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# A *GAL* family of upstream activating sequences in yeast: roles in both induction and repression of transcription

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**Binding sites for the *GAL4*-positive regulatory protein have been identified upstream of six galactose-inducible genes of *Saccharomyces cerevisiae* on the basis of (i) protection in DNAse I footprints, (ii) loss of protection when excess *GAL4*-binding oligonucleotide is added and (iii) homology with a 23-bp dyad-symmetric consensus sequence. Many of the binding sites have been shown to function as upstream activating sequences. The number of binding sites upstream of the various genes ranges from one to four, but a feature is conserved: in cases of multiple sites there is a pair with highest binding affinity located at dyad–dyad distances of 82–87 bp. We suggest that a pair of sites facilitates repression by the *GAL80*-negative regulatory protein, on the basis of (i) a correlation of a pair of sites (or only one) with full (or only partial) repression and (ii) the introduction of a second site abolishing transcription occurring with one.**

*Key words: GAL4/GAL80*/upstream activating sequence/DNase I footprint/yeast

## Introduction

Upstream activating sequences (UASs) are regulatory elements in yeast capable of inducing transcription at variable distances up to several hundred base pairs from a promoter (Guarente, 1984). Current interest in UASs is centered around their mechanism of action at a distance and possible relationship to enhancers in higher organisms. The physiological significance of UASs lies in their role in the coordinate regulation of genes that may be widely dispersed in the genome and transcribed to different extents.

An especially well characterized example of a UAS, located between the *GAL1* and *GAL10* genes of *Saccharomyces cerevisiae*, is responsible for the induction of both genes from $<0.1$ to $>50$ transcripts/cell upon addition of galactose to the growth medium (St. John and Davis, 1981; Guarente *et al.*, 1982; Johnston and Davis, 1984). Induction in galactose and the lack of transcription in its absence depend on protein products of the *GAL4* and *GAL80* genes (Douglas and Hawthorne, 1972; Matsumoto *et al.*, 1980; Oshima, 1982), presumably through interaction of the proteins with the UAS and with one another. An activity has been partially purified from extracts of a *GAL4* overproducer that binds specifically to *GAL1–10* UAS DNA (Bram and Kornberg, 1985). A *GAL4* protein–UAS DNA complex is formed, as shown by its precipitation by antibodies against a *GAL4–URA3* fusion polypeptide (Buchman and Kornberg, in preparation). DNase I footprints reveal two 30-bp sites of *GAL4* binding ~55 bp apart in the UAS. A similar footprint is obtained with a region of DNA upstream of the *GAL7* gene, whose regulation is identical to that of the *GAL1* and *GAL10* genes. The binding sites in the two footprints are closely homologous in sequence but the spacers between the sites are not. Others have presented evidence for *GAL4* binding at these and additional sites *in vivo* (Giniger *et al.*, 1985). Single sites and also the entire *GAL7*-flanking region have been inserted upstream of other promoters and shown to activate their transcription (Lorch and Kornberg, 1985; Giniger *et al.*, 1985). Thus *GAL4* binding is indicative of UAS function *in vivo*.

Why do both the *GAL1–10* and *GAL7* UASs contain multiple *GAL4*-binding sites with a conserved spacing? We have approached this question by investigating the pattern of sites upstream of the *GAL2*, *GAL80* and *MEL1* genes, which are also inducible by galactose in a *GAL4*-dependent manner (Kew and Douglas, 1976; Moreno *et al.*, 1979; Shimada *et al.*, 1984). *GAL80* and *MEL1* differ from other members of the *GAL* family of genes in being transcribed to an appreciable extent in the absence of galactose and induced only 5- and 50-fold, respectively, in its presence (Post-Beittenmiller *et al.*, 1984; Shimada *et al.*, 1984). We report here that UASs associated with *GAL* genes share sequences involved in *GAL4* binding but vary in structure according to the pattern of transcription of the particular genes they regulate.

## Results

### *Single GAL4-binding sites upstream of the GAL80 and MEL1 genes*

Plasmid DNAs containing the *GAL80* and *MEL1* genes were end-labeled in the vicinity of the transcription initiation sites (Figure 1A and B) and *GAL4* binding was revealed by partial DNase I digestion and gel electrophoresis. *GAL4* protein was supplied by a nuclear extract (~10-fold enriched in UAS-specific binding activity over a whole cell extract), giving DNase I footprints (Figure 2) of comparable quality to those obtained (Bram and

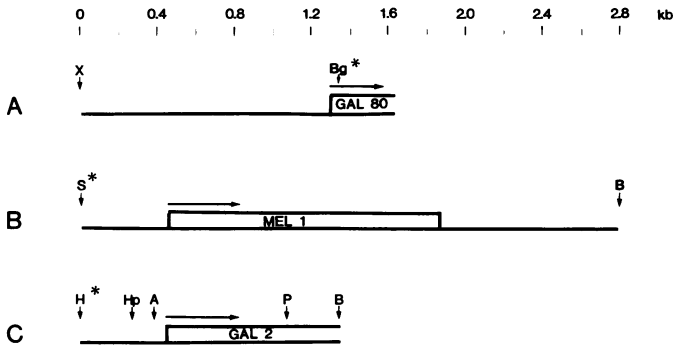


**Fig. 1.** Restriction enzyme sites for DNase I footprint and nitrocellulose filter-binding experiments. Boxed regions begin at transcription initiation sites of the *GAL80* (Nogi and Fukasawa, 1984) and *GAL2* (Figure 6) genes, and at a site 10 bp upstream of the translation initiation site of the *MEL1* gene (Liljeström, 1985). Restriction enzyme sites are abbreviated: A, *Acc*I; B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Hp, *Hpa*I; P, *Pvu*II; S, *Sal*I; X, *Xmn*I. Asterisks indicate sites of labeling in footprint and filter-binding experiments.

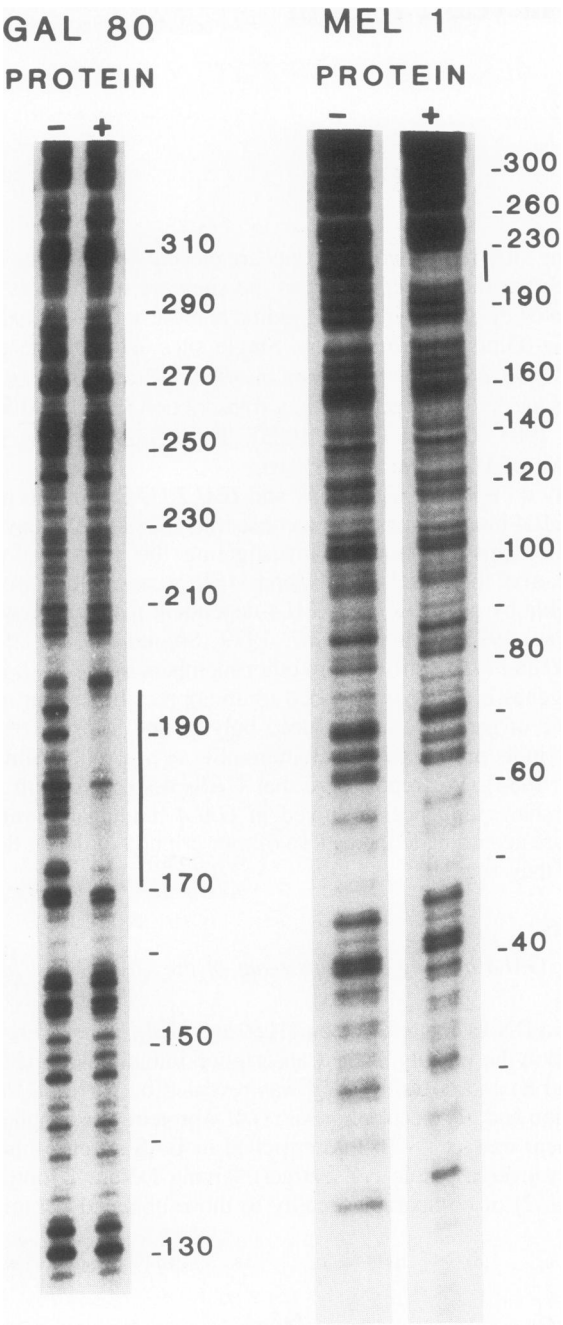


**Fig. 2.** DNase I footprints of *GAL4* protein on *GAL80*- and *MEL1*-flanking regions. *Xmn*I−*Bgl*II and *Sal*I−*Bam*HI fragments, labeled as indicated in Figure 1, were incubated with (+) or without (−) nuclear extract (20 μg protein), treated with DNase I, and analyzed by gel electrophoresis as described (Bram and Kornberg, 1985). DNA lengths in nucleotides indicated to the right of each gel are based on the pattern of φX174 *Hae*III fragments run in the same gel.

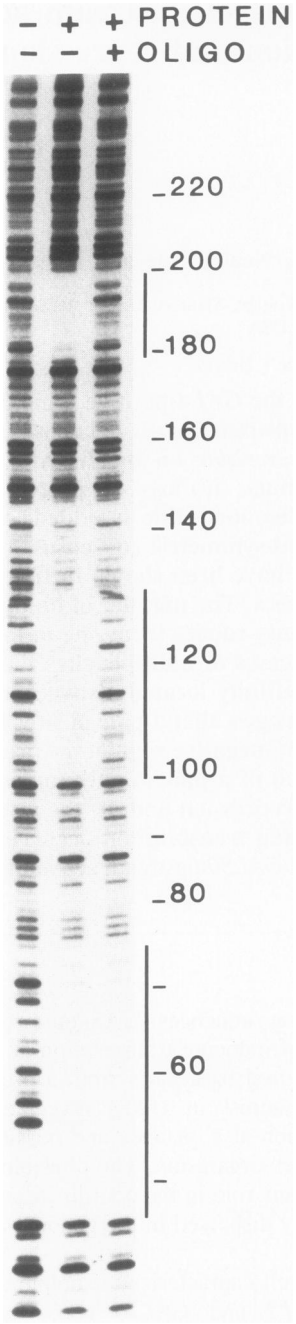


**Fig. 3.** DNase I footprint of *GAL4* protein in *GAL2*-flanking region. The *Hin*dIII−*Acc*I fragment shown in Figure 1 was treated with nuclear extract as in Figure 2 in the presence (+) and absence (−) of 10 ng (150-fold molar excess) of the *GAL4*-binding oligonucleotide (Bram and Kornberg, 1985). DNA lengths were determined as in Figure 2.

Kornberg, 1985) with a phosphocellulose fraction (12-fold enriched). The footprints showed single regions of protection about 30 bp in length, 95 and 230 bp upstream of the *GAL80* and *MEL1* genes, respectively. These regions are homologous in sequence to *GAL4*-binding sites found previously in the *GAL1−10* and *GAL7* UASs (see below).

*A pair of GAL4-binding sites upstream of the GAL2 gene*

The location of *GAL2* sequences within a 1.35-kb genomic clone was unknown so we tested various restriction fragments for *GAL4* binding in a nitrocellullose filter binding assay and identified one for finer mapping in footprint experiments. Restriction fragments were labeled and treated with nuclear extract in the presence and absence of excess unlabeled oligonucleotide containing a *GAL4*-binding sequence (Bram and Kornberg, 1985). Mixtures were passed through nitrocellulose, and filter binding that was abolished by oligonucleotide was judged *GAL4*-specific. A *Hin*dIII−*Acc*I fragment (Figure 1C) showed 10% specific binding, while a *Pvu*II−*Bam*HI fragment showed none. Footprints with DNA labeled at the *Hin*dIII site revealed three 30-bp regions of protein binding, two located 55 bp apart and the third at a

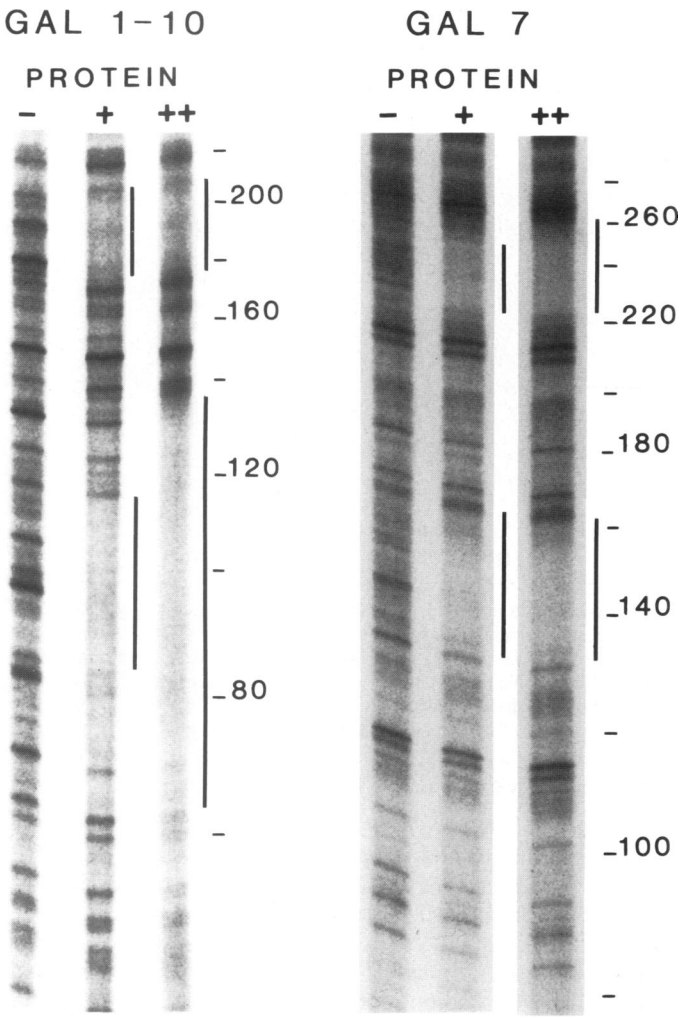


**Fig. 4.** DNase I footprints of *GAL4* protein on *GAL1-10* and *GAL7*-flanking regions. Plasmid DNAs were labeled and treated as described (Bram and Kornberg, 1985) with 5 μg (+) or 15 μg (++) of protein from peak phosphocellulose fractions. DNA lengths were determined as in Figure 2.

distance of 25 bp (Figure 3). The first two regions could be attributed to *GAL4* binding and the third to another factor, because: (i) binding of the first two regions was abolished by the oligonucleotide mentioned above, while binding of the third region was unaffected (Figure 3); (ii) binding of the first two regions could not be detected with a nuclear extract from a *GAL4* deletion mutant, while binding of the third region was still found (data not shown); and (iii) the sequences of the first two regions are homologous with other *GAL4*-binding sites, while the sequence of the third region is unrelated (see below). Transcription mapping of the *GAL2* gene (see below) showed that the first two regions extend from 300 to 410 bp upstream of the transcription initiation site, and the third region lies further upstream. Insertion of the first two regions in front of a heterologous promoter activates its transcription, showing that these *GAL2*-associated sequences function as a UAS (Bram and Kornberg, in preparation).

*Multiple GAL4-binding sites in the GAL1-10 and GAL7 UASs*

Previous footprint experiments on *GAL4* binding to the *GAL1-10* UAS revealed an enlargement of the protected region nearest the *GAL10* gene when higher levels of protein were used (Bram and Kornberg, 1985). We find the 30-bp protected region is extended by 20 bp in both directions when the amount of protein is increased 3-fold (Figure 4). Similar results are obtained with the protected region nearest the *GAL7* gene in the *GAL7* UAS. This region is extended by 10 bp in the direction of the *GAL7* gene when more protein is used (Figure 4). The additional protected sequences in both UASs are homologous with other *GAL4*-binding sequences (see below). In brief, both *GAL1-10* and *GAL7* UASs contain a pair of high-affinity *GAL4*-binding sites, one of which is flanked by low-affinity sites.

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                                                          266
CAATTTCTTT TTCTATTAGT AGCTAAAAAT GGGTCACGTG ATCTATATTC GAAAGGGCCG

                                                          206
GTTGCTCAGG AAGGCACCGG CGGTCTTTCG TCCGTGCGGA GATATCTGCG CCGTTCAGGG

                                                          146
GTCCATGTGC CTTGGACGAT ATTAAGGCAG AAGGCAGTAT CGGGGCGGAT CACTCCGAAC

                                                           86
CGAGATTAGG TAAGCCCTTC CCATCTCAAG ATGGGGAGCA AATGGCATTA TACTCCTGCT

                                                           26
AGAAAGTTAA CTGTGCACAT ATTCTTAAAT TATACAACGT TCTGGAGAGC TACTGTTCAA

AAAACAAACA TTGCGCAGGC TAAAA
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**Fig. 5.** DNA sequence of *GAL2*-flanking region. The 360-bp *Hin*dIII–*Acc*I fragment from pG2P was subcloned in M13mp8 and M13mp9 and the sequences of the two strands were determined as described (Sanger *et al.*, 1977). The sequence of all but ~20 bp at each end is shown. The bars indicate regions of homology to the consensus *GAL4*-binding sequence. The arrows indicate the dyad-symmetric sequence that shows homology to a conserved region of yeast centromeres and binds a novel factor.

*Sequences of GAL4-binding sites*

The sequence of the *GAL4*-binding region upstream of the *GAL2* gene was determined (Figure 5) and combined with published sequences of the other *GAL* and *MEL1* upstream regions to derive a consensus *GAL4*-binding sequence. Two forms of the consensus sequence are presented (Table I), one showing the most common nucleotide(s) at each position, and the second modified to make the sequence dyad-symmetric. The modifications were not extensive, suggesting that the dyad symmetry is significant. The 11 individual *GAL4*-binding sequences compiled here show 15–20 correct matches with the symmetrized consensus sequence of 23 bp. There is good agreement with a symmetrized consensus sequence of 17 bp reported by Giniger *et al.* (1985) while this work was in progress.

The identification of a dyad axis allows a more precise determination of the distance between *GAL4*-binding sites. Thus, the pairs of high affinity sites in the *GAL1-10*, *GAL2* and *GAL7* UASs, separated by spacer regions of ~55 bp, are located at dyad–dyad distances of 82, 83 and 87 bp, respectively.

*Transcriptional regulation by the GAL2 UAS and by a synthetic GAL4-binding site*

Seeking a purpose for the conserved spacing of two high-affinity *GAL4*-binding sites in the *GAL1-10*, *GAL2* and *GAL7* UASs, we noticed a correlation with regulatory behavior: the *GAL1*, *GAL7* and *GAL10* genes are fully repressed in cells grown in glycerol or ethanol in the absence of galactose (conditions of repression by *GAL80* protein), whereas the *GAL80* and *MEL1* genes, apparently controlled by single *GAL4*-binding sites, are expressed at 2–20% of their fully induced levels (Post-Beittenmiller *et al.*, 1984; Shimada *et al.*, 1984). This suggested that two *GAL4*-binding sites are required for complete repression by *GAL80* protein in glycerol or ethanol.

We were prompted to measure the extent of repression with the *GAL2* UAS and with a synthetic *GAL4*-binding site, previous-

**Table I.** *GAL14*-binding sequences

| Binding site | Correct matches | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sequence | | | | | | | | | | | | | | | | | | | | | | | Per 23 bp |
| *GAL10* (proximal-low affinity 1) | G | T | A | C | G | G | A | T | T | A | G | A | A | G | C | C | G | C | C | G | A | G | C | 17 |
| *GAL10* (proximal-high affinity 2) | G | A | G | C | G | G | G | T | G | A | C | A | G | C | C | C | T | C | C | G | A | A | G | 18 |
| *GAL10* (proximal-low affinity 3) | C | G | A | A | G | G | A | A | G | A | C | T | C | T | C | C | T | C | C | G | T | G | C | 19 |
| *GAL1* (proximal) | C | C | T | C | G | C | G | C | C | G | C | A | C | T | G | C | T | C | C | G | A | A | C | 19 |
| *GAL7* (distal) | C | T | T | C | G | G | A | G | C | A | C | T | G | T | T | G | A | G | C | G | A | A | G | 20 |
| *GAL7* (proximal) | G | C | T | C | G | G | A | C | A | A | C | T | G | T | T | G | A | C | C | G | T | G | A | 19 |
| *GAL7* (proximal-low affinity) | C | A | A | C | T | G | T | T | G | A | C | C | G | T | G | A | T | C | C | G | A | A | G | 17 |
| *MEL1* | A | T | T | C | G | G | C | C | A | T | A | T | G | T | C | T | T | C | C | G | A | A | A | 16 |
| *GAL2* (proximal) | T | A | T | C | G | G | G | G | C | G | G | A | T | C | A | C | T | C | C | G | A | A | C | 15 |
| *GAL2* (distal) | C | A | C | C | G | G | C | G | G | T | C | T | T | T | C | G | T | C | C | G | T | G | C | 18 |
| *GAL80* | T | A | C | C | G | G | C | G | C | A | C | T | C | T | C | G | C | C | C | G | A | A | C | 17 |
| Consensus | C | A | T | C | G | G | A | G | G | A | C | T | G | T | C | G | T | C | C | G | A | A | C | |
| | G | T | A | | | | G | C | C | | | A | C | | | C | | | | | | | | |
| Symmetrized consensus | G | T | T | C | G | G | A | G | G | A | C | T | G | T | C | C | T | C | C | G | A | A | C | |
| | C | C | A | | | | | C | C | | | A | | | G | G | | | | | T | G | G | |

The consensus sequence lists nucleotide(s) occurring in six or more of the 11 binding sequences (two nucleotides are shown if the first occurs less than six times). The symmetrized consensus sequence differs from the consensus by omission of the most common nucleotide at position 2 and by inclusion of the second most common nucleotide or reordering of the first and second most common nucleotides at positions 1, 7, 13, 15, 16, 21, 22 and 23. The numbers above the sequences give the correct matches to the symmetrized consensus sequence out of a possible eleven at each position. The numbers to the right of the sequences give the correct matches to the symmetrized consensus sequence of each binding sequence. Sequences are from Johnston and Davis (1984) (*GAL1*, *GAL10*), Nogi and Fukasawa (1983) (*GAL7*), Liljeström (1985) (*MEL1*), Figure 5 (*GAL2*) and Nogi and Fukasawa (1984) (*GAL80*).

**Table II.** Transcriptional regulation of the *GAL2* gene and of a *GAL–HIS* fusion gene under control of synthetic *GAL4*-binding sites

| Gene/ plasmid | UAS | RNA level (%); when grown on: | | |
|---|---|---|---|---|
| | | GAL | GLY | DEX |
| *GAL2* | *GAL2* | 100 | <0.2 | <0.2 |
| p10GH | 1 oligo | 100 | 3.8 | <0.4 |
| $p10^2$GH | 2 oligos | 97 | <0.4 | <0.4 |

*GAL2* transcripts were determined by S1 mapping of total RNA from *S. cerevisiae* strain 5C (a *ura 3-52 his 3Δ200*) grown in YP medium (1% yeast extract, 2% bactopeptone) plus 2% galactose (GAL), 3% glycerol and 2% lactate (GLY) or 2% dextrose (DEX). *GAL–HIS* fusion transcripts were detected by S1 mapping of poly(A)-containing RNA from strain 5C harboring plasmids with a *GAL–HIS* fusion gene under control of one (p10GH) or two ($p10^2$GH) *GAL4*-binding oligonucleotides, grown in minimal medium (0.67% yeast nitrogen base) plus 2% galactose, or 3% glycerol and 2% lactate. Total RNA was prepared by the glass bead method (Carlson and Botstein, 1982). Poly(A)-containing RNA was isolated by two cycles of adsorption and elution from oligo(dT)-cellulose, and represented 2–4% of the total RNA. S1 mapping was performed as described (Berk and Sharp, 1977) with 25 μg of total RNA and 15 ng of pG2P DNA (cleaved with *Bam*HI and 5′ end-labeled with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP), or with 0.7 μg (p1-10GH) or 9 μg (p10GH, $p10^2$GH) of poly(A)-containing RNA and 5 ng of plasmid DNA (cleaved with *Bgl*II and 5′ end-labeled). Quantitation was by densitometry, with RNA levels expressed as percentages of those found upon growth in galactose.

ly unstudied in this regard. Transcription was revealed by S1 mapping and, in the case of the *GAL2* gene, was undetectable in the absence of galactose and was induced >500-fold in its presence (Table II). The case of a synthetic *GAL4*-binding site was investigated with the *GAL4*-binding oligonucleotide mentioned above. When inserted upstream of a *GAL–HIS* fusion gene in a centromeric plasmid, this oligonucleotide-activated gene expression (Lorch and Kornberg, 1985) and transcription persisted in the absence of galactose at ~4% of the fully induced level (Table II). Thus the *GAL2* UAS, with a pair of *GAL4*-binding sites, gave full repression, while a single synthetic site allowed some escape from repression extending the correlation noted from published studies of other *GAL* and *MEL* genes.

*Transcriptional regulation by two synthetic GAL4-binding sites*

Is repression in glycerol or ethanol with the *GAL1–10*, *GAL2* and *GAL7* UASs attributable to a pair of *GAL4*-binding sites or to some other feature, such as spacer or flanking sequences? We addressed this question by inserting a second synthetic *GAL4*-binding site upstream of the *GAL–HIS* gene in the plasmid described above. A 68-bp spacer from a globin cDNA clone was incorporated between the two *GAL4*-binding sites, resulting in a dyad–dyad distance of 93 bp. Insertion of the second site abolished transcription in the absence of galactose but had little effect on transcription in its presence (Table II). Apparently, two *GAL4*-binding sites are sufficient for complete repression by *GAL80* protein, with no requirement for additional sequences. The possibility of a fortuitous effect of the globin spacer sequence remains, however, to be excluded. It further remains to examine the dependence of repression on spacer length.

## Discussion

The *GAL4*-binding regions identified here (Figure 6) may be regarded as UASs on the basis of functional studies: the regions upstream of the *GAL1*, *GAL2*, *GAL7* and *GAL10* genes have been shown to activate transcription from heterologous promoters (Guarente *et al.*, 1982; Johnston and Davis, 1984; Lorch and Kornberg, 1985; Bram and Kornberg, in preparation); the single *GAL4*-binding sites upstream of the *GAL80* and *MEL1* genes have not been tested in this way, but single synthetic sites were shown to activate transcription in studies published while our work was in progress (Giniger *et al.*, 1985; Lorch and Kornberg, 1985).

The requirement for a pair of *GAL4*-binding sites for complete repression by *GAL80* protein may be explained in many ways. For example, a *GAL80* molecule or oligomer may bind simultaneously at two sites (or to a pair of *GAL4* molecules at these sites), raising the affinity of the *GAL80*–DNA (or *GAL80–GAL4*–DNA) interaction. Alternatively, binding of *GAL80* protein at one site may prevent *GAL4* action at an adja-

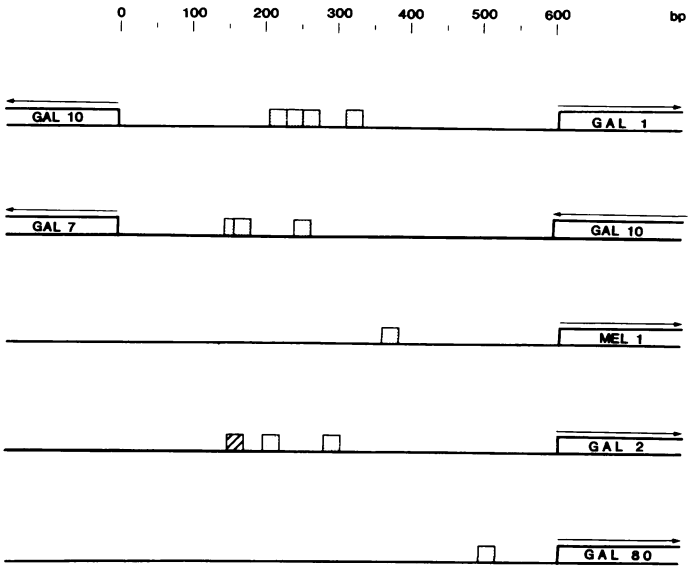


**Fig. 6.** *GAL* family of UASs. Stippled boxes represent *GAL4*-binding sites. The diagnonally lined box represents a region of homology to yeast centromeres that binds a novel factor. Open boxes are as in Figure 1.

cent site, increasing the probability of repression. Pairs of protein binding sites in enhancers may function by similar mechanisms. The long terminal repeated sequences of mouse mammary tumor virus (Schneidereit *et al.*, 1983) and the region upstream of the chicken lysozyme gene (Renkawitz *et al.*, 1984) contain pairs of binding sites for the glucocorticoid receptor, with distances between the sites of 55 and 120 bp, respectively. The SV40 enhancer resides in direct repeats of a 72-bp sequence and represents a locus of repression as well as enhancement of transcription (Borrelli *et al.*, 1984).

In contrast with the importance of multiple *GAL4*-binding sites in repression, there is little effect on induction. Thus the *MEL1* gene, with a single site, is transcribed at a fully induced level comparable with that of the *GAL1*, *GAL7* and *GAL10* genes (Post-Beittenmiller *et al.*, 1984). Moreover, the introduction of a second synthetic site upstream of a *GAL–HIS* fusion gene gave no increase in transcription over that obtained with one (Table II). Finally, two UASs give little more transcription than one (Lorch and Kornberg, 1985). In the light of these observations, additional low-affinity *GAL4*-binding sites in the *GAL1–10* and *GAL7* UASs serve no obvious purpose. The low- and high-affinity sites in the *GAL7* UAS overlap by 16 bp, with a dyad–dyad distance of 7 bp, apparently compatible with *GAL4* binding at both sites (Figure 4) but possibly an impediment to concerted action at these sites.

Although the number of *GAL4*-binding sites has little bearing on expression, their location may be important. Moving a UAS too far from or too close to a promoter may diminish transcription. For example, deletions that bring the *GAL1–10* UAS within 120 bp of an RNA start site on one side (Δ24 and Δ31 in West *et al.*, 1984) reduce transcription from this site without affecting that from the site on the other side. This may explain why the *GAL80* gene, with a single *GAL4*-binding site located 95 bp upstream (Figure 6), is expressed at a much lower level than the *MEL1* gene, with a single site 230 bp upstream.

A most surprising aspect of the results presented here is the occurrence of a binding site for a novel factor ustream of the *GAL2* UAS (Figure 6). The sequence of this site is dyad-symmetric at 18/24 residues, compared with an average of only 10/22 residues for *GAL4*-binding sites. The sequence is homologous to a conserved region of yeast centromeres and competes for binding with centromeres (Bram and Kornberg, in preparation). The location of the sequence adjacent to the *GAL2* UAS and the abundance of the novel factor (comparable with that of *GAL4* protein in an overproducer) suggest a general role related to transcription. One possibility would be a barrier function, preventing transcription of an upstream gene from extending through a UAS, or preventing a UAS from acting on an upstream gene.

## Materials and methods

*Plasmids*

The *GAL80*, *MEL1* and *GAL2* genes, on pRYGAL80-50, pSc483 and pTUG6, were gifts from Drs R.Yocum (Biotechnica International), D.Vollrath (Stanford University) and R.Schekman (University of California, Berkeley), respectively. The subclone, pG2P, was constructed by insertion of a 1.35-kb *Hin*dIII–*Bam*HI fragment of pTUG6 DNA, containing the 5′ half of the *GAL2* gene, into pSP65 (Melton *et al.*, 1984). A yeast centromeric plasmid carrying the synthetic *GAL4*-binding oligonucleotide (p10GH) upstream of the *GAL1* promoter and *HIS3* gene (Lorch and Kornberg, 1985) was a gift from Dr Y.Lorch. For the insertion of a second *GAL4*-binding oligonucleotide, a 68-bp *Eco*RI–*Bam*HI fragment containing residues 293–361 of a rabbit β-globin cDNA clone (Efstratiadis *et al.*, 1977) was ligated with the oligonucleotide, and a 118-bp product consisting of the β-globin fragment with an oligonucleotide on each end was isolated by polyacrylamide gel electrophoresis. The 118-bp product was ligated with a 1.65-kb *GAL–HIS* fragment and a 7.33-kb YCp50 fragment as described (Lorch and Kornberg, 1985) to give $p10^2GH$.

*Protein fractions*

Crude extract, nuclear extract and phosphocellulose column fractions were prepared from *S. cerevisiae* strain Sf657-2D (a *pep4-3 his4-580 ura3-52 gal2⁻ leu2-3, -112*) carrying the *GAL4* gene on a high copy number plasmid (Bram and Kornberg, 1985). Nuclear extract was concentrated by precipitation with 70% saturated ammonium sulfate in buffer A(50). The precipitate was washed with 60% and then 50% saturated ammonium sulfate in buffer A(50), and the 50% wash, containing all the *GAL4* binding activity, was dialyzed for use in binding experiments as described (Bram and Kornberg, 1985).

*Nitrocellulose filter-binding, DNase I footprints*

For filter-binding experiments, pG2P was cleaved with *Hin*dIII and *Bam*HI and labeled with the large fragment of DNA polymerase I and [α-$^{32}$P]dATP in the presence of unlabeled dGTP, dCTP and TTP. The 1.35-kb fragment containing the *GAL2* gene was isolated by agarose gel electrophoresis, digested with *Acc*I or *Pvu*II, and again subjected to electrophoresis in agarose. The resulting fragments were used in binding reactions with crude extract (1 μg of protein). Details of nitrocellulose filter-binding and DNase I footprint experiments were as described (Bram and Kornberg, 1985).

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