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Properties of Initiator-Associated Transcription Mediated by GAL4-VP16

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Transcription associated with a terminal deoxynucleotide transferase gene initiator element is shown to respond to the transcription factor GAL4-VP16 both in vivo and in vitro. High-level transcription requires both an intact initiator element and bound activator. Transcription from this initiator-directed promoter is synergistic in vivo in that five GAL4 DNA binding sites yield 36 times the expression of a single site. Promoters dominated by initiator and TATA elements respond similarly to several GAL4-based activators, including GAL4-Sp1, GAL4-CTF, GAL4(1–147), GAL4-p53, GAL4-C/EBP, and GAL4-ER(EF), as well as GAL4-VP16 and Sp1. These and other similarities suggest that primary activation of TATA- and initiator-dominated promoters occurs at common steps. Since the initial assembly steps do not appear to be common for the two promoter types, the results place interesting constraints on models for how activation occurs.

Gene transcription by RNA polymerase II is directed by a combination of basal and regulatory DNA elements. Many genes contain a TATA box as a basal promoter element which directs downstream transcription initiation. Early studies in the yeast *Saccharomyces cerevisiae* pointed to alternative basal elements that influenced the transcription start site (7, 14, 21, 22, 29). In mammalian cells, such an initiator (Inr) element was originally found in association with a terminal deoxynucleotide transferase (TdT) gene promoter; this Inr element is essential and specifies the transcription start site within itself (23, 33). By far the majority of mammalian studies have concentrated on TATA-dependent promoters, since these were discovered first and thus far constitute the majority of cases. However, there is increasing evidence that Inr elements are quite common and may often occur in conjunction with TATA elements (38, 41). There is only preliminary mechanistic information about Inr-dependent promoters (reviewed in references 5 and 27).

Several studies indicate that the initial steps in the assembly of transcription complexes differ for Inr and TATA promoters, raising the possibility that there are important mechanistic differences between the two types of promoters. Promoters with a strong TATA box begin assembly of basal transcription complexes by the binding of TATA box-binding protein (TBP) and TBP-associated factors (TAFs) (i.e., TFIID) to the TATA box. This is followed by the ordered addition of other essential transcription factors (4, 12, 13, 27). Inr-dependent promoters begin assembly in a different manner, since they lack a sequence to which TBP binds (5). It is not known which proteins initiate assembly at Inr elements, but several candidates, including YY1, TFII I, HIP1, USF, the polymerase itself, and the TAFs, have been proposed (1, 3, 5, 25, 28, 32). Subsequent to the different initiating events, the assembly pathways at Inr-dependent and Inr-independent promoters are believed to converge, since many of the same basal factors are required (reviewed in reference 38).

Despite this important difference in initial steps, it has been found that TBP is also required for transcription of promoters containing an Inr element but lacking a strong TATA element (5, 25, 34). Since TBP does not bind the Inr element directly, it must be recruited to the transcription complex by a different mechanism. In this regard, Inr-dependent transcription resembles RNA polymerase I and III transcription in that these promoters also require proteins to recruit TBP to the DNA template (for a review, see reference 26). TBP, however, is not sufficient for activated transcription at either Inr-dependent or consensus TATA promoters. The role of the required TAFs is not yet clear, since in one case a single purified holo-TFIID complex containing very few TAF proteins could direct both TATA- and Inr-dependent transcription (45), but in another case different TAFs direct TATA and Inr transcription (25).

These comparisons involve primarily the basal transcription factors, since there is very little information concerning the role of activators in specifying transcription within a strong Inr. The Inr is known to be responsive primarily to upstream GC boxes in the absence of a consensus TATA (23, 33). Since regulatory factors are believed to target early steps in the assembly pathway (19, 20, 37, 40) and these steps differ for TATA and Inr promoters, the possibility exists that Inr and TATA promoters respond differently to different classes of activators (see, for example, reference 36).

Surprisingly, there have been no reports of activation at Inr-dependent promoters by the acidic class of activators such as VP16. By contrast, this has been the subject of intense study of TATA-dependent promoters, which has led to proposals for important mechanisms (2, 6, 11, 17, 39). In this report, we investigate the properties of transcription associated with the TdT Inr element and GAL4-VP16. Transcription is shown to occur in a synergistic fashion in vivo, and this closely resembles TATA-dependent activation. In addition, Inr and TATA transcription respond quite similarly to a range of hybrid activators. The results place interesting constraints on mechanisms, since one must reconcile these similar activation properties with different initial assembly steps at promoters containing strong TATA and strong Inr elements.

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MATERIALS AND METHODS

Constructs and materials. Starting plasmids contained the TdT gene Inr element (pSVI) or mutant Inr element (generously provided by Steve Smale), 1 or 5 GAL4 DNA binding sites (pG1 or 5E4T) (kindly provided by Mike Carey), or the luciferase coding region (pGEMluc; Promega). The *Hin*dIII-*Xmn*I fragment of pGEMluc was cloned into the *Hin*dIII-*Xmn*I sites of wild-type or mutant Inr plasmids to make pInr-luc or pR-luc. pG1Iluc, pG15Iluc, and pG5Rluc were constructed by cloning the *Bgl*II-*Nae*I fragment of pInr-luc or pR-luc, which contained the Inr-luciferase gene, into the *Bam*HI-*Pvu*II sites of plasmids pG1E4T and pG5E4T. pG5luc was constructed by ligation of the *Bam*HI-*Nae*I fragment of pGEMluc to the *Bam*HI-*Pvu*II fragment of pG5E4T. pJ6Iluc was made by insertion of the *Hin*dIII-*Xmn*I fragment of pGEMluc into a plasmid containing six GC boxes upstream of the Inr element. pG1Tluc and pG5Tluc were cloned by insertion of the *Hin*dIII-*Xmn*I fragment into the same sites of plasmid pAOT (36) and then cloning the *Bgl*II-*Nae*I fragment into the *Bam*HI-*Pvu*II sites of plasmids pG1E4T and pG5E4T.

The constructs for GAL4 derivatives were kindly provided by M. Ptashne [GAL4(1–147)], R. Tjian (GAL4-Sp1), N. Mermod (GAL4-CTF), A. Berk (GAL4-p53), and P. Chambon [GAL4-ER(EF)]. Purified *Escherichia coli*-expressed GAL4-VP16 protein was generously provided by M. Carey. HeLa extract was prepared as described by Dignam et al. (9) by Y. Jiang of this laboratory.

In vivo transcription assay. For in vivo transcription experiments, reporter plasmids, together with either vector pOR4 plasmid DNA as a negative control or specified effector plasmids, were transfected into CV-1 cell monolayers by the calcium phosphate precipitation method (30). Except as indicated, the ratio of reporter to effector plasmids was 1 to 5. After 48 h, cells were lysed with 600 μl of Promega cell lysis buffer (24); luciferase activity was assayed immediately with a modified Beckman scintillation counter, using 5 to 50 μl of cell extract.

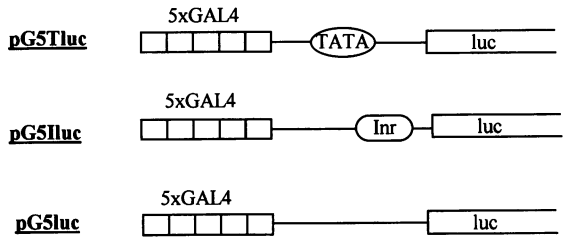
In vitro transcription assay. For in vitro transcription, 50 ng of plasmid DNA was incubated in 40-μl mixtures containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.6), 60 mM KCl, 7.5 mM $MgCl_2$, 12.5% glycerol, 0.6 mM dithiothreitol, 0.5 mM nucleoside triphosphates, and 25 μl of HeLa nuclear extract, in the presence or absence of purified GAL4-VP16 protein (9). After 1 h at 30°C, the reaction was stopped and the transcripts were analyzed by primer extension with reverse transcriptase (10). The products were electrophoresed on a 10% denaturing polyacrylamide gel and autoradiographed.

In vivo RNA start site mapping. Total cytoplasmic RNA was isolated at 48 h posttransfection by the Nonidet P-40 cell lysis method (33). Basically, cells grown on 10-cm-diameter plates were lysed with 0.5 ml of Nonidet P-40 lysis buffer on ice and then centrifuged at 20,000 × *g* for 10 min. The supernatant was extracted with phenol-chloroform-isoamyl alcohol twice. After ethanol precipitation, the pelleted RNA was used for primer extension as described previously (37).

RESULTS

A promoter containing an Inr element can be activated by GAL4-VP16 in vivo. Initially, three reporter plasmids were constructed to test whether GAL4-VP16 could direct transcription in the presence of the strong TdT Inr element in vivo (Fig. 1A). All plasmids contained five GAL4 binding sites upstream of the luciferase reporter gene. One template contained an insert with a consensus TATA element, one contained an insert with the wild-type TdT Inr sequence, and the third contained neither TATA nor Inr sequences. These three reporters were cotransfected into CV-1 cells with a GAL4-VP16 expression construct or a control vector plasmid. Cell extracts were prepared 48 h posttransfection and assayed for luciferase activity (24).

A).



B).

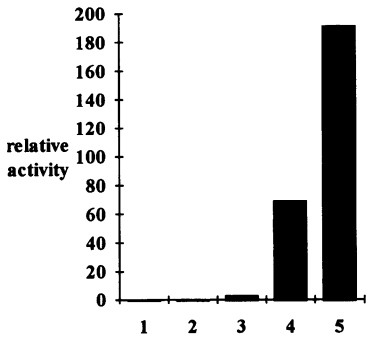


FIG. 1. Activation of TATA and Inr promoters in vivo by GAL4-VP16 construct. (A) Schematic representation of reporter constructs. The TATA box sequence is TATAAA; the Inr sequence is CCCTCATTCT. luc, luciferase. (B) GAL4-VP16 activation of TATA and Inr promoters in vivo. Two micrograms of reporter plasmids was used in each experiment. Columns: 1 and 2, no cotransfected GAL4-VP16 plasmid; 3 to 5, 5 μg of GAL4-VP16 expression plasmid; 1 and 5, pG5Tluc TATA transcription; 2 and 4, pG5Iluc Inr transcription; 3, a construct with no promoter elements (pG5luc). The transcription level was assayed by expression of luciferase activity in vivo.

The result of the experiment is shown in bar graph form in Fig. 1B. In the absence of cotransfected activator GAL4-VP16, only background levels of luciferase activity are detected for templates containing either the TATA insert (column 1) or the Inr insert (column 2). When activator is provided via a cotransfected expression vector, both an Inr element (column 4) and a TATA box (column 5) direct a significant level of luciferase activity. When the activator is present but the template contains neither an Inr nor a TATA insert, only a very low level of luciferase activity is detected (column 3). These comparisons demonstrate that both an inserted basal element and GAL4-VP16 are needed for significant expression and that the basal insert can be either Inr or TATA.

To confirm that the GAL4-VP16 activation is indeed

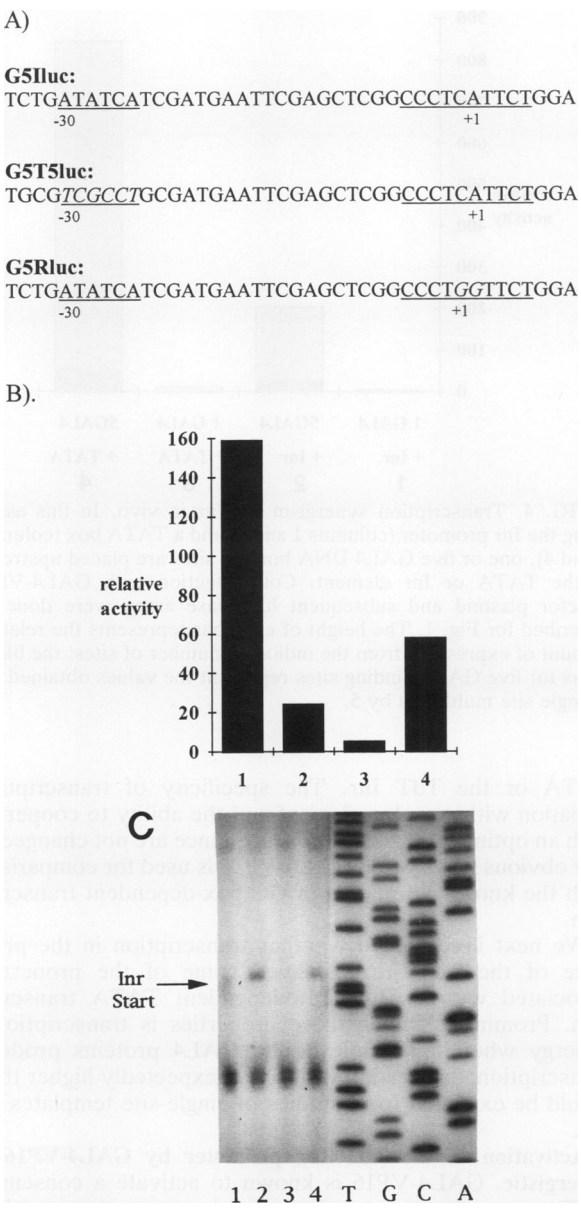


FIG. 2. Transcription activation of Inr mutants by GAL4-VP16 in vivo. (A) Sequences of wild-type and two mutant Inr promoters. (B) Reduction of transcription by mutation in the Inr element, but not as much in the −30 sequence. Columns: 1, wild-type Inr; 2, Inr mutant; 3, template with no element; 4, −30 mutant. (C) In vivo RNA start site mapping. Lanes: 1, wild-type Inr with expression vector alone; 2 to 4, Inr constructs with cotransfected GAL4-VP16; 2, wild-type Inr; 3, mutant Inr; 4, −30 mutant; T, G, C, and A, sequencing ladder.

mediated through the Inr element, two mutants were assayed. The sequences of the wild type and the two mutants are shown in Fig. 2A. G5Rluc has a two-base-pair mutation within critical Inr sequences which was shown to cause a significant reduction of transcription mediated by Sp1 in vitro (34). This change also causes a similar reduction in response to GAL4-VP16 (Fig. 2B). The data show a sevenfold loss of plasmid-directed luciferase activity. The result confirms that expression in this system is dependent on the Inr element.

The RNAs specified by these plasmids were isolated from transfected cells, and the transcription start sites were determined by primer extension analysis. Fig. 2C, lane 2, shows that the wild-type plasmid directs correct initiation within the Inr element (compare with the no-activator control in lane 1). No transcript is seen when RNA from cells transfected with the Inr mutant is analyzed (lane 3), further confirming the requirement for the Inr element in this system.

As a further comparison, we studied plasmid G5T5luc, in which the −30 region has been changed. The wild-type plasmid contains the sequence GATATCAT in place of a TATA box in the −30 region. This sequence has a weak affinity for TBP which is completely abolished (43) when it is changed to GTCGCCTG in G5T5luc. The results show that although there is a modest (twofold) reduction in activity, mutant plasmid G5T5luc retains the ability to direct GAL4-VP16-activated transcription. This expression is quite strong, as luciferase expression from G5T5luc (Fig. 2B, column 4) is approximately 12-fold greater than expression from a template with no elements (Fig. 2B, column 3). G5T5luc RNA mapping in vivo confirms that the RNA produced from this plasmid has the correct start within the Inr element (Fig. 2C, lane 4). We conclude that the Inr controls both the extent of transcription and the specificity of initiation in the system studied in this investigation.

The data also show that when a true TATA sequence is present at −30, transcription is very strong. The comparisons within Fig. 1 indicate that GAL4-VP16 expression mediated by the Inr insert is less than that mediated by the consensus TATA insert. The Inr element leads to 25- to 30-fold increase over the absence of an element, whereas the TATA element leads to a 70- to 100-fold increase. This 3-fold difference between TATA and Inr is modest compared with the 25-fold difference caused by insertion of the Inr into a template with no element. It is also modest compared with the much larger difference caused by GAL4-VP16. Thus, in this system, GAL4-VP16 and either TATA or the Inr are required for high-level expression, and TATA expression is somewhat stronger than that directed by the Inr.

GAL4-VP16 directs transcription initiation within the TdT Inr in vitro. A TATA-less Inr promoter can be activated by upstream GC boxes in the presence of HeLa cell extracts containing basal transcription factors and endogenous Sp1 (23, 33). This in vitro activation leads to transcription initiation within the Inr sequence itself, an observation considered central to understanding the mechanism of action of the Inr element. We now compare the in vitro transcription of an Inr promoter in response to GC boxes and to GAL4-VP16.

Figure 3A, lane 3, shows the transcription pattern from a template containing the Inr insert and upstream GC boxes. A prominent transcript (marked by an arrow) which has initiated within the Inr sequence is detected. This finding reproduces previous observations (34) and confirms that the HeLa extract contains Inr-responsive factors. The in vitro transcription was repeated with a template containing the Inr insert and five upstream GAL4 DNA binding sites. When *E. coli*-expressed GAL4-VP16 is added to the extract with the template, the transcription pattern shown in lane 2 is observed. A prominent band appears within the Inr element at the same position specified by the GC box-Inr template in vitro and by GAL4-VP16 in vivo (23) (Fig. 2C). When the template containing GAL4 sites is used but GAL4-VP16 protein is absent, the transcript is not seen (lane 1), and transcripts are not seen in the presence of 1 μg of α-amanitin

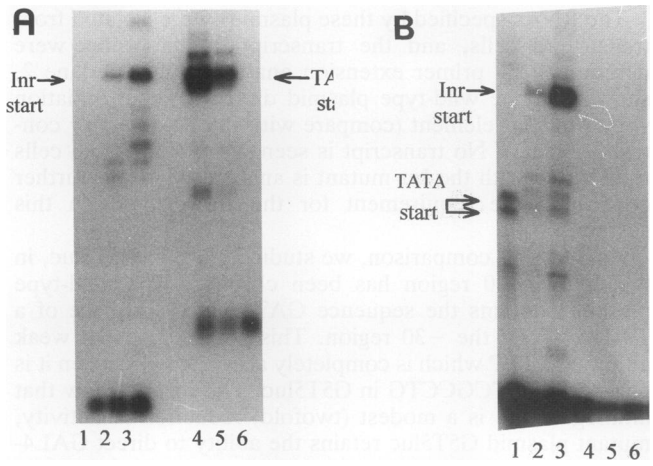


FIG. 3. Activation of the Inr-containing promoter in vitro. (A) Sp1 compared with GAL4-VP16 in vitro. Lanes: 1 to 3, Inr transcription; 4 to 6, TATA box transcription; 1, 2, 5, and 6, constructs with five GAL4 binding sites upstream of the basal element; 3 and 4, templates bearing six GC boxes; 1 and 6, no GAL4-VP16 protein; 2 and 5, GAL4-VP16; 3 and 4, endogenous Sp1 as activator. (B) GAL4-VP16 stimulates transcription from different basal elements in vitro. Lanes: 1 to 3, GAL4-VP16 protein; 4 to 6, no GAL4-VP16 protein; 1 and 4, TATA-mediated transcription; 2 and 5, transcription from the Inr promoter; 3 and 6, transcription from a template with both TATA and Inr elements. In this construct, the sequence in the promoter region is TATAAAAGGGGGTGGGGGGAGCTCG GCCCTCATTCTGGAG. The result has been reproduced with three different HeLa nuclear extract preparations.

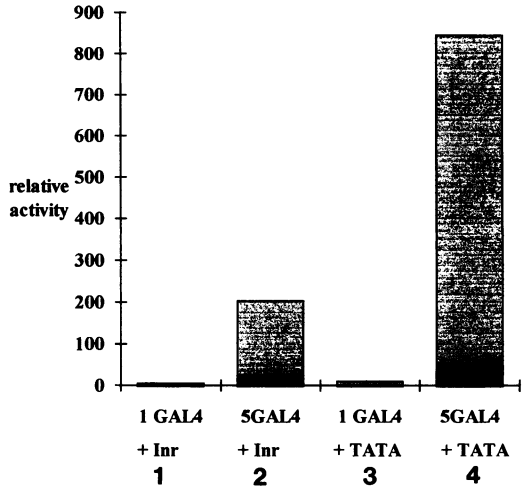


FIG. 4. Transcription synergism for Inr in vivo. In this assay using the Inr promoter (columns 1 and 2) and a TATA box (columns 3 and 4), one or five GAL4 DNA binding sites are placed upstream of the TATA or Inr element. Cotransfection with GAL4-VP16 effector plasmid and subsequent luciferase assay were done as described for Fig. 1. The height of each bar represents the relative amount of expression from the indicated number of sites; the black areas for five GAL4 binding sites represent the values obtained for a single site multiplied by 5.

per ml (not shown). We conclude that bound GAL4-VP16 can cooperate with an Inr element to specify transcription initiation within the Inr in vitro. The activation by GAL4-VP16 is lower than that from Sp1 transcription (compare lanes 2 and 3). This seems to be a property of the activators themselves rather than Inr dependent, since the difference is retained with a TATA-alone template, as shown in Fig. 3A (compare lane 4 [Sp1 transcription] and lane 5 [GAL4-VP16 on the TATA promoter]). This comparison shows that GC box-dependent transcription is stronger than GAL4-VP16 transcription in vitro whether the basal insert is Inr or TATA.

We also compared the changing strength of in vitro transcription when basal elements are changed. Lane 1 of Fig. 3B shows consensus TATA-dependent transcription (TATA start) that is about threefold stronger than Inr-dependent transcription (Inr start; lane 2). Both transcripts require GAL4-VP16 to form (compare with lanes 4 and 5 [lacking GAL4-VP16]). This is quite similar to what was observed in the in vivo experiments described above, in which there was also a threefold difference in the strength of TATA and Inr expression. When consensus TATA and TdT Inr are joined on the same template with the optimal separation of 30 bp, much stronger transcription is observed (Fig. 3B, lane 3, Inr start). This transcription is also heavily dependent on added GAL4-VP16 (compare with lane 6). This cooperation between optimally placed TATA and Inr elements was observed previously for GC box-dependent transcription in vitro (23).

These comparisons essentially show no selectivity of transcription activators for either basal element. GC box transcription is roughly comparably stronger than GAL4-VP16 transcription whether the basal insert is consensus TATA or the TdT Inr. The specificity of transcription initiation within an Inr element and the ability to cooperate with an optimally placed TATA sequence are not changed in any obvious ways when GAL4-VP16 is used for comparison with the known properties of GC box-dependent transcription.

We next investigated whether transcription in the presence of the TdT Inr displayed some of the properties associated with GAL4-VP16-dependent TATA transcription. Prominent among these properties is transcriptional synergy wherein multiple bound GAL4 proteins produce transcriptional activation which is unexpectedly higher than would be expected from studies of single-site templates (6, 18).

Activation of the TdT Inr promoter by GAL4-VP16 is synergistic. GAL4-VP16 is known to activate a consensus TATA promoter synergistically in the sense that multiple binding sites lead to activation that is much stronger than expected on the basis of results from a single site (6, 18). The source of this synergy is not known, but some proposals have important implications for the general mechanism of polymerase II promoter activation (for discussions, see references 10 and 15). We now test whether the TdT Inr promoter exhibits similar behavior in response to GAL4-VP16.

The experiment compares luciferase activity in CV-1 cells, using TdT Inr reporter constructs containing either one or five GAL4 binding sites. The results (Fig. 4) show that a single site yields 5 U of activity (column 1), whereas five sites yield 180 U of activity (column 2). This observation fits the previous definition of synergy quite well, since five times the single site activity is only 25 U (black portion of column 2). A series of experiments with a consensus TATA promoter in parallel transfections confirmed the expected synergy in that case (columns 3 and 4). In the context of a consensus TATA box, the increase was from 10 to 800 U. The synergy is somewhat larger in the context of consensus

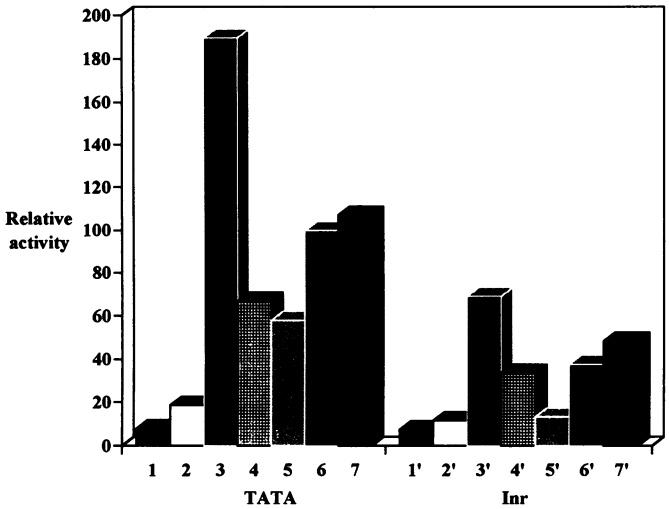


FIG. 5. Transcriptional activation by different activation domains. TATA box- or Inr-containing reporter genes (the same as those used for Fig. 1) were cotransfected with constructs encoding different GAL4 derivatives into CV-1 cells. Columns: 1 and 1', with expression vector pOR4 alone; 2 and 2', with GAL4(1–147); 3 and 3', with GAL4-Sp1; 4 and 4', with GAL4-CTF; 5 and 5', with GAL4-C/EBP; 6 and 6', with GAL4-p53(1–393); 7 and 7', with GAL4-ER(EF).

TATA, but since the absolute amount of synergy depends somewhat on experimental conditions (unpublished data), we do not know whether this difference is meaningful. In any case, the experiment shows that the TdT Inr element can be associated with synergistic transcriptional activation by GAL4-VP16.

Different activation domains show similar profiles at TdT Inr and consensus TATA promoters. Several classes of domains are known to activate transcription from TATA box-containing promoters. To obtain a further indication as to whether an Inr promoter might respond similarly to a range of activators, we tested several activation domains to supplement the results just described. These were the GAL4(1–147) domain and the activation domains from transcription factors CTF (C-terminal proline-rich domain including amino acids 220 to 499), p53 (N-terminal domain with amino acids 1 to 73 or domain containing amino acids 1 to 393), C/EBP (N-terminal domain with amino acids 1 to 9), estrogen receptor (hormone-dependent activation domain EF), and Sp1, all as hybrid GAL4 constructs. In separate experiments, these expression vectors were cotransfected with the Inr-luciferase reporter, and activity was measured after 2 days. A control experiment used the expression vector alone without any encoded proteins.

The results (Fig. 5) show that TdT Inr transcription can be stimulated by most of the activation domains assayed. The GAL4(1–147) DNA binding domain alone does not produce a high level of transcription over the control samples (compare columns 1' and 2'). Expression from the C/EBP N-terminal domain is only slightly higher, but all of the other domains show very significant stimulation (compare lanes 3', 4', 6', and 7' with lane 1'). All of these stimulations by GAL4 hybrids are small compared with that from GAL4-VP16 (see above), which produces 100-fold-higher activity. An exception is the p53 N-terminal 73-amino-acid domain, which produces activation about half of that achieved by GAL4-VP16 (data not shown).

Columns 1 to 7 of Fig. 5 show parallel experiments done with the consensus TATA promoter, which yields a profile similar to that for Inr. In addition, GAL4-VP16 produces about 100-fold-higher activity than do the domains shown, and GAL4-p53(1–73) produces about half of the GAL4-VP16 activity (data not shown). This selectivity does not differ, however, from that associated with the Inr promoter (columns 1' to 7'). We conclude that in this data set, there is no evidence for a differential response to activation domains between promoters dominated by the Inr and TATA elements, though the activation using the Inr is always weaker than that using the TATA element. The same conclusion was reached in vitro on the basis of the comparison between GAL4-VP16 and GC box transcription.

DISCUSSION

This study has examined the properties of GAL4-VP16 activation in the presence of the TdT initiator sequence and in the absence of a consensus TATA box. The in vivo results exhibit properties that are thought to have important implications for deciphering transcriptional control mechanisms, such as transcriptional synergy (6, 18), illustrated by a 36-fold increase in activity when the number of GAL4 binding sites is increased from one to five. Parallel studies showed no significant differences in these properties compared with a consensus TATA promoter. The two types of promoters also showed surprisingly similar responses to different activation domains.

The lack of difference between TdT Inr and consensus TATA promoters is unexpected in view of the differences in critical steps in the assembly of transcription complexes (see the introduction) (reviewed in reference 38). TATA promoters begin assembly by binding the TBP component of TFIID to the TATA box. As discussed above, the lack of a TATA sequence and other evidence indicates that initial recognition of an Inr promoter is not by TBP (3, 5, 28, 32). Inr-dependent transcription initiation resembles polymerase I and polymerase III transcription in the sense that TBP must be brought to the template by other proteins rather than by selective DNA sequence recognition (26). An Inr promoter also appears to have a different requirement for the TAFs, which associate very early in the assembly pathway (25). Despite these critical differences in the early assembly steps, the two promoter types studied here are nearly indistinguishable in assays which measure properties that are thought to be intimately related to the detailed mechanism of transcription complex assembly.

This unexpected similarity places important constraints on the role of activators in promoting assembly of transcription complexes at promoters containing either Inr elements or TATA boxes. These and other strong similarities raise the distinct possibility that the critical steps carried out by the activators are ones that are common in these two cases. Although the details of assembly in the context of an Inr are unknown, it appears that the Inr and TATA pathways converge, likely after recruitment of TFIID to the template (5, 44) and before the DNA is opened (37, 42). Studies of TATA promoter assembly have suggested that activators intervene in the early steps involving TFIID, TFIIA, and TFIIB (16, 19, 20, 31, 35, 37, 40). In view of the differences between recruitment of TBP to the TATA element and to the Inr, it seems unlikely that the activator would perform a common function at this stage. Instead, the common steps are more likely candidates for activation; these primarily involve the action of the TFIIA fraction (on the TBP-TAF

complex) and the subsequent recruitment of factor TFIIB. These considerations are consistent with our previous suggestion that GAL4-VP16 works by cooperating with the bound TFIID complex and the TFIIA fraction to prepare templates for the rapid binding of TFIIB and subsequent factors (37). This process could be the same at Inr and TATA promoters even though different TAFs are used.

One complication in these models is the apparent cooperation between sequences in the TATA position and those in the Inr position, even in cases in which these elements have little resemblance to consensus elements (41, 43). The TdT Inr used here is slightly influenced by sequences near −30 (Fig. 2). This phenomenon has been studied systematically by Zenzie-Gregory et al., who suggested that Inr-dependent transcription activation can be enhanced by TBP binding to non-TATA sequence in the −30 region (43). It is possible that many promoters actually contain sequences, ill matched to the consensus, that nonetheless function in both positions (41, 43).

This view suggests that typical pathways used for transcription initiation may be composites of the extremes noted with consensus TATA and consensus Inr promoters. Our results indicate that important characteristics of the mechanism, such as transcription synergy, are very similar for the TATA and Inr promoters. Since the results also demonstrate similar responses to a set of activators, differential regulation of such promoters might be difficult to achieve by changing regulators. However, since the initiating basal factors may differ, differential regulation of two such promoters might be accomplished by either of two ways. One would be to control the relative amounts of factors capable of initiating TATA versus Inr assembly (8). In this view, the composite nature of many promoters might reflect the fine tuning of transcription levels that could be accomplished by changing the mix of TFIID and Inr-binding factors in different cells and circumstances. Once a promoter is recognized, however, subsequent steps should be very similar regardless of the promoter type. Alternatively, one might control the state of the polymerase itself (3). In either of these contexts, it is interesting that the results of this study imply that differential regulation of Inr and TATA promoters might be easiest to achieve by altering the composition of initiating factors that are part of the basal transcription apparatus.

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REFERENCES

1. **Basu, A., K. Park, M. L. Atchison, R. S. Carter, and N. G. Avadhani.** 1993. Identification of a transcriptional inhibitor element in the cytochrome c oxidase subunit Vb promoter which binds to transcription factors NF-E1 (YY-1, d) and Sp1. J. Biol. Chem. **268:**4188–4196.
2. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61:**1199–1208.
3. **Buermeyer, A. B., N. E. Thompson, L. A. Strasheim, R. R. Burgess, and P. J. Farnham.** 1992. The HIP1 initiator element plays a role in determining the in vitro requirement of the dihydrofolate reductase gene promoter for the C-terminal domain of RNA polymerase II. Mol. Cell. Biol. **12:**2250–2259.
4. **Buratowski, S., S. Hahn, L. Guarente, and P. A. Sharp.** 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. Cell **56:**549–561.
5. **Carcamo, J., L. Buckbinder, and D. Reinberg.** 1991. The initiator directs assembly of a transcription factor IID-dependent transcription complex. Proc. Natl. Acad. Sci. USA **88:**8052–8056.
6. **Carey, M., Y.-S. Lin, M. R. Green, and M. Ptashne.** 1990. A mechanism for synergistic activation of a mammalian gene by Gal4 derivatives. Nature (London) **345:**361–364.
7. **Chen, W., and K. Struhl.** 1985. Yeast mRNA initiation sites are determined primarily by specific sequences, not by the distance from the TATA element. EMBO J. **4:**3273–3280.
8. **Colgan, J., and J. Manley.** 1992. TFIID can be rate limiting in vivo for TATA-containing, but not TATA-lacking, RNA polymerase II promoters. Genes Dev. **6:**304–315.
9. **Dignam, J. D., P. L. Martin, B. S. Shastry, and R. G. Roeder.** 1983. Eukaryotic gene transcription with purified components. Methods Enzymol. **101:**582–598.
10. **Emami, K. H., and M. Carey.** 1992. A synergistic increase in potency of a multimerized VP16 transcriptional activation domain. EMBO J. **11:**5005–5012.
11. **Flanagan, P. M., R. J. Kelleher III, M. H. Sayre, H. Tschochner, and R. D. Kornberg.** 1991. A mediator required for activation of RNA polymerase II transcription in vitro. Nature (London) **350:**436–438.
12. **Flores, O., H. Lu, M. Killeen, J. Greenblatt, Z. F. Burton, and D. Reinberg.** 1991. The small subunit of transcription factor TFIIF recruits RNA polymerase II into preinitiation complex. Proc. Natl. Acad. Sci. USA **88:**9999–10003.
13. **Greenblatt, J.** 1991. RNA polymerase-associated transcription factors. Trends Biochem. Sci. **16:**408–411.
14. **Hahn, S., E. T. Hoar, and L. Guarente.** 1985. Each of three "TATA elements" specifies a subset of the transcription initiation sites at the CYC-1 promoter of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82:**8562–8566.
15. **Herschlag, D., and F. B. Johnson.** 1993. Synergism in transcriptional activation: a kinetic view. Genes Dev. **7:**173–179.
16. **Ingles, C. J., M. Shales, W. D. Cress, S. J. Triezenberg, and J. Greenblatt.** 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature (London) **351:**588–590.
17. **Kelleher, R. J., III, P. M. Flanagan, and R. D. Kornberg.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61:**1209–1215.
18. **Lin, Y.-S., M. Carey, M. Ptashne, and M. R. Green.** 1990. How different eukaryotic transcriptional activators can cooperate promiscuously. Nature (London) **345:**359–361.
19. **Lin, Y.-S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator in vitro. Cell **64:**659–664.
20. **Lin, Y.-S., I. Ha, E. Maldonado, D. Reinberg, and M. R. Green.** 1991. Binding of general transcription factor TFIIB to an acidic activating region. Nature (London) **353:**569–571.
21. **McNeil, J. B., and M. Smith.** 1986. Transcription initiation of the *Saccharomyces cerevisiae* Iso-1-cytochrome *c* gene. Multiple, independent TATA sequences. J. Mol. Biol. **187:**363–378.
22. **Nagawa, F., and G. R. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation sites at the HIS4 gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82:**8557–8561.
23. **O'Shea-Greenfield, A., and S. Smale.** 1992. Roles of TATA and initiator elements in determining the start site location and direction of RNA polymerase II transcription. J. Biol. Chem. **267:**1391–1402.
24. **Promega Corp.** 1991. Protocols and applications guide, p. 294–296. Promega Corp., Madison, Wis.
25. **Pugh, B. F., and R. Tjian.** 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. Genes Dev. **5:**1935–1945.
26. **Rigby, P. W.** 1993. Three in one and one in three: it all depends on TBP. Cell **72:**7–10.
27. **Roeder, R. G.** 1991. The complexes of eukaryotic transcription initiation: regulation of preinitiation complex assembly. Trends Biochem. Sci. **16:**402–408.
28. **Roy, A. L., M. Meisterernst, P. Pognonec, and R. Roeder.** 1991. Cooperative interaction of an initiator-binding transcription factor and the helix-loop-helix activator USF. Nature (London)

354:245–248.

29. **Rudolph, H., and A. Hinnen.** 1987. The yeast PHO5 promoter: phosphate-control elements and sequences mediating mRNA start-site selection. Proc. Natl. Acad. Sci. USA **84:**1340–1344.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Seipel, K., O. Georgive, and W. Schaffner.** 1992. Different activation domains stimulate transcription from remote ('enhancer') and proximal ('promoter') positions. EMBO J. **11:** 4961–4968.
32. **Seto, E., Y. Shi, and T. Shenk.** 1991. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. Nature (London) **354:**241–245.
33. **Smale, S. T., and D. Baltimore.** 1989. The initiator as a transcription control element. Cell **57:**103–113.
34. **Smale, S. T., M. C. Schimidt, A. J. Berk, and D. Baltimore.** 1990. Transcriptional activation by SP1 as directed through TATA or initiator: specific requirement for mammalian transcription factor IID. Proc. Natl. Acad. Sci. USA **87:**4509–4513.
35. **Stringer, K. F., C. J. Ingles, and J. Greenblatt.** 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. Nature (London) **345:**783–786.
36. **Wang, W., and J. D. Gralla.** 1991. Differential ability of proximal and remote element pairs to cooperate in activating RNA polymerase II transcription. Mol. Cell. Biol. **11:**4561–4571.
37. **Wang, W., J. D. Gralla, and M. Carey.** 1992. The acidic activator Gal4-AH can stimulate polymerase II transcription by promoting assembly of a closed complex requiring TFIID and TFIIA. Genes Dev. **6:**1716–1727.
38. **Weis, L., and D. Reinberg.** 1992. Transcription by RNA polymerase II: initiator-directed formation of transcription-competent complexes. FASEB J. **6:**3300–3309.
39. **White, J. H., C. Brou, J. Wu, N. Burton, J.-M. Egly, and P. Chambon.** 1991. Evidence for a factor required for transcriptional stimulation by the chimeric acidic activator GAL4-VP16 in Hela cell extracts. Proc. Natl. Acad. Sci. USA **88:**7674–7678.
40. **White, J., C. Brou, J. Wu, Y. Lutz, V. Moncollin, and P. Chambon.** 1992. The acidic transcriptional activator GAL-VP16 acts on performed template-committed complexes. EMBO J. **11:**2229–2240.
41. **Wiley, S. R., R. J. Kraus, and J. E. Mertz.** 1992. Functional binding of the "TATA" box binding component of transcription factor TFIID to the −30 region of TATA-less promoters. Proc. Natl. Acad. Sci. USA **89:**5814–5818.
42. **Ying, J., S. T. Smale, and J. D. Gralla.** 1993. A common ATP requirement for open complex formation and transcription at promoters containing initiator or TATA elements. J. Biol. Chem. **268:**6535–6540.
43. **Zenzie-Gregory, B., A. Khachi, I. P. Garraway, and S. T. Smale.** 1993. Mechanism of initiator-mediated transcription: evidence for a functional interaction between the TATA-binding protein and DNA in the absence of a specific recognition sequence. Mol. Cell. Biol. **13:**3841–3849.
44. **Zenzie-Gregory, B., A. O'Shea-Greenfield, and S. T. Smale.** 1992. Similar mechanisms for transcription initiation mediated through a TATA box or an initiator element. J. Biol. Chem. **267:**2823–2830.
45. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964–1974.