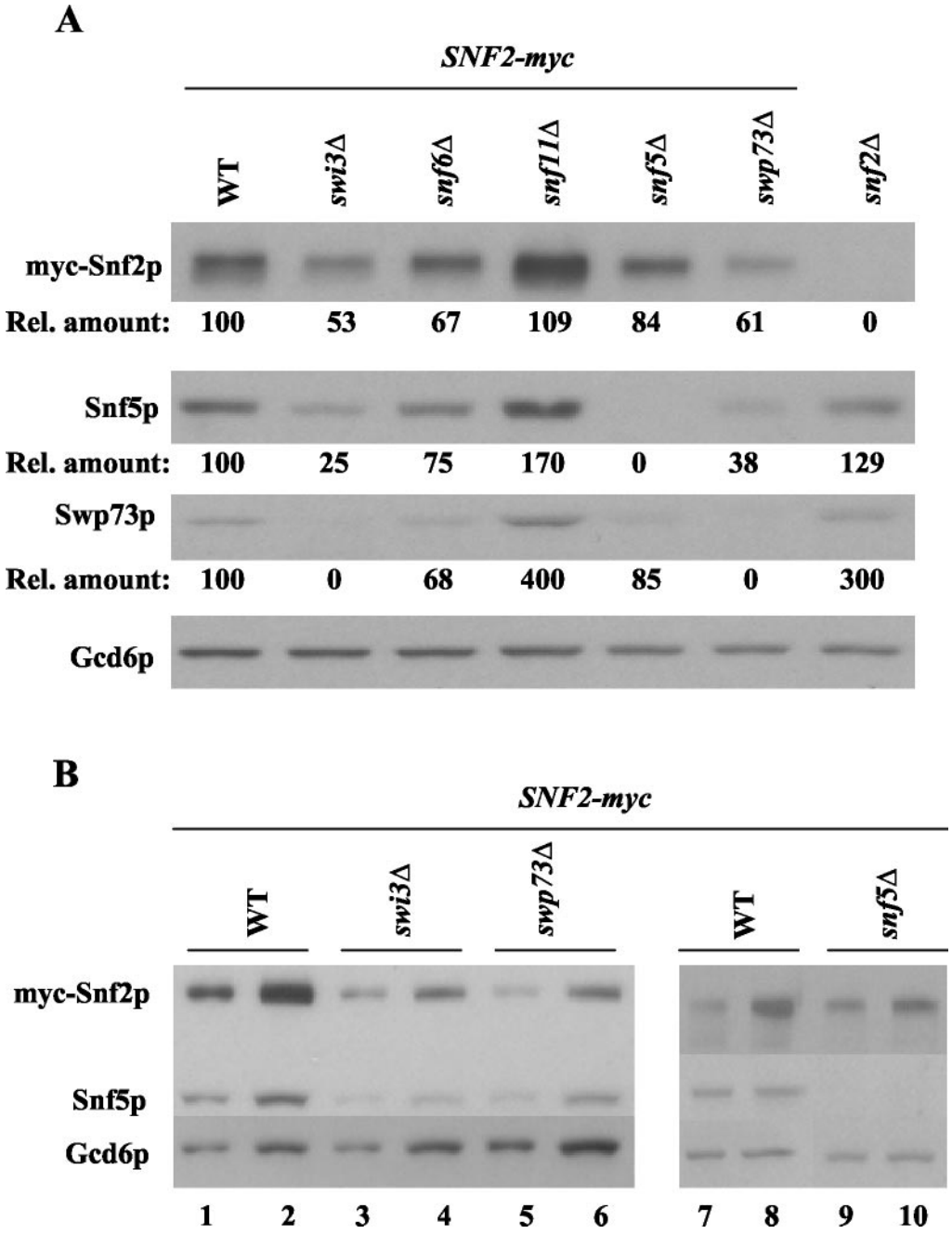

FIG. 6. Western blot analysis of SWI/SNF subunits in *swi*/*snf* mutants. (A) The *GCN4* strains containing *SNF2-myc* and either WT SWI/SNF subunits or the indicated *swi*/*snf* deletions were grown in SC-ILV, and total proteins were extracted as described in Materials and Methods. Aliqouts with equal amounts of total proteins were separated by SDS-PAGE by using 10% gels, transferred to a nitrocellulose membrane, and probed with antibodies against myc, Snf5p, or Swp73p, as indicated on the left of the blot. Probing with Gcd6p antibodies provided a loading control. The Western blotting signals were quantified by video densitometry by using NIH Image software, normalized for the Gcd6p signals, expressed relative to the normalized value measured in WT cells, and listed below the corresponding blots for myc-Snf2p, Snf5p, and Swp73p (Rel. amount). (B) The *swp73*Δ *and swi3*Δ strains exhibit similar reductions in SWI/SNF subunit levels. The analysis described for panel A was repeated for the WT, *swi3*Δ, *swp73*Δ, and *snf5*Δ strains harboring *SNF2-myc*, loading two different amounts of extract in adjacent lanes; thus, the sizes of samples in lanes 1, 3, 5, 7, and 9 were 50% of those loaded in lanes 2, 4, 6, 8, and 10.

precipitation analysis that the association between myc-Snf2p and Snf5p was impaired in our *snf6*Δ and *swi3*Δ strains, and we obtained the same result for the *swp73*Δ mutant (Fig. 7A). By contrast, myc-Snf2p/Snf5p association was intact in *snf11*Δ cells, as was the myc-Swi3p/Snf5p association in *snf2*Δ cells (Fig. 7B). The latter finding is in accordance with our Western blot analysis of *snf2*Δ cells showing high-level expression of SWI/SNF subunits (Fig. 6A) and our ChIP data showing little or no defect in SWI/SNF recruitment in the *snf2*Δ mutant (Fig. 4B through F and 5A and B). To account for the previous data

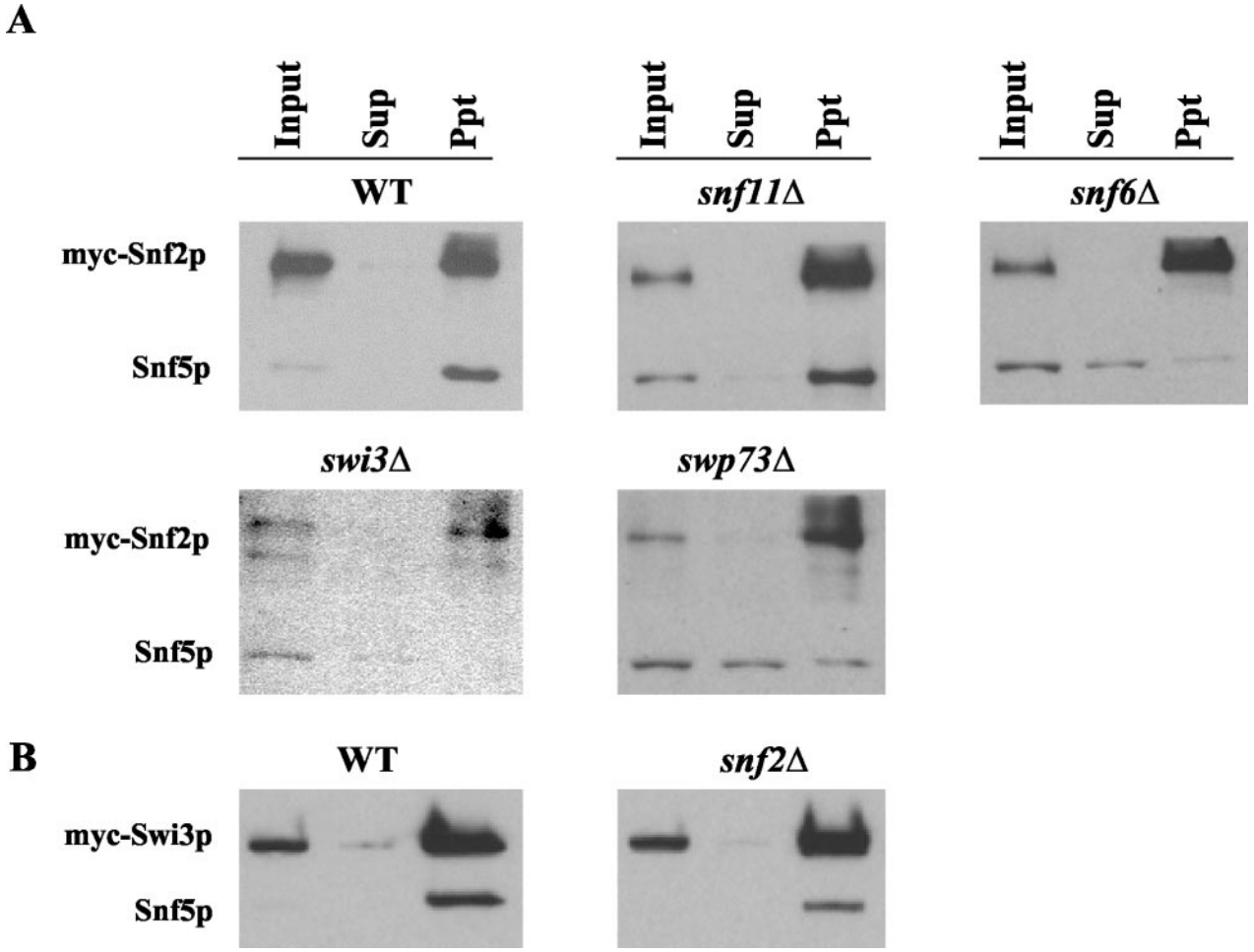

FIG. 7. Examination of SWI/SNF complex integrity in *swi/snf* mutants by coimmunoprecipitation analysis. (A) *SNF2-myc* strains containing WT SWI/SNF subunits or the indicated *swi/snf* deletions were grown in YPD, and WCEs were prepared as described in Materials and Methods. Aliqouts containing 0.3 to 0.5 mg of protein were immunoprecipitated with mouse monoclonal myc antibodies. Ten percent of the input samples (Input), 100% of the immunoprecipitates (Ppt), and 10% of the supernatant (Sup) fractions were resolved by 4 to 20% SDS-PAGE and subjected to Western blot analysis using monoclonal mouse antibodies against myc or rabbit antibodies against Snf5p. The upper and lower portions of each membrane were probed separately with different antibodies. (B) The same kind of analysis as that described for panel A was carried out by using the WT or *snf2*Δ strains harboring *SWI3-myc*.

indicating disruption of SWI/SNF in *snf2*Δ cells (40), we propose that SWI/SNF is largely intact in *snf2*Δ cells and dissociated in vitro under the conditions of gel filtration analysis. The fact that the SWI/SNF complex is disrupted (Fig. 7A) and subject to proteolysis (Fig. 6B) in *swp73*Δ cells yet shows no defect in recruitment by Gcn4p to *ARG1* could indicate that a low level of intact SWI/SNF complex remains in this strain that is sufficient for nearly WT recruitment by Gcn4p to *ARG1* but inadequate for SWI/SNF recruitment to *SNZ1* (Fig. 5A and B). It is also possible that SWI/SNF subcomplexes which presumably occur in *swp73*Δ cells can be recruited independently of one another by Gcn4p, at least to *ARG1* (see Discussion).

The results presented above suggest that efficient recruitment of Snf2p and Snf5p by Gcn4p in vivo is strongly dependent on their association with other subunits in the SWI/SNF complex. If so, then overexpressing these proteins individually from high-copy-number plasmids should not increase their level of recruitment by Gcn4p. To test this prediction, we introduced a multicopy *SNF2-myc* plasmid, or empty vector, into the otherwise WT strain harboring *SNF2-myc.* Likewise, we transformed the WT *SNF5-myc* strain with a multicopy *SNF5-myc* plasmid or with vector alone. In both instances, increasing the gene dosage of *SNF2-myc* or *SNF5-myc* led to overexpression of the cognate-tagged protein (Fig. 8A). Importantly, overexpression of myc-Snf2p led to a decrease, rather than an increase, in its recruitment to *ARG1* (Fig. 8B, compare lanes 1 and 2). Overexpression of myc-Snf5p had no impact on the amount of this protein that was recruited by Gcn4p to *ARG1* (Fig. 8B, compare lanes 6 and 7). To explain the negative effect of myc-Snf2p overexpression on its recruitment, we suggest that increasing the amount of Snf2p leads to the formation of Snf2p-containing subcomplexes that lack one or more other subunits required for efficient recruitment of SWI/SNF by Gcn4p.

The multicopy *SNF2-myc* plasmid also led to greater-than-WT levels of myc-Snf2p in the *snf6*Δ mutant, compensating for the slight effect of this mutation on myc-Snf2p expression (Fig. 8A, compare lanes 3 through 5). Nonetheless, the

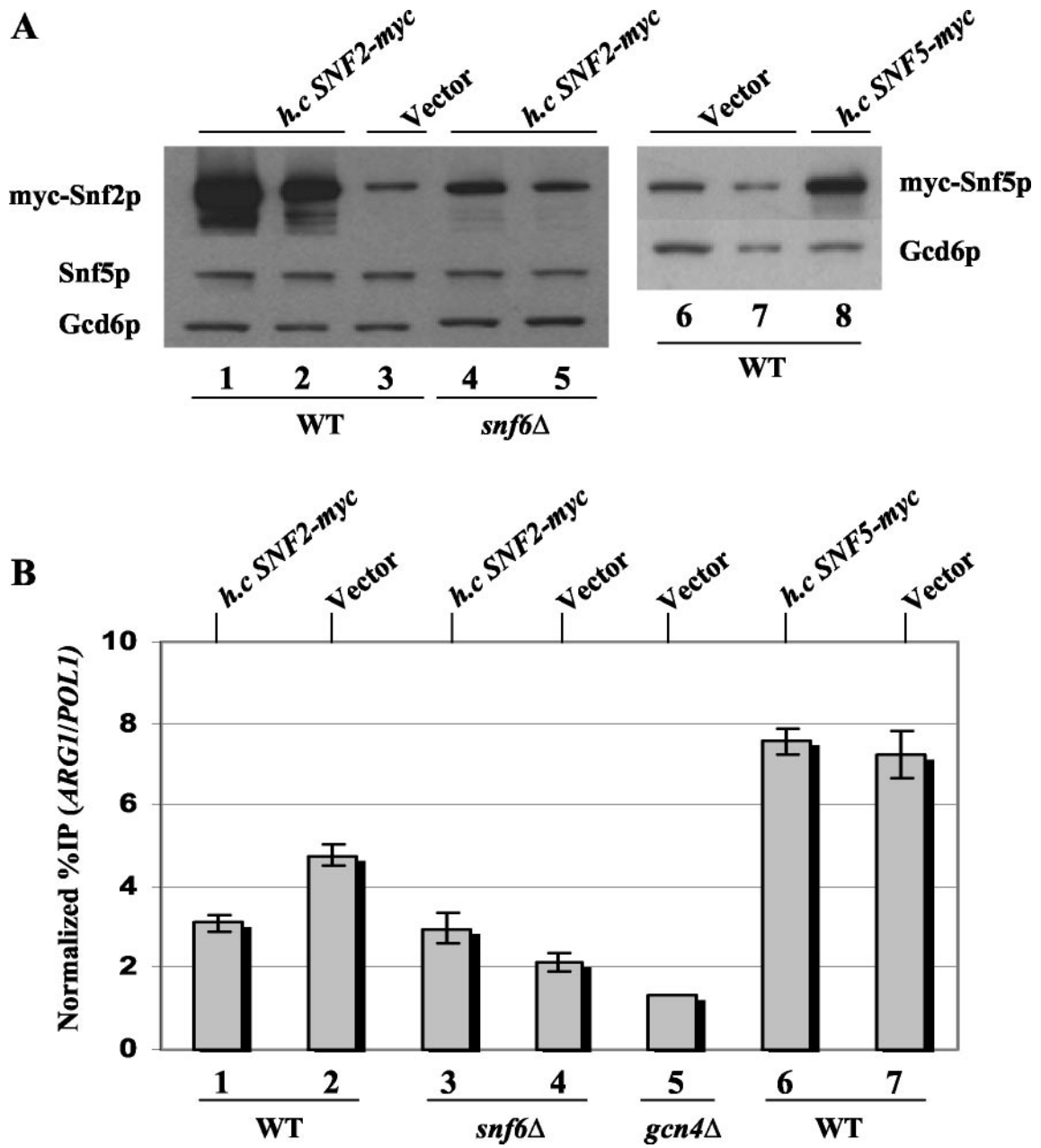

FIG. 8. Overexpression of myc-Snf2p or myc-Snf5p does not increase their recruitment to *ARG1*. (A) The *SNF2-myc* (SY1), *SNF5-myc* (SY162), and *snf6*Δ *SNF2-myc* (SY3) strains described in the legends to Fig. 1 and 4 were transformed with high-copy-number plasmid pSY286 or pSY287, harboring *SNF2-myc* or *SNF5-myc*, respectively, or with empty vector YCp50 and subjected to Western blot analysis as described in the legend to Fig. 6. Lanes 1 through 3, *SNF2-myc* strain transformed with the plasmids indicated at the top; lanes 4 and 5, *snf6*Δ *SNF2-myc* strain transformed with pSY286; lanes 6 through 8, *SNF5-myc* strain transformed with the plasmids indicated at the top. The samples in lanes 2, 5, and 7 contained 50% of those loaded in lanes 1, 4, and 6, respectively. (B) ChIP analysis was performed on the transformants described for panel A. Lanes 1 through 4, transformants of the *SNF2-myc* and *snf6*Δ *SNF2-myc* strains harboring the plasmids indicated at the top; lanes 6 and 7, transformants of the *SNF5-myc* strain harboring the plasmids indicated at the top; lane 5, transformant of the *gcn4*Δ *SNF2-myc* strain containing YCp50.

recruitment of myc-Snf2p was impaired in this transformant relative to that seen in the WT strain with native levels of myc-Snf2p (Fig. 8B, compare lanes 2 and 3). We found by coimmunoprecipitation analysis that overexpressing myc-Snf2p in the *snf6*Δ mutant slightly increased SWI/SNF complex formation (data not shown), most likely accounting for the small increase in myc-Snf2p recruitment observed in this situation (Fig. 8B, compare lanes 3 and 4). The results shown in Fig. 8 support our conclusion that Snf6p contributes to SWI/SNF recruitment in a manner that exceeds its contribution to SWI/SNF subunit expression, presumably by promoting SWI/SNF complex integrity (see Discussion).

Recruitment of SWI/SNF by Gcn4p requires SRB mediator subunits and a non-Gcn5p function of SAGA. We next turned

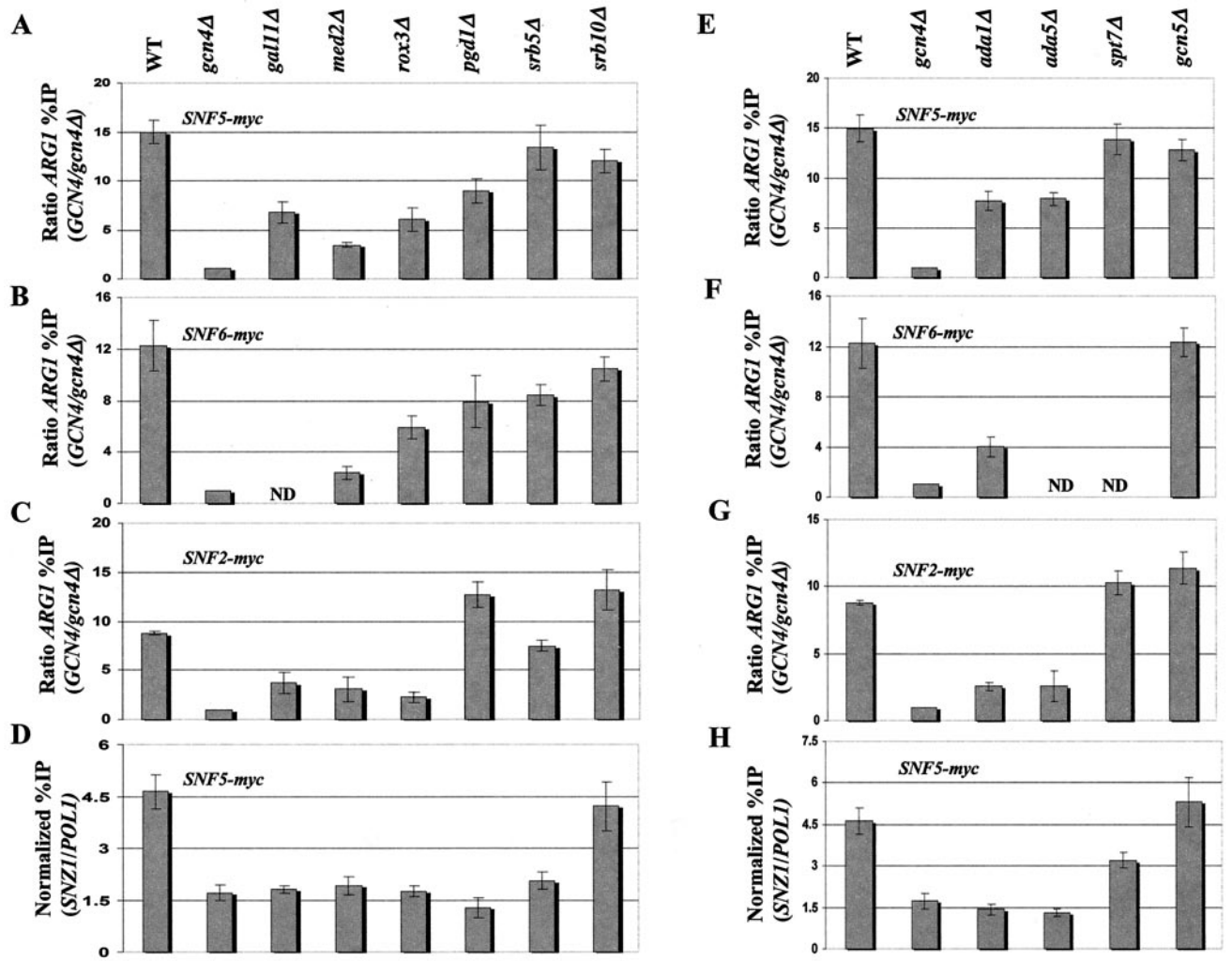

FIG. 9. Subunits of mediator and SAGA are required for optimal recruitment of SWI/SNF by Gcn4p. (A through C and E through G) We generated a panel of strains harboring the chromosomal myc-tagged *SWI/SNF* alleles designated in the upper left of each histogram from *GCN4* strains containing all WT coactivator subunits (WT) or the indicated deletion alleles (shown at the top) that remove subunits of mediator (A through C) or SAGA (E through G) and from the *gcn4*Δ strain containing all WT coactivator subunits (*gcn4*Δ) (see Table 1 for a list of strains). High-copy-number plasmid pHQ1239 was introduced into the *GCN4* strains, and empty vector YCp50 was introduced into the *gcn4*Δ strains. The resulting transformants were subjected to ChIP analysis to measure binding of the myc-tagged SWI/SNF subunit to *ARG1*, as described in the legend to Fig. 1. (D and H) The strains described for panels A and E that harbor the *SNF5-myc* allele were subjected to ChIP analysis to measure binding of myc-Snf5p to *SNZ1*. ND, not determined.

to the question of whether recruitment of the SWI/SNF complex is dependent on SRB mediator. To address this possibility, we myc_{13}-tagged Snf2p, Snf5p, or Snf6p in deletion mutants lacking single subunits of SRB mediator (*gal11*Δ, *med2*Δ, *rox3*Δ, *pgd1*Δ, *srb5*Δ, or *srb10*Δ). All of these strains showed defects in transcriptional activation of Gcn4p-dependent reporters and selected Gcn4p target genes in vivo (53). The resulting set of tagged strains was analyzed by ChIP assays as described above. Deletion of *GAL11*, *MED2*, and *ROX3* had relatively strong effects on recruitment of SWI/SNF subunits to *ARG1* (Fig. 9A through C). Deletion of *SRB5* or *SRB10* produced either a modest reduction or had no effect on recruitment of SWI/SNF, while deletion of *PGD1* gave various results (Fig. 9A through C). Interestingly, Gal11p, Med2p, Rox3p, Pgd1p, and Srb5p were all strongly required for efficient recruitment of myc-Snf5p to *SNZ1*, as deletion of each protein lowered recruitment to nearly the same low level seen in the *gcn4*Δ strain (Fig. 9D).

We carried out similar experiments to address the importance of SAGA subunits Ada1p, Ada5p, Spt7p, and Gcn5p in recruitment of SWI/SNF to *ARG1* and *SNZ1*. Deletions of these proteins also impair transcriptional activation of Gcn4p-dependent reporters and certain Gcn4p target genes (53). The results revealed a moderate to strong requirement for Ada1p and Ada5p in recruitment of SWI/SNF subunits to *ARG1* (Fig. 9E through G) and a complete dependence on both proteins for SWI/SNF recruitment to *SNZ1* (Fig. 9H). By contrast, Spt7p was dispensable for recruitment of SWI/SNF subunits to *ARG1* and made only a small contribution to SWI/SNF recruitment at *SNZ1*. Gcn5p was dis-

pensable for a WT level of SWI/SNF recruitment at both genes (Figs. 9E through H).

It is important to note that ChIP experiments using *GCN4-myc$_{13}$* derivatives of the mediator and the SAGA mutants analyzed in Fig. 9 revealed no reductions in myc-Gcn4p binding at *ARG1* or *SNZ1* in any of these strains (Qiu and Hinnebusch, unpublished). Thus, the diminished recruitment of SWI/SNF in mediator and SAGA mutants cannot be attributed to reduced binding of the activator Gcn4p at the target promoters. It was also important to consider the possibility that expression of the tagged SWI/SNF subunit or the integrity of the SWI/SNF complex was compromised in the mediator and SAGA mutants. To eliminate this possibility, we conducted Western blot analysis of myc-Snf2p and Snf5p levels in WCEs prepared from the relevant mutants. In addition, we compared the amounts of Snf5p that coimmunoprecipitated with Snf2-myc in the mediator and SAGA mutants with those of WT. The results showed little or no effect of the mediator or SAGA mutations on the steady-state levels of myc-Snf2p or Snf5p or the integrity of the SWI/SNF complex (data not shown). We conclude that recruitment of the intact SWI/SNF complex by Gcn4p is strongly enhanced by SRB mediator and SAGA.

DISCUSSION

The results presented in this report increase our understanding of the recruitment of SWI/SNF by the activator Gcn4p in several important respects. First, we provide the strongest evidence to date that Gcn4p recruits the entire SWI/SNF complex, not just the catalytic subunit Snf2p, to authentic target genes in vivo. Second, we show that recruitment of SWI/SNF is dependent on the hydrophobic clusters in the activation domain that are required for activation by Gcn4p in vivo (22) and for the interaction between Gcn4p and purified SWI/SNF in vitro (35). It was possible that low constitutive binding of SWI/SNF to the target promoters, with attendant nucleosome remodeling, would be required for high-level binding of Gcn4p to its recognition sites. Our results eliminate this possibility at *ARG1* and *SNZ1* by showing that a WT level of Gcn4p binding occurs at these promoters in the *snf2*Δ mutant. It could also be proposed that another subunit of SWI/SNF, e.g., Snf5p or Swi3p, would enhance Gcn4p binding through direct physical contact with the activation domain in the *snf2*Δ mutant, given that the SWI/SNF complex remains intact and binds to *ARG1* and *SNZ1* in *snf2*Δ cells. At odds with this possibility, we observed nearly WT levels of Gcn4p binding to both promoters in mutants lacking Swi3p, Snf6p, Snf11p, Snf5p, or Swp73p. Thus, the simplest explanation for our results is that Gcn4p binds to *ARG1* and *SNZ1* independently of nucleosome remodeling by SWI/SNF and then recruits SWI/SNF via interactions with the hydrophobic clusters in the Gcn4p activation domain. Because the gcn4-14Ala protein showed a WT level of binding to *ARG1* and *SNZ1*, it appears that Gcn4p binding is not enhanced by any coactivators that interact with the Gcn4p activation domain, including SAGA, SRB mediator, RSC, and CCR4-NOT (11, 53).

Snf2p is dispensable, but Snf5p, Snf6p, and Swi3p are required for optimal recruitment of SWI/SNF by Gcn4p. Our results also illuminate several aspects of the mechanism of SWI/SNF recruitment by Gcn4p. Because Snf2p, Snf5p, and Swi1p interact with the Gcn4p activation domain in vitro (37), we sought to determine whether these subunits are necessary or sufficient for recruitment of native SWI/SNF by Gcn4p in vivo. We found that deletion of *SNF2* did not significantly reduce the recruitment of other SWI/SNF subunits by Gcn4p to *ARG1* and *SNZ1*. (We verified the absence of both *SNF2* coding sequences and immunologically detectable Snf2p in the *snf2*Δ strain.) Additionally, the excess Snf2p present in a strain overexpressing this subunit alone was not recruited to *ARG1*, suggesting that Snf2p cannot be recruited directly by Gcn4p in vivo. In contrast to these results, deletion of *SNF5* decreased Gcn4p-dependent recruitment of other SWI/SNF subunits to *ARG1* by 50 to 80%, with an average reduction of 70%. Moreover, deletion of *SNF5* severely impaired SWI/SNF recruitment to *SNZ1*, lowering the binding of myc-Snf6p and myc-Swi1p to the same low level observed in *gcn4*Δ cells. These recruitment defects are substantially greater than the ~15% reductions in subunit expression we observed in *snf5*Δ cells, suggesting that Snf5p makes a contribution to SWI/SNF recruitment beyond its role in maintaining WT levels of SWI/SNF subunits.

One way to account for the role of Snf5p in SWI/SNF recruitment is to propose that Snf5p is a direct target of Gcn4p (37). However, we found that Snf5p cannot be efficiently recruited by Gcn4p in a *snf6*Δ mutant, in which Snf5p expression is only slightly reduced, and that excess Snf5p produced in WT cells was not recruited by Gcn4p. To explain these findings, we propose that the Gcn4p-Snf5p interaction is only one of several contacts between Gcn4p and the SWI/SNF complex and that this interaction is not strong enough to support recruitment of Snf5p outside of intact SWI/SNF. Because SWI/SNF is disrupted in the *snf6*Δ mutant, Gcn4p cannot interact simultaneously with Snf5p and other targets in the complex, such as Snf2p or Swi1p, leading to defective recruitment of Snf5p in the *snf6*Δ strain. Similarly, deletion of *SNF5* disrupts the complex and prevents concerted interaction of Gcn4p with multiple other subunits in addition to abolishing the contact with Snf5p.

Ostensibly at odds with the idea that complex integrity is required for SWI/SNF recruitment by Gcn4p, the *swp73*Δ mutation did not impair the recruitment of SWI/SNF to *ARG1*, even though it disrupted interaction between myc-Snf2p and Snf5p, as indicated by coimmunoprecipitation experiments. However, *swp73*Δ did reduce the recruitment of SWI/SNF to *SNZ1*. One way to explain these findings is to propose that a low level of the SWI/SNF complex lacking only Swp73p persists in *swp73*Δ cells and is recruited efficiently by Gcn4p to *ARG1* because it contains multiple contact sites for Gcn4p. Indeed, a low level of myc-Snf2p/Snf5p association was detected in the *swp73*Δ extracts in our coimmunoprecipitation assays. By contrast, low levels of otherwise intact SWI/SNF complexes lacking only Snf5p, Snf6p, or Swi3p may not exist in *snf5*Δ, *snf6*Δ, or *swi3*Δ mutants. The inability of Gcn4p to recruit the putative SWI/SNF complex lacking only Swp73p to *SNZ1* in *swp73*Δ cells may be related to the fact that binding of Myc-Gcn4p and recruitment of WT SWI/SNF to *SNZ1* occurs at only one-fourth or one-third, respectively, of the levels seen at *ARG1*. SAGA and SRB mediator are also recruited at lower levels to *SNZ1* than to *ARG1* (data not shown). Thus, the low concentration of intact SWI/SNF complexes in *swp73*Δ cells

may be insufficient for their recruitment to *SNZ1* because of reduced levels of Gcn4p and other coactivators bound at the promoter.

Another way to account for the recruitment of SWI/SNF subunits to *ARG1* in *swp73*Δ cells would be to propose that particular subcomplexes remaining in *swp73*Δ cells retain sufficient contacts with Gcn4p to support their recruitment to *ARG1* but not to *SNZ1*. By contrast, the *snf5*Δ, *snf6*Δ, and *swi3*Δ mutations would produce different, more defective subcomplexes containing too few contacts for Gcn4p. The latter suggestion is consistent with the fact that Snf5p and Swi3p belong to the evolutionarily conserved core of the SWI/SNF complex (42) and that Swi3p and Snf6p are the only two subunits present in two copies per complex (48). Hence, Snf5p, Snf6p, and Swi3p may play greater roles than Swp73p in linking together multiple subunits of the SWI/SNF complex.

Our finding that Snf2p is dispensable for SWI/SNF recruitment by Gcn4p differs from results described recently for SWI/SNF binding at the *SUC2* and *SER3* promoters. At *SER3*, Snf2p functions in repression in a manner largely independent of other SWI/SNF subunits. In this instance, Snf2p was crucial for Snf5p binding at *SER3*, whereas Snf5p was dispensable for Snf2p binding to the promoter (33). Thus, the relative importance of Snf2p and Snf5p for recruitment seems to be reversed between *SER3* and *ARG1*/*SNZ1*. It is unknown whether Snf2p is actively recruited to *SER3*; perhaps it binds directly to acetylated histone tails through its bromodomain (17). At *SUC2*, it was found that Snf2p and Snf5p are mutually interdependent for promoter binding, and it was concluded there, as in our study, that an intact complex is crucial for recruitment of SWI/SNF to this promoter (13). It remains possible that the optimal rate of SWI/SNF recruitment by Gcn4p is dependent on Snf2p, even if the final extent of binding is not significantly impaired in *snf2*Δ cells. It was conceivable that Snf2p would be required for efficient SWI/SNF recruitment at lower levels of promoter occupancy by Gcn4p than were examined in our experiments; however, we found recently that deleting *SNF2* does not decrease SWI/SNF recruitment to *ARG1* in cells expressing native levels of Gcn4p (data not shown).

Recruitment of SWI/SNF is dependent on particular mediator and SAGA subunits but independent of Gcn5p. We found that deletions of the Gal11p, Med2p, and Rox3p subunits of SRB mediator led to substantial reductions in recruitment of SWI/SNF subunits Snf5p, Snf6p, and Snf2p to *ARG1* and *SNZ1*. None of these mutations reduced binding of Gcn4p itself to *ARG1*, nor did they produce any obvious reductions in the integrity or abundance of the SWI/SNF complex. Previously, we reported ChIP data showing that SRB mediator is recruited by Gcn4p to *ARG1* in vivo (53). Thus, our findings are consistent with the idea that SWI/SNF binding is enhanced by prior recruitment of mediator to the same promoter. Given that Gcn4p can interact independently with purified SWI/SNF and SRB mediator complexes in vitro (29, 35) and considering that SWI/SNF copurifies with holoenzyme under certain conditions (56), it is possible that these coactivators cooperate in binding simultaneously to Gcn4p at the promoter. This model would be analogous to the activator-dependent cooperative assembly of human mediator and TFIID on promoters observed in vitro (23). Consistent with this idea, Snf2p binding and Snf2p-dependent remodeling at *RNR3* were shown to be impaired by Ts^- mutations in the PolII subunit Rpb1p and the mediator subunit Srb4p (46). Our finding that Pgd1p and Srb5p are required for SWI/SNF recruitment to *SNZ1* but not to *ARG1* might indicate that more extensive contacts between SWI/SNF and mediator, or a higher level of mediator recruitment, are required to stabilize SWI/SNF binding when there is a lower level of activator associated with the promoter, as occurs at *SNZ1* versus at *ARG1*.

We found that SAGA subunits Ada1p and Ada5p are additionally required for WT recruitment of SWI/SNF by Gcn4p at *ARG1* and *SNZ1*, whereas Gcn5p is dispensable for SWI/SNF binding at both genes. Hassan et al. reported that the Snf2p bromodomain is required for recruitment of Snf6p to the *SUC2* promoter in vivo and for cell growth on a carbon source (raffinose) requiring *SUC2* induction (17). These results are consistent with the idea that histone acetylation by Gcn5p can enhance SWI/SNF recruitment through interaction of the Snf2p bromodomain with acetylated histones. In contrast, deletion of the Snf2p bromodomain had no apparent effect on transcriptional activation by Gcn4p unless it was combined with mutations in SAGA subunits Gcn5p or Tra1p (17). This observation fits with our findings that Snf2p and Gcn5p are dispensable for recruitment of SWI/SNF by Gcn4p in otherwise WT cells. It is possible that H3 acetylation by Gcn5p would enhance the rate of SWI/SNF recruitment or that it becomes critical for recruitment in mutants lacking other coactivator functions. Furthermore, it was shown that H3 acetylation by Gcn5p promotes nucleosome remodeling by SWI/SNF independently of SWI/SNF recruitment (54).

Although the HAT activity of Gcn5p is dispensable, other functions of SAGA dependent on Ada1p and Ada5p are needed for optimal recruitment of SWI/SNF by Gcn4p. Ada1p and Ada5p are required with Spt7p for integrity of SAGA during purification, and mutations in these three subunits have more severe phenotypes than do mutations in other nonessential SAGA subunits, including Gcn5p. These findings indicate that SAGA has critical functions beyond HAT activity that are disrupted when Ada1p, Ada5p, or Spt7p is deleted (21, 32, 50). Consistently, it was shown that Ada5p and Spt3p, but not Gcn5p, are required for TBP recruitment by Gal4p to the promoter (3, 12). Our results indicate that enhancing SWI/SNF recruitment can be viewed as another important non-HAT function of SAGA.

The dependence of SWI/SNF recruitment on SAGA could be a manifestation of the cooperative assembly of coactivator complexes proposed above to explain the stimulatory effect of mediator subunits on SWI/SNF recruitment. While it might seem difficult to accommodate several large coactivator complexes simultaneously at the same promoter, Gcn4p binds as a homodimer and frequently has multiple binding sites in the promoter. Hence, several Gcn4p activation domains may function simultaneously, each tethering a different coactivator to the promoter. The fact that mutations in TFIID subunits impaired SWI/SNF binding at *RNR3* could indicate a role for TBP in SWI/SNF recruitment (46). Thus, the *ada1*Δ and *ada5*Δ mutations might reduce SWI/SNF recruitment by Gcn4p indirectly by impairing TBP recruitment. Given the stimulatory role of SRB mediator in TBP recruitment (28, 30), the same indirect mechanism could explain the requirement for mediator subunits in SWI/SNF recruitment by Gcn4p. Ad-

ditional ChIP experiments are needed to distinguish between these hypotheses.

The fact that *spt7*Δ had no effect on SWI/SNF recruitment at *ARG1* and a significantly smaller effect at *SNZ1* than *ada1*Δ and *ada5*Δ seems inconsistent with the idea that SAGA is disrupted by all three of these SAGA subunit deletions. To explain our findings, we propose that a subcomplex containing Ada1p and Ada5p persists in *spt7*Δ cells and retains the function of SAGA involved in promoting SWI/SNF recruitment by Gcn4p. It is noteworthy that partial SAGA complexes were identified recently in *ada1*Δ and *ada5*Δ mutants (57). The requirement for Spt7p to achieve WT recruitment of SWI/SNF at *SNZ1* suggests that the putative SAGA subcomplex in *spt7*Δ cells is inferior to WT SAGA in supporting SWI/SNF recruitment by Gcn4p.

The requirement for particular subunits of the mediator or SAGA for SWI/SNF recruitment by Gcn4p could have several underlying causes. One simple possibility is that these subunits are specifically required for recruitment of the mediator or SAGA itself to the promoter by Gcn4p. Another possibility is that the required subunits provide points of contact between the mediator and SWI/SNF or between SAGA and SWI/SNF. Finally, the required subunits of the mediator or SAGA may be needed for chromatin modification or recruitment of another factor, e.g., TBP, which in turn promotes SWI/SNF binding to the promoter (46). These various possibilities are presently under investigation.

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