Regardless of the funds that may be allocated to the development and application of new methods of teaching, it seems obvious that children's success in mathematics and other subjects will depend on greater awareness and an increased willingness by American parents to be of direct assistance to their children. Schools may be improved, but the task of helping children reach higher levels of achievement cannot be accomplished without more cooperation and communication between the school and the home. Further, without greater acknowledgement of the importance of the elementary school years to children's education in mathematics and science, legislation to improve instruction in secondary schools may result in little more than exercises in remediation for most children.

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7. Statistical analyses of the achievement tests indicated good reliability. Tests of the reliability of the mathematics test yielded values that ranged from 0.92 to 0.95 when the Cronbach α statistic was computed separately by grade and country. The coefficients of concordance for the three parts of the reading test ranged from 0.91 to 0.94 when computed separately for each country. Results for standardized tests of mathematics and reading were not obtained for purposes of comparison with our tests. The standardized achievement tests, if available, were not comparable among the three countries; the results often were not current; and they were group tests, rather than individually administered tests such as those used in this study.
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Research Articles

Separation of DNA Binding from the Transcription-Activating Function of a Eukaryotic Regulatory Protein

LIAM KEEGAN, GRACE GILL, MARK PTASHNE

The yeast GAL4 protein (881 amino acids) binds to specific DNA sites upstream of target genes and activates transcription. Derivatives of this protein bearing as few as 74 amino terminal residues bind to these sites but fail to activate transcription. When appropriately positioned in front of a gene these derivatives act as repressors. These and related findings support the idea that GAL4 activates transcription by touching other DNA-bound proteins.

TRANSCRIPTION OF MANY EUKARYOTIC GENES DEPENDS ON DNA sites located far from the gene (*1*). For example, midway between the divergently transcribed *GAL1* and *GAL10* genes of yeast, about 250 bp from each, is a region called the *GAL* upstream activation site (UAS_G). The UAS_G is essential for the transcriptional activation of both genes by GAL4 protein (GAL4) (*2–8*). If the UAS_G is placed as far as 600 bp from the transcription start site of a *GAL* gene, or of a heterologous gene, GAL4 activates transcription of that gene (*9*).

On the basis of chemical probe experiments on intact cells, Giniger *et al.* (*10*) have argued that GAL4 binds to four related 17-bp sequences in UAS_G. One synthetic 17-bp sequence is sufficient to mediate significant GAL4-dependent activation of downstream genes. This sequence is a near-consensus of the natural sites, and is called (for convenience) the 17mer (*11*). Bram and Kornberg (*12*) have shown that GAL4 binds to specific sites in UAS_G.

How does a DNA-bound protein such as GAL4 activate transcription at a site several hundred base pairs away? Specifically, is the binding of GAL4 sufficient for upstream activation? Here we show (i) that GAL4 binds to the UAS_G and to the 17mer in vitro, and (ii)

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that just the first 74 amino acids of GAL4 protein fused to β-galactosidase (β-gal), direct binding to the 17mer and to four sites in UAS_G. (iii) We show that several GAL4 derivatives bearing 74 or more amino terminal residues of GAL4 fail to activate transcription in vivo. Instead, (iv) these GAL4 derivatives repress transcription, when bound to an appropriately positioned site, blocking activation from a heterologous UAS. We conclude that DNA binding of GAL4 protein per se does not activate transcription.

Binding of GAL4 and GAL4 derivatives in extracts to specific DNA sequences. We produced GAL4 in *Escherichia coli* to about 0.01 to 0.1 percent of total cellular protein, as measured by Western blot (immunoblot) analysis (Fig. 1 and legend) (*13*). Extracts of these *E. coli* cells contain a UAS_G-specific DNA binding activity detected by a nitrocellulose filter binding assay (Fig. 2) (*12, 14*). Plasmid DNA bearing the synthetic GAL4 binding site (the 17mer) was also specifically bound by these extracts, but the same plasmid

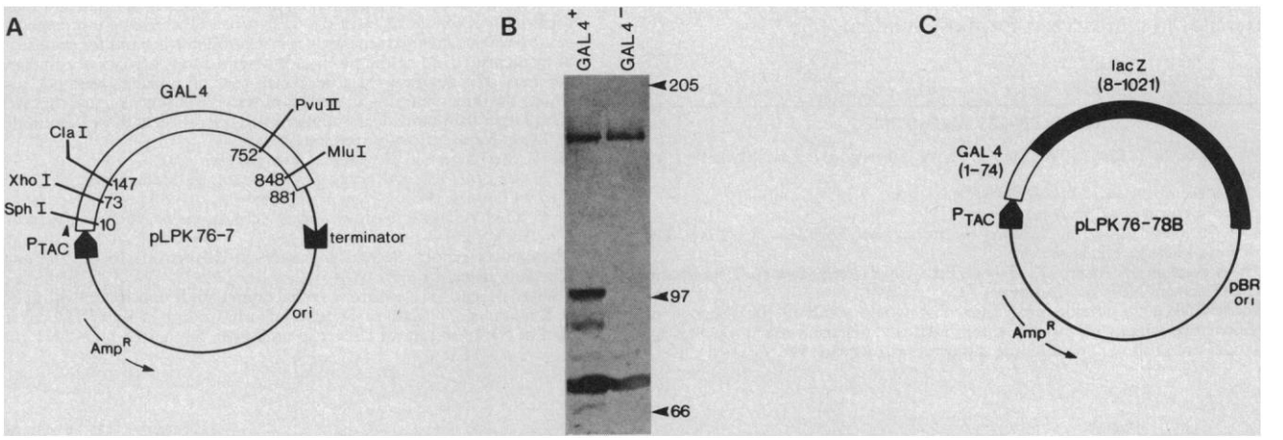


Fig. 1. Production of GAL4 and GAL4-β-gal fusion proteins in *E. coli*. (A) A plasmid that directs synthesis of GAL4 from the *tac* promoter in *E. coli* (*13*). The second codon of *GAL4* has been joined to the *E. coli lac* translation initiation codon (*27*). Sites are shown in *GAL4* at which amino terminal GAL4 coding sequences have been fused in other constructs to *E. coli lacZ*. The number below each site denotes the codon at which it occurs. (B) Detection of GAL4 protein in total cell lysates of *E. coli* with an antiserum to GAL4 (Western blot) (*8*). Total cell lysates, prepared from cells bearing pLPK76-7 ($GAL4^+$) or its parent plasmid lacking *GAL4* ($GAL4^-$), were resolved by electrophoresis through a 6 percent polyacrylamide–sodium dodecyl sulfate (SDS) gel and transferred to nitrocellulose. GAL4 was then visualized, with the use of rabbit antiserum to GAL4, and horseradish peroxidase–coupled goat antiserum to rabbit immunoglobulin G. (C) The plasmid that directs synthesis of the GAL4(1–74)-β-gal fusion protein in *E. coli*. All plasmids producing GAL4 derivatives were maintained in the bacterial strain SF1148 [Δ(*lac pro*) X_{111} *rpsE thi* val^R/*F' lac* ^{Q1}*lacZ*::*Tn5*], and protein production was induced as described (*13*).

Fig. 2. Binding of GAL4 in *E. coli* extracts to specific DNA sites shown by the nitrocellulose filter assay (*12*, *14*). (A) Retention of DNA fragments bearing or lacking (UAS_G) by extracts of *E. coli* cells that produce ($GAL4^+$) or do not produce ($GAL4^-$) the yeast activator protein (*28*). The binding reactions were filtered through a nitrocellulose membrane and the retained DNA (^{32}P-labeled) was measured. The purified DNA fragments were pBR + UAS_G, cleaved and ^{32}P-labeled at the Eco RI site, and its backbone fragment labeled at Bgl II. On the horizontal axis is indicated the amount of total extract protein present in each 20-μl binding reaction. Retention is expressed as the percentage of the total ^{32}P in each reaction that was retained on the filter. Retention of the labeled fragement in the absence of extract was always less than 5 percent. (B) Retention of two purified DNA fragments that differ only in that one bears and the other does not bear the synthetic GAL4 site-of-action (the 17mer), in the assay described above. The DNA fragments were pUC18 + 17mer and pUC18, cleaved, and ^{32}P-labeled at their only Hind III sites.

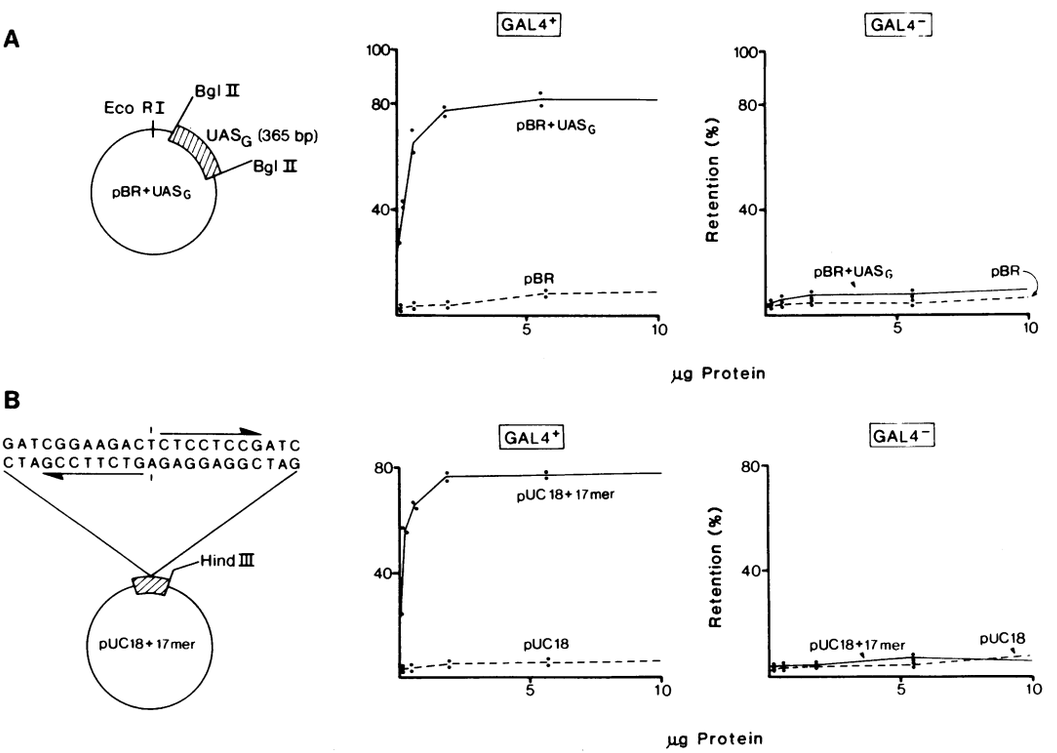


Table 1. Transcriptional activation of a *GAL1-lacZ* hybrid gene by GAL4 derivatives produced in yeast. Plasmids that produce different GAL4 derivatives have been introduced into two yeast strains that differ only in that one (YM335::171) contains, and the other (YM335) does not contain the *GAL1-lacZ* hybrid gene (*30*). For each GAL4 derivative, the difference between the β-galactosidase activity in YM335::171 and that in YM335 is a measure of the degree of transcriptional activation of the *GAL1* promoter.

GAL4 derivative	Yeast strain	
	YM335::171	YM335
GAL4 (pGG2)	1300	0
GAL4(1–848)-β-gal (pGG16)	200	5
GAL4(1–753)-β-gal (pGG13)	15	15
GAL4(1–147)-β-gal (pGG14)	20	20
GAL4(1–74)-β-gal (pGG15)	5	5
GAL4(1–98) (pGG17)	0	0
No GAL4 (pGG1)	0	0

DNA lacking the 17mer insert was not. Control extracts from otherwise isogenic cells that lacked GAL4 did not contain this binding activity.

We also produced in *E. coli* four proteins having 74, 147, 753, or 848 amino terminal residues of GAL4 fused to *E. coli* β-galactosidase (GAL4-β-gal fusions) (Fig. 1C). UAS_G-specific binding activity was detected, by the nitrocellulose filter binding assay, in extracts of *E. coli* cells containing any one of these fusion proteins. Extracts of cells that produced either native β-galactosidase, or a β-gal-GAL4 fusion protein bearing carboxyl terminal residues 147 to 881 of GAL4 fused to the amino terminus of β-gal, did not bind UAS_G in this assay (Fig. 3).

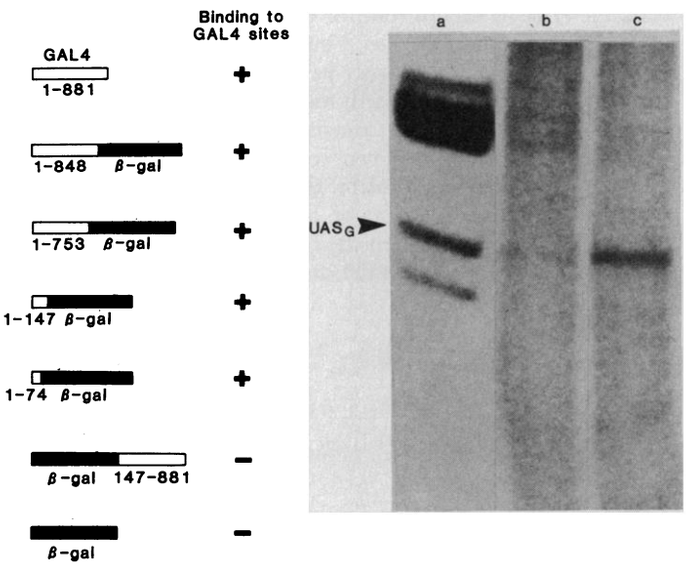


Fig. 3 (left). Summary of filter binding assays on extracts of *E. coli* cells that produce different GAL4 derivatives. Assays were carried out as in Fig. 2B. The positive (+) and negative (−) results were essentially like those observed in the experiments of Fig. 2 (*28*). As controls, extracts of cells producing GAL4 or β-galactosidase alone were prepared and assayed side by side with each test extract. Fig. 4 (right). Precipitation of a specific GAL4-β-gal-DNA complex from *E. coli* extracts with antiserum to β-galactosidase. (Lane a) Hae III digest of pBR + UAS_G. The 306-bp fragment bearing UAS_G is indicated. For the other lanes in this experiment crude extract (50 μl) containing the GAL4(1–147)-β-gal fusion protein was first precipitated, with antiserum to λ-repressor + *Staphylococcus* A in one case and with antiserum to β-galactosidase + *Staphylococcus* A in the other. The precipitates were resuspended and a Hae III digest of pBR + UAS_G was added. The mixtures were centrifuged, the pellet was washed, and the bound DNA was recovered and resolved by gel electrophoresis. (Lane b) Immunoprecipitation with antiserum to λ-repressor. (Lane c) Immunoprecipitation with antiserum to β-galactosidase.

Two additional experiments support the conclusion that the first 74 amino acids of GAL4 are responsible for the DNA-binding activity observed in our extracts. First, prior treatment of extracts containing the GAL4(1–147)-β-gal fusion protein with antiserum to β-galactosidase eliminated all UAS_G-specific DNA-binding activity detectable by the filter binding assay. As expected, the antiserum to β-galactosidase had no effect on the DNA-binding activity of extracts bearing intact GAL4. Second, antiserum to β-galactosidase specifically precipitated DNA fragments bearing UAS_G from a mixture of DNA fragments incubated with extract containing the GAL4(1–147)-β-gal fusion protein (*15*) (Fig. 4). No DNA fragment was precipitated preferentially by an antiserum to λ repressor (Fig. 4).

Binding of purified GAL4-β-gal fusion proteins to specific DNA sequences. A fusion protein [GAL4(1–74)-β-gal], having only the first 74 amino acids of GAL4 fused to β-gal, bound specifically to DNA containing the 17mer, as assayed by protection of this sequence from digestion by deoxyribonuclease I (DNase I) (*16*). As is shown in Fig. 5A, a 50 percent pure preparation (*17*) of this protein protected a 27-bp segment of DNA roughly centered on the 17mer. The protected region extends 4 bp 5′ to the 17mer and 6 bp 3′ to it along one DNA strand. A similar preparation of the

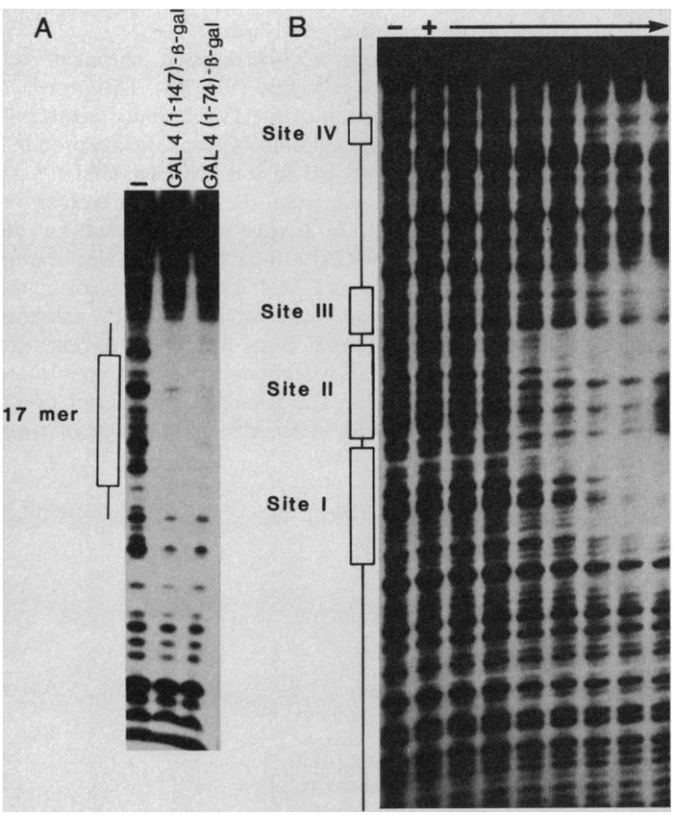
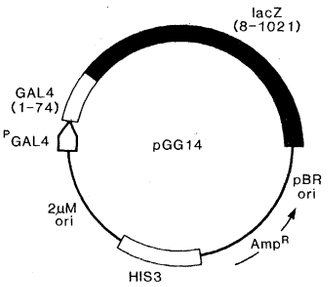


Fig. 5. Protection of synthetic and natural GAL4 binding sites from DNase I cleavage by purified GAL4-β-gal fusion proteins. (A) DNAse I cleavage pattern of a DNA fragment bearing the 17mer ($10^{-10}M$), in the absence (−), or presence of purified fusion proteins at 10^{-7} to $10^{-8}M$ binding activity (*29*). The DNA fragment, derived from pUC18 + 17mer, is ^{32}P-labeled at an Eco RI site 20 bp from the 17mer. (B) DNase I cleavage pattern of a DNA fragment ($10^{-10}M$) bearing (UAS_G) in the absence (−) or in the presence (+) of purified GAL4(1–147)-β-gal fusion protein. The boxes on the left indicate the positions of the GAL4 binding sites proposed by Giniger *et al.* Protein concentrations increase rightward in twofold steps from $10^{-9}M$ binding activity. The DNA fragment used here is derived from pBR + UAS_G and is 3′ end-labeled at the Cla I site of pBR322 (which is 60 bp from site I).

Fig. 6. The plasmid that directs synthesis of the GAL4(1–74)-β-gal fusion protein in yeast (*30*).



GAL4(1–147)-β-gal fusion protein behaved identically in this experiment.

Protection of UAS_G from DNase I digestion by the GAL4(1–147)-β-gal protein is shown in Fig. 5B. The protection pattern is consistent with the idea that GAL4 recognizes the four sites in UAS_G identified by Giniger *et al.* (*10*), and is similar to that observed by Bram and Kornberg (*12*). At low-protein concentrations a 27-bp segment was protected which was centered on site II of Giniger *et al.* With increasing protein concentrations, two additional sequences were protected, one on either side of site II. The boundaries of the protected region are as expected if the protein fills three adjacent sites (first II, then I and III), each of which is centered on a sequence related to the 17mer.

At low-protein concentrations, a 27-bp segment, separated from site II by some 60 bp, was also protected (Fig. 5B). This protected region is centered on the position of site IV. A similar protection pattern was observed with the GAL4(1–74)-β-gal fusion protein.

Positive control by GAL4-β-gal fusion proteins. GAL4-β-gal fusion proteins were expressed from the GAL4 promoter on multicopy plasmids (Fig. 6), in a yeast strain deleted for the chromosomal *GAL4* gene. The GAL4-β-gal fusion proteins bearing 753, 147, or 74 amino acids of GAL4 did not complement the chromosomal Δ*gal4* mutation for anaerobic growth on galactose minimal plates. The GAL4(1–848)-β-gal fusion did complement the Δ*gal4* mutation in this assay although the cells grew more slowly than did wild-type cells. The failure of the shorter fusion proteins to complement Δ*gal4* is not due to a failure of these proteins to enter the nucleus, since Silver *et al.* (*8*) have shown that these fusion proteins are specifically localized to the nucleus in yeast.

In order to measure more sensitively any activation of *GAL1* transcription by these GAL4 derivatives a *GAL1-lacZ* hybrid gene with an intact *GAL1* promoter (*4*) was integrated into the chromosome of the Δ*gal4* strain. Fusion proteins bearing 753, 147, or 74 amino acids of GAL4 evidently failed to activate transcription of the *GAL1-lacZ* hybrid gene (Table 1). In each case the residual β-galactosidase activities observed could be accounted for entirely by the GAL4-β-gal fusion proteins themselves (Table 1).

A small amino terminal portion of GAL4, GAL4(1–98), also failed to activate *GAL1-lacZ* transcription in this assay. The GAL4(1–98) derivative was expressed at high levels from the yeast *ADH1* promoter and, unlike the GAL4-β-gal fusion derivatives, it did not itself contribute β-galactosidase activity that might have obscured a very weak activation of the *GAL1-lacZ* gene. The GAL4(1–848)-β-gal fusion protein activated transcription of the *GAL1-lacZ* hybrid gene, although less efficiently than did GAL4 protein.

Negative control by GAL4-β-gal fusion proteins. The GAL4-β-gal fusion proteins that were deficient in transcriptional activation of the *GAL1* promoter did nevertheless bind specifically to GAL4 binding sites in vivo. This conclusion follows from an experiment based on the observation of Brent and Ptashne (*18*) that a prokaryotic repressor, LexA, produced in yeast, blocks transcription of a gene if a *lexA* operator is placed between the gene and its UAS. We reasoned that a GAL4 derivative able to bind to its recognition site but defective in positive control would also act as a negative regulator under similar circumstances.

To test for specific binding of GAL4 derivatives in vivo, we first place the *HIS4* UAS upstream of a *GAL1-lacZ* hybrid gene from which UAS_G had been deleted. The 89-bp DNA fragment that we call UAS_H has been shown to have all the upstream sequences necessary for transcriptional activation of *HIS4* under conditions of histidine starvation (*19*). It contains three copies of a consensus sequence that has been shown to be the binding site for the transcriptional activator protein GCN4 (*20*). As expected, the UAS_H activated transcription of the *GAL1-lacZ* gene. We then inserted two 17mers between the *HIS4* and *GAL1* sequences and integrated the construct into the chromosome of the Δ*gal4* strain.

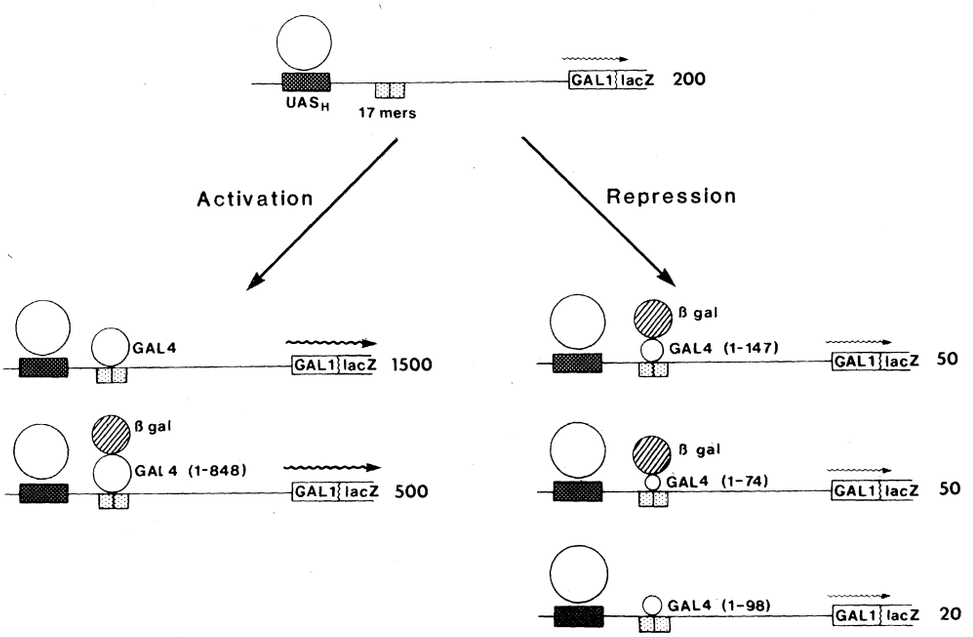


Fig. 7. Summary of transcriptional activation and repression by GAL4 and GAL4 derivatives produced in yeast. The figure shows the effect of different GAL4 derivatives on the expression of a *GAL1-lacZ* fusion gene whose UAS_G has been replaced by the UAS_H, which ordinarily controls transcription of the *HIS4* gene. Starvation for histidine stimulates transcription to the level shown. The construct, in yeast strain YM335::GG9 (*30*), also contains two 17mers between UAS_H and the transcription start. GAL4 binds to these sites and stimulates transcription and the GAL4 derivatives either stimulate or repress transcription by interfering with activation by UAS_H. In this experiment only ~50 bp separate the 17mer from UAS_H on one side and from the *GAL1 TATA* sequence on the other.

In the absence of GAL4 protein or its derivatives, the inserted 17mers had no effect on the transcription of this gene. GAL4 activated transcription of the *GAL1* promoter in this construct, as indicated by the large increase in total β-galactosidase activity (Fig. 7). The GAL4(1–848)-β-gal fusion protein also activated transcription but less efficiently than did GAL4 (Fig. 7).

In contrast to the result with intact GAL4 protein, the GAL4(1–147)-β-gal and GAL4(1–74)-β-gal fusion proteins not only failed to increase total β-galactosidase activity but actually decreased it. The β-galactosidase activity contributed by the fusion proteins themselves (Table 1) has not been subtracted and therefore the approximately fourfold decrease in total β-galactosidase activity shown in Fig. 7 is an underestimate of the degree of repression. In the absence of any inserted 17mers these fusion proteins had no effect in this experiment, an indication that specific DNA binding was required for repression. A twofold decrease in total β-galactosidase activity was observed for these two fusion proteins with a single inserted 17mer. For reasons that we do not understand, the GAL4(1–753)-β-gal protein did not exhibit the negative regulatory effect of the shorter fusion proteins, even though it did bind specifically in vitro, and is specifically localized to the nucleus in vivo (*8*).

Also shown in Fig. 7 is the result of an experiment in which the GAL4(1–98) fragment was expressed from the *ADH1* promoter in cells containing the UAS_H-17mers-*GAL1-lacZ* construct. In this case, the factor of repression was tenfold. In the absence of 17mers, the GAL4(1–98) fragment had no effect on the level of β-galactosidase activity.

DNA binding and gene activation. The results presented here complement and extend those of Brent and Ptashne (*11*), who replaced the first 74 amino acids of GAL4 with the DNA-binding domain of a bacterial repressor, LexA. This LexA-GAL4 fusion protein, when synthesized in yeast, activated transcription of a gene placed downstream from a *lexA* operator. The LexA-GAL4 hybrid did not activate transcription from UAS_G, implying that the DNA binding specificity of GAL4 had been changed without destroying its activation function. Here, we have shown the converse, namely that GAL4 derivatives bearing various amino terminal portions of the protein recognize GAL4 binding sites in DNA but lack the activation function.

Our experiments show that the first 74 of the 881 amino acids of GAL4 determine its DNA-binding specificity (*21*). GAL4 derivatives bearing as few as 74 amino terminal amino acids of GAL4 fused to β-galactosidase bound to UAS_G in vitro, and the DNase I protection pattern observed was consistent with the proposal of Giniger *et al.* (*10*), that four sites are recognized. Moreover these derivatives bound specifically in vivo, as shown by the fact that, when the GAL4 binding site was appropriately positioned, two of these derivatives of GAL4 acted as repressors. Similar results were obtained with a fragment consisting of the first 98 amino acids of GAL4.

The bacteriophage λ repressor activates transcription of its own gene when bound to a site that is immediately upstream of its promoter, P_{RM} (*22*). Positive control mutants of λ repressor bind normally to the repressor binding sites but fail to stimulate transcription from P_{RM} (*23*). These mutants lie on a surface of repressor that, on the basis of other experiments, could touch RNA polymerase bound to P_{RM}. The properties of these mutants argue against the idea that positive control results from an alteration in DNA conformation caused by λ repressor binding. The derivatives of GAL4 that bind to upstream activation sequences but are defective in the transcriptional activation function are analogous to the positive control mutants of λ repressor.

Our results are consistent with the idea that transcription-activation by GAL4, like transcription-activation by λ repressor involves contacts between DNA binding proteins (*16*, *23*), the role of sequence-specific DNA binding being simply to place GAL4 on the DNA in the vicinity of the promoter. These findings argue against any model of upstream activation that proposes that binding of the activator protein per se creates or stabilizes an unusual DNA structure, which is then recognized as an entry site by RNA polymerase (*24*). Our experiments do not exclude the possibility that a part of GAL4, not required for specific sequence recognition, changes DNA structure and that somehow this change stimulates transcription. We consider this possibility remote (*25*).

We prefer the idea that GAL4, bound to an upstream activation site, interacts with some other protein bound near the transcription start site, the intervening DNA being looped out or combined with other proteins (*26*). According to this view our DNA-bound GAL4 derivatives function as repressors either by excluding binding of some other protein, or by hindering formation of complexes between proteins bound to separated sites.

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21. Inspection of the sequence of the first 74 amino acids of GAL4 does not reveal an obvious homology to the helix-turn-helix unit used by some DNA-binding proteins to recognize specific DNA sequences [R. T. Sauer, R. R. Yocum, R. R. Doolittle, M. Lewis, C. O. Pabo, *Nature* (*London*) **298**, 447 (1982); R. P. Wharton and M. Ptashne, *ibid.* **316**, 601 (1985); J. E. Anderson, M. Ptashne, S. C. Harrison, *ibid.*, p. 596]. This sequence does, however, contain a lysine-rich, six-cysteine motif that is also present near the amino termini of certain other yeast-positive control proteins (PPRI and ARGRII) (P. Messenguy, *Eur. J. Biochem.*, in press). Cysteine–lysine–arginine–rich regions have been noted in some other DNA-binding proteins, such as human glucocorticoid receptor [C. Weinberger *et al.*, *Nature* (*London*) **318**, 670 (1985)] and in the *Xenopus laevis* 5S RNA gene transcription factor TFIIIA, where nine repeats of such a region are thought to be DNA-binding structures, each folded around a zinc atom [J. Miller, A. D. McLachlan, A. Klug, *EMBO J.* **4**, 1609 (1985)].
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27. pLPK76-7 was constructed, in two steps, from pRW76 [R. Wharton, thesis, Harvard University (1985)], which provides an Nco I site at the *lac* translation initiation codon. First, the carboxyl terminal part of *GAL4*, from the Sph I site at codon 10 to the Hind III site downstream of the coding sequence, was inserted

downstream of the *tac* promoter in pRW76. Then the region between the Nco I and Sph I sites was replaced by an oligonucleotide encoding *GAL4* sequences from codon 2 to the Sph I site, to yield pLPK76-7. The transcriptional terminator was derived from the P22 phage *ant* gene. The β-gal-GAL4(147–881) fusion is formed at the Eco RI site 17 codons from the end of *E. coli lacZ*, and is expressed from the *lac* promoter.

28. Soluble extracts for filter-binding assays were prepared from cells from 250-ml cultures of SF1148/pLPK76-7 (GAL4$^+$) and SF1148/pRW76 (GAL4$^-$); the cells were resuspended in 2 ml of buffer A(200) [buffer A, as modified from Bram and Kornberg (*9*), is 25 m*M* Hepes, *p*H 7.5; 5 m*M* $MgCl_2$; 0.1 m*M* EDTA; 5 m*M* 2-mercaptoethanol; 10 percent glycerol; 1 m*M* phenylmethylsulfonyl fluoride; 2 μ*M* pepstatin; 0.6 μ*M* leupeptin with KCl at the concentration given in parentheses)] and lysed by sonication below 10°C. The protein content of the cleared lysates was determined by the protein-dye binding method of M. M. Bradford [*Anal. Biochem.* 72, 248 (1976)], with bovine serum albumin as standard, and was typically ~15 mg/ml. In filter-binding experiments with pUC18 and pUC18 + 17mer DNA's we included 5 m*M* IPTG (isopropyl-β-thio-D-galactopyranoside) to reduce binding of *lac* repressor present in the extract to the *lac* operator on these plasmids. We estimated that the affinity of the GAL4-β-gal fusions for GAL4 binding sites was not more than tenfold lower than that of intact GAL4.

29. DNase I protection experiments were carried out in buffer A(50) containing sheared salmon testis DNA (50 μg/ml). Conditions for the footprinting experiments were established as follows: Filter-binding curves of the type shown in Fig. 2B were carried out at 17mer fragment concentrations from 10^{-13} to 10^{-8} *M*. The GAL4(1–147)-β-gal fusion concentration required for half-maximal retention was independent of 17mer concentration when this was at or below 10^{-10} *M*, but shifted up threefold at 10^{-9} *M* 17mer. Therefore, at the 17mer concentration used in the footprinting experiments (10^{-10} *M*), the ratio of specifically bound to free 17mer is independent of the 17mer concentration [A. D. Johnson, C. O. Pabo, R. T. Sauer, *Methods Enzymol.* 65, 839 (1980)]. The specific-binding activity of different preparations is now routinely quantitated by measuring the retention of 17mer by an appropriate dilution of the preparation under conditions of stoichiometric complex formation, at 10^{-8} *M* 17mer. For GAL4 and GAL4 derivatives, the concentration required to half-maximally occupy the 17mer is lower than that required to half-maximally occupy any of the natural sites in UAS_G.

30. The plasmid has been adapted for propagation in histidine-selective media, by replacement of LEU2 in a construct described in Silver *et al.* (*8*), with a *HIS3* gene. The plasmid pGG17 encodes GAL4(1–98) expressed from the *ADH1* promoter. The parental yeast strain in these experiments was YM335 (*a Δ-gal4 ura3-52 ade2-101 lysl his3 met*) obtained from M. Johnston [M. Johnston and R. Davis, *Mol. Cell. Biol.* 4, 1440 (1984)]. The plasmid pRY171 (*4*), which bears a *GAL1-lacZ* fusion but no 2 μ*M* origin of replication, was linearized at its only Apa I site in *URA3* [T. Orr-Weaver, J. Szostak, R. Rothstein, *Proc. Natl. Acad. Sci. U.S.A.* 78, 6354 (1981)], and introduced into cells of the YM335 strain to yield YM335::171. The YM335::GG9 recombinant was constructed as follows: The intact *HIS4* UAS (*18*), extending from 109 to 198 bp upstream of the *HIS4* transcription start site, was inserted, in its normal orientation, 128 bp upstream of the GAL1 start site, (in pLR1Δ20) (*5*). Two copies of a 30-bp DNA fragment containing the 17mer were then installed between the *HIS4* and *GAL1* sequences. The construct was then chromosomally integrated in YM335 (legend to Table 1). Plasmids (Fig. 6) that produce *GAL4*-β-gal fusion proteins were then introduced into these strains by the lithium acetate procedure [H. Ito, Y. Fukuda, K. Murata, A. Kimura, *J. Bacteriol.* 53, 163 (1983)]. Individual transformants were grown to 2 × 10^7 cell/ml in minimal medium lacking histidine [F. Sherman, G. Fink, J. Hicks, Eds., *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, rev. ed., 1983)], containing, 2 percent galactose, 3 percent glycerol, and 2 percent lactate as sodium lactate, *p*H 6. The β-galactosidase assays were performed in triplicate on cultures grown from separate transformant colonies. The standard errors were <20 percent.

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Crystal Structure of Cd,Zn Metallothionein

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The anomalous scattering data from five Cd in the native protein were used to determine the crystal structure of cadmium, zinc (Cd,Zn) metallothionein isoform II from rat liver. The structure of a 4-Cd cluster was solved by direct methods. A 2.3 Å resolution electron density map was calculated by iterative single-wavelength anomalous scattering. The structure is folded into two domains. The amino terminal domain (β) of residues 1 to 29 enfolds a three-metal cluster of one Cd and two Zn atoms coordinated by six terminal cysteine thiolate ligands and three bridging cysteine thiolates. The carboxyl terminal domain (α) of residues 30 to 61 enfolds a 4-Cd cluster coordinated by six terminal and five bridging cysteine thiolates. All seven metal sites have tetrahedral coordination geometry. The domains are roughly spherical, and the diameter is 15 to 20 Å; there is limited contact between domains. The folding of α and β is topologically similar but with opposite chirality. Redundant, short cysteine-containing sequences have similar roles in cluster formation in both α and β.

Metallothioneins are low molecular weight, cysteine-rich proteins that bind metal ions (*1–4*). Metallothioneins and their genes have several potential kinds of physiological activity in that (i) the genes are induced by metal ions and glucocorticoid hormones; (ii) transcription is modulated during embryonic development; (iii) the genes may be involved in control of cell differentiation and proliferation; (iv) the proteins may function to activate Zn requiring apo-enzymes and regulate cellular metabolism; and (v) the proteins may act as free-radical scavengers, and they are synthesized in response to ultraviolet (UV) radiation (*5*). The metallothionein primary sequences are highly conserved, especially at positions of cysteine residues (*1, 6, 7*).

Metallothionein isoform II (MT II) from rat liver contains 61 amino acids including 20 cysteines (*7*). This molecule can be cleaved into two domains: residues 1 to 29 (denoted β, and having 9 cysteines) and residues 30 to 61 (denoted α, with 11 cysteines) (*8*). Nuclear magnetic resonance (NMR) with ^{113}Cd spectra demonstrate that Cd is bound to the protein in clusters of four and three

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