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# A Critical Role for Chromatin in Mounting a Synergistic Transcriptional Response to GAL4-VP16

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**The role of chromatin in mounting a synergistic transcriptional response to GAL4-VP16 was investigated. Strong synergy was observed when chromatin templates were used in vitro. The synergy was severely reduced when naked DNA templates were transcribed. In vivo synergy was strong when nonreplicating templates were used. However, the use of replicating templates, which involved transient disruptions of chromatin, led to strong reductions in synergy. In both of these low-synergy responses, transcription levels were high. We infer that strong synergy has a requirement for chromatin that may be understood in terms of the competition between multiple activator molecules and histone cores for promoter DNA.**

Transcription by RNA polymerase II can be activated by many sequence-specific DNA binding proteins (for a review, see reference 34). Regulation can be achieved by the combinatorial effects of these transcription factors. Genes are rarely associated with a single regulatory DNA binding site, and single sites usually activate transcription poorly. High-level expression can be obtained when multiple sites are present, either for the same or for different transcription factors. Multiple sites can exert a stimulatory effect greater than that expected from the sum of single sites, a phenomenon known as transcriptional synergy. Examples include transcriptional activation by GAL4 and its derivatives, by Sp1, by steroid hormone receptors, and by Oct-2 (1, 5, 6, 9, 13, 27, 28, 31, 36, 38, 39, 46).

The mechanisms that may contribute to synergistic activation have been studied and discussed at length (5, 14, 17, 25, 33, 44). In several cases in which factors exhibit synergy, it has been shown that these factors assist each other in binding to DNA. For example, Sp1 synergy relies on cooperative binding with factors such as Ets1, OTF-1, and bovine papillomavirus E2 (15, 20, 25). Other examples include OTF-1 interacting with hormone receptors and the assembly of factors of the same type at multiple binding sites (3, 4, 24, 32, 44, 51). These examples have in common the proposal that synergy arises from the need for multiple sites to assist the binding of required factors to the promoter.

An alternative model for synergy that does not require cooperative binding has been proposed. In this case, synergy arises from a proposed need for the activator to contact simultaneously more than one general transcription factor in order for preinitiation complexes to form (5). Thus, a single bound activator is ineffective because it cannot make both contacts simultaneously; multiple bound factors can do this easily, accounting for the synergistic response (5, 17). This model is based largely on numerous studies of GAL4-VP16 and is supported by studies demonstrating GAL4-VP16 interactions with multiple general transcription factors, including TATA box-binding protein, TATA box-binding protein-associated factors, TFIIB, and polymerase (16, 19, 26, 30, 37, 41).

Because GAL4-VP16 does not bind to naked DNA in a cooperative fashion in vitro (5), this multiple-contact hypothesis has been preferred over cooperative-binding models. One problem is that when multiple activation domains are brought to the DNA via a single site, synergistic activation is not commonly observed. For example, Emami and Carey (14) found that GAL4-VP16 hybrid proteins containing multiple VP16 activation domains stimulated transcription synergistically, but only when at least two GAL4 DNA binding sites were present. Previously, Oliviero and Struhl (35) had found that synergistic activation by a Jun/Fos heterodimer with multiple GCN4 acidic domains also could not occur from a single DNA site.

A third possible model arises from a variety of studies showing a complex interplay between GAL4 and chromatin templates (for examples, see references 11, 23, 43, and 48). In a critical experiment, Taylor et al. (43) confirmed that GAL4 did not bind cooperatively to naked DNA but found that it did bind cooperatively to chromatin in vitro. Because the synergism of the transcription response has not been compared under these two conditions, it is possible that chromatin mediates the synergistic response. In this paper, we find significant support for this third model for activation and discuss how such synergy might arise.

## MATERIALS AND METHODS

**Plasmid constructs and materials.** $pG_{1,2,5}$Tluc was constructed by cloning the *Bgl*II-*Nae*I fragment of pTluc, which contained the TATA box and luciferase coding region, into *Bam*HI-*Pvu*II sites of $pG_{1,2,5}$E4T that contained one, two, or five GAL4 DNA binding sites (from M. Carey). Plasmid $pOG_{1,2,5}$Tluc was constructed by inserting the *Xmn*I-*Stu*I fragment of $pG_{1,2,5}$Tluc into *Xmn*I-*Ssp*I sites of pOR4 (from P. Tegtmeyer) so that the resulting plasmids contained a complete simian virus 40 (SV40) replication origin (including GC boxes and one copy of enhancer) 2 kbp away from GAL4 DNA binding sites and TATA boxes.

Effector plasmid pSGVP, containing the GAL4-VP16 coding region, was from M. Ptashne. To avoid replication competition with reporter plasmids that contained the same SV40 replication origin, the *Bgl*II-*Alw*NI fragment of pSGVP containing the coding region of GAL4-VP16 was cloned into the *Bam*HI-*Alw*NI sites of pUCE3. The resulting construct had the

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activator protein under the control of the E3 promoter, and the plasmid did not contain an SV40 origin.

Purified *Escherichia coli*-expressed GAL4-VP16 protein was provided by M. Carey. HeLa cell nuclear extract was prepared according to the method of Dignam et al. (12) by Y. Jiang of this laboratory.

**In vivo expression assay.** CV-1 cells were maintained in Dulbecco's modified Eagle's medium containing 5% calf serum. COS-7 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. The indicated amounts of reporter and effector plasmids were transfected into CV-1 or COS-7 cells by the standard calcium phosphate precipitation method. Cell extracts were obtained 2 days after transfection, and luciferase activity was assayed by the standard method (Promega).

**In vitro transcription assay.** For in vitro transcription (12), 50 ng of plasmid DNA was incubated in 40-μl mixtures containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [pH 7.6]), 60 mM KCl, 7.5 mM $MgCl_2$, 12.5% glycerol, 0.6 mM dithiothreitol, 0.5 mM nucleotide triphosphates, 25 μl of HeLa cell nuclear extract, and 200 ng of GAL4-VP16 protein. After 1 h at 30°C, the reaction was stopped and the transcripts were analyzed by primer extension with reverse transcriptase. The products were electrophoresed on a 10% denaturing polyacrylamide gel and autoradiographed. For quantitation of the transcription activity, both scintillation counting of the gel slice containing the signal and densitometer scanning of film were done.

**Preparation of oocyte extract and in vitro chromatin assembly.** *Xenopus laevis* oocyte extract was prepared according to the method of Shimamura et al. (40). Basically, oocytes were digested with 0.15% type II collagenase (Sigma) in OR-2 buffer (final concentrations of 5 mM HEPES, 1 mM $Na_2HPO_4$, 82.5 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$) at room temperature for several hours until the oocytes were dispersed. The oocyte extract was then prepared by ultracentrifugation in extraction buffer (20 mM HEPES [pH 7.5], 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 10 mM β-glycerophosphate, 0.5 mM dithiothreitol) in a Beckman SW50.1 rotor for 30 min at 40,000 rpm at 4°C. The clear supernatant in the middle phase was extracted and stored at −70°C.

Chromatin was assembled on nonradioactive plasmid DNA according to the method of Shimamura et al. (40). In a 50-μl final reaction volume, 150 ng of DNA was incubated with 5 μl of 10× chromatin assembly buffer (30 mM ATP, 50 mM $MgCl_2$, 400 mM creatine phosphate), 5 μl of 10-ng/μl creatine phosphokinase, and 30 μl of oocyte extract. After 2 h at 37°C, the assembled templates were isolated by passage through a Sepharose CL-4B column (49) at 4°C. The template structure was assayed by micrococcal nuclease digestion, which revealed a nucleosome repeat pattern. In vitro transcription of one-third of the sample immediately followed the isolation of DNA templates.

**In vitro DNase footprinting.** Fifteen nanograms of naked DNA or assembled chromatin template was incubated with 200 ng of GAL4-VP16 protein at 30°C for 1 h. The 20-μl reaction volume was brought to 180 μl with buffer A (150 mM sucrose, 80 mM NaCl, 35 mM HEPES [pH 7.4], 5 mM $KH_2PO_4$, 5 mM $MgCl_2$, 2 mM $CaCl_2$). DNA was then digested with $10^{-4}$ (chromatin template) or $10^{-5}$ (naked DNA) mg of DNase per ml at room temperature for 2 min. The reaction was stopped by EDTA, and DNA was extracted by phenol and chloroform before precipitation. The extension signal was amplified by PCR with a labeled primer and revealed by gel electrophoresis and autoradiography.

TABLE 1. Similar synergistic activation patterns by different amounts of activators[a]

| Reporter | Amt of activator plasmid (μg) | Relative luciferase activity (U) | Activation ratio | Synergy |
|---|---|---|---|---|
| 1× GAL4 | 0.5 | 1.6 | | |
| 2× GAL4 | 0.5 | 17 | 11 | 5.4 |
| 5× GAL4 | 0.5 | 81 | 51 | 1.9 |
| 1× GAL4 | 1 | 2.0 | | |
| 2× GAL4 | 1 | 27 | 13 | 6.5 |
| 5× GAL4 | 1 | 90 | 45 | 1.4 |
| 1× GAL4 | 2 | 2.2 | | |
| 2× GAL4 | 2 | 39 | 18 | 9 |
| 5× GAL4 | 2 | 180 | 82 | 1.9 |
| 1× GAL4 | 5 | 2.7 | | |
| 2× GAL4 | 5 | 75 | 28 | 14 |
| 5× GAL4 | 5 | 460 | 170 | 2.4 |

[a] One microgram of reporter plasmid was cotransfected with the indicated amount of activator plasmid into CV-1 cells. Luciferase activity was assayed 2 days after transfection by scintillation counting. The fold activation was defined as activity achieved at two (2×) or five (5×) sites divided by the activity of one (1×) GAL4 site. Transcription synergism was defined as the fold increase in activation divided by the fold increase in number of sites, which yielded the same result as that calculated according to the method of Herschlag and Johnson (17). The experiments have been repeated at least twice.

## RESULTS

**Strong synergy in vivo is primarily associated with increasing the number of sites from one to two.** Previous studies of GAL4-VP16 synergy in vivo have indicated that the response is not uniform as the number of binding sites increases (5, 14). We investigated this phenomenon systematically with CV-1 cells. Plasmids containing different numbers of GAL4 binding sites upstream from a TATA-dependent promoter were assayed for expression in vivo. One microgram of these reporter plasmids containing the luciferase gene linked to one, two, or five GAL4 DNA binding sites was cotransfected with 2 μg of GAL4-VP16 expression plasmid. Two days later, expression was measured via the luciferase activity assay. Control comparisons with the reporter gene with no binding sites or with cotransfected expression vector DNA alone showed only very low background levels (data not shown).

Table 1, rows 7 to 9 (2 μg of activator plasmid), shows how activity varies with the number of GAL4 binding sites in this experiment. A single binding site yielded 2 U of activity, whereas two sites yielded 39 U. This nearly 20-fold increase upon doubling the number of sites is clearly synergistic. Increasing the number of sites from two to five yielded an increase from 39 U to 180 U. In this case, the inclusion of 2.5 times the number of sites has yielded an approximately 4.5-fold increase in expression. Therefore, the synergistic effect is very small.

These experiments were repeated with amounts of GAL4-VP16 plasmid that varied over a 10-fold range. Using a range could cover a variety of hypothetical situations, including limiting amounts of GAL-VP16 at the low end and squelching at the high end. The data in Table 1 show that, for all constructs, activity increases with the amount of GAL4-VP16 effector plasmid. This demonstrates that the effects seen over this range correspond to those for a situation in which the availability of GAL4-VP16 effectively limits expression.

In all four sets of conditions, transcriptional synergy was strong when one and two sites were compared and weak when two and five sites were compared. In this context, we define synergy as the fold increase in activation divided by the fold increase in the number of sites. The identical conclusion is

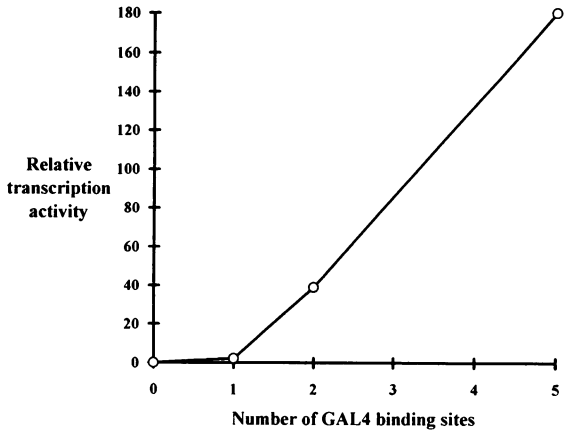


FIG. 1. Synergistic transcription activation in vivo. The relative transcription activation from luciferase assays is plotted against the number of GAL4 binding sites.

reached when synergy is defined with a construct with zero sites as a standard (17), because the two ratios are simply related by a constant. In the four conditions tested, the amounts of synergy observed when comparing one and two sites were 5, 7, 9, and 14. There appears to be a trend of increasing synergy as the amount of GAL4-VP16 is increased over this range. By contrast, when the synergy arising from further increasing the number of sites from two to five was calculated under the same four conditions, very low values of about 2, 2, 2, and 2 were obtained (Table 1). The absolute amount of activation from the construct containing five sites increased sixfold as the amount of expression plasmid was increased from 0.5 μg to 5 μg; this confirms that the lack of strong synergy upon increasing the number of sites from two to five is not due to a lack of available activator over this range of conditions. These data indicate that the synergistic response under these conditions is associated primarily with the increase in the number of sites from one to two.

This view is extended in Fig. 1, which plots the activation observed versus the number of sites from one of the four experiments described above (2 μg of expression plasmid). This form of presentation illustrates that the synergy apparent in Table 1 is essentially a consequence of the extremely low level of activity of a single-site construct. After one achieves a high level of activity with two sites, the response to additional sites is linear. Similar plots for the other experiments show similar behavior (not shown).

**DNA replication reduces transcription synergy.** As discussed above, chromatin can cause a general repression of transcription and may also influence the cooperativity of protein binding (10, 11, 22, 43, 50). We used DNA replication to transiently disrupt chromatin structure and see if the synergy changes. During replication, competition between the binding of transcription factors and the formation of nucleosomes on the DNA is established. During this process, genes that have been repressed by chromatin structure can be activated (for example, see references 21 and 42). This activation could have a component that resulted from the interaction of transcription factors with DNA that was not fully chromatinized in vivo. In the case of GAL4-VP16, this transient disruption of chromatin in vitro plays a role in its ability to activate transcription (21). Therefore, we explored whether actively replicating templates would still exhibit synergy.

In this experiment, the SV40 replication origin was inserted into the series of GAL4 reporter vectors used above. This insertion allows the plasmids to replicate in COS-7 cells (data not shown). The origin was inserted 2 kbp away from the GAL4 sites, and the plasmids bearing one, two, or five GAL4 sites replicated to the same extent to give the same final amount (reference 18 and data not shown). One microgram of these reporter plasmids was cotransfected with 2 μg of effector plasmids into COS-7 cells or into CV-1 cells for comparison. Two days later, both sets of transfected cells were lysed and luciferase activity was assayed. The results are shown in Table 2.

TABLE 2. Loss of synergistic activation by replication in vivo[a]

| Reporter | CV-1 cells | | | COS-7 cells | | |
|---|---|---|---|---|---|---|
| | Luciferase activity (U) | Activation ratio | Synergy | Luciferase activity (U) | Activation ratio | Synergy |
| 1× GAL4 + ori | 2.9 | | | 10 | | |
| 2× GAL4 + ori | 23 | 8 | 4.0 | 28 | 2.8 | 1.4 |
| 5× GAL4 + ori | 94 | 32 | 1.6 | 96 | 9.6 | 1.4 |

[a] Plasmids containing the SV40 replication origin (ori) were transfected into either CV-1 or COS-7 cells, and luciferase activity was assayed as described for Table 1.

These experiments show that there is little or no synergy when these replicating templates are used. A two-site construct gives 28 U of activity, compared with 10 U of activity from a single-site construct; the synergy is 1.4-fold (Table 2). A similar value is obtained when the number of sites is increased from two to five. The reduction of synergy is not related to the ratio of reporter to activator; varying the amount of reporter plasmid did not alter the synergy (data not shown). The significance of this large reduction in synergy is tempered somewhat by the lessened synergy observed with these plasmids in CV-1 cells (fourfold in Table 2); the lessened synergy in CV-1 cells may result from a disturbance in chromatin

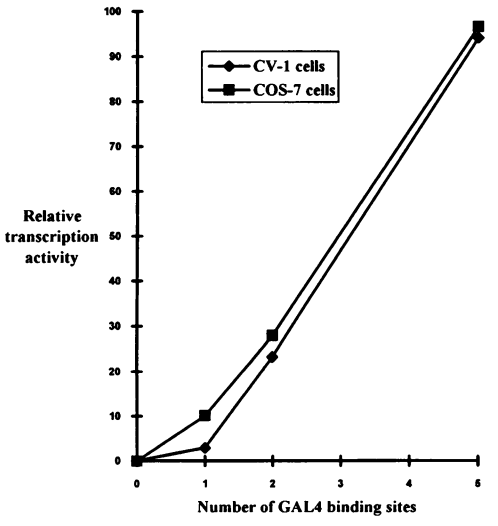


FIG. 2. Replication reduces transcription synergy. Relative transcription activity with replicating plasmids in CV-1 or COS-7 cells is plotted against the number of GAL4 sites.

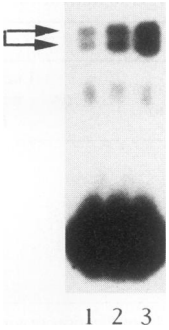


FIG. 3. Reduced transcription synergy in vitro. In vitro transcription in HeLa cell extract with one (lane 1), two (lane 2) or five (lane 3) GAL4 binding sites is shown. Arrows indicate the expected transcripts.

associated with transcription from the SV40 early promoter within the replication origin.

Interestingly, the difference between replicating and nonreplicating conditions is almost exclusively in the one-site constructs. That is, the two- and five-site constructs give essentially identical activities in the two cell lines (approximately 25 and 95 U, respectively). However, the COS-7 cells support three to four times the amount of expression from the single-site construct that CV-1 cells support (Fig. 2). Thus, the loss of synergy associated with replication appears to be exclusively due to a gain in the ability to transcribe with a single GAL4 site. This differs somewhat from the result of an in vitro experiment in which a five-site chromatin template could not be transcribed unless it was replicated (21). Such severe repression is not apparent in our in vivo experiment, which probably reflects more balanced competition for DNA between histones and activator than existed in the in vitro condition.

To rule out the possibility that SV40 large T antigen in COS-7 cells is responsible for the observed reduction of synergy from one to two sites, we transfected the plasmids without a replication origin into COS-7 cells. The expression from these plasmids mimics the synergistic activation observed in CV-1 cells in that the two GAL4 sites show 13-fold more expression than one site (data not shown). This confirms that the reduction of synergy is due to DNA replication.

If this reduction in synergy is due to replication allowing GAL4 interactions with chromatin-disrupted DNA, then naked DNA templates in vitro should show reduced synergy. We therefore extended our analysis of synergy to transcription in vitro, for which the existing data are significantly less extensive than those for transcription in vivo.

**The level of synergy is low when naked DNA templates are used in vitro.** The in vitro transcription experiments used 50 ng of the same plasmid DNA templates (without replication origin) as assayed in vivo. We found that transcription of these templates by using HeLa cell nuclear extract was strongly dependent on added GAL4-VP16 protein (see reference 7 and data not shown). The results with different numbers of GAL4 sites are shown in Fig. 3.

The data show that transcription is primarily nonsynergistic in that it increases roughly in proportion to the number of activator binding sites. When the number of sites was increased from one to two, analysis showed that the amount of transcript approximately doubled (compare lanes 1 and 2 in Fig. 3). Increasing the number of GAL4 DNA binding sites from two to five also yielded approximately double the amount of transcript (compare lanes 3 and 2 in Fig. 3). When the data are plotted (Fig. 5A) in the same form as that used for the in vivo data (Fig. 1), the contrasting lack of synergy in the in vitro experiment is apparent. Instead, one observes only the roughly linear increase characteristic of the nonsynergistic phase of the in vivo curve (qualitatively consistent with the GAL4-VP16 activation data of reference 5).

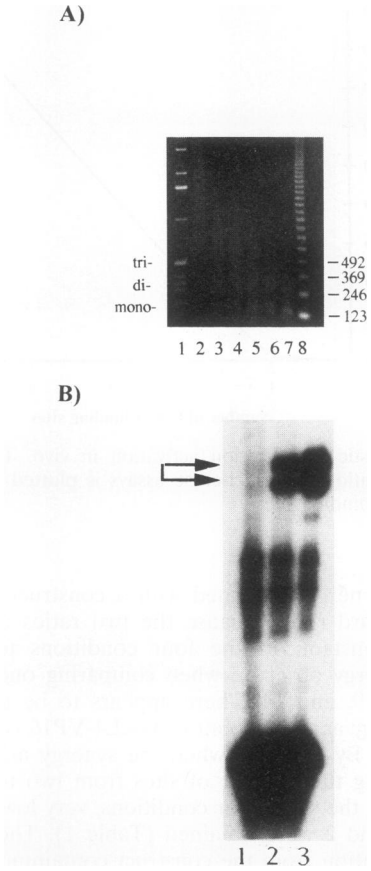


FIG. 4. Transcription synergy is restored by assembly of naked DNA into chromatin structure in vitro. (A) Analysis of assembled chromatin template by micrococcal nuclease digestion. Lanes 1 and 8 are 1-kbp and 123-bp molecular size markers, respectively. Lanes 2 to 7 are assembled template digested with 0.3 U of micrococcal nuclease per μl for 1, 4, 6, 15, 20, and 40 min, respectively. The mono-, di-, and trinucleosome positions are indicated. (B) In vitro transcription with the isolated assembled template. Lanes 1, 2, and 3 are templates containing one, two, and five GAL4 DNA binding sites, respectively.

The results suggest that the loss of synergy associated with the replicating templates in vivo could be accounted for by interactions with transiently dechromatinized templates. This model implies that restoration of chromatin in vitro would restore the transcription synergy. We now test this possibility.

**Chromatin assembly in vitro can restore synergistic transcriptional activation.** The same plasmid DNA templates studied as naked DNA were assembled into chromatin structures with an *X. laevis* oocyte extract under standard conditions for chromatin assembly (see Materials and Methods). The assembled templates were separated from the extract by gel filtration chromatography. We confirmed that chromatin assembly had occurred as expected on the basis of micrococcal nuclease digestion patterns (Fig. 4A and data not shown). The nucleosome ladder is formed after chromatin assembly, and the average length of the nucleosome repeat is 140 to 150 bp.

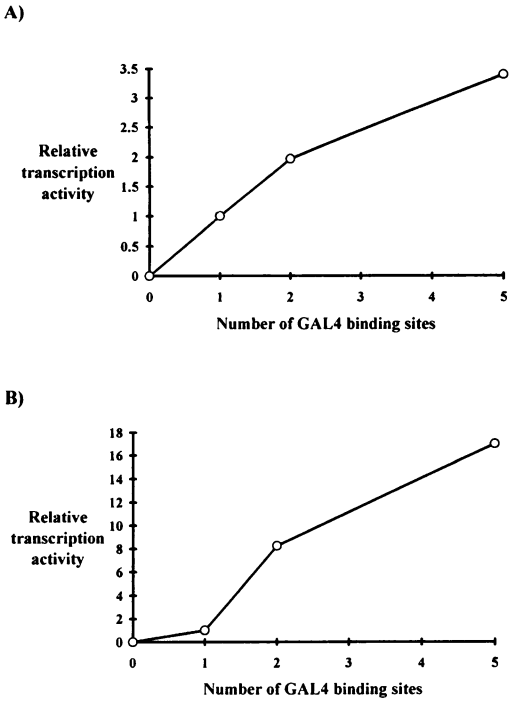


FIG. 5. Quantification of in vitro transcription. In vitro transcription activity of a naked DNA template (A) or a chromatin template (B) is plotted against the number of GAL4 binding sites. Activity measured by densitometer scanning and scintillation counting of gel slices gave similar results.

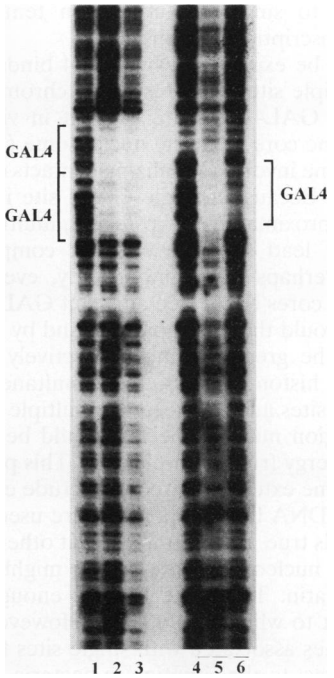


FIG. 6. In vitro DNase footprinting. Lanes 1, 2, and 3 are footprinting of a two-GAL4-site template, while lanes 4, 5, and 6 are from a one-GAL4-site template. Lanes 1 and 4 are naked DNA digestion, lanes 2 and 5 are naked DNA with GAL4-VP16, and lanes 3 and 6 are assembled chromatin incubated with GAL4-VP16.

These chromatin templates were transcribed in vitro under conditions identical to those used to transcribe naked DNA. The autoradiograph in Fig. 4B shows that the same transcript is produced as that on naked DNA (Fig. 3). However, comparison of results achieved with templates with one, two, and five binding sites demonstrates that chromatin assembly has restored the synergistic activation that was absent with naked DNA templates. That is, the level of transcription from a chromatin template containing one GAL4 site (lane 1) was extremely low, whereas transcription from the two-site template (lane 2) was quite strong.

Quantitative analysis showed a 10-fold increase for the two-site template over that for the one-site template (Fig. 5B), indicating a synergy factor of 5. This is less than the average 10-fold synergy observed in vivo under most conditions and is comparable to the weakest synergy obtained in vivo (Table 1). Increasing the number of sites from two to five gave a two- to threefold increase in the amount of transcript. These results are quite comparable to those observed in vivo, in which the introduction of a second site led to strong synergy whereas the introduction of three more sites led to activation increases in which synergy was weak. We conclude that assembly of these templates into chromatin rescues their ability to mount a synergistic transcriptional response to GAL4-VP16.

As discussed above, the source of synergy could be the cooperative binding of GAL4 derivatives to chromatin templates but not to naked DNA (43). We repeated aspects of this experiment in our system, which uses a different chromatin assembly process. DNase footprinting of GAL4-VP16 binding to naked DNA and to chromatin was done, and the results are shown in Fig. 6. Under these conditions, a template containing two GAL4 sites is fully protected on naked DNA and on chromatin (lanes 2 and 3). By contrast, a template with one GAL4 site showed much stronger protection on naked DNA (lane 5) than on chromatin (lane 6). We conclude that, under the conditions of chromatin assembly used here, GAL4-VP16 binding is selectively reduced on a one-site chromatin template. This is consistent with previous reports of cooperative GAL4 binding to chromatin (43) and helps explain the low level of transcription from the one-site chromatin template.

## DISCUSSION

The results presented here support the view that chromatin plays a critical role in allowing the mounting of a strong synergistic transcriptional response by GAL4-VP16. The results show that synergy on chromatin templates is predominantly a consequence of a low level of activity when templates contain a single GAL4 site. When chromatin is disrupted transiently by DNA replication, a single-site construct becomes much more active, leading to a strong reduction in synergy. The level of synergy was found to be low on naked DNA in vitro (consistent with the twofold effect shown in reference 5) but was greatly increased when chromatinized templates were used (also consistent with the in vivo results in reference 5).

These results are also consistent with those from a variety of previous studies of the interaction of GAL4 with chromatin (see the introduction). GAL4-VP16 can counteract the repressive effect of chromatin on transcription (10, 11, 22, 50). Thus, an important role of GAL4 is to function as an antirepressor. However, in vitro GAL4 binds poorly to single-site chromatin constructs but binds well to multiple-site constructs, effects not seen on naked DNA (Fig. 6 and reference 43). Thus, transcription synergy can be explained, at least in part, by GAL4-VP16

binding poorly to single-site chromatin templates, thereby limiting the transcription response.

It remains to be explained why GAL4 binds and functions well when multiple sites are present on chromatin templates. When multiple GAL4 sites are present in vivo, GAL4 can displace a histone core from the nucleosome (2). The binding of the nucleosome involves stabilizing contacts over a region of at least 140 bp. Because a single GAL4 site involves contact with only an approximately 20-bp region, multiple GAL4 sites would naturally lead to more effective competition against nucleosomes. Perhaps more importantly, even transient release of histone cores could free adjacent GAL4 sites simultaneously; these could then be rapidly bound by multiple GAL4 proteins, with the greater number effectively preventing rebinding by the histones. Thus, the simultaneous freeing of multiple GAL4 sites and the need for multiple bound proteins to keep the region nucleosome free could be the sources of binding and synergy from multiple sites. This process could be mimicked to some extent in vitro when crude extracts containing nonspecific DNA binding proteins are used.

If this model is true, then it implies that other activators that need to bind a nucleosome-free region might bind cooperatively to chromatin. There are not yet enough examples to know the extent to which this applies. However, the rarity of mammalian genes associated with single sites for activation is in striking contrast to the situation in bacteria, in which single sites are common (8). The lack of stable nucleosomes in bacteria may provide an environment in which activation from single sites is easy.

This model must be evaluated in the context of a variety of convincing studies indicating that GAL4-based activators can interact with multiple components of the general transcription machinery, a requirement for alternative models for synergy (see the introduction and references 16, 19, 26, 29, 30, 37, 41, 45, and 47). Further evaluation of the contribution from the multiple-contact model will require establishing correlations between conditions that promote simultaneous factor binding and conditions that promote synergy. Studies in vitro with highly purified systems lacking nonspecific DNA binding proteins will be very useful in this evaluation.

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