Chromatin Remodeling by Transcriptional Activation Domains in a Yeast Episome*

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We examine the generality of transcription factor-mediated chromatin remodeling by monitoring changes in chromatin structure in a yeast (Saccharomyces cerevisiae) episome outside of the context of a natural promoter. The episome has a well-defined chromatin structure and a binding site for the transcription factor GAL4 but lacks a nearby functional TATA element or transcription start site, so that changes in chromatin structure are unlikely to be caused by transcription. To separate changes caused by binding and by activation domains, we use both GAL4 and a chimeric, hormone-dependent activator consisting of the GAL4 DNA-binding domain, an estrogen receptor (ER) hormone-binding domain, and a VP16 activation domain (Louvion, J.-F., Havaux-Copf, B. and Picard, D. (1993) Gene (Amst.) 131, 129–134). Both GAL4 and GAL4-VP16 show very little perturbation of chromatin structure in their nonactivating configurations. Substantial additional perturbation occurs upon activation. This additional perturbation is marked by changes in micrococcal nuclease cleavage patterns, restriction endonuclease accessibility, and DNA topology and is not seen with the nonactivating derivative GAL4-ER. Remodeling by GAL4-ER-VP16 is detectable within 15 min following hormone addition and is complete within 45 min, suggesting that replication is not required. We conclude that activation domains can exert a major influence on chromatin remodeling by increasing binding affinity and/or by recruitment of other chromatin remodeling activities and that this remodeling can occur outside the context of a bona fide promoter.

Transcriptional induction in eukaryotes requires binding of activators to promoter elements termed enhancers (called upstream activator sequences [UASs]) in yeast. Binding of a transcriptional activator in its activating form results in recruitment or reconfiguration of a preinitiation complex in a form competent to allow initiation and elongation by RNA polymerase II. These events occur in the context of chromatin, which means that histone proteins are available to compete with activators and components of the preinitiation complex for binding to DNA. One function of transcriptional activators may be to help overcome nucleosomal repression of transcription, particularly at the site of preinitiation complex formation (1).

Gene activation is often accompanied by perturbations in chromatin structure. For some genes, this perturbation appears to be essential for proper transcriptional regulation (2–4). In some instances, specific effects of activation domains have been observed. The yeast activator GAL4 can bind to promoter sites in both activating and nonactivating forms; this has allowed the detection of subtle effects on chromatin structure in the GAL1–10 promoter that require an unmasked activation domain (5, 6). Another transcriptional activator, PHO4, perturbs chromatin structure at the PHO5 promoter in an activation domain-dependent manner (7). The thyroid hormone receptor-retinoid X receptor heterodimer binds without perturbing chromatin structure in its unliganded state; the addition of hormone results in a local perturbation of chromatin structure in the TRβ4 gene promoter (8).

The above examples of chromatin remodeling by transcription factors occur at natural promoters. Although chromatin remodeling under activating conditions can occur in nonfunctional promoters having mutated TATA boxes (3, 9), other cis-acting promoter elements could still be involved in this process. Perturbation of a positioned nucleosome by GAL4 has been examined outside of the context of a natural promoter in a plasmid episome in yeast (10). In this study, the GAL4 site was near the center of a positioned nucleosome (when GAL4 was absent); GAL4 expression resulted in micrococcal nuclease (Mnase) cleavage sites, indicative of nucleosome perturbation. This perturbation appeared stronger, as assessed by the intensities of the Mnase cleavages, under activating conditions.

The mechanism by which an activation domain causes chromatin remodeling remains unknown. To examine the generality of transcription factor-mediated chromatin remodeling and to increase our understanding of this important step in transcriptional activation, we have examined the ability of GAL4 and a chimeric activator, GAL4-VP16, to perturb chromatin structure in the yeast episome TALS, which has an exceptionally well defined chromatin structure. In yeast haploid a cells, the repressor complex a2MCM1 helps to incorporate TALS into strongly positioned nucleosomes (11). One of these nucleosomes contains a GAL4 binding site, which derives from the GAL3 promoter (12). We show that both GAL4 and GAL4-ER-VP16 perturb TALS chromatin most strongly in their activating configurations, and we discuss possible mechanisms by which an unmasked transcriptional activation domain could remodel chromatin in vivo.

EXPERIMENTAL PROCEDURES

Plasmids—Introduction of TA17380 into yeast has been described (10); TALS (11) and TALS4 (13), in which the UAS Gal4 has been removed by mutagenesis (generous gift of M. Klodde), were excised from pUC19 sequences by digestion with HindIII and religated before introduction into yeast. GAL4-VP16 was expressed from the plasmid pHCA, here referred to as pRS313GAL4-ER-VP16 (Ref. 14; generously provided by D. Picard) or from pRS416GAL4-ER-VP16, which was con-
structed by ligation of the Clal-Spe1 fragment encompassing the GAL4-ERVP16 gene from pRS313GAL4-ERVP16 into pRS416 (Ref. 15). The expression vector for GAL4-ER was constructed by deletion of the SacI fragment bearing the VP16 coding sequence from the expression vector for GAL4-VP16. For experiments in which perturbation of chromatin by GAL4-VP16 was monitored, GAL4 was contained in a copy GAL4 expression vector pRS425GAL4 (10). The plasmid pCl1 (16) was used to express GAL4 from the ADH1 promoter. The reporter construct used for β-galactosidase assays was pRS314-17380lacZ (20).

Yeast Transformations, Cell Growth, and β-Galactosidase Assays—Plasmids were transformed into yeast cells (strain YNN282 [MATa trp1; ura3–32 lys2–801 ade2–1]) (11), strain FY24 (MATa ura3–52 trp1; lys2–801 ade2–1) (11), strain KY24 (MATa ura3–52 trp1; lys2–801 ade2–1) (17), or the gal80- strain KY245 (MATa gal80A his4–801 lys2–1762 trp1; lys2–801 ade2–1); generous gift of Karen Arndt) by a modification (18) of the method of Itu et al. (19). Cells were grown in dropout media (Bio 101) in the presence of 2% glucose, 1.5% raffinose, or 2% galactose. Cells were grown in dropout media (Bio 101) in the presence of 2% glucose, 1.5% raffinose, or 2% galactose. Yeast cells (0.4–1.0 liter) were

| GAL4-ERVP16 β-estradiol Carbon source Activity | Miller units |
|-----------------------------------------------|--------------|
|                                             | 7 ± 2 (5)    |
| –                                             | 7 ± 2 (5)    |
| +                                             | 16 ± 9 (7)   |
| –                                             | 535 ± 110 (7) |
| +                                             | 800 ± 60 (8) |
| –                                             | 770 ± 95 (8) |
| +                                             | 345 ± 25 (6) |

To determine whether GAL4-ERVP16 binds to chromatin in vivo in the absence of hormone, we compared transcriptional activation of a UASGAL-lacZ reporter by GAL4 in the presence and absence of the GAL4-ERVP16 expression vector. β-Galactosidase activity induced by GAL4 (via growth of cells in galactose) was decreased in the presence of GAL4-ERVP16 (Table I), suggesting that GAL4-ERVP16 could compete with GAL4 for site occupancy in the absence of hormone. To determine whether GAL4-ERVP16 could bind to a nucleosomal site in the absence of hormone, we examined its effect on the chromatin structure of TA17A60 (10). TA17A60 was a TRPIARSI-based plasmid in which a single strong GAL4 binding site has been introduced such that the site is incorporated near the center (i.e., near the pseudosyndeyd) of a positioned nucleosome in cells grown in glucose, conditions under which GAL4 is expressed at extremely low levels (29). Yeast cells harboring TA17A60 were grown in the presence of GAL4-ERVP16 with and without hormone and in the absence of GAL4-ERVP16 in glucose (GAL4 nearly absent) and in galactose (GAL4 present in its activating configuration). Nuclei were prepared and treated with micrococcal nuclease (MNotase), and the cleavage sites were mapped relative to an EcoRV restriction site (25, 26). Regions of about 150 base pairs that are protected from MNotase digestion in chromatin but are cleaved as naked DNA are diagnostic of positioned nucleosomes (30, 31); in the case of TA17A60 and its progenitor TRPIARSI, the assignment of positioned nucleosomes I and II (Fig. 1) is also based on a substantial body of earlier work (10, 30, 31).

In cells grown in glucose without the GAL4-ERVP16 expression vector, GAL4-ERVP16 is absent and GAL4 is expressed at very low levels, and positioned nucleosomes are present as expected (Fig. 1, lanes 1 and 2). In galactose medium, GAL4 expression is induced, and MNotase cleavage sites are generated in the regions of both nucleosomes I and II, as observed previously (Fig. 1, lanes 3–4; Ref. 10). These new cleavage sites are also seen in naked DNA treated with MNotase (10) and indicate that...
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GAL4 is binding to its site and perturbing nucleosome positioning. The same cleavage sites are seen, albeit more weakly, when GAL4-ER-VP16 is expressed in cells grown in glucose medium and hormone is not added (Fig. 1, lanes 5–7), indicating that GAL4-ER-VP16 can bind under nonactivating conditions. Interestingly, the addition of 0.1 μM β-estradiol caused enhanced cleavage in the regions of nucleosomes I and II in TA17Δ80 (compare the relative intensities of cleavage sites marked by stars with the site marked by a filled circle, lanes 5–9), suggesting that binding of GAL4-ER-VP16 was increased or altered by the addition of hormone.

These results are consistent with previous findings that binding of GAL4 to TA17Δ80 under activating conditions (in galactose) allowed strong MNase cleavage in the regions of nucleosomes I and II, whereas ectopic expression of GAL4 in glucose, in which it is repressed by GAL80, or expression of the very weakly activating derivative, GAL4(1–147)H, allowed only weak MNase cleavage (10). We wondered whether a GAL4 binding site embedded in chromatin in a different context would show a similar effect of an unmasked activation domain, so we examined another yeast episome having a GAL4 binding site in a positioned nucleosome, TALS. The TALS episome is packaged into strongly positioned nucleosomes in yeast α cells by the α2-MCM1 complex in conjunction with SSN6 and TUP1 (Refs. 11 and 32; see Fig. 2). Nucleosome IV of TALS, immediately adjacent to the α2-MCM1 operator, contains a strong binding site for GAL4, which derives from the GAL3 promoter (12) and is in a region of nucleosome IV inaccessible to Escherichia coli Dam methyltransferase expressed in yeast (33). As in TA17Δ80, the GAL4 binding site in this plasmid is removed from its natural context (because the 3′ portion of the GAL3 promoter, including the TATA box, is not present in TALS, and sequences farther than 60 base pairs upstream of the UAS_GAL do not contribute to GAL3 expression (12)). Nearly the entire TALS plasmid is packaged into strongly positioned nucleosomes in yeast α cells, so we thought it would provide a potentially interesting template to examine the effects of GAL4 binding and activation domain unmasking on chromatin structure.

Nuclei from yeast cells harboring TALS were prepared and digested with MNase, and cleavage sites were mapped counterclockwise from the EcoRV site (Fig. 2). Comparison of the digestion patterns of chromatin from cells lacking the GAL4-ER-VP16 expression vector and grown in glucose (lane 9) with naked DNA (lane 11) shows that TALS is packaged into strongly positioned nucleosomes, as observed previously (11). (Although the sample in lane 9 is heavily digested, the cleavage sites are identical to those seen in lighter digests (Ref. 11 and data not shown).) When GAL4-ER-VP16 is expressed, a new cleavage site is generated in the region of nucleosome IV, close to the GAL4 binding site, suggesting that GAL4-ER-VP16 can bind and perturb nucleosome positioning in TALS even in its nonactivating form (Fig. 2, lanes 2–4; see the band marked by

bands that are cleaved more strongly than the empty star. Cleavage at these sites indicates that nucleosome positioning has been perturbed. The cleavage site corresponding to the distal border of nucleosome II is marked with a filled circle. Locations of nucleosomes I and II in cells grown in glucose are indicated; the box attached to nucleosome I represents the GAL4 binding site. Lane 8 was taken from a different exposure than lanes 5–7 and 9. Lower panel, densitometric scans of the salient regions of lanes 1 and 4 (upper traces, labeled glucose and galactose, respectively) and lanes 7 and 8 (lower traces, labeled −E2 and +E2, respectively). The filled circle and starred peaks correspond to those in the upper panel, and the regions corresponding to nucleosomes I and II are indicated; the black box attached to nucleosome I represents the GAL4 binding site.

**Fig. 1. Nucleosome perturbation in the yeast episome TA17Δ80 by ligand-activated and nonactivated GAL4 ER-VP16.**

Upper panel, nuclei were prepared from yeast cells (strain FY24) harboring TA17Δ80 and either pRS425GAL4 (left part, lanes 1–4) or pRS416GAL4-ER-VP16 (right part, lanes 5–9) and digested with micrococcal nuclease. Cells were grown in glucose (lanes 1–2 and 5–9) or galactose (lanes 3–4) in the absence (lanes 1–7) or presence (lanes 8–9) of β-estradiol, as indicated. Micrococcal nuclease cleavage sites were mapped clockwise relative to the EcoRV site as indicated. Samples were digested with increasing amounts of micrococcal nuclease from the outer lanes of each panel toward the centers. Bands indicated by stars are cleaved in naked DNA (data not shown and Ref. 10) and protected by nucleosomes I and II (lanes 1 and 2); the filled stars correspond to
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FIG. 2. Nucleosome perturbation in the yeast episome TALS by ligand-activated and nonactivated GAL4-ER VP16 and by GAL4 in galactose. Nuclei were prepared from yeast cells harboring TALS and pRS13GAL4-ER VP16 (strain YNN282, lanes 1–8) or TALS and pRS425GAL4 (strain FY24, lanes 9 and 10) and digested with micrococcal nuclease at 0 (lanes 1 and 8), 5 (lanes 2 and 7), 20 (lanes 3, 6, 9, and 10), or 50 units/ml (lanes 4 and 5). Naked DNA was isolated from FY24 cells harboring TALS and digested at 10 units/ml MNase. MNase cleavage sites were mapped counterclockwise relative to the EcoRV site as indicated. The marker lane (M) contains a ΦX174HaeIII digest. The arrowhead indicates a cleavage site that is not cut in cells lacking GAL4-ER VP16 and grown in glucose (compare lane 9) and that is cut more strongly in cells containing GAL4-ER VP16 when hormone is present than when it is absent, and the filled circle indicates a cleavage site only cut in the presence of GAL4-ER VP16 plus hormone or in cells grown in galactose (lane 10). The band marked by an asterisk corresponds to supercoiled plasmid not cut by EcoRV. Location of nucleosomes II–V in cells grown in glucose medium (ellipses) and the α2-MCM1 operator (rectangle between nucleosomes IV and V) are indicated to the left; only nucleosome IV is shown on the plasmid map at the top. The rectangle in nucleosome IV represents the GAL4 binding site.

an arrowhead, which is absent from lane 9). A parallel culture incubated for 4 hours in the presence of 0.1 μM β-estradiol showed further changes in chromatin structure (Fig. 2, lanes 5–7); the cleavage indicated by the arrowhead is enhanced relative to the site below it, and a new site is generated in the vicinity of nucleosome III (filled circle). A weak cleavage site is also generated near the center of the region protected by nucleosome IV, which is also cut only weakly in naked DNA (lane 11).

GAL4 in galactose perturbs the MNase cleavage pattern of TALS chromatin similarly to GAL4-ER VP16 in the presence of hormone. GAL4 can also bind to DNA in a nonactivating configuration in the absence of galactose, when its activation domain is masked by the repressor protein GAL80 (34, 35). Binding of nonactivating GAL4 in vivo has been detected by dimethyl sulfate footprinting (36), photofootprinting (37), and perturbation of positioned nucleosomes (10). When galactose is present, a conformational change unmasks the GAL4 activation domain (38). To determine whether GAL4, like GAL4-ER VP16, would perturb TALS chromatin differently in its activating and nonactivating configurations, we performed MNase digests of TALS chromatin from cells grown in glucose (GAL4 nearly absent), galactose (GAL4 present in its nonactivating configuration), and galactose (GAL4 present in its activating configuration). We also analyzed chromatin from cells in which GAL4 was ectopically expressed from the ADH1 promoter in glucose. The high levels of GAL4 expressed from the ADH1 promoter titrate GAL80 (35), but most (>75%; data not shown) of the GAL4 molecules are still repressed by GAL80 under these conditions.

GAL4 under activating and nonactivating conditions. Yeast cells (strain YNN282, lanes 1–3, or strain FY24, lanes 4–7) harboring TALS and pH 825GAL4 (lanes 4–5) or pCL1, which constitutively expresses GAL4 from the ADH1 promoter (lanes 6–7) were grown in medium containing glucose, raffinose, or galactose as indicated, and micrococcal nuclease cleavage sites in TALS chromatin were mapped clockwise relative to the EcoRV site as indicated using 2 (lanes 5 and 6), 5 (lanes 4 and 7), or 20 units/ml MNase (lanes 1–3). The locations of nucleosomes III–V in cells grown in glucose medium (ellipses) and the α2-MCM1 operator (rectangle between nucleosomes IV and V) are indicated on the sides; only nucleosome IV is shown on the plasmid map at the top. The rectangle in nucleosome IV represents the GAL4 binding site. The filled circles indicate cleavage sites cut more strongly in raffinose than glucose and more strongly in galactose than raffinose.
measured plasmid topology of TALS isolated from cells harboring GAL4-ER-VP16 in the presence and absence of β-estradiol. Since each nucleosome introduces one negative supercoil into plasmid DNA (39, 40), any loss of nucleosomes accompanying binding of GAL4-ER-VP16 in either its activating or nonactivating forms should change plasmid topology.

DNA was rapidly isolated from yeast cells harboring TALS under conditions that rapidly inactivate topoisomerase, so plasmid topology reflects that present in vivo (28, 41, 42). Samples were electrophoresed on chloroquine-containing gels to resolve individual topoisomers, blotted, and hybridized with a probe specific for TALS. Under the conditions used (40 μg/ml chloroquine diphosphate), TALS topoisomers migrated as positively supercoiled molecules, so that faster migrating bands represent more positively supercoiled species. Fig. 4 shows that in the absence of GAL4-ER-VP16, administration of 0.1 μM β-estradiol had no effect on TALS topology (lanes 1 and 2). Expression of GAL4-ER-VP16 in the absence of hormone had no effect on TALS topology (lane 3), despite its effect on the MNase cleavage pattern (Fig. 2, lanes 2–4 compared with lane 9). Hormone administration caused a loss of nearly one negative supercoil (Fig. 4, lane 4; Table II), suggesting loss of one nucleosome in a majority of the TALS minichromosomes. Similarly, growth in raffinose, in which GAL4 is expressed but does not activate transcription due to its repression by GAL80, resulted in minimal topological perturbation (compare lane 5 with lane 1), whereas growth in galactose, where GAL4 is present in its activating form, caused a loss of nearly one negative supercoil (lane 6). When the same experiment was done in a gal80− strain, loss of negative supercoiling was seen in cells grown in raffinose compared with cells grown in glucose, and no change was seen between cells grown in raffinose and galactose (data not shown). GAL4 expressed ectopically in glucose medium, in which it is repressed by GAL80, had only a minimal effect on TALS topology, as did the very weakly activating derivative GAL4(1–147)H, which lacks the GAL4 activation domain (data not shown). A derivative of TALS lacking the GAL4 binding site, TALS4, showed no topological shift between glucose and galactose (Fig. 4, lanes 9 and 10) and was not affected by hormone addition in the presence of GAL4-ER-VP16 (data not shown). Results from several such experiments are tabulated in Table II, providing quantitative support for the visual evidence of Fig. 4.

**Restriction Enzyme Accessibility Modulated by Binding of Activating and Nonactivating GAL4 and GAL4-ER-VP16**—If nucleosome IV of TALS is lost from a majority of minichromosomes upon binding of GAL4-ER-VP16 in the presence of β-estradiol, but only from a minority in the absence of hormone, we would expect restriction endonuclease sites in this region to be only moderately more accessible in the presence of GAL4-ER-VP16 without hormone than in its absence and considerably more accessible following hormone addition. We have used an SstI site that is close to the center of the region protected by nucleosome IV to test this hypothesis. Yeast nuclei from cells harboring TALS were treated with SstI, and aliquots were stopped after 15- and 30-min incubation with enzyme. Cleavage of the nucleosome IV SstI site was increased only slightly after 30 min compared with after 15 min (Fig. 5), and a control plasmid with an SstI site added at 15 min was nearly completely cleaved by 30 min, demonstrating that SstI activity was not lost during the incubation (Fig. 5, lane 13 and data not shown). Together these results suggest that almost all of the accessible SstI sites in the TALS minichromosomes were cleaved by the end of the 30-min reaction.

TALS minichromosomes from cells grown in glucose and lacking GAL4-ER-VP16 were cleaved very little by SstI (Fig. 5, lanes 10 and 11). Cleavage was increased little if at all by expression of GAL4-ER-VP16 (lanes 1 and 2) but was increased substantially and reproducibly by incubation with β-estradiol (lanes 4 and 5). Similarly, expression of GAL4 under nonactivating conditions by growth of cells in raffinose caused a very modest increase in SstI accessibility (Fig. 5, lanes 12 and 13), whereas expression of the activating form of GAL4 by growth in galactose consistently increased accessibility (Fig. 5, lanes 14 and 15). Thus, restriction enzyme accessibility is consistent with increased disruption of nucleosome IV accompanying unmasking of the activation domains of GAL4 or GAL4-ER-VP16.

**Effect of the Activation Domain on Hormone-dependent Perturbation of TALS Chromatin by GAL4-ER-VP16**—The data

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**TABLE II**  
Alterations in linking number of TALS minichromosomes upon binding or activation of GAL4 or GAL4-ER-VP16

Values shown were obtained by taking the differences between the centers of the Gaussian distribution of topoisomers under the conditions indicated for each column. For instance, the average linking number in the presence of GAL4 ER-VP16 was increased by 0.7 turns when β-estradiol was present compared with when it was absent (column 2). The numbers in parentheses indicate the number of independent measurements for each value.

| Activator          | GAL4-ER-VP16 | GAL4-ER-VP16+ER2α | GAL4: glu/raffβ | GAL4: glu/galβ |
|--------------------|-------------|-------------------|----------------|---------------|
| Linking number shift | 0.0 ± 0.2 (3) | 0.7 ± 0.1 (4) | 0.24 ± 0.15 (5) | 0.7 ± 0.2 (4) |

α ER2, 0.1 μM β-estradiol.

β Cells were grown in glucose (no GAL4), raffinose (GAL4 expressed but repressed by GAL80), or galactose (GAL4 expressed and activation-competent).
presented so far suggest that the activation domains of GAL4 and GAL4-ER-VP16 affect their interaction with TALS chromatin. However, it has been reported that DNA binding of intact ER and derivatives is affected by hormone addition (13, 43, 44). To separate effects exerted by the hormone-binding domain of GAL4-ER-VP16 on the DNA-binding domain from those due to unmasking of the activation domain, we decided to examine the effect of eliminating the activation potential of GAL4-ER-VP16. We therefore excised the VP16 coding region from our expression vector to create an expression vector for GAL4-ER. GAL4-ER is unable to activate transcription from the simple UAS<sub>GAL</sub>-lacZ reporter used in our assay (Table III), in agreement with Louvion et al. (14). It does, however, measurably inhibit transcription of the UAS<sub>GAL</sub>-lacZ reporter by GAL4 (Table III), suggesting that it is expressed at levels similar to that of GAL4-ER-VP16. GAL4-ER slightly affects TALS chromatin in the absence of hormone, as assayed by micrococcal nuclease digestion (Fig. 6, lanes 9 and 10; compare with Fig. 2, lane 9, and Fig. 3, lane 3), similar to what was observed for GAL4-ER-VP16 (Fig. 2). However, in contrast to GAL4-ER-VP16, hormone addition in the presence of GAL4-ER does not further perturb the MNase cleavage pattern (Fig. 6, lanes 9–12 versus lanes 3–6). Consistent with this lack of effect, hormone addition does not alter supercoiling (Fig. 4, lanes 7–8) or appreciably increase restriction enzyme accessibility (Fig. 5, lanes 6–9). We conclude that it is not a hormone-induced conformational change in GAL4-ER-VP16 per se but unmasking of the VP16 activation domain upon ligand binding by the ER hormone binding domain that results in chromatin perturbation.

Kinetics of Chromatin Remodeling by GAL4-ER-VP16—The topological analysis presented above entails rapid isolation of DNA from small (10-ml) volumes of yeast cells. We took advantage of this protocol to examine the kinetics of TALS chromatin remodeling by GAL4-ER-VP16 following hormone administration. Yeast cells harboring TALS and the expression vector for GAL4-ER-VP16 were grown, and DNA was isolated from 10- or 20-ml aliquots immediately before (t = 0) and at intervals following the addition of β-estradiol to 0.1 μM. Topological distributions of TALS from one representative experiment are shown in Fig. 7A, and results from three independent experiments are combined graphically in Fig. 7B. A substantial change in TALS topology is seen 15 min after hormone addition, and the topological shift is essentially complete after 30

![FIG. 5. Increased accessibility of SstI to nucleosome IV in TALS by binding of GAL4 or GAL4-ER-VP16 in their activating configurations. Nuclei were prepared from yeast cells (strain YNN282) harboring TALS alone, TALS and pRS313GAL4-ER-VP16, or pRS313GAL4-ER as indicated. Nuclei were digested for 15 or 30 min with SstI, and the purified DNA was secondarily digested with EcoRV and then analyzed by indirect end-labeling. The bands indicated Parent correspond to TALS cut only with EcoRV, and the SstI-cut band is also indicated. The bands indicated by the arrowhead (lanes 1, 2, and 5) correspond to supercoiled DNA present because of incomplete digestion by EcoRV, and the filled circles in lane 13 indicate bands due to a naked control plasmid added at 15 min to this sample; the upper band is uncut, and the lower band is cut by SstI. The marker lane (M) contains a φX/HaeIII digest.](image55x559)

![FIG. 6. No nucleosome perturbation in the yeast episome TALS by β-estradiol addition in the presence of GAL4-ER. Upper panel, nuclei were prepared from yeast cells (strain YNN282) harboring TALS and pRS313GAL4-ER-VP16 or pRS313GAL4-ER in the presence or absence of β-estradiol as indicated and digested with micrococcal nuclease at 0 (lanes 2, 7, 8, and 13), 4 (lane 1, which is a naked DNA digest), 5 (lanes 3, 6, 9, and 12), or 20 units/ml (lanes 4, 5, 10, and 11). MNase cleavage sites were mapped counterclockwise relative to the EcoRV site as indicated. Arrowheads indicated cleavages enhanced by hormone addition in the presence of GAL4-ER-VP16. Location of nucleosomes II–V in unperturbed TALS chromatin (ellipses) and the α2-MCM1 operator (rectangle between nucleosomes IV and V) are indicated to the left; only nucleosome IV is shown on the plasmid map at the top. The rectangle in nucleosome IV represents the GAL4 binding site. Lower panel, densitometric scans of lanes 3 and 5 (labeled +E2 and +E2, respectively). The peaks indicated by the arrowhead are enhanced in the presence of hormone and correspond to the bands marked by arrowheads in the upper panel; the left peak (upper band in the upper panel) is more clearly resolved in gels that have been electrophoresed for longer times. Nucleosomes III and IV, the GAL4 binding site in nucleosome IV, and the α2-MCM1 operator (hatched rectangle) are also indicated.](image313x299)

![TABLE III GAL4-ER is transcriptionally inactive](image313x318)
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**DISCUSSION**

Chromatin Perturbation Caused by Activation Domain Unmasking—In this paper we have used the yeast episomes TA17Δ80 and TALS as reporters to monitor changes in chromatin structure caused by binding of GAL4 and GAL4-ER-VP16 in their activating and nonactivating configurations. The chromatin remodeling we have observed in both of these plasmids occurs outside the context of natural promoters. TALS (11) contains a single GAL4 binding site from the GAL3 promoter (UASg1 in Ref. 2) but lacks downstream promoter elements, including the TATA element and transcription start site. Furthermore, chromatin structure in TALS in yeast α cells is dominated by the α2 operator (11), and in the region perturbed by GAL4 and GAL4-ER-VP16, which corresponds to the region upstream of the GAL4 binding site in the GAL3 promoter, it is very different in TALS than in the endogenous GAL3 promoter whether GAL4 is present or absent.2 Thus, the results reported here suggest that the chromatin remodeling observed in natural promoters (2, 5–8) reflects a general property of at least some transcriptional activators that does not depend on collaboration of particular cis-acting promoter elements. The precise nature of the remodeling, however, depends on the chromatin structure that is perturbed. For example, substantial perturbation by GAL4 is seen with TALS and TA17Δ80, both of which have nucleosomal GAL4 binding sites, but only modest perturbation is seen in the GAL1 promoter, in which the GAL4 binding sites are nonnucleosomal (5, 6).

The GAL4 binding site in TALS is located 33–49 base pairs from the edge of nucleosome IV, which is adjacent to the α2-MCM1 nucleosome-positioning element (22). This region is inaccessible to *E. coli* Dam methyltransferase expressed in yeast (33). Nevertheless, the GAL4 DNA-binding domain can evidently gain access to this region, although whether it does so in the presence of a fully formed nucleosome or at a point in the cell cycle when nucleosome structure is perturbed, such as during DNA replication, has not been determined. Similarly, a GAL4 binding site near the center of nucleosome I of the TRP1ARS1 derivative TA17Δ80 can be accessed by GAL4 (Ref. 10; Fig. 1), although this region is also inaccessible to *E. coli* Dam methyltransferase (33).

Some perturbation of the chromatin structure of TA17Δ80 (Fig. 1) and TALS (Fig. 2) is caused by both GAL4 and GAL4-ER-VP16 in their nonactivating configurations, as evident by a slightly altered MNase digestion pattern. Binding by nonactivating GAL4-ER-VP16 and GAL4-ER is also demonstrated by interference with GAL4 activation (Tables I and III). This may represent transient or unstable binding under these conditions. It seems reasonable that some interaction must occur between the factor and its binding site even without an activation domain, since otherwise it is difficult to understand how the factor in its activating configuration makes its initial interaction (i.e. it seems unlikely that a single factor such as GAL4 would cause a global alteration in chromatin structure that allows the factor to interact with its binding sites in chromatin). Unmasking of the GAL4 activation domain by growth in galactose results in altered micrococcal nuclease cleavage of TALS chromatin, increased restriction enzyme accessibility to the *SacI* site in nucleosome IV, and a loss of negative superhelicity. The addition of hormone leads to similar changes mediated by GAL4-ER-VP16. Similarly, chromatin structure in TA17Δ80 shows increased perturbation by GAL4 and GAL4-ER-VP16 in their activating compared with their nonactivating forms (Fig. 1; Ref. 10). The effect of hormone addition on TALS chromatin is not seen with GAL4-ER, showing that this effect is not a consequence of unmasking an occluded DNA-binding domain.

Two pathways by which GAL4 or GAL4-ER-VP16 could alter TALS chromatin structure in an activation domain-dependent manner are by increasing factor binding and by recruiting a chromatin remodeling activity. Increased or more stable binding could result in increased loss/perturbation of nucleosome IV in TALS and nucleosomes I and II in TA17Δ80, because the GAL4 binding site is nucleosomal. This mechanism is suggested by experiments showing that occupancy of low and moderate affinity GAL4 binding sites is increased in the presence of a nearby TATA box in yeast, suggesting direct or indirect cooperative interactions between the GAL4 activation domain and TBP (45), and by in vivo footprinting data showing that an Oct-2 POU DNA-binding domain occupies binding sites in yeast more efficiently when it carries an activation domain

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2 M. Ryan and R. H. Morse, unpublished observations.
Similarly, GAL4 binding in mammalian cells is enhanced by the presence of an activation domain (47). This increased binding could be caused by an interaction between the activation domain and a protein or proteins involved in transcriptional activation, such as TBP, TFIIIB, or the RNA polymerase II holoenzyme (48–50). The interacting protein could contact DNA sequences near the GAL4 binding site and increase the effective affinity of GAL4 through cooperative interactions. Both the interactions between the contacted protein(s) and DNA and the increased effective affinity of the activator for its site could inhibit histones from occupying these sequences and thereby alter chromatin structure. One possible difficulty with this mechanism is that chromatin remodeling in TALS occurs outside of the context of a natural promoter, and therefore, presumably, high affinity binding sites for components involved in transcription are lacking. A near consensus TATA box, TATATA, is present 330 base pairs from the UASGAL-distal to the α2-MCM1 operator; however, activation domain-dependent changes in TALS chromatin structure are still observed when this sequence is mutated (data not shown). It is conceivable that nonspecific interactions between proteins recruited by the activation domain and sequences near the GAL4 binding site are sufficiently avid to increase GAL4 or GAL4-ERV-VP16 binding. Since TBP has a high affinity for nonspecific sites in DNA (51) and interacts with the GAL4 (48, 50) and VP16 (49) activation domains in vitro, it remains a good candidate for such interactions.

This first mechanism does not involve any interactions specifically directed toward chromatin; rather, protein-DNA interactions are postulated to be sufficient to alter the ability of histones to occupy particular sequences. However, strong evidence exists for proteins that have evolved to contend with chromatin templates (52), and it is possible that an unmasked activation domain acts to recruit such proteins. For example, the GAL4 DNA binding domain has been shown to interact with ADA2 in vitro (48), which is part of a complex in yeast containing GCN5 (53). Since yeast GCN5 is homologous to Tetrakymena histone acetyltransferase A and itself possesses histone acetyltransferase activity (54), recruitment of the ADAGCN5 complex could lead to alterations in chromatin structure. Another likely candidate for this role is the SWI-SNF complex (52), which in one in vitro study has been shown to cooperate with GAL4 derivatives to alter nucleosome structure and enhance GAL4 binding in a manner dependent on the strength of the activation domain attached to the GAL4 DNA-binding domain (55). It is of course also possible that both pathways contribute to the activation domain-dependent changes in chromatin structure we have reported here. For example, an activation domain may help basal transcription factors compete with histones for occupancy of sequences near the activator binding site (1), and this effect may also depend on auxiliary factors such as the SWI-SNF complex (56).

Whatever the mechanism by which the GAL4-ERV-VP16 activation domain remodels chromatin, it is apparently able to do so in the absence of replication (Fig. 7). Chromatin remodeling by PHO4 (57), by the glucocorticoid receptor (58), and by the thyroid hormone receptor-retinoid X receptor heterodimer (8) also occurs independent of replication. The ability to remodel chromatin independent of replication may prove to be a general property of transcriptional activators.

**Implications for the Mechanism of Hormone Activation of Estrogen Receptor**—Our results suggest that GAL4-ERV-VP16 can bind to a UASGAL in the absence of hormone, albeit weakly, but is still unable to activate transcription (Table I; Figs. 1 and 2). This suggests that the ER hormone-binding domain can mask activator function even when bound at a promoter, as also proposed for intact ER expressed in yeast (59) and mammalian cells (60). Remarkably, this occurs with a heterologous activation domain. Hormone administration induces substantial changes in chromatin structure, consistent with previous observations of hormone-induced alterations in chromatin structure mediated by ER derivatives expressed in yeast (44, 61). We suggest that upon hormone addition, unmasking the VP16 activation domain results in increased binding to chromatin or in recruitment of chromatin remodeling activity. An alternative explanation is that the unliganded hormone-binding domain of the ER partially masks the attached DNA-binding domain, as has been suggested for the intact ER (13, 44), and that hormone binding relieves this masking. However, if this were the case, the GAL4-ER chimera should have shown increased perturbation of chromatin upon hormone addition, and this was not observed.

**Implications for Other Systems**—As discussed in the introduction, activation domain-dependent perturbation of chromatin structure has been observed at the GAL1 and PHO5 promoters (2, 7). In both cases, perturbation is seen even when the TATA box is mutated. This could be explained by recruitment of factors that can modify chromatin components, as discussed above. It could also be explained by nonspecific interactions between TBP and DNA or by interactions between proteins recruited by the activation domain and sequences outside the TATA box. These latter interactions may be strong; Fedor and Kornberg (5) reported that coding sequences linked to the GAL1-10 promoter were transcribed under inducing conditions even in the absence of the GAL1 TATA and initiation sequences.

It has also been reported that PHO4 can bind to the nucleosomal site in the PHO5 promoter in the absence of the binding site located in the nearby linker region between nucleosomes (7, 62). This binding requires overexpression of PHO4 and requires PHO4 to carry an activation domain; it also results in disruption of all four positioned nucleosomes in the PHO5 promoter. This would again be consistent with an increased affinity of PHO4 for its binding site caused by recruitment of another DNA-binding protein by the PHO4 activation domain or by recruitment of chromatin-modifying machinery.

One important difference between the two mechanisms that we have suggested for activation domain-dependent chromatin remodeling is that one is passive in the sense that histones “get out of the way” as a consequence of binding of other factors (e.g., TBP or TFIIIB), without invoking any special chromatin remodeling activity, whereas the second explicitly invokes such an activity. Determining what proteins are required for the activation-domain-dependent changes in chromatin structure we have reported here should shed light on this problem and should also provide new insight into mechanisms of transcriptional activation in vivo.

**Acknowledgments**—We thank Robert Simpson (National Institutes of Health) for generous support and encouragement, Michael Kladde for valuable discussions, Sharon Roth for a critical reading of the manuscript, and Rachael Jones and Peter Bocca for excellent technical help. Didier Picard, Michael Kladde, and Karen Arndt are thanked for providing strains and/or plasmids. We gratefully acknowledge the use of the Wadsworth Center’s molecular genetics core facility.

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