To gain a better understanding of the role of chromatin in the regulation of transcription by RNA polymerase II, we examined the relation between promoter structure and the ability of Gal4-VP16 to function with chromatin templates assembled in vitro. First, to investigate whether there are synergistic interactions among multiple bound factors, we studied promoter constructions containing one or five Gal4 sites and found that a single recognition site is sufficient for Gal4-VP16 to bind to chromatin, to induce nucleosome repositioning, and to activate transcription. Notably, we observed that Gal4-VP16 binds to a single site in chromatin with affinity comparable with that which it binds to naked DNA, even in the absence of ATP-dependent nucleosome remodeling activity. Second, to explore the relation between translational nucleosome positioning and transcriptional activation, we analyzed a series of promoter constructions in which nucleosomes were positioned by Gal4-VP16 at different locations relative to the RNA start site. These experiments revealed that the positioning of a nucleosome over the RNA start site is not an absolute barrier to transcriptional activation. Third, to determine the contribution of core promoter elements to transcriptional activation with chromatin templates, we tested the ability of Gal4-VP16 to activate transcription with TATA box- versus DPE-driven core promoters and found that the TATA box is not required to achieve transcriptional activation by Gal4-VP16 with chromatin templates. These results suggest that a single protomer of a strong activator is able to bind to chromatin, to induce nucleosome remodeling, and to activate transcription in conjunction with a broad range of chromatin structures and core promoter elements.

The regulation of gene transcription is often a critical step in cellular responses to developmental and environmental cues. Transcription by RNA polymerase II involves the action of promoter- and enhancer-binding factors, co-activators and co-repressors, and the basal transcriptional machinery (for reviews, see Refs. 1–5). In addition, the structure of the chromatin template is important for the proper regulation of transcriptional activity (for reviews, see Refs. 6–19).

The biochemical analysis of chromatin templates has been a useful approach to the study of the role of chromatin structure in the regulation of transcription. In reconstituted biochemical systems, basal transcription by the general transcriptional machinery is strongly repressed (>100-fold) by the packaging of DNA into chromatin, whereas many promoter- and enhancer-binding factors are able to activate transcription with nucleosomal templates. It thus appears that at least one function of sequence-specific activators is to counteract the repression of basal transcription by chromatin. Notably, other treatments that reduce transcriptional activity from non-chromatin templates in vitro, such as template dilution, addition of histone H1, addition of chromatin assembly extract in the absence of histones, or high salt concentrations, do not faithfully reproduce the regulation observed with chromatin (for example, see Ref. 20). It therefore appears that a key and unique feature of chromatin is its specific ability to repress basal, but not activated transcription. Yet, the role of chromatin structure in transcriptional regulation is likely to be more complex than a matter of repression and derepression. For instance, the packaging of DNA into chromatin appears to facilitate transcription by promoting interactions among transcription factors (for example, see Refs. 21–24).

In particular, the use of chromatin templates prepared with the Drosophila S-190 assembly extract (22, 25) yields an experimental system that appears to recreate the mechanism of transcriptional regulation with a good degree of accuracy, especially when compared with effects seen with non-chromatin DNA templates (for instance, see Refs. 20 and 26–32). For example, the magnitude of transcriptional activation with such chromatin templates more closely resembles that seen in vivo than that observed with naked DNA templates. In addition, the requirement for a transcriptional activation region in activators (such as Gal4 derivatives (20), LEF-1 (32), NF-κB1 proteins (30), CREB (28), and estrogen receptor (27)) with chromatin templates more closely resembles that seen in vivo than the less stringent requirement for an activation region that is often observed with non-chromatin templates. The available evidence suggests that the presence of ATP-utilizing nucleosome remodeling factors (for recent reviews, see Refs. 8–11, 13, 16, 18, 19, and 33) in the S-190 extract is important for transcription of the chromatin templates (20, 29, 34).

These and other findings have led us to explore, in greater detail, the relation between promoter structure and transcriptional activation by the Gal4-VP16 activator (35, 36) with chromatin templates assembled with the S-190 extract.
investigate whether synergistic interactions among multiple DNA-bound activators are essential for high levels of transcriptional activation in chromatin, we tested the ability of a single Gal4-VP16 protomer (i.e. dimer (37)) to bind to chromatin, to activate transcription, and to induce nucleosome remodeling. Second, to test the relation between nucleosome positioning and transcriptional activation, we systematically varied the spacing between the Gal4 binding sites and the core promoter and analyzed the transcriptional properties of the corresponding chromatin templates. Third, we examined elements of the core promoter and tested the ability of Gal4-VP16 to activate transcription in conjunction with TATA box-driven versus DPE-directed core promoters.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The pGIE-n series of plasmids, which contain the adenovirus E4 core promoter with five tandem Gal4 sites (CAGGAGGACAGTACCCG; derived from pG2E4T (38)) located at various distances (n + 21 bp) upstream of the TATA box, were constructed by inserting defined fragments (synthesized by polymerase chain reaction) of Drosophila TFIIIB genomic DNA (39) between the Gal4 sites and the TATA box. The genomic DNA inserts (of length = n bp) were a nested series of fragments that were oriented in the 5’→3′ direction relative to the TATA box. For the single binding site plasmids (pG1E-n series), the five tandem Gal4 sites were excised from the pGIE-n series plasmids and replaced with a single copy of the same Gal4 recognition sequence. The Gal4 site from the Saccharomyces cerevisiae MEL1 gene is as follows: CGCGCATACTGTCCTCCG (40). For comparison of the minimal promoters, five tandem Gal4 sites (also derived from pG2E4T) were placed upstream of the previously described minimal core promoter constructions (41, 42) to give the G5min series. The G5min plasmids were the gift of Drs. Catherine George and Thomas Burkh (University of California, San Diego), and some of the pGIE-n series plasmids were the gift of Dr. Michael Bulger (University of California, San Diego). All plasmids were purified by alkaline lysis methodology followed by two sequential CsCl gradients.

**Chromatin Assembly**—Chromatin was assembled with plasmid DNA, Drosophila core histones, Drosophila histone H1, and S-190 assembly extract derived from Drosophila embryos, as described previously (20, 25, 43). Histone H1 was included, where indicated, at 200 nm concentration in the assembly reactions, which results in approximately 1 molecule of H1 per nucleosome core incorporated into the chromatin. When Gal4-VP16 was added during assembly, the Gal4-VP16 was added either after assembly, the activator was added 4.5 h after the initiation of the assembly reaction (which was complete within 4 h), and the resulting mixture was incubated for an additional 30 min prior to further analysis. In each experiment, all of the samples were assembled for the same amount of time and analyzed in a parallel fashion.

The periodicity of the nucleosome arrays and the efficiency of chromatin assembly were tested by micrococcal nuclease digestion analysis (with the requirement that we observed at least 7, but typically 9 to 12, repeats) and by DNA supercoiling analysis in the presence of 1.6 μM chloroquine, as described previously (20, 25, 43). The chromatin assembly process was consistent in the presence or the absence of an activator as well as with different plasmid constructions. The presence of histone H1 increased the repeat length from approximately 170 to approximately 200 bp and caused a slight reduction in the extent of DNA supercoiling, as expected from the increase in the repeat length.

**In Vitro Transcription**—Chromatin was transcribed with a Drosophila nuclear extract (SNF), as described previously (20, 43). A mixture of buffer, ribonucleoside triphosphates, and extract was added to duplicate aliquots of assembled chromatin (each containing 75 ng of DNA). The transcripts were detected by primer extension analysis, and the data were quantitated with a Fuji BAS1000 phosphoimager and Mac-BAS software. The transcription reactions for pGIE-n plasmids were analyzed with the E4 primer (5’-CTTCAGAAGGCCAGCCTAACAGTGT-3’), whereas the transcription reactions for the G5min plasmids were analyzed with the M13 reverse sequencing primer (5’-CTCGGGACATTCTGTAATCAGTGGAAACATTTCCAGCCAGGAAGGATCGGTGACTGTACTGTTGCTGTCGAG-3’). Transcript labeling analysis (44, 45) in the absence of transcription extract, as described previously (20, 43). Chromatin or naked DNA was digested with micrococcal nuclease, and the DNA (125 ng for each sample) was deproteonized and then digested with BglII and AluNI. Southern blot analysis of the resulting DNA fragments with probes that hybridize to either end of the region yielded approximately the same number of nucleosomes (data not shown). Although the rearrangement of nucleosome positioning was readily detectable by this indirect end-labeling analysis with either one or five Gal4 sites, the disruption of the regularity of the nucleosome array, as revealed by the micrococcal nuclease ladder disruption assay (which reveals changes in nucleosome periodicity within specific regions of chromatin; for example, see Refs. 20 and 46), was observed with five Gal4 sites but not with one Gal4 site (data not shown). These results suggest that the periodicity of the array is not significantly disrupted by the binding of a single Gal4-VP16 dimer (see also Ref. 47).

**RESULTS**

**Gal4-VP16 Binds with Comparable Affinity to Naked DNA or to Chromatin, Even in the Absence of ATP-dependent Remodeling Activities**—We first examined the extent to which the binding affinity of Gal4-VP16 to chromatin is affected by the packaging of DNA into chromatin that is reconstituted with the S-190 extract. In earlier experiments with either mononucleosome or poly-nucleosome templates, the packaging of DNA into chromatin has been generally observed to cause a substantial reduction in the affinity of binding of Gal4 derivatives to DNA (reviewed in Refs. 10, 13, 18, and 49). For instance, in the biochemical analysis of chromatin templates that were prepared by the ATP-independent deposition of histones with a heat-treated Xenopus egg extract (containing nucleoplasmin and N1/N2), it was found that the binding of Gal4-AH (which has the same Gal4 DNA binding region as Gal4-VP16) is strongly inhibited by nucleosomes (~100-fold inhibition with a single Gal4 site and ~10-fold inhibition with five Gal4 sites (50)). Because we were using nucleosome arrays assembled with ATP-utilizing factors that are likely to be involved in the physiological assembly process (for discussion of ATP-dependent versus ATP-independent histone deposition processes, see Ref. 51), we felt that we might potentially observe different properties of Gal4-VP16 binding than those seen previously with either mononucleosomes or with poly-nucleosomes obtained by the ATP-independent deposition of histones.

To this end, we carried out a primer extension DNA I footprinting analysis of preassembled chromatin templates containing a single Gal4 binding site. In these experiments, the promoter constructions were assembled into chromatin by using the S-190 extract in conjunction with purified core histones and histone H1 (22, 25). Chromatin assembled with the S-190 extract has been shown to have pronounced alterations in nucleosome positions by several criteria, which include micrococcal nuclease digestion analysis and electron microscopy (22, 25). Then, Gal4-VP16 (at the indicated concentrations) was added to the preassembled chromatin (in the continued presence of the S-190 extract), and the samples were subjected to DNA I footprinting analysis. In parallel, non-chromatin (naked DNA) templates were also tested.

As shown in Fig. 1A, the affinity of Gal4-VP16 to chromatin templates containing a single consensus Gal4 binding site is nearly as high as the affinity of Gal4-VP16 to the corresponding naked DNA templates. These results indicate that the-packag-
The affinity of Gal4-VP16 binding to chromatin is similar to the affinity of its binding to naked DNA. Chromatin was assembled onto template DNAs containing either a single consensus Gal4 site (A) or a Gal4 site located upstream of the S. cerevisiae MEL1 gene (B). Gal4-VP16 was added at the indicated concentrations either to preassembled chromatin samples or to the corresponding naked DNAs and allowed to bind to the templates for 30 min. Where indicated, preassembled chromatin was incubated with apyrase prior to the addition of Gal4-VP16. The resulting chromatin or naked DNA samples were subjected to primer extension DNase I footprinting.

To test whether the presence of multiple binding sites leads to cooperative binding, we examined the binding of Gal4-VP16 to a single Gal4 site in chromatin (Figs. 1 and 2), we sought to examine whether a single Gal4-VP16 protomer would be sufficient to achieve transcriptional activation with preassembled chromatin templates. It was possible, for instance, that synergistic interactions between chromatin-bound Gal4-VP16 activation domains are required to achieve strong transcriptional activation.

To test whether a single DNA-bound activator can mediate transcriptional activation with chromatin templates, we compared the transcriptional properties of promoters that contained zero, one, or five consensus Gal4 binding sites located upstream of the adenovirus E4 core promoter. As in the DNase I footprinting experiments, the promoter constructions were assembled into chromatin by using the S-190 extract in conjunction with purified core histones and histone H1. Then, Gal4-VP16 (at the indicated concentrations) was added to the preassembled chromatin (in the continued presence of the S-190 extract), and the samples were subjected to in vitro transcription analysis.

With the chromatin templates (Fig. 3A), high levels of transcriptional activation were achieved with either one Gal4 site (64-fold activation at 25 nM Gal4-VP16) or five Gal4 binding sites (340-fold activation at 25 nM Gal4-VP16). In addition,
similar results were obtained in the absence of the linker histone H1, except that the overall amount of transcription (with the same dose response) was about 2- to 5-fold higher in the absence of H1 (data not shown). Therefore, we conclude that a single Gal4-VP16 protomer is sufficient to achieve strong activation of transcription in vitro with chromatin templates.

With the naked DNA templates (Fig. 3B), there was a high level of basal transcription in the absence of Gal4-VP16 and relatively low levels of transcription activation. With naked DNA templates containing a single Gal4 binding site, there was less than 3-fold activation of transcription by Gal4-VP16 at 25 nM concentration, which is in contrast to the 64-fold activation seen with chromatin templates at the same concentration of Gal4-VP16. Thus, the packaging of the template into chromatin is important to observe high levels of transcriptional activation by a single Gal-VP16 dimer.

**Nucleosome Positioning and Transcriptional Activation by Gal4-VP16**—To test the relation between nucleosome positioning and transcriptional activation, we studied a series of promoter constructions that contain variable lengths of DNA inserted between the adenovirus E4 TATA box and one or five Gal4 binding sites. In these constructions, the spacer DNA fragments are a nested series of sequences with identical sequences adjacent to the TATA box and different sequences adjacent to the upstream Gal4 sites.

With this protomer series, we initially investigated whether...
the binding of one or five Gal4-VP16 protomers is able to induce nucleosome positioning over the differing adjacent DNA sequences. To this end, we carried out micrococcal nuclease digestion and indirect end-labeling analyses. In these experiments, Gal4-VP16 (25 nM) was added to chromatin that was previously assembled with the S-190 extract (in the continued presence of the S-190, but in the absence of *in vitro* transcription extract), and then the resulting nucleosome positioning that was induced by Gal4-VP16 was examined by partial micrococcal nuclease digestion and indirect end-labeling analysis (Fig. 4A). In addition, control experiments were performed with naked DNA templates (Fig. 4B) to identify preferred sites of micrococcal nuclease cleavage with each of these DNA templates.

It is particularly useful to examine the results with the 100-bp spacer DNA construction. First, with each of the naked DNA templates (including the 100-bp spacer construction), there is a strong preferential micrococcal nuclease cleavage site that is approximately coincident with the +1 transcription start site (Fig. 4B). Then, examination of this approximately +1 hypersensitive site with the chromatin templates (Fig. 4A) reveals complete protection of this hypersensitive site with the five Gal4 site templates and extensive but incomplete protection of the hypersensitive site with the one Gal4 site template. Thus, these data indicate that there is a positioned nucleosome centered over the transcription start site in chromatin that is assembled with the +100-bp spacer construction containing five Gal4 sites.

These data, which are summarized in Fig. 4C, indicate that either one or five Gal4-VP16 dimers can induce the positioning of about one or two nucleosomes on each side of the Gal4 sites. Thus, a single Gal4-VP16 dimer is sufficient to remodel chromatin, although it can also be seen that the nucleosome positioning that is induced with five Gal4 sites is more distinct than that achieved with a single Gal4 site. In addition, the positioning of nucleosomes adjacent to the Gal4 sites occurred with different flanking DNA sequences. Hence, these results provide additional evidence that transcription factor binding can be a dominant factor, relative to the DNA sequence, in nucleosome positioning. In the interpretation of these experiments, it is additionally relevant to note that the nucleosome remodeling reactions were carried out in the absence of *in vitro* transcription extract. Therefore, the changes in chromatin structure were not a consequence of the transcription process.

Next, we examined the transcriptional properties of this series of chromatin templates containing positioned nucleosomes. In these experiments, Gal4-VP16 was first added to newly assembled chromatin to induce nucleosome positioning, as depicted in Fig. 4C, and then, *in vitro* transcription reactions were carried out with a nuclear extract containing the basal transcription factors and coactivators. As shown in Fig. 5A, the chromatin templates exhibit about an 8-fold decrease in transcriptional activity when the spacer DNA between the TATA box and the proximal Gal4 site is increased from 0 (with the promoter in a nucleosome region) to 100 bp (with the promoter approximately at the center of a nucleosome). Then, a relatively constant level of transcription is seen with spacer DNAs ranging in length from 100 to 250 bp, even though the transcriptional positioning of nucleosomes relative to the RNA start site varies considerably in these constructions (Fig. 4C). Notably, the amount of transcription from the 100-bp spacer template, which contains a nucleosome centered over its RNA start site, is comparable with the amount of transcription from the chromatin templates containing spacer DNA lengths of 150, 200, or 250 bp. In addition, parallel results were obtained with a series of analogous DNA constructions containing a single Gal4 binding site (data not shown), except that the magnitude of transcriptional activation with one Gal4 site was less than that with five Gal4 sites.

In control experiments with naked DNA templates (Fig. 5B), the magnitude of transcriptional activation by Gal4-VP16 de-
naked DNAs (the DPE, which is typically located at about 30 relative to the TATA box and the histone-like TAFII60-TAFII40 subunits of TFIIID (of *Drosophila*)). Both the TATA box and the DPE are recognition sites for binding of the TFIIID basal transcription factor, with the TBP subunit of TFIIID contacting the TATA box and the histone-like TAFII60-TAFII40 subunits of TFIIID (of *Drosophila*) contacting the DPE. In many respects, the DPE, which is typically located at about +30 relative to the transcription start site, is a downstream analog of the TATA box.

Biochemical analyses of transcription with chromatin templates have thus far been carried out with TATA box-driven core promoters—mainly, the adenovirus E4 and HIV-1 promoters. However, the promoters of many genes lack TATA boxes, and therefore, we were interested in testing the properties of core promoters in which TFIIID binds via the downstream promoter region is in a nucleosome-free region.

Transcriptional Activation by Gal4-VP16 with Chromatin Templates Containing TATA Box-Versus DPE-driven Core Promoters—To complement the analysis of the location and number of the activator binding sites, we examined the effects of variation of the core promoter itself. Two major types of core promoters are those in which TFIIID binds via the TATA box element and those in which TFIIID binds via the downstream promoter element, DPE (41, 53, 54). Both the TATA box and the DPE are recognition sites for binding of the TFIIID basal transcription factor, with the TBP subunit of TFIIID contacting the TATA box and the histone-like TAFII60-TAFII40 subunits of TFIIID (of *Drosophila*) contacting the DPE. In many respects, the DPE, which is typically located at about +30 relative to the transcription start site, is a downstream analog of the TATA box.

Biochemical analyses of transcription with chromatin templates have thus far been carried out with TATA box-driven core promoters—mainly, the adenovirus E4 and HIV-1 promoters. However, the promoters of many genes lack TATA boxes, and therefore, we were interested in testing the properties of core promoters in which TFIIID binds via a DPE rather than TATA box. To this end, we analyzed a variety of naturally occurring core promoters onto which five Gal4 sites were placed upstream of the transcription start site. The core promoters were classified as follows: TATA box only; TATA box + Initiator (Inr) element; and Inr + DPE.

The different core promoter constructions were assembled into chromatin and subjected to *in vitro* transcription analysis (Fig. 6A). In the absence of Gal4-VP16, the packaging of the various DNA templates into chromatin resulted in strong repression of basal transcription. When, however, Gal4-VP16 was added to the preassembled chromatin prior to carrying out the *in vitro* transcription reactions, strong activation of transcription was observed with all of the different core promoters. In addition, the relative strengths of each of the different promoters in Gal4-VP16-activated chromatin were similar to those in Gal4-VP16-activated naked DNA templates (compare Fig. 6, A with B).

**DISCUSSION**

Gal4-VP16 Binds to a Single Site in Naked DNA or in Chromatin with Comparable Affinity—We observed that Gal4-VP16 binds to a single consensus site in chromatin or to the natural *MEL1* site in chromatin with nearly the same affinity as it binds to naked DNA, even in the absence of ATP-dependent chromatin remodeling activities (Fig. 1). This facile binding of Gal4-VP16 to chromatin is in contrast to the greater than 100-fold repression of basal transcription (in the absence of activator) that is seen upon packaging of DNA into chromatin (Fig. 3) (20). These findings indicate that ATP-dependent chromatin remodeling activities, such as SWI-SNF complex, NURF, CHRAC, or ACF, are not required for efficient binding of Gal4-VP16 to its recognition site in chromatin. These biochemical data are also in accord with the recent observation *in vivo* in *S. cerevisiae* that the binding of the Gal4 activator to a site in a nucleosome is not significantly reduced upon mutation of the SWI1 gene, which encodes an essential component of the SWI-SNF complex (55). It should also be considered, however, SWI1 mutations have been reported to inhibit Gal4 binding in a different context *in vivo* (56). We do not expect every transcription factor to function similarly. For instance, while NF-E2 and TTF-1 are able to bind chromatin in the absence of ATP (26, 57), HSF and p18/MafG require cofactors to bind to chromatin (26, 58). In addition, it has been suggested that SWI-SNF complex facilitates general transcription factor binding to nucleosomes (55, 59).
We have found that a single recognition site is not only sufficient for Gal4-VP16 to bind to its recognition site in chromatin (Figs. 1 and 2), but it is also able to induce a rearrangement of nucleosome positioning (Fig. 4) and to activate transcription (Fig. 3). In the remodeled chromatin, the Gal4-VP16 is bound to DNA in a nucleosome-free region in which the single Gal4 site is located between flanking nucleosomes. These results are consistent with the known characteristics of Gal4 activation in vivo in yeast with a single high-affinity binding site (60–62) and further support the notion that a single activator can achieve high levels of transcriptional activation in the apparent absence of synergism with other DNA-bound activators. This potency of a single Gal4-VP16 protomer is in contrast to the synergy that we have previously observed with multiple, natural factors bound to a natural transcriptional unit (HIV-1 LTR promoter) by using this same system (30). While it might be thought that the observed facile binding of Gal4-VP16 to chromatin is in contradiction to other biochemical data (for instance, see Ref. 50), it is pertinent to note that the experiments in this report were carried out with chromatin that was assembled onto nonrepetitive DNA with ATP-utilizing factors (in the S-190 extract) that are likely to be involved in the physiological assembly process, whereas in contrast, other experiments in which the binding of Gal4 derivatives was analyzed in vitro were generally carried out with nucleosomes that were formed by an ATP-independent histone deposition process. There are differences, such as in the periodicity of nucleosome spacing, that are seen with the different types of chromatin that are obtained with the ATP-dependent versus the ATP-independent processes. In addition, we had previously observed significant differences in the extent of transcriptional repression by histone H1 with chromatin that was reconstituted by an ATP-independent process relative to chromatin that was assembled with the S-190 extract (for example, compare Refs. 20, 22, and 63). These results collectively indicate that there are differences, such as in the arrangement or folding of nucleosomes in the chromatin fiber, that lead to differences in the biochemical properties of chromatin assembled with the S-190 extract relative to chromatin that is reconstituted by an ATP-independent histone transfer process.

Nucleosome Positioning and Transcriptional Activation—The finding that the positioning of a nucleosome at the RNA start site is not an absolute barrier to transcription (Figs. 4 and 5) is consistent with data indicating that a variety of promoters contain nucleosomes positioned over their start sites. These results, combined with the observation that transcription factor-induced chromatin remodeling contributes to transcriptional activation in vitro (20, 29, 64), suggest that rearrangement of a nucleosome comprising the core promoter can be an important step in transcriptional activation.

In some respects, these biochemical experiments with the Gal4-VP16 activator are analogous to in vivo studies of the yeast Mata2 repressor, in which it was observed that transcriptional repression by Mata2 protein is insensitive to the translational positioning of a nucleosome in the core promoter region (65). It thus appears that specific nucleosome positioning can contribute to the transcriptional functions of Gal4-VP16, but is not absolutely necessary for activation. Notably, even when the promoter was located between nucleosomes (as in the 200-bp...
space construction), activation was found to be diminished relative to the 0-bp spacer construction (Fig. 5); perhaps the linker region between the flanking nucleosomes is too small to accommodate a transcription complex. More generally, these and other results collectively suggest that the specific translational positioning of nucleosomes is not necessarily a dominant factor in the determination of the amount of gene activity.

Variation of Core Promoter Elements—We found that chromatin-mediated repression of basal transcription occurs with both TATA box- and DPE-driven core promoters and that Gal4-VP16 is able to function with both of these types of core promoters to mediate transcriptional activation in chromatin (Fig. 6). To date, experiments involving transcriptional activation with chromatin templates in vitro have involved the use of TATA box-containing promoters, and these results indicate that a TATA box is not required to achieve transcriptional activation by Gal4-VP16 with chromatin templates. Given the differences in the mechanisms of basal transcription with TATA- versus DPE-driven core promoters, it seems possible that there are activators that function differently with these two types of core promoters.

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