

## SWI-SNF Complex Participation in Transcriptional Activation at a Step Subsequent to Activator Binding

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**The SWI-SNF complex in yeast and related complexes in higher eukaryotes have been implicated in assisting gene activation by overcoming the repressive effects of chromatin. We show that the ability of the transcriptional activator GAL4 to bind to a site in a positioned nucleosome is not appreciably impaired in *swi* mutant yeast cells. However, chromatin remodeling that depends on a transcriptional activation domain shows a considerable, although not complete, SWI-SNF dependence, suggesting that the SWI-SNF complex exerts its major effect at a step subsequent to activator binding. We tested this idea further by comparing the SWI-SNF dependence of a reporter gene based on the *GAL10* promoter, which has an accessible upstream activating sequence and a nucleosomal TATA element, with that of a *CYC1-lacZ* reporter, which has a relatively accessible TATA element. We found that the *GAL10*-based reporter gene showed a much stronger SWI-SNF dependence than did the *CYC1-lacZ* reporter with several different activators. Remarkably, transcription of the *GAL10*-based reporter by a GAL4-GAL11 fusion protein showed a nearly complete requirement for the SWI-SNF complex, strongly suggesting that SWI-SNF is needed to allow access of TFIID or the RNA polymerase II holoenzyme. Taken together, our results demonstrate that chromatin remodeling *in vivo* can occur by both SWI-SNF-dependent and -independent avenues and suggest that the SWI-SNF complex exerts its major effect in transcriptional activation at a step subsequent to transcriptional activator-promoter recognition.**

Transcriptional activation in eukaryotes requires activator proteins and the preinitiation complex, including TATA-binding protein (TBP) and RNA polymerase, to recognize their respective binding sites in a gene promoter. Each of these interactions can be strongly inhibited *in vitro* by incorporating the relevant recognition site into a nucleosome (20, 26, 37, 44, 51, 53, 59, 78, 84). Since eukaryotic DNA is packaged into nucleosomes, this creates a potential problem. One solution would be for eukaryotic promoters to be constitutively free of nucleosomes, but this is not the case (79). Another would be for the cell to have evolved chromatin remodeling activities which would allow activators and other components of the preinitiation complex to recognize their sites in chromatin. Several candidate activities have been identified (9, 12, 21, 28, 35, 73–75, 80), but the roles they play *in vivo* are poorly understood.

One candidate for helping transcriptional activation occur in the context of chromatin *in vivo* is the SWI-SNF complex, first identified in the yeast *Saccharomyces cerevisiae*. This large complex, of about  $2 \times 10^6$  Da, contains at least 11 distinct polypeptides (8, 61, 72) and is conserved across most of the eukaryotic kingdom (60, 80). Transcriptional induction of a variety of genes is impaired in yeast cells lacking components of the SWI-SNF complex (62, 87), and genetic and biochemical evidence suggests that the SWI-SNF complex may aid transcriptional induction by helping to counteract the repressive effects of packaging DNA into chromatin. Suppressors of the *swi* phenotype in yeast include deletion of one of the two sets of genes encoding histones H2A and H2B, as well as point mutations in histones H3 and H4 that increase the accessibility of nucleosomal DNA (25, 38, 63, 81, 87). Furthermore, the

chromatin structure of the active *SUC2* promoter, whose activity is strongly SWI-SNF dependent, is altered in *swi* cells but is partially restored in suppressor mutants (18a, 25, 86). Finally, *in vitro* studies have shown that the yeast SWI-SNF complex or its mammalian homolog can alter nucleosomal structure in a way that facilitates the binding of derivatives of the transcriptional activator GAL4 to nucleosomal sites (14, 80). Although the ratio of SWI-SNF to nucleosomes in these experiments was vastly greater than that found *in vivo*, this work nonetheless demonstrated that the SWI-SNF complex can alter nucleosome structure in a way that facilitates protein binding. A key finding was that this alteration was ATP dependent (14), since SWI2-SNF2, a critical component of the complex, has ATPase activity that is essential for its *in vivo* activity (34, 43).

The SWI-SNF complex could support transcriptional induction in chromatin by facilitating factor binding, as suggested by the *in vitro* experiments in which SWI-SNF assists the binding of GAL4 derivatives to nucleosomal sites (14, 80). Alternatively, it could assist subsequent steps such as recruitment of TATA-binding protein (26) or RNA polymerase, as suggested by a report that SWI-SNF proteins are associated with the RNA polymerase II holoenzyme (82). It could even assist in elongation through nucleosomes (5), although if this were its only point of action, it is difficult to understand how a tethered SWI-SNF component could activate transcription (41, 42).

We have previously shown that GAL4 can bind to its recognition sequence in a nucleosome in yeast with concomitant nucleosome disruption (55, 69). We have also shown that perturbation of nucleosome positioning can depend on the presence of an activation domain (69). Here we assess whether either of these events requires participation by the SWI-SNF complex. Our results suggest that in genes requiring SWI-SNF for maximal activation, the SWI-SNF complex exerts its effect principally at a step that occurs subsequent to transcriptional activator binding. By inference, the results also suggest that

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

Strain	Genotype	Reference
FY24	<i>MAT<math>\alpha</math> ura3-52 trp1<math>\Delta</math>63 leu2<math>\Delta</math>1</i>	83 (isogenic to FY23)
YNN282	<i>MAT<math>\alpha</math> trp1-<math>\Delta</math> his3-<math>\Delta</math>200 ura3-52 lys-801<sup>a</sup> ade2-1<sup>o</sup></i>	65
CY296	<i>MAT<math>\alpha</math> gal4<math>\Delta</math>::LEU2 lys2-801 ade2-101 leu2-<math>\Delta</math>1 his3-<math>\Delta</math>200 ura3-<math>\Delta</math>99 trp1-<math>\Delta</math>99</i>	7
CY297	<i>MAT<math>\alpha</math> gal4<math>\Delta</math>::LEU2 swi1<math>\Delta</math>::LEU2 lys2-801 ade2-101 leu2-<math>\Delta</math>1 his3-<math>\Delta</math>200 ura3-<math>\Delta</math>99</i>	7
CY297b	<i>MAT<math>\alpha</math> gal4<math>\Delta</math>::LEU2 swi1<math>\Delta</math>::LEU2 lys2-801 ade2-101 leu2-<math>\Delta</math>1 his3-<math>\Delta</math>200 ura3-<math>\Delta</math>99 trp1</i>	This work

some other chromatin-remodeling activity is likely to assist in the initial binding of transcriptional activators to their recognition sites in at least some promoters.

#### MATERIALS AND METHODS

**Strains, media, and genetic methods.** The *S. cerevisiae* strains used are derivatives of S288C and are listed in Table 1. Strain CY297b was derived from CY297 (7) by two-step deletion of the *TRP1* gene (1). The yeast strains were grown at 30°C in complete synthetic dropout media (Bio 101) (60) containing 2% glucose, 1.5% raffinose, or 2% galactose and were transformed by a modification (24) of the method of Ito et al. (27).

**Plasmids.** The episome TA17 $\Delta$ 80 was introduced into yeast by first excising bacterial sequences from two pUC19-based plasmids carrying complementary pieces of TA17 $\Delta$ 80 and ligating those pieces, as described previously (55). TALS was introduced after excision of the complete yeast sequence from a pUC19 vector and religation (65).

To create the *lacZ* reporter plasmid 314-17 $\Delta$ 80lacZ $\Delta$ Nco (bearing the reporter gene *GAL4-CYC1-lacZ*), a *PstI* fragment encompassing the *lacZ* gene and *CYC1* TATA box from plasmid pLG669-Z (23) was ligated into the *PstI* site of pRS104-17 $\Delta$ 80 (55) at 823 map units of the TRP1ARS1 episome; the *KpnI-HindIII* fragment of the resulting plasmid containing the *lacZ* gene and TRP1ARS1 sequences was then inserted into the polylinker of the CEN-containing shuttle vector pRS314 (67) to create pRS314-17 $\Delta$ 80lacZ. Finally, the *URA3* gene contained in this plasmid was removed by excision of a 1.7-kb *NcoI* fragment to yield pRS314-17 $\Delta$ 80lacZ $\Delta$ Nco. The *GAL4* binding site is 400 bp upstream of the first *lacZ* ATG.

The *GAL10-MEL1* fusion gene was carried on plasmid pBM150SKMEL1. The *MEL1* gene coding sequences were amplified with primers 5'-ATAATTTCTTA CTGGATCCTAGGAGAGCAACGGAAATTCAAAGCAA-3' and 5'-AAAATT GAAGAGAATTCGGGCAAAAATTTGGTACCAATGCATCCAA-3'. Restriction sites (*Bam*HI and *Eco*RI or *Eco*RI and *Kpn*I; underlined bases) were introduced to facilitate cloning. The PCR product was digested with *Eco*RI, which removes the *MEL1* transcription start site, and the fragment was cloned into pBluescript (SK-) to generate SKMEL1. To confirm that *MEL1* coding sequences in SKMEL1 were functional, a *Bam*HI-*Kpn*I fragment from SKMEL1 was subcloned into a yeast expression vector and the resulting plasmid was transformed into yeast. SKMEL1 plasmids having functional *MEL1* coding sequences were isolated as *Eco*RI fragments and cloned into pBM150 (29) to generate pBM150SKMEL1.

The reporter plasmids carrying *GAL4-CYC1-lacZ* and *GAL10-MEL1* were introduced into CY296 and CY297b (Table 1) along with expression vectors for *GAL4-ftz*<sup>(3-413)</sup> (17), *GAL4-ER-VP16* (48, 69), or *GAL4-GAL11*. The *GAL4* gene was introduced as a 3.6-kb *Bam*HI fragment (40) carried on the CEN-containing shuttle vector pRS416 (13) in cells harboring the *GAL4-CYC1-lacZ* reporter, or on the 2 $\mu$ m plasmid pRS426 (13) in cells harboring the *GAL10-MEL1* reporter. To construct the expression plasmid for *GAL4-GAL11*, the coding sequence for amino acids 1 to 93 of *GAL4* (which includes the DNA-binding and dimerization domains) fused to the *ADH1* promoter was amplified from pRS313GAL4-ER-VP16 (48) with primers 5'-ACACTTGAGCTCGTCCGACTGACCCGGGCAATGCTTTTATATC-3' and 5'-CAAGGTACCAATAAA TGATGGTAAATG-3'. The PCR product was digested with *Kpn*I and *Sac*I (underlined) and ligated into pRS416 (13) to yield pRS416-GAL4DBD. The coding sequence for *GAL11* amino acids 799 to 1081 (4) was amplified with primers 5'-AGGTCTGAGCTCAATACCGCTAAGTCAACC-3' and 5'-AGGT TCACCGAGCTCTCAGCACTAGCTAACC-3'. The resulting fragment was cut with *Sac*I (underlined sites) and ligated with *Sac*I-cut pRS416-GAL4DBD. Clones with the correct orientation of the *GAL11*-encoding sequences were identified and subcloned into pRS413 (13).

**$\beta$ -Galactosidase and  $\alpha$ -galactosidase (*MEL1*) assays.** Cells harboring *lacZ* reporters were grown to an absorbance at 600 nm ( $A_{600}$ ) between 0.5 and 1.5 in galactose medium for *GAL4* activation or in glucose medium for activation by *GAL4-ER-VP16*, *GAL4-ftz*, and *GAL4-GAL11*; for activation by *GAL4-ER-VP16*, 0.1  $\mu$ M  $\beta$ -estradiol was added and growth was continued for 4 h before assaying for  $\beta$ -galactosidase activity as described previously (52, 64). To assay for  $\alpha$ -galactosidase (the *MEL1* gene product) activity, we combined features of an assay involving cell extracts (30, 33) and the commonly used permeabilized cell assay used to measure  $\beta$ -galactosidase activity (64). Yeast cultures (5 to 10 ml) were grown in galactose (for *GAL4*) or raffinose (for *GAL4-ER-VP16*, *GAL4-*

*ftz*, and *GAL4-GAL11*) to an  $A_{600}$  between 0.5 and 1.5. A 1-ml volume of cells was centrifuged, and the pellet was taken up in 200  $\mu$ l of 20 mM HEPES (pH 7.5)–10 mM dithiothreitol–0.002% sodium dodecyl sulfate. One or two drops of chloroform was added with a Pasteur pipette, and the sample was vortexed for 10 s. After a 5-min preequilibration at 30°C, 800  $\mu$ l of a 7 mM solution of *p*-nitrophenyl- $\alpha$ -galactose (Sigma) in 61 mM citric acid–77 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 4) was added and incubation at 30°C was continued. At various times, 100- $\mu$ l aliquots were added to 900  $\mu$ l of 0.1 M Na<sub>2</sub>CO<sub>3</sub> to stop the reaction and develop the yellow color characteristic of the cleaved *p*-nitrophenyl- $\alpha$ -galactose (which remains colorless at pH 4). The terminated reaction products were centrifuged for 5 min in a microcentrifuge, and the  $A_{400}$  was determined. Units of  $\alpha$ -galactosidase activity are given as [ $A_{400}/(A_{600} \times \text{time in minutes})] \times 1,000$ , using samples taken at time points such that the  $A_{400} < 3.0$ .

**Indirect end-label analysis of plasmid chromatin.** Yeast nuclei (66) were prepared from 1 liter of cells grown to an  $A_{600}$  between 0.6 and 1.6, and 300- $\mu$ l aliquots were digested with micrococcal nuclease (MNase) for 10 min at 37°C at concentrations varying from 0 to 50 U/ml. Alternatively, spheroplast lysates (32) were prepared from 100 to 200 ml of cells and treated for 5 min at 37°C with 0 to 400 U of MNase per ml. The cleavage patterns did not vary within this range of concentrations. For digestion of naked DNA, samples were treated with proteinase K, extracted with phenol-chloroform, taken up in 300  $\mu$ l of 150 mM NaCl–5 mM KCl–1 mM EDTA–20 mM Tris.HCl (pH 8.0)–2 mM CaCl<sub>2</sub>–5 mM MgCl<sub>2</sub> or in 300  $\mu$ l of 10 mM HEPES (pH 7.5)–2 mM CaCl<sub>2</sub>–5 mM MgCl<sub>2</sub>, and digested as above. The digestions were stopped by the addition of 55  $\mu$ l of 5% sodium dodecyl sulfate–5 mg of proteinase K per ml to the 300- $\mu$ l reaction mixtures and processed as described previously (55, 69). MNase cleavage patterns were analyzed by indirect end labeling as previously described (55, 69). The probes used were the 231-bp *EcoRV-HindIII* fragment (see Fig. 1) and the 200-bp *EcoRV-XbaI* fragment (see Fig. 2) from TRP1ARS1, both prepared by PCR; the 276-bp *Bam*HI-*Sall* fragment from pBM150SKMEL1 (see Fig. 4); and a 252-bp *PvuII-Clal* fragment from pRS314 (see Fig. 5). The probes were labeled by random priming. Marker lanes contained 0.5 ng of a  $\Phi$ X/HaeIII digest, and labeled  $\Phi$ X/HaeIII DNA was included with the hybridization probe. Chromatin was prepared from at least two independent clones for all MNase analyses.

**Topoisomer analysis.** DNA from three independent clones was prepared as described previously (54) from 10-ml cultures grown to an  $A_{600}$  of 0.6 to 1.2 by rapid glass bead lysis. Purified DNA was electrophoresed at 2.5 V/cm for 18 to 20 h on 1.5% agarose gels containing 40  $\mu$ g of chloroquine diphosphate (Sigma) per ml, transferred to nylon membranes, and hybridized with probes specific for TRP1ARS1 sequences as described above. The Gaussian centers of the topoisomer distributions were calculated as described previously (54) with images scanned on a Molecular Dynamics PhosphorImager. Topological changes in *swi1 $\Delta$*  cells were measured in strain CY297b; changes in *SWI1*<sup>+</sup> cells were measured in FY24 cells for *GAL4*-induced changes and in YNN282 cells for *GAL4-ER-VP16*-induced changes. Topological changes induced by *GAL4* and *GAL4-ER-VP16* are identical between YNN282 and FY24 cells (reference 69 and data not shown).

## RESULTS

**Activator binding to nucleosomal sites in *swi1 $\Delta$*  yeast cells.** To test whether the SWI-SNF complex is required for binding of a transcriptional activator to a nucleosomal site in yeast, we examined the disruption of nucleosome positioning by *GAL4* binding in the yeast episome TA17 $\Delta$ 80. This plasmid contains a *TRP1* marker and a single strong *GAL4*-binding site of 17 bp, which is located near the middle of a strongly positioned nucleosome when *GAL4* is absent or present in very small amounts. The growth of cells in galactose, which induces *GAL4* expression, or exogenous expression of *GAL4* or its derivatives results in perturbation of this nucleosome by *GAL4* (55).

We introduced TA17 $\Delta$ 80 into *SWI1*<sup>+</sup> and *swi1 $\Delta$*  yeast strains, both derived from the parent strain S288C. *SWI1* is essential to SWI-SNF complex integrity, since the complex cannot be iso-

lated intact from *swi1Δ* cells (61); furthermore, *swi1Δ*, *swi2Δ*, and *swi3Δ* cells have very similar phenotypes, indicating that loss of any of these proteins inactivates the complex (62). Nucleosome positioning in TA17Δ80 was examined in *SWI<sup>+</sup>* and *swi1Δ* yeast cells grown in glucose or galactose by MNase cleavage followed by indirect end labeling (56, 85). Two regions of about 150 bp are cleaved by MNase as naked DNA but protected against cleavage in chromatin from yeast cells grown in glucose, indicating the presence of two positioned nucleosomes, one of which includes the GAL4-binding site (Fig. 1A, lanes 1 to 5) (55). These regions show enhanced cleavage by MNase in *SWI<sup>+</sup>* cells grown in galactose (lanes 6 and 7), indicating that GAL4 is able to compete successfully against the histones for occupancy of its site, as observed previously (55). Protection against MNase cleavage is also seen in *swi1Δ* cells grown in glucose, indicating that nucleosome positioning is not generally affected by the SWI-SNF complex (lane 8; we note that the site between nucleosomes I and II is cleaved very weakly in this sample, but this site shows some variability in its cleavage from sample to sample and in general is not cleaved more weakly in *swi1Δ* yeast cells than in wild-type cells). In galactose, MNase cleavages in the regions protected by nucleosomes I and II in glucose are observed (lanes 9 and 10). The MNase cleavage pattern from cells grown in galactose is very similar between *SWI<sup>+</sup>* and *swi1Δ* cells (Fig. 1B), and we have found this to be reproducibly the case. We conclude that the binding of GAL4 to a nucleosomal binding site, with concomitant chromatin remodeling, can occur independently of the SWI-SNF complex in vivo.

GAL4 synthesis is also stimulated by growth in raffinose (by relief of glucose repression [22, 57]); however, in the absence of galactose, the GAL4 activation domain is masked by GAL80 and it cannot activate transcription (31, 50). GAL4 produced by growth in raffinose can also perturb nucleosome positioning in TA17Δ80 in both wild-type and *swi1Δ* yeast cells (lanes 11 to 18). Perturbation of TA17Δ80 chromatin by GAL4 in raffinose is somewhat weaker than in galactose for both *SWI<sup>+</sup>* and *swi1Δ* yeast cells, consistent with previous results obtained with *SWI<sup>+</sup>* yeast cells (55). Thus, binding of the transcription factor GAL4 in both its activating and nonactivating configurations can occur at a nucleosomal site, with concomitant nucleosome perturbation, in yeast cells lacking a functional SWI-SNF complex.

**Role of the SWI-SNF complex in activation domain-dependent chromatin remodeling.** Although the SWI-SNF complex is not needed for GAL4 to gain access to a strong binding site in chromatin in vivo (Fig. 1), this does not imply that chromatin remodeling by the SWI-SNF complex is not important for transcriptional activation. Other proteins, including TBP and RNA polymerase, must also bind their sites in chromatin, and they could require chromatin remodeling mediated by the SWI-SNF complex to do so. To examine whether chromatin perturbation occurring subsequent to GAL4 binding is assisted by the SWI-SNF complex, we took advantage of our recent observations of activator-dependent changes in chromatin structure in another yeast episome, TALS (69).

The TALS episome is packaged into strongly positioned nucleosomes in yeast haploid  $\alpha$  cells (36, 65, 66). TALS also contains a GAL4-binding site derived from the *GAL3* promoter, although the region of the *GAL3* promoter downstream of this site is absent (3); this GAL4-binding site is packaged into a nucleosome in yeast  $\alpha$  cells grown in glucose (36, 65, 66). Using the chimeric, hormone-dependent activator, GAL4-ER-VP16, we have shown that the chromatin structure of the episome is only minimally affected in the absence of hormone (69). The addition of  $\beta$ -estradiol in the presence of GAL4-ER-VP16 results in remodeling of TALS chromatin, as as-

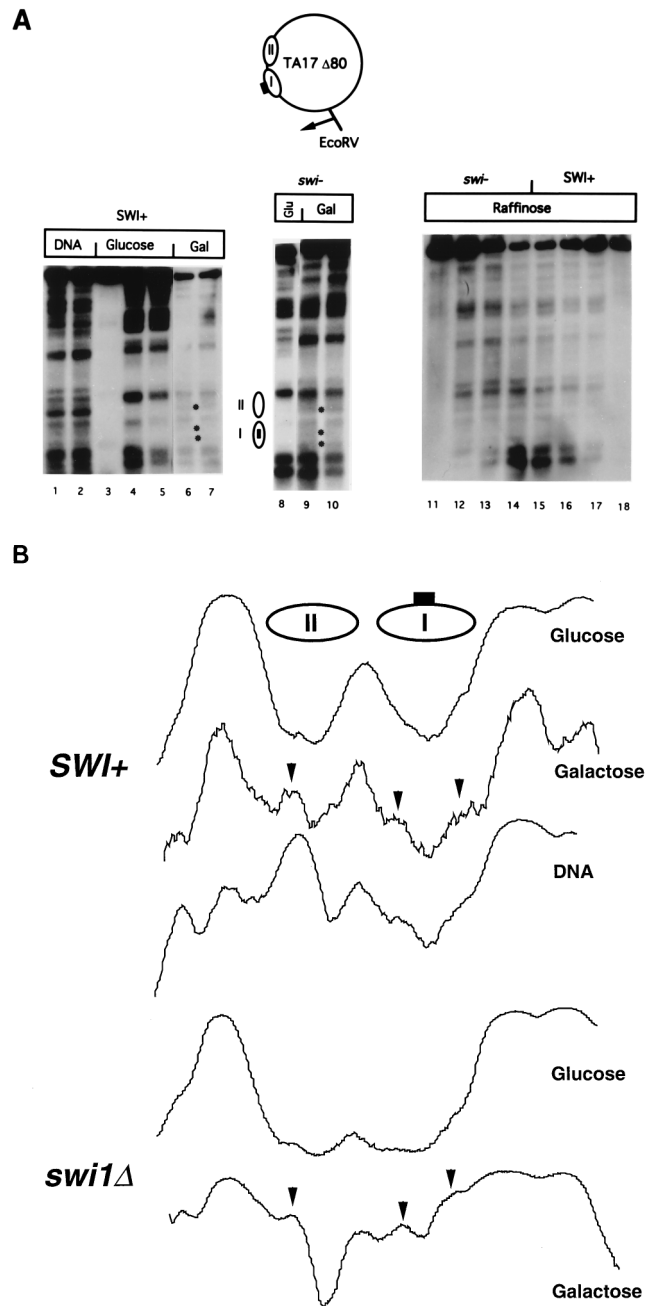


FIG. 1. Perturbation of positioned nucleosomes in the yeast episome TA17Δ80 by GAL4 binding in *SWI<sup>+</sup>* and *swi1Δ* yeast cells. (A) MNase cleavage sites in chromatin from *SWI<sup>+</sup>* and *swi1Δ* cells grown in glucose, raffinose, or galactose medium were mapped relative to the *EcoRV* site as indicated. Samples were digested with MNase at 4 U/ml (lane 1), 10 U/ml (lane 2), 0 U/ml (lanes 3, 11, and 18), 2 U/ml (lanes 4, 6, 12, and 17), 5 U/ml (lanes 5, 7, 8, 10, 13, and 16), or 20 U/ml (lanes 9, 14, and 15). Bands cleaved in chromatin from cells grown in glucose are marked by asterisks to the right of lanes 6 and 9. Locations of nucleosomes I and II in cells grown in glucose are indicated; the box in nucleosome I represents the GAL4-binding site. (B) Densitometric scans of MNase cleavage patterns in the vicinity of nucleosomes I and II. Scans represent, in descending order, lanes 4, 6, 1, 8, and 10 in panel A. Arrowheads indicate cleavage sites induced in galactose. Note that the cleavage sites in the region of nucleosome I, which are cleaved weakly in chromatin from cells grown in galactose, are also weakly cut in naked DNA.

essed by MNase cleavage, restriction endonuclease accessibility, and topological analysis. Similarly, the endogenous activator GAL4 only slightly affects TALS chromatin structure under nonactivating conditions but has considerable effect under activating conditions (69). Remodeling depends on the presence of both the activator and the GAL4-binding site.

We detected remodeling of TALS chromatin by GAL4·ER·VP16 upon addition of hormone in both *SWT*<sup>+</sup> and *swi1Δ* cells as determined by alterations in the MNase cleavage pattern (Fig. 2). Perturbation is seen in the regions flanking nucleosome IV, which contains the GAL4-binding site. First, the relative intensity of the two cleavage sites between nucleosomes IV and V changes upon hormone addition, with cleavage at the site proximal to nucleosome IV being relatively enhanced in the presence of hormone (Fig. 2A, compare lanes 5 to 7 with lanes 2 to 4 for *SWT*<sup>+</sup> cells and compare lanes 11 and 12 with lanes 9 and 10 for *swi1Δ* cells; Fig. 2B). Second, a new site is cleaved at the edge of nucleosome III in the presence of hormone at the expense of the cleavage site between nucleosomes III and IV (Fig. 2). Similar changes were observed in both *SWT*<sup>+</sup> and *swi1Δ* cells when GAL4 was used as the activator (data not shown). Since the GAL4-binding site in TALS is nucleosomal in the absence of GAL4 (69), these results again indicate that GAL4 (and GAL4·ER·VP16) can bind to a nucleosomal site without help from the SWI-SNF complex, corroborating the results of Fig. 1. Furthermore, these results indicate that activation domain-dependent changes in chromatin structure can occur in yeast in the absence of a functional SWI-SNF complex.

Although TALS chromatin is clearly perturbed by GAL4·ER·VP16 in the presence of β-estradiol in *swi1Δ* cells, there were some indications that this perturbation was less dramatic than in *SWT*<sup>+</sup> cells. For example, we often observed nearly complete replacement of the cleavage site between nucleosomes III and IV with the new site at the edge of nucleosome III in *SWT*<sup>+</sup> cells in the presence of hormone-activated GAL4·ER·VP16, whereas this shift was reproducibly seen only partially in *swi1Δ* cells (Fig. 2B, compare the shift in the peak indicated by the dotted line in traces derived from *SWT*<sup>+</sup> and *swi1Δ* cells). However, these are subtle changes and are certainly not subject to quantitative analysis. Therefore, to obtain a more quantitative comparison of activator-dependent changes in TALS chromatin structure in *swi* and *SWT*<sup>+</sup> cells, we examined plasmid topology.

Since each nucleosome confers one negative supercoil on closed circular DNA, nucleosome loss results in an increased linking number (19, 68). We previously demonstrated that both GAL4 and GAL4·ER·VP16, in their activate configurations, cause an average increase in linking number (loss of negative supercoiling) of 0.7 per molecule of TALS (69). To compare this effect on TALS topology in *SWT*<sup>+</sup> and *swi1Δ* cells, DNA was rapidly harvested from the appropriate cells under activating and nonactivating conditions (i.e., glucose versus galactose for GAL4 and plus or minus hormone for GAL4·ER·VP16) and treated to inactivate topoisomerases (so that the distribution of supercoiled plasmids reflected the *in vivo* distribution). The DNA was electrophoresed in agarose containing 40 μg of chloroquine diphosphate per ml, conditions under which the plasmids migrate as positively supercoiled topoisomers. These topoisomer distributions conform to Gaussian distributions, which allows their centers to be measured precisely from the relative intensities of individual topoisomers (54). Consequently, differences in topology can also be measured with precision. Quantitation of several independent experiments yielded values for the loss of negative supercoiling in TALS in *SWT*<sup>+</sup> and *swi1Δ* cells (summarized in Fig. 3). The

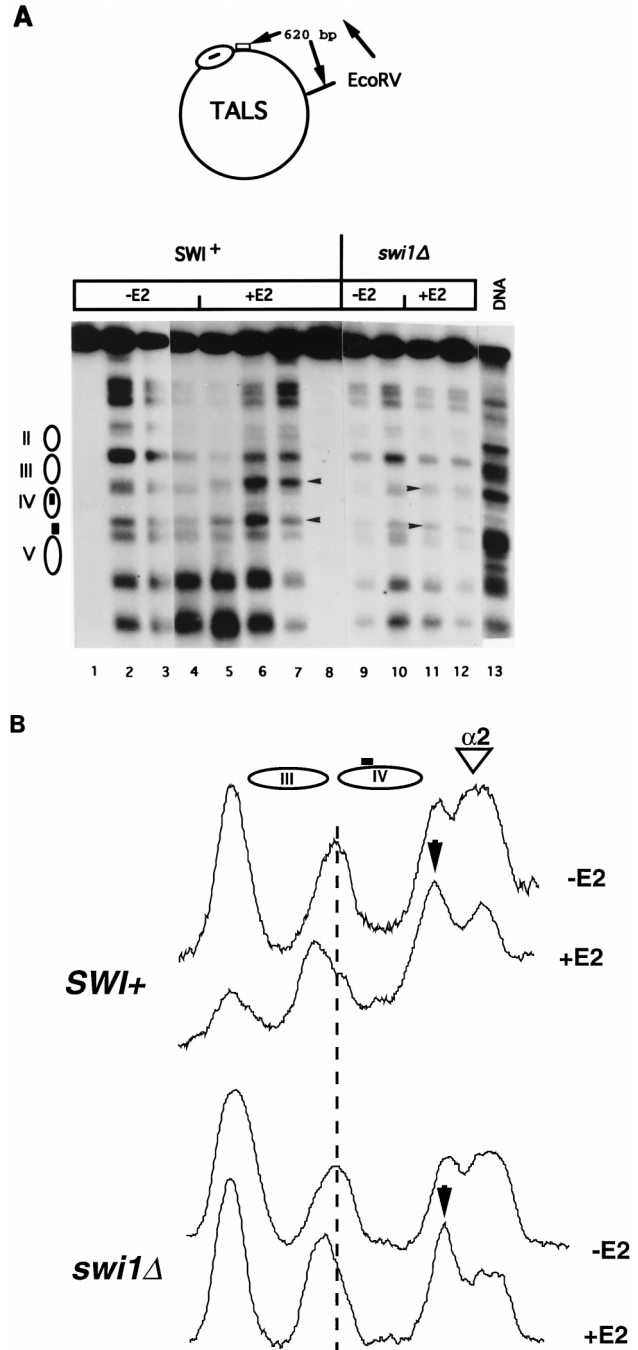


FIG. 2. (A) Perturbation of TALS chromatin by the GAL4·ER·VP16 activation domain in *SWT*<sup>+</sup> and *swi1Δ* yeast haploid α cells. MNase cleavage sites in cells harboring TALS and expressing GAL4·ER·VP16, either incubated for 4 h with 0.1 μM β-estradiol (+E2 lanes) or not (-E2 lanes), were mapped relative to the *EcoRV* site as indicated. Samples were digested with MNase at 0 U/ml (lanes 1 and 8), 5 U/ml (lanes 2 and 7), 20 U/ml (lanes 3, 6, 9, and 12), 50 U/ml (lanes 4, 5, 10, and 11), or 10 U/ml (lane 13). Control samples (0 U/ml) for the *swi1Δ* samples were identical in appearance to those shown for the *SWT*<sup>+</sup> samples (data not shown). Arrowheads indicate bands cleaved preferentially under activating (+E2) conditions. The locations of nucleosomes II to V are indicated; the box in nucleosome IV represents the GAL4-binding site, and the box between nucleosomes IV and V represents the α2/MCM1 operator. (B) Densitometric scans of MNase cleavage patterns from lanes 4, 5, 10, and 11. The arrowhead indicates the lower of the two hormone-induced enhanced cleavage sites seen in the panel A, and the dotted line allows visualization of the slight shift in position of the cleavage site corresponding to the upper arrowhead in panel A.

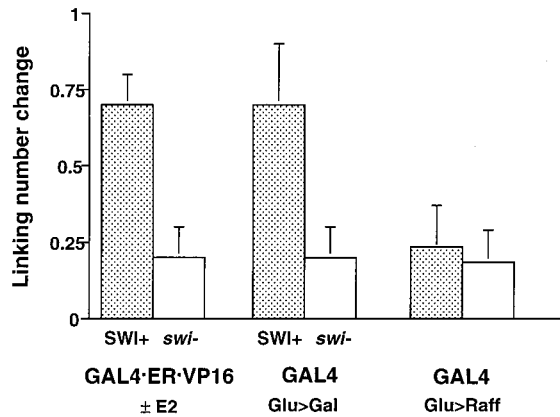


FIG. 3. Topological perturbation of TALS chromatin in *SWI*<sup>+</sup> and *swi1* $\Delta$  yeast cells by GAL4 and by GAL4·ER·VP16. Linking-number changes induced by hormone addition in the presence of GAL4·ER·VP16 or by growth in galactose (Gal) or raffinose (Raff) compared to glucose (Glu) in the presence of the endogenous *GAL4* gene are indicated along with standard errors.

hormone-dependent alteration in TALS topology in the presence of GAL4·ER·VP16 is substantially reduced in *swi1* $\Delta$  cells (Fig. 3,  $P < 0.001$  for the null hypothesis that the difference between *SWI*<sup>+</sup> and *swi1* $\Delta$  cells is not significant [49]). Similarly, alteration of TALS topology by GAL4 under activating conditions is reduced in *swi1* $\Delta$  compared to *SWI*<sup>+</sup> cells (Fig. 3,  $P < 0.01$ ). Under nonactivating conditions (cells grown in raffinose), a small change in topology is seen in both *SWI*<sup>+</sup> and *swi1* $\Delta$  cells (Fig. 3), possibly indicating that this effect is due to SWI-SNF-independent binding of nonactivating GAL4 complexed with GAL80. Taken together, the results of Fig. 2 and 3 indicate that SWI-SNF contributes to but is not absolutely required for activator-dependent remodeling of TALS chromatin. This conclusion is consistent with the partial dependence upon the SWI-SNF complex for transcriptional activation observed in previous studies (7, 18, 25, 42, 62) as well as in the present work (see Fig. 6).

**SWI-SNF dependence of two promoters differing in chromatin structure at proximal elements.** The results presented so far suggest that an activator can bind to a strong binding site in chromatin independently of the SWI-SNF complex, whereas chromatin remodeling occurring subsequent to transcriptional activator binding depends at least partly on SWI-SNF. We would therefore predict that a promoter with an organized chromatin structure that includes the TATA element and transcription start site would show a relatively strong SWI-SNF dependence compared to a promoter having more accessible proximal elements. We would also predict that a promoter having an accessible upstream activating sequence (UAS) could show a strong SWI-SNF dependence if its proximal elements were present in positioned nucleosomes. In contrast, if the primary function of the SWI-SNF complex were to allow activator access to chromatin, such a promoter should not show any SWI-SNF dependence at all.

To test this idea, we compared transcription from two promoters in *SWI*<sup>+</sup> and *swi1* $\Delta$  yeast cells. One reporter gene was a GAL4-*CYC1-lacZ* fusion, having a single strong binding site for GAL4 upstream of the *CYC1* promoter sequence and a *lacZ* coding sequence, and the other was a fusion of the *GAL10* promoter to the *MEL1* coding sequence. The native *GAL10* promoter contains four GAL4-binding sites in its UAS (which is shared with the *GAL1* promoter) in a nucleosome-free region, but downstream sequences, including the TATA ele-

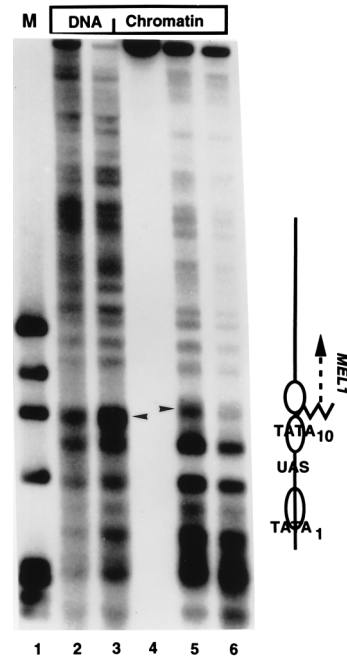


FIG. 4. Indirect end-label analysis of the chromatin structure of the *GAL10-MEL1* gene fusion. MNase cleavage sites in naked DNA or chromatin, as indicated, were mapped relative to a *SalI* site 830 bp upstream of the *GAL10* TATA element, on the *GAL1* side of the promoter in plasmid pBM150SKMEL1. Samples were digested with 4 (lane 2), 10 (lane 3), 0 (lane 4), 150 (lane 5) or 300 (lane 6) U of MNase per ml. Note the strong cleavage in naked DNA (arrowhead, lanes 2 and 3), which is protected in chromatin; a new cleavage (arrowhead, lanes 5 and 6) is present slightly higher on the gel, corresponding to the nucleosome-sized protected region containing the *GAL10* TATA element. The overall pattern of cleavages in the vicinity of the UAS and *GAL10* TATA is identical to that seen in the endogenous *GAL1-10* promoter (data not shown). The locations of relevant promoter elements are indicated on the right; the jagged line represents the site of the fusion between the *GAL10* promoter and the *MEL1* coding sequence. Lane 1 contains  $\Phi$ X/*Hae*III markers.

ment, are packaged in a highly organized array of positioned nucleosomes (10, 46, 47). We fused the *GAL10* promoter with the *MEL1* coding sequence, which encodes  $\alpha$ -galactosidase. The activity of this enzyme can be quantified by a simple colorimetric assay (see Materials and Methods), similar to that commonly used for  $\beta$ -galactosidase. An indirect end-label assay of MNase cleavage sites revealed that the chromatin structure of the *GAL10* promoter is well preserved in the *MEL1* fusion (Fig. 4). The accessible UAS is not cut by MNase in either chromatin or naked DNA (the *GAL10* UAS is cut by DNase I in chromatin and naked DNA [46]) and is flanked by protected regions of about 150 bp, characteristic of positioned nucleosomes. The *GAL10* TATA element is near the center of such a protected region, and a *Ban*II site 23 bp from the TATA element is also strongly protected in chromatin (data not shown). In contrast, one of the two major TATA elements of the *CYC1* promoter (45) in the GAL4-*CYC1-lacZ* reporter gene is in a region highly accessible to MNase (Fig. 5).

We examined transcription from the *GAL10-MEL1* and GAL4-*CYC1-lacZ* reporter genes in *SWI*<sup>+</sup> and *swi1* $\Delta$  cells by using several different activation domains fused to the GAL4 DNA-binding domain as activators, including the native activator GAL4. Figure 6 shows that neither GAL4, GAL4·ER·VP16, nor GAL4-ftz, which differ substantially in their ability to activate transcription, exhibit strong SWI-SNF dependence at the GAL4-*CYC1-lacZ* promoter. In contrast, the ability of GAL4 to activate transcription from the *GAL10-MEL1* fusion

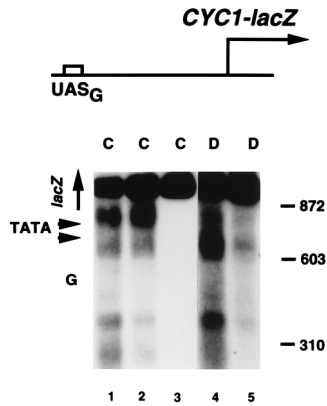


FIG. 5. Indirect end-label analysis of chromatin structure of 314-17Δ80lacZ ΔNco, which bears the GAL4-CYC1-lacZ reporter gene. MNase cleavage sites in chromatin (lanes C) and DNA (lanes D) were mapped relative to a PvuII site that is 400 bp upstream of the GAL4-binding site (UAS<sub>G</sub>). Cell lysates (32) or DNA was digested with MNase at 20 U/ml (lane 1), 5 U/ml (lane 2), 0 U/ml (lane 3), 4 U/ml (lane 4), or 10 U/ml (lane 5). The location of the GAL4 site is indicated by G, and the locations of the two major TATA elements in the CYC1 promoter (45) and the lacZ gene are also indicated. Lanes 1 to 3 and lanes 4 and 5 were taken from separate gels which ran identically (as shown by size markers).

gene was reduced about fourfold in *swi1Δ* relative to *SWI*<sup>+</sup> yeast cells, consistent with previous work showing that *GAL10* mRNA levels are reduced several fold in *swi1Δ* yeast cells (62). Even greater (8- to 12-fold) reduction in α-galactosidase ac-

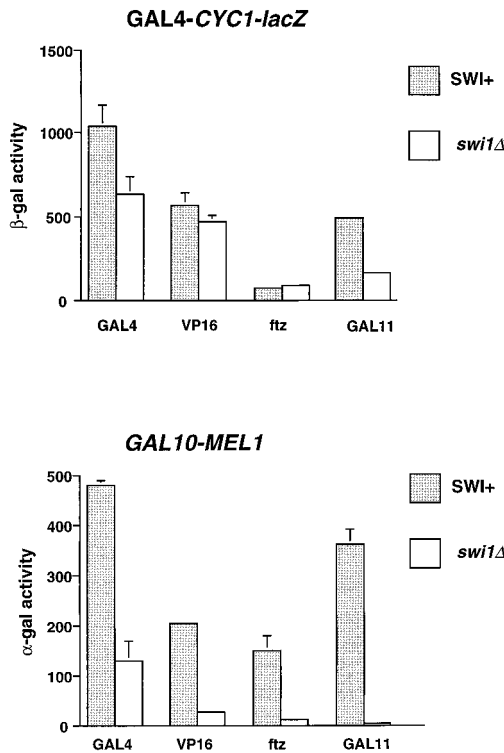


FIG. 6. Activity of the two reporter genes, GAL4-CYC1-lacZ and GAL10-MEL1, induced by different activation domains in *SWI*<sup>+</sup> (strain CY296) and *swi1Δ* (strain CY297b) cells. The activators used were GAL4, GAL4-ER-VP16, GAL4-ftz, and GAL4-GAL11. Activities were measured for at least three independent clones for each sample. Some standard errors were too small for the error bars to be seen in the graphs.

tivity from *GAL10-MEL1* was seen in *swi1Δ* cells with GAL4-ER-VP16 and GAL4-ftz, indicating that the reduction in activity in *swi1Δ* cells seen with GAL4 was not due to indirect effects on genes required for galactose induction (2). These results indicate that a promoter having an accessible UAS and highly ordered chromatin structure around the TATA element and transcription start site (*GAL10-MEL1*) shows a stronger SWI-SNF dependence than one having an accessible proximal promoter region.

We also examined transcription from these two promoters by recruiting the RNA polymerase II holoenzyme via a GAL4-GAL11 fusion protein (4, 15). Transcriptional activation of the GAL4-CYC1-lacZ reporter by GAL4-GAL11 was comparable to activation by GAL4, consistent with the results of earlier work (4, 15, 18), and was reduced about threefold in *swi1Δ* compared to *SWI*<sup>+</sup> cells, indicating that the SWI-SNF dependence of transcription from this promoter is slightly increased when normal activator function is bypassed by holoenzyme recruitment (Fig. 6). Transcription of *GAL10-MEL1* by GAL4-GAL11 in *SWI*<sup>+</sup> cells was also robust (Fig. 6). Remarkably, the transcription of this reporter by GAL4-GAL11 in *swi1Δ* cells was almost abolished (Fig. 6). Consistent with this result, GAL4-GAL11 strongly supports the growth of *gal4 SWI*<sup>+</sup> yeast in galactose medium but does not support the growth of *gal4 swi1Δ* yeast in galactose (data not shown). The nearly complete SWI-SNF dependence of *GAL10-MEL1* transcription by GAL4-GAL11 supports the idea that the SWI-SNF complex exerts its effect at a step subsequent to transcriptional activator binding by two lines of reasoning. First, if SWI-SNF was needed principally to allow activator binding, the same SWI-SNF dependence should be observed for a given promoter no matter which activation domain is tethered to the GAL4 DNA-binding domain, which is not consistent with the very different dependencies on the SWI-SNF complex seen for GAL4 (about 4-fold) and GAL4-GAL11 (about 50-fold). Second, if GAL4-GAL11 activates transcription by directly recruiting the RNA polymerase II holoenzyme (15), the near-complete SWI-SNF dependence seen at the *GAL10-MEL1* promoter with this protein suggests a stringent requirement for the SWI-SNF complex in order for the basal transcription machinery (holoenzyme or TFIID) to gain access to the proximal promoter. This would be consistent with the SWI-SNF complex being associated with the holoenzyme (82), although this is at present somewhat controversial (9).

DISCUSSION

The SWI-SNF complex is required in vivo for maximal transcriptional induction of a number of promoters and for chromatin remodeling at the *SUC2* promoter in yeast (see Introduction). In vitro, the SWI-SNF complex can enable the binding of GAL4 to a single site in a nucleosomal template (14). These findings suggest a model in which the SWI-SNF complex assists transcriptional activation in vivo by helping activator proteins bind to their sites in chromatin (60). Alternatively, the SWI-SNF complex could be recruited by activators, for example in association with the RNA polymerase II holoenzyme (82), and could assist in steps subsequent to activator binding, such as binding of TBP and RNA polymerase II to their sites in chromatin.

We have tested these ideas by using templates with defined chromatin structures to examine transcription and chromatin perturbation induced by GAL4 and derivatives thereof in vivo. Our results indicate that activator binding to nucleosomal sites can occur essentially independently of SWI-SNF and that the SWI-SNF complex is likely to play its major role in overcoming

the repressive effects of chromatin at steps subsequent to activator binding during transcriptional activation. First, perturbation of a positioned nucleosome containing a single strong GAL4-binding site by GAL4 occurs essentially independently of SWI-SNF (Fig. 1). Second, activation domain-dependent chromatin remodeling showed substantial SWI-SNF dependence (Fig. 3), although it was not eliminated entirely (Fig. 2), consistent with the partial dependence on SWI-SNF observed for transcriptional activation (7, 18, 25, 42, 62) (Fig. 6). Finally, a *GAL10-MEL1* fusion, which has a nonnucleosomal UAS and a highly organized chromatin structure in the region of the TATA element and transcription start site, shows a strong SWI-SNF dependence with GAL4, GAL4-ER-VP16, and GAL4-ftz and a nearly complete SWI-SNF dependence with GAL4-GAL11 (Fig. 6), consistent with a requirement for SWI-SNF activity at a step subsequent to activator binding.

**GAL4 binding to a nucleosomal site in *swi1*Δ yeast cells.** We have shown that GAL4 can bind to a nucleosomal site with concomitant nucleosome perturbation in *swi* yeast cells. We cannot rule out that the SWI-SNF complex enhances GAL4 binding to TA17Δ80 to a minor degree which our assays are not sensitive enough to measure; nor can we exclude that examination of other transcription factors, or weaker GAL4-binding sites, might show an increased dependence on the SWI-SNF complex. In fact, GAL4 binding to a pair of weak sites is impaired in *swi* yeast cells in at least one instance (7). One way this could be reconciled with the present work is if stable binding to weak sites were especially dependent on the activation domain. Indeed, evidence exists that transcription factor binding and chromatin perturbation depends on activation domains *in vivo* (6, 69–71). Furthermore, stable binding of GAL4 to low- and moderate-affinity binding sites has been shown to be strengthened by the presence of a nearby TATA element (76). A likely explanation for this effect, which requires the presence of an activation domain, is that binding to low- and moderate-affinity sites is enhanced by cooperative interactions between GAL4 and general transcription factors such as TFIID. If interactions involving the latter were diminished in *swi* cells, GAL4 binding could be affected. This explanation might also help to account for factor occupancy being diminished only about 2-fold in *swi* cells while transcription from the same reporter was decreased by 25-fold (7).

Recently, it has been shown that chromatin remodeling of the *PHO5* promoter by the transcription factor PHO4 can occur in *swi* yeast cells (18). This example differs from the work described here in that PHO4 binding first occurs at a site between two positioned nucleosomes, which then allows nucleosome disruption and binding to the nucleosomal site (77). It seems likely that this disruption may differ mechanistically from that of nucleosome I of TA17Δ80 by GAL4 (Fig. 1), in which binding to a nucleosomal site occurs directly. Different mechanisms for the two systems are also suggested by the different results obtained with PHO4-GAL11 with the *PHO5* promoter (weak SWI-SNF dependence, similar to that seen with PHO4) from those obtained with GAL4-GAL11 with the *GAL10-MEL1* reporter (nearly complete dependence, and much stronger dependence than seen with GAL4). However, the conclusion that chromatin remodeling by activators can occur by a pathway independent of SWI-SNF *in vivo* is consistent with our findings. Further work is required to elucidate the mechanism by which GAL4 can access a nucleosomal site *in vivo* and to determine whether any of several other candidate chromatin-remodeling activities (9, 12, 28, 73, 75) contribute to chromatin remodeling during transcription factor binding *in vivo*.

**A role for SWI-SNF in activation domain-dependent remodeling of chromatin.** We found that activation domain-dependent perturbation of TALS chromatin, especially as measured by a change in topology, was reduced in *swi1*Δ yeast (Fig. 3) but not eliminated (Fig. 2 and 3). This perturbation occurs outside of the context of a natural promoter (69), suggesting that the differences seen in *swi* cells are unlikely to be a consequence of defects in transcription. Similarly, the chromatin structure of the derepressed *SUC2* promoter is altered by mutations in *SNF2-SWI2* or *SNF5*, and the differences between *SWI*<sup>+</sup> and *swi* cells are observed even when the TATA element has been mutated (25), although a direct effect of an activator on the chromatin structure of this locus has not been established. These findings suggest that transcriptional activators have a general capability to remodel chromatin in the vicinity of their binding sites and that this ability is impaired but not abolished in the absence of the SWI-SNF complex.

We have examined the changes in the chromatin structure of the *GAL10-MEL1* promoter which accompany activation in *SWI*<sup>+</sup> yeast cells in the hope of learning more about the specific remodeling which occurs at this strongly SWI-SNF-dependent promoter. However, in agreement with observations made with the *GAL10* promoter (11, 16, 47), we found only subtle changes in chromatin structure in the *GAL10* promoter upon activation, although changes in the MNase cleavage pattern of the transcribed *MEL1* coding sequence were seen (data not shown). Nevertheless, the apparent incorporation of the TATA element into a positioned nucleosome in the repressed promoter (10, 14, 43) (Fig. 4), in contrast to the accessible TATA element in the much less SWI-SNF-dependent GAL4-*CYC1-lacZ* reporter (Fig. 5), suggests that TBP recruitment could be an important step requiring chromatin remodeling by the SWI-SNF complex. Consistent with this notion, the *SUC2* promoter, which also displays a strong SWI-SNF dependence (25, 58), appears to have its TATA element incorporated into a nucleosome under repressed conditions (18a, 25, 86). The *PHO5* promoter, which has a nucleosomal TATA element and which shows little SWI-SNF dependence for its activation (18), appears to deviate from this rule. Perhaps in this gene the disruption that accompanies PHO4 binding to its nucleosomal site near the TATA element is sufficient to allow TBP access, or it may be that PHO4 is able to recruit other chromatin-remodeling activities that obviate the need for the SWI-SNF complex. Alternatively, chromatin remodeling by the SWI-SNF complex may be required for the binding of general transcription factors or RNA polymerase subsequent to TFIID binding.

Participation of the SWI-SNF complex in transcriptional activation in a step that depends on both the activation domain and the promoter is consistent with previous work (39, 42, 62). One mechanism which could account for this dependency would be recruitment by activation domains, directly or indirectly, of an RNA polymerase II holoenzyme which includes the SWI-SNF complex (82). This would also account for the complete SWI-SNF dependence of *GAL10-MEL1* transcription by GAL4-GAL11. However, the extent to which the SWI-SNF complex is associated with the RNA polymerase II holoenzyme *in vivo* remains to be resolved (9).

If the role of the SWI-SNF complex is entirely to remodel chromatin to allow access to DNA by proteins involved in transcriptional activation, the nearly complete SWI-SNF dependence of *GAL10-MEL1* transcription by GAL4-GAL11 also implies a strong requirement for chromatin remodeling for transcriptional activation at this promoter. Since GAL4 showed only partial SWI-SNF dependence at this promoter (Fig. 6), we infer that GAL4 must be able to remodel chroma-

tin by some alternative pathway. It is possible that activators are generally capable of recruiting more than one chromatin-remodeling activity via more than one pathway. For example, SWI-SNF might be recruited along with the RNA polymerase holoenzyme (82) and the SAGA complex via adaptor complexes (21). Other identified chromatin-remodeling activities (9, 28, 73, 75) might be recruited by presently undefined pathways. Whether these many candidate activities are partly redundant or have evolved to operate at specific promoters or during specific steps of transcriptional activation or are involved in other processes which must contend with nucleosomal templates, such as replication and repair, remains to be determined.

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