

# Chromatin remodelling at the *PHO8* promoter requires SWI–SNF and SAGA at a step subsequent to activator binding

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**The SWI–SNF and SAGA complexes possess ATP-dependent nucleosome remodelling activity and histone acetyltransferase (HAT) activity, respectively. Mutations that eliminate the ATPase activity of the SWI–SNF complex, or the HAT activity of SAGA, abolish proper chromatin remodelling at the *PHO8* promoter *in vivo*. These effects are mechanistically distinct, since the absence of SWI–SNF freezes chromatin in the repressed state, while the absence of Gcn5 permits a localized perturbation of chromatin structure immediately adjacent to the upstream trans-activator binding site. However, this remodelling is not propagated to the proximal promoter, and no activation is observed under all conditions. Furthermore, Pho4 is bound to the *PHO8* promoter in the absence of Snf2 or Gcn5, confirming a role for SWI–SNF and SAGA in chromatin remodelling independent of activator binding. These data provide new insights into the roles of the SWI–SNF and SAGA complexes in chromatin remodelling *in vivo*.**

**Keywords:** GCN5/gene regulation/histone acetylation/*PHO8*/SNF2

## Introduction

Chromatin structure plays a dynamic role in the regulation of transcription (Felsenfeld, 1996; Peterson, 1996; Wolffe, 1997; Gregory and Hörz, 1998). Nucleosomes can preclude transcription factor access and prevent the assembly of the transcription machinery on to the DNA (Paranjape *et al.*, 1994; Workman and Kingston, 1998). To orchestrate transcriptional activation within this repressive chromatin environment, the cell contains factors that are thought to bring about the opening of chromatin, thus assisting the function of transactivators and/or the general transcription machinery.

The highly conserved ATP-dependent SWI–SNF complex is an example of such a chromatin remodelling factor (see Peterson and Tamkun, 1995). This complex has been implicated in the transcriptional activation of a number of diversely regulated genes such as *INO1*, *SUC2* and *HO* (Winston and Carlson, 1992; Peterson and Tamkun, 1995). The phenotypes resulting from the elimination of components of the SWI–SNF complex can

be suppressed by histone mutations or alterations of histone dosage, formally connecting this complex with a chromatin-associated role (Hirschhorn *et al.*, 1992; Kruger *et al.*, 1995). Furthermore, *in vivo* chromatin analysis suggests that the chromatin alterations that occur on activation of the *SUC2* promoter are SWI–SNF dependent (Hirschhorn *et al.*, 1992; Gavin and Simpson, 1997; Wu and Winston, 1997).

In addition to remodelling machines, the cell also contains activities that can directly modify the histones themselves (Grunstein, 1997). Histones can be acetylated on their N-terminal tails, and this acetylation is broadly correlated with transcriptionally active DNA regions (Allfrey *et al.*, 1964). The yeast Gcn5 protein, originally identified as a transcriptional coactivator (Berger *et al.*, 1992; Georgakopoulos and Thireos, 1992; Marcus *et al.*, 1994), has been shown to possess histone acetyltransferase (HAT) activity (Brownell *et al.*, 1996), and participates in at least two large protein complexes (Ada and SAGA) (Grant *et al.*, 1997; Pollard and Peterson, 1997; Saleh *et al.*, 1998). The HAT activity of Gcn5 is required for its function in transcriptional regulation (Kuo *et al.*, 1996, 1998; Candau *et al.*, 1997; Wang *et al.*, 1998), and elegant *in vitro* experiments have shown that transactivator proteins can target these HAT complexes to chromatin templates, bringing about transcriptional activation in an acetyl-CoA-dependent manner (Utley *et al.*, 1998). Histone acetylation alters the dynamic properties of the nucleosome fibre creating a more accessible substrate for transactivators (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1996) and the general transcription machinery (Ura *et al.*, 1997). These effects are believed to be the result of reduced histone–DNA affinity (see Wade *et al.*, 1997; Tse *et al.*, 1998) and/or nucleosome–nucleosome interactions (Luger *et al.*, 1997). *In vivo*, absence of Gcn5 HAT activity has been shown to prevent the complete remodelling of chromatin at the yeast *PHO5* promoter under conditions of sub-maximal activation (Gregory *et al.*, 1998b). Thus, the SAGA and/or Ada complexes directly affect the remodelling of chromatin.

To investigate the roles played by SWI–SNF and SAGA in the regulation of transcription through chromatin modulation *in vivo*, we have extended our study to the yeast *PHO8* promoter, which undergoes pronounced chromatin remodelling on activation, which is distinct, however, from that observed at *PHO5* (Barbaric *et al.*, 1992). This gene encodes a phosphate repressible alkaline phosphatase (Kaneko *et al.*, 1985, 1987) that is regulated through Pho4, and the same signal transduction system as the *PHO5* promoter (Vogel and Hinnen, 1990). Under repressing conditions the *PHO8* promoter is organized into an array of nucleosomes that are remodelled upon activation (Barbaric *et al.*, 1992). However, there are important differences to the *PHO5* promoter: (i) the Pho4-

dependent chromatin remodelling observed at *PHO8* is partial even under fully activating conditions; (ii) this remodelling is Pho2 independent (Barbaric *et al.*, 1992); and (iii) while two UAS elements cooperatively activate the *PHO5* promoter (Barbaric *et al.*, 1996, 1998), a single high affinity Pho4 binding site (UASp2) located within a hypersensitive site is sufficient for activation and chromatin remodelling (M.Münsterkötter, S.Barbaric and W.Hörz, manuscript in preparation). The co-ordinate regulation of two different promoters by the same signal transduction pathway provides a unique opportunity to study in parallel their individual requirements for chromatin modulating complexes.

Here we show that absence of SWI–SNF components or mutation of the ATPase activity of the SWI–SNF complex results in the complete loss of chromatin remodelling at the *PHO8* promoter under inducing conditions. Our experiments also demonstrate the requirement for members of the SAGA complex (Gcn5, Spt3 and Spt7) for transcriptional activation of the *PHO8* promoter. This transcriptional effect is linked to repressive chromatin at the *PHO8* promoter, as deletion of the HAT Gcn5 results in severely impaired chromatin modulation even under maximal induction. This effect appears to be a direct result of the loss of HAT activity, since mutations that abolish Gcn5 HAT activity result in the same defect in activation and chromatin remodelling. Although mutations of the SWI–SNF and SAGA complexes both result in the failure to remodel *PHO8* promoter chromatin properly, the effects of mutations in these complexes are distinct. The absence of SWI–SNF function results in the complete loss of remodelling, whereas the absence of Gcn5 permits only a partial and localized perturbation of the chromatin upstream of the proximal promoter. Furthermore, *in vivo* dimethyl sulfate (DMS) footprinting demonstrates Pho4 DNA binding to the essential UASp2 element of the *PHO8* promoter in both  $\Delta gcn5$  and  $\Delta snf2$  strains. Thus, SAGA and SWI–SNF are shown to act differently but are both necessary for chromatin remodelling at the *PHO8* promoter *in vivo*.

## Results

### **Histone H4 depletion derepresses the *PHO8* promoter**

Activation of the yeast *PHO8* gene is accompanied *in vivo* by the remodelling of chromatin across the promoter (Barbaric *et al.*, 1992). By employing a yeast strain in which the sole copy of histone H4 is under *GAL* promoter control, nucleosome depletion can be achieved by the addition of glucose (Han and Grunstein, 1988; Han *et al.*, 1988). This leads to the activation of a range of genes, notably *PHO5* and *HIS3*, a result attributed to the relief of the repressive chromatin structure across the proximal promoter of these genes. In the analogous experiments with *PHO8*, nucleosome loss under otherwise repressing conditions derepresses this promoter, generating 47 U of  $\beta$ -galactosidase activity compared with the wild-type induced level of 105 U (uninduced level 9 U). This activity was independent of the presence or absence of the UAS elements normally responsible for induction. In comparison with the *PHO5* promoter, which reaches only ~15% of the fully induced level under the same conditions

(Han *et al.*, 1988 and data not shown), this demonstrates the relative importance of histones in the repression of the *PHO8* promoter, and highlights the functional role of chromatin remodelling at this locus.

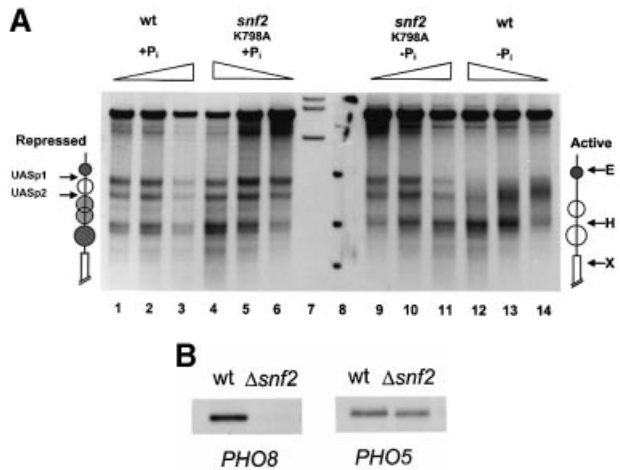
### **SWI–SNF ATPase activity is required for chromatin remodelling at the *PHO8* promoter *in vivo***

To determine whether chromatin remodelling of the *PHO8* promoter requires the SWI–SNF complex, a  $\Delta snf2$  strain was shifted to phosphate starvation medium and the chromatin structure resolved by DNase I analysis. Despite the fact that these were maximally inducing conditions, the *PHO8* promoter failed to undergo any detectable perturbation of the chromatin structure in the absence of Snf2 (data not shown). An identical result was obtained with a strain mutated for a second component of the complex, Swi1, confirming that this effect is specific to the SWI–SNF complex (data not shown).

To ascertain whether the need for SWI–SNF was attributable to the ATP-dependent chromatin remodelling activity observed *in vitro*, an *SNF2* mutant strain (K798A) was employed (a generous gift from C.L.Peterson). A normal SWI–SNF complex is formed in strains expressing the mutant protein. However, it is unable to hydrolyse ATP (Laurent *et al.*, 1993; Côté *et al.*, 1994; Richmond and Peterson, 1996). Furthermore, this mutation impairs transcription of the *GAL*, *HO* and *SUC2* genes to the same extent as the deletion of individual components of the complex (Richmond and Peterson, 1996).

The chromatin structure of the *PHO8* promoter in the ATPase defective mutant (*snf2*K798A) was determined by DNase I analysis under both repressed (+P<sub>i</sub>) and inducing (–P<sub>i</sub>) conditions (Figure 1). The result was the same as for the  $\Delta snf2$  strain, i.e. except for some very subtle changes in relative band intensities, chromatin at this promoter is locked in the inactive configuration characteristic of the repressed promoter (compare *snf2*K798A –P<sub>i</sub> with both wt +P<sub>i</sub> and wt –P<sub>i</sub>). For example, the unshaded nucleosome flanked on each side by Pho4 UAS elements (Figure 1A, schematic on the left) persists on induction in the absence of Snf2 ATPase activity (lanes 9–11), whereas in the wild type the protection due to this nucleosome is completely lost (lanes 12–14). Taken together, these results show that chromatin remodelling of the *PHO8* promoter is dependent upon the SWI–SNF complex and that the ATP-dependent functions of this complex are stringently required to achieve remodelling *in vivo*.

We next wished to correlate this dramatic effect on chromatin opening with transcription. *LacZ*-based reporter plasmids have been employed successfully to monitor both *PHO5* and *PHO8* promoter activity in a number of mutant and wild-type backgrounds (Barbaric *et al.*, 1998 and this study). However, in a  $\Delta snf2$  background, such reporter plasmids were found to reflect *PHO8* promoter strength inaccurately. We therefore measured directly mRNA levels from phosphate-starved wild-type and  $\Delta snf2$  cells and probed for both *PHO8*- and *PHO5*-specific transcripts. The results of this analysis are shown in Figure 1B. As previously observed by measuring acid phosphatase (Gaudreau *et al.*, 1997), the absence of Snf2 has little effect upon the level of *PHO5* expression. However, when these samples were probed for *PHO8*



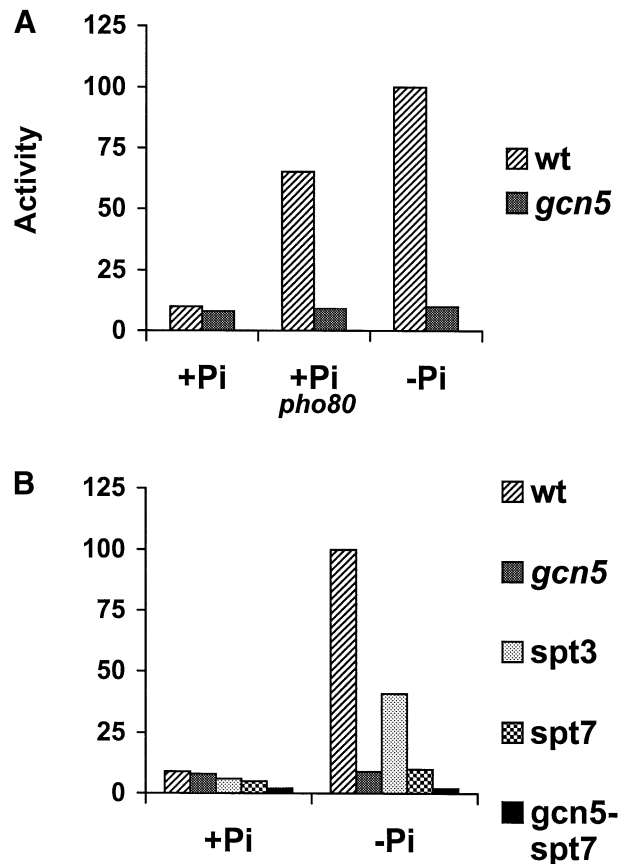
**Fig. 1.** SWI-SNF ATPase activity is required for chromatin opening at the *PHO8* promoter *in vivo*. (A) Nuclei isolated from a wt (CY396) or an *snf2*K798A (CY397) strain grown in the presence or absence of phosphate were treated with 0.25 (lanes 1, 6, 9 and 14), 0.5 (lanes 2, 5, 10 and 13) and 1 (lanes 3, 4, 11 and 12) U/ml of DNase I for 20 min at 37°C. DNA was isolated, digested with *Bgl*II, analysed on a 1.5% agarose gel, blotted and hybridized with the *Pvu*II-*Xho*I fragment (Barbaric *et al.*, 1992). Lane 8 contains restriction nuclease double digests of purified genomic DNA with *Bgl*II and either *Eco*RV (E), *Hind*III (H) or *Xho*I (X), which serve as markers. Lane 7 is a marker lane for rehybridization with an alternative probe for a different locus. Schematic representations of the repressed and active promoters with respect to the position of the UAS elements and marker fragments are shown to the right and left of the figure, respectively. See Figure 4C for nucleosome numbering and shading. (B) Northern analysis of total mRNA isolated under phosphate starvation conditions from the strains indicated. The left panel shows the signal for *PHO8*, and the right panel shows *PHO5* as control. Phosphoimager analysis of the *PHO8* panel quantifies the  $\Delta snf2$  mRNA signal as <15% of wild type (data not shown).

mRNA, the mutant strain showed a greatly reduced signal that was close to the transcript level at high phosphate (not shown). These results demonstrate the functional relevance of the chromatin transition at the *PHO8* promoter (Figure 1A), and indicate a crucial role for the SWI-SNF complex in the remodelling process.

#### Gcn5-SAGA dependence of the *PHO8* promoter

The dependence of the *PHO8* promoter on the HAT Gcn5 and on other components of the SAGA complex was determined by assaying the *PHO8* promoter in various genetic backgrounds. As shown in Figure 2A, this promoter, unlike *PHO5*, demonstrates an absolute dependence upon Gcn5 not only under sub-maximal activation conditions ( $\Delta pho80$ , +P<sub>i</sub>) as is the case for *PHO5* (Gregory *et al.*, 1998b), but also under maximally inducing (-P<sub>i</sub>) conditions, with the activity of the  $\Delta gcn5$  strain close to the repressed values. As previously observed for *PHO5*, there is also a small but reproducible effect on the basal activity of the promoter.

This study was extended to include two further components of the SAGA complex, Spt3 and Spt7 (Figure 2B). While these proteins are present in the SAGA complex along with Gcn5 they possess no intrinsic HAT activity (Grant *et al.*, 1997). The results shown indicate a strong influence of the loss of Spt7, and an appreciable effect of the absence of Spt3 on *PHO8* expression under induced conditions (-P<sub>i</sub>). Additionally, a slight reduction in basal promoter activity at high phosphate (+P<sub>i</sub>) is



**Fig. 2.** Requirements for Gcn5 and SAGA at the *PHO8* promoter. (A) The effect of Gcn5 on promoter activity under repressing (+P<sub>i</sub>), sub-maximally inducing (+P<sub>i</sub>,  $\Delta pho80$ ) and fully activated conditions (-P<sub>i</sub>). (B) Effects of mutations in the SAGA complex on *PHO8* promoter activity.  $\beta$ -galactosidase activity data are shown for the activation of *lacZ* reporter plasmids driven by the *PHO8* promoter, under repressing (+P<sub>i</sub>) or inducing (-P<sub>i</sub>) conditions for the strains indicated. Strains in (A) are YS18 (wt), YS31 ( $\Delta pho80$ ), YS5189 ( $\Delta gcn5$ ) and YS5319 ( $\Delta gcn5/\Delta pho80$ ). Strains employed in (B) are wt (FY86),  $\Delta gcn5$  (YS5189),  $\Delta spt3$  (FY294),  $\Delta spt7$  (FY963) and  $\Delta spt3/\Delta gcn5$  (FY1441). The values are the average of at least three independent measurements.

observed. Under phosphate starvation conditions (-P<sub>i</sub>), the  $\Delta spt7$  strain has an effect of similar severity to the loss of Gcn5, consistent with the loss of integrity of the SAGA complex in the absence of this protein (Grant *et al.*, 1997), while the double disruption (Spt7/Gcn5) renders the promoter transcriptionally silent. Interestingly, the finding that the absence of Spt3 has a less severe effect is consistent with the persistence of SAGA HAT activity in this background (Sterner *et al.*, 1999). Taken together, these data support a pivotal role for SAGA in the activation of the *PHO8* promoter *in vivo*.

#### Gcn5 HAT activity is required for the activation of the promoter

To address the question of whether the requirement for Gcn5 and SAGA is due to its intrinsic histone modification activity, activation of the *PHO8* promoter was studied in strains containing mutants of Gcn5 that abolish the catalytic HAT activity of the protein (Gregory *et al.*, 1998b; Wang *et al.*, 1998). These mutants are only defective in HAT function and behave as wild type with respect to formation of the SAGA complex. The results

**Table I.** Activation of the *PHO8* promoter is dependent on Gcn5 HAT activity

Mutant	HAT activity (%)	Promoter ( $\beta$ -gal) activity	
		High $P_i$	No $P_i$
yGCN5	100	12	98
$\Delta$ gcn5	22	9	12
KQL	23	11	9
PKM	19	7	9
PKE	114	9	101
YIA	62	5	30
YDR	100	11	104

The mutations and resulting strains have been described previously (Gregory *et al.*, 1998b; Wang *et al.*, 1998).

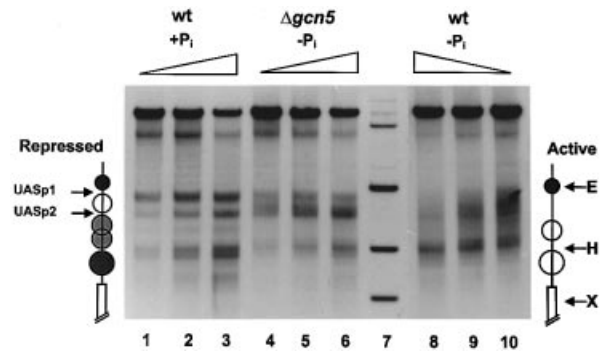
for this experiment are shown in Table I. Mutants YDR and PKE, which show 100% catalytic HAT activity, support wild-type levels of induction, whereas KQL and PKM, which possess only a residual HAT activity equivalent to  $\Delta$ gcn5, display absolutely no increase in promoter activity under fully activating conditions. Furthermore, the YIA mutant, which is intermediate in HAT activity, is also intermediate in promoter activity. These results provide a strong correlation between the histone acetylation activity of Gcn5 and the ability to activate transcription at the *PHO8* promoter.

#### **Absence of Gcn5 activity severely compromises nucleosome remodelling of the *PHO8* promoter**

The absence of promoter activation in strains deleted for *GCN5* or *SPT7* implicates the SAGA complex in the induction of the *PHO8* promoter. Furthermore, the identical phenotype obtained in strains expressing Gcn5 proteins defective in their catalytic HAT activity suggests that this effect is mediated directly through the acetylation function of this complex. To reveal the extent of chromatin opening in the absence of Gcn5, nuclei were isolated from wt and  $\Delta$ gcn5 cells under induced conditions, and treated with DNase I to determine the nucleosomal organization of the promoter. The results of this assay are shown in Figure 3. The absence of Gcn5 strongly impairs chromatin opening of the *PHO8* promoter (compare  $\Delta$ gcn5  $-P_i$  with wt  $+P_i$  and  $-P_i$ ). Under repressing conditions, the chromatin patterns of the wt and the  $\Delta$ gcn5 strain are indistinguishable (data not shown). However, only partial remodelling occurs in the  $\Delta$ gcn5 strain under activating conditions, as described in more detail in the following section. In addition, the HAT mutants KQL and PKM, which were shown to prevent the transcriptional activation of the *PHO8* promoter (Table I), also result in a similar defect in the remodelling of the *PHO8* promoter chromatin structure (data not shown). These results are in accord with the effects of these mutants on transcription and chromatin remodelling of the *PHO5* promoter (Gregory *et al.*, 1998b). Thus, loss of Gcn5 HAT activity is sufficient to impede complete chromatin remodelling of the *PHO8* promoter.

#### **Differential effects of SWI-SNF and Gcn5 on chromatin remodelling in vivo**

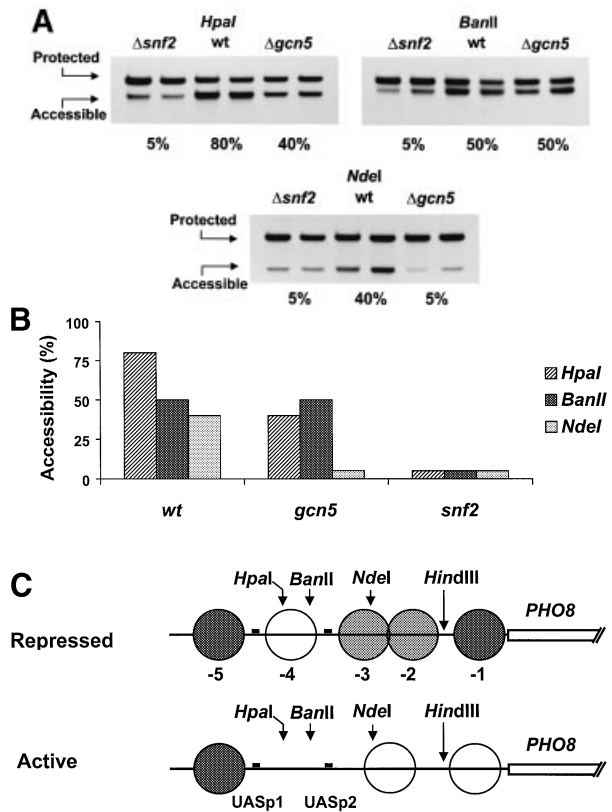
Although the opening of chromatin is severely perturbed in both *snf2* and *gcn5* mutant strains, their effects are



**Fig. 3.** Gcn5 is required for chromatin opening of the *PHO8* promoter *in vivo*. Nuclei isolated from wt (CY337) or  $\Delta$ gcn5 (CY53379) strains grown in the presence or absence of phosphate were treated with 0.25 (lanes 1, 4 and 10), 0.5 (lanes 2, 5 and 9) and 1 (lanes 3, 6 and 8) U/ml of DNase I for 20 min at 37°C. DNA was isolated and treated as in Figure 1. Lane 7 contains restriction nuclease double digests of purified genomic DNA with *Bgl*III and either *Eco*RV (E), *Hind*III (H) or *Xho*I (X), which serve as markers. Schematic representations of the repressed and active promoters with respect to the position of the UAS elements and marker fragments are shown to the right and left of the figure, respectively. See Figure 4C for nucleosome numbering and shading.

not identical. As can be observed by comparison of the DNase I analyses for these mutants, the chromatin structure of the *snf2*K798A strain (Figure 1, lanes 9–11) is practically indistinguishable from the wild-type repressed pattern (lanes 1–3), while the  $\Delta$ gcn5 structure (Figure 3, lanes 4–6) shows broadening of the DNase I hypersensitivity in the region of UASp2, suggesting a localized chromatin perturbation. This remodelling does not, however, lead to activation as the promoter is transcriptionally inactive in the absence of Gcn5 (see Figure 2A and B).

To define further the effects of these mutations on the remodelling process, a restriction enzyme analysis of the promoter was performed. The results for this assay are shown in Figure 4A and B, while Figure 4C shows a schematic of the promoter nucleosomal organization under both repressed and active conditions in relation to the positions of the restriction sites analysed. The *Ban*II site, which is some 40 bp upstream of the UASp2 element and contained within nucleosome –4, displays low accessibility in the  $\Delta$ snf2 strain (5%), whereas in both the wt and  $\Delta$ gcn5 strains this site shows >50% accessibility. However, the *Hpa*I site located at the centre of nucleosome –4, which is also poorly accessible (5%) in the  $\Delta$ snf2 strain, is only some 30–40% accessible in the  $\Delta$ gcn5 strain in comparison with the wt strain (70%). These results are consistent with partial remodelling of nucleosome –4 in the absence of Gcn5 and are in agreement with the DNase I analysis that shows an increase in DNase I sensitivity immediately adjacent to the UASp2 element but persistent protection further upstream (Figure 3). Importantly, this increase in accessibility is not propagated downstream towards the core promoter, since the *Nde*I site located within the –3 nucleosome demonstrates only 5% accessibility in both the  $\Delta$ gcn5 and  $\Delta$ snf2 strains (compared with 40% for the wild type), demonstrating the localized nature of the perturbation in the absence of Gcn5. It should be stressed that this site never reaches >50% accessibility under inducing conditions in a wild-type strain, consistent with the incomplete remodelling characteristic of the *PHO8*

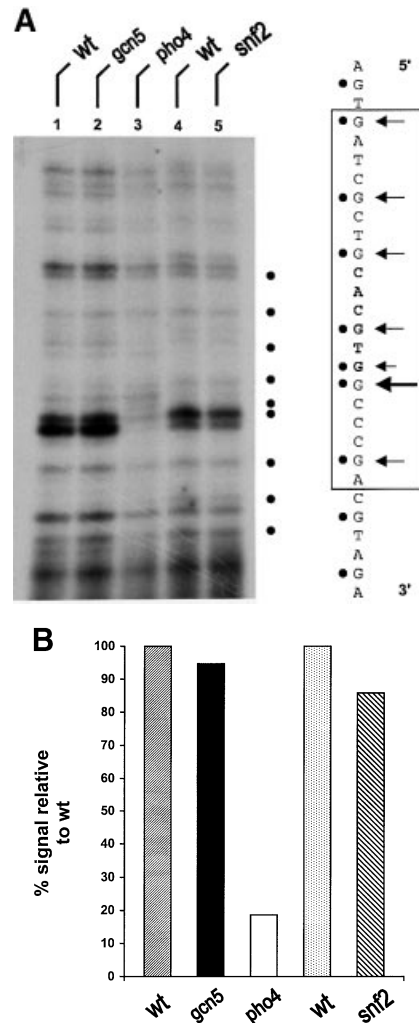


**Fig. 4.** Differential effects of SWI-SNF and SAGA on chromatin remodelling at the *PHO8* promoter. (A) Nuclei isolated from the strains indicated above, grown in the absence of phosphate, were treated with 50 U (left) or 200 U (right) of the restriction enzyme indicated, and incubated for 30 min at 37°C. In order to monitor the extent of cleavage of *HpaI* and *NdeI*, DNA was isolated, cleaved with *BglIII* and *EcoRV*, separated on a 1.5% agarose gel, blotted and hybridized with the *PvuII-XhoI* probe (Barbaric *et al.*, 1992). For *BanII* the isolated DNA was cleaved with *SacI* and *XhoI* and hybridized with probe D (Barbaric *et al.*, 1992). In all cases, the appearance of the lower band represents the cleaved product. (B) A graphical representation of the data shown in (A). (C) A schematic representation of the positions of the restriction sites with respect to the nucleosomal organization of the repressed and active *PHO8* promoter is shown. Stable nucleosomes (solid circles), partially stable (shaded circles) and unstable nucleosomes (open circles) are indicated (Barbaric *et al.*, 1992).

promoter (Barbaric *et al.*, 1992). This overall reduction in accessibility in the mutant strains is not due to the randomization of nucleosomes as previously observed at the *PHO5* promoter, since the *HindIII* site, which is constitutively accessible, remains equally cleavable in the  $\Delta gcn5$  and  $\Delta snf2$  strains, excluding the possibility that all nucleosomes have lost their positioning (data not shown). Furthermore, the DNase I patterns obtained do not support the general loss of nucleosomal organization (Figure 3) but suggest a structure very similar to native closed chromatin. Thus, loss of *Gcn5* permits a greatly reduced level of chromatin remodelling, which is restricted to the region directly flanking the *UASp2* element, while the absence of *Snf2* abolishes chromatin remodelling at this promoter altogether.

#### SWI-SNF and SAGA act at a point after transactivator binding

To begin to determine the mechanistic requirements for the SWI-SNF and SAGA complexes at the *PHO8*



**Fig. 5.** *Pho4* can bind to *PHO8* *UASp2* *in vivo* in the absence of *Gcn5* or *Snf2*. (A) wt strains (lanes 1 and 4) or strains lacking *Pho4* (lane 3), *Gcn5* (lane 2) or *Snf2* (lane 5) were grown in the absence of phosphate, treated with DMS and analysed with a *PHO8* *UASp2*-specific primer as described previously (Gregory *et al.*, 1998a). The positions of the guanine residues within the *UASp2* element are marked with dots next to the gel and correspondingly on the sequence in which the *in vitro* DNase I footprint of *Pho4* is boxed (Barbaric *et al.*, 1992). The thick arrow indicates a guanine residue that becomes hypersensitive to DMS treatment upon *Pho4* binding, observed as an enhancement of the signal from this residue. The short arrow indicates a protected guanine, while the medium size arrows indicate residues whose reactivity with DMS is unaffected by *Pho4* binding. All strains are additionally  $\Delta cpf1$  to eliminate binding of *Cpf1* to this site. (B) Quantification of *Pho4* occupancy at the *PHO8* promoter. Values are shown for the guanine that becomes hypersensitive to methylation on *Pho4* binding and are relative to the respective wt strain (100%). They have been corrected for background signals and loading differences by comparison with a region of each lane unaffected by *Pho4* occupancy. Results shown are the average of two independent experiments. Identical results were obtained for the lower band, which also increases in intensity upon *Pho4* binding (data not shown).

promoter, the ability of *Pho4* to bind to the essential *UASp2* element of the promoter was assayed by *in vivo* DMS footprinting. The methylation patterns obtained by this analysis are shown in Figure 5A. First, by comparing a *pho4* strain (Figure 5A, lane 3) with the wt strain (lanes 1 and 4) one can identify a pair of guanine residues that are altered in their sensitivity to DMS. The upper band is weakly protected while the lower becomes strongly

hypersensitive to DMS on Pho4 binding, resulting in a strongly enhanced signal from this residue (and also an apparent signal from the cytosine residue following). The methylation pattern observed in the absence of Gcn5 (Figure 5A, lane 2) also shows the characteristic protection and hypermethylation of this pair of guanine residues in comparison with the *pho4* strain (lane 3), and there is little difference between the absence and presence of Gcn5 (compare lanes 1 and 2). The methylation pattern obtained in the absence of Snf2 (Figure 5A, lane 5) is almost identical to that observed in the wild type (lane 4); both show the characteristic modifications associated with Pho4 binding. Quantitative phosphoimager analysis of the bands that become hypersensitive upon Pho4 binding is shown in Figure 5B. The values for the *gcn5* and *snf2* strains range from 83 to 95% of those obtained for the wild type. This demonstrates that the binding of Pho4 to *PHO8* UASp2 *in vivo* is not significantly affected by Snf2 or Gcn5.

## Discussion

### **SWI–SNF-dependent chromatin remodelling *in vivo***

The SWI–SNF complex is a highly conserved protein complex that has been proposed to function in transcription by overcoming the repressive nature of chromatin (Winston and Carlson, 1992). This premise is supported by a wealth of *in vitro* data documenting the ATP-dependent chromatin remodelling functions of the complex (Laurent *et al.*, 1993; Peterson and Tamkun, 1995; Richmond and Peterson, 1996) as well as *in vivo* data that link SWI–SNF functions to chromatin components (Peterson and Tamkun, 1995; Kingston *et al.*, 1996; Peterson, 1996; Gavin and Simpson, 1997). However, direct demonstrations of the effect of SWI–SNF on the ability to remodel promoter chromatin structure *in vivo* are limited to a single promoter (Hirschhorn *et al.*, 1992; Wu and Winston, 1997). The chromatin remodelling observed at the *PHO8* promoter under inducing conditions is dependent upon the ATPase activity of the SWI–SNF complex (Figure 1A). In contrast, the *PHO5* promoter is SWI–SNF independent (Figure 1B and Gaudreau *et al.*, 1997), although under the control of the identical genes in the signal transduction network. This serves to exclude the possibility of indirect effects upon the phosphatase system, and current experiments are focused upon resolving the factors that determine the contrasting behaviour of these promoters.

### **SAGA-dependent chromatin perturbation**

The loss of SAGA subunits (Gcn5 and Spt7) eliminates transcriptional activation of the *PHO8* promoter (Figure 2). Furthermore, this effect appears to be mediated through chromatin, since deletion of either *GCN5* itself or mutation of residues critical for its HAT activity are equally able to interfere with complete chromatin opening (Table I, Figure 3 and data not shown). Thus, histone acetylation directly affects the ability to remodel chromatin *in vivo*. Importantly, remodelling of chromatin is not abolished completely in *gcn5* mutants, instead these strains demonstrate an inability to extend a restricted local perturbation of the chromatin adjacent to the UASp2 element into the remodelling of the entire promoter (Figures 3 and 4).

Although the formal possibility remains that histones are not the relevant substrate for this effect (Gu and Roeder, 1997; Imhof *et al.*, 1997), transcription-linked, Gcn5-dependent histone acetylation has been demonstrated *in vivo* (Kuo *et al.*, 1996, 1998). Moreover, the fact that under identical conditions Pho4 can successfully remodel and activate the *PHO5* promoter again argues against an indirect effect on the activator and the signal transduction system.

### **Mechanistically distinct roles for the SWI–SNF and SAGA complexes**

The data presented here define an essential function for both the histone acetylation activity of the SAGA complex and the ATP-dependent nucleosome remodelling activity of the SWI–SNF complex in the remodelling of chromatin at the native *PHO8* promoter. Furthermore, the fact that mutations in these complexes result in quite different effects on chromatin remodelling suggests mechanistically distinct roles for SWI–SNF and SAGA in overcoming a repressive chromatin structure, a result consistent with the different catalytic activities of the two complexes.

Interestingly, our *in vivo* DMS footprinting analyses demonstrate that Pho4 is able to access and bind to the *PHO8* UASp2 element to a comparable extent irrespective of the presence or absence of Gcn5 or Snf2 (Figure 5A and B). These data rule out the possibility that the effect of Gcn5 and Snf2 at the *PHO8* promoter is indirect via Pho4 expression and/or nuclear localization and/or covalent modification (Komeili and O'Shea, 1999). Also, the genome-wide analysis conducted in the laboratory of R.Young, which showed that the mRNA levels of Pho4 are not affected by either a *snf2* or a *gcn5* mutation (Holstege *et al.*, 1998), argue against an effect of these mutations on Pho4 expression. Instead, our results are consistent with earlier work suggesting a role for SWI–SNF after activator binding (Ryan *et al.*, 1998).

A previous *in vivo* analysis identified two criteria that influence promoter SWI–SNF dependence: the affinity of the binding site and its nucleosomal location (Burns and Peterson, 1997). Thus, a low-affinity binding site contained within a positioned nucleosome produced a strong SWI–SNF dependence. These data supported earlier *in vitro* experiments showing that the main function of SWI–SNF was to help an activator target a nucleosomal binding site (Côté *et al.*, 1994; Utley *et al.*, 1997). Our *in vivo* experiments, however, demonstrate that SWI–SNF and SAGA are not needed for the binding of Pho4 to its site, a result consistent with the inter-nucleosomal nature of the UASp2 element at *PHO8* (Barbaric *et al.*, 1992). Consequently, at the *PHO8* promoter an essential functional role for the SWI–SNF and SAGA complexes is at a subsequent chromatin remodelling step. Given that Pho4 can bind in the absence of these complexes, it would be in a perfect position to recruit such activities directly to the promoter.

It has been suggested that SAGA-dependent genes also require the SWI–SNF complex for activation (Pollard and Peterson, 1997, 1998; Roberts and Winston, 1997). This observation holds true for the *PHO8* promoter, although the features of this promoter that confer such double dependency are currently unknown. Experiments are in progress to elucidate the properties of this promoter that

define the *in vivo* requirement for the SWI-SNF and SAGA chromatin-associated complexes.

## Materials and methods

### Yeast strains and media

All *Saccharomyces cerevisiae* strains used in this study have been described previously: CY337, CY407, CY396 and CY397 (Richmond and Peterson, 1996) (gifts from C.L.Peterson); FY86, FY294, FY963 and FY1441 (Roberts and Winston, 1997) (gifts from F.Winston); YS18, YS31, YS5189 and YS5319 (Gregory *et al.*, 1998b). Gcn5 HAT domain mutant strains (Wang *et al.*, 1998) were described previously (Gregory *et al.*, 1998b). A *gcn5* derivative of CY337 (CY53379) was created for this study as described previously (Gregory *et al.*, 1998b). Yeast strains were grown in YNB medium supplemented with the required amino acids (high phosphate conditions) or in phosphate-free synthetic medium (Svaren *et al.*, 1995). Phosphate was shown to be the sole limiting nutrient by control experiments in which inorganic phosphate was supplemented to starved cells allowing them to resume growth.

### Plasmids

The *PHO8-lacZ* reporter was constructed by exchanging the *Bam*HI-*Bam*HI *PHO5* promoter fragment of the previously described *PHO5-lacZ* reporter plasmid (Straka and Hörz, 1991) against a PCR-generated 920 bp *PHO8* promoter fragment, described elsewhere (M.Münsterkötter, S.Barbaric and W.Hörz, manuscript in preparation).

### Functional assays and chromatin analysis

$\beta$ -galactosidase activity measurements (Straka and Hörz, 1991), nuclei preparation (Almer *et al.*, 1986), restriction nuclease and DNase I digestion of isolated nuclei (Svaren *et al.*, 1995) were performed as previously described. Restriction nuclease accessibility values were determined by phosphoimaging the Southern blots and calculating the ratios of the two fragments in each lane. All activity values shown are the average of at least three independent measurements (SD <20% for  $\beta$ -galactosidase). *In vivo* DMS footprinting was carried out as described previously (Gregory *et al.*, 1998a) and quantified with a Fuji phosphor-imager. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions.

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