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Isolation and preliminary characterization of the *GAL4* gene, a positive regulator of transcription in yeast

(recombinant DNA/mRNA mapping/eukaryotic gene regulation)

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ABSTRACT **The *GAL4* locus encodes a positive regulator of the inducible galactose and melibiose genes of yeast. Using the yeast plasmid vector YEp13, we have cloned *GAL4* by complementation of a *gal4* mutation. Restriction endonuclease mapping of subclone DNA has delimited the region sufficient for complementation to a 3.2-kilobase segment of DNA. The *GAL4* mRNA is 2.8 kilobases long, sufficient to encode a protein as large as 105,000 daltons. The concentration of the *GAL4* transcript is about 0.1 per cell and is almost identical in galactose-induced and noninduced cells. This result is consistent with a previously proposed model in which the activity of the GAL4 protein and not the transcription of the *GAL4* gene is modulated by galactose induction.**

One of the most interesting examples of eukaryotic gene regulation involves the gene cluster *GAL1*, *-7*, and *-10* in yeast, which encodes galactokinase, α-D-galactose-1-phosphate uridyl transferase, and uridine diphosphogalactose-4-epimerase (1). In response to galactose, these genes undergo a coordinate 1,000-fold induction (2). Douglas and Hawthorne (1) discovered that at least two additional unlinked genes, *GAL4* and *GAL80*, are involved in regulating this induction. *gal80* mutants are constitutively induced for *GAL1*, *-7*, and *-10*, suggesting that the *GAL80* product acts as a repressor of the cluster. *gal4* mutants are noninducible, indicating that the *GAL4* product functions in a positive manner to induce the *GAL1*, *-7*, and *-10* genes. *gal4 gal80* double mutants are noninducible, consistent with the idea that *GAL4* function is required for induction regardless of whether or not *GAL80* product is present.

Douglas and Hawthorne (1) proposed that the *GAL4* protein allows transcription of *GAL1*, *-7*, and *-10* and that the *GAL80* protein represses the transcription of *GAL4*. Galactose induction would inactivate the *GAL80* protein, switching on the *GAL4* gene whose product in turn switches on the *GAL1*, *-7*, and *-10* genes. However, several lines of evidence indicate that the *GAL4* protein is made constitutively and is not induced by galactose as part of a regulatory cascade (3, 4). Oshima and coworkers (5, 6) presented genetic evidence for interaction between the *GAL4* and *GAL80* proteins and, along with Perlman and Hopper (4), proposed a model in which the *GAL80* protein exerts its negative effect by binding to *GAL4* protein to prevent it from inducing transcription. Galactose induction would then lead to inactivation of *GAL80* protein, allowing *GAL4* protein to turn on transcription.

Several groups have described a general procedure for the cloning of yeast genes by complementation of mutations using yeast plasmid vectors (7–9). By selecting for complementation of a *gal4* mutation, we have isolated the *GAL4* gene from a yeast genomic library. Isolation of the gene has enabled us to identify the *GAL4* mRNA and to study its expression. Johnston and Hopper (10) have carried out related studies independently.

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MATERIALS AND METHODS

Enzymes and Reagents. Materials were from the following sources: T4 DNA ligase, P-L Biochemicals; bacterial alkaline phosphatase, BAPF, Worthington; avian myeloblastosis virus reverse transcriptase, Life Sciences (St. Petersburg, FL); agarose, Marine Colloids (Rockland, ME); galactose (glucose-free), Sigma. (*N*-3-Nitrobenzoxymethyl)pyridinium chloride was a gift from T. Helentjaris.

Yeast Strains and Media. Haploid *Saccharomyces cerevisiae* strains 95-4A (α *arg4-17 his5-2*), 95-4B (*a ade6 his5-2 leu1-2*), DC5 (*a leu2-3 2-112 his3 can1*), R440-8C (*a gal4-6 gal80-1 leu1-2 his5-2*), R171-11C (α *gal80-1 arg4-17 his5-2*), and GAL4-4 (α *gal4-4 leu1-2 his5-2*) were generously provided by J. Broach. *gal4-4* is a missense mutation. Strain S32A (α *gal4-4 leu2-3 2-112*) was obtained by sporulation and tetrad dissection of a diploid from the mating of DC5 and GAL4-4. *GAL4*-transformed strains G202, G213, G215, G501, G515, G525, and G528 were obtained by transformation of S32A with the respective YEp13-derived *GAL4* plasmids: YEpG202, YEpG213, etc. Yeast strains were grown at 30°C on 2% peptone/1% yeast extract plus 2% carbon source or on 0.67% yeast nitrogen base plus amino acids and 2% carbon source (plus 2% agar for plates).

Nucleic Acid Purification. Yeast DNA was prepared as described by Nasmyth and Reed (9) except that DNA for use in construction of the yeast library was also purified by equilibrium centrifugation in CsCl. Plasmid DNA from *Escherichia coli* was prepared as described (11). DNA fragments were purified from SeaPlaque agarose gels according to Weislander (12).

RNA was purified from yeast cells as follows (R. Elder, personal communication). A 200-ml culture was grown to a density of 1–2 × 10^7/ml and the cells were pelleted and washed with 40 ml of water. Cells were suspended in 5 ml of 0.5 M NaCl/0.2 M Tris, pH 7.5/0.01 M EDTA/1% $NaDodSO_4$. Five milliliters of phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol) and 10 g of glass beads were added, and the mixture was agitated with a vortex mixer for 2–2½ min at room temperature. The mixture was centrifuged and the aqueous phase was reextracted with phenol/chloroform/isoamyl alcohol until the interface was clean. The RNA in the aqueous phase was precipitated overnight at −20°C with 2 vol of 95% ethanol/0.05% diethylpyrocarbonate. RNA was enriched for poly(A)$^+$ sequences by a single passage over poly(U)-Sepharose as described by St. John and Davis (13).

Gel Transfer and Hybridization. RNA was fractionated by electrophoresis in methylmercury hydroxide agarose gels, transferred to diazobenzyloxymethyl-paper (DBM-paper), and hybridized with ^{32}P-labeled DNA as described by Alwine *et al.* (14). Yeast colony hybridizations were performed according to Hinnen *et al.* (15). Unless otherwise noted, all autoradiographs were exposed on X-Omat AR5 film at −70°C with Dupont

Abbreviations: kb, kilobases; DBM-paper, diazobenzyloxymethyl-paper; bp, base pair(s).

Lightning Plus intensifier screens.

For use as a hybridization probe, DNA was labeled with [α-^{32}P]dCTP to a specific activity of 5×10^8 cpm/μg by nick translation (16) or by reverse transcription using random calf thymus DNA primers (17). The primers (100 μg) and 0.1 μg of the fragment to be labeled were denatured together in 5 μl of 0.1 M NaOH, neutralized with 0.5 μl of 1.8 M Tris (pH 7.4), and added directly to the reverse transcription reaction mixture.

RESULTS

Construction and Screening of the Plasmid Library. The hybrid yeast–bacterium plasmid YEp13 (7) which contains the bacterial ampicillin- and tetracycline-resistance genes and the yeast *LEU2* gene were used for construction of a yeast genomic library as described by Nasmyth and Reed (9). DNA from 95-4A, which contains the wild-type allele of *GAL4*, was partially digested with *Sau*3A endonuclease and ligated into the single *Bam*HI site in the tetracycline-resistance gene of YEp13. This mixture was used to transform *E. coli* strain C600, selecting for ampicillin resistance. Plasmid DNA was prepared from a pool of 13,000 ampicillin-resistant colonies which constituted the "library" of plasmid DNA and was used to transform yeast spheroplasts from strain S32A (*α gal4-4 leu2-3 2-112*) (18). Selection for Leu$^+$ and Gal$^+$ should yield the desired clone with *LEU2* from the vector and *GAL4* from the insert. However, simultaneous selection for both markers was unsuccessful. Apparently, spheroplasts do not regenerate in the absence of an immediately usable carbon source (glucose). Therefore, the spheroplasts were first regenerated for 2 days on medium lacking leucine but with 2% glucose, to select for Leu$^+$. The microcolonies were then overlaid with the same medium with 2% galactose in place of glucose to select for Gal$^+$ colonies. Approximately 10^4 Leu$^+$ transformants yielded 2 Gal$^+$ Leu$^+$ colonies which, after purification, were designated strains G202 and G501.

Both strains G202 and G501 coreverted to Leu$^-$ and Gal$^-$ at a high frequency when grown on medium containing leucine and glucose, suggesting that *LEU2* and *GAL4* might be carried together on an unstable plasmid, as expected. These plasmids were rescued by using DNA prepared from G202 and G501 to transform *E. coli* C600 to ampicillin resistance. Plasmid DNA from the resulting ampicillin-resistant transformants was tested for the ability to transform yeast S32A to Leu$^+$ Gal$^+$, and high frequency cotransformation (10^4/μg of DNA) was observed in both cases. Thus, the initial G202 and G501 strains carried plasmids capable of replicating in both *E. coli* and yeast and capable of complementing the *gal4-4* mutation. Restriction endonuclease mapping (Fig. 1) and Southern transfer DNA hybridization analysis (data not shown) revealed that these plasmids, named YEpG202 and YEpG501, consisted of the YEp13 vector with 20- and 15-kilobase (kb) inserts, respectively, and that the two inserts contained 4 kb of overlapping homologous sequences which, we presume, contain *GAL4*.

Subcloning and Mapping. Subclones derived from YEpG202 and 501 were obtained by ligation of *Sau*3A partial digests of these plasmids to *Bam*HI-digested YEp13 followed by direct transformation of yeast S32A. Leu$^+$ Gal$^+$ transformants were selected as before and those with the smallest inserts as determined by hybridization to Southern transfers of subclone DNA (data not shown) were chosen for further study. Plasmids from these strains were transformed back into *E. coli* C600 and purified for restriction endonuclease mapping. Fig. 1 shows the maps of the inserts of the smallest subclones obtained. All contained a 3.2-kb region delimited by the left end of YEpG525 and the right end of YEpG528. This 3.2-kb region should contain the entire *GAL4* gene.

To map the limits of *GAL4* further, two deletions of

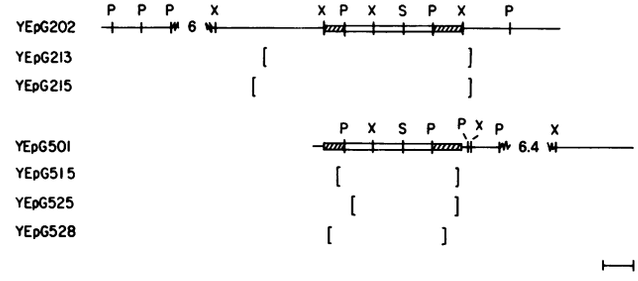

FIG. 1. Restriction maps of the yeast insert DNA from *GAL4* clones and subclones. Subclone maps are denoted by brackets below the clone from which they were derived. Sequence homology between YEpG202 and YEpG501 is shown as an open bar; the hatched bar indicates uncertainty as to the positions of the ends of this homology. The numbers in the gaps are length in kb. P, *Pvu* II; S, *Sal* I; X, *Xho* I. Scale = 1 kb.

YEpG525 were constructed *in vitro* as shown in Fig. 2. YEpG4dP was constructed by fusing the *GAL4* and pBR322 *Pvu* II sites such that yeast sequences distal to the *Pvu* II site were deleted. YEpG4dH was constructed by fusing the two *Hpa* I sites in the insert, resulting in the deletion of about 400 base pairs (bp). Both deletion plasmids failed to complement *gal4-4* when transformed into yeast S32A. It follows that the end points of *GAL4* must lie between the *Pvu* II site and the YEpG528 insert junction at one end and between the proximal *Hpa* I site and the YEpG525 insert junction on the other. This 1.6- to 3.2-kb region spans the center of the yeast DNA insert of YEpG525.

The restriction maps of YEpG202 and 501 diverge outside of the 4-kb region of common homology. Genomic restriction digestion patterns (data not shown) corresponded to the map of YEpG501 and not to that of YEpG202. It is likely that, in the construction of the YEp13 yeast library, many noncontiguous *Sau*3A fragments were ligated together to form plasmid inserts with restriction maps not found in the yeast genome. This is the probable origin of plasmid YEpG202.

Genetic Identity of the *GAL4* Clone. To verify that the clones that complemented the *gal4-4* mutation contained the bona fide *GAL4* gene (instead of a gene that fortuitously conferred a Gal$^+$ phenotype) a nonreplicating *GAL4* plasmid was allowed to integrate into the yeast genome under conditions that should favor homologous recombination and was then mapped genetically. pG525 (Fig. 2), which contains only pBR322 and the putative *GAL4* region sequences, was used to transform S32A selecting for Gal$^+$. Because a cut in that region of the plasmid DNA which is homologous to a portion of the yeast genome results in a higher frequency of integration (19), the plasmid was first digested with *Xho* I which cleaved the plasmid once and in the *GAL4* sequence. Gal$^+$ transformants were obtained at a frequency of 100/μg of DNA. As a control, transformation of S32A with 10 μg of pBR322 yielded no Gal$^+$ transformants.

The site of plasmid integration was mapped for one transformant. This strain (*α leu2-3 2-112 gal4-4 pBR322 GAL4*) was mated with 95-4B (*a his5-2 ade6 leu1-2*). A Leu$^+$ diploid was isolated, sporulated, and dissected. All 17 tetrads analyzed were of the parental configuration with respect to *GAL4*. That is, all spores were Gal$^+$, indicating tight linkage between the integrated *GAL4* plasmid allele and the *gal4-4* mutant allele. Linkage of pBR322 sequences to the plasmid *GAL4* allele in the integrant was demonstrated in a cross with a *gal4* strain. R440-8C (*a gal4-6 gal80-1 leu1-1 his5-2*) was mated with the integrated *GAL4* transformant, and a Leu$^+$ diploid was selected, sporulated, and dissected. Colony hybridization (15) was done on the tetrads by using pBR322 as a probe. All markers segregated 2:2, and pBR322 sequences were tightly linked to the *GAL4* allele

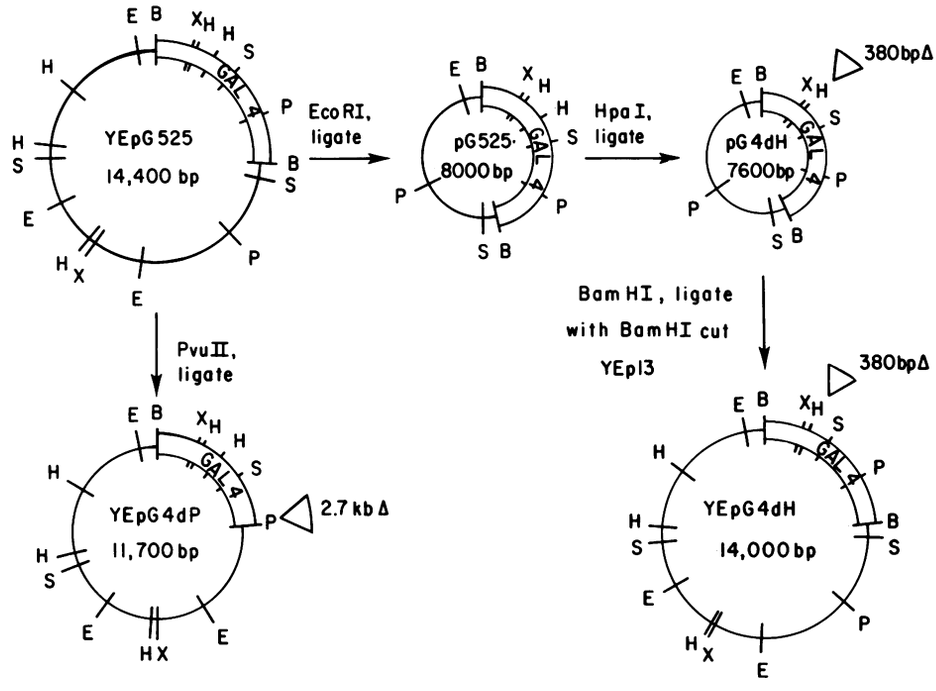

FIG. 2. Construction of plasmids pG525, YEpG4dH, and YEpG4dP. YEpG525 is YEp13 with a 3.7-kb *Sau*3A fragment of *GAL4* inserted which regenerates *Bam*HI sites. *Eco*RI digestion of YEpG525 removed yeast 2-μm plasmid and *LEU2* sequences to yield pG525. *Pvu* II digestion removed a portion of *GAL4* and most of the tetracycline-resistance gene to give YEpG4dP. YEpG4dH contains a 400-base-pair (bp) deletion of *GAL4* sequences between *Hpa* I sites. B, *Bam* HI; E, *Eco* RI; H, *Hpa* I; P, X, and S, as in Fig. 1.

because all 15 tetrads analyzed were of the parental configuration for *GAL4* and pBR322 sequence homology. This mapping demonstrates that the insert DNA of pG525 is homologous to the *GAL4* locus.

GAL4 Region Transcripts. In order to investigate transcription of the *GAL4* region, cloned *GAL4* DNA was used as a hybridization probe to detect homologous RNAs which had been transferred to DBM-paper after size fractionation by electrophoresis in 1.2% agarose gels containing methylmercury hydroxide. We assumed that the *GAL4* transcripts must map entirely within the 3.2-kb region between the left end of YEpG525 and the right end of YEpG528 because these positions delimit the smallest region known to complement a *gal4* mutation. The *GAL4* transcript must also be homologous to most or all of the 1.6-kb region between the *Pvu* II site and the proximal *Hpa* I site because deletions constructed *in vitro* (Fig. 2) that fail to complement *gal4-4* demonstrate that this region is within *GAL4*. Plasmid pG525 was divided into five fragments to use as hybridization probes for mapping. The ^{32}P-labeled fragments numbered I–V were used to probe poly(A)$^+$RNA from 95-4A (wild type) or G525 (*GAL4*-transformed) cells grown in medium lacking leucine and containing galactose.

First, consider the transcripts appearing in the wild-type lanes of Fig. 3*B*. A 2.8-kb transcript is homologous to fragment III and to adjacent fragments II and IV. A 1.8-kb fragment is homologous to fragment II and possibly to fragment III. This suggests that these 1.8- and 2.8-kb transcripts overlap at the junction of II and III. A more likely possibility is that the apparent weak hybridization of fragment III to the 1.8-kb species is due to slight contamination of the probe with fragment II. Because the 1.8-kb transcript has little if any detectable homology to fragment III and is 700 bp longer than fragment II (1.1 kb) we conclude that this transcript must extend 700 bp or more off of the left side of the restriction map in Fig. 3*A*.

The 1.8-kb RNA cannot be the *GAL4* transcript because 40% of its length is homologous to DNA outside of *GAL4* (as defined above). In contrast, the 2.8-kb RNA is homologous to the center of *GAL4* and is large enough to cover the 1.6-kb region known to be necessary for complementation. If the complete *GAL4* gene is necessary for complementation, the entire 2.8-kb transcript should map to the left of the YEpG528 end point denoted by the arrow in Fig. 3*A*. This would place the left end of the transcript at least several hundred base pairs to the left of the *Hpa* II site at the junction of fragments II and III. This positioning is consistent with the limited hybridization of fragment II to the 2.8-kb transcript. The approximate positions of the 2.8-kb *GAL4* mRNA and the nearby 1.8-kb transcript are shown in Fig. 3*A*.

Anticipating the possibility of overproduction of *GAL4* mRNA, we examined the transcription pattern of the *GAL4*-transformed strain G525. Fragment V, constituting the pBR322 sequences to the right of the *GAL4* insert, is homologous to the 1.8- and 2.8-kb species in the samples from *GAL4*-transformed cells (Fig. 3*B*). These are abundant species because only 1/50th the amount of RNA was compared to the wild type samples in Fig. 3*B*, and at this level the pG525 insert-specific RNAs were not detected (fragments II, III, and IV). Note that fragment V, containing only pBR322 sequences, shows no homology to any transcripts in wild type yeast. Thus, the abundant 1.8- and 2.8-kb transcripts present in the *GAL4*-transformed strain are transcribed from pBR322 sequences of the *GAL4* plasmid (YEpG525) contained in that strain and it is only coincidental that these RNAs are almost the same size as the 2.8-kb *GAL4* mRNA and the adjacent 1.8-kb transcript. The transcription of pBR322 sequences in yeast has been reported (20).

The *GAL4* region transcripts of the *GAL4*-transformed strain can be seen in Fig. 3*C*. Here, all lanes contained 50 times more RNA than did the samples from *GAL4*-transformed cells in Fig. 3*B*. The 2.8-kb *GAL4* mRNA is apparently overproduced about 15-fold compared to wild type. Part or all of this more-intense 2.8-kb band conceivably could be due to contamination of the hybridization probe with pBR322 sequences and not from transcription of plasmid copies of the *GAL4* gene. However, both the 1.8- and 2.8-kb species should be equally affected, but this

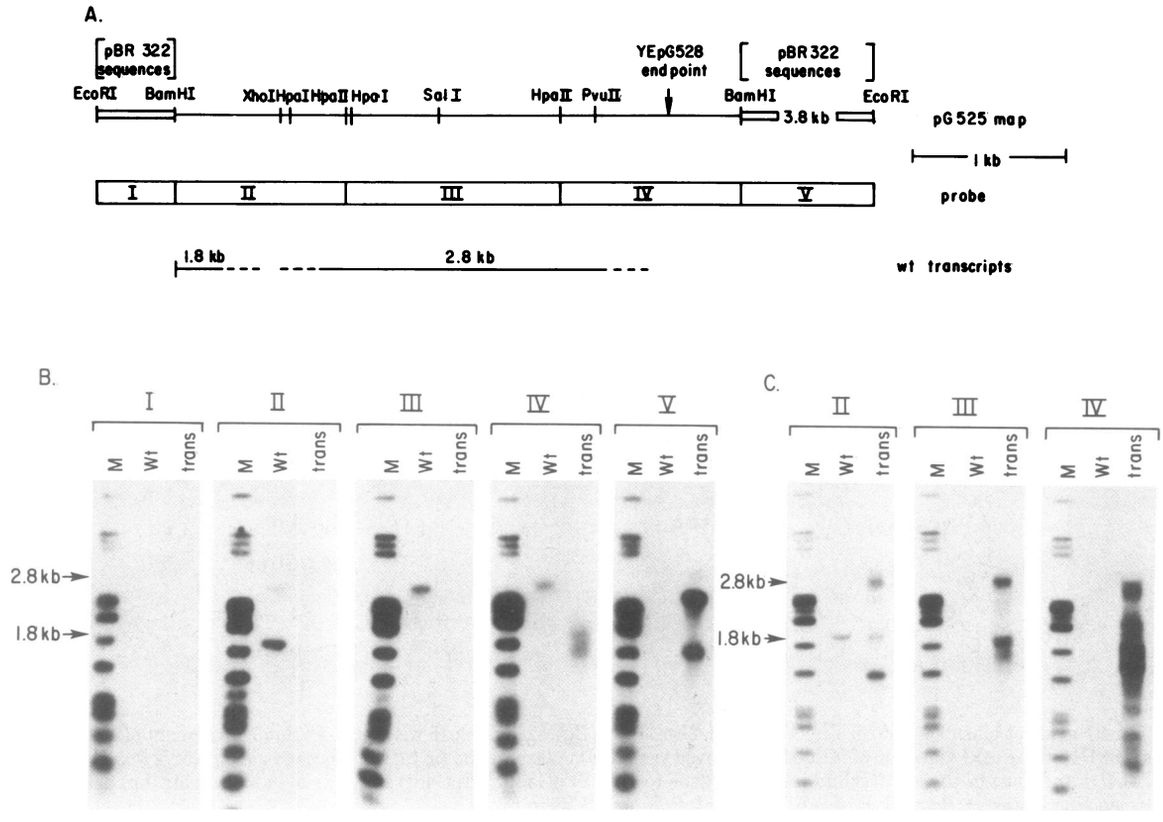

FIG. 3. Mapping of *GAL4* region transcripts. (*A*) Position of the 1.8- and 2.8-kb transcripts on the restriction map of the 3.7-kb yeast DNA insert of pG525. The precise end points of the RNAs are uncertain as indicated by the dashed lines. The positions of restriction fragments I, II, III, IV, and V used as hybridization probes are shown. ↓, Righthand end of yeast sequences in the plasmid YEpG528; wt, wild type. (*B* and *C*) Pattern of hybridization of fragments I, II, III, IV, and V to poly(A)$^+$RNA from 95-4A and G525 grown on media containing galactose and lacking leucine. Poly(A)$^+$RNA was fractionated by electrophoresis on a methylmercury hydroxide 1.2% agarose gel and transferred to DBM-paper. Identical transfers were hybridized with ^{32}P-labeled fragments I–V as indicated. Gel lanes were loaded as follows: M, *Hpa* I-digested phage T7 DNA labeled by incubation with [α-^{32}P]ATP and T4 polynucleotide kinase; Wt, 5 μg of poly(A)$^+$RNA from yeast 95-4A; trans, 0.1 μg of poly(A)$^+$RNA from yeast G525 in *B* and 5 μg of the same RNA in *C*. All fragments were purified by electrophoresis in SeaPlaque gels and labeled with [α-^{32}P]dCTP by reverse transcriptase extension of random calf thymus DNA primers to a specific activity of 5×10^8 cpm/μg. Exposures; *B*, 6 days; *C*, 3 days.

was not the case because hybridizations with fragment II (Fig. 3*C*) gave 1.8-kb bands of intensities equal in wild type and *GAL4*-transformed lanes, both probably due to transcription from the chromosomal copy of this unidentified gene.

To determine whether *GAL4* is expressed constitutively or if it is regulated at the transcriptional level by galactose induction, we measured *GAL4* mRNA levels in induced and noninduced cells. Fig. 4 shows a DBM-paper transfer of poly(A)$^+$RNA (fractionated as before) hybridized to ^{32}P-labeled *GAL4* and *URA3* DNAs. The galactose and glycerol lanes contained 1.6 and 2.9 times as much *GAL4* mRNA as did the glucose lane. There actually was more *GAL4* mRNA present during growth on a noninducing carbon source (glycerol) than during growth on galactose. This shows that induction of *GAL1*, *-7*, and *-10* does not result from increased transcription of *GAL4*. RNA from a *gal80* strain grown on YEP glycerol is shown in lane E. There was 0.69 times as much *GAL4* mRNA present in the *gal80* lane as in the wild-type glycerol-grown lane. The approximately equivalent levels of *GAL4* mRNA in the noninduced and induced strains is consistent with *GAL80* regulation of the activity and not the synthesis of the *GAL4* gene product. There also is no evidence for any influence of *GAL4* on its own synthesis because a *gal4* mutant (lane F) contained about the same level of *GAL4* mRNA as did the wild type (lane B) and *gal80* strains (lane E). Glucose caused a slight repression of *GAL4* synthesis as can be seen by the lower levels in lane A and in lane D.

In Fig. 4, levels of *URA3* mRNA (≈900 bp) varied as much as 2-fold depending upon the carbon source used. Normalization of the *GAL4* mRNA levels to those for *URA3* altered the results quantitatively but not qualitatively. It has been reported that *URA3* constitutes 1.3×10^{-4} of yeast mRNA (21). From

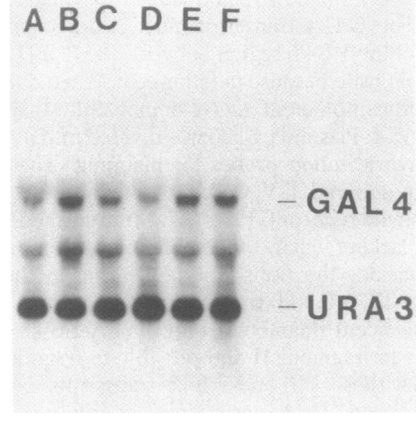

FIG. 4. *GAL4* region transcripts. Poly(A)$^+$RNA (5 μg per lane) from yeast cells grown on YEP plus a carbon source was fractionated by electrophoresis in a methylmercury hydroxide 1.2% agarose gel, transferred to DMB-paper, and hybridized with pG525 insert DNA and a fragment internal to the *URA3* gene, both labeled to 5×10^8 cpm/μg by reverse transcriptase extension of random calf thymus DNA primers with [α-^{32}P]dCTP. Lanes: A, 95-4A, glucose; B, 95-4A, glycerol; C, 95-4A, galactose; D, 95-4A, glucose plus galactose; E, R171-11c (*α gal80-1 arg4-17 his5-2*), glycerol; F, S32A (*α gal4-4 leu2-2 2-112*), glycerol. The exposure was for 24 hr.

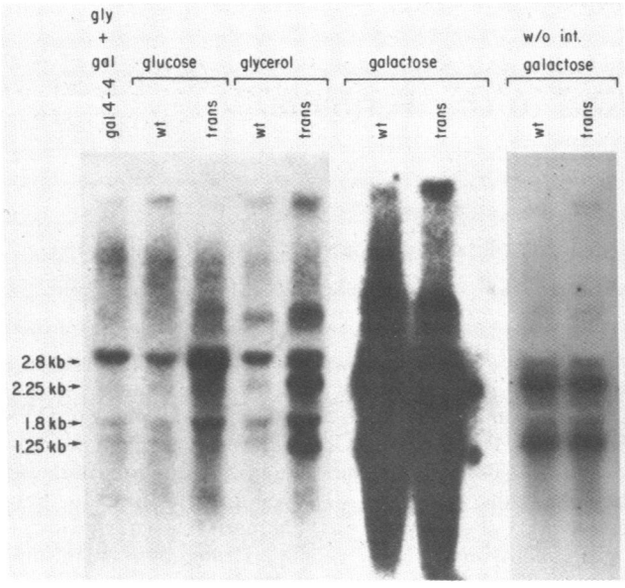

FIG. 5. Transcription of the *GAL7* and *-10* genes in a *GAL4*-transformed strain. wt, Wild type; trans, *GAL4*-transformed. Total cellular RNA (10 μg per lane) was fractionated by electrophoresis in methylmercury hydroxide/1.2% agarose gels and transferred to DBM-paper. The filters were hybridized with a *GAL7*- and *GAL10*-containing 5.4-kb *Eco*RI fragment from the plasmid Sc4811 labeled to a specific activity of 5×10^8 cpm/μg by nick-translation with [α-^{32}P]dCTP. RNAs were prepared from yeast strains grown on media lacking leucine and containing the indicated carbon source. Lanes were loaded with RNA from the following yeast strains: gal4-4, S32A; wt, 95-4A; trans, G525. The film was exposed for 21 hr. A duplicate exposure (last two lanes at right) of the galactose lanes was made without intensifying screens to show clearly the 1.25-kb *GAL7* and 2.25-kb *GAL10* transcripts. The galactose-induced samples were run separately from the glucose and glycerol samples, ruling out the possibility that the bands appearing in the *GAL4*-transformed strains are due to spillover from the galactose lanes.

comparison with *URA3*, we calculate *GAL4* mRNA to be 1.6×10^{-5} of the mRNA during growth on galactose.

Transcription of the *GAL1*, *-7*, and *-10* Genes. Johnston and Hopper (10) found that strains that contain *GAL4* carried on a plasmid also have increased levels of α-galactosidase and uridyl transferase. In confirmation of this, we found that the *GAL7* and *GAL10* mRNAs are partially induced in a *GAL4*-transformed strain (Fig. 5). G525, the *GAL4* plasmid-containing strain, was derived by transformation of S32A with the plasmid YEpG525. The *GAL4*-transformed strain was induced by galactose to the same degree as the wild type strain but not by growth on glucose. However, when grown on glycerol, the *GAL4*-transformed strain expressed these genes at a level intermediate between noninduced and fully induced. The *GAL4*-transformed strain grown on glycerol had less *GAL7* and *GAL10* mRNA, by a factor of 0.05 to 0.04, than did the same strain or wild type grown on galactose. It has been estimated that *GAL1*, *-7*, and *-10* genes are induced 1,000-fold by galactose (5). The *GAL7* and *GAL10* mRNAs then are present in about 40- to 50-fold higher amounts in the *GAL4*-transformed strains than in wild type when these strains are grown under noninducing conditions on glycerol. The *GAL7* and *GAL10* mRNAs were not detectable in glucose-grown *GAL4* transformed or wild type cells.

DISCUSSION

As a first step toward characterizing the *GAL4* protein we were interested in identifying the *GAL4* mRNA. Two poly(A)$^+$RNAs, 1.8 and 2.8 kb, are homologous to our smallest complementing *GAL4* subclone. The 2.8-kb transcript maps across the center of *GAL4* whereas the 1.8-kb transcript extends outside of *GAL4* to the left as oriented in Fig. 3A. Therefore, we conclude that the 2.8-kb species is the *GAL4* transcript. At 2.8 kb, the *GAL4* message could encode a protein as large as 105,000 daltons.

Douglas and Hawthorne (1) proposed that regulation of *GAL1*, *-7*, and *-10* was due to modulation of *GAL80* repression of *GAL4* synthesis. Recent evidence suggests that the *GAL4* protein is synthesized constitutively and not under the control of *GAL80* (3, 4). In agreement with this, we found that the *GAL4* mRNA is present in noninduced cells in approximately the same concentrations as in induced cells. In glycerol-grown cells (noninduced) there is actually more *GAL4* mRNA than there is in galactose-grown cells. Based on comparisons with *URA3* mRNA [1.3×10^{-4} poly(A)$^+$RNA (21)] we estimate the level of *GAL4* mRNA in galactose-grown cells to be 1.6×10^{-5} of total mRNA [$\approx$0.1 mRNA per cell (22)]. The low abundance of *GAL4* mRNA is consistent with the function of *GAL4* protein as a regulatory protein sufficient at low concentrations.

Using cloned copies of the *GAL4* gene, Johnston and Hopper (10) found that increased *GAL4* gene dosage results in higher-than-normal levels of several galactose pathway enzymes. We have confirmed this for the *GAL7* and *GAL10* mRNAs and, in addition, have found that *GAL4* mRNA appears to be overproduced 15-fold in a *GAL4* plasmid-containing strain. It seems likely that *GAL80* repressor can be titrated by excess *GAL4*, resulting in the partial induction of the *GAL7* and *GAL10* genes.

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