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# Regulation of the galactose pathway in *Saccharomyces cerevisiae*: Induction of uridyl transferase mRNA and dependency on *GAL4* gene function

(*in vitro* translation/immunoprecipitation/*GAL* gene cluster/positive regulation)

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**ABSTRACT** In *Saccharomyces cerevisiae*, utilization of galactose requires four inducible enzyme activities. Three of these activities (galactose-1-phosphate uridyl transferase, EC 2.7.7.10; uridine diphosphogalactose 4-epimerase, EC 5.1.3.2; and galactokinase, EC 2.7.1.6) are specified by three tightly linked genes (*GAL7, GAL10,* and *GAL1,* respectively) on chromosome II, whereas the fourth, galactose transport, is specified by a gene (*GAL2*) located on chromosome XII. Although classic genetic analysis has revealed both positive and negative regulatory genes that coordinately affect the appearance of all four enzyme activities, neither the basic events leading to the appearance of enzyme activities nor the roles of the regulatory genes have yet been determined. Regulation of inducible enzyme activity could be mediated by events related to transcription, translation, or enzyme activation. For the purpose of studying galactose pathway induction and its regulation, we have developed an immunoprecipitation assay that enables us to detect the *GAL7* specified uridyl transferase polypeptide in yeast extracts and among the polypeptides synthesized in an RNA-dependent *in vitro* translation system. Use of this immunoprecipitation assay in conjunction with *in vivo* labeling experiments demonstrates the presence of [$^3$H]leucine-labeled transferase in extracts prepared from cells grown in galactose but not from cells grown in glucose. This galactose-specific induction of transferase polypeptide is mediated by the *de novo* appearance of a functional mRNA species whose synthetic capacity is detectable by the combination of *in vitro* translation and immunoprecipitation. The appearance of functional transferase mRNA depends on wild-type expression of the regulatory gene, *GAL4.* Cells carrying a nonsense mutation in the *GAL4* gene fail to produce the transferase mRNA, whereas a nonsense suppressor of the *GAL4* mutant regains the galactose-specific mRNA response. Our results establish that the induction of the *GAL7* specified uridyl transferase activity is mediated by *de novo* appearance of a functional mRNA and that this galactose-specific response is dependent on a wild-type *GAL4* gene product.

In the simple eukaryote, *Saccharomyces cerevisiae*, the utilization of carbon from exogenously provided galactose requires the expression of an inducible enzyme system consisting of a specific galactose transport activity (1, 2) and the Leloir pathway enzymes galactokinase (EC 2.7.1.6), galactose-1-phosphate uridyl transferase ("transferase," EC 2.7.7.10), and uridine diphosphogalactose 4-epimerase ("epimerase," EC 5.1.3.2) (3–6). The conversions catalyzed by these inducible enzymes produce glucose 1-phosphate which is then converted to glucose 6-phosphate by a constitutively produced isoenzyme activity of phosphoglucomutase (EC 2.7.5.1) (7, 8). Carbon originally in the form of galactose is thus made available for subsequent glycolysis.

Genetic control of the inducible galactose pathway enzymes involves the four structural genes *GAL1, GAL10, GAL7,* and *GAL2* and four regulatory genes, *GAL4, GAL81* (*c*), *GAL80* (*i*), and *GAL3.*‡ Mutations in *GAL1, GAL10, GAL7,* and *GAL2* affect the individual appearance of galactokinase, epimerase, transferase, and galactose transport activities, respectively (6). Mutations defining the *GAL1, GAL10,* and *GAL7* genes have invariably been recessive, and they map in three tightly linked complementation groups near the centromere of chromosome II (6, 9, 10). Mutations identified at the *GAL2* locus are also recessive and map on chromosome XII (6, 10).

Of the four regulatory genes *GAL4, GAL81, GAL80,* and *GAL3,* only *GAL3* has been mapped relative to known centromere-linked genes (10). *GAL4* and *GAL81* are tightly linked to each other, but all other pairwise combinations among the four regulatory genes indicate no linkage to one another or to any of the galactose pathway structural genes (6, 11).

The *GAL4* gene is defined by pleiotropic recessive mutations resulting in a coordinate loss of all four inducible enzyme activities (6, 11). More recently it has been shown that the *GAL4* gene controls inducibility of a fifth enzyme activity, an α-galactosidase (EC 3.2.1.22), which is required for utilization of melibiose (12). The *GAL4* locus probably codes for a polypeptide because some *gal4* mutations are suppressible by unlinked nonsense suppressors (D. Hawthorne, personal communication). The expression of the *GAL4* gene appears to be under the direct control of the tightly linked *GAL81* region and indirectly under the control of the unlinked *GAL80* gene (11, 13, 14). The *GAL81* region is characterized by a mutation, $GAL81^C$ (designated *C* by Douglas and Hawthorne), that results in constitutive production of the galactose pathway enzyme activities (11). This constitutive phenotype is expressed only when the $GAL81^C$ mutation occurs in a *cis* configuration with respect to a functional *GAL4* gene (11). The *GAL80* locus is defined by three allelic configurations: *GAL80,* inducible; *gal80,* constitutive; and $GAL80^S$, noninducible (*gal80* is recessive to *GAL80* and $GAL80^S$ is dominant to *GAL80*) (13, 14). The *cis* dominant mutation $GAL81^C$ is epistatic to the $GAL80^S$ allele (14). Although suppressible (nonsense) mutations occur in the *GAL80* gene (14), none has been found in the *GAL81* region (D. Hawthorne, personal communication), thus suggesting that the *GAL80* gene, but not the *GAL81* region, codes for a polypeptide. The *GAL3* gene is defined by recessive mutations that produce an increase in the time required for induction of galactose pathway enzyme activities (11, 15, 16).

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

‡ The *c* and *i* designations will be replaced by *GAL81* and *GAL80,* respectively, in order to conform to the standard three-letter nomenclature.

Douglas and Hawthorne (11) have proposed a model for control of galactose pathway gene expression based in part on the Jacob–Monod operon concept of genetic regulation in bacteria. The Douglas–Hawthorne model proposes that the *GAL80* gene encodes a repressor protein whose site of action is the *GAL81* region. The *GAL81* region is conceived to be essentially like a bacterial operator region in that it is the site of repressor recognition and controls the expression of a contiguous gene, in this case the *GAL4* gene. The *GAL4* gene product is viewed as a diffusible positive intermediary that controls the synthesis of the galactose enzymes at the level of mRNA synthesis or at some subsequent step in protein synthesis.

Confirmation of the Douglas–Hawthorne model would provide support for the notion that eukaryotic cells utilize genetic control circuits similar to those well known in prokaryotes. A first step toward a test of the model requires direct evidence that inducibility and control of inducibility in the galactose system is based on the appearance of new mRNA species. We provide evidence here that induction of *GAL7* specified uridyl transferase activity is based on *de novo* synthesis of uridyl transferase polypeptide and on the *de novo* appearance of a uridyl transferase mRNA species detectable by an *in vitro* translational assay. Data are also presented that indicate that the appearance of this mRNA species is under the control of the *GAL4* gene.

## MATERIALS AND METHODS

**Yeast Strains and Media.** Strain 108-3c, *a, trp1, ura1,* is a haploid wild-type galactose fermenter obtained from Howard Douglas' stock collection. Strain 3430-3c obtained from D. C. Hawthorne is a haploid galactose nonfermenter carrying an amber(*a*) nonsense mutation in *GAL4*; its genotype is *gal4* (*a*), *mal, his, trp2, met8*(*a*), *ade5,7*(*a*), *lys2, tyr7*(*a*). Strain 3430-3c-R2S is a spontaneous galactose-fermenting revertant of 3430-3c. Strain 3665-1c, a haploid carrying an ochre (*o*) nonsense mutation in *GAL7*, was obtained from Donald Hawthorne. The amber mutations and ochre mutation in these strains have been shown to be suppressed by standard UAG (SUP-a) or UAA (SUP-o) suppressors, respectively (D. Hawthorne, personal communication).

YEP medium contained 10 g of Bacto-yeast extract and 20 g of Bacto-peptone per liter. YEPD, YEP-galactose, and YEP-acetate, were prepared by the addition of dextrose, galactose, or acetate, respectively, to YEP to yield a final 2% (wt/vol) solution of the sugar. YEP-ethanol was prepared by addition of absolute ethanol to YEP to yield a final 2% (vol/vol) solution of ethanol. Complete defined media contained (per liter of final solution): 6.7 g of yeast nitrogen base without amino acids, 30 mg of leucine, 300 mg of threonine, 20 mg of tyrosine, 20 mg of methionine, 20 mg of histidine, 20 mg of arginine, 30 mg of lysine, 20 mg of phenylalanine, 25 mg of tryptophan, 20 mg of adenine, 10 mg of uracil, and 20 g of a carbon source.

**Materials.** L-[$^{35}$S]Methionine at 400 Ci/mmol was obtained from Amersham/Searle. L-[$^{3}$H]Leucine at 55 Ci/mmol was obtained from New England Nuclear. The sodium salts of ATP and GTP were obtained from Boehringer Mannheim, and phosphocreatine was from Gibco. Phosphocreatine kinase and spermidine (free base) were obtained from Sigma. Commercial raw wheat germ was a gift from Ray Erikson and came originally from the Peavey Co., Denver, CO. Goat antiserum raised against rabbit IgG was obtained from Miles Laboratory. Sephadex G-25 was purchased from Pharmacia Fine Chemicals. Acrylamide and *N,N'*-methylene bisacrylamide (electrophoresis grade) were purchased from Bio-Rad Laboratories. BDH specially pure sodium dodecyl sulfate ($NaDodSO_4$) was purchased from Gallard Schlesinger. All inorganic salts were of ACS analytical grade.

**Selection of *GAL4* Revertants.** Stationary phase cells of strain 3430-3c grown in YEPD were washed, resuspended in water, and plated on YEP-galactose plates at $9 \times 10^5$ cells per plate. Large colonies appearing within 4–7 days at 30° were transferred to YEPD plates, allowed to grow at 30°, and finally replica-plated to YEP-galactose plates and to defined media plates containing glucose but lacking tryptophan, adenine, tyrosine, methionine, or lysine. Candidates for amber-suppressing mutants were those that displayed growth on YEP-galactose plates or on plates lacking methionine, adenine, or tyrosine, respectively, but no growth on plates lacking tryptophane or lysine. These candidates were streaked for single colonies on YEP-galactose plates and retested as above. The suppressed revertant, 3430-3c-R2S, of 3430-3c (*gal4* amber) was chosen from these tests.

**Purification of Galactose-1-Phosphate Uridyl Transferase.** Galactose-1-phosphate uridyl transferase was purified from cells of strain 108-3c grown in YEP-galactose by using a slightly modified version of a procedure to be described in detail elsewhere.

**Preparation of Immune Serum and the Gamma Globulin Fraction.** The purified uridyl transferase sample was adjusted to 50 mM $Na_2HPO_4/NaH_2PO_4$ (pH 7.2) and 150 mM NaCl and an aliquot was mixed with an equal volume of complete Freund's adjuvant. The resultant mixture was emulsified by sonication and an aliquot of this emulsion containing 100 μg of protein was injected intradermally into four places on the back of a New Zealand White rabbit. Injections (40–50 μg of protein) were repeated 4 wk later. On the 10th day after this booster the rabbit was bled from the ear vein. The gamma globulin fraction was prepared from the serum as described (17, 18).

**Preparation of RNA.** The preparation of polysomal RNA was carried out as described (19). To extract total cellular RNA, the polysome isolation step was omitted. Poly(A)-containing RNA was purified from polysomal RNA by essentially the procedure of Firtel and Lodish (20).

**Preparation and Use of the Wheat Germ Cell-Free Protein-Synthesis System.** Wheat germ extracts were prepared by a modification (21) of several procedures (19, 22–24). Total cellular, polysomal, and poly(A)-containing RNA were routinely assayed in 50-μl reaction mixtures containing [$^{35}$S]methionine and all other reaction components at levels described (21).

Aliquots for the determination of radioactivity in trichloroacetic acid-precipitable material and for sodium dodecyl sulfate/slab gel electrophoresis were processed as described (25).

***In Vivo* Labeling.** Cells were grown in YEP-galactose, YEP-ethanol, or YEP-acetate with shaking at 30°. At a density of $5 \times 10^7$ cells per ml, 2 ml of the culture was quickly filtered on a Millipore filter, washed with $H_2O$, and dispensed into 2 ml of complete medium lacking leucine and containing the appropriate carbon source. [$^{3}$H]Leucine (50 μCi) was added and the cells were incubated with shaking at 30°. At the end of 30 min, the cells were harvested by centrifugation, washed once with cold $H_2O$, resuspended in 1 ml of 10 mM sodium phosphate, pH 7.2/150 mM NaCl, and homogenized with glass beads in the Bronwill homogenizer. Cell debris and glass beads were separated from the supernatant by centrifugation.

**Immunoprecipitation Assay.** *Method A.* This was the double antibody method (indirect method) using goat anti-rabbit gamma globulin. The equivalence point for this reaction

was determined routinely. The details have been described (21)..

*Method B.* In this direct precipitation method using only rabbit antibody, 7–10 μg of purified uridyl transferase in 5 μl was added to 50 μl of cell extract; immediately following, this 10 μl of antiserum containing 250 μg of protein was added, and the reaction mixture was incubated at room temperature for 1 hr. The resulting immunoprecipitates were washed by pelleting through a discontinuous gradient of sucrose as detailed by Palmiter (26). The washed immunoprecipitates were prepared for electrophoresis or for radioactivity determinations.

**NaDodSO$_4$/Slab Gel Electrophoresis and Autoradiography.** Aliquots of the translation reaction mixtures and aliquots of resuspended immunoprecipitates were diluted 1:1 with double-strength electrophoresis sample buffer (27), boiled for 3 min, and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography. Conditions of electrophoresis, gel drying, and autoradiography were essentially those described by Studier (27).

## RESULTS

**Galactose-Induced Synthesis of Uridyl Transferase Polypeptide.** When immunoprecipitates of extracts from cells grown on galactose-containing media in the presence of [$^3$H]-leucine were electrophoresed on NaDodSO$_4$/10% polyacrylamide gels, a major band comigrating with purified uridyl transferase was observed after autoradiography of the dried slab gels (Fig. 1). This band was not observed in the immunoprecipitates of extracts from cells grown on media containing acetate as the sole carbon source rather than galactose. When the trichloroacetic acid-insoluble radioactivity recovered in washed immunoprecipitates was expressed as a percentage of the total radioactivity incorporated in extracts, values of 1% and 0.15% were obtained for galactose- and acetate-grown cell extracts, respectively.

**Galactose-Induced Appearance of Uridyl Transferase mRNA.** Total cellular, polysomal, or poly(A)-containing RNA prepared from cells grown on YEP-galactose, YEP-glucose, or YEP-ethanol was programmed in the wheat germ cell-free translation system in the presence of [$^{35}$S]methionine. After *in vitro* translation, aliquots of the reaction mixtures were used in either the direct or indirect immunoprecipitation assay. Only those immunoprecipitates obtained from translation reactions programmed with RNA from galactose-grown cells revealed the presence of uridyl transferase polypeptide among the *in vitro* synthesized polypeptides (Fig. 2). The uridyl transferase mRNA activity detectable by this method is in the poly(A)-containing yeast mRNA fraction because more than 70% of the activity was bound to poly(U)-Sepharose under conditions in which less than 20% of the ribosomal RNA was bound.

Because of the lack of complete specificity of our double immunoprecipitation assay, several other bands corresponding to radioactive polypeptides synthesized in the *in vitro* translation reaction are seen. The identity of the uridyl transferase band was confirmed by immunocompetition and by use of a known chain termination (nonsense) mutation in the uridyl transferase structural gene. Both galactokinase and uridyl transferase purified from *S. cerevisiae* were tested individually for ability to compete with the candidate transferase band in the immunoprecipitation. Only purified uridyl transferase resulted in the disappearance of the [$^{35}$S]methionine-labeled band observed on NaDodSO$_4$ gel autoradiograms at the position of authentic uridyl transferase (Fig. 3).

The second method of confirmation made use of the fact that chain termination mutations produce shorter than full-length

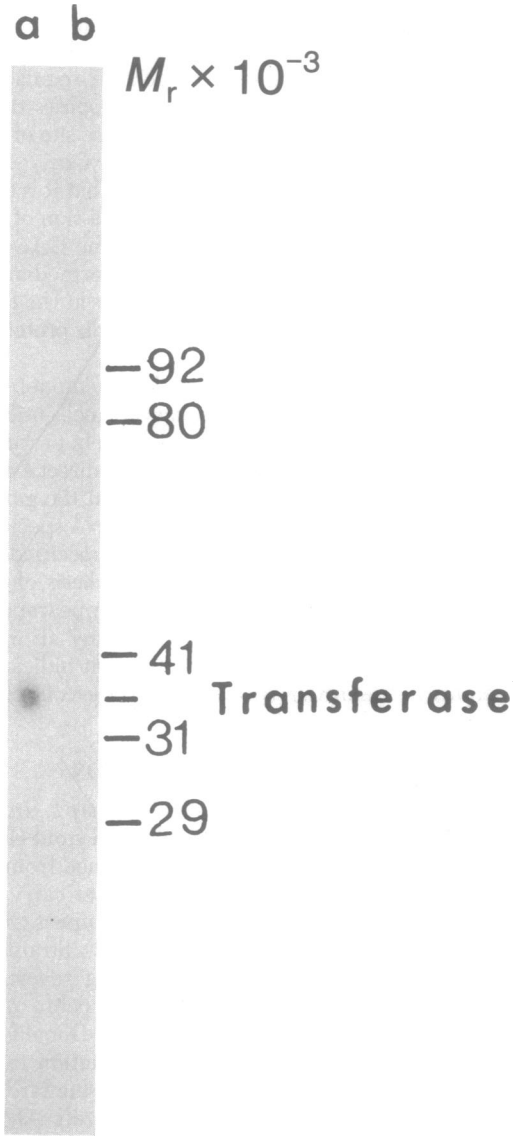


FIG. 1. Radioautogram of a NaDodSO$_4$/10% polyacrylamide gel illustrating the galactose-specific induction of *in vivo* uridyl transferase synthesis. Cellular proteins of strain 108-3c were labeled with L-[$^3$H]leucine in complete medium lacking leucine. Immunoprecipitates were obtained from cell extracts by the direct method (single antibody) and were prepared for electrophoresis by boiling in electrophoresis sample buffer for 3 min. Lanes: a, immunoprecipitate from extracts of galactose-grown cells; b, immunoprecipitate from extracts of acetate-grown cells. Preimmune serum yielded the same result as shown in lane b when used against extracts from either galactose- or acetate-grown cells. The numbers at the right indicate the molecular weights of commercial protein markers. The indicated position of purified uridyl transferase was determined by staining the gel with Coomasie brilliant blue (not shown).

polypeptides that may or may not retain crossreactivity to antibodies produced against the wild-type polypeptide. The uridyl transferase band should either be missing or be shifted to a smaller molecular weight position on the gel when the RNA used to program the cell-free synthesis is extracted from a mutant carrying a nonsense mutation in the known uridyl transferase gene (*GAL7*). The identity of the uridyl transferase band among the immunoprecipitated polypeptides was confirmed by such an experiment (Fig. 3, lanes g and h). As an additional control, 25 nonsense mutations in the *GAL1* gene were tested and shown to have no effect on the uridyl transferase band (not shown).

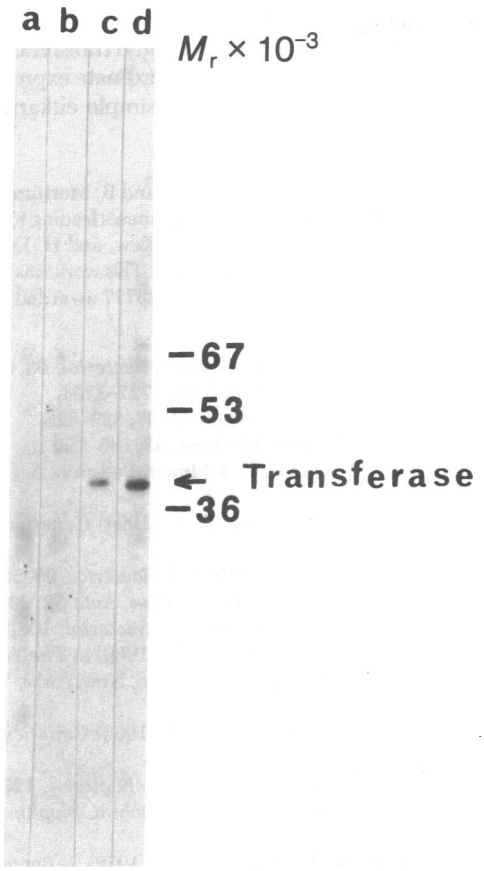


FIG. 2. Radioautogram of a NaDodSO$_4$/10% polyacrylamide gel illustrating galactose-induced appearance of uridyl transferase mRNA activity detectable by *in vitro* translation and immunoprecipitation. Total and poly(A)-containing RNA was prepared from cells of strain 108-3c grown on YEP-galactose or YEP-glucose and programmed in the wheat germ cell-free translation system in the presence of [$^{35}$S]-methionine. Immunoprecipitation of aliquots of the translation reaction mixtures was performed by using either preimmune gamma globulin or gamma globulin raised in rabbits against purified uridyl transferase. The direct method (single antibody) of immunoprecipitation was used. Lanes: a, total RNA from glucose-grown cells (immune gamma globulin); b, total RNA from galactose-grown cells (preimmune gamma globulin); c, total RNA from galactose-grown cells (immune gamma globulin); d, poly(U)-Sepharose-bound poly(A)-containing RNA from galactose-grown cells (immune gamma globulin).

***GAL4* Function Is Required for the Appearance of Galactokinase mRNA.** Because the *GAL4* gene function is required for the induction of galactose enzyme activities, it was of considerable interest to ascertain whether *GAL4* function is required for appearance of functional galactokinase mRNA detectable by the *in vitro* translation and immunoprecipitation assay. For this purpose we used the galactose nonfermenting strain, 3430-3c, known to contain an amber mutation in the *GAL4* gene (D. Hawthorne, personal communication). This mutant was compared to a selected galactose-fermenting revertant (3430-3c-R2S) which was shown to carry an amber suppressor mutation that simultaneously suppressed the *gal4*, *met8*, *ade*, and *tyr7* amber mutations. RNA extracted from separate cultures of 3430-3c and the revertant 3430-3c-R2S grown in YEP-ethanol plus 2% glucose or 2% galactose was programmed in the cell-free translation system in the presence of [$^{35}$S]methionine. Uridyl transferase mRNA activity was detectable only in the RNA sample extracted from the revertant strain grown on galactose (Fig. 4).

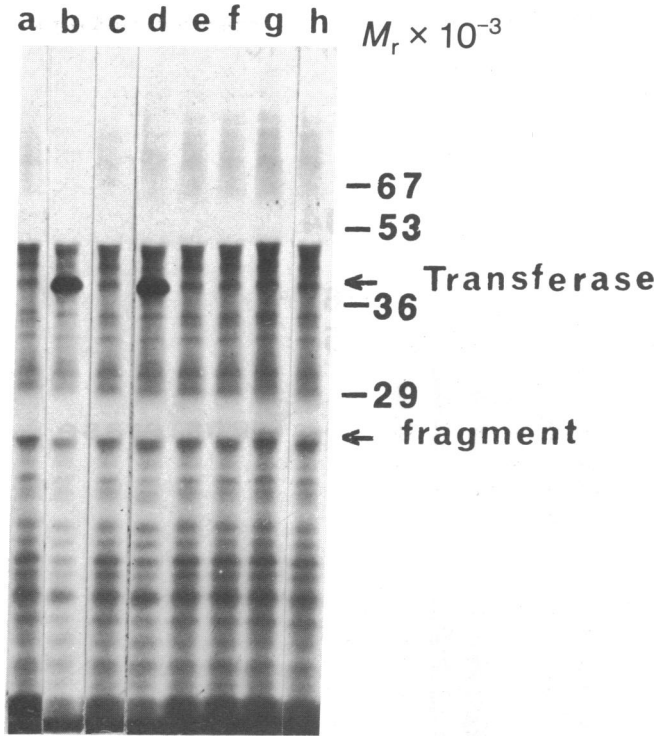


FIG. 3. Radioautogram of a NaDodSO$_4$/10% polyacrylamide gel illustrating the specificity of the induced appearance of uridyl transferase mRNA activity detectable by *in vitro* translation coupled with the indirect method (double antibody) of immunoprecipitation. Polysomal RNA prepared from cells grown in either YEP-glucose or YEP-galactose was programmed in the wheat germ cell-free translation system in the presence of [$^{35}$S]methionine. Aliquots of the translation reaction mixtures were challenged with gamma globulin obtained from rabbits immunized with purified uridyl transferase. The immunocompetition experiments involved the addition of 0.05 $\mu$g of purified uridyl transferase or 0.05 $\mu$g of purified galactokinase to the reaction mixtures immediately prior to the addition of rabbit gamma globulin. Lanes: a, RNA derived from YEP-glucose-grown cells of strain 108-3c; b, RNA derived from galactose-grown cells of strain 108-3c; c, as in b except uridyl transferase was added; d, as in b except galactokinase was added; e and f, RNA derived from strain 3665-1c grown in YEP-ethanol plus glucose; g and h, RNA derived from strain 3665-1c grown in YEP-ethanol plus galactose.

## DISCUSSION

The results presented here provide strong evidence that the induction of the galactose pathway *GAL7* gene product, uridyl transferase, is based on *de novo* synthesis of inducible transferase polypeptide and on *de novo* appearance of a functional transferase mRNA detectable by the *in vitro* translation assay. Similar data (unpublished) have been obtained for the galactokinase enzyme encoded by the *GAL1* gene. The simplest interpretation of these results is that the induction of enzyme activity in the galactose system is mediated primarily by events at the transcriptional or immediate post-transcriptional level rather than primarily at the level of enzyme activation or stabilization. Our results do not distinguish among the following possibilities: induction mediated by transcription initiation, anti-termination of transcription, stabilization of constitutively produced but rapidly degraded transferase transcripts, or processing (maturation) of constitutively produced precursor transcripts. These possibilities cannot be distinguished on the basis of our functional assay (*in vitro* translation coupled with immunoprecipitation) but can be distinguished by use of a physical assay for transferase mRNA sequences.

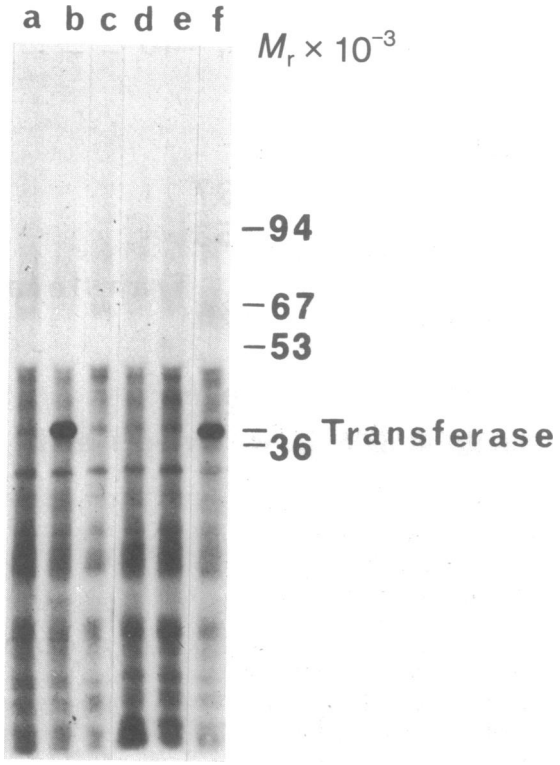


FIG. 4. Radioautogram of a NaDodSO$_4$/10% polyacrylamide gel illustrating the dependency of the galactose-induced appearance of uridyl transferase mRNA activity on wild-type expression of the *GAL4* gene. The general method was identical to that described for Fig. 3. Only gamma globulin raised against purified uridyl transferase was used. The RNA used for *in vitro* translation was total cellular RNA. Lanes: a, strain 108-3c grown on YEP-ethanol plus glucose; b, strain 108-3c grown on YEP-ethanol plus galactose; c, strain 3430-3c grown on YEP-ethanol plus glucose; d, strain 3430-3c grown on YEP-ethanol plus galactose; e, strain 3430-3c-R2S grown on YEP-ethanol plus glucose; f, strain 3430-3c-R2S grown on YEP-ethanol plus galactose.

Although the precise role of the *GAL4* gene product in the induction process is not known, the results presented here demonstrate that the *GAL4* gene function is required for the appearance of a *GAL7* specific mRNA detectable by "cell-free translation." The *GAL4* gene product must therefore play a key role in the mechanism involved in the appearance of that mRNA. A more detailed determination of the role of *GAL4* must await the development of a physical assay for the *GAL7* specified sequences and the identification and isolation of the *GAL4* gene product.

Because the galactose system is characterized by coordinate expression and regulation of the four inducible enzyme activities, coordinate expression and regulation are predicted at the mRNA level. Essentially identical results have been found for the induction of galactokinase mRNA and its control by the *GAL4* gene as presented here for the uridyl transferase mRNA (unpublished data), thus confirming coordinate expression and regulation at the mRNA level in this simple eukaryotic system.

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1. Douglas, H. C. & Condie, F. (1954) *J. Bacteriol.* **68**, 662–670.
2. Cirillo, V. P. (1968) *J. Bacteriol.* **95**, 1727–1731.
3. Kosterlitz, H. W. (1943) *Biochem. J.* **37**, 322–326.
4. Leloir, L. F. (1951) *Arch. Biochem.* **33**, 186–190.
5. Kalckar, H. M., Braganca, C. & Munch-Peterson, A. (1953) *Nature* **172**, 1038.
6. Douglas, H. C. & Hawthorne, D. C. (1964) *Genetics* **49**, 837–844.
7. Bevan, P. & Douglas, H. C. (1969) *J. Bacteriol.* **98**, 532–535.
8. Douglas, H. C. (1961) *Biochim. Biophys. Acta* **52**, 209–211.
9. Bassel, J. & Mortimer, R. K. (1971) *J. Bacteriol.* **108**, 179–183.
10. Mortimer, R. K. & Hawthorne, D. C. (1969) in *The Yeasts*, eds. Rose, A. H. & Harrison, J. S. (Academic, New York), Vol. 1, pp. 385–460.
11. Douglas, H. C. & Hawthorne, D. C. (1966) *Genetics* **54**, 911–916.
12. Kew, O. M. & Douglas, H. C. (1976) *J. Bacteriol.* **125**, 33–41.
13. Douglas, H. C. & Pelroy, G. (1963) *Biochim. Biophys. Acta* **68**, 155–156.
14. Douglas, H. C. & Hawthorne, D. C. (1972) *J. Bacteriol.* **109**, 1139–1143.
15. Winge, O. & Roberts, C. (1948) *C. R. Trav. Lab. Carlsberg. Ser. Physiol.* **24**, 135–263.
16. Spiegelman, S., Sussman, R. & Pinska, E. (1950) *Proc. Natl. Acad. Sci. USA* **36**, 591–606.
17. Palmiter, R. D., Oka, T. & Schimke, R. T. (1971) *J. Biol. Chem.* **246**, 724–737.
18. Palacios, R., Palmiter, R. D. & Schimke, R. T. (1972) *J. Biol. Chem.* **247**, 2316–2321.
19. Gallis, B. M., McDonnell, J. P., Hopper, J. E. & Young, E. T. (1975) *Biochemistry* **14**, 1038–1046.
20. Firtel, R. A. & Lodish, H. F. (1973) *J. Mol. Biol.* **79**, 295–314.
21. Hopper, J. E., Bostian, K. A., Rowe, L. B. & Tipper, D. J. (1977) *J. Biol. Chem.* **252**, 9010–9017.
22. Stern, H. (1957) *Nature* **179**, 160–161.
23. Shih, D. S. & Kaesberg, P. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1799–1803.
24. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2330–2334.
25. Hopper, J. E., Ko, G. & Young, E. T. (1975) *J. Mol. Biol.* **94**, 539–554.
26. Palmiter, R. D. (1974) *J. Biol. Chem.* **249**, 6779–6787.
27. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 237–248.