

RNA Polymerase II Holoenzyme Recruitment Is Sufficient to Remodel Chromatin at the Yeast *PHO5* Promoter

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Summary

We examine transcriptional activation and chromatin remodeling at the *PHO5* promoter in yeast by fusion proteins that are thought to act by recruiting the RNA polymerase II holoenzyme to DNA in the absence of a classic activating region. These hybrid proteins (e.g., Gal11+Pho4 or Gal4(58–97)+Pho4 in the presence of a *GAL11P* allele) efficiently activated transcription and remodeled chromatin. Similar chromatin remodeling was observed at a *PHO5* promoter deleted for TATA and thus unable to support transcription. We conclude that recruitment of the holoenzyme or associated proteins suffices for chromatin remodeling. We also show that the SWI/SNF complex is required neither for efficient transcription of the wild-type *PHO5* nor the *GAL1* promoters, and we observe nearly complete chromatin remodeling at *PHO5* in the absence of Snf2.

Introduction

Induction of the *PHO5* gene in yeast is correlated with chromatin remodeling (Almer and H^orz, 1986; Almer et al., 1986). The repressed *PHO5* promoter bears a positioned array of nucleosomes interrupted only by a 70 bp region hypersensitive to nucleases (Figure 1). Upon activation of the *PHO5* promoter by phosphate starvation, the two pairs of nucleosomes flanking this region are no longer detectable by nuclease accessibility assays. Transcriptional activation and chromatin disruption are dependent on the basic–helix–loop–helix activator Pho4, which binds to two sites in the *PHO5* promoter: UASp1, which is present within the short nucleosome free region, and UASp2, which lies within nucleosome –2. Chromatin remodeling, which can occur in the absence of DNA replication (Schmid et al., 1992), requires the activating region of Pho4 (Svaren et al., 1994). However, nucleosome disruption occurs even if transcription is prevented by a deletion of the TATA element (Fascher et al., 1993), and similar experiments have shown that transcriptional initiation and elongation are not required for chromatin disruption at other promoters (Hirschhorn et al., 1992; Axelrod et al., 1993; Verdin et al., 1993). These results have led to the suggestion that binding of an activator to a promoter causes chromatin remodeling by a mechanism that is independent of interactions with the transcriptional machinery. According to this

idea, only after nucleosome clearance would the activation domains interact with the transcriptional machinery and facilitate formation of an initiation complex on the promoter (Kwon et al., 1994; Bunker and Kingston, 1996).

To test this model, we used a form of gene activation in yeast that requires no classic activating region. The experiments of Barberis et al. (1995) and of Farrell et al. (1996) show that fusion of a DNA-binding domain to a component of the RNA polymerase II holoenzyme—a large complex that contains in addition to the polymerase many of the basal transcription factors including a group of Srb proteins and Gal11 (Koleske and Young, 1994; Kim et al., 1994)—can create an activator that bears no classic activating region. For example, LexA+Gal11, which comprises the DNA-binding domain of LexA fused to Gal11, is a particularly powerful activator, and a variety of experiments indicate that this fusion protein activates transcription, when bound to LexA sites, by inserting its Gal11 component into the holoenzyme and recruiting the complex to DNA. A variety of other components of the transcriptional machinery, when fused to a DNA-binding domain, have been shown to work as transcriptional activators (Laurent et al., 1991; Jiang and Stillman, 1992; Chatterjee and Struhl, 1995; Klages and Strubin, 1995; Xiao et al., 1995; Apone et al., 1996). Another case of gene activation that bypasses the requirement for a classic activating region is found with yeast strains bearing the *GAL11P* mutation. The single amino acid change introduced into Gal11 by this mutation creates a fortuitous interaction between Gal11P and a fragment of the dimerization region (residues 58–97) of Gal4, and that interaction triggers gene activation when the dimerization fragment is tethered to DNA. The strength of the interaction of various *Gal11P* alleles with the Gal4 dimerization fragment and mutants thereof, as determined in vitro, correlates with the level of gene activation measured in vivo. These and other experiments argue that in this case, as well as with fusion proteins, gene activation is effected by recruitment of the holoenzyme.

The SWI/SNF complex has been shown to be required for the activation of certain genes in yeast, and it is thought to counteract a repressive chromatin structure (Winston and Carlson, 1992). Several in vitro studies have shown that this protein complex from yeast, as well as a similar complex isolated from human cells, is capable of catalyzing an ATP-dependent disordering of nucleosome structure that facilitates binding of transcriptional activators and TATA box-binding protein (Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994). Kwon et al. (1994) reported that an activation domain enhanced binding of Gal4 derivatives to nucleosomal templates in the presence of human SWI/SNF fractions, whereas Côté et al. (1994) found that an activation domain did not enhance binding of Gal4 derivatives in the presence of purified yeast SWI/SNF. The yeast SWI/SNF complex has recently been reported to be associated with the holoenzyme (Wilson et al., 1996), but this finding is disputed (Cairns et al., 1996).

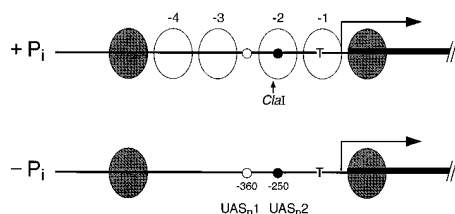


Figure 1. Chromatin Structure at the *PHO5* Promoter

Nucleosomes -1 , -2 , -3 , and -4 are remodeled upon activation of the promoter by phosphate starvation conditions (Almer et al., 1986). The small circles mark UASp1 (open) and UASp2 (solid), which are Pho4-binding sites found by *in vitro* (Vogel et al., 1989) and *in vivo* (Venter et al., 1994) footprinting experiments. The positions are listed relative to the coding sequence (solid bar). T denotes the TATA box (Rudolph and Hinnen, 1987). The location of a Clal site at -275 relative to the coding region is shown.

We show here that hybrid proteins bearing the DNA-binding region of Pho4 fused to Gal11 or Srb2 (Gal11+Pho4 Δ 2 and Srb2+Pho4 Δ 2) activate transcription and remodel chromatin at the *PHO5* promoter. Similar results are obtained in a Gal11P strain with a fusion protein containing the DNA-binding domain of Pho4 and the dimerization region of Gal4. We show that at a *PHO5* promoter deleted for its TATA sequence, Gal11+Pho4 Δ 2, although unable to induce transcription, nevertheless efficiently remodels chromatin. We also show that mutation of a component of the SWI/SNF complex has only a small effect on activation by Pho4 or by Gal11+Pho4 Δ 2 at the wild-type *PHO5* promoter. Similarly, there is little effect on activation by Gal4 or by the fusion protein Gal11+Gal4 (which bears Gal11 sequences in place of the activating region of Gal4) at a wild-type *GAL1* promoter. As expected from these results, Pho4 remodels chromatin at *PHO5* in a *snf2* strain almost as efficiently as in a wild-type strain. We describe modifications that render the *GAL1* promoter sensitive to mutation of *SWI1* (Peterson and Tamkun, 1995; C. Peterson, personal communication) and find that even under conditions of SWI/SNF dependence, Gal11+Gal4 activates transcription efficiently. We discuss the implications of these results for our understanding of the mechanism of chromatin remodeling associated with gene activation.

Results

Transcriptional Activation and Chromatin Remodeling by Pho4 Fusion Proteins

The nucleosome structure of the repressed *PHO5* promoter is shown in Figure 1. Activation of the promoter by Pho4 is associated with the concomitant disruption of nucleosomes -1 through -4 as measured by accessibility to DNase I and with various restriction enzymes (Almer and Hörz, 1986; Almer et al., 1986). Chromatin disruption is also observed at a *PHO5* promoter in which the TATA element is deleted and which is hence unable to support transcription (Fascher et al., 1993). A deletion derivative of Pho4 (Pho4 Δ 2) containing its DNA-binding domain but lacking the activating region is competent to bind UASp1 yet fails to disrupt nucleosome structure.

Binding of Pho4 Δ 2 to UASp2 is prevented by the persistence of nucleosome -2 (Venter et al., 1994).

Previous work has shown that VP16-Pho4 Δ 2, in which the VP16 activation domain was substituted for the native Pho4 domain, activated transcription and disrupted chromatin (Svaren et al., 1994). In several of the experiments reported here, we use similar fusion proteins in which one or another fragment of Gal11 is fused to Pho4 Δ 2. Barberis et al. (1995) showed that fusion proteins bearing a carboxy-terminal fragment of Gal11 (e.g., LexA-Gal11(799–1081)) were potent transcriptional activators, whereas an amino-terminal fragment of Gal11 (e.g., LexA+Gal11(141–846)) activated significantly less well. Furthermore, immunoprecipitation experiments showed that the carboxyl portion of Gal11 interacts with the holoenzyme particularly efficiently. The extent of transcription of the *PHO5* gene was determined by assaying cells for acid phosphatase, the product of the *PHO5* gene. To assay chromatin disruption of the *PHO5* promoter, we used nuclease digestion of isolated nuclei to determine the accessibility to DNase I and/or the accessibility of a Clal site within nucleosome -2 , which has proven to be a reliable and quantifiable assay of chromatin opening (Svaren et al., 1995).

As shown in Figure 2, expression of Gal11+Pho4 Δ 2 fusion proteins bearing carboxyl fragments of Gal11 in place of the Pho4 activating region efficiently activated transcription and disrupted nucleosome structure at the *PHO5* promoter. Transcriptional activation (Figure 2A) and chromatin remodeling (Figure 2B) were observed, with the fusion proteins expressed at either high or low levels from 2 μ or ARS-CEN plasmids, respectively. Very little transcription or chromatin remodeling was elicited by the Pho4 DNA-binding domain alone (line 2) or by a Gal11+Pho4 Δ 2 fusion bearing an amino-terminal fragment of Gal11 (line 5). A DNase I protection assay was used to probe the chromatin structure (Figure 2C). The resulting pattern shows an extensive hypersensitive region with clearly defined boundaries, indistinguishable from the one obtained with wild-type Pho4 because of the selective disruption of nucleosomes -1 to -4 (Almer and Hörz, 1986; Almer et al., 1986).

Another example of an activator lacking a classic activating region is a fusion of the holoenzyme component Srb2 to a DNA-binding domain. Figure 3 summarizes data showing that fusion of Srb2 to the DNA-binding domain of Pho4 also creates an activator that efficiently activates transcription and disrupts nucleosome structure. This result with the Srb2 fusion is of particular interest in light of the analysis of *srb2-1*. That mutation (P14H) was isolated as a dominant suppressor of a mutant yeast bearing a deletion of part of the carboxyl terminus of the largest subunit of RNA polymerase II (Koleske et al., 1992). Farrell et al. (1996) have shown that LexA+Srb2-1 activates more efficiently than LexA+Srb2 in that mutant strain, whereas the two activate equally in a wild-type strain (Farrell et al., 1996). These results are those expected if LexA+Srb2-1, bound to LexA sites, activates by inserting the Srb2 moiety into the holoenzyme and recruiting that structure to DNA. Moreover, the mutant LexA+Srb2 (P14A) fails to activate in either wild-type or mutant yeast (Farrell et al., 1996), a result indicating that a point mutation at

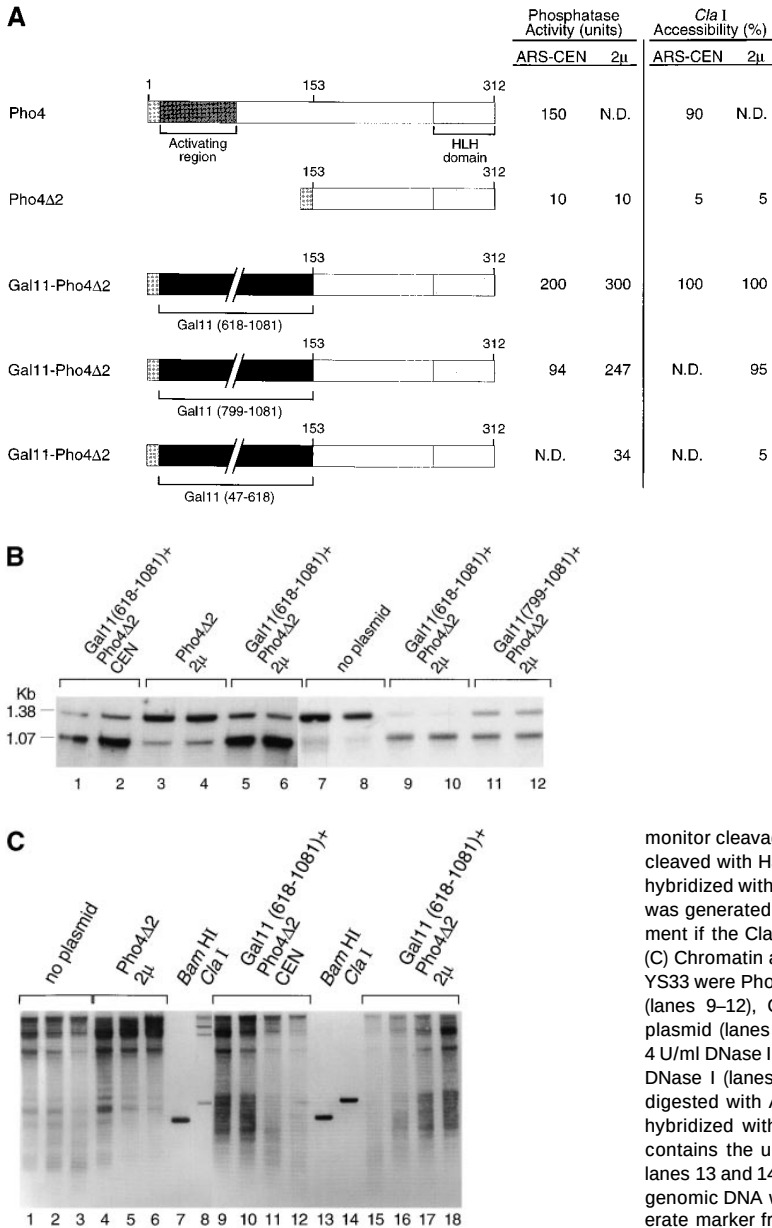


Figure 2. Transcriptional Activation and Chromatin Remodeling by Gal11+Pho42 Fusion Proteins

The strains used in this experiment are derivatives of YS33 (Svaren et al., 1995) and are deleted for the chromosomal *PHO4* and *PHO80* genes. The latter mutation renders the promoter constitutively active, even in the presence of inorganic phosphate, provided that a functional Pho4 derivative is present. (A) Transcriptional activity of the Pho4 derivatives. All of the derivatives were expressed from the *PHO4* promoter either on a low (ARS-CEN) or high (2 μ) copy vector. Activity was measured as acid phosphatase units produced by the product of the *PHO5* gene directly off its chromosomal locus under high phosphate conditions in a *pho80* background, and the extent of nucleosome disruption was measured by percentage accessibility of the *Clal* recognition sequence at position -275 in chromatin digestion experiments (see B).

(B) Chromatin analysis by digestion with *Clal*. Expression plasmids present in YS33 were Gal11(618-1081)+Pho4 Δ 2(CEN) (lanes 1 and 2), Pho4 Δ 2 (lanes 3 and 4), Gal11(618-1081)+Pho4 Δ 2(2 μ) (lanes 5 and 6 and lanes 9 and 10), Gal11(799-1081)+Pho4 Δ 2(2 μ) (lanes 11 and 12), or no plasmid (lanes 7 and 8). Nuclei containing approximately 10 μ g of DNA were digested for 60 min at 37°C in 200 μ l with 50 U *Clal* (lanes 1, 3, 5, 7, 9, and 11) or 200 U *Clal* (lanes 2, 4, 6, 8, 10, and 12). To

monitor cleavage of the *Clal* site at position -275, DNA was isolated, cleaved with *HaeIII*, analyzed in a 1% agarose gel, and blotted and hybridized with probe D (Almer et al., 1986). A 1.38 kb *HaeIII* fragment was generated if *Clal* did not cleave and a 1.07 kb *HaeIII/Clal* fragment if the *Clal* site had been accessible.

(C) Chromatin analysis by DNase I. Expression plasmids present in YS33 were Pho4 Δ 2(2 μ) (lanes 4-6), Gal11(618-1081)+Pho4 Δ 2(CEN) (lanes 9-12), Gal11(618-1081)+Pho4 Δ 2(2 μ) (lanes 15-18), or no plasmid (lanes 1-3). Nuclei were digested for 20 min with 1, 2, and 4 U/ml DNase I (lanes 1-3 and 6-4, respectively) or 1, 2, 4 and 8 U/ml DNase I (lanes 9-12 and 18-15, respectively). DNA was isolated, digested with *Apal*, separated in a 1.5% agarose gel, blotted, and hybridized with probe D. The *Apal* site is at position -1340 and contains the upstream *HaeIII* site used in (B). Lanes 7 and 8 and lanes 13 and 14 contain restriction nuclease double digests of YS33 genomic DNA with *Apal/BamHI* and *Apal/Clal*, respectively, to generate marker fragments.

position 14 (introduced by site directed mutagenesis) can disrupt interaction with the holoenzyme and thereby abolish activation by the fusion protein.

Transcriptional Activation and Chromatin Remodeling at the *PHO5* Promoter by DNA-Tethered Gal4(58-97) in Gal11P Cells

For the experiment of Figure 4, the Gal4 dimerization region (residues 58-97) was fused to the Pho4 DNA-binding domain, and the construct was introduced into yeast strains expressing either wild-type Gal11 or Gal11P. In the presence of Gal11P, but not in the presence of wild-type Gal11, the hybrid activator activated transcription and remodeled chromatin almost as efficiently as did wild-type Pho4 (Figure 4A, lines 1 and 3, and Figure 4B). The DNA-binding domain of Pho4 was

inactive in these assays in a Gal11P cell (data not shown).

Chromatin Remodeling at a *PHO5* Promoter Deleted for the TATA Box

To determine whether the TATA region is required for chromatin opening by Gal11+Pho4 fusion proteins, we assayed the ability of Gal11+Pho4 Δ 2 to trigger chromatin remodeling at a *PHO5* promoter from which 20 base pairs, including the TATA region, were deleted. Neither Pho4 nor Gal11+Pho4 Δ 2 activated this promoter (Figure 5B), but both of these activators efficiently rendered the *PHO5* promoter accessible to *Clal* digestion (Figure 5C). This result with Pho4 is essentially the same as reported previously (Fascher et al., 1993). Similar results were obtained with another *PHO5* reporter plasmid from

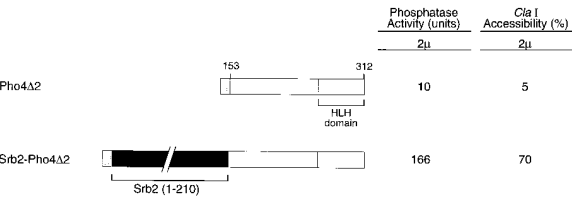


Figure 3. Srh2+Pho4Δ2 Can Activate Transcription and Disrupt Nucleosomes at the *PHO5* Promoter
Another component of the RNA polymerase II holoenzyme can activate the *PHO5* promoter when tethered to the Pho4 DNA-binding domain and trigger nucleosome disruption. Srh2+Pho4Δ2(2μ) (see Experimental Procedures) was expressed in YAG28, a derivative of YS33 (see Table 1). Activation and disruption were measured as described in the legend to Figure 2B.

which 91 base pairs, including the TATA region and 86 adjacent base pairs, have been deleted (data not shown). In this deletion the complete absence of transcription had originally been demonstrated by S1 and primer extension analysis (Rudolph and Hinnen, 1987).

Deleting *GAL11* Does Not Impair Chromatin Remodeling at the *PHO5* Promoter
Deleting *GAL11* from yeast cells causes a general decrease in cellular transcription (Suzuki et al., 1988; Himelfarb et al., 1990; Long et al., 1991), but the effect of a *GAL11* disruption on the *PHO5* promoter has not been assayed previously. Figure 6A shows that deletion of *GAL11* resulted in a significant decrease in transcription at the *PHO5* promoter (420 units in wild-type cells to 130 units in *gal11* cells), consistent with the pleiotropic

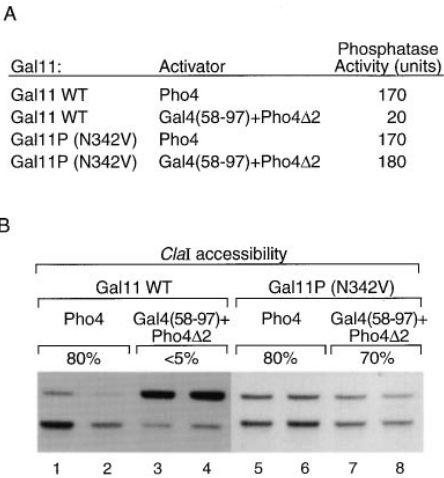


Figure 4. DNA-Tethered Gal4(58-97) Can Activate Transcription and Disrupt Nucleosomes at the *PHO5* Promoter in Gal11P Cells
Gal4(58-97)+Pho4Δ2 can activate transcription and disrupt nucleosomes at the *PHO5* promoter in the presence of Gal11P, a variant of Gal11 with a valine residue at position 342 instead of asparagine. Activation (A) and disruption (B) were measured as described in the legend to Figure 2B. YAG29 (see Table 1) containing the following plasmids was used in this experiment: pSO23 and Pho4(2μ) (lanes 1 and 2); pSO23 and Gal4(58-97)+Pho4Δ2(2μ) (lanes 3 and 4); pSO32 and Pho4(2μ) (lanes 5 and 6); and pSO32 and Gal4(58-97)+Pho4Δ2(2μ) (lanes 7 and 8).

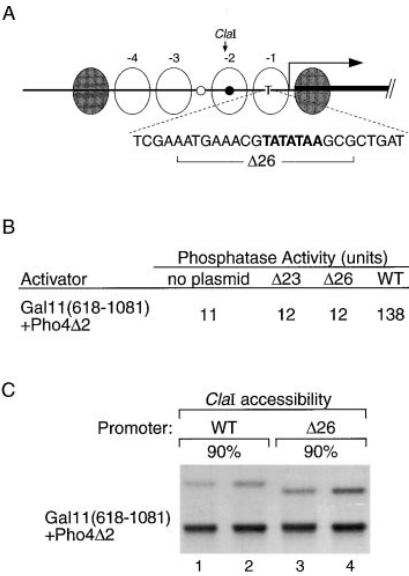


Figure 5. The TATA Box Is Not Required for Nucleosome Disruption by Gal11+Pho4Δ2
Promoter activation and nucleosome disruption by Gal11(618-1081)+Pho4Δ2(2μ) were measured with the *PHO5* reporter plasmids, pCBwt and pCBΔ26 (Fascher et al., 1993) in YAG31. (A) Promoter structure of pCBΔ26 indicating the TATA region that has been deleted. (B) Chromatin analysis was performed as described in the legend to Figure 2B.

function of the Gal11 protein. However, chromatin disruption at the *PHO5* promoter was virtually unaffected (Figure 6B) indicating that the Pho4 protein does not need Gal11 to disrupt chromatin and that the transcriptional defect of the promoter caused by the lack of Gal11 involves a step subsequent to chromatin opening.

SWI/SNF Requirement of the *PHO5* and *GAL1* Promoters
The SWI/SNF complex is thought to counteract a repressive chromatin structure (Winston and Carlson, 1992). The recent report that the SWI/SNF proteins are part of the holoenzyme (Wilson et al., 1996) makes this connection even more intriguing for our studies (but see Cairns et al., 1996). We therefore tested to what extent the *PHO5* promoter depended on the SWI/SNF complex. To do so, we measured *PHO5* activation elicited by Pho4 and by Gal11+Pho4Δ2 in a strain disrupted for the *SNF2* gene, a disruption that has been shown to inactivate SWI/SNF function (Peterson and Tamkun, 1995). As shown in Figure 7A, with both activators, Pho4 and Gal11+Pho4Δ2, phosphatase levels reached about 70% of the levels measured in parallel in an isogenic wild-type strain. Moreover, under activating conditions, there was almost complete opening of the promoter by Pho4 in the *snf2* strain (compare lanes 5-7 and 8-10), the slight difference to the wild-type promoter being consistent with the 30% decrease in promoter strength. Under repressing conditions, the typical nucleosomal array was observed (Figure 7B, lanes 1-3); the pattern was indistinguishable from the usual wild-type pattern under high phosphate conditions.

The wild-type *GAL1* promoter is also affected to only

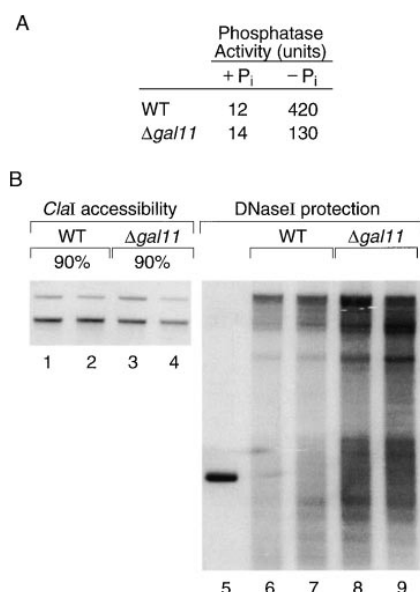


Figure 6. Transcriptional Activation and Chromatin Disruption in a *gal11* Strain

Phosphatase activity (A) and nucleosome disruption as measured by Clal and DNase I accessibility (B) were assayed in YS18 (lanes 1 and 2 and lanes 6 and 7) and YS185 (lanes 3 and 4 and lanes 8 and 9) as described in the legend to Figure 2. Lane 5 contains Apal/Clal-digested genomic DNA.

a small degree by a *SNF/SWI* disruption (Figure 7C). Consistent with the results of Peterson and Tamkun (1995) and C. Peterson (personal communication), we have found, however, that deleting sites 1 and 2 of the *UAS_G* renders activation of that promoter by Gal4 sensitive to a *swi1* mutation by about a factor of 10. Figure 7C shows that activation of that mutant promoter by Gal4(1–100)+Gal11(799–1081) was also reduced approximately 10-fold by mutation of *SWI1*. Thus, the function provided by *SWI/SNF*, whatever it may be, can also be provided to an activator (i.e., Gal4+Gal11) lacking a classic activating region.

Discussion

In the experiments reported here, we used three “non-classic” activators (Gal11+Pho4 Δ 2, Srb2+Pho4 Δ 2, and Gal4(58–97)+Pho4 Δ 2/Gal11P) to target the RNA polymerase II holoenzyme to the *PHO5* promoter in the absence of a classic activating region. We have shown that, in each case, *PHO5* transcription is activated and furthermore, that the chromatin structure of the promoter is disrupted in a manner very similar to the disruption observed upon natural activation of the *PHO5* promoter by wild-type Pho4. The finding that prevention of *PHO5* transcription by deletion of the TATA box does not alter the ability of the Gal11+Pho4 Δ 2 fusion to open chromatin shows that transcription initiation by the holoenzyme is not required for chromatin disruption by the hybrid protein; it also argues that chromatin is disrupted in an early step of the transcriptional activation process.

It remains to be seen whether other specific promoter sequences are required (in addition to activator binding sites) for chromatin disruption. Our results imply that even at a promoter deleted for TATA, the holoenzyme or some component thereof can be recruited to DNA and that this recruitment suffices to remodel chromatin. We suggest therefore that the chromatin remodeling observed with natural activators, even at promoters deleted for TATA (Hirschhorn et al., 1992; Axelrod et al., 1993; Fascher et al., 1993), can be effected by recruitment of the holoenzyme and/or associated factors, a possibility also discussed by Struhl (1996).

Consistent with the view that transcription per se is not required for chromatin remodeling is our observation (in addition to that in the TATA mutant) in a strain deleted for *GAL11*. In that strain, wild-type Pho4 remodels chromatin with full efficiency despite a 3- to 5-fold defect in transcriptional activation when compared with its effects in wild-type cells. The result also shows that Gal11 itself is not required for chromatin remodeling. Consistent with this view, Sakurai et al. (1993) have shown that Gal11 functions as a positive cofactor of basal and activator-induced transcription in an in vitro system in which nucleosomes are absent. Sakurai et al. (1996) have suggested that Gal11 stimulates promoter activity by enhancing an association of TFIIE with the preinitiation complex.

It is not clear whether nucleosome disruption is intrinsic to holoenzyme components that have so far been identified. The recent finding by Wilson et al. (1996) that the *SWI/SNF* complex is associated with the holoenzyme (but see Cairns et al., 1996) makes it a prime candidate for such an activity. We have found, however, that a mutation of the *SNF2* gene, which in other experiments has been shown to disable *SWI/SNF* function as does mutation of *SWI1* (Peterson and Tamkun, 1995), has only a minor effect on activation and chromatin disruption of the *PHO5* promoter. Similarly, we have found that transcriptional activation at the *GAL1* promoter, by either Gal4 or by a hybrid protein bearing Gal11 in place of the activating region of Gal4, is virtually unaffected by disruption of *SWI1*. Thus, at these promoters, efficient transcription and, as explicitly shown in the Pho4 case, nucleosome disruption do not require *SWI/SNF*. Consistent with the results of Peterson and Tamkun (1995) and C. Peterson (personal communication), we have found that transcription of the *GAL1* promoter can be rendered sensitive to *swi1* by weakening the affinity of the *UAS* at *GAL1* for the Gal4 DNA-binding domain (see Figure 7C). Thus, with such promoter constructs, *SWI/SNF* contributes significantly to transcriptional activation by Gal4+Gal11. Our experiments indicate that this contribution of *SWI/SNF*, by whatever mechanism it may occur, does not require specific targeting by direct contact with a classic activating region.

The results presented here have general implications for current models concerning the mechanism of chromatin disruption during transcriptional activation. One model consistent with our results would be that the transcriptional machinery competes with nucleosomes for DNA access, and when the total energy of the protein–DNA interactions is sufficiently high, no extra function is required for nucleosome remodeling. Polach and

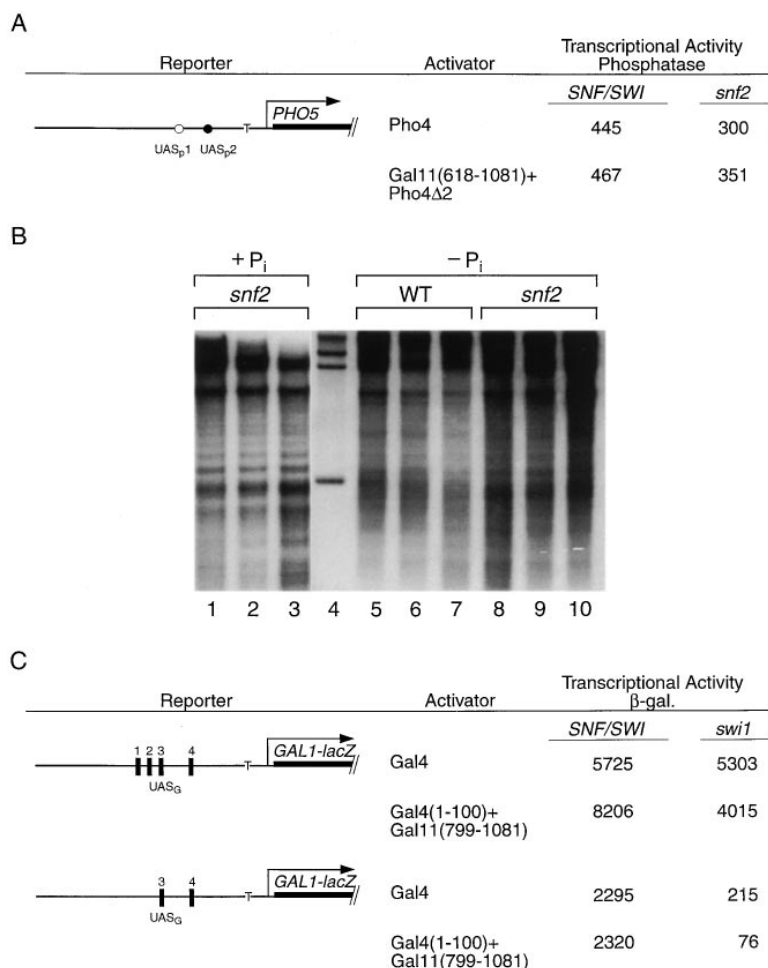


Figure 7. Effects of a *SNF/SWI* Mutation at the *PHO5* and *GAL1* Promoters

(A) Phosphatase activity of the chromosomal *PHO5* locus was measured at low phosphate conditions in CY337 (*SNF2*) and CY407 (*snf2*) in the case of Pho4 and in CY338 (*pho4*, *SNF2*) and CY408 (*pho4*, *snf2*) in the case of Gal11(618-1081)+Pho4Δ2.

(B) The chromatin structure under phosphate starvation conditions was analyzed by DNase I digestion in CY337 (lanes 5-7) and CY407 (lanes 8-10) as described in the legend to Figure 2C. Lanes 1-3 show an analysis of CY407 at high phosphate conditions. Lane 4 contains *Apal/ClaI*-digested genomic DNA.

(C) β-galactosidase activity of *GAL1-lacZ*-based reporters was measured in YAG70 (*SWI1*) and YAG71 (*swi1*) in the case of the wild-type *UAS_G*; CY533 (*SWI1*) and CY535 (*swi1*) were used to measure transcription of a reporter containing sites 3 and 4 of the *UAS_G*.

Widom (1995, 1996) have argued for such a mechanism based on studies performed *in vitro*. Consistent with this idea, Marsolier et al. (1995) have described an example *in vivo* of competition between the transcriptional machinery and nucleosomes at the *SNR6* gene, the outcome of which is strongly affected by the strength of the interaction between a key component (TFIIIC) and DNA. Perhaps, as suggested by Kingston et al. (1996) and Struhl (1996), at weak promoters where the total energy is not sufficient, components in addition to the holoenzyme (e.g., *SWI/SNF*) would be required.

In an alternative model, the holoenzyme may always require factors that specifically labilize histone-DNA interactions. If so, at the wild-type *PHO5* and *GAL1* promoters, those special factors would not include the *SWI/SNF* complex, at least not *Snf2* or *Swi1*. There appear to be several distinct activities, however, capable of labilizing histone-DNA interactions in yeast in an ATP-dependent fashion, and they may turn out to be components of the holoenzyme or may be associated with it transiently in a way that is functionally significant. For example, a nucleosome remodeling factor that has been found in *Drosophila* contains a member of the *Snf2* family, *Iswi* (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995) and not *Brahma*, the *Drosophila* homolog of *Snf2*. There are many *Snf2*-related proteins in yeast, so there may well be a family of such activities. Histone acetylation may also regulate disruption (Vidal and Gaber, 1991;

Brownell and Allis, 1996; Brownell et al., 1996; Taunton et al., 1996), and acetylases, such as the *Ada/Gcn5* complex (Brownell and Allis, 1996; Brownell et al., 1996), might be recruited to a promoter by the holoenzyme during transcriptional activation. Work is in progress to distinguish among these possibilities with use of the chromatin disruption assay at the *PHO5* promoter.

Experimental Procedures

Genetic Methods

Cells were grown and assayed for acid phosphatase activity as described by Svaren et al. (1994). Plasmids expressing wild-type Pho4 and Pho4Δ2 also have been described by Svaren et al. (1994). All of the constructs expressing fusions to Pho4Δ2 were constructed by polymerase chain reaction using Pfu polymerase (Stratagene). ARS-CEN plasmids expressing wild-type Gal11 (pSO23) and Gal11P (N342V) alleles (pSO32) were a gift from S. Farrell, and both are expressed off the *GAL11* promoter. Plasmids expressing wild-type Gal4 and Gal4(1-100)+Gal11(799-1081) were a gift from R. Reece and A. Barberis, respectively; both of these fusions are expressed by the β-actin promoter and have ARS-CEN replicating origins. Details of plasmid construction are available upon request. The yeast strains used in this study are described in Table 1.

Isolation of Yeast Nuclei, Nuclease Digestion, Gel Electrophoresis, Hybridization, and DNA Probes

All methods used have been described previously (Almer et al., 1986) or are explained in the legends. Biodyne B nylon membranes (Pall, Dreieich, Germany) were used for Southern transfer. Probes

Table 1. Yeast Strains Used in This Study

Strain	Genotype	Source
CY337	<i>MATa, ura3-52, lys2-801, ade2-101, leu2-Δ1, his3-Δ200</i>	C. Peterson
CY407	<i>MATa, ura3-52, lys2-801, ade2-101, leu2-Δ1, his3-Δ200, snf2::HIS3</i>	C. Peterson
CY338	<i>MATa, ura3-52, lys2-801, ade2-101, leu2-Δ1, his3-Δ200, Δpho4::LEU2</i>	This study
CY408	<i>MATa, ura3-52, lys2-801, ade2-101, leu2-Δ1, his3-Δ200, snf2::HIS3, Δpho4::LEU2</i>	This study
CY533	<i>MATa, ura3-52, his3-Δ200, leu2Δ1, Δgal4::LEU2, pEG28::URA3</i>	C. Peterson
CY535	<i>MATa, ura3-52, his3-Δ200, leu2Δ1, Δswi1::LEU2, Δgal4::LEU2, pEG28::URA3</i>	C. Peterson
YAG23	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, Δtrp1, Δpho4::ura3Δ5, Δpho80::HIS3</i>	This study
YAG28	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, Δtrp1, Δpho4::ura3Δ5, Δpho80::HIS3, lys2Δ385</i>	This study
YAG29	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, Δtrp1, Δpho4::ura3Δ5, Δpho80::HIS3, Δgal11::TRP1</i>	This study
YAG31	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, Δtrp1, Δpho4::ura3Δ5, Δpho80::HIS3, lys2::Δ385, Δpho5::URA3</i>	This study
YAG70	<i>MATa, ura3-52, his3-Δ200, leu2Δ1, Δgal4::LEU2, pRY131Δ2μ::URA3</i>	This study
YAG71	<i>MATa, ura3-52, his3-Δ200, leu2Δ1, Δswi1::LEU2, Δgal4::LEU2, pRY131Δ2μ::URA3</i>	This study
YS18	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5</i>	Svaren et al., 1994
YS33	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, Δpho4::ura3Δ5, Δpho80::HIS3</i>	Svaren et al., 1994
YS185	<i>MATα, his3-11, his3-15, leu2-3, leu2-112, can^R, ura3Δ5, lys2::URA3, Δgal11::LYS2</i>	This study

were labeled by the random primer method (Feinberg and Vogelstein, 1983).

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