Eukaryotic gene expression starts off from a largely obstructive chromatin substrate that has to be rendered accessible by regulated mechanisms of chromatin remodeling. The yeast PHO5 promoter is a well known example for the contribution of positioned nucleosomes to gene repression and for extensive chromatin remodeling in the course of gene induction. Recently, the mechanism of this remodeling process was shown to lead to the disassembly of promoter nucleosomes and the eviction of the constituent histones in trans. This finding called for a histone acceptor in trans and thus made histone chaperones likely to be involved in this process. In this study we have shown that the histone chaperone Asf1 increases the rate of histone eviction at the PHOS promoter. In the absence of Asf1 histone eviction is delayed, but the final outcome of the chromatin transition is not affected. The same is true for the coregulated PHO8 promoter where induction also leads to histone eviction and where the rate of histone loss is reduced in asf1 strains as well, although less severely. Importantly, the final extent of chromatin remodeling is not affected. We have also presented evidence that Asf1 and the SWI/SNF chromatin remodeling complex work in distinct parallel but functionally overlapping pathways, i.e. they both contribute toward the same outcome without being mutually strictly dependent.

The DNA of eukaryotic cells is compacted in the nucleus into a complex structure called chromatin. The first level of chromatin organization is formed by the nucleosome, which consists of a histone octamer core organizing ~1.7 turns of double-stranded DNA around its surface (1). DNA that is wound around a histone octamer in a canonical nucleosome is much less accessible for most DNA-interacting factors than DNA in the linker regions between nucleosomes.

It is now widely accepted not only that nucleosomes serve a structural role for the compaction of eukaryotic DNA but also that the obstructive nature of the nucleosomal histone-DNA interactions is a means to regulate the expression of genetic information (2–4). This mode of regulation involves changes in chromatin structure at, for example, promoter or enhancer regions. A hallmark of such regulatory changes is the switch of DNA regions from a state that is protected from nucleases to a state that is sensitive, or even hyper sensitive, to nucleases.

**The Histone Chaperone Asf1 Increases the Rate of Histone Eviction at the Yeast PHOS and PHO8 Promoters**

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To understand the process of regulation through chromatin structure it is therefore crucial to study the molecular mechanisms that lead to the inducible generation of hypersensitive sites. To this end, the PHOS promoter in yeast became a classical model system (5). In its repressed state this promoter region is organized into four positioned nucleosomes with a short hypersensitive site in the middle. Upon activation by phosphate starvation this characteristic chromatin organization becomes remodeled into an extended hypersensitive region (6). The promoter nucleosomes in this induced state are completely disassembled as assayed by the loss of histone DNA contacts (7–9). Recently, we and others showed that the loss of nucleosomal organization corresponds to a movement of histones away from the promoter in trans (10, 11), raising the mechanistic question of where the histones go.

Free histones together with DNA are notoriously aggregation prone, and it is assumed that they occur in the cell mainly in complex with nucleic acids or histone chaperones (12–14). Histone chaperones are a diverse family of proteins that interact with various kinds of histones and probably serve mainly as histone donors and acceptors during nucleosome assembly or disassembly processes. In addition, they can have regulatory functions as in the case of the Hir proteins or Asf1 (15, 16). Histone chaperones appear to form a redundant network as yeast strains deleted in multiple histone chaperone genes are viable (13). Nonetheless, histone chaperones can also be specific for certain histone variants (17) or for certain processes, e.g. for replication-dependent or -independent chromatin assembly (18, 19). Chromatin remodeling complexes may work more or less specifically with, or may even contain, histone chaperones (14, 20–23). Therefore, histone chaperones are likely candidates for the histone acceptor in the process of histone eviction in trans upon remodeling of PHOS promoter chromatin.

In this study we investigated the effect of mutations in genes coding for various histone chaperones on the induction of the PHOS gene. Of all five tested histone chaperones, only the lack of Asf1 showed an effect on PHOS induction. The rate of chromatin remodeling was delayed, but, importantly, the final extent of chromatin remodeling and gene expression was not compromised.

We also extended our study to the PHO8 gene that codes for an alkaline phosphatase and is coregulated with PHOS by the same trans-activator Pho4 (24, 25). Here as well, chromatin remodeling upon induction led to histone loss from the promoter region and Asf1 contributed to the rate of histone eviction. Because PHO8 induction is strictly dependent on Snf2 (26), we conclude that Asf1 may cooperate with the remodeling complex SWI/SNF. However, Asf1 and Snf2 do not exclusively work in the same pathway, as we observed a synthetic effect of combined snf2 and asf1 mutations for the case of PHOS induction.

**MATERIALS AND METHODS**

**Yeast Strains, Plasmids, and Media—**Yeast strain W303 asf1::HIS3 was a gift from Mary Ann Osley. Strains W303 asf1::KAN^† (alias
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MAR101), W303 cac1Δ::TRP1, W303 hir1::HIS3, W303 hir2::HIS3, W303 hir3::HIS3, and W303 asf1::KAN, hir3::HIS3 (alias YAG120) were a gift from Alain Verreault. Strains PKY028 (wt, W303), PKY937 (asf1Δ), and PKY1019 (asf1::caci) were gifts from Paul Kaufman (27). Strain BY4741 asf1::KAN was obtained from EUROSCARF (web.uni-frankfurt.de/fb15/mikro/euroscarf). Using the primers Asf1-fwd 5′-GGTGGCGTTCTTTTGTG-3′ and Asf1-rev 5′-GGAGAGGTGTCTCCGGTTC-3′, we generated an asf1::KAN disruption construct by PCR with genomic DNA of BY4741 asf1::KAN as template. The ASF1 gene in the strains CY337 (α ura3Δ-S2, lys2Δ-801, ade2Δ-101, leu2Δ1, his3Δ200 (28)), CY407 (CY337 snf2Δ::HIS3), and YS31 (29) was disrupted by linear transformation with this PCR product, yielding the strains CY337 asf1::KAN, CY407 asf1::KAN, and YS31 asf1::KAN, respectively. The disruption of the locus was confirmed by genomic PCR with the above primers and digestion of the PCR product with HindIII and Clal, thus detecting an introduced Clal site.

Plasmids pPpho4-lacZ, pPpho5v33-lacZ, and pPggl1-lacZ were as described (24, 30). Yeast strains were grown in yeast extract/peptone/dextrose/adenine (YPD) or in yeast nitrogen base (YNB) medium supplemented with the required amino acids (high phosphate conditions), and PHO5 induction was initiated by transferring cells to phosphate-free synthetic medium (6). Galactose induction was done as described (30).

Functional Assays—Acid phosphatase and β-galactosidase assays were done as described (30).

Chromatin Analysis—Nuclei preparation and chromatin analysis by restriction enzymes or DNaseI as well as indirect end labeling, gel electrophoresis, and blotting procedures were as described (6, 31, 32). Quantification of restriction enzyme accessibility assays was done using phosphorimaging (Fuji FLA3000, AIDA™ software). The probe for the PHOS locus corresponds to the Apal-BamHI fragment upstream of the PHOS promoter (probe D in Ref. 6), and Apal (for DNaseI mapping) or HaeIII (for restriction enzyme accessibility) was used for secondary cleavage. The probe for the PHO8 locus corresponds to the PvuII-Xhol fragment downstream of the PHO8 promoter (25), and BglII (DNaseI mapping) or BglII and EcoRV (restriction analysis) were used for secondary cleavage.

Chromatin Immunoprecipitation—Yeast cultures of a density of 1–2 × 10^6 cells/ml were treated with 1% formaldehyde for 20 min at room temperature. Cross-linking was quenched by adding glycine to 125 mM final concentration. Cells were washed twice in ice-cold 0.9% NaCl, resuspended in HEG150 buffer (150 mM NaCl, 50 mM HEPES, pH 7.6, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) and treated with a French Press onding and restriction enzyme digestion, both under repressive conditions. Pho4 and histones H3 and H4, respectively, were gifts from A. Verreault. The anti-Pho4 antibody was commercially prepared (Abcam) using purified Pho4. Immunoprecipitated DNA was quantitated in duplicates by the ABI PRISM 7000 sequence detection system using the following amplicons: PHOS UAsp2-A, 5′-GAAATAG-GCAATCTCTAAATGATATCGA-3′; PHOS UAsp2-B, 5′-GAAAACA-CAGGGACCAAGAATCATATAAAT-3′; PHOS UAsp2 probe, 5′-FAM-ACCTTG6CCTACATCAGGGACTAGC-3′-TAM; ACT1-A, 5′-TGGATTTCCGGTGATGGTTTGTGTT-3′; ACT1-B, 5′-TCAAATAG-GGTTAGGTAGAGA-3′; ACT1-probe, 5′-FAM-CTCACGTGTT-

FIGURE 1. The kinetics of PHOS induction are delayed in an asf1 strain. Acid phosphatase activity was monitored during the induction of a wt (CY337; closed circles) and the isogenic asf1 strain (open circles) in phosphate-free medium. Error bars indicate the S.D. of two to four independent experiments per time point.

TCCAATTACCCTGTTT-3′-TAM; PHOS upstream ORF-A, 5′-TATTTATTTTTAGCCGCTTTTG-3′; PHOS upstream ORF-B, 5′-CAATCTTGTGCACTGGCTAGT3′; PHOS upstream ORF probe, 5′-FAM-CCAATGCGAGTCATCCCTTGGAGCAGCA-3′-TAM; PHO8 UAsp2-A, 5′-TGGCCGCTATTGTTGCTAGC-3′; PHO8 UAsp2-B, 5′-AGCGCGAAAGGTCATCTAC-3′; PHO8 UAsp2-probe, 5′-FAM-ATCGCTGACAGTGGCCCGA-3′-TAM.

RESULTS

The Kinetics of PHOS Induction Are Delayed in the Absence of the Histone Chaperone Asf1—In the course of our mechanistic studies of chromatin opening at the PHOS promoter, we examined a possible role of histone chaperones in the process that leads to histone eviction in trans (10, 11). Therefore we tested whether induction of Pho5 activity, i.e. secreted acid phosphatase activity, was impaired in strains that are singly disrupted in various of the known histone chaperone genes in yeast: hir1Δ, hir2Δ, hir3Δ, or asf1Δ, as well as the double mutants asf1hir1Δ and asf1::caciΔ (12–14). All these strains could be induced to wild type levels of Pho5 activity by overnight incubation in phosphate-free medium (Fig. 1 and data not shown). As complete remodeling of the PHOS promoter chromatin is a prerequisite for induction of Pho5 activity (34), this result shows that neither of the tested histone chaperones is essential for chromatin remodeling at the PHOS promoter.

The histone chaperone Asf1 was recently reported to be required for remodeling at the PHOS promoter (35). Therefore we compared the chromatin structure of asf1Δ and wild type (wt)2 strains by DNaseI mapping and restriction enzyme digestion, both under repressive conditions and after overnight incubation in phosphate-free medium. Under both conditions the asf1Δ mutant showed the same results as the wt strain (data not shown). We conclude that Asf1 is necessary neither for the assembly of the repressed state nor for chromatin remodeling upon induction.

We and others observed in the past that the absence of chromatin-related factors like Gcn5 or Snf2, although not essential for chromatin opening at the PHOS promoter, could lead to a delay in the induction process2 (7, 30, 36, 37). We also tested this possibility for the above histone chaperone mutant strains by monitoring induction kinetics. The strain deleted in the ASF1 gene showed a marked delay in PHO5

2The abbreviations used are: wt, wild type; ChIP, chromatin immunoprecipitation; ORF, open reading frame.

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induction (Fig. 1). An equivalent delay was observed in two other strain backgrounds if deleted for **ASFI** (W303 and BY4741, data not shown). The **cac1** mutant also showed a significant delay in **PHO5** induction. However, by monitoring restriction enzyme accessibility during induction kinetics (see below), we found that the delay in induction was not because of retardation on the level of chromatin opening (data not shown) as was analogously observed by Adkins et al. (35) in histone-loss kinetics during induction of a **cac2** mutant. Accordingly, the induction of phosphatase activity in the **asf1,cac1** double mutant was more delayed than in either single mutant but did not show a significant further delay on the level of chromatin opening as compared with the **asf1** single mutant (data not shown). All other tested mutants did not significantly affect **PHOS** induction (data not shown), and we focused on further studies on the role of **Asf1**.

**The Absence of Asf1 Delays the Kinetics of PHO5 Induction on the Level of Chromatin Opening**—We tested whether the kinetic effect of the **asf1** mutation as measured on the level of **PHOS** activity was because of a kinetic delay at the chromatin transition step. The course of chromatin remodeling at the **PHOS** promoter during induction kinetics in **asf1** and wt strains was monitored by measuring the accessibility of the intranucleosomal **ClaI** site in the **PHOS** promoter region (6, 32). A clear delay in nucleosome remodeling was observed (Fig. 2A). In addition, we followed the loss of histones from the **PHOS** promoter region by ChIP assay either with antibodies directed against the C terminus of histone H3 in the **CY337** background (Fig. 2B) or with antibodies directed against the C terminus of histone H4 in the W303 background (data not shown). The ChIP assay was internally controlled for region specificity as amplicons corresponding to an open reading frame upstream (“upstream ORF”) of **PHOS** (Fig. 2B) did not detect significant changes in histone levels (7). This chromatin assay also showed a clear delay for the **asf1** strain in both cases, but after prolonged incubation in phosphate-free medium no difference between the wt and **asf1** strains was observed (Fig. 2B). Therefore, both quantitative chromatin assays confirmed that the rate of chromatin opening, rather than only other steps downstream in the gene expression process, is affected in the absence of **Asf1**.

It was shown previously that stable binding of **Pho4** to its intranucleosomal binding site **UASp2** requires chromatin opening (38). We therefore hypothesized that the observed delay in chromatin remodeling should also cause a delay in **Pho4** binding to the promoter. Indeed, by ChIP assay using anti-**Pho4** antibodies, we found in **asf1** strains that **Pho4** recruitment to the promoter was equally delayed as chromatin remodeling (Fig. 2C). Equivalent results were obtained in a W303 background (data not shown).

The Kinetic Delay in **asf1** Strains Is Not Due to Effects Upstream of the Chromatin Remodeling Process—Previously, we took advantage of the **PHOS** promoter variant 33 (**P<sub>pho5v33</sub>**), in which both **Pho4** binding sites are replaced by Gal4 binding sites, to control for effects that a mutation might have upstream of the chromatin remodeling step at the **PHOS** promoter (30, 39). In such a variant, the same chromatin transition and resulting promoter activation take place upon galactose induction as with the wt promoter upon phosphate starvation (40). Therefore, any mutation that is thought to directly affect the chromatin remodeling step should also affect the chromatin transition in this variant.

The induction of the promoter **P<sub>pho5v33</sub>** through the Gal pathway was similarly delayed in an **asf1** strain (Fig. 3A) as the induction of the wt promoter through the Pho pathway (Fig. 1). This delay was not because of possible effects on the Gal signaling pathway or on Gal4 activity as the induction kinetics of the **Gal1** promoter was unaffected by the **asf1** mutation (Fig. 3B). The **asf1** strain even showed a much higher extent of final induction for the **P<sub>gal1</sub>-lacZ** construct than the wild type strain (data not shown). We note that **asf1** strains tend to yield higher final values than the wt strains for basal and fully induced levels of **PHOS** induction (Fig. 1) or for final **Pho4** binding (Fig. 2C). However, such higher expression levels are not associated with changes in the chromatin structure (data not shown) and are probably due to downstream effects on gene expression.

**The Combined Disruption of the SNE2 and ASFI Genes Has a Synthetic Effect on the Induction Kinetics of PHOS**—So far, no chromatin remodeler has been identified that is essential for opening **PHOS** promoter chromatin. However, the absence of Snf2 leads to a marked kinetic delay in **PHOS** induction (7, 36, 37), indicating a significant role of the SWI/SNF complex in chromatin remodeling at the **PHOS** promoter. We wanted to test whether the SWI/SNF complex cooperates with **Asf1** in this remodeling process and, if so, whether they function...
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The kinetic delay in asf1 strains is not due to effects upstream of the chromatin remodeling process. A, the wt (CY337; closed circles) and isogenic asf1 strains (open circles), both carrying the plasmid pP\textsubscript{PHO5v33-lacZ}, were monitored for β-galactosidase activity during the time course of galactose induction. B, as in panel A, but cells contained the plasmid pP\textsubscript{gal1-lacZ}. Error bars indicate the S.D. of two to six independent experiments per time point. o/n, overnight induction times (15–18 h).

Deletion of the negative regulator Pho80 has previously been shown to lead to full opening of the chromatin structure at the PHO5 promoter even in the absence of Snf2.

We constructed an asf1,snf2 double disruption strain and compared PHOS induction kinetics in this strain with the kinetics in the single mutants (Fig. 4A). While the kinetics of PHOS induction were strongly delayed in the snf2 strain, the additional absence of Asf1 caused a significant further delay, showing that Asf1 contributes to the rate of the alternative, Snf2-independent remodeling process. After overnight induction, however, the final activity level of the double mutant was similar to that of the single mutants (data not shown), arguing for full opening of PHOS promoter chromatin even in the absence of both Snf2 and Asf1.

Deletion of the negative regulator Pho80 has previously been shown to lead to full opening of the chromatin structure at the PHOS promoter even under otherwise repressive high phosphate conditions (41, 42). Under such conditions the absence of Asf1 had no effect, or even slightly increased Pho5 activity, and the absence of Snf2 led to a modest decrease in activity. However, the level of induction of the asf1,snf2 double mutant was strongly reduced in comparison to that of the snf2 mutant (Fig. 4B). This demonstrated again a synthetic effect of this double deletion, suggesting that under such conditions the contribution of Asf1 to chromatin remodeling becomes important only in the absence of Snf2, i.e. as part of an alternative pathway. Nonetheless, the double mutant was still able to significantly induce PHOS in a pho80 background under high phosphate conditions as compared with the corresponding repressed PHO80 strain (compare asf1,snf2,pho80 to asf1,snf2 in Fig. 4B).

The Kinetics of PHO8 Induction Are Also Delayed at the Level of Chromatin Remodeling in the Absence of Asf1—Induction of the PHO8 promoter is triggered by the transactivator Pho4 as well and also leads to a pronounced chromatin transition (25). Intriguingly, chromatin remodeling and the induction of this promoter depend critically on the activities of Snf2 and Gcn5 (26), much in contrast to the chromatin transition at the PHOS promoter.

Adkins et al. (35) reported that Asf1 was essential for induction of PHO8. We measured PHO8 promoter activity by using a PHO8 promoter-driven lacZ reporter gene because alkaline phosphatase activity in yeast is due not only to the PHO8 but also to the PHO13 gene product (43). The PHO8 induction kinetics in phosphate-free medium were delayed in an asf1 strain, whereas the final level of activity was close to wild type levels (Fig. 5A).

We have shown here that induction of the PHO8 gene also led to histone depletion in the promoter region in agreement with recently published data (35). Furthermore, the kinetics of histone loss in asf1 strains were significantly delayed in comparison to wt strains (Fig. 5B), corresponding with the delay in promoter induction. The same immunoprecipitated DNA as in Fig. 2B was used, making additional control experiments not necessary. Normalization of the data in Figs. 2B and 5B to the pre-induction values allows one to compare more directly the kinetic delay of histone loss at the PHOS and PHO8 promoter regions (Fig. 5C). For unknown reasons, the histone occupancy at the PHO8 promoter prior to induction as measured by ChIP started off with lower
shows positions of fragments generated with restriction enzymes that cut in the region p in an extended hypersensitive site (CY337; promoter region is not affected in an remodelig in the absence of Asf1."

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the accessibility of restriction enzymes in the PHO8 promoter region that are known to change due to the chromatin transition upon induction (25). After 2 h of induction all three restriction sites tested were less accessible in asf1 than in wt cells (Fig. 5D). Nonetheless, we confirmed in several strain backgrounds by Hpal restriction site accessibility that remodeling in asf1 cells, even though delayed after 2 h of induction in phosphate-free medium, reached wild type levels after overnight induction (Fig. 5E). In addition, the constitutive induction of an asf1,pho80 double mutant in high phosphate medium led to the same open chromatin structure. Furthermore, the DNaseI pattern of the asf1 mutant after full induction was indistinguishable from the wild type pattern (Fig. 5F).

As was the case for the PHOS promoter, at the PHO8 promoter no significant effect on the level of chromatin opening was observed with the hir3 or cac1 histone chaperone mutant strains (data not shown). Altogether, these data demonstrate that the rate of chromatin remodeling at the PHO8 promoter was compromised in the absence of Asf1, although not as strongly as at the PHOS promoter (compare Figs. 2A and 5E and Figs. 2B and 5B), and that full remodeling was achieved after prolonged incubation.

Asf1 Is Critically Required for PHOS Induction Only under Submaximal Inducing Conditions—As already mentioned, Adkins et al. (35) reported that Asf1 is essential for PHOS and PHO8 induction, whereas we have shown here that full induction of both promoters was achieved in asf1 strains. Adkins et al. use phosphate-depleted medium for induction, and it is known that the phosphate depletion protocol (44) generates low phosphate medium that contains residual amounts of phosphate, in contrast to the no phosphate synthetic medium used in our study. Therefore, the apparent discrepancy between the results from our and the Tyler group could be due to the different induction media used.

Dhasarathy and Kladde (36) showed recently that the cofactors Gcn5 and Snf2, which are not necessary for complete PHOS induction and chromatin opening under fully inducing conditions (7, 30, 36, 37), become essential under submaximal inducing conditions. The submaximal induction conditions were generated by allowing various amounts of residual phosphate in the induction medium. We used the same approach to examine the Asf1 requirement for PHOS induction at various low phosphate concentrations. By increasing the phosphate concentration the induction of PHOS was more severely affected in the asf1 mutant than in the wt cells (Fig. 6). At certain amounts of phosphate the induction of the asf1 mutant was entirely repressed, whereas induction of the wt remained mostly unaffected.

FIGURE 5. The kinetics of PHO8 induction are also delayed at the level of chromatin remodeling in the absence of Asf1. A, \(\beta\)-galactosidase activity was measured for the wt (CY337; closed circles) and isogenic asf1 strain (open circles), both carrying the plasmid pPHO8-lacZ, during induction kinetics in phosphate-free medium. Error bars indicate the variation of two independent experiments. B, histone H3 occupancy at the PHO8 promoter during induction kinetics for wt (closed circles) and asf1 strain (open circles). The same immunoprecipitated DNA as in Fig. 2B was used for ChIP analysis but with primers for an amplicon in the PHO8 promoter region. Equivalent results were obtained using the W303 background and anti-histone H4 antibodies (not shown). C, the data for the PHOS promoter in Fig. 2B and for the PHO8 promoter in panel B were normalized to the respective values at 0 h induction. D, using a nuclei preparation after 2 h of induction as in Fig. 2A, the accessibility of the indicated restriction sites in the PHO8 promoter region was assayed. The left and right lanes for each restriction enzyme show the results obtained with 0.3 or 1.2 units/\(\mu\)l, respectively. Numbers underneath the lanes give the percentage of cleavage as quantitated by phosphorimaging analysis. E, nuclei of wt (black bars) and asf1 mutant (white bars) after 2 h and after overnight (o/n) induction in phosphate-free medium were analyzed for Hpal restriction enzyme accessibility. Error bars indicate the S.D. of three independent experiments per time point and include results with CY337, W303, and BY4741 as strain backgrounds. Results with YS31 (pho80) under high phosphate conditions (constitutive induction) were also included with the o/n induction data set. F, the chromatin structure of the repressed (+P) and fully induced (−P) state at the PHOS promoter region is not affected in an asf1 strain. Nuclei prepared with wt (CY337) and asf1 mutant grown in full medium (+P) or incubated overnight in phosphate-free medium (−P) were analyzed by limited DNaseI digestion and indirect end labeling. Wedges on top of the lanes denote increasing DNaseI concentrations. Ovals on the left of the gel mark the positions of nucleosomes 1 to 4 in the promoter region in the repressed state. The coding region is represented by a black vertical line (PHO8 ORF). The arrows point to short hypersensitive sites (sHS) that correspond to accessible upstream activating sequence elements in the repressed state. Upon induction remodeling results in an extended hypersensitive site (eHS, dashed line on the right). The marker lane (M) shows positions of fragments generated with restriction enzymes that cut in the region of the PHO8 promoter (XhoI, HindIII, and EcoRV).

DISCUSSION

In this study we have shown that the histone chaperone Asf1 increases the rate of histone eviction during induction of the yeast PHOS and PHO8 promoters. At both promoters the kinetics of chromatin remodeling and consequent induction were delayed in asf1 strains. Such a phenotype is reminiscent of earlier findings with PHOS induction in strains deleted in the genes for the histone acetyltransferase Gcn5 or the ATPase subunit Snf2. Both deletions also lead to a kinetic delay in PHOS induction on the chromatin level (7, 30, 36, 37). Interestingly, in the case of the coregulated PHO8 promoter that is critically dependent on Snf2 and Gcn5 (26) no mutations causing a kinetic delay are known so far. The asf1 mutation described here is the first of this kind.

Importantly, prolonged induction of the asf1 mutant under fully inducing conditions, i.e. in the absence of phosphate, led to the full extent of remodeling, and the resulting final activity levels were as high as in wt.
or even higher than in wt strains. Therefore there is an alternative, Asf1-independent pathway for chromatin remodeling at the PHOS and PHO8 promoters. This is not unexpected as histone chaperones constitute a notoriously redundant system in yeast (13). However, the normal mechanism of chromatin remodeling at both promoters in the wt situation appears to have some kind of specificity for Asf1 as the absence of four other histone chaperones; i.e., Hir1, Hir2, Hir3, or Cac1, did not show any effect.

A role for a histone chaperone in remodeling of PHOS promoter chromatin agrees with the prediction that a remodeling mechanism leading to histone eviction in trans (10, 11) should, in some way or other, involve a histone acceptor in trans as well. An involvement of histone chaperones would be less likely if histones were to leave the promoter region by a mechanism in cis. In turn, we use this argument to suggest that the observed contribution of the histone chaperone Asf1 to PHO8 promoter chromatin remodeling speaks for a mechanism of histone eviction in trans at this promoter as well.

Interestingly, induction of the GAL10 promoter was also shown to lead to the depletion of histones (45, 46), but we have shown here that Asf1 does not influence the rate of induction of the coregulated GAL1 promoter. This may suggest that histone eviction at the GAL promoters does not occur via a trans pathway or that there is a specificity for a different histone chaperone. It clearly shows that Asf1 does not necessarily have a role in transcriptional activation in general.

Recently, another study also addressed the role of Asf1 in the induction of PHOS and PHO8 (35). The authors came to the conclusion that neither the PHOS nor the coregulated PHO8 gene could be induced in asf1 strains at all and therefore considered Asf1 as essential for transcriptional activation of these genes. As outlined above, our results are in agreement with the general conclusion that Asf1 plays a role in chromatin remodeling and the resulting activation of both genes, but we have shown here that chromatin remodeling at both genes can be achieved to wild type levels in the absence of Asf1. The apparent discrepancy between the two data sets can be explained by differences in the induction conditions used. It is reported that the extent of induction of the PHO regulon can be controlled by the phosphate concentration in the medium and that submaximally inducing conditions (low instead of no phosphate) lead to more pronounced dependencies of the PHOS gene on cofactors like, for example, Snf2 or Gcn5 (36). We obtained analogous results comparing the induction of PHOS in an asf1 and wt strain at various levels of residual phosphate in the induction medium. There were intermediary induction conditions in which a wt strain is substantially induced while an asf1 mutant is uninduced. Adkins et al. made use of induction medium that is prepared by a chemical depletion protocol (44). In contrast to phosphate-free synthetic medium, such phosphate-depleted medium contains residual amounts of phosphate and may even allow slow but continued cell proliferation. Therefore, such medium probably corresponds to intermediary inducing conditions as described above and by two other groups (36, 47) and result in the more stringent dependence on Asf1. In our experiments, we examined the requirement for Asf1 during induction in phosphate-free medium that led cells to go into growth arrest after overnight incubation (6), or we used the pho80 mutation that allows constitutive PHO induction even for continuously dividing cells in high phosphate media (41, 42). Both protocols led to complete opening of the PHOS and PHO8 promoters even in the absence of Asf1.

Our findings are in agreement with the recent results of a genome-wide screen for yeast mutants defective for PHOS regulation (48). This screen identified nine new genes that were not known before to be involved in PHOS regulation. However, also in this study phosphate-free synthetic medium was used and the ASFI gene was not found to be essential for PHOS induction, either in the screen or when tested individually and in various strain backgrounds.

It is reported and discussed that chromatin remodeling complexes, for example the SWR or Ino80 complexes that catalyze the exchange of histones, work in concert with histone chaperones (14, 21, 22). The same is likely to be true for chromatin remodelers that catalyze the eviction of histones in trans. This eviction mechanism has not yet been properly reconstituted in vitro; however, there is ample evidence for its relevance in vivo (45, 49), and chromatin remodeling at the PHOS and PHO8 promoters were among the first examples (7, 9–11).

The induction of both PHOS and PHO8 is mediated by Snf2 with PHO8 induction being totally abolished (26) and PHOS induction severely compromised in the absence of Snf2 (7, 36, 37). Here, we have provided evidence that the SWI/SNF complex can work together with the histone chaperone Asf1, suggesting that the SWI/SNF complex is an example of a chromatin remodeler that can catalyze histone eviction in trans in vivo. However, our results indicate that the role of Asf1 in nucleosome eviction is not exclusively connected with the remodeling function of the SWI/SNF complex, i.e. that the SWI/SNF complex and the histone chaperone Asf1 work in distinct parallel pathways that functionally overlap. The SWI/SNF complex and Asf1 can cooperate with alternative histone chaperones or remodelers, respectively. The argument is as follows. Chromatin remodeling at the PHO8 promoter has to proceed via a SWI/SNF-dependent pathway. This pathway is delayed in asf1 strains, implying that Asf1 usually contributes to the outcome of this pathway but can be replaced by some other factor. Conversely, chromatin remodeling at the PHOS promoter usually involves a SWI/SNF-dependent pathway as well but can also be achieved in the absence of Snf2 through another pathway, albeit at a slower rate. This alternative pathway in turn can also cooperate with Asf1, as a deletion of Asf1 in a snf2 background leads to a synthetic kinetic effect with even slower induction rates and even lower steady state induction levels during induction via the pho80 mutation in high phosphate media. Strictly speaking, we cannot be sure whether the alternative pathways make use of alternative remodelers or histone chaperones at all. However, the alternative pathways in each case led to the same final outcome of remodeled chromatin structure, suggesting that the main features of the overall mechanism, e.g. histone eviction in trans through the combined
action of a chromatin remodeler and a histone chaperone, are maintained.

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