Borrower: FBA

Lending String:
FTU,*FDA,FWA,FHM,FEC,FXG,FQG,GJG,GMU,MWR,NMW,NNM,MUM,LWA,LLM

Patron:

Journal Title: Cell.

Volume: 52 **Issue:** 2
Month/Year: 1988 **Pages:** 169-78

Article Author: Webster N

Article Title: The yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator.

Imprint: [Cambridge, Mass., Cell Press, etc.]

ILL Number: 134047248

Call #: 570.5 C39

Location: DIRAC

Charge
Maxcost: 60.00IFM

Shipping Address:
State Library of Florida
Interlibrary Loan Grant
R. A. Gray Building
Tallahassee, FL 32399-0250
2 1254 00152 7603

Fax: 850-245-6744
Odyssey:
Ariel:
Email: Linda.Pulliam@dos.myflorida.com

Cell, Vol. 52, 169–178, January 29, 1988, Copyright © 1988 by Cell Press

The Yeast UAS_G Is a Transcriptional Enhancer in Human HeLa Cells in the Presence of the GAL4 *Trans*-Activator

Nicholas Webster,* Jia Rui Jin,* Stephen Green,* Melvyn Hollis,† and Pierre Chambon*

* Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS
Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM
Institut de Chimie Biologique
Faculté de Médecine
11, Rue Humann
67085 Strasbourg Cedex France

† Department of Biochemistry and Molecular Biology
Harvard University
Cambridge, Massachusetts 02138

Summary

The yeast *trans*-activator protein GAL4, when expressed in HeLa cells, stimulates transcription from several class B (II) eukaryotic promoters containing GAL4 binding sites either as the full UAS_G or as synthetic 17-mers. The characteristics of this activation are indistinguishable from those of the SV40 enhancer. Transcription was similarly stimulated from either complex promoter regions containing multiple upstream elements or from a simple promoter region composed of only a TATA box. Addition of a 17-mer GAL4 binding site to the SV40 enhancer resulted in a synergistic enhancement of transcription in the presence of GAL4. Furthermore, chimeras of the human estrogen receptor DNA binding domain and either GAL4 or GCN4 activating "acidic" regions can activate a promoter region controlled by an estrogen-responsive enhancer. Together, these data indicate that the molecular mechanisms responsible for transcriptional enhancement have been conserved from yeast to man.

Introduction

The control of gene transcription is achieved through the interaction of *trans*-acting proteins with *cis*-acting DNA promoter elements. The promoter of genes transcribed by RNA polymerase B (II) in higher eukaryotes can be divided into several components, namely, a positioning element (e.g., TATA box), upstream elements, and enhancer elements (for reviews and references, see Chambon et al., 1984; Serfling et al., 1985; Wasylyk, 1987; Hatzopoulos et al., 1987). The TATA box, located approximately 30 bp upstream of the cap site, interacts with the TATA box factor and is necessary for faithful and efficient initiation of transcription. However, the promoter regions of some genes, notably housekeeping genes, do not contain a TATA box (Reynolds et al., 1984). Upstream elements, located in the −40 to −110 region, bind specific *trans*-acting factors and are required for efficient transcription. These elements vary among promoters both in number and position with respect to the start site, and are interchangeable between different promoters (for reviews, see McKnight and Tjian, 1986; Dynan, 1987; Maniatis et al., 1987). Enhancer elements (for reviews and references, see Serfling et al., 1985; Wasylyk, 1987, Hatzopoulos et al., 1987) stimulate initiation of transcription from homologous or heterologous, natural or cryptic promoter elements, whether or not the activated elements contain a TATA box or a functional upstream element (Hen et al., 1982; Wasylyk et al., 1983). Enhancers are composed of one or more motifs, sometimes repeated, which are binding sites for factors that act synergistically and may be cell-specific (for references, see Nomiyama et al., 1987). Enhancers exhibit an exceptional positional flexibility in that they stimulate transcription irrespective of their orientation or distance both upstream or downstream of the cap site, although very often the efficiency of stimulation decreases with increasing distance (Wasylyk et al., 1983; Wasylyk et al., 1984).

To date, few regulatory proteins interacting with upstream or enhancer elements have been characterized in higher eukaryotes. Recent studies (for references, see Petkovich et al., 1987) have shown that members of a family of nuclear receptors whose function is dependent on the binding of specific ligands (e.g., steroid and thyroid hormones and retinoic acid) represent *trans*-acting regulatory factors that activate transcription of target genes by binding to a specific promoter element which exhibits the properties of an enhancer (for references, see Yamamoto, 1985; Kumar et al., 1987; Martinez et al., 1987). Amino acid sequence comparison of these receptors, together with mutational analyses, have defined two conserved important functional domains: a DNA binding domain (region C), which determines the receptor specificity for target gene transcription, and a functionally independent ligand-binding domain (region E) (for references, see Kumar et al., 1986; Kumar et al., 1987). In vivo studies have shown that human estrogen receptor (hER) mutants, in which most or all of the hormone-binding domain is removed, retain only about 5% of the transcriptional activity of the wild-type receptor, and yet appear to bind efficiently to estrogen responsive elements both in vivo (Kumar et al., 1987) and in vitro (V. Kumar and P. Chambon, unpublished data), thus suggesting that the hormone binding domain (region E) may play an important role in the activation function of the hER.

The organization of the class B (II) promoters in the lower eukaryote Saccharomyces cerevisiae shows similarities to that of the corresponding promoters of higher eukaryotes. Yeast promoters are composed of initiation, TATA, and upstream activator sequence (UAS) elements (for review and references, see Struhl, 1987a). TATA elements are necessary, but not sufficient, for efficient initiation of transcription from most yeast genes. However, in contrast to higher eukaryotes, the distance between yeast TATA elements and mRNA start sites ranges between 40–120 bp depending on the promoter. UAS elements, which are required for efficient transcription, exhibit some

of the properties of mammalian enhancer elements since they function irrespective of their orientation and, to some extent, of their distance (up to at least 600 bp). Like enhancer elements, UAS elements can be DNA binding sites for *trans*-acting regulatory proteins, such as the yeast transcriptional activators GAL4 and GCN4. The GAL4 protein binds four related 17 bp sites within the region (UAS_G) required for activation of the divergently transcribed galactose metabolizing genes GAL10 and GAL1 (Giniger et al., 1985; Bram et al., 1986; and references therein). Moreover, a synthetic oligonucleotide, the 17-mer, which is a near consensus of these sites, binds GAL4 in vitro and activates transcription in vivo (Giniger et al., 1985). The GCN4 protein induces transcription of several coregulated genes in response to amino acid starvation (e.g., HIS3) and binds to specific UAS elements located upstream of these genes (see Hope and Struhl, 1986). The DNA binding and transcriptional activation domains of both GAL4 and GCN4 are clearly separable functional domains. The 74 N-terminal amino acids of GAL4 (Keegan et al., 1986) and the 60 C-terminal amino acids of GCN4 (Hope and Struhl, 1986) are sufficient for DNA binding, but fail to activate transcription. Moreover, a hybrid protein, in which the DNA binding domain of the E. coli LexA repressor replaces the GAL4 DNA binding domain (Brent and Ptashne, 1985), is able to activate transcription from a promoter in which UAS_G is replaced by the LexA operator. A similar result was obtained in the case of GCN4 (Brent and Ptashne, 1985; Hope and Struhl, 1986). Dissection of the transcriptional activation function of GAL4 has revealed two relatively short regions (region I, residues 148–196, or region II, residues 768–881) that can activate transcription (Ma and Ptashne, 1987a). The transcriptional activation function of GCN4 has been ascribed to a short region of 35–40 amino acids of which a 19 amino acid core retains some activation function (Hope and Struhl, 1986). In both GAL4 and GCN4, the activating regions have an acidic character, but do not share any obvious sequence similarity (Ma and Ptashne, 1987a). Recently, Ma and Ptashne (1987b) have shown that other apparently unrelated acidic amino acid sequences can stimulate transcription when fused to the DNA binding region of GAL4.

The present study was undertaken with the aim of further characterizing the domains responsible for the DNA binding and transcriptional activation functions of hER and investigating whether similar mechanisms may be involved in activation of transcription in mammalian and yeast cells following interaction of enhancer or UAS elements with their cognate factors. As a first step in such a study, it was necessary to investigate whether GAL4 itself could function as a transcriptional activator in mammalian cells. We report here that the yeast transcriptional activator GAL4 can efficiently stimulate transcription in human HeLa cells from several higher eukaryotic promoter regions to which either the UAS_G or a synthetic 17-mer has been added. A similar result is described in the accompanying paper by Kakidani and Ptashne (1988). We also show that chimeric proteins generated by fusion of the hER DNA binding domain and either GAL4 or GCN4 activating regions can efficiently activate transcription controlled by an estrogen-responsive enhancer element.

Results

UAS_G Functions as an Enhancer Element in the Presence of GAL4 for the HSV-1 Thymidine Kinase and the Rabbit β-Globin Gene Promoters in HeLa Cells

We investigated first whether GAL4, when expressed in mammalian cells, could activate transcription of reporter genes containing GAL4-responsive elements. The GAL4 coding sequence was inserted into the expression vector pKCR2 downstream of the SV40 early promoter and the β-globin splice site and upstream of the β-globin/SV40 polyadenylation signals. As a negative control, the GAL4 coding sequence was also inserted in the reverse orientation (see Experimental Procedures).

Suitable reporter plasmids (UAS-tk-CAT and UASR-tk-CAT) were constructed by insertion of the UAS_G from the GAL1–GAL10 divergent promoter in both orientations upstream of the herpes simplex virus (HSV) thymidine kinase (tk) promoter, which is located upstream of the E. coli chloramphenicol acetyltransferase (CAT) gene in plasmid pBLCAT8+ (see Figure 1A and Experimental Procedures). CAT activity was measured following cotransfection into HeLa cells of activator and reporter plasmids, along with a reference plasmid that contains the β-galactosidase gene under the control of the Rous sarcoma virus promoter (RSV-LacZ). All CAT assays were standardized for β-galactosidase activity and quantified by scintillation counting. CAT activity was observed in HeLa cells cotransfected with the UAS-tk-CAT reporter plasmid and the GAL4 expression vector (Figure 1B, lane 3), but not with either the perental expression vector pKCR2 (lane 8) or pKCR2 containing the GAL4 coding sequence in the reverse orientation (data not shown). Furthermore, GAL4 stimulated expression of the reporter gene irrespective of the orientation of the UAS element (compare UAS-tk-CAT and UASR-tk-CAT), although stimulation was slightly (~30%) stronger in the GAL10 orientation (see legend to Figure 1 and compare lanes 3 and 4). We are unable to differentiate as to whether this reflects a preferential activation by GAL4 in a particular orientation of UAS_G or a distance effect due to asymmetric distribution of the four GAL4 binding sites in UAS_G.

To demonstrate that the GAL4 binding sites in UAS_G were necessary and sufficient for stimulation, a single 17-mer near-consensus GAL4 binding site (17M) was inserted upstream of the tk promoter (17M-tk-CAT; Figure 1A). The sequence of this binding site is 5′-CGGAGTACTGTCCTCCG-3′ and contains a single base change (G→T) from the perfect palindromic consensus to create a ScaI site. In vitro binding studies have shown that the DNA binding fragment of GAL4 (1–147) binds to this sequence about 3-fold more tightly than it does to the 17-mer (Giniger et al., 1985) used in the accompanying paper of Kakidani and Ptashne (1988) (M. Hollis and M. Ptashne, unpublished data). The presence of this single binding site in close apposition to the tk promoter was sufficient for GAL4 to stimulate CAT activity to a level similar (55%) to that seen with the whole UAS_G (see legend to Figure 1 and compare lanes 3 and 5). The last construction 17MX2-tk-CAT, which contains a tandem repeat of a perfect palindromic 17-mer (5′-CGGAGGACTGTCCTCCG-3′, 17MX) inserted upstream of the tk promoter, showed an even stronger stimulation with GAL4 (see legend to Figure 1 and compare lanes 3, 5, 6, and 7).

In order to estimate the relative magnitude of the stimulation by GAL4, we compared the level of GAL4-induced CAT activity to that induced by the estrogen receptor (HEO) using the estrogen-responsive reporter gene vit-tk-CAT, which contains the ERE of the Xenopus vitellogenin A2 gene (Klein-Hitpass et al., 1986). Previous studies have shown that maximal stimulation of 1 μg of vit-tk-CAT occurs with 100 ng of HEO (Kumar et al., 1987). Since here 1 μg of HEO was cotransfected with 1 μg of vit-tk-CAT, it is clear that GAL4 can stimulate the HSV tk promoter as strongly as the estrogen receptor (see also Figure 5B). A 50- to 100-fold stimulation was observed (see legend to Figure 1).

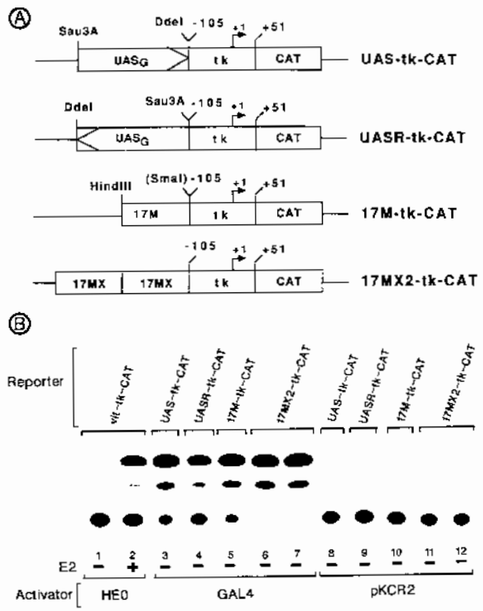

Figure 1. GAL4 Stimulates CAT Activity from GAL4-Responsive Reporter Genes in HeLa Cells

(A) Schematic organization of the GAL4-responsive reporter genes. The *cis*-acting GAL4 responsive elements, UAS_G and 17-mers (17M and 17MX; see Experimental Procedures), are inserted in a polylinker located at −105 upstream of the HSV tk cap site. The orientation of UAS_G in UAS-tk-CAT and UASR-tk-CAT is indicated by the chevron that points toward the GAL10 gene of the natural GAL1–GAL10 promoter region. The transcription start site of the tk promoter (+1) is indicated by an arrow.

(B) Induction of CAT activity. HeLa cells were cotransfected with a reporter gene (1 μg) and either an activator gene (HEO or GAL4) or the parental vector pKCR2 (1 μg), together with 4 μg of the internal control plasmid RSV-lacZ. Estradiol (E2, 10^{-8} M) was added as indicated to activate the estrogen receptor (HEO). Quantitation of labeled acetylated chloramphenicol in parallel experiments where less than 50% of the chloramphenicol substrate was utilized indicated the following relative levels of CAT activities: UAS-tk-CAT, 100%; UASR-tk-CAT, 75%; 17M-tk-CAT, 53%; 17MX2-tk-CAT, 124%. The basal activity in the absence of GAL4 was of the order of 1%.

To demonstrate that GAL4 could stimulate faithful transcription from a higher eukaryotic gene promoter, we constructed a series of reporter plasmids to determine directly, at the RNA level, the extent of stimulation by GAL4. The first series of reporters were constructed using the vector pG1 (Sassone-Corsi et al., 1985), which contains the rabbit β-globin gene under its own promoter with upstream sequences to −109. The entire UAS_G and "ScaI site" 17-mer (17M) were cloned into pG1 (Figure 2A) and each was cotransfected with the GAL4 expression vector. Cytoplasmic RNA was analyzed by quantitative S1 nuclease mapping using a 5′ end-labeled DNA probe and the plasmid pAO (Zenke et al., 1986), which contains the SV40 early promoter, as a cotransfected reference plasmid. GAL4 stimulated faithful β-globin gene transcription from either UAS-pG1 (Figure 2B, lane 1) or 17M-pG1 (lane 3), whereas no stimulation was observed using either the "reverse" GAL4 expression vector (GAL4R) (lane 2) or pG1, which lacks a GAL4-responsive element (lane 4). Comparison of the level of β-globin RNA transcribed from the GAL4-responsive reporter genes and from pG1B (lane 5), which contains the SV40 enhancer with a single 72 bp sequence inserted immediately upstream of the β-globin promoter (see Figure 2A), indicated that (GAL4-UAS_G)-induced transcription was 25% (UAS-pG1) to 65% (17M-pG1) of that achieved with the SV40 enhancer. Note that the activation of transcription was higher with the 17-mer than with UAS_G, irrespective of the amount of transfected reporter gene (compare lanes 1 and 3 with lanes 10 and 11).

Since one characteristic of an enhancer is its positional flexibility (see Introduction), a second series of reporter plasmids containing UAS_G or the "ScaI site" 17-mer were constructed using the vector pG2 (Hen et al., 1986), which contains the β-globin gene upstream flanking sequences to −425 (Figure 2A). In each case, the insertion of either the UAS_G, 17-mer, or SV40 enhancer element at position −425 resulted in a decrease of β-globin transcription when compared with the corresponding pG1 constructs (Figure 2B). No orientation effect of the UAS_G was observed at −425, in contrast to the CAT results presented earlier where UAS_G was in close apposition to the tk promoter. Presumably, the asymmetry of the location of the GAL4 binding sites in UAS_G is less important at a distance. The decrease due to moving the control elements further upstream was moderate for both the pG2 constructs containing UAS_G (60% of that seen at −109, compare lanes 1 and 6) or the SV40 enhancer (45%, compare lanes 5 and 9), but was striking with the 17-mer (6%, compare lanes 3 and 8). Thus, it appears that a single 17-mer is a weaker enhancer element than the full UAS_G.

Interestingly, when we inserted UAS_G at position +475 downstream of the β-globin cap site (+1) in plasmid pG2 (Figure 3A, pG2-UAS), a stimulation of transcription in the presence of GAL4 was also observed (Figure 3B, compare lanes 2 and 3). However, the level of transcribed RNA ap-

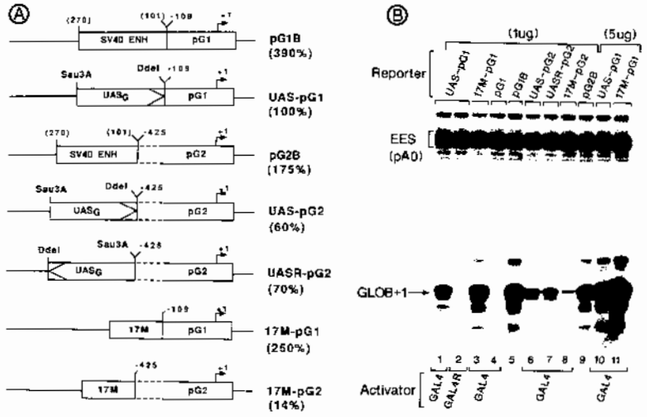

Figure 2. GAL4 Stimulates Faithful Transcription from a Rabbit β-Globin Promoter

(A) Schematic organization of the GAL4-responsive reporter genes (UAS-pG1, UAS-pG2, UASR-pG2, 17M-pG1, 17M-pG2, same symbols as in Figure 1) and the SV40 enhancer (ENH)-driven plasmids pG1B and pG2B. The *cis*-acting elements (UAS$_G$, 17M, SV40 ENH) are positioned in polylinkers at −109 (with respect to the globin cap site) in the pG1 constructs and at −425 in the pG2 constructs. The level of transcription observed, as determined by densitometric scanning of the autoradiograms, relative to UAS-pG1 after correction for transcription from the reference plasmid pAO, is indicated in parentheses. The rabbit β-globin gene transcription start site (+1) is indicated with an arrow. SV40 ENH corresponds to the SV40 sequence between coordinates 101 and 270, but with a single 72 bp element (Zenke et al., 1986).

(B) Quantitative S1 nuclease analysis of transcription from the reporter and reference plasmids shown in (A). Either 1 μg or 5 μg of reporter gene was cotransfected with 1 μg of the GAL4 activator plasmid, or the negative control GAL4R, together with 0.2 μg of the reference plasmid pAO. Cytoplasmic RNA was prepared and hybridized with a 5′ end-labeled probe complementary to pAO (see Experimental Procedures). After S1 nuclease digestion, the protected probe was analyzed on a 6% sequencing gel. EES: early–early start site (+1) of the SV40 early promoter of the reference plasmid pAO. GLOB +1: start site of β-globin promoter within the reporter genes.

peared to be ~20% of that seen for UAS$_G$ at −425 (Figure 3B, compare lanes 1 and 3).

To investigate whether the yeast UAS$_G$ 17-mer and the SV40 enhancer could cooperate to activate the β-globin promoter, we constructed 17M-pG2B (Figure 3A) in which a "ScaI site" 17-mer was located upstream of the SV40 enhancer. A 2-fold stimulation of transcription by GAL4 was observed when compared with pG2B or 17M-pG2B cotransfected with the control GAL4R expression vector after correction for transcription from the cotransfected reference plasmid pAO (compare lanes 5, 6, and 7 in Figure 3B). Note that this increase is much higher than the corresponding sum of the individual transcription from 17M-pG2 (lane 4) and pG2B (lane 5), and approximately the same as that observed by duplication of the 72 bp sequence in the SV40 enhancer (Zenke et al., 1986). Thus it appears that a yeast UAS$_G$ motif, the 17-mer, can cooperate synergistically with the SV40 enhancer.

Transcription from a TATA Box Element Associated with a 17-mer Is Stimulated by GAL4

We show above that GAL4, together with either the yeast UAS$_G$ or a synthetic 17-mer, can stimulate transcription from the strong, and relatively complex, HSV-tk and β-globin promoters. To investigate whether GAL4 could also stimulate transcription from a minimal promoter region, we constructed a series of reporter plasmids containing simply the TATA box and cap site elements (positions +33 to −34) of the adenovirus-2 major late promoter (Ad2MLP) located upstream of a promoterless rabbit β-globin gene truncated at position −9 (Figure 4A). "ScaI" 17-mers were ligated at position −71 into a polylinker sequence located upstream of the TATA box. GAL4-induced transcription from the Ad2MLP was dependent upon both the presence of GAL4 and a GAL4-responsive element (Figure 4B, compare lanes 1, 2, and 4). Using transcription from pG1B as an internal control, it is clear that the extent of transcription from the "monomeric" (17M-TATA-GLOB, lane 2) or "dimeric" (17M2-TATA-GLOB, lane 3) 17-mer reporter genes was much weaker than that obtained with UAS$_G$ located upstream of the tk promoter (see Figure 5B, lane 7, and below). Note that this lower level of transcription most probably does not correspond to a lower activation by GAL4, but to the very low activity of the truncated Ad2MLP promoter (no signal whatsoever could be detected for the TATA-GLOB plasmid in lane 1). Interestingly, transcription from the 17M2-TATA-GLOB reporter, which contains a dimer of the 17-mer whose centers of symmetry are separated by 28 bp, was approximately 8-fold greater than that seen with the 17M-TATA-GLOB reporter that contains a single 17-mer sequence (see legend to Figure 4A). These results suggest that a GAL4-responsive element can cooperate with an isolated TATA box element to stimulate transcription, and that two GAL4-responsive elements can act synergistically.

Chimeric Proteins Containing the Human Estrogen Receptor DNA Binding Domain and Either the GAL4 or GCN4 Activation Domains Stimulate Transcription from an Estrogen-Responsive Element

The above results demonstrate that GAL4, expressed in mammalian cells, stimulates transcription from a variety of promoters. In order to demonstrate that the same GAL4 domains are responsible for transcriptional activation in both yeast and mammalian cells, we constructed the chimeric activator plasmid ER-GAL4 (281/768) (see Figure 5A). This plasmid contains the DNA sequences encoding the N-terminal half of the human estrogen receptor (hER) (amino acids 1–281) that includes the DNA binding (but not the hormone-binding) domain (Kumar et al., 1987)

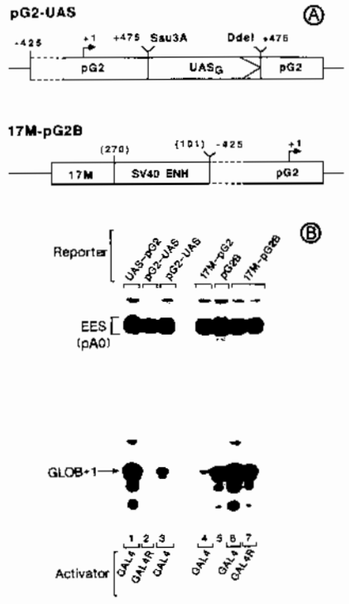

Figure 3. A 3′-Located UAS$_G$ Stimulates Transcription and a 5′-Located 17-mer Cooperates with the SV40 Enhancer

(A) Schematic organization of reporter genes. pG2-UAS: UAS$_G$ was inserted into pG2 (see Figure 2) 3′ to the rabbit β-globin promoter at position +475. 17M-pG2B: the 17-mer (17M) was inserted upstream of the SV40 enhancer in pG2B (see legend for Figure 2).

(B) Quantitative S1 nuclease analysis. The reporter gene (5 μg for lanes 1 to 3 and 1 μg for lanes 4 to 7) was cotransfected with 1 μg of the relevant activator gene together with 0.2 μg of pAO as a reference gene (see legend to Figure 2). The 5′ end-labeled probe was the same as in Figure 2. Quantitation by densitometric scanning of the autoradiograms indicated the following values after correction for transcription from the reference gene pAO: Lanes 1 and 3 (relative to UAS-pG2), UAS-pG2, 100%, pG2-UAS, 20%; lanes 4 to 7 (relative to pG2B), pG2B, 100%; 17M-pG2, 7%; 17M-pG2B+GAL4 (lane 6), 390%; 17M pG2B+GAL4R (lane 7), 210%.

fused to sequences of GAL4 that encode the C-terminal activation region II (amino acids 768–881) (Ma and Ptashne, 1987a). When cotransfected with the estrogen-responsive reporter gene vit-tk-globin (see above), ER-GAL4 stimulated transcription to a level ~50% of that seen with the wild-type estrogen receptor (Figure 5B, lane 5). Note that the N-terminal half of the chimera, which is equivalent to the previously described hER mutant HE15 (Kumar et al., 1987), has only approximately 5% the stimulatory activity seen with HEO (Figure 5B, lane 4).

Interestingly, this activating region of GAL4 is acidic in character, a property that has also been noted for the activating region of GCN4 (see Introduction). To test if the 19 amino acid core of this latter region is also active in mammalian cells, we fused the DNA sequences encoding these 19 amino acids to those encoding the N-terminal half of the hER described above (see ER-GCN4, Figure 5A). When cotransfected with the estrogen-responsive reporter gene vit-tk-globin (Figure 5C) or vit-tk-CAT (Figure

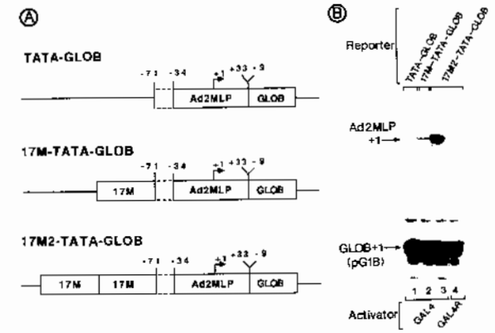

Figure 4. GAL4 Cooperates with a Minimal Promoter

(A) Schematic organization of the reporter genes. The 17-mer GAL4-binding site 17M is present as either a monomer (17M-TATA-GLOB) or dimer (17M2-TATA-GLOB) upstream of the Ad2MLP (−34 + 33 region) that contains just the TATA box and cap site (+1). GLOB represents the promoterless rabbit β-globin gene truncated at position −9.

(B) Quantitative S1 nuclease analysis of trancription from the reporter genes shown in (A). Five micrograms of reporter gene was cotransfected with 1 μg of the activator gene together with 0.5 μg of pG1B (see legend to Figure 2) as a reference gene. A 5′ end-labeled probe complementary to the TATA-GLOB plasmid was used. The start sites of GAL4-induced transcription (Ad2MLP, +1) and reference transcription from pG1B (GLOB, +1) are indicated. Densitometric scanning of the autoradiograms indicated the following levels of relative transcription after correction for transcription from the reference gene pG1B. TATA-GLOB, <5%; 17M-TATA-GLOB, 100%; 17M2-TATA-GLOB, 840%.

5D), ER-GCN4 stimulated transcription from the tk promoter to a level that was approximately 15% of that observed with HEO (Figure 5C, compare lanes 2 and 4; Figure 5D, compare lane 2 with lanes 5 and 6). Again, the stimulation brought about by HE15 using vit-tk-globin (Figure 5C, lane 3) or vit-tk-CAT (Figure 5D, lanes 3 and 4) was about 5% of that seen with HEO. Thus, the acidic regions of GAL4 and GCN4 responsible for activation of transcription in yeast are also functional in human HeLa cells.

It should be noted that we have not determined either the levels of the activator proteins ER-GAL and ER-GCN4 in the above experiments or whether the maximum level of stimulation by these activators was reached. However, previous work has shown (Kumar et al., 1987) that, under the present conditions, the stimulation of transcription of vit-tk-globin by HEO or HE15 is maximal.

Discussion

The Yeast UAS$_G$ Exhibits Properties Indistinguishable from Those of Higher Eukaryotic Enhancers in HeLa Cells Producing the GAL4 Activator

The results displayed in Figures 1 and 2 demonstrate that the yeast UAS$_G$ element activates transcription from either the HSV tk or rabbit β-globin gene promoters when the GAL4 activator is synthesized in HeLa cells. Moreover, the same UAS$_G$ motifs appear to be responsible for transcriptional activation both in yeast and HeLa cells, since stimulation of transcription by GAL4 could also be medi-

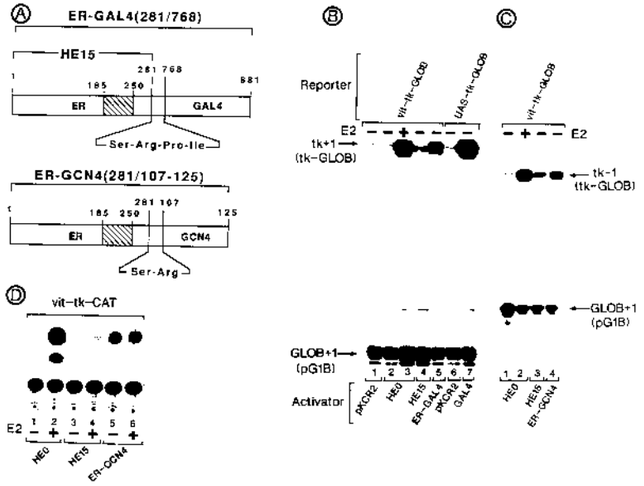

Figure 5. GAL4 and GCN4 Activating Regions Stimulate Transcription in HeLa Cells

(A) Schematic organization of the activators. ER-GAL4 (281/768): the N-terminal half (HE15 [amino acids 1–281]; Kumar et al., 1987) of the human estrogen receptor (ER) containing the DNA-binding domain (shown shaded; amino acids 185–250) is fused to the activating region II of GAL4 (amino acids 768–881). ER-GCN4 (281/107–125): HE15 is fused to the core of the activating region of GCN4 (amino acids 107–125). (B) Quantitative S1 nuclease analysis of transcription using either the estrogen-responsive (vit-tk-GLOB) or GAL4-responsive (UAS-tk-GLOB) reporter genes. Five micrograms of reporter gene was cotransfected with 1 μg of the relevant activator plasmid together with 0.5 μg of pG1B as a reference gene. A 5′ end-labeled probe complementary to tk-globin (Kumar et al., 1987) was used. The start sites of the reporter (tk +1) and reference (GLOB +1) transcripts are indicated. Estradiol (E2) was added as indicated. Quantitation by densitometric scanning of the autoradiograms indicated the following relative levels of transcription after correction for transcription from the reference gene pG1B: HEO(+E2), 100%; HE15, 3%; ER-GAL4, 50%; GAL4, 270%. (C) Quantitative S1 nuclease analysis of transcription using the estrogen-responsive reporter gene vit-tk-globin and either HEO, HE15, or ER-GCN4. Five micrograms of vit-tk-globin was cotransfected with 1 μg of the relevant activator plasmid together with 0.5 μg of pG1B as a reference gene. Estradiol (E2, 10^{-8} M) was added as indicated. The 5′-end-labeled probe and symbols were as in (B). Quantitation by densitometric scanning of this and similar autoradiograms gave the following relative level of transcription after correction for transcription from the reference gene pG1B: HEO(+E2), 100%; HE15, 5%; ER-GCN4, 13%. (D) Stimulation of the estrogen-responsive reporter gene vit-tk-CAT by HEO, HE15, or ER-GCN4. Estradiol (E2, 10^{-8} M) was added as indicated. Other details are as in Figure 1. Quantitation yielded the following relative values: HEO(+E2), 100%; HE15, 4%; ER-GCN4, 14%.

ated by a synthetic 17-mer whose sequence was similar to those of the four motifs recognized by GAL4 in UAS_G.

The enhancing properties of UAS_G in HeLa cells are in all respects indistinguishable from those of the SV40 enhancer, which is typical of higher eukaryotic enhancers (see Introduction). UAS_G stimulates transcription equally well in either orientation relative to the HSV tk or β-globin promoters, and moving it 300 bp further upstream resulted in only a moderate decrease of transcription. In all cases the stimulatory activity of UAS_G on the β-globin promoter was of the same order of magnitude as that achieved by the SV40 enhancer on the same promoter. The observation (Figure 2) that a single 17-mer was more active than the full UAS_G element when in close apposition to the β-globin promoter, but much less active when moved upstream by 300 bp, reflects most probably both the known decrease in activity of enhancers as they are moved away from the activated promoter elements (Wasylyk et al., 1983; Wasylyk et al., 1984; Augereau and Wasylyk, 1984), and the well-known synergism that occurs between the individual motifs that may be present in an enhancer element (Zenke et al., 1986; Schirm et al., 1987; Ondek et al., 1987; Nomiyama et al., 1987; see Introduction for references). In this respect, we note that UAS_G contains four GAL4 binding sites similar to the 17-mer that act synergistically (Giniger and Ptashne, 1988), but that even in our constructions, where UAS_G is in close apposition to the β-globin promoter, the nearest GAL4 binding site is located 80 bp upstream of the 5′ end of this promoter.

Interestingly, a stimulation of transcription was also observed when UAS_G was located downstream of the β-globin gene promoter, although the amount of synthesized RNA was lower than that observed with UAS_G situated at a comparable distance upstream of this promoter (Figure 3). Whether this difference reflects a lower efficiency of activation by a "downstream" UAS_G or a decrease in stability of the modified β-globin RNA is unknown. Previous experiments using yeast promoters in yeast cells have failed to reveal a "downstream effect" of UAS elements, which was thought to reflect a significant difference between yeast UAS and higher eukaryotic enhancer elements (for references, see Struhl, 1987a). Our results indicate that in this respect also, there may not be any intrinsic difference between UAS and enhancer elements.

In common with higher eukaryotic enhancers, UAS_G

or the 17-mer can stimulate transcription from different promoters, i.e., the HSV tk and the β-globin promoters, which contain unrelated upstream elements. Kakidani and Ptashne (1988) show that the unrelated MMTV promoter can also be stimulated by GAL4. Moreover, as in the case of SV40 (Hen et al., 1982; Wasylyk et al., 1983; Wasylyk, et al., 1984) or other mammalian (Wirth and Baltimore, 1987) enhancers, GAL4 can activate transcription controlled by a TATA box in the absence of any upstream promoter element (Figure 4). Note in this respect that a 17-mer dimer located upstream of the Ad2MLP TATA box was approximately 8-fold better than a 17-mer monomer for stimulation of transcription, suggesting again that multimerized 17-mer motifs, like mammalian enhancer motifs, act synergistically in HeLa cells. It is worth mentioning here that the TATA box–less promoter of the housekeeping gene HMG-CoA reductase (Reynolds et al., 1984), bearing a UAS_G approximately 300 bp upstream of the mRNA start site, could also be stimulated by GAL4 in HeLa cells (our unpublished data). This is reminiscent of the property of the SV40 enhancer which can activate transcription controlled by the Sp1-binding GC boxes of the SV40 early promoter, in the absence of any nearby TATA box (Barrera-Saldana et al., 1985; Takahashi et al., 1986).

Finally, the similarity between the action of UAS and higher eukaryotic enhancer motifs is further demonstrated by the observation that a UAS_G 17-mer and the SV40 enhancer can synergistically stimulate transcription from the β-globin promoter in HeLa cells synthesizing GAL4 (Figure 3). A similar synergism between a 17-mer and the glucocorticoid responsive element of MMTV is reported in the accompanying paper by Kakidani and Ptashne (1988).

The Same Activating Regions of the Yeast GAL4 and GCN4 Activators Are Responsible for Enhancement of Transcription in Yeast and in HeLa Cells

As recalled in the Introduction, the DNA-binding and activation functions of GAL4 and GCN4 in yeast are separable. In both cases, the activating regions have been characterized using hybrid proteins containing the DNA binding domain of the E coli repressor LexA and various GAL4 or GCN4 segments, together with an appropriate "reporter" gene bearing the LexA operator. Our results show clearly that the same GAL4 and GCN4 regions that are responsible for activation of transcription in yeast also activate transcription in HeLa cells when fused to a truncated hER (HE15) that contains the DNA binding domain but has little of the activation functions of the receptor (Kumar et al., 1987). It is noteworthy that the GAL4 activating region II, which is quite efficient in yeast (Ma and Ptashne, 1987a), is also very efficient at stimulating transcription from the tk promoter controlled by the vitellogenin ERE when fused to the hER DNA-binding domain [ER-GAL4 (281/768); Figure 5]. That the GCN4 19 amino acid long sequence also stimulates transcription in HeLa cells is even more striking, since this acidic stretch represents only the core of the GCN4 activating region in yeast (Hope and Struhl, 1986). The fact that the chimeric protein containing the hER DNA-binding domain and GAL4 activating region II stimulates ERE-controlled transcription almost as efficiently as the wild-type estrogen receptor (HEO), whereas a truncated hER (HE15) containing the DNA binding domain but not the hormone-binding domain stimulates transcription only very poorly, clearly supports our previous conclusion (Kumar et al., 1987) that the hormone-binding domain of hER is not required for the DNA binding domain to bind to an ERE, but is important for efficient activation of transcription. The observation that the hER DNA binding domain can function when linked to totally unrelated activating regions in chimeric activators should be useful to further characterize this DNA binding domain.

It has been pointed out previously that both the GAL4 and GCN4 activating regions correspond to acidic regions, and several unrelated short acidic regions have been shown to function in yeast as substitutes for the GAL4 activating regions (Ma and Ptashne, 1987b). No obvious sequence homology is apparent when comparing these acidic regions, but the possible presence of an amphipathic α-helix is a feature common to many of them, including the GAL4 region I and GCN4 activating regions (Giniger and Ptashne, 1987). However, the activating region II of GAL4 does not appear to be capable of forming such an amphipathic α-helix (Giniger and Ptashne, 1987). Region E of the hER, which is essential for efficient activation of transcription (see above and Kumar et al., 1987), may also contain a putative α-helix with amphipathic character (amino acids 469 to 486). In this respect, hybrid proteins resulting from the fusion of the GAL4 DNA binding domain and various segments of region E should be useful in identifying the activating domain present in this region of the hER.

Molecular Mechanism of Transcriptional Enhancement in Eukaryotes

The observation that a yeast UAS element and its cognate activator protein can stimulate higher eukaryotic promoters in human HeLa cells with characteristics indistinguishable from those of a higher eukaryotic enhancer element indicates that these elements are functionally identical, and suggests very strongly that the underlying molecular mechanisms of stimulation of transcription are similar in both cases. This suggestion is further supported by the finding that the same activating regions of the yeast activators GAL4 and GCN4 function in yeast and human cells when linked to heterologous DNA binding domains. Based on studies performed with the SV40 enhancer, Takahashi et al. (1986) have suggested that protein factors bound to enhancer elements stimulate transcription by interacting with proteins bound to the other promoter elements. Ptashne (1986) has suggested that DNA-bound GAL4 could stimulate transcription in yeast by a similar mechanism. If this were true, it would follow that the same type of contacts exist in yeast and in HeLa cells between GAL4 and these other proteins. In this respect, it would be interesting to investigate whether there is any stereoalignment requirement for GAL4 to stimulate transcription from higher eukaryotic promoter elements. Since a promoter region composed of a simple TATA box linked to a 17-mer

GAL4 recognition site can still be stimulated by GAL4, it is clear that proteins interacting with higher eukaryotic promoter upstream elements are not necessary targets for GAL4. Protein interactions may thus involve some fundamental components of the transcription machinery, such as the RNA polymerase or those factors that are absolutely required for initiation of transcription, e.g., the TATA box factor (note, however, the stimulation of the TATA box–less HMG-CoA reductase promoter mentioned above). A high degree of conservation of the salient features of these factors during evolution is not inconceivable, since RNA polymerase B (II) itself appears to be well conserved between yeast and higher eukaryotes (see Ahearn et al., 1987 for references). Enhancer factors may also have been conserved during evolution, as suggested by the homology between the yeast GCN4 activator and the oncogene JUN, which appears to be related to the higher eukaryotic enhancer factor AP1 (see Struhl, 1987b and Short, 1987 for references). Further studies with higher eukaryotic enhancer factors are necessary to investigate the possible presence and function of acidic regions similar to those present in the yeast activators. It should be stressed, however, that alternative mechanisms may exist whereby UAS or enhancer elements, together with their cognate factors, may function without directly interacting with components of the transcription machinery (e.g., interaction with the nuclear matrix or modification of chromatin structure; for reviews and references, see Chambon et al., 1984; Wasylyk, 1987; Hatzopoulos et al., 1987). Nevertheless, our results indicate that the mechanisms that are involved in transcriptional enhancer function must be very similar in yeast and man.

Experimental Procedures

Plasmid Constructions

All DNA constructions were performed using standard procedures (Maniatis et al., 1982). The activator plasmids pKCR2-GAL4 and pKCR2-GAL4R were constructed by ligating the 2.9 kb HindIII fragment of pLKC15 (Silver et al., 1984) into the EcoRI site of the expression vector pKCR2 (Breathnach and Harris, 1983) using synthetic HindIII–EcoRI linkers. For reporter plasmids UAS-tk-CAT and UASR-tk-CAT (see legend to Figure 1), the UAS_G of the GAL1–GAL10 divergent promoter was excised as a 365 bp BglII fragment from plasmid pRY24 (Yocum et al., 1984) and inserted in both orientations into the BamHI site of plasmid pBLCAT8$^+$ (Klein-Hitpass et al., 1986). 17M-tk-CAT was constructed by ligation of the HindIII–SmaI fragment of pMH100 (which contains in pUC18 the synthetic "ScaI site" 17-mer 5′-CGGAGTACTGTCCTCCG-3′ [17M]) into the polylinkar of pBLCAT8$^+$ (at −114 with respect to the tk cap site), which had been blunt-ended with Klanow enzyme at the BamHI site and then cut at the HindIII site. A perfect palindromic synthetic 17-mer (17MX) within the following sequence 5′-AGCTTCGGAGGACTGTCCTCCG-3′ was ligated as a dimer into the polylinker HindIII site (−140) of pBLCAT8+ to generate 17MX2-tk-CAT (Figure 1A). To prepare reporter genes for the S1 nuclease analysis (Figure 2A), the 365 bp BglII UAS_G fragment was subcloned in both orientations into the BamHI site of Bluescribe M13$^+$ (Stratagene) to give BSM-UAS and BSM-UASR. UAS_G was excised from BSM-UAS with SmaI and XbaI, and inserted into the SmaI and XbaI sites of the polylinker of plasmid pG1 (Sassone-Corsi et al., 1985) to yield UAS-pG1. For the pG2 (Hen et al., 1986) vectors, the UAS_G fragment was excised from BSM-UASR and BSM-UAS with SacI and XbaI, then ligated into the SacI and XbaI sites of the polylinker of pG2 to give UAS-pG2 and UASR-pG2, respectively (see legend to Figure 2). The "ScaI site" 17-mer (17M) was excised from pMH100 as above and ligated into the HindIII and SmaI sites of pG1 to give 17M-pG1. The remaining reporter genes containing the "ScaI site" 17-mer (17M) were constructed by ligating synthetic oligonucleotides corrasponding to the sequence 5′-CTAGAGGTCGGAGTACTGTCCTCCGACT-3′ into tha polylinker XbaI sites of pG2, pG2B, and TATA-GLOB (see legend to Figure 4) to produce 17M-pG2, 17M-pG2B, 17M-TATA-GLOB, and 17M2-TATA-GLOB, respectively. TATA-GLOB was a kind gift from Dr. J. White. The 3′ reporter gene, pG2-UAS (Figure 3), consists of the 365 bp BglII UAS_G fragmant inserted into the unique BamHI site (+475) of pG2. The chimeric activator plasmid ER-GAL4(281/768) (Figure 5) was constructad by ligating the GAL4 580 bp NarI–HindIII fragment (containing activating region II) from pKCR2-GAL4 into the ClaI–HindIII sites of the hER mutant HE25 (Kumar et al., 1987). For the chimeric activator plasmid ER-GCN4 (281/107-125) (Figure 5), 7 synthetic oligonucleotides corresponding to the 19 amino acid core of the GCN4 activating region (amino acids 107 to 125; see Hope and Struhl, 1986) were "shotgun" ligated into the XhoI–SacI sites of the hER mutant HE22 (Kumar et al., 1987). All constructions were checked both by restriction enzyme analysis and dideoxy sequencing prior to transfection.

HeLa Cell Transfections and CAT Assay

HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum that had been treated with dextran-coated charcoal to remove steroid hormones, as previously described (Kumar et al., 1986). Cells were transfected at 30%–50% confluenca in 9 cm Petri dishes with a total of 20 μg of DNA using the calcium phosphate precipitation technique (Kumar et al., 1986). For the CAT assays, 1 μg of each of the activator and reporter plasmids, together with 4 μg of tha reference RSV-lacZ plasmid (see Bonnerot et al., 1987) and 14 μg of carrier DNA (Bluescribe M13$^-$), were transtected per plate. The precipitate was removed after 24 hr, and the cells refed with medium. When appropriate, estradiol was added to the medium to 10^{-8} M. After a further 24 hr, the cells were harvested, rinsed, lysed by five cycles of freeze-thaw, and centrifuged at 10,000 × g for 20 min. The β-galactosidase activity was measured in the extracts by the method of Herbomel et al. (1984). Extracts corresponding to 5 U of β-galactosidase activity were used for the CAT assay with 0.1 μCi [^{14}C] chloramphenicol (50 mCi/mmol, Amersham) for 1 hr at 37°C, followed by chromatography on 0.2 mm silica gel TLC plates (Merck Kieselgel 60F$_{254}$). For quantification of the results, the CAT assay was run with 0.2 μCi [^{14}C] chloramphenicol at 37°C for 30 min, the acetylated bands excised, and counted in 5 ml scintillant. Undar these conditions, the assay was within the linear range.

RNA Preparation and Quantitative S1 Nuclease Mapping

HeLa calls wera transfected as above with 1 μg of activator plasmid, 1 or 5 μg of reporter plasmid (as indicated in tha legends to the figures), and 0.2 or 0.5 μg of the reference plasmids pAO (Zenke et al., 1986) or pG1B, raspectively. Carrier DNA (as above) was added to 20 μg. Cells were harvested 40–48 hr after transfection (10^{-8} M estradiol was added 24 hr after transfection, when appropriate), and the cytoplasmic RNA isolated by 0.5% Nonidet P40 lysis (Groudine et al., 1981). Single-stranded DNA probes were prepared by primer extension along single-stranded M13 DNA templates containing either the 1142 bp HindIII–DraI fragment from vit-tk-globin (Kumar et al., 1987), the ~1470 bp EcoRI fragment from pAO (Zenke et al., 1986), or the ~1250 bp EcoRI fragment from the Ad2MLP TATA-GLOB plasmid. The primer used was complementary to bases +39 to +60 of the rabbit β-globin gene (Zenke et al., 1986) and was 5′ end-labeled with γ-[^{32}P]ATP prior to extension. The labeled DNA was cut with EcoRI and the single-stranded probes isolated from a 6% sequencing gel by electroalution. For quantitative S1 nuclease analysis (Zenka et al., 1986), 10 μg of RNA was hybridized with 20,000–30,000 cpm of probe in 50% (v/v) formamide/0.4 M NaCl/40 mM PIPES (pH 6.5)/1 mM EDTA overnight at 42°C, then digested with 100 U of S1 nuclease (Appligene) at 25°C for 3 hr in 30 mM NaAc (pH 4.5)/3 mM $ZnSO_4$/300 mM NaCl. The protected fragments were run on a 6% sequencing gel and quantified by densitometric scanning of the autoradiograms exposed for various periods.

Acknowledgments

We are grateful to V. Kumar for gifts of HE15, HE22, and HE25; to L. Keegan, R. Yocum, and M. Ptashne for gifts of LKC15 and pRY24; to G. Ryffel for gifts of pBLCAT8+ and vit-tk-CAT; and to J. White and M. Ponglikitmongkol for TATA-GLOB. We thank F. Ruffenach and A. Staub for oligonucleotide synthesis, E. Scheer for technical assistance, the cell culture group for HeLa cells, C. Werlé and B. Boulay for illustrations, and the secretarial staff for assembling the manuscript. N. W. is supported by a Royal Society European Exchange fellowship. The present work was supported by grants from the INSERM (grants CNAMTS), the CNRS (AIV), the Ministère de la Recherche et de l'Enseignement Supérieur, the Fondation pour la Recherche Médicale, and the Association pour la Recherche sur le Cancer, and by a National Institutes of Health grant (GM32308) to M. Ptashne.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 4, 1987.

References

Ahearn, J. M., Jr., Bartolomei, M. S., West, M. L., Cisek, L. J., and Corden, J. L. (1987). Cloning and sequence analysis of the mouse genomic locus encoding the largest subunit of RNA polymerase II. J. Biol. Chem. *262*, 10,695–10,705.

Augereau, P., and Wasylyk, B. (1984). The MLV and SV40 enhancers have a similar pattern of transcriptional activation. Nucl. Acids Res. *12*, 8801–8818.

Barrera-Saldana, H., Takahashi, K., Vigneron, M., Wildeman, A., Davidson, I., and Chambon, P. (1985). All six GC-motifs of the SV40 early upstream element contribute to promoter activity *in vivo* and *in vitro*. EMBO J. *4*, 3839–3849.

Bonnerot, C., Rocancourt, D., Briand, P., Grimber, G., and Nicolas, J. F. (1987). A β-galactosidase hybrid protein targeted to nuclei as a marker for devalopmental studies. Proc. Natl. Acad. Sci. USA *84*, 6795–6799.

Bram, R. J., Lue, N. F., and Kornberg, R. D. (1986). A GAL family of upstream activating sequences in yeast: roles in both induction and repression of transcription. EMBO J. *5*, 603–608.

Breathnach, R., and Harris, B. A. (1983). Plasmids for the cloning and expression of full-length double stranded cDNAs under control of the SV40 early or lata gane promoter. Nucl. Acids Res. *11*, 7119–7136.

Brent, R., and Ptashne, M. (1985). A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell *43*, 729–736.

Chambon, P., Dierich, A., Gaub, M. P., Jakowlev, S., Jongstra, J., Krust, A., LePannec, J. P., Oudet, P., and Reudelhuber, T. (1984). Promoter elements of genes coding for proteins and modulation of transcription by oestrogens and progestarone. In Recent Progress in Hormone Research, Volume 40, R. O. Greep, ed. (New York: Academic Press), pp. 1–42.

Dynan, W. S. (1987). What mechanisms underlie tissue-spacific gene transcription. Trends Ganet. *3*, 121–122.

Giniger, E., Varnum, S. M., and Ptashne, M. (1985). Specific DNA binding of GAL4, a positive regulatory protein of yeast. Cell *40*, 767–774.

Giniger, E., and Ptashne, M. (1987). Transcription in yeast activated by a putative amphipathic α helix, linked to a DNA binding unit. Nature *330*, 670–672.

Giniger, E., and Ptashne, M. (1988). Cooperative DNA binding of the yeast transcriptional activator, GAL4. Proc. Natl. Acad. Sci. USA *85*, 382–386.

Groudine, M., Peretz, M., and Weintraub, H. (1981). Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. *1*, 281–288.

Hatzopoulos, A. K., Schlokat, U., and Gruss, P. (1987). Enhancers and other cis-acting regulatory sequences. In Eukaryotic RNA Synthesis and Processing, Horizons in Molecular Biology Series, B. D. Hames and D. M. Glober, eds. (Arlington, VA: IRL Press), in press.

Hen, R., Sassone-Corsi, P., Corden, J., Gaub, M. P., and Chambon, P. (1982). Sequences upstream from the TATA box are required *in vivo* and *in vitro* for efficient transcription from the adenovirus-2 major late promoter. Proc. Natl. Acad. Sci. USA *79*, 7132–7136.

Hen, R., Borrelli, E., Fromental, C., Sassone-Corsi, P., and Chambon, P. (1986). A mutated polyoma virus enhancer which is active in undifferentiated embryonal carcinoma cells is not repressed by the adenovirus-2 E1A products. Nature *321*, 249–251.

Herbomel, P., Bourachot, B., and Yaniv, M. (1984). Two distinct enhancers with different cells specificities coexist in the regulatory region of polyoma. Cell *39*, 653–662.

Hope, I. A., and Struhl, K. (1986). Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. Cell *46*, 885–894.

Kekidani, H., and Ptashne, M. (1988). GAL4 activates gene expression in mammalian cells. Cell *52*, 161–167.

Keegan, L., Gill, G., and Ptashne, M. (1986). Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. Science *231*, 699–704.

Klein-Hitpaß, L., Schorpp, M., Wagner, U., and Ryffel, G. (1986). An estrogen-responsive element derived from the 5′ flanking region of the Xenopus vitellogenin A2 gene functions in transfected human cells. Cell *46*, 1053–1061.

Kumar, V., Green, S., Staub, A., and Chambon, P. (1986). Localisation of the oestradiol-binding and putative DNA-binding domains of the human oestrogen receptor. EMBO J. *5*, 2231–2236.

Kumar, V., Graen, S., Stack, G., Berry, M., Jin, J.-R., and Chambon, P. (1987). Functional domains of the human estrogen receptor. Cell *51*, 941–951.

Ma, J., and Ptashne, M. (1987a). Deletion analysis of GAL4 defines two transcriptional activating segments. Cell *48*, 847–853.

Ma, J., and Ptashne, M. (1987b). A new class of yeast transcriptional activators. Cell *51*, 113–119.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Maniatis, T., Goodbourn, S., and Fischer, J. A. (1987). Regulation of inducible and tissue-specific gene expression. Science *236*, 1237–1244.

Martinez, E., Givel, F., and Wahli, W. (1987). The estrogen-responsive element as an inducible enhancer: DNA sequence requirements and conversion to a glucocorticoid-responsive element. EMBO J. *6*, 3719–3727.

McKnight, S., and Tjian, R. (1986). Transcriptional selectivity of viral genes in mammalian cells. Cell *46*, 795–805.

Nomiyama, H., Fromental, C., Xiao, J. H., and Chambon, P. (1987). Coll-specific activity of the constituent elements of the SV40 enhancer. Proc. Natl. Acad. Sci. USA *84*, 7881–7885.

Ondek, B., Shepard, A., and Herr, W. (1987). Discrete elements within the SV40 enhancer region display different cell-specific enhancer activities. EMBO J. *6*, 1017–1025.

Petkovich, M., Brand, N. J., Krust, A., and Chambon, P. (1987). A human retinoic acid recaptor which belongs to the family of nuclear receptors. Nature *330*, 444–450.

Ptashne, M. (1986). Gene regulation by proteins acting nearby and at a distance. Nature *322*, 697–701.

Reynolds, G. A., Basu, S. K., Osborne, T. F., Chin, D. J., Gil, G., Brown, M. S., Goldstein, J. L., and Luskey, K. L. (1984). HMG CoA reductase: a negatively regulated gene with unusual promoter and 5′ untranslated regions. Cell *38*, 275–285.

Sassone-Corsi, P., Duboule, D., and Chambon, P. (1985). Viral enhancer activity in teratocarcinoma cells. Cold Spring Harbor Symp. Quant. Biol. *L*, 747–752.

Serfling, E., Jasin, M., and Schaffner, W. (1985). Enhancers and eukaryotic gene transcription. Trends Genet. *1*, 224–230.

Schirm, S., Jiricny, J., and Schaffner, W. (1987). The SV40 enhancer can be dissected into multiple segments, each with a different cell type specificity. Genes and Development *1*, 65–74.

Short, N. J. (1987). An oncogene caught red-handed? Nature *330*, 209–210.

Silver, P. A., Keegan, L. P., and Ptashne, M. (1984). Amino terminus of the yeast GAL4 gene product is sufficient for nuclear localization. Proc. Natl. Acad. Sci. USA *81*, 5951–5955.

Struhl, K. (1987a). Promoters, activator proteins, and the mechanism of transcriptional initiation in yeast. Cell *49*, 295–297.

Struhl, K. (1987b). The DNA-binding domains of the jun oncoprotein and the yeast GCN4 transcriptional activator proteins are functionally homologous. Cell *50*, 841–846.

Takahashi, K., Vigneron, M., Matthes, H., Wildeman, A., and Chambon, P. (1986). Stereospecific alignments are required for initiation from the SV40 early promoter. Nature *319*, 121–126.

Wasylyk, B. (1987). Transcription elements and factors of RNA polymerase B promoters of higher eukaryotes. CRC Crit. Rev. Biochem., in press.

Wasylyk, B., Wasylyk, C., Augereau, P., and Chambon, P. (1983). The SV40 72 bp repeat preferentially potentiates transcription starting from proximal natural or substitute promoter elements. Cell *32*, 503–514.

Wasylyk, B., Wasylyk, C., and Chambon, P. (1984). Short and long range activation by the SV40 enhancer. Nucl. Acids Res. *12*, 5589–5608.

Wirth, T., Staudt, L., and Baltimore, D. (1987). An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity. Nature *329*, 174–178.

Yamamoto, K. R. (1985). Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. *19*, 209–252.

Yocum, R. R., Hanley, S., West, R., Jr., and Ptashne, M. (1984). Use of lacZ fusions to delimit regulatory elements of the inducible divergent GAL1–GAL10 promoter in Saccharomyces cerevisiae. Mol. Cell. Biol. *4*, 1985–1998.

Zenke, M., Grundström, T., Matthes, H., Wintzerith, M., Schatz, C., Wildeman, A., and Chambon, P. (1986). Multiple sequence motifs are involved in SV40 enhancer function. EMBO J. *5*, 387–397.