Mutations in the AF-2/Hormone-binding Domain of the Chimeric Activator GAL4-Estrogen Receptor-VP16 Inhibit Hormone-dependent Transcriptional Activation and Chromatin Remodeling in Yeast*

(Received for publication, August 25, 1998, and in revised form, October 14, 1998)

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GAL4-estrogen receptor-VP16 (GAL4-ER-VP16), which contains the GAL4 DNA-binding domain, the human ER hormone binding (AF-2) domain, and the VP16 activation domain, functions as a hormone-dependent transcriptional activator in yeast (Louvion, J.-F., Havaux-Copf, B., and Picard, D. (1993) Gene (Amst.) 131, 129–134). Previously, we showed that this activator can remodel chromatin in yeast in a hormone-dependent manner. In this work, we show that a weakened VP16 activation domain in GAL4-ER-VP16 still allows hormone-dependent chromatin remodeling, but mutations in the AF-2 domain that abolish activity in the native ER also eliminate the ability of GAL4-ER-VP16 to activate transcription and to remodel chromatin. These findings suggest that an important role of the AF-2 domain in the native ER is to mask the activation potential of the AF-1 activation domain in the unliganded state; upon ligand activation, a conformational change releases AF-2-mediated repression and transcriptional activation ensues. We also show that the AF-2 domain, although inactive at simple promoters on its own in yeast, can enhance transcription by the MCM1 activator in hormone-dependent manner, consistent with its having a role in activation as well as repression in the native ER.

With the determination of the partial crystal structure (LBD) of four nuclear receptors, including the liganded estrogen receptor (8–11), the mechanism of action of this superfamily is coming into focus. The ligand-binding domain of nuclear receptors appears to act as a switch or a “mouse-trap.” The ligand binds to a pocket in the receptor, tripping a conformational change that rearranges helix 12 to form a “lid” over the agonist. Helix 12 contains the AF-2 core, and Wurtz et al. (12) suggest that realignment may create a new surface for interactions with co-activators and/or break connections with repressors. Brzozowski et al. (8) have shown that an estrogen antagonist prevents the alignment of helix 12, providing further evidence that activation requires this new surface.

However, it is still not clear how tripping the mouse-trap of the LBD leads to activation of the receptor. It is certain that the AF-2 core is vital for productive interaction with agonist. Danielian et al. (13) demonstrated that mutations in this core significantly reduce ligand-dependent transcription, without affecting steroid or DNA binding. The co-activators RIP 140 and TIF2 likewise require AF-2 core activity to interact with nuclear receptors (14, 15), while SRC-1 also requires a lysine residue in helix 3 (16).

Experiments utilizing the modular domains of nuclear receptors are instructive in elucidating the mechanisms of receptor activation and transcription. Previously, using a chimeric transcription factor, GAL4-ER-VP16, we demonstrated that disruption of chromatin structure required an unmasked activation domain (17). GAL4-ER-VP16 is composed of the yeast GAL4 DNA-binding domain, the human estrogen receptor hormone binding/AF-2 domain, and the viral VP16 activation domain (18). This hormone-dependent transcriptional activator perturbed chromatin in a yeast episome, outside of the context of a bona fide promoter, only when both hormone and the VP16 activation domain were present. Likewise, the yeast GAL4 protein significantly disrupted chromatin structure only when in an active form.

Here, we further show that a weakened VP16 domain in GAL4-ER-VP16 does not abrogate its ability to disrupt chromatin. Conversely, mutations that abolish AF-2 activity without compromising hormone binding or binding to DNA in the intact estrogen receptor (13) also eliminate the ability of GAL4-ER-VP16 to activate a lacZ reporter and disrupt chromatin structure without interfering with the ability of the chimeric receptor to bind hormone or to bind to DNA. These results suggest that the AF-2 domain has a repressive function in the unliganded ER, which can act even on a heterologous activation domain, and that mutations in the AF-2 domain can prevent ligand from “unlocking” this configuration. Additionally, these results support the tight correlation between an intact activation domain and chromatin remodeling observed previously (17, 19–21). We also show that, while eliminating
the VP16 moiety results in a factor incapable of independently activating transcription or remodeling our chromatin reporter. GAL4-ER can still synergize with a proximal activator.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Yeast Transformation—The plasmid TALS was transformed into yeast following digestion with HindIII and religation to remove pUC19 sequences (22). GAL4-ER-VP16 was expressed from the plasmid pHCA (generously provided by D. Picard; Ref. 18). Mutations in GAL4-ER-VP16 were introduced by polymerase chain reaction. GAL4-ER-VP16 mutants were subcloned into pRS414 following digestion with ClaI and SpeI. The coding sequence for the VP16 activation domain was removed by SacI digestion of the parent GAL4-ER-VP16 construct, followed by religation. The β-galactosidase reporter plasmid pRS314–178αlacZ contains a single GAL4 binding site upstream of the bacterial lacZ gene (23). The plasmid pRS314a2GAL4lacZΔNco contains the lacZ gene and CYC1 TATA box from the plasmid pL6G69-Z (24) fused to a SalI fragment containing the α2/MCM1 operator from the STE6 promoter and a 17-bp near-consensus GAL4 binding site (25), carried on the CEN-containing shuttle vector pRS314 (26). The GAL4 binding site is 515 bp upstream of the transcription start site for lacZ in this plasmid, and the edge to edge distance between the GAL4 and MCM1 binding sites is 71 bp. LexA-ER was made by replacing the GAL4 DNA-binding domain in pHCA with the bacterial LexA DNA-binding domain. The reporter plasmid pSH18–34 contains eight LexA binding sites driving the VP16 activation domain (27). Strains used were YNN282 (MATa trpl-1 his3-200 ura3-52 lys-801 ade2-101) (22), CY296 (MATa gal4Δ::LEU2 lys2–801 leu2–Δ1 his3-200 ura3-5299) (28) and YJ0 (MATa gal4Δ gal80Δ ura3-52 leu2–3, 112 his3 trpl ade2–101), generously provided by Stephen Johnston. Cells were grown in dropout media (Bio 101) with 2% glucose or galactose. β-Estradiol (Sigma) from a 5 mM ethanol stock was added to final desired concentration 3–4 h before cells were harvested for DNA purification or determination of β-galactosidase activity. β-Galactosidase activity was assayed as described (29) and reported in Miller units: 1000 × A660/A492 × time (min) × volume (ml).

Analysis of Plasmid Chromatin—Yeast cells (1 liter) were grown at 30 °C to A600 between 0.6 and 1.6. Yeast nuclei were prepared as described previously (17, 30) and digested with varying concentrations of micrococcal nuclease (MNase) (Worthington) for 10 min at 37 °C. MNase digestion was monitored by indirect end labeling (Duralon UV, Stratagene) and Southern analysis performed. Probes were EcoRV to XhoI fragments from TALS prepared by polymerase chain reaction. Indirect end label analysis was done in two independent experiments for each of the AF-2 mutants examined here, as well as for the parent F442P receptor.

Topoisomser Analysis—DNA was prepared by pelleting 10 ml of cell cultures grown to A600 = 0.6–1.2, resuspending in 500 μl of 10 mM Tris, 1 mM EDTA, and rapidly lysing with glass beads in the presence of 100 μl of 5% SDS, 5 mg/ml protease K. Purified DNA was separated on gel at 4 V/cm for 5–5.5 h. The DNA was transferred to nylon membranes (Duralon UV, Stratagene) and Southern analysis performed. Probes were EcoRV to XhoI fragments from TALS prepared by polymerase chain reaction. Indirect end label analysis was done in two independent experiments for each of the AF-2 mutants examined here, as well as for the parent F442P receptor.

RESULTS

A Slight Reduction in the Activating Potential of GAL4-ER-VP16 Does Not Affect Its Ability to Remodel Chromatin—We previously used the chimeric transcription factor GAL4-ER-VP16 to demonstrate activation domain-dependent chromatin disruption in the yeast episome TALS, which contains a strong binding site for GAL4 within a positioned nucleosome (17). Since it has been reported that the DNA binding activity of the intact estrogen receptor is affected by hormone addition (33–35), we wished to separate possible effects of the hormone-binding domain of GAL4-ER-VP16 on its DNA-binding domain from those due to unmasking of the activation domain. We first examined the effect of reducing the activation potential of GAL4-ER-VP16 by introducing a point mutation into the VP16 activation domain, F442P, which reduces activity of the mutant than of the parental GAL4-ER-VP16. All measurements were performed with cells grown in glucose medium, and are averages of at least five independent determinations; standard errors are indicated or else are too small to be visible.

\[ A_{\text{wild type}} > A_{\text{F442P}} \]

We next assessed the ability of GAL4-ER-VP16 derivatives. Activation of pRS314–178αlacZ, a CYC1-lacZ reporter gene with a single strong GAL4 binding site (UAS17), by derivatives of GAL4-ER-VP16 was monitored in the absence or presence of β-estradiol (≥ E2). The VP16 activation domain was either absent or present as wild type or with the F442P mutation, as indicated, and the AF-2 domain was either present as wild type or mutant, or absent, as indicated. The first two columns show measurements done in the absence of GAL4-ER-VP16. All measurements were performed with cells grown in glucose medium, and are averages of at least five independent determinations; standard errors are indicated or else are too small to be visible.

\[ A_{\text{wild type}} > A_{\text{F442P}} \]

We next assessed the ability of GAL4-ER-VP16(F442P) to remodel chromatin structure in the TALS reporter plasmid. The TALS minichromosome is packaged into strongly positioned nucleosomes in yeast (20). Furthermore, Student’s t test yields \( p < 0.001 \) for the null hypothesis that the two activators are equally active.)

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end label analysis (31, 32). The pattern of MNase cleavages in TALS chromatin induced by GAL4-ER-VP16(F442P) changed upon the addition of β-estradiol (Fig. 2, left panel). The lowest arrowhead marks a site of enhanced cleavage seen when cells are grown with hormone. The upper two arrowheads mark cleavage sites seen only in the presence of hormone. These hormone-dependent alterations in TALS chromatin demonstrate perturbation of nucleosome IV, which contains the GAL4 binding site, and the adjacent nucleosome III, and are indistinguishable from those seen previously with GAL4-VP16 (17).

Perturbation of chromatin structure in a closed circular minichromosome can result in a change in the distribution of supercoiled topoisomers, since each nucleosome in the plasmid constrains one negative supercoil (38). To investigate the effect of GAL4-ER-VP16(F442P) on TALS topology, DNA from cells expressing the chimeric factor was rapidly harvested and digested with micrococcal nuclease. MNase cleavage sites were mapped counterclockwise from the EcoRV site, as indicated. MNase concentrations used for the chromatin samples were from left to right (left panel), 0, 2, 5, 20, 20, 5, 2, and 0 units/ml for both samples in the right panel. Naked DNA (left panel) was digested at 1 and 4 units/ml MNase. The arrowheads indicate cleavage sites that are enhanced or cut only when hormone is present. Location of the unperturbed nucleosomes II–V (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.

A Functional AF-2 Core Is Required for Both Transcriptional Activity and Chromatin Disruption by GAL4-ER-VP16—The hormone-binding domain of the estrogen receptor also contains an activation domain (AF-2). The AF-2 domain apparently lacks a universal ability to activate transcription on its own; although it can activate transcription from a complex promoter, it does not activate transcription from
A β-galactosidase reporter gene driven by LexA-ER-VP16 via eight LexA binding sites was used with the indicated concentrations of β-estradiol in the presence of the wild type or mutant GAL4-ERVP16(F442P) receptors indicated. β-Galactosidase activities were measured and normalized to those obtained in the presence of the empty vector pRS414 (defined as 1.00 in the first column). Each value is derived from six independent measurements. WT, wild type.

### Table II

| Competition for limiting β-estradiol by GAL4-ERVP16(F442P) receptors reduces transcription of a β-galactosidase reporter gene by LexA-ERVP16 |
|---|
| WT | L539A/L540A | M543A/L544A |
| 2.5 nM β-estradiol | 1.00 | 0.62 ± 0.04 | 0.05 ± 0.05 |
| 250 nM β-estradiol | 1.00 | 0.90 ± 0.03 | 0.82 ± 0.03 |

### Table I

| Alterations in linking number of TALS minichromosomes by parent and mutant GAL4-ERVP16(F442P) receptors |
|---|
| WT | L539A/L540A | M543A/L544A |
| Linking number shift | 0.6 ± 0.2 (3) | 0.07 ± 0.14 (4) | 0.03 ± 0.02 (3) |

Values shown were obtained by taking the differences between the centers of the Gaussian distributions of TALS topoisomers in the presence and absence of 100 nM β-estradiol for each of the indicated GAL4-ERVP16(F442P) receptors. The number of independent measurements used to determine each value is given in parentheses.

a simple promoter lacking binding site(s) for other activators (6, 17, 18).

Either of two pairs of mutations within AF-2 (L539A/L540A and M543A/L544A of the human ER) almost totally abolishes transcriptional activation by the estrogen receptor in mammalian cells, without affecting DNA or ligand binding (13). In order to assess the requirements for AF-2 activity in the context of the chimeric activator, we introduced these mutations into GAL4-ERVP16(F442P) and expressed the resulting mutant proteins in yeast.

To demonstrate that the mutant GAL4-ERVP16 receptors still effectively bind hormone, we first attempted binding assays using radiolabeled β-estradiol with both intact yeast cells (40) or yeast cellular extracts (41). Unfortunately, although binding was easily measured and could be competed with unlabeled β-estradiol, identical results were obtained whether or not the yeast cells expressed GAL4-ERVP16. As an alternative, we therefore performed an in vivo competition assay. YJ0 cells harboring an expression vector for LexA-ERVP16 and a β-galactosidase reporter containing eight LexA binding sites were transformed with expression plasmids for mutant and wild type GAL4-ERVP16(F442P) or an empty vector control. LexA-ERVP16 is identical to GAL4-ERVP16, except it contains the LexA DNA-binding domain in place of that for GAL4, and functions as a hormone-dependent transcriptional activator via LexA binding sites. At limiting hormone concentrations, we expected that the GAL4-ERVP16 chimeras might compete with LexA-ERVP16 for the available β-estradiol and hence decrease transcription of the β-galactosidase reporter gene.

As shown in Table II, β-galactosidase activity induced by LexA-ERVP16 in the presence of 2.5 nM β-estradiol was indeed reduced by about 40% in cells harboring pRS414GAL4-ERVP16(F442P) compared with cells harboring the empty vector pRS414. Similarly, the chimeric GAL4-ERVP16(F442P) receptors containing mutated AF-2 domains also reduced β-galactosidase activity induced by LexA-ERVP16 by about 50% (Table II). In contrast, β-galactosidase activity induced by LexA-ERVP16 was reduced only slightly by either the wild type or mutant GAL4-ERVP16(F442P) receptors at 250 nM β-estradiol (Table II), supporting the interpretation of the results at 2.5 nM β-estradiol as being due to competition for limiting hormone. Thus, these results indicate that, as for the intact ER (13), the L539A/L540A and M543A/L544A mutations in the AF-2 domain do not affect hormone binding in the GAL4-ERVP16 chimeras.

When GAL4-ERVP16(F442P) harboring either the L539A/L540A or M543A/L544A mutation were assayed for transcriptional activity using the UAS17-lacZ reporter, both were found to be completely inactive (Fig. 1). The L539A/L540A and M543A/L544A mutations also result in nearly complete inactivation of GAL4-ERVP16 (i.e. with completely active VP16) (Fig. 1 and data not shown). The data of Table II suggest that the mutated proteins were expressed at levels comparable to the parent GAL4-ERVP16(F442P). To provide further evidence for their expression and to show that the AF-2 mutants were capable of binding to a GAL4 binding site, we examined the ability of unliganded GAL4-ERVP16, GAL4-ERVP16(F442P), and the AF-2 mutants derived from the latter to interfere with transcriptional activation by native GAL4 in cells grown in galactose-containing media (Fig. 4). Both unliganded mutant and wild-type receptors interfered with activation by GAL4, indicating that they occupy the UAS17 to similar extents. As a control, we examined transcription of a LexA-lacZ reporter by a fusion protein containing the LexA DNA-binding domain and the GAL4 activation domain and found that transcription was unaffected by the presence of unliganded GAL4-ERVP16 (data not shown). Thus, even in the context of a heterologous activation domain [VP16 or VP16(F442P)], the chimeric receptor requires an active AF-2 function to activate transcription.

We next assessed the effect of the AF-2 mutation on the ability of the chimeric activator to remodel chromatin. In contrast to GAL4-ERVP16(F442P), GAL4-ER(L539A/L540A)VP16 (F442P) does not alter the MNase cleavage pattern of TALS chromatin upon addition of β-estradiol (Fig. 2). Consistent with this result, the distribution of supercoiled TALS topoisomers is not affected by hormone induction in the presence of the AF-2 mutants (Fig. 3, lanes 3 and 4; Table I). We conclude that the AF-2 mutations, which do not affect DNA binding or hormone binding (13), abolish the ability of GAL4-ERVP16(F442P) both to activate transcription and to remodel chromatin.

### Hormone-dependent Synergy between the AF-2 Domain and the Yeast Activator MCM1—To investigate the effect of a non-activating GAL4-ERVP16 derivative with an intact regulatory (ER) domain, we excised the VP16 moiety to recover GAL4-ER. GAL4-ER is unable to activate transcription from the UAS17-lacZ reporter in our assay (Fig. 1 and Ref. 17). It does, however, measurably inhibit transcription by endogenous GAL4, showing that it is expressed and capable of DNA binding (17). GAL4-ER binding slightly affects TALS chromatin, but addition of hormone does not induce any further changes, as assayed by MNase digestion, restriction enzyme accessibility, or plasmid topology (17).

To discern whether the presence of hormone could increase DNA binding of GAL4-ER, we utilized a different lacZ reporter, pRS314-e2GAL4lacZαNco. In addition to a single GAL4 binding site in the promoter, this reporter has a binding site for the α2 and MCM1 proteins further upstream. In yeast α cells, which lack α2 protein, MCM1 binds to this site and activates transcription (Fig. 5; Ref. 42). Non-activating proteins binding at the GAL4 site are expected to interfere sterically with activation by MCM1 (43). Indeed, GAL4-ER measurably reduced lacZ activity (Fig. 5) in the absence of hormone, as did GAL4-ER(L539A/L540A) and GAL4-ER(M543A/L544A). However, the addition of hormone did not result in a greater reduction of lacZ activity by GAL4-ER, as would be expected if

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3 B. Balasubramanian and R. H. Morse, unpublished results.
lacZ reporter gene with a single strong GAL4 binding site (UAS17), were VP16.

Yeast cells containing pRS314–17, a CYC1-lacZ reporter gene with a single strong GAL4 binding site (UAS17), were grown in glucose (first column) or galactose in the presence or absence of GAL4-VP16 derivatives as indicated. All measurements were done in the absence of hormone so that activation was solely due to GAL4. Values are averages of two (last two columns) or more measurements, and standard errors are indicated.

DNA-binding of transcriptionally inert GAL4-ER were increased by hormone binding. Instead, lacZ activity was increased nearly 2-fold (Fig. 5). This level is higher than that seen in the absence of any GAL4-ER, indicating that it cannot be attributed to loss of GAL4-ER binding, but rather must reflect enhanced transcription from the combined effects of MCM1 and ligand-bound GAL4-ER. We observed a similar effect in a different yeast strain, YJ0 (data not shown). This increase in activity required a functional AF-2 core, as neither GAL4-ER(L539A;L540A) nor GAL4-ER(M543A;L544A) increased MCM1-activated transcription in the presence of β-estradiol (Fig. 5).

DISCUSSION

The results presented here support the view of the hormone binding/AF-2 domain of the human estrogen receptor as a modular entity that is capable of regulating the activity of a heterologous activation domain, in agreement with previous results (Ref. 18 and references therein). We further show that the hormone-dependent release of the heterologous VP16 activation domain from ER-mediated repression is abolished by mutations in the AF-2 domain (Fig. 1), which do not affect DNA binding or ligand binding (Figs. 4 and 5; Table II; Ref. 13), suggesting that these mutations lock the AF-2 domain in a repressive configuration. These findings suggest that an important role of the AF-2 domain in the native ER is to mask the activation potential of AF-1 in the unliganded state; upon ligand activation, a conformational change releases AF-2-mediated repression and transcriptional activation ensues. Transcriptional activation by the native ER and other nuclear hormone receptors in their native, physiological contexts is clearly more complicated than this, with ligand binding resulting in release of co-repressors and recruitment of co-activators (7). These additional complexities are absent in the heterologous system studied here, and their relative importance therefore cannot be assessed; nevertheless, the work reported here indicates a role for the unliganded AF-2 domain in directly preventing activation by a linked activation domain that is likely to be relevant to its normal function in the intact ER.

We also show that the AF-2 domain, although inactive at simple promoters on its own in yeast (Refs. 17 and 18; Fig. 1), can increase transcription by the MCM1 activator in a hormone-dependent fashion (Fig. 5). This would be consistent with the AF-2 domain contributing to activation as well as repression in the native ER, as suggested by previous work (2–4). Finally, we demonstrate that the AF-2 mutations which abolish transcriptional activation by GAL4-ER-VP16 also abolish chromatin remodeling (Figs. 2 and 3), supporting our previous conclusion that chromatin remodeling by GAL4-ER-VP16 and by GAL4 requires an unmasked transcription activation domain (17).

Considerable progress has been made toward understanding the mechanism whereby the binding of a ligand to the estrogen receptor promotes transcription. Structural studies have shown that ligand binding causes a conformational change within the ligand-binding domain, repositioning several helices, most importantly helix 12 (8–11). It has been proposed that the proper placement of helix 12 creates a surface that interacts with coactivators (12). Although the evidence for coactivators in mammalian cells is overwhelming (7), any mechanism for ER activation must also include AF-1, which can function alone (3, 5) and which synergizes with AF-2, even when expressed as a separate protein (4). The activation potential of AF-1 must therefore somehow be nullified in the absence of hormone in the intact ER. Furthermore, GAL4-ER-VP16 is virtually inactive in the absence of hormone (17, 18), indicating that AF-2 can prevent even a heterologous activation domain from functioning in the absence of ligand.

![Fig. 4. Inhibition of GAL4 activation by derivatives of GAL4-ER-VP16](image)

![Fig. 5. Effect of GAL4-ER derivatives on MCM1-activated transcription of pRS314+2GAL4lacZΔNco in the presence and absence of hormone.](image)
The unliganded AF-2 domain could act as a modular repressor domain by interacting with another protein(s) that could prevent the receptor from binding to its cognate site. Steroid hormone receptors can complex with many proteins, including hsp90, which could prevent the estrogen receptor from binding to promoter sites (1). However, Lee et al. (44) have demonstrated that this mechanism cannot provide a complete explanation by designing chimeric receptors that do not bind hsp90, yet still display hormone dependence. They fused a VP16 activation domain N-terminal to the GAL4 DNA-binding domain and several truncations of the ER ligand-binding domain. These chimeras displayed 15–35% of the activity of VP16-GAL4 in the absence of hormone, but activity was enhanced 4-fold by estradiol. These results indicate that at least part of the hormone dependence of the AF-2 domain is independent of sequestration by hsp90, consistent with previous suggestions (45, 46). Even more directly, we have found that GAL4-ERVP16 and GAL4-ER bind DNA in the absence of β-estradiol, as inferred from their ability to inhibit activation by GAL4 (Fig. 4; Ref. 17) and by MCM via steric interference (Fig. 5), as well as the ability of unliganded GAL4-ERVP16 to perturb chromatin structure, albeit weakly (17).

The AF-2 domain of the unliganded receptor could interact with a repressor. For example, the thyroid hormone receptor binds the repressor SMRT, although its exact mechanism of action is unknown (47). However, the presence of a repressor in yeast that would act on the AF-2 of the mammalian receptor seems unlikely, although certainly not impossible (48, 49). More tellingly, we do not see evidence of trans-repression (e.g. of MCM1), as we would expect from a repressor that contacts general transcription factors or modifies chromatin (as expected for a histone deacetylase; Ref. 50).

We propose that the unliganded AF-2 domain presents a surface that binds nearby activation domains (AF-1 or VP16), preventing them from contacting normal targets. If the unliganded AF-2 surface resembled such a target, it would explain why AF-2 inhibits a heterologous activator. This is also consistent with the differences between GAL4-ERVP16, which is essentially inactive in the absence of hormone, and VP16-GAL4-ER, which retains 15–35% of its hormone-stimulated activity even in the absence of hormone (44); the conformation of the activation domain with respect to AF-2 determines the extent of inhibition. We suggest that, in yeast, the ligand-binding domain principally regulates the linked activation domain, which, when unmasked, recruits general transcription factors and/or chromatin remodeling complex(es). When the core AF-2 is mutated, the second activation domain cannot be released from its repression by AF-2, and no remodeling or transcription is seen. This hypothesis does not dismiss the role of co-activators like SRC-1 and RIP-1 (as expected for a hormone-stimulated receptor).

The chromatin remodeling activity tightly correlates with the transcriptional potential of the receptor, consistent with findings with the thyroid hormone receptor and the yeast transcriptional activators Gal4p and Pho4p, as well as with our previous work (17, 19–21). GAL4-ER was not able to activate transcription or cause chromatin remodeling (17), and the AF-2 mutants used in this paper cause simultaneous extinction of transcriptional activation and chromatin remodeling by GAL4-ERVP16(F442P). Thus, chromatin remodeling appears generally to be intimately linked to transcriptional activation. Uncoupling these functions, other than by the trivial routes of disabling transcription by crippling critical promoter elements or downstream components of transcription (as with α-amanitin), is likely to require considerable ingenuity (51, 52).

The increase in MCM1-activated transcription caused by ligand-bound GAL4-ER was unexpected, and suggests that there can exist a threshold below which an “activator” cannot function on its own, although it can still synergize with another (weak) activator. Similar results have been reported using the vitellogenin A1 io promoter (53). This promoter contains estrogen response elements which cannot activate the promoter on their own, but which contribute to transcription by Sp1. One mechanism suggested to account for this synergy was that Sp1 could interact with the same transcriptional machinery as the liganded estrogen receptor at the downstream estrogen response elements. In our system, it may be that the AF-2 domain alone does not interact strongly enough with the transcriptional machinery in yeast to activate transcription in isolation, but does interact sufficiently to stabilize interactions created by another activator (e.g. MCM1) and thus to increase levels of transcription. This mechanism does not differ in principle from mechanisms proposed to account for synergy between activators, but adds the proviso that one activator can be so weak that it cannot activate transcription by itself and yet still can enhance transcription if another, stronger activator acts at the same promoter.

Acknowledgments—We thank the Wadsworth Center Molecular Genetic Core Facility for sequencing and oligonucleotide synthesis. We thank Peter Bocca for excellent technical assistance, Dr. Stephen John-son for providing the yeast strain YJ0, Drs. Bhuvana Saburabramanian and Steve Hanes for gifts of plasmids, Dr. John Gierthy for advice and helpful discussions, and Dr. Trevor Archer for a critical reading of the manuscript.

REFERENCES

1. Beato, M., Herrlich, P., and Schütz, G. (1995) Cell 83, 851–857
2. Webster, N. J. G., Green, S., Jin, J. R., and Chambon, P. (1988) Cell 54, 199–207
3. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., and Chambon, P. (1989) Cell 59, 477–487
4. Kraus, W. L., McInerney, E. M., and Katzenellenbogen, B. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12134–12138
5. Pham, T. A., Hwang, Y.-P., Santiso-Mere, D., McDonnell, D. P., and O’Malley, B. W. (1992) Mol. Endocrinol. 6, 1043–1050
6. Metzger, D., Losson, R., Bornert, J.-M., Lemoine, Y., and Chambon, P. (1992) Nucleic Acids Res. 20, 2813–2817
7. Torchia, J., Glass, C., and Rosenfeld, M. G. (1998)Curr. Opin. Cell Biol. 10, 337–383
8. Brazowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., Bonn, T., Engstrom, O., Ohman, L., Greene, G. L., Gustafsson, J.-A., and Carlquist, M. (1997) Nature 389, 753–758
9. Bourguet, W., Ruff, M., Chambon, P., Grønemeyer, H., and Moras, D. (1995) Nature 375, 377–382
10. Renaud, J.-P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Grønemeyer, H., and Moras, D. (1995)Nature 387, 681–689
11. Wagner, R. L., Apliette, J. W., Britton, M. E., West, B. L., Baxter, J. D., and Fletterick, R. J. (1995) Nature 378, 690–697
12. Wurtz, J.-M., Bourguet, W., Renaud, J.-P., Vivat, V., Chambon, P., Moras, D., and Grønemeyer, H. (1996)Nat. Struct. Biol. 3, 87–94
13. Danielian, P. S., White, R., Lees, J. A., and Parker, M. G. (1992)EMBO J. 11, 1025–1033
14. Joyeux, A., Cavailles, V., Balaguer, B., and Nicolas, J. C. (1997) Mol. Endocrinol. 11, 193–202
15. Voegel, J. J., Heine, M. J. S., Zechel, C., Chambon, P., and Grønemeyer, H. (1990)EMBO J. 15, 3667–3675
16. Henrutt, P. M. A., Kalkhoven, E., and Parker, M. G. (1997)Mol. Cell Biol. 17, 1832–1839
17. Stafford, G. A., and Morse, R. H. (1997)J. Biol. Chem. 272, 11526–11534
18. Legrosion, J.-P., Havaux-Copé, B., and Picard, D. (1993)Gene (Amst.) 131, 129–134
19. Axelrod, J. D., Reagan, M. S., and Majors, J. (1993)Genes Dev. 7, 857–869
20. Svanen, J., Schmitz, J., and Horz, W. (1994)EMBO J. 13, 4856–4862
21. Miller, J. H. (1972)Experiments in Molecular Genetics, Cold Spring Harbor Library Press, Cold Spring Harbor, NY
22. Shimizu, M., Roth, S. Y., Stent-Gyorgyi, C., and Simpson, R. T. (1991)EMBO J. 10, 3033–3041
31. Nedospasov, S. A., and Georgiev, G. P. (1980) *Biochem. Biophys. Res. Commun.* **92**, 532–539
32. Wu, C. (1980) *Nature* **286**, 854–860
33. Gilbert, D. M., Losson, R., and Chambon, P. (1992) *Nucleic Acids Res.* **20**, 4525–4531
34. Kladde, M. P., Xu, M., and Simpson, R. T. (1996) *EMBO J.* **15**, 6290–6300
35. Cheskis, B. J., Karathanasis, S., and Lyttle, C. R. (1997) *J. Biol. Chem.* **272**, 11384–11391
36. Berger, S. L., Pina, B., Silverman, N., Marcus, G. A., Agapite, J., Regier, J. L., Triezenberg, S. J., and Guarente, L. (1992) *Cell* **70**, 251–265
37. Cooper, J. P., Roth, S. Y., and Simpson, R. T. (1994) *Genes Dev.* **8**, 1400–1410
38. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., and Chambon, P. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72**, 1843–1847
39. Morse, R. H. (1991) *J. Mol. Biol.* **222**, 133–137
40. Shiau, S.-P., Glasebrook, A., Harkikar, S. D., Yang, N. N., and Hershberger, C. L. (1996) *Gene (Amst.)* **179**, 205–210
41. Lyttle, C. R., Damian-Matsumura, P., Juul, H., and Butt, T. R. (1992) *J. Steroid Biochem. Mol. Biol.* **42**, 677–685
42. Keleher, C. A., Goutte, C., and Johnson, A. D. (1988) *Cell* **53**, 927–936
43. Keegan, L., Gill, G., and Ptashne, M. (1984) *Science* **221**, 699–704
44. Lee, H. S., Aumais, J., and White, J. H. (1996) *J. Biol. Chem.* **271**, 25727–25730
45. McDonnell, D. P., Nawaz, Z., and O’Malley, B. W. (1991) *Mol. Cell. Biol.* **11**, 4350–4355
46. Reese, J. C., and Katzenellenbogen, B. S. (1992) *Mol. Cell. Biol.* **12**, 4531–4538
47. Chen, J. D., and Evans, R. M. (1995) *Nature* **377**, 454–457
48. Baniamad, C., Nawaz, Z., Baniamad, A., Gleeson, M. G. G., Tsai, M.-J., and O’Malley, B. W. (1995) *Mol. Endocrinol.* **9**, 34–43
49. McDonnell, D. P., Vegeto, E., and O’Malley, B. W. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10563–10567
50. Kadosh, D., and Struhl, K. (1997) *Cell* **89**, 365–371
51. Maclay, P. C., Saven, J., Martin, S. R., Horw, W., and Goding, C. R. (1998) *Mol. Cell. Biol.* **18**, 5818–5827
52. Fryer, C. J., and Archer, T. K. (1998) *Nature* **393**, 88–91
53. de Medeiros, S. R. R., Krey, G., Hishi, A. K., and Wahl, W. (1997) *J. Biol. Chem.* **272**, 18250–18260
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J. Biol. Chem. 1998, 273:34240-34246.
doi: 10.1074/jbc.273.51.34240

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