Promoter Occupancy Is a Major Determinant of Chromatin Remodeling Enzyme Requirements

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Chromatin creates transcriptional barriers that are overcome by coactivator activities such as histone acetylation by Gcn5 and ATP-dependent chromatin remodeling by SWI/SNF. Factors defining the differential coactivator requirements in the transactivation of various promoters remain elusive. Induction of the *Saccharomyces cerevisiae PHO5* promoter does not require Gcn5 or SWI/SNF under fully inducing conditions of no phosphate. We show that *PHO5* activation is highly dependent on both coactivators at intermediate phosphate concentrations, conditions that reduce the nuclear concentration of the Pho4 transactivator and severely diminish its association with *PHO5* in the absence of Gcn5 or SWI/SNF. Conversely, physiological increases in Pho4 nuclear concentration and binding at *PHO5* suppress the need for both Gcn5 and SWI/SNF, suggesting that coactivator redundancy is established at high Pho4 binding site occupancy. Consistent with this, we demonstrate, using chromatin immunoprecipitation, that Gcn5 and SWI/SNF are directly recruited to *PHO5* and other strongly transcribed promoters, including *GAL1-10*, *RPL19B*, *RPS22B*, *PYK1*, and *EFT2*, which do not require either coactivator for expression. These results show that activator concentration and binding site occupancy play crucial roles in defining the extent to which transcription requires individual chromatin remodeling enzymes. In addition, Gcn5 and SWI/SNF associate with many more genomic targets than previously appreciated.

The incorporation of regulatory elements into nucleosomes interferes with their function by obstructing their accessibility to *trans*-acting factors (47). Several highly conserved multisubunit complexes, termed coactivators or chromatin modifiers and remodelers, act in concert with site-specific activators to help the transcriptional apparatus contend with chromatin structure (41). One class of coactivators contains an ATPase subunit (e.g., Swi2/Snf2 of SWI/SNF) that uses the energy derived from ATP hydrolysis to disrupt histone-DNA interactions (38, 64). A second class of coactivators post-translationally modifies specific amino acid residues of the basic core histone proteins, e.g., acetylates lysines in the histone amino termini (26). In yeast, this class includes the SAGA (for "Spt-Ada-Gcn5 acetyltransferase") and NuA4 (for "nucleosomal acetyltransferase histone H4") complexes, which primarily acetylate histones H3 and H4 via their respective catalytic subunits Gcn5 and Esa1 (9, 19, 54). Gcn5 and SWI/SNF are partially redundant, performing independent but overlapping functions during transcriptional activation (7, 49, 51, 53, 56, 58, 66).

Although distinct programs of recruitment of chromatin remodelers and other multiprotein complexes have been reported for various promoters (2, 13, 55), a common theme has emerged. Each transcriptional program is generally initiated by one or more site-specific activator proteins that access metazoan enhancers or upstream activating sequences (UASs) in yeast. Activation domains then mediate the high-affinity interaction and hence "recruitment" of specific chromatin modifiers

and remodelers, which do not bind DNA with specificity (15, 23). Ultimately, changes in chromatin structure or remodeling facilitate the assembly of the transcription preinitiation complex onto the core promoter (2, 30, 37).

Although much is known about how coactivators are recruited, the reason why promoters vary in their requirements for chromatin modifiers and remodelers is unresolved. Given the central role of site-specific activators in coactivator recruitment, it seems reasonable that various activation domain subclasses might interact with and hence recruit distinct coactivators. However, acidic activators interact directly with a similar subset of chromatin-associated activities, including yeast NuA4, SAGA, and SWI/SNF as well as their human counterparts (15, 23, 27, 43, 67). The apparent absence in vitro of distinct interaction preferences among this subset of coactivators is consistent with in vivo studies suggesting that a variety of natural and chimeric activators are able to recruit overlapping sets of coactivators (7, 12, 49, 53, 56, 58, 66).

Recently, a few studies have suggested that promoter architecture, i.e., the relative location of *cis*-regulatory sequences with respect to nucleosomes, orchestrates a specific coactivator recruitment program and hence requirements for individual coactivator complexes (37, 52, 53, 56). Thus, in some cases, promoters with a nucleosomal TATA (yeast *SUC2* and human beta interferon) require Gcn5 and SWI/SNF for activation (2, 21, 24). These coactivator dependencies are alleviated at other promoters where TATA is either naturally accessible or exposed artificially (37, 53, 56). However, the well-studied *GAL1* and *PHO5* promoters, at which TATA is occluded by nucleosomes, require neither SWI/SNF nor Gcn5 under fully activating conditions (6, 14, 16, 20, 42, 48). Interestingly, a prerequisite for both SWI/SNF and Gcn5 is imposed on *GAL1* and *PHO5* activation in mitosis (32, 42), possibly because the chro-

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matin architecture is condensed. However, many promoters have an absolute requirement for these coactivators in interphase, indicating that additional factors must play a role in determining a promoter's need for specific chromatin modifiers and remodelers.

While *PHO5* induction does not require these coactivators in strict genetic terms, we and others have shown that both Gcn5 and SWI/SNF are needed to achieve full rates of initial promoter activation (4, 5, 42). Further, under fully activating conditions of complete P_i starvation, *PHO5* expression depends on Gcn5 when the promoter is weakened by mutations in either of the two UASs (20). Lastly, growth of yeast in rich medium, which is limiting for P_i, leads to partial activation of *PHO5* in mitosis (~10% of the full activity achieved overnight in no-P_i medium) that is highly dependent on Gcn5 and SWI/SNF (42). These observations are consistent with the hypothesis that *PHO5* promoter induction requires these remodelers when low levels of activator are associated with the promoter.

Testing this hypothesis, here we show that *PHO5* transactivation is strongly reduced in the absence of either Gcn5 or SWI/SNF at low levels of UAS-bound Pho4. By contrast, the requirement for either remodeler is alleviated when Pho4 binding site occupancy is increased, suggesting that functional redundancy is established at promoters with robust activator interactions. Thus, we also find significant recruitment of Gcn5 and SWI/SNF to several promoters known to exhibit strong activator binding and transcription at which they are currently thought not to function. These results define a critical role for activator concentration and promoter occupancy in determining the extent to which transactivation depends on specific chromatin modifiers and remodelers. Moreover, our data suggest that Gcn5 and SWI/SNF have many genomic targets and support a model in which high levels of promoter-bound activator drive the genetic redundancy that is observed between various coactivators.

MATERIALS AND METHODS

Yeast media, growth conditions, rAPase activity assays, and Northern blotting. Defined, P_i-free medium (pH 5.5) was prepared as described previously (42), except that it was supplemented with complete synthetic mix (CSM) as indicated by the manufacturer (Bio 101). All starter cultures were grown in this medium with KH_2PO_4 added back to 13.4 mM to supply inorganic phosphate (P_i). To determine activation time courses, cells were washed and transferred to defined medium containing 13.4 mM KCl (no P_i). For dose responses, cells were washed with defined, P_i-free medium with CSM and transferred for 12 h to the same medium containing the indicated concentrations of KH_2PO_4 or KCl to bring the potassium ion concentration to 13.4 mM. Repressible acid phosphatase (rAPase) activity and *PHO5* transcript levels were assayed as previously described (42).

Pho4 cellular localization. For green fluorescent protein (GFP) studies, strains were grown for 12 h in defined medium with 13.4, 0.2, or 0 mM P_i. Cells (1 ml) were sonicated, washed with 1× phosphate-buffered saline (PBS), and fixed with 70% ethanol for 20 min. The cells were washed again with 1× PBS, resuspended in 10 μl of 1-μg/ml 6-diamidino-2-phenylindole (DAPI), and incubated at room temperature for 12 min. The cells were then washed with 1× PBS and viewed with an Axiovert 135 with a 100× Plan-Apochromat oil immersion objective (Carl Zeiss MicroImaging). Representative cell images were collected using Zeiss Axiovision ver. 3.1.

ChIP analysis. Chromatin immunoprecipitation (ChIP) analysis was performed as described previously (11), except that the cells were cross-linked for 15 min at room temperature with 1% formaldehyde. After cross-linking and cell lysis, total-cell lysates containing soluble and pelleted chromatin were resolubilized and sheared by sonication. Aliquots of the fixed and sheared chromatin were deproteinized and analyzed by agarose gel electrophoresis to determine the amount of chromatin and verify shearing to an average length of 500 bp. Similar amounts of cross-linked chromatin were immunoprecipitated using rabbit A-14 anti-myc antibody (2 μl; SC-789; Santa Cruz Biotechnology). For *PHO5* sequences, a single primer pair (ADO236, CATGTAAGCGGACGTC [−456 to −441 relative to the *PHO5* ATG translation start], and LFO740, GCCTTGCC AAGTAAGGTGAC [−173 to −154]) was used to amplify sequences from the endogenous UASs of the *PHO5* promoter as well as a negative control *PHO5* promoter (*pho5* ΔUASs) by quantitative competitive PCR. This negative control contains *PHO5* sequences from −1537 to +9 with two 50-bp deletions (encompassing UASp1 and UASp2 from −401 to −352 and −258 to −209, respectively) and was integrated either by gene replacement of (strains ADY2459 and ADY2461) or by loop in at (strains ADY2695, ADY2701, ADY2719, ADY2727, ADY2915, ADY2921, and ADY2923) the *CAN1* locus (11). Likewise, LFO644 (GGAAATGTAAAGAGCCCC [−547 to −530]) and LFO645 (TTGAAGGT TTGTGGGG [−270 to −255]) were used to simultaneously amplify the endogenous UAS_G region of the *GAL1-10* promoter and a negative control *gal1-10* $ΔUAS_G$ promoter. This negative control comprises the entire *GAL1-10* intergenic region (−698 to +36 relative to the *GAL1* ATG) with a deletion of all four Gal4 sites (UAS_G, −453 to −336), which was integrated by loop in at *CAN1*. Primers used for amplification of various yeast promoters (*EFT2*, *PYK1*, *RPL19B*, and *RPS22B*) were previously described (50).

Western blotting. Yeast cells (100 ml) were grown in defined medium with or without P_i to an optical density at 600 nm of ~1 and Western blot analyses were performed using standard techniques. Briefly, cells were lysed by addition of 0.3 g of ice-cold glass beads (425 to 600 μm) and 500 μl of lysis buffer and vortexing twice for 1 min. Cell debris was pelleted, total protein was quantified using the bicinchoninic acid assay kit (Pierce), and 70 μg of protein per lane was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (37.5:1 acrylamide-to-bisacrylamide ratio; 10% polyacrylamide). After transfer to a polyvinylidene difluoride (PVDF) membrane (Amersham Pharmacia), the blot was incubated overnight with rabbit anti-FLAG antibody (Sigma; F-742) and then with horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (Amersham Pharmacia). Protein was detected with the ECL PLUS kit (Amersham Pharmacia) and visualized using a Storm 860 phosphorimager. The blot was reprobed with mouse monoclonal anti-yeast Pgk1 (3-phosphoglycerate kinase) antibody (Molecular Probes, 22C5-D8) followed by HRP-conjugated anti-mouse immunoglobulin G.

RESULTS

SWI/SNF physically associates with the induced *PHO5* promoter prior to Gcn5. Promoters differ vastly in their requirements for SWI/SNF and Gcn5 (7, 25, 59). With respect to *PHO5*, prior reports have shown that, after extended times under fully activating conditions (no-P_i medium), there is no major effect on *PHO5* transcription in either *swi2Δ* or *gcn5Δ* strains (4, 5, 16, 20, 42). However, the kinetics of *PHO5* induction are strongly dependent on Gcn5 (4, 42) and SWI/SNF (42). Ada2 is recruited to the *PHO5* promoter by Pho4 as a component of SAGA (4); however, direct recruitment of SWI/SNF to *PHO5* has not been shown.

We performed ChIP experiments on 13myc-tagged strains (Table 1) to assay for Gcn5 and Swi2 association at *PHO5*. ChIP analysis of the region of the *PHO5* promoter (Fig. 1A) encompassing both UASp1 and UASp2 was performed at various times after shifting the cells to medium that lacks P_i. The two myc-tagged strains and the parent, untagged strain were assayed internally and in parallel for rAPase activity and were shown to exhibit induction profiles that are essentially identical (Fig. 1B). Since these strains also have *PHO3* (coding for constitutive acid phosphatase) deleted, the measured activities essentially reflect *PHO5* expression (31, 42). Besides the expected kinetic lag relative to accumulation of *PHO5* transcript, we and others have shown that rAPase activities accurately reflect the state of *PHO5* activation following P_i starvation (5, 42). The immunoprecipitated DNA was analyzed by quantita-

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype[a,b]
ADY2459	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R GCN5-13myc-kanMX4 can1Δ::pho5*[pro] *ΔUASs-LEU2*
ADY2461	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R SWI2-13myc-kanMX4 can1Δ::pho5*[pro] *ΔUASs-LEU2*
ADY2695	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R 3myc-PHO4 swi2Δ::kanMX4 CAN1:pho5*[pro] *ΔUASs-LEU2*
ADY2701	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R CAN1:pho5*[pro] *ΔUASs-LEU2*
ADY2719	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R 3myc-PHO4 gcn5Δ::kanMX4 CAN1:pho5*[pro] *ΔUASs-LEU2*
ADY2727	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R 3myc-PHO4 CAN1:pho5*[pro] *ΔUASs-LEU2*
ADY2915	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R CAN1:gal1-10*[pro] *ΔUAS_G-LEU2*
ADY2921	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R GCN5-13myc-kanMX4 CAN1:gal1-10*[pro] *ΔUAS_G-LEU2*
ADY2923	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R SWI2-13myc-kanMX4 CAN1:gal1-10*[pro] *ΔUAS_G-LEU2*
ADY3035	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R* [pRS426 *GPD*[pro]*-PHO4-URA3*]
DNY2049	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R pho4Δ::kanMX4 bar1Δ::R-URA3-R can1Δ::PHO4-GFP-K. lactis LEU2*
DNY2232	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R pho4Δ::kanMX4 bar1Δ::R-URA3-R gcn5Δ::kanMX4 can1Δ::PHO4-GFP-K. lactis LEU2*
MRY2985	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R swi2Δ::kanMX4* [pRS316 *PHO4-GFP-URA3*]
MRY3049	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R* [pRS416 *TEF1*[pro]*-FLAG-PHO4-URA3*]
MRY3053	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R swi2Δ::kanMX4* [pRS416 *TEF1*[pro]*-FLAG-PHO4-URA3*]
MRY3055	*MAT**a** leu2Δ0 lys2Δ0 ura3Δ0 pho3Δ::R gcn5Δ::kanMX4* [pRS416 *TEF1*[pro]*-FLAG-PHO4-URA3*]

[a] The superscript 'pro' indicates promoter.
[b] R is a *Zygosaccharomyces rouxii* recombinase site that remains after intramolecular recombination.

tive competitive PCR (Fig. 1C, left). As can be seen in Fig. 1C (right), both SWI/SNF and Gcn5 are enriched over time at the endogenous *PHO5* promoter compared to the internal negative control locus, *pho5* ΔUASs, lacking both UASp1 and UASp2. The fold enrichments of *PHO5* compared to negative control sequences (normalized to the same ratio in the respective input sample) are shown in Fig. 1D. Similar P_i starvation-dependent enrichments for these coactivators compared to another negative control region in the *WHI4* ORF are also observed (data not shown). Gcn5 and SWI/SNF are significantly enriched at the wild-type *PHO5* promoter at 4 h of P_i starvation, consistent with an early role for both coactivators in chromatin remodeling and promoter activation. This is the first demonstration of the physical presence of Gcn5 and Swi2 (or any SWI/SNF subunit) at *PHO5*, since SWI/SNF recruitment has previously been detected only at the *PHO84* promoter (57). Since Ada2 was previously shown to associate with the *PHO5* promoter as a part of SAGA (4), it is likely that Gcn5 association is also occurring via SAGA. Maximal recruitment of both complexes requires many hours (≥12 h) of P_i deprivation, consistent with the delay in activation observed in *gcn5*Δ (4, 5, 42) or *swi2*Δ (42) strains.

Maximal association of Pho4 at *PHO5* requires many hours following P_i starvation. Since Pho4, the principal PHO transcriptional activator, recruits chromatin remodeling complexes via its acidic activation domain (4, 44), we examined the time course of Pho4 binding at the *PHO5* promoter following P_i starvation. A 3myc-Pho4 strain that is not defective in *PHO5* activation kinetics compared to the wild type was used. The time course of induction of rAPase activity in no-P_i medium is shown in Fig. 2A. As shown in Fig. 2B, ChIP analysis of internal aliquots of cells revealed that Pho4 binding is not detected before P_i starvation and is first significant (~2.3-fold enrichment) after 2 h in P_i-free medium. Additionally, as shown above for Gcn5 and SWI/SNF, many hours of P_i withdrawal are required for high levels of the activator to associate with the *PHO5* promoter. In accord with the recruitment paradigm, these results demonstrate that Pho4 binding precedes the recruitment of Gcn5 and Swi2. However, since transport of Pho4

from the nucleus to cytoplasm is complete by 1 h at very low or no P_i (5, 28), it is surprising that so many hours are required for Pho4 binding to plateau at the *PHO5* promoter.

***PHO5* activation requires chromatin remodelers at low Pho4 binding site occupancy.** Increased association of Pho4 with the *PHO5* promoter during activation is probably due in part to further occupancy of the low-affinity, histone-free UASp1 as Pho4 accumulates in the nucleus (57). Additional interaction of Pho4 with the high-affinity UASp2, which is essential for *PHO5* activation (63), is thought to require disruption of nucleosome −2 (60). In support of this, the absence of the activity of various remodeling enzymes leads to modest (57) or severe (45) decreases in Pho4 binding and promoter induction. In contrast, recent evidence suggests that Pho4 interacts with UASp2 in the absence of chromatin remodeling (1, 61).

Our ChIP results, showing that recruitment of Pho4, Gcn5, and Swi2 all peak at ≥12 h of *PHO5* induction, are most consistent with a model in which nucleosome −2 is disrupted over time to enable access of Pho4. Moreover, we hypothesized that the combined action of distinct classes of coactivator complexes might be required to achieve full induction at lower levels of Pho4 site occupancy when, on average, only UASp1 rather than the nucleosomal UASp2 is occupied. Conversely, high-level association of Pho4 and hence recruitment of coactivators might suppress the need for a particular chromatin-associated complex.

To test this hypothesis, we regulated the nuclear concentration of Pho4 to effect different steady-state levels of UAS occupancy at the *PHO5* promoter in wild-type, *gcn5*Δ, and *swi2*Δ cells. The nucleocytoplasmic distribution of Pho4 is controlled through its phosphorylation by the Pho80-Pho85 cyclin–cyclin-dependent kinase (28, 29, 31, 46). Under conditions of high P_i availability, phosphorylation blocks nuclear import and promotes nuclear export, leading to cytoplasmic localization of Pho4. When P_i is limiting, *PHO4* expression is not affected (34), Pho80-Pho85 activity is inhibited, and unphosphorylated Pho4 accumulates in the nucleus (35). Thus, the most physiologically relevant way to regulate nuclear levels of
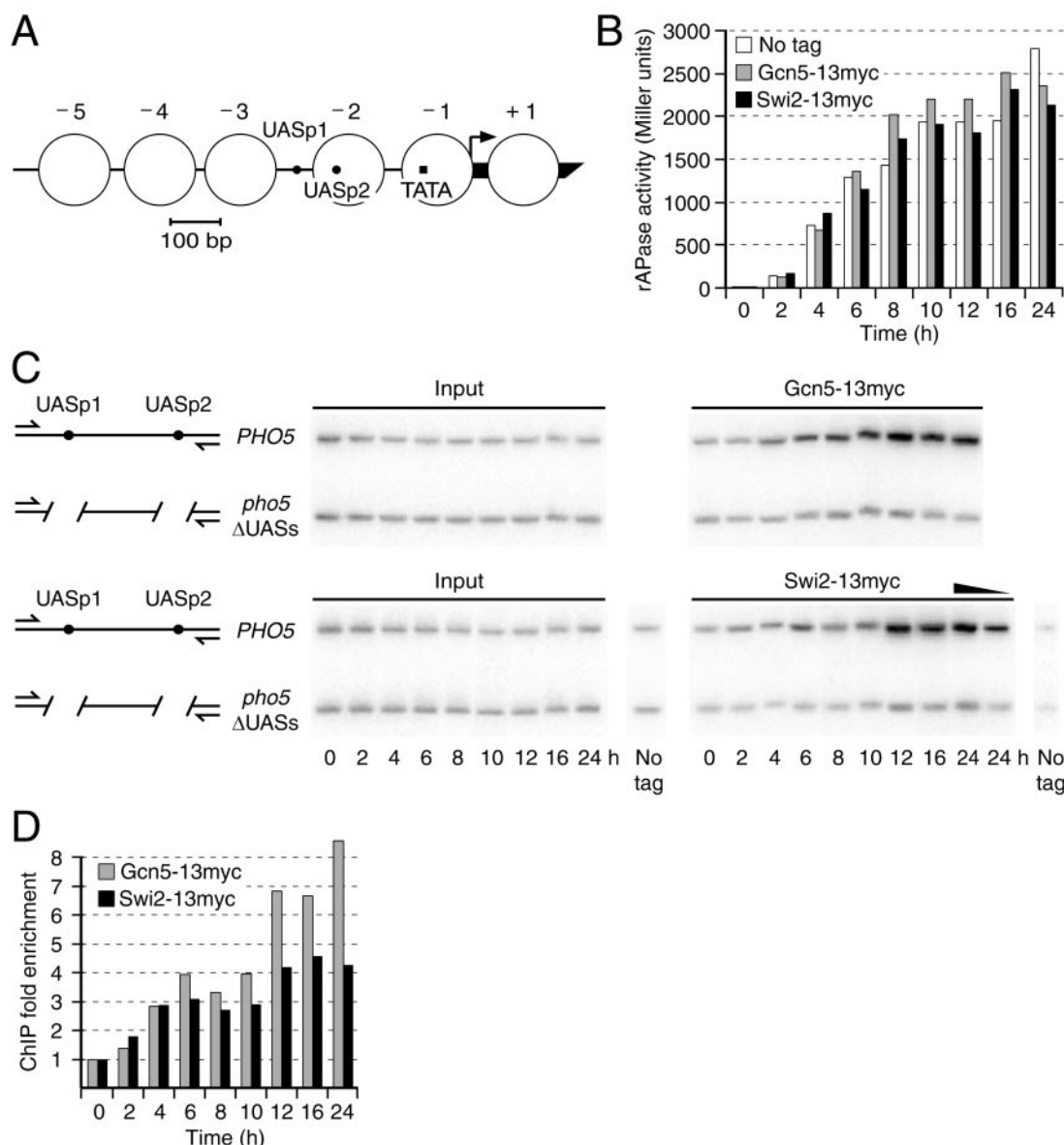
FIG. 1. SWI/SNF and Gcn5 associate with the *PHO5* promoter following P$_i$ starvation. (A) The *PHO5* promoter. Filled circles, UASp1 and UASp2; filled square, TATA element; large open circles, positioned nucleosomes −5 to +1 (3). (B) Time course of *PHO5* activation following transfer from high- to no-P$_i$ medium. Wild-type (No tag), Gcn5-13myc (ADY2459), and Swi2-13myc (ADY2461) cells were harvested at the indicated times and assayed for rAPase activity. (C) Internal aliquots of cells from the Gcn5- and Swi2-13myc cultures assayed in the experiment in panel B were subjected to ChIP analysis (see Materials and Methods) at the indicated times of induction. The presence of *PHO5* and negative control (*pho5* ΔUASs) sequences was analyzed by quantitative competitive PCR as depicted at left. The negative control promoter has deletions of both UASp1 and UASp2 (filled circles) and thus is unable to bind Pho4 (11). The gels show the radiolabeled PCR products amplified from either nonimmunoselected input DNA (middle; diluted 1:200) or DNA immunoselected with anti-myc antibody from formaldehyde-cross-linked chromatin (right) obtained from each strain at each time point. A wild-type strain (ADY2701) was starved of P$_i$ for 24 h and carried in parallel through all steps to serve as an untagged (No tag) specificity control. The PCR of the 24-h sample (below and at the right side of the ramp) included half as much immunoselected DNA compared to all other reactions and demonstrates amplification linearity. (D) The ratio of *PHO5* to negative control (*pho5* ΔUASs) product in each myc-tagged strain normalized to the same ratio in the input samples indicates the relative enrichment of each coactivator at the promoter. The data are representative of two independent experiments.

Pho4 is to grow cells in the presence of different concentrations of P$_i$ (62), leading to different degrees of Pho80-Pho85 activity and redistribution of Pho4 between the nucleus and cytoplasm. We first tested and found that the nuclear level of Pho4-GFP increased in a graded manner across the population of cells at successively lower concentrations of P$_i$ (Fig. 3). This excludes

the alternative scenario of an all-or-none binary response where the fraction of cells with nucleus-localized Pho4 increases as the concentration of P$_i$ is decreased.

Having shown that the nuclear concentration of Pho4 can be controlled in a graded manner, we examined *PHO5* expression and Pho4 binding site occupancy as a function of P$_i$ concen-
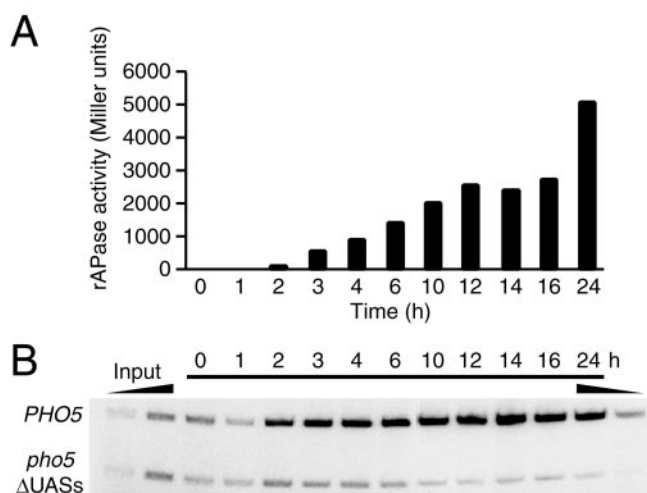
FIG. 2. Pho4 binding at *PHO5* increases for many hours after P_i starvation. After shifting strain ADY2727 (3myc-Pho4) from high- to no-P_i medium, cells were internally assayed at the indicated times for rAPase activity (A) and 3myc-Pho4 binding by ChIP analysis (B). The first two lanes contain nonimmunoselected (input) samples, whereas immunoselected DNA was assayed in all other lanes. Quantitative competitive PCR amplification of the endogenous *PHO5* and negative control (*pho5* ΔUASs) promoters was performed as in the experiment in Fig. 1C. The PCR amplifications analyzed in the first and last lanes contained five-fold less input DNA (taken from the 24-h culture and diluted 1:500) or immunoselected DNA, respectively. The data are representative of three independent experiments.
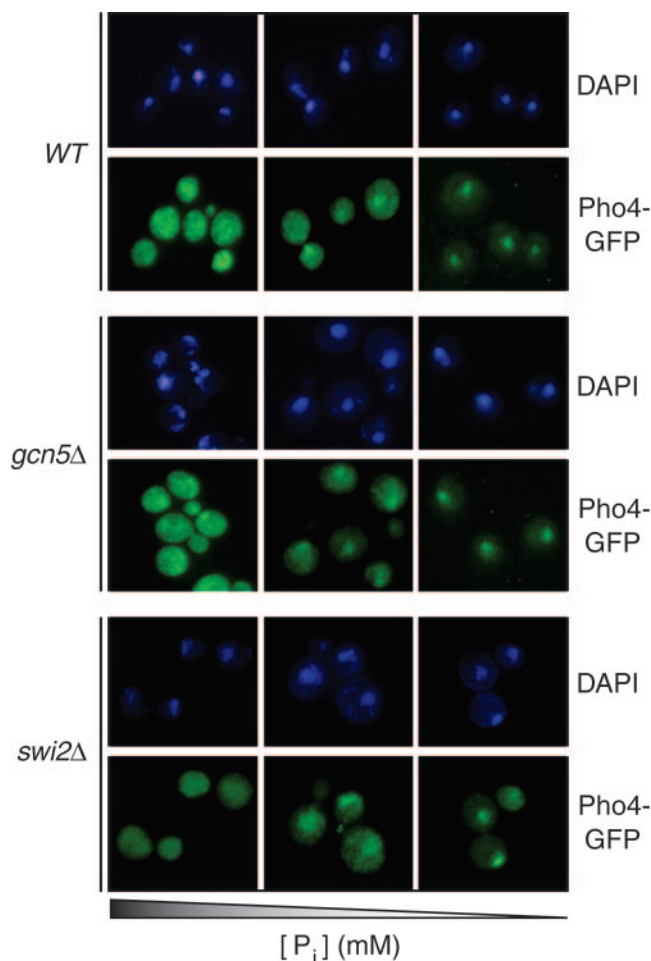

FIG. 3. The Pho4 nuclear concentration increases in a graded manner with the severity of P_i deprivation in the absence or presence of Gcn5 and SWI/SNF. Wild-type (*WT*; DNY2049), *gcn5*Δ (DNY2232), and *swi2*Δ (MRY2985) strains were grown for 12 h in defined minimal medium containing either 13:4 mM (left), 0.2 mM (middle), or 0 mM (right) P_i. The cells were washed with 1× PBS and either stained with DAPI to visualize nuclear DNA or visualized directly for Pho4-GFP fluorescence. Note the similar levels of fluorescence in wild-type, *gcn5*Δ, and *swi2*Δ cells and the increasing nuclear focus of Pho4-GFP as the P_i concentration decreases from left to right. The data are representative of three independent experiments.

tration in wild-type, *gcn5*Δ, and *swi2*Δ strains (Fig. 4). The cells were internally assayed for rAPase activity (and/or *PHO5* mRNA) and subjected to ChIP analysis after 12 h, when steady-state levels of Pho4 binding are achieved (Fig. 2B). At lower P_i availability (0.01 and 0 mM), when Pho4 is mainly nuclear, *PHO5* expression showed essentially no dependence on either Gcn5 or SWI/SNF. However, at higher concentrations of P_i ($\geq$0.2 mM), when the nuclear levels of Pho4 are relatively low, both mutant strains showed severely reduced rAPase activity compared to the wild type (Fig. 4A and C). Northern analysis of the *PHO5* transcript showed the same result as the assayed rAPase activities (Fig. 4E). ChIP analysis showed that, in the wild-type strain, Pho4 binding occurred at 0.25 mM P_i. By comparison, a lower P_i concentration (0.1 mM P_i), i.e., more nuclear Pho4, was needed for the activator to associate with *PHO5* at similar levels in the *gcn5*Δ and *swi2*Δ strains (Fig. 4B and D). There was also a clear delay in the kinetics of Pho4 binding at the *PHO5* promoter in *gcn5* and *swi2* mutants (data not shown).

Loss of Gcn5 and SWI/SNF could indirectly lead to a shift in the *PHO5* response to P_i deprivation by affecting Pho4 protein levels. This seemed unlikely, however, since there were no apparent differences in the fluorescent intensity of Pho4-GFP between wild-type, *gcn5*Δ, and *swi2*Δ strains (Fig. 3). Furthermore, the levels of rAPase activity were the same in wild-type and coactivator mutant cells at 0.01 mM and 0 mM P_i (Fig. 4A and C). Nevertheless, we directly determined by Western blotting that levels of a fully active FLAG-tagged version of Pho4 were unaffected in wild-type, *gcn5*Δ, and *swi2*Δ strains in medium containing or lacking P_i (Fig. 5). We conclude that the

degree of Pho4 binding site occupancy at *PHO5* is a crucial determinant of the promoter's need for the chromatin remodelers Gcn5 and SWI/SNF in activation, ranging from essentially complete dependence to independence at low and high levels of promoter occupancy, respectively. Moreover, Gcn5 and SWI/SNF are required for maximal association of Pho4 with the *PHO5* promoter at intermediate concentrations of P_i. This probably reflects the necessity for Gcn5 and SWI/SNF activity in exposing the high-affinity UASp2 in nucleosome -2 (see Discussion).

Gcn5 and SWI/SNF associate with additional strongly transcribed promoters. Previous microarray studies suggested that deletion of *GCN5* or *SWI2/SNF2* affects the transcript levels of less than 5% of yeast genes (25, 59). However, we have shown that both Gcn5 and SWI/SNF associate with the *PHO5* pro-
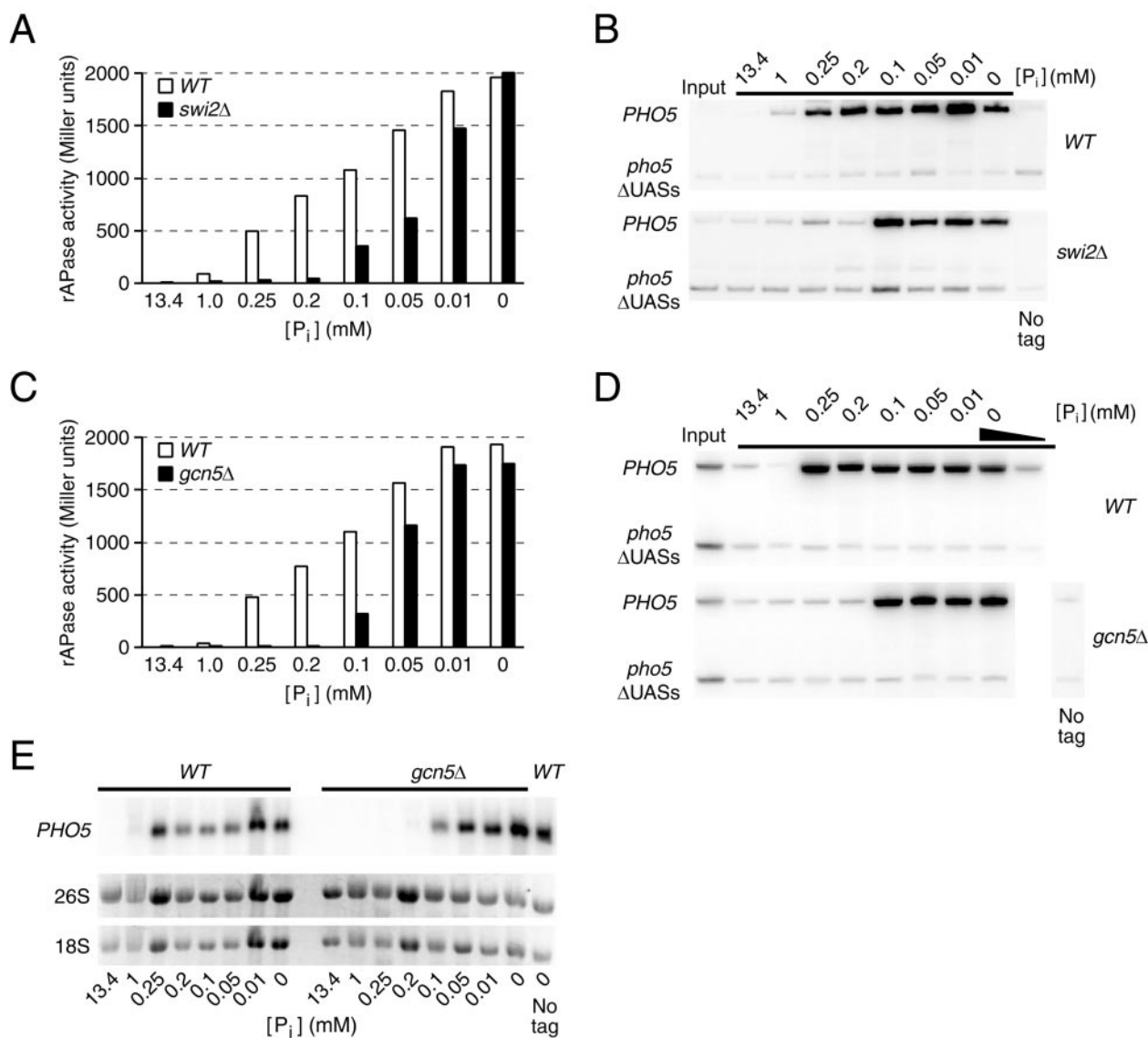
FIG. 4. *PHO5* activation is markedly dependent on Gcn5 and SWI/SNF at low nuclear concentrations of Pho4 and decreased binding site occupancy. Wild-type (*WT*, ADY2727), *swi2Δ* (ADY2695), and *gcn5Δ* (ADY2719) strains containing 3myc-Pho4 were grown 12 h in medium with the indicated concentrations of P_i and were internally assayed for rAPase activity (A and C), 3myc-Pho4 binding by ChIP (B and D), and *PHO5* mRNA by Northern blotting (E). In the ChIP analyses, the first lane contains nonimmunoselected 0 mM P_i sample (input), whereas immunoselected DNA was assayed in all other lanes. Quantitative competitive PCR amplification of the endogenous *PHO5* and negative control (*pho5* ΔUASs) promoters was done as for the experiment in Fig. 1C. The PCR sample analyzed in the last lane marked by the ramp in panel D contained half as much immunoselected DNA (from the 0 mM P_i culture) as did all other samples. The untagged (No tag) specificity controls were a wild-type strain (ADY2701) that was grown in 0 mM P_i for 12 h and carried in parallel through all experimental steps. The internal rAPase (A and C) and ChIP (B and D) assays using 3myc-Pho4 strains are very reproducible, and those shown are representative of three independent experiments. Identical rAPase activity results were obtained in two additional, independent experiments with a nontagged Pho4 strain. In one further experiment, relative to the wild type, single *gcn5Δ* and *swi2Δ* strains (each expressing a nontagged allele of *PHO4*) exhibited dramatic decreases in rAPase activity at ≥0.2 mM P_i after growth for 16 h.

moter under conditions of high transactivator binding when neither remodeler is required for transcriptional activity. Thus, we hypothesized that Gcn5 and SWI/SNF are also recruited to other highly transcribed promoters that do not require their activities for transcription. The *GAL1-10* promoter, for example, has an upstream regulatory region (UAS_G) that contains four binding sites (two high-affinity and two low-affinity sites) for the strong acidic activator Gal4. Although Gal4 is not an

abundant activator protein, high-level occupancy of UAS_G occurs due to DNA binding cooperativity (17). Previous studies have shown that Gal4 recruits Gcn5 as a component of the SAGA complex to UAS_G of *GAL1-10* (6, 33), but physical association of SWI/SNF has not been demonstrated. Deletion of genes coding for Gcn5 or SWI/SNF subunits only modestly affects *GAL1* expression (6, 14, 48, 59).

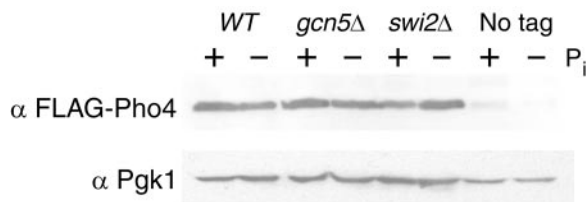As above, we performed ChIP with strains expressing Swi2-

FIG. 5. Deletion of *GCN5* or *SWI2/SNF2* does not affect Pho4 protein levels. Wild-type (*WT*; MRY3049), *gcn5*Δ (MRY3055), and *swi2*Δ (MRY3053) strains expressing *FLAG-PHO4* or a strain expressing untagged (No tag) *PHO4* (ADY3035) were grown for 12 h in the presence (+) or absence (−) of P_i before whole-cell extracts were isolated. Equivalent amounts of total protein were analyzed by immunoblotting and probing with anti-FLAG antibody (top). The blot was reprobed with monoclonal anti-yeast Pgk1 antibody (bottom) to provide a loading control.

13myc and Gcn5-13myc that, in addition to the normal genomic *GAL1-10* locus, also contain a negative control locus, *gal1-10* Δ*UAS*$_G$. This control comprises the entire *GAL1-10* promoter with UAS$_G$ (all four Gal4 sites) deleted, eliminating Gal4 binding and hence recruitment of chromatin modifiers and remodelers. Figure 6A demonstrates that both SWI/SNF and Gcn5 are recruited to the endogenous *GAL1-10 UAS*$_G$ in galactose medium when the promoter is transcriptionally active but not in repressive glucose medium. Strong recruitment of both coactivators lends further support to the results of studies suggesting that Gcn5 and SWI/SNF perform partially redundant functions at the *GAL1* promoter (7, 49, 51). Moreover, *GAL1* expression becomes strongly dependent on Gcn5 and SWI/SNF after deletion of the two high-affinity Gal4 sites of UAS$_G$ (16, 22, 39), consistent with our working model that high levels of activator binding establish functional redundancy.

We further hypothesized that promoters regulated by the abundant general regulatory factors Abf1 and Rap1, which strongly associate at their target genes (40), would thus recruit significant levels of multiple coactivators. In doing so, our model predicts that functional redundancy might be established, thereby alleviating the need for a single, specific coactivator. Strong Abf1 and Rap1 targets include the RNA polymerase II-transcribed promoters of genes coding for ribosomal proteins, additional aspects of protein synthesis, and glycolytic enzymes (36, 50). Rap1 target genes are generally transcribed at extremely high rates during growth in rich medium, averaging 45 mRNAs per h compared to 7 mRNAs per h for all yeast genes, accounting for an estimated 37% of total RNA polymerase II-derived transcripts (25).

To test this hypothesis, we used ChIP analysis to assay for the association of Gcn5 and SWI/SNF at representative Rap1 targets for which transcript levels are unaffected in *gcn5*Δ or *swi2*Δ strains (25, 59), including *RPL19B* (ribosomal protein), *PYK1* (glycolysis), and *EFT2* (protein synthesis). Consistent with our working model, in Fig. 6B, D, and E, each of the Rap1 target genes shows significant association with Gcn5 and SWI/SNF relative to the *gal1-10* Δ*UAS*$_G$ negative control, at which the coactivators were not detected (Fig. 6A). Both coactivators were also recruited to the *RPS22B* promoter (Fig. 6C), which is a target for Abf1 but not Rap1 (36, 50). This is consistent with studies showing that the activation domains of Abf1 and
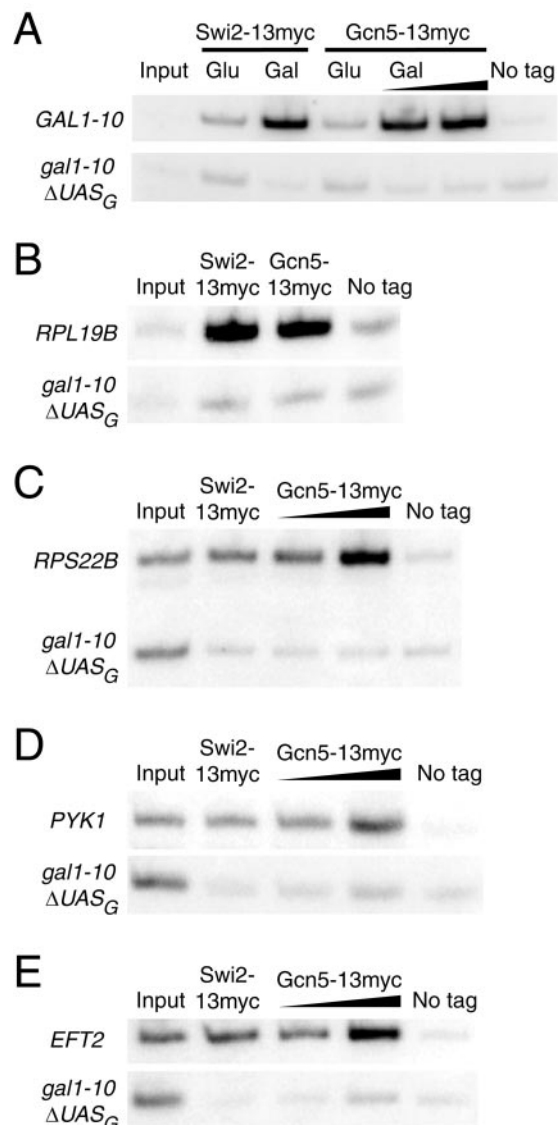

FIG. 6. SWI/SNF and Gcn5 are recruited to strongly transcribed promoters that do not require them for transcription. Swi2-13myc (ADY2923) and Gcn5-13myc (ADY2921) strains were grown in parallel in YPD, washed, and then resuspended in either YPD (glucose, Glu) or YPG (galactose, Gal) medium for another 6 h before ChIP analysis was performed. Nonimmunoselected (lane 1, input) and immunoselected (all other lanes) DNA was analyzed by PCR using primers for the *GAL1-10* (A), *RPL19B* (B), *RPS22B* (C), *PYK1* (D), and *EFT2* (E) promoters as well as a negative control, mutated promoter (*gal1-10* Δ*UAS*$_G$). (A) Quantitative competitive PCR analysis of *GAL1* using a single PCR primer pair amplifying both *GAL1* and the control locus was performed as for *PHO5* in Fig. 1C. Also, the input sample was taken from the YPG-grown Gcn5-13myc culture, and the untagged (No tag) control strain (ADY2915) was grown in YPG in parallel to the other strains. (B to E) Analysis of only the YPD-grown, Gcn5-13myc and Swi2-13myc samples is shown and the experimental and *gal1-10* Δ*UAS*$_G$ loci were assayed in the same PCR amplification by including two primer pairs, one specific for each locus. For simplicity, the low-level signal at the endogenous *GAL1-10* locus is not shown in panels B through E. Also, the input sample was taken from the YPD-grown Gcn5-13myc culture and the untagged (No tag) control strain was grown in YPD in parallel with the other strains.

Rap1 are interchangeable and that both factors can function with core promoters from nonribosomal genes (10, 12, 18). Thus, we conclude that Gcn5 and SWI/SNF associate with a variety of heavily transcribed promoters, despite the observation that they are not needed for expression (25, 59).

DISCUSSION

We found that the extent to which *PHO5* induction requires activity of the Gcn5 histone acetyltransferase and SWI/SNF remodeler is strongly related to the nuclear concentration of the acidic activator Pho4 and thus the levels of promoter-bound transactivator. Maximal Pho4 binding at *PHO5* requires P_i deprivation for $\geq$12 h (Fig. 2); the approximate time needed to overcome the kinetic delay in *PHO5* activation in single *gcn5* and *swi2/snf2* mutants (4, 5, 42). This suggests that each remodeling enzyme is needed primarily when Pho4 binding is limiting. Indeed, *PHO5* activation is markedly dependent on both Gcn5 and SWI/SNF at low steady-state promoter occupancy (Fig. 4). Conversely, high nuclear levels of Pho4 lead to marked increases in Pho4 binding and promoter activation in the absence of either Gcn5 or SWI/SNF. Our data suggest, therefore, that the necessity for a specific remodeler can be circumvented by driving promoter occupancy, thereby establishing functional redundancy through increased recruitment of coactivators that normally associate with the promoter. In support of this model, Gcn5 and SWI/SNF are strongly recruited to *PHO5* when Pho4 occupancy is high and to representative promoters that are robustly occupied by the abundant transactivators Abf1 or Rap1 (Fig. 6).

Association of Pho4 with *PHO5* UASp2 requires chromatin modifiers and remodelers. When yeast cells are deprived of P_i, Pho4 is imported into the nucleus and activates genes in the PHO cluster (35). It is thought that at the *PHO5* promoter, Pho4 first binds cooperatively with the homeodomain factor Pho2 to the accessible, low-affinity UASp1 (CACGTT) and then to the high-affinity UASp2 (CACGTG), located in nucleosome −2 in the repressed promoter (60). The absolute correlation of *PHO5* induction with chromatin disruption has led to the widely accepted view that binding of Pho4 to UASp2 requires remodeling of nucleosome −2. Consistent with this view, several chromatin modifiers and remodelers are recruited to *PHO5*, including SAGA (4) (Fig. 1), NuA4 (45), and INO80 com (57). We report for the first time that SWI/SNF is also brought directly to the activated *PHO5* promoter (Fig. 1).

Our results provide further evidence that efficient association of Pho4 with UASp2 requires chromatin remodeling. The resolution of the ChIP analysis precludes assignment of the relative amounts of Pho4 bound to UASp1 versus UASp2, since they are only 103 bp apart. However, our results in Fig. 4 are clearly consistent with initial, limited Pho4 binding at the nonnucleosomal low-affinity UASp1 followed by a large cooperative increase in binding on chromatin remodeling and exposure of the high-affinity UASp2, as we observed previously (11). The requirement for a higher nuclear concentration of Pho4 in *gcn5* and *swi2/snf2* mutants suggests that SAGA and SWI/SNF facilitate high-level binding of the activator to UASp2 in nucleosome −2 (Fig. 4) (57). Furthermore, in the absence of Esa1 histone acetyltransferase activity, *PHO5* chromatin remodeling and activation under P_i-free conditions is severely deficient and Pho4 binding achieves only about 10% of wild-type levels (45). This suggests that Pho4 binds approximately nine times better to the high-affinity UASp2 than to the low-affinity UASp1 in vivo, in good agreement with gel shift experiments using purified Pho4 protein (65). The need for Esa1 can be overcome only by Pho4 overexpression. By contrast, we and others find that the loss of *PHO5* induction in *gcn5* and *swi2/snf2* mutants can be fully suppressed by wild-type levels of Pho4 expression following many hours in P_i-free medium (4, 5, 16, 20, 42). Taken together, this suggests a greater need for acetylation by Esa1 in NuA4 than by Gcn5 in SAGA for *PHO5* induction (45). However, a striking finding of our work is that, even in the presence of a wild-type copy of *ESA1*, *PHO5* induction requires Gcn5 and SWI/SNF at intermediate P_i concentrations (0.2 to 0.25 mM [Fig. 4]). Thus, in the absence of efficient chromatin remodeling, increased activator concentration is again required to achieve high levels of activator binding.

High activator binding site occupancy confers functional redundancy for coactivators. Yeast genes have been classified into three major groups with respect to their need for Gcn5 and SWI/SNF, i.e., those requiring both, either, or neither activity, suggesting that the remodelers have overlapping but independent functions (7, 25, 59). Our results with the *PHO5* system show that increases in coactivator recruitment (Fig. 1) correlate well with the time-averaged level of activator binding or promoter occupancy (Fig. 2). We propose that when UASp1 is primarily occupied (rather than UASp2) at intermediate P_i concentrations, the activities of SAGA and SWI/SNF, and perhaps INO80 com and NuA4 (45, 57), are requisite for *PHO5* promoter induction. Thus, Gcn5 and SWI/SNF function is required at low levels of activator binding, which probably reflects natural conditions of P_i depletion in which P_i is not completely absent.

Increasing the nuclear level of Pho4 at successively lower concentrations of P_i (Fig. 3) is an effective means of mounting a physiological response of the appropriate magnitude (Fig. 4). Under extreme conditions of sustained growth in the absence of P_i, a robust level of activator binding drives the recruitment of multiple remodeling activities by simple chemical principles, ensuring chromatin disruption and increased transcription. In good agreement with this model, we have observed a strong correlation between the extents of promoter occupancy and chromatin disruption by using a galactose-regulated allele of *PHO4* (S. Hoose, A. Dhasarathy, W. Jessen, and M. P. Kladde, unpublished data). Moreover, loss of Gcn5 and SWI/SNF activity delays chromatin remodeling and activation of *PHO5* following P_i starvation (4, 5, 42). However, at higher levels of Pho4 binding, sufficient amounts of chromatin modifiers and remodelers are recruited to suppress the transcriptional defects of single *gcn5* and *swi/snf* mutants. In such cases, Gcn5 and SWI/SNF appear to be fully redundant; however, it is equally plausible that recruitment of Esa1 in NuA4 and the INO80 complex establish the functional redundancy at the induced *PHO5* promoter.

Possible global roles for coactivators. We show substantial recruitment of Gcn5 and Swi2/Snf2 to *PHO5*, *GAL1*, genes involved in protein synthesis (*RPL19B*, *RPS22B*, and *EFT2*), and a glycolytic promoter (*PYK1*) (Fig. 6), supporting the view that these coactivators play widespread roles in transcription

(7). Recruitment occurs despite observations that the transcript levels of each of these genes are unaffected or modestly decreased in *gcn5* or *swi2/snf2* mutants (14, 25, 59). Esa1 is also recruited to *PHO5* and ribosomal promoters (45, 50). It was previously inferred that the abundant ribosomal promoter activators Abf1 and Rap1 could recruit SAGA to the core promoters of natural and chimeric reporter genes (12). Our finding that Gcn5 directly associates with *RPL19B* and *RPS22B* supports this conclusion and shows that SWI/SNF is directly recruited as well.

Why might chromatin modifiers and remodelers be recruited to such strongly transcribed promoters? Robust recruitment and retention of coactivators may ensure that an active chromatin configuration is established at critical promoters following nascent chromatin deposition in S phase. Alternatively, increasing evidence suggests that chromatin remodelers are continuously required because there is a rapid, dynamic equilibrium between active and repressive chromatin structures (7, 8, 58). By our working model, the position of this equilibrium is set by the "recruitment potential" of bound upstream activators and thus the level of coactivator recruitment. Furthermore, we suggest that changes in activator or coactivator concentration, activator DNA binding domain/binding site affinity, and binding cooperativity modulate the transcriptional requirements for individual chromatin modifiers and remodelers at various promoters. Additionally, weakening an activation domain renders reporters with nonnucleosomal TATA elements or those with nucleosomal TATA elements more dependent on Gcn5 and SWI/SNF function (56), presumably due to a decreased ability to recruit coactivators. We propose that each of these factors must be evaluated to fully delimit the coactivator requirements of a given promoter.

ACKNOWLEDGMENTS

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ERRATUM

Promoter Occupancy Is a Major Determinant of Chromatin Remodeling Enzyme Requirements

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Volume 25, no. 7, p. 2698–2707, 2005. Page 2699, column 2: The lead-in to the first section in Results should read as follows. "SWI/SNF and Gcn5 physically associate with the induced *PHO5* promoter."