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Specific protein binding to far upstream activating sequences in polymerase II promoters

(*Saccharomyces cerevisiae*/*GAL4* gene/*GAL1*, *GAL7*, and *GAL10* promoters/nitrocellulose filter binding/DNA-protection mapping)

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ABSTRACT **A binding activity specific for the upstream activating sequence of the *GAL1–GAL10* promoter of *Saccharomyces cerevisiae* has been purified 220-fold on the basis of a nitrocellulose filter-binding assay. The binding activity is enriched in a nuclear preparation and is likely to be the *GAL4* gene product. DNase I-protection mapping patterns reveal binding to two 30-base-pair regions at the boundaries of the sequence. A nearly identical mapping pattern is obtained with the coordinately regulated *GAL7* promoter. The four 30-base-pair regions of binding in the two promoters are closely homologous, with a core consensus sequence of C-G-$\genfrac{}{}{0pt}{}{C}{G}$-$\genfrac{}{}{0pt}{}{T}{G}$-C-A-A-C-A-G-T-G-C-T-C-C-G-A-A-$\genfrac{}{}{0pt}{}{G}{C}$-G-A-T. A synthetic oligonucleotide with such a sequence competes with the upstream activating sequence in the binding reaction.**

Two classes of regulatory region have been found associated with promoters for transcription by RNA polymerase II. The first, located 30 to 100 base pairs (bp) upstream of the start site of transcription, is involved in the activation of heat shock and metallothionein genes and also in the expression of viral genes (1). Proteins that bind specifically to such regions of DNA and that potentiate transcription *in vitro* have been partially purified (2–4). These proteins may function in a manner analogous to the catabolite activator protein of *Escherichia coli*, which binds at a similar distance from sites of transcription initiation (5). A second class of regulatory region is distinguished by its capacity to activate transcription at a variable, often quite large, distance. Such regions have been found hundreds to thousands of base pairs from transcription initiation sites in viral and immunoglobulin genes, where they are referred to as enhancers (1); in the case of yeast genes, such regions have been termed upstream activating sequences (UASs) (6, 7). Little is known of the proteins that interact with these sequences. Here we describe the partial purification and characterization of a binding activity specific for UASs associated with the galactose-inducible genes of yeast.

The *GAL1*, *GAL7*, and *GAL10* genes, which encode enzymes of the galactose utilization pathway in *Saccharomyces cerevisiae*, are coordinately regulated (8, 9). On addition of galactose to the growth medium, expression of these genes is induced about 1000-fold at the transcriptional level (10–12). Deletion analysis of the region between the *GAL1* and *GAL10* genes, which contains both promoters, has defined a stretch of about 100 bp some 200–300 bp upstream of the transcription initiation site that is required for induction and is termed UAS_G (6, 13). Genetic studies have revealed a positive regulatory protein, encoded by the *GAL4* gene (14, 15), also required for induction and thought to interact with UAS_G. A negative regulatory element has also been identified, the *GAL80* gene product, that opposes GAL4 protein action (9, 14, 16, 17).

The *GAL4* gene appears to be expressed constitutively but at a very low level (18–22). To facilitate isolation of the GAL4 protein, we have introduced the gene on a multicopy plasmid and exploited the presumptive interaction of the protein with UAS_G DNA in a nitrocellulose filter-binding assay.

MATERIALS AND METHODS

Plasmid DNAs. Plasmid pSJ4 (18), which contains the *GAL4* gene, was provided by J. E. Hopper (Pennsylvania State University). The shuttle vector pJDB207 (23) was a gift from J. D. Beggs (Imperial College of Science and Technology, London). Plasmid pLG4 was constructed by excision of the *GAL4* gene from pSJ4 with *Bam*HI and *Hin*dIII restriction endonucleases and ligation into the corresponding sites of pJDB207. The *GAL1–GAL10* UAS_G was subcloned from pBM125 (13) (a gift from Mark Johnston, Washington University, St. Louis, MO). The 250-bp *Dde* I/*Eco*RII restriction fragment indicated in Fig. 1*A* was modified with synthetic *Bam*HI and *Eco*RI octanucleotide linkers and inserted into the corresponding sites of pBR322, yielding plasmid pGF1. A control plasmid, pBRd, identical to pGF1 except lacking the yeast sequences, was made by cleaving pBR322 with *Eco*RI and *Bam*HI endonucleases, filling in the ends with the large fragment of DNA polymerase I, adding *Bam*HI linkers, and cyclizing the resulting DNA. Plasmid pSc4813 (24), containing the *GAL7* promoter sequences (Fig. 1*B*), was a gift from T. P. St. John (Stanford University).

Yeast Strains and Media. Sf657-2D (a *pep4-3 his4-580 ura3-52 gal2⁻ leu2-3, -112*) was provided by C. Fields (University of California, San Francisco). The pleiotropic mutation pep4-3 (25) gives reduced levels of three proteases, as well as RNase and phosphatase, and was used to minimize proteolysis during preparation of cell extracts. YNN267 (a *ura3-52 his3Δ200 ade2-101 lys2-801 met⁻, gal4Δ537*) was a gift from Mark Johnston. BY2 is the designation for Sf657-2D transformed with plasmid pLG4 by the LiOAc procedure (26).

Cells were generally grown in YP medium (1% yeast extract, 2% bactopeptone) containing 2% glucose or sucrose. Sf657-2D is derived from S288C and cannot grow on galactose as sole carbon source. Induction with this strain was therefore carried out with 2% galactose in the presence of 2% sucrose, known to have a minor catabolite repression effect (20). BY2 was grown in minimal medium (0.67% yeast nitrogen base plus uracil and histidine); in some large-scale preparations, the cells were transferred from minimal to YP medium for the last 10 generations to maximize cell yield.

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Abbreviations: bp, base pair(s); UAS, upstream activating sequence.

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Cell Extracts. Extracts with optimal levels of specific binding activity were prepared as follows. BY2 cells were grown at 30°C in minimal medium/2% sucrose to stationary phase (A_{600} value of 1.5), then diluted 1:250 into YP medium/2% sucrose and grown to an A_{600} value of 5. Galactose was added to a final concentration of 2%, and the cells were allowed to grow for an additional 30 min. Cells were harvested by centrifugation at 5000 rpm for 5 min, washed with cold water, and then suspended with an amount of buffer A(50)† equal in volume to the cell pellet. The cells were frozen dropwise in liquid nitrogen, thawed overnight at 4°C, and broken with an equal volume of glass beads (0.45-mm diameter) using eight 30-sec pulses of a bead beater (Biospec Products, Bartlesville, OK). Solid ammonium sulfate was added to 0.3 M, and the lysate was stirred for 30 min at 4°C. (Omission of this salt extraction step gave extracts having a much lower activity.) The cell lysate was centrifuged in a Sorvall SS-34 rotor at 10,000 rpm for 10 min at 4°C; the supernatant was decanted and centrifuged in a Beckman Ti60 rotor at 50,000 rpm for 3 hr at 0°C. The supernatant was again collected and was dialyzed twice against 5 vol of buffer A(50) in Spectrapor 6 tubing (M_r cutoff, 30,000) for 3 hr at 4°C. The final extract contained 20–40 mg of protein per ml, a yield of 0.5–1 g of protein per liter of cells. Extracts could be frozen in liquid nitrogen and thawed numerous times without any loss of binding activity.

Nuclear Extracts. Cells were grown as described above. Spheroplasts were prepared and nuclei were isolated as described (27). The nuclear pellet was suspended in 10 vol of buffer A(50), and binding activity was released by the addition of ammonium sulfate to 0.3 M. This nuclear extract was centrifuged and dialyzed as described above.

Assay of UAS_G-Binding Activity. Plasmids pGF1 and pBRd were linearized by cleavage with *Bam*HI endonuclease. The recessed 3′ ends were filled in with the large fragment of DNA polymerase I in the presence of [α-^{32}P]dATP (28). pSc4813 was cleaved with *Xmn* I, treated with T4 DNA polymerase in the presence of [α-^{32}P]dATP (28), and digested with *Ava* I endonuclease, and the fragment containing the *GAL7* promoter sequences was isolated by electrophoresis in a 1% agarose gel.

Binding reactions were carried out in 20 μl of buffer A(50) with 4 fmol of radioactive DNA, 1 μg of sonicated double-stranded salmon sperm DNA as carrier, 0.5% galactose, and 0.1–40 μg of protein. The addition of galactose had no appreciable effect on the binding; it was included in case a galactose-dependent phenomenon became manifest in the course of purification. The reaction mixtures were kept at 22°C for at least 10 min, diluted with 1 ml of buffer A(50) and immediately passed through presoaked 13-mm nitrocellulose filters (Millipore) at a pressure of 5 in of Hg. The extent of binding was determined by liquid scintillation counting of the filters. For each set of experiments, two controls were done: the total number of counts was determined by spotting the reaction mixture directly onto nitrocellulose, and samples without added protein were filtered. The background value was generally 2–7% of the total. Each determination was carried out in duplicate and the average value was taken (the range of error was <5%). One unit of activity is the percent pGF1 DNA bound minus the percent pBRd DNA bound, divided by 50%.

Fractionation of UAS_G-Binding Activity. Phosphocellulose (P11) was prepared according to Whatman. Double-stranded DNA-cellulose prepared according to Alberts and Herrick (29) was provided by Martha Fedor. Protein determination was by the method of Bradford (30) with bovine serum albumin as standard.

Crude extract (12 ml) was loaded at 1 ml/min onto a 40-ml phosphocellulose column (2.5-mm diameter) equilibrated with buffer A(50). The column was washed with 100 ml each of buffer A(50) and buffer A(250), and then UAS_G-binding activity was eluted with 100 ml of buffer A(400). The activity of alternate fractions was measured, and peak fractions were pooled and dialyzed against buffer A(50) for 3 hr. For DNase I-protection mapping experiments, fractions from the phosphocellulose column were precipitated by saturation with ammonium sulfate, resuspended in a small volume of buffer A(50), and dialyzed. Alternatively, the protein was further purified and concentrated by loading at 5 ml/hr onto a 2.7-ml DNA-cellulose column (1-cm diameter) in buffer A(50), washing with 7 ml of buffer A(50), and eluting with a 14-ml 0.1–0.6 M KCl gradient. The activity of alternate fractions was measured, and the active fractions were dialyzed against buffer A(50) for 3 hr, frozen in small aliquots, and stored in liquid nitrogen.

DNase I-Protection Mapping. Reaction mixtures were prepared as described for the filter-binding assay. Following the 10-min incubation at 22°C, the mixtures were treated with 1–2 ng of DNase I (Worthington) freshly diluted in 5 μl of 50 mM KCl/25 mM Hepes, pH 7.5/5 mM $MgCl_2$/3 mM $CaCl_2$/20% glycerol for 30 sec at 22°C. The mixtures were then treated with 100 μl of 1% Sarkosyl/100 mM NaCl/100 mM Tris, pH 8.0/10 mM EDTA containing proteinase K at 0.5 mg/ml and tRNA at 50 μg/ml for 15 min at 37°C, heated for 5 min at 90°C, and extracted with phenol/chloroform, 1:1 (vol/vol). DNA was precipitated with ethanol, washed with 75% ethanol, suspended in 4 μl of 80% formamide/0.1× TBE buffer (TBE buffer is 90 mM Tris borate, pH 8.3/2.5 mM EDTA)/0.15% xylene cyanol FF/0.15% bromphenol blue, heated for 60 sec at 100°C, cooled quickly in ice, and electrophoresed in 0.4-mm-thick 5–6% polyacrylamide gels containing 7 M urea and TBE buffer. The gels were dried and autoradiographed with Lightning plus intensifying screens at −70°C.

Synthetic Oligonucleotides. Two complementary oligonucleotides were synthesized by Vernon Oi (Becton Dickenson) using the phosphite triester method (31) and purified by electrophoresis in 15% polyacrylamide gels containing 7 M urea and TBE buffer (28). The complementary strands were annealed by mixing equal amounts of the two 25-mers (at 0.1 mg/ml), heating for 5 min at 65°C, and then cooling slowly to room temperature. The resulting double-stranded oligonucleotide could be labeled and ethanol precipitated with efficiencies comparable with those of plasmid DNA.

RESULTS

Assay of UAS_G-Binding Activity. Specific DNA-binding factors in subcellular fractions can be detected by nitrocellulose filter binding and DNase I-protection mapping. Filter binding is more useful as an assay because it is rapid and quantitative, and attempts at protection mapping with crude extracts and UAS_G DNA have been unsuccessful (see below). The filter-binding assay involves mixing ^{32}P-labeled plasmid DNA with carrier DNA and the protein fraction in question, followed by incubation and filtration through nitrocellulose. Plasmid DNA containing a 250-bp insert of UAS_G from the *GAL1–GAL10* promoter (Fig. 1*A*) showed more binding than vector DNA (plasmid without the insert) in assays of crude extracts from cells containing a high copy number of the *GAL4* gene. The difference in binding between the two DNAs (UAS_G-specific binding) was proportional to the amount of extract, up to ≈50% of total DNA bound (Fig. 2). Specific binding was dependent on the copy number of the *GAL4* gene: it was barely detectable with extracts of strains

†Buffer A: 25 mM Hepes, pH 7.5/5 mM $MgCl_2$/0.1 mM EDTA/0.5 mM dithiothreitol/10% glycerol/1 mM phenylmethylsulfonyl fluoride/2 μM pepstatin A/0.6 μM leupeptin containing KCl at the concentration (mM) given in parentheses.

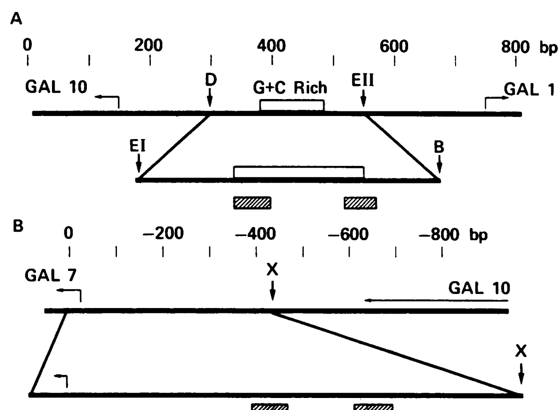

FIG. 1. Sequence organization of the *GAL1–GAL10* (*A*) and *GAL7* (*B*) promoter regions. The upper lines show organization of chromosomal DNA, the lower lines show fragments used in this work. Filled boxes indicate protected regions in the DNase I-protection maps of Fig. 3. Numbering schemes are from refs. 13 and 32. Restriction enzyme sites are abbreviated as follows: D, *Dde* I; EI, *Eco*RI; EII, *Eco*RII; B, *Bam*HI; X, *Xmn* I.

containing a single copy of the gene or lacking the gene altogether (Table 1). Specific binding was greater when cells were grown in galactose, as compared with glucose, a repressor of galactose-inducible genes (33, 34) (Table 1). The amount of specific binding activity extracted from a nuclear preparation was comparable with that from whole cells, consistent with nuclear localization of the binding factor.

For detection of UAS_G-specific binding activity in crude extracts, it was essential that (*i*) plasmids derived from pBR322 have residues 1–375 deleted, to avoid interference by an uncharacterized activity in yeast extracts that binds to this region of bacterial DNA (data not shown); (*ii*) a 1000-fold excess of carrier DNA be included to reduce the level of nonspecific binding; (*iii*) a higher ionic strength be provided than in previous filter-binding experiments—for example, those on lac repressor–operator interaction (35); and (*iv*) an incubation period of at least 5 min be allowed for specific

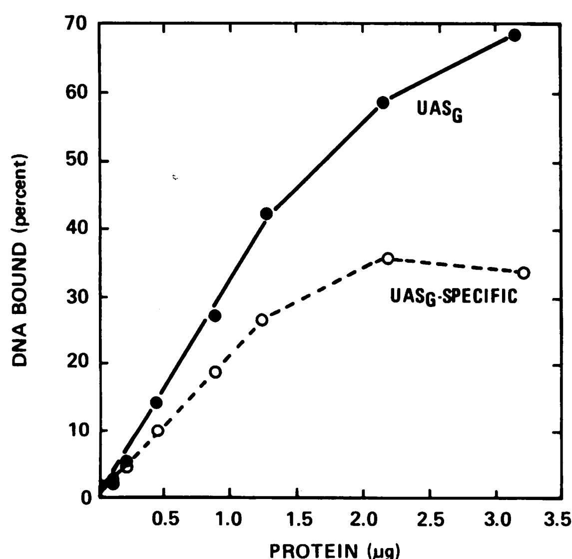

FIG. 2. Assay of UAS_G-binding activity. ^{32}P-labeled pGF1 (UAS_G DNA) and pBRd (vector DNA) were treated with crude extract from strain BY2 grown in minimal medium plus sucrose and galactose and were assayed by nitrocellulose filter binding. The percentage of pGF1 DNA bound (UAS_G, —) and the difference between the percentages of pGF1 and pBRd DNAs bound (UAS_G-specific, ---) are shown as functions of the amount of extract used.

Table 1. Dependence of UAS_G-binding activity in crude extracts on dosage of the *GAL4* gene and on induction with galactose

Strain	Relevant genotype	Growth medium	Specific activity, units/μg: Glucose	Specific activity, units/μg: Sucrose/galactose
YNN267	*gal4Δ*	YP	ND	0.06
Sf657-2D	*GAL4*$^+$	YP	ND	0.05
BY2	(*GAL4*) × 50*	YP	0.09	0.30
BY2	(*GAL4*) × 50*	Minimal	0.22	0.64

Specific activity was determined using glucose or sucrose/galactose as carbon source. ND, not determined.
*Strain BY2 carries the *GAL4* gene on pJDB207, which is typically maintained at about 50 copies per cell (23).

binding (the nonspecific reaction, by contrast, was complete in <1 min).

Fractionation of UAS_G-Binding Activity. The filter-binding assay monitored the fractionation of UAS_G-binding activity (Table 2). When a crude extract was applied to phosphocellulose and washed with buffer containing 0.25 M KCl, 95% of the total protein was removed. UAS_G-specific binding activity was eluted with 0.4 M KCl, dialyzed, and applied to DNA-cellulose. More than 90% of the total protein flowed through the column; on gradient elution, a major peak of protein was removed between 0.2 and 0.3 M KCl; UAS_G-specific binding activity was recovered between 0.25 and 0.35 M KCl. The ratio of UAS_G plasmid to vector DNA binding in this partially purified fraction was 5- to 10-fold greater than that in the crude extract (data not shown). UAS_G-specific binding activity appeared to be susceptible to proteolysis, inasmuch as both phenylmethylsulfonyl fluoride and a mixture of pepstatin A and leupeptin were required for stability of the activity during fractionation. For example, when pepstatin A and leupeptin were omitted, the activity in the phosphocellulose fractions decayed with a half-time of ≈2 hr at 0°C, compared with no loss of activity in the presence of the protease inhibitors.

DNase I-Protection Mapping of UAS_G-Binding Activity. The 250-bp segment of UAS_G DNA from the *GAL1–GAL10* promoter used in the filter binding experiments was end-labeled and treated with protein fractions in the same manner as for filter binding. The pattern of protection from DNase I was determined by brief digestion followed by gel electrophoresis. Two regions of the *GAL1–GAL10* promoter were protected by peak fractions from either the phosphocellulose or the DNA-cellulose columns (Fig. 3), whereas no pattern of protection was obtained with crude extracts. The protected regions are ≈30 bp long and ≈55 bp apart. (The *GAL10*-proximal protected region appeared slightly larger when higher levels of protein were used.)

Since the *GAL1*, *GAL7*, and *GAL10* genes are coordinately regulated (8, 9), the filter binding and DNase I-protection maps of *GAL7* and *GAL1–GAL10* promoter DNAs were compared. In the absence of evidence from deletion analysis

Table 2. Fractionation of UAS_G-binding activity

Step	Vol, ml	Total units	Protein, mg/ml	Yield, %	Specific activity, units/mg
Crude extract	12	3600	21	(100)	14.3
Phosphocellulose	55	2420	0.25	67	176
DNA-cellulose	2	380	0.06	10	3167

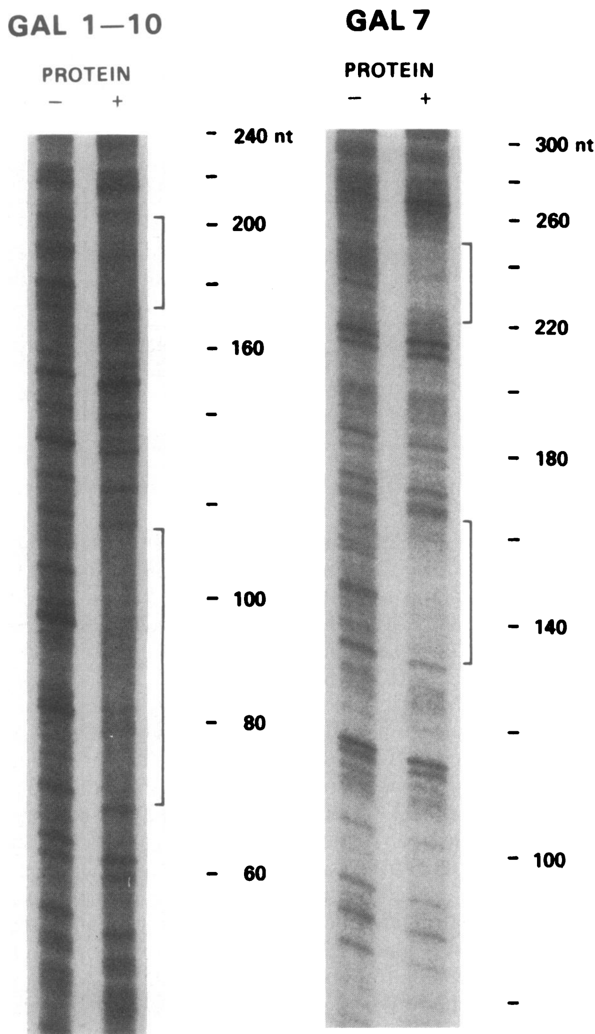

FIG. 3. DNase I-protection maps of UAS_G-binding activity on *GAL1–GAL10* and *GAL7* promoters. The pGF1 insert of *GAL1–GAL10* DNA was labeled at the *Eco*RI site (Fig. 1*A*), and a segment of *GAL7* DNA extending from the *Xmn* I site at residue −405 to an *Ava* I site within the *GAL7* gene was labeled at the *Xmn* I site (Fig. 1*B*). Labeled DNAs (4 fmol) were incubated with (+) or without (−) peak phosphocellulose fractions (10 μg protein). The DNA lengths indicated to the right of each gel are based on the pattern of ϕX174 *Hae* III fragments run in the same gel. nt, Nucleotides.

for the occurrence or location of a UAS_G in the *GAL7* promoter, we used a segment extending 405 bp upstream of the transcription initiation site (Fig. 1*B*). The behavior of this DNA in filter-binding experiments with crude extracts was the same as for the *GAL1–GAL10* promoter: the amount of binding was twice that of vector DNA with an extract of strain BY2 grown on YP medium plus sucrose and galactose but only 20% greater than vector DNA with the same amount of extract from the same strain grown in the presence of glucose. DNase I-protection maps of peak phosphocellulose and DNA-cellulose fractions on *GAL7* promoter DNA were also similar to those obtained with the *GAL1–GAL10* promoter (Fig. 3). Two regions of protection ≈30 bp long and 55 bp apart, extending from 155 to 270 bp upstream of the *GAL7* transcription initiation site, were observed.

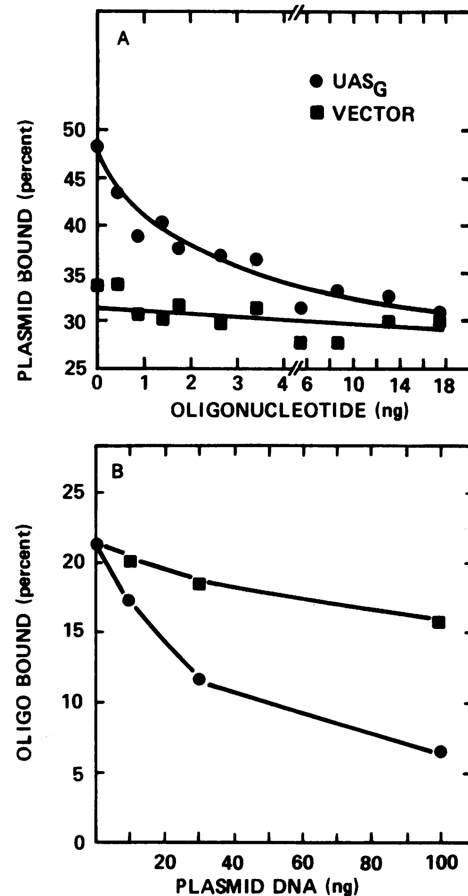

FIG. 4. Competition between synthetic oligonucleotide and promoter DNA for UAS_G-binding activity. Labeled plasmid DNAs as in Fig. 2 and unlabeled oligonucleotide (*A*) or unlabeled plasmid DNAs and labeled oligonucleotide (*B*) were treated with crude extract from strain BY2 grown in minimal medium plus sucrose and galactose (36 μg of protein) and assayed by nitrocellulose filter binding.

The four regions of protection in the *GAL1–GAL10* and *GAL7* protection maps are not only similar in size and spacing but also closely homologous in sequence (Table 3). They show 18–22 correct matches with a consensus sequence of 23 residues. The four sequences are identical at 9 positions within a stretch of 18 residues.

Competition Between a Synthetic Oligonucleotide and Promoter DNA for UAS_G-Binding Activity. The activities detect-

Table 3. Homology within protected regions in DNase I-protection maps

Core protected region	Nucleotides	Sequence	Correct matches per 23 bp
GAL1 proximal	465 to 487	C-G-C-G-C-C-G-C-A-C-T-G-C-T-C-C-G-A-A-C-A-A-T	18
GAL7 distal	−270 to −292	C-G-C-T-C-A-A-C-A-G-T-G-C-T-C-C-G-A-A-G-T-A-T	22
GAL7 proximal	−183 to −205	C-G-G-T-C-A-A-C-A-G-T-T-G-T-C-C-G-A-G-C-G-C-T	19
GAL10 proximal	383 to 405	C-G-G-G-T-G-A-C-A-G-C-C-C-T-C-C-G-A-A-G-G-A-A	18
Consensus		C-G-(C-T / G-G)-C-A-A-C-A-G-T-G-C-T-C-C-G-A-A-(G / C)-G-A-T	
Synthetic oligonucleotides		G-A-T-C-C-G-G-G-T-G-A-C-A-G-C-C-C-T-C-C-G-A-A-G-G G-C-C-C-A-C-T-G-T-C-G-G-G-A-G-G-C-T-T-C-C-T-T-A-A	

ed by DNase-protection mapping and filter binding appear to be the same. First, the degree of protection in the maps corresponded with the amount of filter binding across the peak of activity from the DNA-cellulose column (data not shown). Second, one of the sequences protected in the maps was shown to compete with UAS_G plasmid DNA in filter-binding experiments: a synthetic oligonucleotide, containing 23 residues of the *GAL10*-proximal mapping region, inhibited the filter binding of UAS_G DNA but had no effect on the binding of vector DNA (Fig. 4*A*); conversely, UAS_G DNA inhibited filter binding of the oligonucleotide, while vector DNA had a lesser effect (Fig. 4*B*).

DISCUSSION

Our results have revealed both a probable UAS component of the *GAL7* promoter, corresponding to the UAS_G of the *GAL1–GAL10* promoter described by others (6, 13) and a binding activity specific for UAS_Gs. The binding phenomenon seems likely to play a role in positive regulation of the *GAL1*, *GAL7*, and *GAL10* genes because the binding activity (*i*) is detected only in extracts of cells containing multiple copies of the *GAL4* gene, a positive regulatory element of the GAL system; (*ii*) is increased in cells grown in galactose, which activates transcription of the *GAL* genes; (*iii*) may be detected with both galactose-inducible promoters tested and reveals common features of these promoters; and (*iv*) confers protection against DNase I digestion in a 115-bp region of the *GAL1–GAL10* promoter that completely overlaps the 108-bp region shown to be required for induction by deletion mapping. The binding activity is in all likelihood a product of the *GAL4* gene, but alternatives such as a secondary component elicited by a *GAL4* product remain to be excluded. The amounts of DNA specifically bound by crude extracts lead to an estimate of ≈100 copies of binding protein per cell of a strain with 50–100 copies of the *GAL4* gene. Assuming a molecular weight of 100,000 [as expected (36, 37) for a single polypeptide chain of the GAL4 protein], ≈30,000-fold purification would be required to reach homogeneity. The lower level of binding activity in extracts of cells grown in glucose may reflect the action of the *GAL80* gene product.

The DNase I-protection maps obtained with the *GAL1–GAL10* and *GAL7* promoters are remarkably alike. Two 30-bp regions with similar sequences the same distance apart are protected in both cases. Moreover, the two protected sequences occur in direct repeat orientation in both promoters. This may indicate that the bidirectional *GAL1–GAL10* promoter represents a single regulatory element, rather than two closely linked promoters. It follows that the putative UAS component of the *GAL7* promoter may also be capable of activating transcription in both directions. The question arises of whether a single protein molecule or oligomer binds simultaneously to both 30-bp sites in a UAS_G. Such binding, with no interaction between the sites, would have an affinity constant equal to the square of that for binding at one site. Our competitive binding studies argue against this possibility, since the affinity for UAS_G DNA is at most an order of magnitude or two greater than the affinity for an oligonucleotide containing a single binding site. It may be that the sites can function independently, although a cooperative interaction involving additional components is not excluded.

There is no apparent homology of the two 55-bp regions between the protected sequences in the *GAL1–GAL10* and *GAL7* footprints. This is in keeping with the disparity in (G+C)/(A+T) ratio of the two 55-bp regions (61% G+C in the *GAL1–GAL10* promoter, 34% G+C in *GAL7*) and may indicate that it is more the length than the sequence of these regions that is important for UAS function.

The DNase I-protection maps reported here correspond in a striking way with results of DNase I digestion of yeast chromatin (unpublished work). The region between the two protected sequences in the maps is particularly susceptible to digestion in chromatin. Further studies of the UAS_G-binding activity may shed light on the role of DNase I-hypersensitive sites in the gene activation process.

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