Activation domain-mediated enhancement of activator binding to chromatin in mammalian cells

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ABSTRACT DNA binding by transcriptional activators is typically an obligatory step in the activation of gene expression. Activator binding and subsequent steps in transcription are repressed by genomic chromatin. Studies *in vitro* have suggested that overcoming this repression is an important function of some activation domains. Here we provide quantitative *in vivo* evidence that the activation domain of GAL4-VP16 can increase the affinity of GAL4 for its binding site on genomic DNA in mammalian cells. Moreover, the VP16 activation domain has a much greater stimulatory effect on expression from a genomic reporter gene than on a transiently transfected reporter gene, where factor binding is more permissive. We found that not all activation domains showed a greater activation potential in a genomic context, suggesting that only some activation domains can function *in vivo* to alleviate the repressive effects of chromatin. These data demonstrate the importance of activation domains in relieving chromatin-mediated repression *in vivo* and suggest that one way they function is to increase binding of the activator itself.

Binding of transcriptional activators to DNA is frequently inhibited by the presence of nucleosomes. GAL4 derivatives, glucocorticoid receptor, progesterone receptor, USF, Sp1, and Myc/Max heterodimers are all able to bind to nucleosomal DNA, but the affinity of these activators for nucleosomal DNA is decreased by up to three orders of magnitude over that observed on naked DNA (1–12). Other activators, such as NF1 and human heat shock factor 1, cannot bind to nucleosomal DNA under conditions tested so far, raising the possibility that nucleosomal inhibition of binding might prevent the function of these activators under certain circumstances (3, 10, 13). The mechanisms that facilitate activator binding to chromatin are therefore a key aspect of transcriptional control in eukaryotes.

Several considerations suggest that activation domains might increase the ability of activators to bind to nucleosomal DNA. Activation domains can contact general transcription factors (GTFs; for review, see refs. 14 and 15), leading to the possibility that cooperative interactions between activators and GTFs might facilitate activator binding in an activation-domain-dependent manner. Activation domains are also capable of alleviating the repressive effects of nucleosomes on transcription *in vitro* (16–18), and can alter chromatin structure in intact yeast cells (19, 20). Thus, activation domains might be able to direct remodeling of chromatin, which in turn might increase binding by the activator itself.

Recent studies in yeast and mammals support the hypothesis that active mechanisms exist for facilitating activator binding to chromatin. The yeast and human SWI/SNF complexes are able to disrupt nucleosome structure and increase binding affinity of GAL4 derivatives in an ATP-dependent manner (21–23). Human SWI/SNF fractions had a greater stimulatory effect on GAL4 binding to nucleosomal DNA when the GAL4

derivative contained either the AH or the VP16 transcriptional activation domain, suggesting a possible interaction between hSWI/SNF and activation domains in facilitating activator loading (23). It is therefore possible that direct contacts between activation domains and nucleosome remodeling complexes such as SWI/SNF, in addition to direct contacts between activation domains and GTFs, might increase the ability of activators to bind in chromatin. Studies in yeast cells have shown an effect of activation domains on binding by activators *in vivo* (refs. 24 and 25; G. A. Stafford and R. Morse, unpublished observations), although this effect has been ascribed to interactions with factors binding to the TATA sequence (25).

We demonstrate here that the VP16 activation domain can enhance the ability of the corresponding GAL4 derivative to bind to sites located in genomic chromatin in mammalian cells. In addition, we have measured the relative activation potentials of these GAL4 derivatives and others containing different activation domains on chromosomal and transiently transfected reporters. We found that the relative potencies of these activators were dramatically different when compared under these two conditions, such that only certain activation domains were effective in stimulating transcription from the genomic reporter. Thus, certain activation domains such as VP16 are highly potent in a chromatin context *in vivo*, and part of that potency may result from stimulation of activator binding.

MATERIALS AND METHODS

Generation of Cell Lines. BALB/c 3T3 cells were electroporated with pX₆G₂CAT (26) plus a neomycin marker. Clones were isolated in DMEM/10% calf serum with 1 mg/ml G418 (GIBCO) and the reporter copy number in each clone was determined by Southern blot analysis. Two independently derived clonal cell lines, termed XG4 and XG6, were electroporated with pTet-tTA and a puromycin marker. Clones were selected in the above medium containing 0.4 mg/ml G418, 2 μg/ml puromycin (Sigma), and 2 μg/ml tetracycline (Sigma) and tested for Tet induction as described (27). Highly inducible clones were electroporated with Tet-regulated GAL4 protein expression constructs, pTet-spliceGAL4-VP16 or pTet-spliceGAL4(1–94) (28–30), plus a hygromycin marker. Clones were selected in the above medium plus 0.4 mg/ml hygromycin B (Calbiochem). GAL4-VP16 and GAL4(1–94) cell lines showing the highest levels of inducible electrophoretic mobility-shift assay (EMSA) activity were chosen for further studies.

Nuclear Extract Preparation. Dishes (10 cm) of subconfluent cells were harvested following induction for 40 hr in medium containing a given amount of Tet (see Fig. 1B). Nuclear extracts were prepared and EMSA reactions were

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Abbreviations: GTF, general transcription factor; EMSA, electrophoretic mobility-shift assay; TBP, TATA box-binding protein; CAT, chloramphenicol acetyltransferase; LMPCR, ligation-mediated PCR. ‡To whom reprint requests should be addressed at: Department of Molecular Biology, Wellman 10, Massachusetts General Hospital, Boston, MA 02114. e-mail: kingston@frodo.mgh.harvard.edu.

performed and resolved as described (31). EMSA reactions (25 μ l) included 10 μ g of protein and a 1.3×10^{-13} mol (5.2×10^{-9} M) radiolabeled probe containing a single GAL4 site; specificity of binding was determined by competition with wild-type and mutant probes (data not shown). GAL4 specific antisera (Santa Cruz Biotechnology) was used to determine the presence of GAL4 in the shifted complexes (data not shown). Quantification of the number of moles of specifically shifted probe was done on a PhosphorImager (Molecular Dynamics) with IMAGEQUANT software (Molecular Dynamics). The values determined in this way were reproducible and comparable between separate EMSAs. For the calculations in Fig. 3, we estimated 4×10^5 cells per 10 μ g of nuclear extract used in each EMSA reactions.

In Vivo Footprinting. In parallel with cells harvested for EMSA, cells were permeabilized with 0.1 mg/ml lysolecithin (Sigma) and *in vivo* footprinting was performed on monolayer cells in a volume of 1.5 ml with 0.05 mg/ml DNase I (Boehringer Mannheim) for 2 min at room temperature (32). Genomic DNA was purified and used as template for ligation-mediated PCR (LMPCR) with Vent polymerase (New England Biolabs) as described (33). Genomic DNA (8 μ g) was used in each reaction with the set of three nested primers shown in Fig. 2A; 20 cycles of PCR followed by seven rounds of radiolabeled-primer extension were performed to visualize the footprinted region. Quantification of footprints was by phosphorimager as described by Brenowitz *et al.* (34). Briefly, protection was calculated as the ratio of the sum of values for boxed groups of bands over each GAL4 site divided by the sum of values for standards corresponding to similar groups of bands 5' (above) and 3' (below) the GAL4 sites. Protection values did not change when either one or the other GAL4 site value or standard value was used. LMPCR of *in vitro* GAL4 and TBP footprinting used $\approx 10^{-17}$ mol of treated plasmid DNA, which approximates the number of integrated template molecules per genomic DNA sample. Reconstitution of TATA box-binding protein (TBP) footprinting was performed with 10^{-7} to 10^{-8} M yeast TBP (depending upon the reaction) and 10^{-10} M DNA. Bound and digested DNA was mixed with 2.5 μ g *in vitro* DNase-treated genomic DNA and subjected to LMPCR.

Transfections. XG4 and XG6 cell lines were transfected by the calcium phosphate method (34). The DNAs in cotransfection precipitates included a GAL4-luciferase reporter, the given GAL4 activator expression construct, and a human growth hormone (hGH; Nichols Diagnostic, San Juan Capistrano, CA) expression construct to normalize transfection efficiency. Chloramphenicol acetyltransferase (CAT) expression was measured by the phase extraction protocol (34) and luciferase expression was measured according to manufacturer's instructions (Promega). The GAL4-CAT reporter contains two GAL4 sites located 20 bp upstream of the human

hsp70 TATA element and transcription start site (26). The GAL4-luciferase reporter had the same two GAL4 sites positioned 50 base pairs upstream of a synthetic TATA element and transcription start site. Transfected reporter values are the average of two experiments and are similar to values obtained with a transient CAT reporter in BALB/c 3T3 cells (data not shown; ref. 26). Genomic CAT activity measurement were from three individual experiments; in Table 1, experiments 1 and 2 were performed on XG4 cells and experiment 3 was performed on XG6 cells.

RESULTS

System for Analysis of Factor Binding in Mammalian Cells.

To measure activator binding *in vivo*, we established mammalian cell lines that contained a genomic reporter gene and a system, based on the Tet repressor (27), that permitted the regulated expression of GAL4 proteins over a wide concentration range (Fig. 1A). BALB/c 3T3 cells were stably transfected with a CAT reporter gene containing the human *hsp70* TATA element and two GAL4 sites, and clonal cell lines were isolated. Two independent cell lines, XG4 and XG6, were used in all experiments described herein. These lines had reporter copy numbers of less than 10 integrated at single, distinct genomic loci (data not shown) and had undetectable CAT reporter activity in the absence of a GAL4 activator (Table 1 and see below). We established that the integrated reporter in both XG4 and XG6 was assembled into nucleosomes characteristic of genomic chromatin by *in vivo* micrococcal nuclease digests of these cell lines and Southern blot analysis (data not shown).

XG4 and XG6 were used to establish derivative lines that could be induced to express varying amounts of GAL4 proteins. First, XG4 and XG6 were stably transfected with pTet-tTA, which expresses a protein containing the DNA-binding domain of the Tet repressor and the VP16 transcriptional activation domain (tTA). The presence of tetracycline inhibits DNA binding by this fusion protein (27). Therefore, by varying the concentration of Tet in the growth media, we could control the level of DNA binding of tTA, thereby regulating the expression of a promoter that is regulated by tTA. We isolated clonal XG4/tTA and XG6/tTA clonal lines that had low tTA activity in the presence of Tet and high activity in the absence of Tet. These lines were then stably transfected with tTA-regulated expression vectors encoding either the GAL4 DNA-binding and dimerization domains [amino acids 1-94: GAL4(1-94)] or a fusion of GAL4(1-147) and the VP16 transcriptional activation domain (GAL4-VP16; Fig. 1A). Clonal lines of GAL4(1-94)- and GAL4-VP16-expressing cells were selected based on the Tet-inducibility and maximum expression level as determined by EMSA and CAT assay (see below). In this way, we created four clonal cell lines: sibling

Table 1. Activator strength on transfected and genomic reporters

GAL4 Activator	Transfected luciferase (XG4)	Genomic CAT			Activator strength ratio	
		Experiment 1 (XG4)	Experiment 2 (XG4)	Experiment 3 (XG6)	Genomic	Transfected
1. VP16	(100)	(100)	(100)	(100)	VP16	1
2. 1-94	11+/-1	<0.01	<0.01	<0.01	1-94	<0.001
3. HSF1	320+/-1	32	68	57	HSF1	0.2
4. AH	19+/-2	0.04	0.03	0.02	AH	0.002
5. Jun	63+/-5	0.90	0.50	N.D.	Jun	0.01
6. (-)	9+/-0	<0.01	<0.01	<0.01		

Relative activator strength was based on reporter expression as measured by CAT and luciferase activity. Values of relative strength on the transfected and the integrated GAL4 reporters were standardized to GAL4-VP16, which was defined as 100 units. Activity from the genomic CAT reporter in cells transfected with GAL4-AH was low but detectable (1- to 2-fold above background), whereas GAL4(1-94), as well as untransfected XG cells, had no detectable (N.D.) activity. Ratio values of activator strength on genomic versus transfected reporters were calculated for each GAL4 activator from the corresponding CAT and luciferase values; the average of XG4 genomic CAT reporter expression values from experiments 1 and 2 was divided by expression values for the transiently transfected luciferase reporter in XG4. GAL4-VP16 is defined as 1 (100/100). GAL4(1-94) had no detectable activity on the genomic reporter, therefore the ratio value given is a maximum.

FIG. 1. (A) Schematic showing the generation of mammalian cell lines. Clonal cell lines were isolated at each step. Line 1, XG cell lines carry a genomic CAT reporter with two GAL4 sites. Line 2, XG/tTA cells also express the Tet-regulated activator tetR-VP16 (tTA). Line 3, sibling cell lines with tTA-regulated expression of either GAL4-VP16 or GAL4(1-94) were derived from single XG/tTA lines. (B) EMSA measurement of GAL4-VP16 and GAL4(1-94) in nuclear extracts from cells induced with the indicated concentration of Tet in the growth media. The amount of GAL4 protein dimers is given in femtomoles based on the quantification of the amount of probe in the shifted complex.

derivatives of XG4/tTA in which variable levels of GAL4(1-94) and GAL4-VP16 could be expressed and similar sibling derivatives of XG6/tTA. Thus, we established an *in vivo* system in which to analyze the binding of GAL4 with and without an activation domain in identical genomic environments.

The choice of these GAL4 derivatives for our analysis of *in vivo* binding to genomic chromatin was based on the consideration that these proteins have been extensively characterized biochemically and have served as models for both transcriptional and DNA-binding studies. GAL4(1-94) and GAL4-VP16 are essentially identical in their binding affinities, both

on naked DNA and on nucleosomal templates *in vitro* (3, 23, 29). This characterization provided a context in which to evaluate the *in vivo* activities of these proteins.

Measurement of DNA-Binding Activities and Genomic Site Occupancy. Cells were maintained in Tet to keep GAL4 derivative expression repressed. In the experimental protocol, parallel cultures of cells were induced with various concentrations of Tet, thereby providing a range of steady-state levels of either GAL4(1-94) or GAL4-VP16 protein. Steady-state levels of GAL4 protein were reached by 24 hr of induction (data not shown) and cells were harvested at 40 hr. GAL4 protein levels were quantified by EMSA using a radiolabeled single GAL4 site probe and were measured in femtomoles of shifted probe (Fig. 1B). This enabled us to determine the relative amount of active protein per cell number. Measurements of relative protein as determined by EMSA were reproducible between assays within two orders of magnitude, and were linear over three orders of magnitude. We used EMSA as our primary assay for GAL4 because it measured function and because it provided the level of sensitivity necessary for a complete footprint titration experiment; we found that EMSA was two to three orders of magnitude more sensitive than Western blot analysis (data not shown). To confirm that EMSA and Western blot analysis would give consistent measurements of GAL4(1-94) and GAL4-VP16 levels, we tested cell cultures that expressed high levels of each derivative using both assay protocols, and obtained similar results with each protocol (data not shown).

Promoter occupancy by GAL4 proteins was measured by *in vivo* DNase I footprinting. In parallel with nuclear extract preparation for EMSA, induced cell cultures were permeabilized with lysolecithin, a mild ionic detergent, and treated with DNase I. Genomic DNA was isolated and used as template for LMPCR. This enabled us to amplify and visualize the genomic sequences containing the GAL4 sites (see Fig. 2A for primer locations). We found that lower concentrations of GAL4-VP16 than of GAL4(1-94) were required to bind and fully occupy the GAL4 sites *in vivo* (Fig. 2B; EMSA analysis of duplicate samples is shown in Fig. 1B). Whereas full site occupancy by GAL4(1-94) required 25 fmol and did not occur at 13 fmol (lanes 7 and 8), GAL4-VP16 was seen to fully occupy these sites at only 3.3 fmol (lane 3). We quantified the *in vivo* difference in binding between GAL4-VP16 and GAL4(1-94) in numerous experiments with both the XG4 and XG6 cell lines, and correlated the percent occupancy of the GAL4 binding sites with amount of GAL4 protein (Fig. 3 Upper Left and Upper Right). There was a 7.5-fold (XG4) or a 10-fold (XG6) difference in the concentration of GAL4-VP16 and GAL4(1-94) required for half-maximal occupancy of the GAL4 binding sites (Fig. 3 Lower). The concordance of data points from independent induction protocols with each cell line argues for the reliability of the measurement procedures used here (Fig. 3). Therefore, these data suggest that the presence of an activation domain enhances the ability of GAL4 to bind to sites located in genomic chromatin.

We have observed *in vivo* an activation domain-dependent increase in binding affinity of GAL4-VP16 relative to that of GAL4(1-94). This is in contrast to what is seen in purified systems *in vitro*, where GAL4(1-94) and GAL4-VP16 have nearly equivalent affinities for naked or nucleosomal DNA (3, 4, 30). However, increased binding of GAL4-VP16 relative to GAL4(1-94) has been seen *in vitro* in the presence of human SWI/SNF, a complex that can mediate chromatin remodeling (23). Thus, SWI/SNF or a similar activity provides a possible molecular basis for the differential binding by GAL4-VP16 and GAL4(1-94) *in vivo*. An alternative explanation is that cooperative interactions between the activation domain and GTFs mediate the enhanced site occupancy by GAL4-VP16. Cooperative binding should also result in a corresponding increase in occupancy of the TATA region. However, no

FIG. 2. (A) Schematic of genomic GAL4-CAT reporter and LMPCR primers. GAL4-CAT has two consensus GAL4 sites located 20 base pairs upstream of the human *hsp70* TATA element; *hsp70* sequences extend to +165 and are followed by the CAT gene. LMPCR primers correspond to noncoding strand sequences; primer 1 is located at the 5' end of the CAT gene and primers 2 and 3 have 3' ends located at +20 and +5 relative to the *hsp70* transcription start site. (B) LMPCR/*in vivo* footprinting of genomic GAL4 sites and TATA region following the induction of GAL4-VP16 or GAL4(1-94) shown in Fig. 1B. Parallel samples for EMSA and footprinting were prepared from duplicate cell culture plates. Lanes 13 and 14 show LMPCR of *in vitro* footprint reactions with and without purified GAL4(1-94); these were used to standardize *in vivo* footprinting in each experiment. Lanes 15-17 show LMPCR/*in vitro* footprinting with purified TBP at a saturating concentration (10^{-7} M) and dilutions thereof; lane 18 shows a parallel reaction with no TBP. Reactions in lanes 15-18 were mixed with 2.5 μ g genomic DNA and analyzed by LMPCR. "2 x GAL4 sites" and "TATA" and the corresponding bars span the DNA sequences of each.

footprints over the TATA region were observed, even in cells where the site occupancy by GAL4-VP16 was greater than 95% (Fig. 2B). To ensure that we could visualize TBP binding with our methodology, we performed reconstruction experiments in which promoter DNA bound by TBP *in vitro* was DNase treated, mixed with genomic DNA, and subjected to LMPCR. TBP protection was observed under these conditions (Fig. 2B, lanes 15-18). The lack of any detectable TATA footprint adjacent to the GAL4 sites *in vivo* argues against strict cooperativity causing increased binding by GAL4-VP16.

Activation Domain Enhanced Binding Correlates With Transcriptional Potency in Chromatin. Stably replicating chromatin is more refractory to factor binding and transcriptional activation than transiently introduced, nonreplicating templates (35, 36). The ability of an activation domain to facilitate activator binding to a genomic reporter might therefore be reflected in an increased function of the activation domain on an integrated reporter as compared with a transient reporter. We tested this by transiently cotransfecting parental XG4 cells, which contain the integrated CAT reporter but do not express GAL4, with a GAL4-luciferase reporter and a GAL4 activator. We compared the ability of the activator to stimulate expression from the transient reporter and the integrated reporter in the same cells. There was a substantial

difference in the relative strength of GAL4-VP16 on the two reporters: on the transfected luciferase reporter, GAL4-VP16 activated expression by only 9-fold over levels obtained with GAL4(1-94), whereas on the integrated reporter, GAL4-VP16 activated expression more than 10,000-fold over GAL4(1-94) (Table 1). This difference in fold activation by VP16 in these two *in vivo* contexts is analogous to what is seen *in vitro*, where GAL4-VP16 has significantly greater activity on a template that has been assembled into nucleosomes as compared with that on a naked DNA template (16-18, 37).

We also analyzed GAL4 fusion proteins containing the activation domains from HSF1, Jun, and the artificial AH domain (31, 38, 39). GAL4-HSF1, like GAL4-VP16, was a potent activator on both the transient reporter and the genomic reporter, whereas the relative strength of GAL4-Jun and GAL4-AH was reduced by at least two orders of magnitude on the integrated reporter (Table 1). The ability of an activator to function on the integrated reporter was quantified using the ratio (genomic reporter activation)/(transient reporter activation) (Table 1). By this measure, the VP16 activation domain has a value of 1 since its level of stimulation was set as the standard on each reporter. In comparison, the HSF1 activation domain, which was the most potent activator on the transient reporter, was 5-fold less potent on the genomic

FIG. 3. *In vivo* differences in site occupancy between GAL4-VP16 and GAL4(1-94). (Upper Left) Plots of percent occupancy versus protein concentration for GAL4-VP16 (■) and GAL4(1-94) (△) derivatives of XG4. Samples were from a total of five induction experiments. (Upper Right) Similar plots for XG6 derivatives. (Lower) Calculated concentrations for GAL4-VP16 and GAL4(1-94) required for half-maximal protection of the genomic GAL4 sites. Calculations were based on the equations that described each plot line as determined using CRICKETGRAPH software.

reporter, while the AH activation domain was 500-fold reduced in its function in the genomic context. The weaker activators GAL4-AH and GAL4(1-94) are expressed at somewhat higher levels than GAL4-VP16 and GAL4-HSF1 after transfection (data not shown; E. Newton and M. Green, unpublished data), indicating that differences in activator function are not caused by differences in expression level. These data suggest that activation domain function is significantly affected by reporter context, and that only certain activation domains are capable of a high level of function in a genomic context.

DISCUSSION

Current models for transcriptional activation propose that activators function by recruiting GTFs and by overcoming the inhibitory effects of genomic chromatin structure. Sequence-specific binding by activators can be inhibited by chromatin structure. Thus, it is likely that activator binding, in addition to GTF binding, can be a rate-determining step in the transcription of promoters in chromatin and that the ability of an activator to stimulate transcription will be affected by its ability to overcome chromatin-mediated inhibition of binding. Activator binding to chromatin appears to depend in part on the inherent function of the DNA-binding domain (3, 10). We show here that activation domains also play an important role in modulating the ability of an activator to bind to mammalian chromatin *in vivo*.

We have not yet determined the mechanism(s) underlying our *in vivo* observations; however, we favor the hypothesis that activation domains help recruit a nucleosome remodeling activity such as SWI/SNF, and that this recruitment facilitates

activator binding by causing the disruption of chromatin structure underlying that activator binding site. The activation domain function that we have measured *in vivo* and the proposed involvement of SWI/SNF or a related activity in this function is consistent with the *in vitro* observation that the ability of hSWI/SNF to facilitate activator binding is enhanced by the presence of an activation domain (23). Moreover, the *in vitro* results were obtained with a fractionated system containing only highly enriched hSWI/SNF fractions and purified activators and nucleosomes, suggesting that the activation domain and SWI/SNF may interact directly.

It appears that the activation domain effect on activator binding is not likely to result from simple cooperative binding between the activation domain and GTFs bound at the TATA sequence (25, 40): The TATA sequence was not occupied detectably under conditions where the activator binding site is fully bound (Fig. 2B). Apparently, a high level of expression from this integrated reporter can be achieved by only infrequent occupancy of the promoter by GTFs. (We have determined by S1 analysis that transcription from this promoter is initiated at the correct start site and that it is dependent on the presence of a GAL4 activator; data not shown). Nonetheless, it remains possible that interactions with the general machinery cause an alteration in chromatin structure that increases stable binding by the activator and that this is not reflected in an increased occupancy of the TATA sequence.

Recently, a potential connection between the general transcription machinery and SWI/SNF has been made (41). A protein complex termed mediator is associated with the C-terminal domain of yeast RNA polymerase holoenzyme and is required for activated transcription *in vitro*, suggesting that it

interacts with activation domains (42–44). The yeast SWI/SNF complex can be isolated in association with the mediator fraction. Thus, an activator could recruit SWI/SNF together with the RNA polymerase holoenzyme, thereby bringing about chromatin remodeling and GTF recruitment in a concerted step. If, in mammalian cells, SWI/SNF is associated with the holoenzyme, it is possible that the effects of the VP16 activation domain on binding observed above are caused by contact with the general machinery and consequent SWI/SNF recruitment. This model could be consistent with the observation that the TATA element was not occupied on our reporter constructs, since TFIID may not be stably associated with the holoenzyme and might need to be recruited subsequently.

We observed a dramatic difference in the ability of activation domains to function on a genomic reporter and on a transiently transfected reporter. One interpretation of this finding is that the chromatin structure of the genomic reporter is more refractory to the function of the transcriptional apparatus than is the structure of the transient reporter, and that only certain activation domains can relieve the repressive effect of genomic chromatin. We cannot rule out the possibility that a difference in copy number between the transient reporter and the integrated reporter could contribute to this result. The interpretation that chromatin structure is a primary determinant of the difference in responsiveness to GAL4 activators between the integrated and transient reporters is supported by the observation that transfected DNA is more permissive to activator binding than is genomic DNA (36). In addition, the requirement for activator function is less strict on transfected DNA where non-activator mediated transcription appears to be more permissive. Thus, in a genomic context where activator function is essential ("basal" expression from the constructs tested here was undetectable; Table 1), the potency of an activation domain might reflect its ability to facilitate activator binding, as shown above, and its ability to alleviate the repressive effects of chromatin on transcription initiation, as demonstrated *in vitro* (17, 18, 37, 45). This is consistent with observations regarding PHO4 in *Saccharomyces cerevisiae*, where it was shown that activation domain potency as measured by reporter expression correlated with PHO4 derivative binding and chromatin remodeling (20).

We suggest that the differences in activator potency that we observed may reflect the capability of some activation domains, such as VP16, to stimulate multiple steps necessary to overcome the repressive effects of chromatin, whereas activation domains like AH appear to be less capable of counteracting these effects. The fact that AH is highly active in some *in vitro* transcription assays, and is equally competent in GTF recruitment to VP16, suggests that these shared functions are not sufficient to activate transcription in the context of chromatin. The observation that the HSF1 activation domain was the most potent on the transient reporter yet less active relative to VP16 on the genomic reporter argues further for specific activation domain functions being required to stimulate transcription in chromatin.

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- Cordingley, M. G., Riegel, A. T. & Hager, G. L. (1987) *Cell* **48**, 261–270.
- Perlmann, T. & Wrangé, Ö. (1988) *EMBO J.* **7**, 3073–3079.
- Taylor, I. C. A., Workman, J. L., Schuetz, T. J. & Kingston, R. E. (1991) *Genes Dev.* **5**, 1285–1298.
- Workman, J. L. & Kingston, R. E. (1992) *Science* **258**, 1780–1784.
- Vettese-Dadey, M., Walter, P., Chen, H., Juan, L.-J. & Workman, J. L. (1994) *Mol. Cell. Biol.* **14**, 970–981.
- Walter, P. P., Owen-Hughes, T. A., Côté, J. & Workman, J. L. (1995) *Mol. Cell. Biol.* **15**, 6178–6187.
- Li, Q. & Wrangé, Ö. (1993) *Genes Dev.* **7**, 2471–2482.
- Wechsler, D. S., Papoulas, O., Dang, C. V. & Kingston, R. E. (1994) *Mol. Cell. Biol.* **14**, 4097–4107.
- Mymryk, J. S., Berard, D., Hager, G. L. & Archer, T. K. (1995) *Mol. Cell. Biol.* **15**, 26–34.
- Pina, B., Bruggemeier, U. & Beato, M. (1990) *Cell* **60**, 719–731.
- Chen, H., Li, B. & Workman, J. L. (1994) *EMBO J.* **13**, 380–390.
- Li, B., Adams, C. C. & Workman, J. L. (1994) *J. Biol. Chem.* **269**, 7756–7763.
- Archer, T. K., Cordingley, M. G., Wolford, R. G. & Hager, G. L. (1991) *Mol. Cell. Biol.* **11**, 688–698.
- Kingston, R. E. & Green, M. R. (1994) *Curr. Biol.* **4**, 325–332.
- Tjian, R. & Maniatis, T. (1994) *Cell* **77**, 5–8.
- Workman, J. L., Taylor, I. C. A. & Kingston, R. E. (1991) *Cell* **64**, 533–544.
- Kamakaka, R. T., Bulger, M. & Kadonaga, J. T. (1993) *Genes Dev.* **7**, 1779–1795.
- Lorch, Y., LaPointe, J. W. & Kornberg, R. D. (1992) *Genes Dev.* **6**, 2282–2287.
- Axelrod, J. D., Reagan, M. S. & Majors, J. (1993) *Genes Dev.* **7**, 857–869.
- Svaren, J., Schmitz, J. & Hörz, W. (1994) *EMBO J.* **13**, 4856–4862.
- Cote, J., Quinn, J., Workman, J. L. & Peterson, C. L. (1994) *Science* **265**, 53–60.
- Imbalzano, A. N., Kwon, H., Green, M. R. & Kingston, R. E. (1994) *Nature (London)* **370**, 481–485.
- Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. & Green, M. R. (1994) *Nature (London)* **370**, 477–481.
- Tanaka, F. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 4311–4315.
- Vashee, S. & Kodadek, T. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 10683–10687.
- Bunker, C. A. & Kingston, R. E. (1994) *Mol. Cell. Biol.* **14**, 1721–1732.
- Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5547–5551.
- Shockett, P., Difilippantonio, M., Hellman, N. & Schatz, D. G. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 6522–6526.
- Sadowski, I., Ma, J., Triezenberg, S. & Ptashne, M. (1988) *Nature (London)* **335**, 563–564.
- Carey, M., Kakidani, H., Leatherwood, J., Mostashari, F. & Ptashne, M. (1989) *J. Mol. Biol.* **209**, 423–432.
- Green, M., Schuetz, T. J., Sullivan, E. K. & Kingston, R. E. (1995) *Mol. Cell. Biol.* **15**, 3354–3362.
- Zhang, L. & Gralla, J. D. (1989) *Genes Dev.* **3**, 1814–1822.
- Garrity, P. A. & Wold, B. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1021–1025.
- Brenowitz, M., Senear, D. F. & Kingston, R. E. (1987) *Current Protocols in Molecular Biology*, eds Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley, New York), pp. 12.4.1–12.4.16.
- Almouzni, G. & Wolffe, A. P. (1993) *Genes Dev.* **7**, 2033–2047.
- Archer, T. K., Lefebvre, P., Wolford, R. G. & Hager, G. L. (1992) *Science* **255**, 1573–1576.
- Laybourn, P. J. & Kadonaga, J. T. (1991) *Science* **254**, 238–245.
- Baichwal, V. R., Park, A. & Tjian, R. (1992) *Genes Dev.* **6**, 1493–1502.
- Giniger, E. & Ptashne, M. (1987) *Nature (London)* **330**, 670–672.
- Lieberman, P. M. & Berk, A. J. (1991) *Genes Dev.* **5**, 2441–2454.
- Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. S., Kingston, R. E. & Young, R. A. (1996) *Cell* **84**, 235–244.
- Kim, Y.-J., Björklund, S., Li, Y., Sayre, M. H. & Kornberg, R. D. (1994) *Cell* **77**, 599–608.
- Koleske, A. J. & Young, R. A. (1994) *Nature (London)* **368**, 466–469.
- Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. & Ptashne, M. (1995) *Cell* **81**, 359–368.
- Workman, J. L., Taylor, I. C. A., Kingston, R. E. & Roeder, R. G. (1991) *Methods Cell Biol.* **35**, 419–447.