MOLECULAR AND CELLULAR BIOLOGY, May 1984, p. 939-946
0270-7306/84/050939-08$02.00/0

Vol. 4, No. 5

Glucose Represses Transcription of *Saccharomyces cerevisiae* Nuclear Genes That Encode Mitochondrial Components

ELIZABETH SZEKELY AND DONNA L. MONTGOMERY*

Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

Received 28 November 1983/Accepted 21 February 1984

By Northern blot hybridization analysis, we demonstrated that the steady-state levels of mRNAs specifying the α subunit of ATPase, the β subunit of ATPase, and the ATP/ADP translocator are all reduced in cells grown in glucose-rich medium. The extent to which glucose represses the levels of α, β, and translocator mRNAs varies from strain to strain, from 2.5- to 7-fold. Furthermore, by hybridization experiments with an excess of DNA, we showed that glucose represses the rates of synthesis of these mRNAs. The kinetics of repression and derepression of transcription were also studied. Finally, a mutant was characterized which appears to be defective in derepression of transcription of the genes encoding the α and β ATPase subunits as well as the ATP/ADP translocator.

Glucose is the most convenient fermentable carbon source for the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* grown in glucose-rich medium represses the synthesis of functional components of several pathways that are not required for glycolysis. Numerous mitochondrial functions are repressed, including components of the tricarboxylic acid cycle, respiratory chain, and the ATPase complex (17, 18, 25). Mitochondrial protein synthesis is also greatly diminished in glucose-grown cells (10). In addition, many cytoplasmic functions involved in gluconeogenesis and the uptake and metabolism of alternate sugars are repressed when glucose is available (1, 7, 8, 18). Glucose represses these numerous functions at least at two levels. The nuclear genes encoding certain mitochondrial components (e.g., cytochrome *c*, subunits of the ubiquinol-cytochrome *c* reductase complex, subunits of cytochrome *c* oxidase, and the mitochondrial RNA polymerase) and many cytoplasmic enzymes (e.g., enzymes involved in maltose, sucrose, and galactose utilization) have all been shown to be repressed at the level of transcription (6, 11, 12, 16, 23, 27, 28). In addition, certain cytoplasmic enzymes are inactivated by proteolytic cleavage when glucose is present (9).

Genetic studies of catabolite repression in *S. cerevisiae* have produced mutants in which some, but not all, glucose-repressible enzymes are resistant to the effect of glucose (2, 5). As a result of these studies, there is general agreement that the regulatory network controlling metabolic functions in response to glucose is a branched pathway involving multiple regulatory factors. We are focusing our studies on one branch of this pathway: glucose repression of mitochondrial components that are encoded by nuclear genes. We are interested in defining the molecular mechanism that controls mitochondrial functions in response to glucose and identifying specific regulatory factors that are involved in this process. In this report, we demonstrate that glucose coordinately represses the rates of transcription of the genes encoding the α and β ATPase subunits and the ATP/ADP translocator. In addition, we describe the kinetics of accumulation of mRNAs specifying these mitochondrial components after release from glucose repression. Finally, we characterize a mutant that appears to be defective in a function required for derepression of transcription of the genes encoding the α and β ATPase subunits, as well as the ATP/ADP translocator.

MATERIALS AND METHODS

Strains and growth conditions. The following previously described *S. cerevisiae* strains were used in this study: DC5 (3), D273-10B (21), and dRZ1 (28). A collection of ten nuclear *pet* mutants derived from D273-10B, which were defective in multiple mitochondrial functions, were obtained from A. Tzagoloff (see reference 26 for a description of the selection procedure used to obtain these mutants). The petite strains D273-10B-1(ρ^-) and CB11(ρ^0) were also obtained from A. Tzagoloff.

Cells were grown at 30°C with vigorous aeration in YP medium (1% yeast extract, 2% peptone) containing either 5% glucose, 3% glycerol–3% ethanol, 2% galactose, or 2% raffinose. In the RNA-labeling experiments, cells were grown in complete medium lacking uridine (28), supplemented with either 5% glucose or 2% raffinose.

RNA preparation. Total cellular RNA was extracted from cells at logarithmic growth (~2 × 10^7/ml) by the glass bead disruption method described previously (28).

Agarose gels and Northern blot hybridizations. Glyoxal-denatured RNA samples were electrophoresed in 1.1% agarose and transferred to filter paper by the method described by P. Thomas (24), with the modification that Biodyne nylon membrane filters (Pall Ultrafine Filtration Corp., Glen Cove, N.Y.) were substituted for nitrocellulose filter paper.

Radioactively labeled probes were prepared from plasmid DNAs by nick translation (19) with [α-^{32}P]dATP (>400 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Hybridization conditions were the same as previously described (24). When blots were to be reused, old hybridization probes were first removed from the filters by washing with 100% formamide at 42°C for 1 h. Damp filters were wrapped in plastic wrap and autoradiographed at −80°C by using DuPont Cronex Lightning-Plus intensifying screens. Autoradiograms were scanned by a Quick Scann R & D densitometer.

RNA labeling and DNA-excess hybridization. Cells were grown for >16 h at 30°C to a cell density of ~2 × 10^7/ml and then 200 μCi of [^{3}H]uridine (42 Ci/mmol; ICN Chemical and Radioisotope Division, Irvine, Calif.) was added per 2 ml of culture for the designated time. Incorporation was stopped by the addition of 3 volumes of ice-cold RNA extraction buffer (28). Specific activities of [^{3}H]RNAs were ~10^5 cpm/

* Corresponding author.

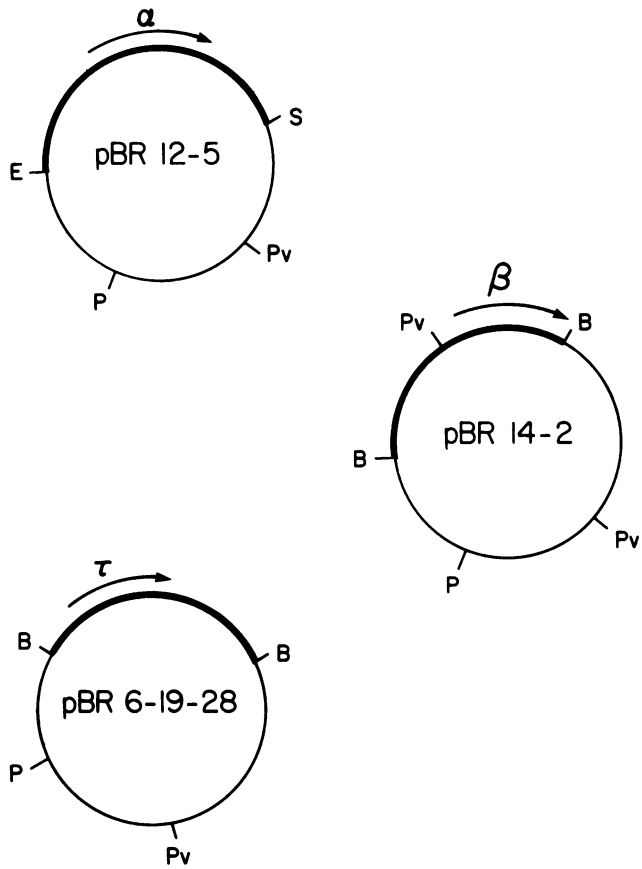

FIG. 1. Recombinant plasmids used for hybridization probes. The restriction maps of pBR322 recombinant plasmids containing yeast genes encoding the α and β ATPase subunits and the ATP/ADP translocator are shown. The approximate positions of the genes encoding these proteins (α, β, and τ) are indicated above the inserts (heavy line) of each plasmid. The sizes of the inserts in plasmids pBR12-5, pBR14-2, and pBR6-19-28 are 2.8, 2.2, and 2.6 kilobases, respectively. Restriction endonuclease sites are indicated as follows: E, *Eco*RI; P, *Pst*I; Pv, *Puv*II; S, *Sal*I; and B, *Bam*HI.

μg for labeling times of 5 min. A previously described procedure (13) was followed for the preparation of DNA filters, hybridization of labeled RNA, and washing of the filters, with the modification that hybridization times were 24 h. After the final wash, the filters were rinsed in 95% ethanol, air dried, and then assayed for radioactivity in a scintillation counter.

Kinetics of derepression. To study the kinetics of accumulation of α, β, and τ mRNAs after release from glucose repression, cells were transferred from repressing growth conditions to derepressing conditions by the following method. dRZ1 cells were grown in medium containing 5% dextrose for >16 h at 30°C with vigorous shaking. When a cell density of ~2 × 10^7/ml was reached, cells were pelleted at room temperature in a clinical centrifuge and suspended to a cell density of 1 × 10^7/ml in warm (30°C) medium containing either 2% raffinose or 5% dextrose, and then vigorous shaking at 30°C was resumed. Immediately after suspension (time zero), RNA was extracted from a 2-ml portion of the dextrose-grown cells. At 1-h intervals thereafter, for 5 h, RNA was prepared from 2-ml portions of cells transferred to medium containing 2% raffinose. (Control experiments indicated that the method followed here to pellet and suspend cells had no effect on the growth rate of the cells.)

RESULTS

Glucose represses steady-state levels of α, β, and τ mRNAs. Does glucose control mitochondrial functions in *S. cerevisiae* by a common mechanism? In an effort to answer this question, we wanted to see whether other nuclear-coded mitochondrial components, besides cytochrome *c* (28) and the subunits for the ubiquinol-cytochrome *c* reductase complex (27), for example, were also repressed by glucose at the level of transcription. We used the cloned genes for the α and β subunits of the mitochondrial ATPase and the ATP/ADP translocator as hybridization probes to test whether glucose had any effect on the steady-state levels of mRNAs specifying these mitochondrial components. These three genes were initially isolated from a gene bank of *S. cerevisiae* DNA contained in the *S. cerevisiae*-bacterial shuttle vector YEp13 (15, 20; M. Takeda and M. Douglas, manuscript in preparation). DNA fragments containing either part or all of the coding regions for these genes were subsequently recloned into the plasmid vector pBR322. The resulting recombinant plasmids are shown in Fig. 1: pBR12-5 contains the α subunit gene of ATPase; pBR14-2 contains part of the β subunit gene of ATPase; and pBR6-19-28 contains the translocator gene. These plasmids, hereafter referred to as the α, β, and τ plasmids, were used as hybridization probes in all of the experiments reported here.

Initially, we measured the steady-state levels of α, β, and τ mRNAs in repressed and derepressed cells. Total RNA was extracted from DC5 cells grown under glucose-repressing (5% dextrose) and derepressing (3% glycerol–3% ethanol) conditions and electrophoresed in 1.1% agarose. Northern blots were prepared from three identical gels, and the blots were hybridized with radioactively labeled α, β, and τ plasmid DNAs. The results of these hybridization experiments are shown in Fig. 2. Each autoradiogram shows only one band, indicating that the α subunit mRNA (α mRNA), the β subunit mRNA (β mRNA), and the translocator mRNA (τ mRNA) are the only RNA species that hybridize at detectable levels to the plasmid probes. The sizes of the α, β,

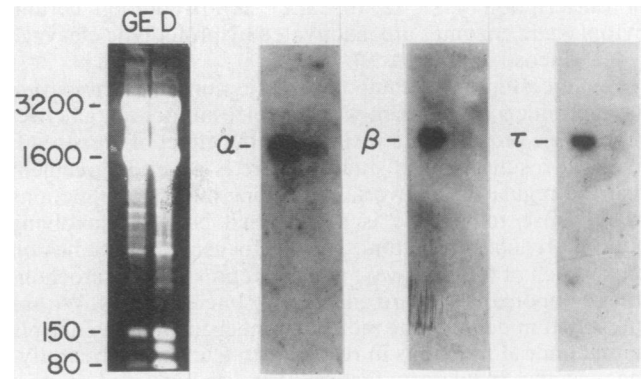

FIG. 2. α, β, and τ mRNA levels in DC5 cells grown under derepressing and glucose-repressing conditions. RNA was extracted from DC5 cells grown in 3% glycerol–3% ethanol (GE) and 5% dextrose (D). Ten micrograms of each RNA preparation was electrophoresed in 1.1% agarose. The ethidium bromide-stained gel profile that was obtained is shown in the left panel. (The positions of the RNA size markers are indicated in nucleotides.) Blots were prepared from three such identical gels and then probed with radioactively labeled α, β, and τ plasmid DNAs. The autoradiograms that were obtained from these hybridizations are shown in the right three panels, and the bands corresponding to the α, β, and τ mRNAs are indicated.

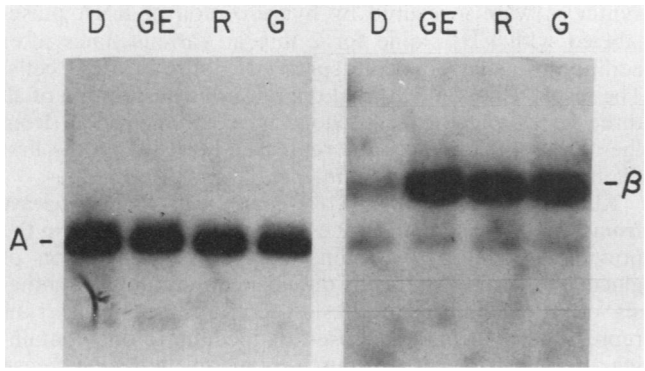

FIG. 3. Effect of carbon source on β mRNA levels in DC5 cells. RNA was extracted from DC5 cells grown in 5% dextrose (D), 3% glycerol–3% ethanol (GE), 2% raffinose (R), and 2% galactose (G). A Northern blot, containing ~10 μg of each RNA preparation, was probed with radioactively labeled pYactI and β plasmid DNAs, in succession. The results of these hybridizations are shown in the left and right panels, respectively, and the bands corresponding to actin (A) and β mRNAs are indicated. (The band intensities on both autoradiograms were quantitated densitometrically, and the values obtained for the relative β/A band intensities are presented in Table 1, line 1.)

and τ mRNAs were approximated to be 1,800, 2,000 and 1,900 nucleotides, respectively. The results of these hybridizations clearly demonstrate that the levels of α, β, and τ mRNAs are all reduced in cells grown under glucose-repressed conditions.

We wanted to quantitate the relative steady-state levels of α, β, and τ mRNA in different yeast strains grown under different conditions. First, blots were prepared from RNA extracted from DC5 cells grown in 5% dextrose and from cells grown in the following derepressing carbon sources: 3% glycerol–3% ethanol, 2% raffinose, and 2% galactose. The blots were probed initially with radioactively labeled pYactI, a recombinant plasmid containing the yeast actin gene (14). This control hybridization was performed to measure the relative amounts of total mRNA in each lane of the blot. (The actin mRNA levels should be representative of total mRNA levels since actin mRNA synthesis is not affected by growth in different carbon sources; R. Ng, personal communication.) The same blots were then reprobed with radioactively labeled α, β, and τ plasmid DNAs. The results of the Northern hybridizations with pYactI and the β plasmid as probes are shown in Fig. 3. The bands on these autoradiograms were scanned by a densitometer. The values obtained for the β mRNA band intensities were divided by the actin mRNA band intensities to quantitate the relative β mRNA levels in each lane. The results from this analysis (see Table 1, line 1) indicate that the β mRNA levels in DC5 cells grown under derepressing conditions are ca. sevenfold higher than the levels measured in glucose-repressed cells. Similar values were obtained when the relative levels of α and τ mRNAs were measured in DC5 cells (data not shown). Next, these hybridization experiments were repeated using RNA isolated from two other yeast strains, D273-10B and dRZ1. The relative levels of β mRNA in these strains grown in the presence of different carbon sources are shown in Table 1, lines 2 and 3. The β mRNA levels measured in D273-10B cells grown under derepressing conditions are about 2.5 times higher than the levels measured in repressed cells, whereas the β mRNA levels measured in dRZ1 cells grown in the presence of derepressing carbon sources are ca. sixfold higher than the levels measured in cells grown in the presence of glucose. Similar values were again obtained when the relative levels of α and τ mRNA were measured in these two strains (data not shown). The results from these experiments thus demonstrate that there is a strain dependence in the extent to which glucose affects the levels of mRNAs encoding the α and β ATPase subunits and the ATP/ADP translocator.

TABLE 1. Effect of carbon source on β mRNA levels in different strains

Strain	Relative levels of β mRNA[a] in cells grown in medium containing:			
	Dextrose	Glycerol/ ethanol	Raffinose	Galactose
DC5	1	6.9	6.5	7.3
D273-10B	1	2.6	3.3	2.2
dRZ1	1	6.6	6.2	5.8

[a] The method used to obtain values for relative levels of β mRNA is described in the legend to Fig. 3.

Glucose represses the rates of transcription. To determine whether the decreased steady-state levels of α, β, and τ mRNAs in glucose-repressed cells were due to decreased rates of transcription, hybridization experiments with an excess of DNA were performed with pulse-labeled RNA. dRZ1 cells were grown in 5% dextrose and 2% raffinose, a derepressing carbon source, and RNA was pulse-labeled with [^{3}H]uridine for 5 min, purified, and then hybridized to α, β, and τ plasmid DNAs immobilized on nitrocellulose

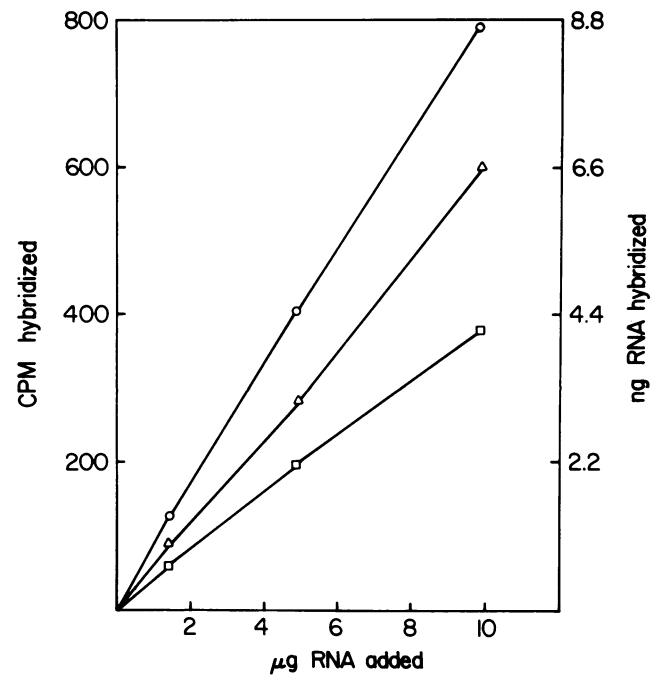

FIG. 4. Hybridization of [^{3}H]RNA to DNA filters. dRZ1 cells were grown in a medium containing 2% raffinose to a cell density of 2×10^7/ml. A 2-ml sample of cells was labeled with [^{3}H]uridine for 5 min, and then RNA was prepared. The specific activity of the extracted RNA was 9.9×10^4 cpm/μg. The values for cpm hybridized were determined by subtracting the counts per minute bound to a control filter containing pBR322 DNA from the counts per minute bound to a filter containing α (○), β (□), or τ (△) plasmid DNA.

TABLE 2. Effect of carbon source on the rates of synthesis of α, β, and τ mRNAs

DNA filter and carbon source	Labeling time (min)	Cpm added (10^5)	Cpm hybridized[a]	Fraction cpm hybridized[b] (10^{-4})
pBR12-5 (α)				
Raffinose	5	2.2	436	19.8
	10	5.6	1,047	18.7
Glucose	5	5.8	191	3.3
	10	8.2	369	4.5
pBR14-2 (β)				
Raffinose	5	2.2	235	10.7
	10	5.6	689	12.3
Glucose	5	5.8	156	2.7
	10	8.2	164	2.0
pBR6-19-28 (τ)				
Raffinose	5	2.2	387	17.6
	10	5.6	896	16.0
Glucose	5	5.8	232	4.0
	10	8.2	369	4.5

[a] The values for counts per minute hybridized were calculated by subtracting the counts per minute bound to a control filter containing pBR322 DNA from those bound to the pBR12-5, pBR14-2, or pBR6-19-28 DNA filter.

[b] These values were obtained by dividing the counts per minute hybridized by the counts per minute added.

filters. To ensure that the hybridizations were performed under conditions of excess DNA, saturation curves were determined for each batch of filters. Different amounts of [^{3}H]RNA were added to identical filters, the total counts per minute that hybridized was determined, and the results were plotted (Fig. 4). Since a linear plot was obtained for each set of hybridizations, it was concluded that hybridization conditions with an excess of DNA were maintained. All subsequent experiments were performed with RNA concentrations in this nonsaturating range.

To ensure that the rates of transcription and not the steady-state levels of α, β, and τ mRNAs were measured, the cells were pulse-labeled with [^{3}H]uridine for 5 and 10 min. If the labeling of the mRNAs achieved the steady state within 10 min, then the fraction of total [^{3}H]RNA that bound to the DNA filters would decrease from 5 to 10 min, since the specific activity of α, β, and τ mRNAs would not be increasing at the same rates as the specific activity of bulk, stable cellular RNA. Alternatively, if the steady-state levels of mRNAs were not achieved by 10 min, the specific activity of α, β, and τ mRNAs would be increasing at the same rate as that of bulk RNA. As can be seen from the data presented in Table 2, in all cases the fraction of hybridized [^{3}H]RNA was the same (within experimental error) at 5 and 10 min. This ratio, the fraction of [^{3}H]RNA that hybridized specifically to α, β, and τ DNA filters, represents the relative rates of synthesis of the α, β, and τ mRNAs, respectively. A comparison of these ratios (see Table 2) clearly indicates that the rates of synthesis of the α, β, and τ mRNAs are reduced by four- to fivefold in glucose-grown cells compared with raffinose-grown cells. This difference in transcription rates corresponds closely to the differences in steady-state mRNA levels measured in dRZ1 cells (see Table 1, line 3).

We wanted to also measure the kinetics of glucose repression, that is, to see how fast the synthesis of α, β, and τ mRNAs was diminished after glucose was added to derepressed cells. To do this, the rates of α, β, and τ mRNA synthesis were measured by hybridization of RNA pulse-labeled with [^{3}H]uridine for 2 min at various times after addition of glucose to a derepressed culture of dRZ1 cells. The results (Fig. 5) show that the rates of transcription of all three genes encoding mitochondrial components fall from the derepressed level to the repressed level within the first 2.5 min after glucose addition.

Kinetics of accumulation of α, β, and τ mRNAs after release from glucose repression. In the experiments described in the previous section, we demonstrated that the addition of glucose to derepressed cells diminished the rates of synthesis of α, β, and τ mRNAs. Therefore, the transfer of repressed cells from a glucose-rich medium to one containing a derepressing carbon source should result in an increase in the rates of synthesis of α, β, and τ mRNAs and a concommitant increase in the steady-state levels of these RNAs. To monitor the time course of accumulation of α, β, and τ mRNAs after release from glucose repression, RNA was extracted from dRZ1 cells before release and at 1-h intervals for 5 h postrelease. Northern blots were prepared from the extracted RNAs and hybridized to radioactively

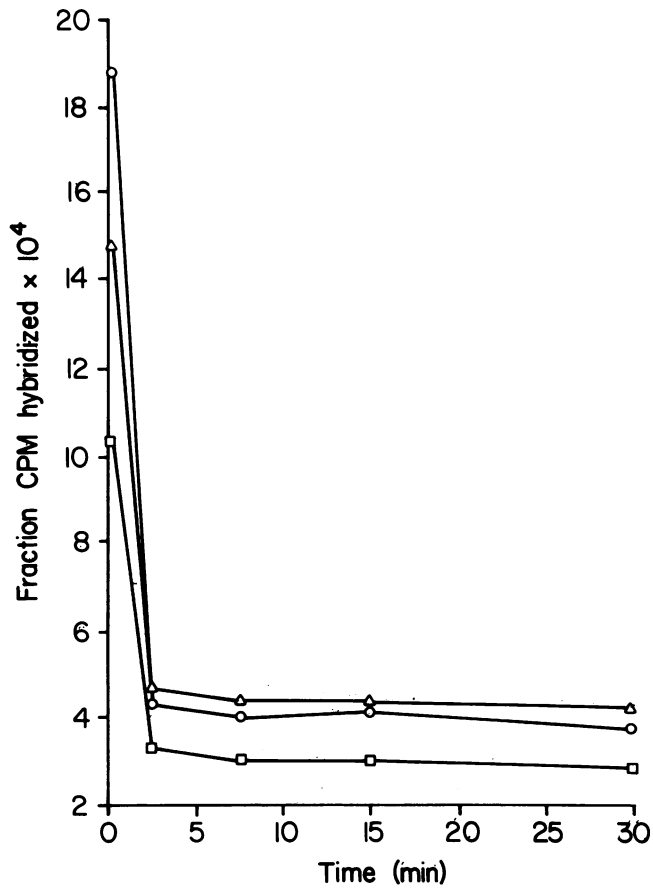

FIG. 5. Kinetics of glucose repression of α, β, and τ mRNA synthesis. dRZ1 cells were grown for 16 h in medium containing 2% raffinose to a cell density of ~2 × 10^7/ml. A 2-ml sample was labeled with [^{3}H]uridine for 2 min. An equal volume of medium containing 10% glucose was added to the remaining culture, and at various times afterwards, 4-ml samples were labeled with [^{3}H]uridine for 2 min. RNA was prepared from the samples, and the fraction of total [^{3}H]RNA that hybridized specifically to α (○), β (□), and τ (△) plasmid DNA filters was determined. Glucose was added at time zero.

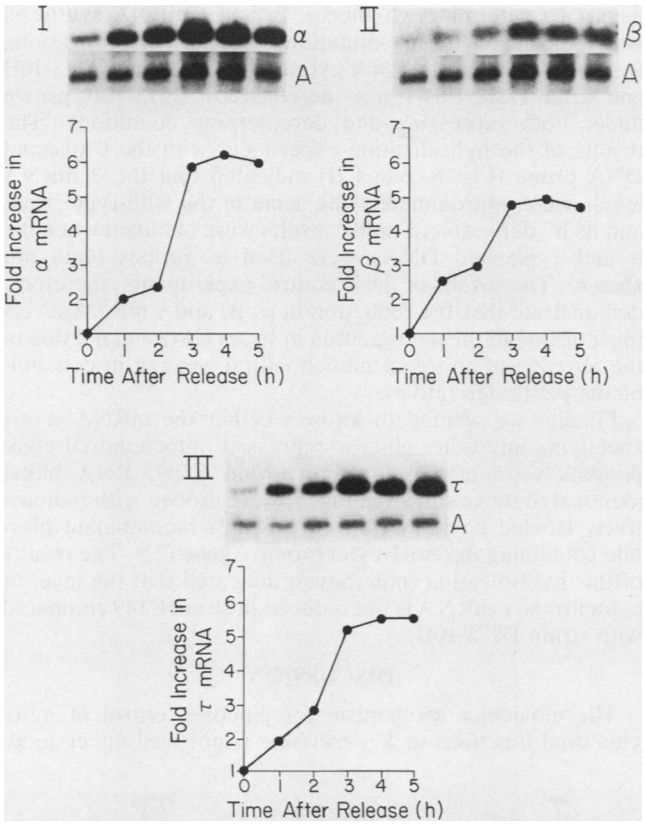

FIG. 6. Kinetics of accumulation of α, β, and τ mRNAs after release from glucose repression. RNA was extracted from dRZ1 cells before and after release from glucose repression at 1-h intervals for 5 h by the procedure described in the text. Northern blots, containing ~10 μg of each RNA preparation, were first hybridized with radioactively labeled pYactI plasmid DNA and then rehybridized with α, β, and τ plasmid DNA probes; the results of these hybridizations are shown in panels I, II, and III, respectively, and the bands corresponding to actin (A), α, β, and τ mRNAs are indicated. The relative intensities of the bands in each set of autoradiograms were quantitated densitometrically, and the results are presented beneath the corresponding autoradiograms.

labeled α, β, and τ plasmid DNAs, as well as to pYactI (the control probe). The autoradiograms that were obtained are shown in Fig. 6. The relative amounts of α, β, and τ mRNAs were quantitated densitometrically, as before. The results were plotted and are shown beneath the autoradiograms in Fig. 6. It is apparent from this analysis that there is a coordinate increase in the accumulation of all three mRNAs and that the steady-state levels for α, β, and τ mRNAs are achieved by 3 h.

Mutant strain C149. Since similar kinetics were observed for the derepression of transcription of the genes encoding the α and β ATPase subunits and the ADP/ATP translocator, it seemed likely that common regulatory factors may control the expression of these and perhaps other genes encoding mitochondrial components in the absence of glucose. We therefore wanted to identify mutants that may be defective in common regulatory factors controlling derepression of transcription. With this aim in mind, a collection of 10 nuclear *pet* mutants with pleiotropic defects in mitochondrial functions were screened by Northern hybridization for lowered steady-state levels of α, β, and τ mRNAs under derepressing conditions of growth. These *pet* mutants were deficient in mitochondrial protein synthesis and in one or more other mitochondrial activities (for example, cytochrome oxidase, cytochrome *c* reductase, and mitochondrial ATPase; A. Tzagoloff, personal communication). The mutant strains, along with the wild-type parental strain, D273-10B, were grown in 2% galactose, a derepressing carbon source. At exponential growth, RNA was extracted, and Northern blots were prepared. These blots were then hybridized to radioactively labeled α, β, and τ plasmid DNAs and also to pYactI (the control probe). The relative levels of α, β, and τ mRNAs in the mutant strains were quantiated densitometrically and compared with levels measured in the wild-type parental strain. As a result of this analysis, one mutant, C149, was found to have reduced levels of all three mRNAs, whereas all the others showed normal levels. The relative levels of α, β, and τ mRNAs observed in the C149 mutant were lower than the levels measured in strain D273-10B by ca. threefold (Fig. 7). The lowered levels of these RNAs observed in the C149 mutant correspond closely to the diminished α, β, and τ mRNA levels observed in the glucose-repressed state of D273-10B, the wild-type parental strain (see Table 1, line 2).

The rates of synthesis of the α, β, and τ mRNAs were compared in strains C149 and D273-10B. Both strains were grown in 2% raffinose and were pulse-labeled with [^{3}H]uridine for 5 and 10 min. The results of these experiments (Table 3) clearly demonstrate that the relative rates of synthesis of α, β, and τ mRNAs are reduced by ca. fourfold in strain C149 relative to strain D273-10B. This value closely approximates the threefold difference in the steady-state levels of α, β, and τ mRNAs observed between strain C149 and the wild-type parental strain, D273-10B.

The observation that both the steady-state levels and the rates of synthesis of α, β, and τ mRNAs are reduced in strain C149 are consistent with the idea that strain C149 is defective in a glucose-responsive transcription factor involved in the derepression pathway. To further test this idea, we compared the levels of α, β, and τ mRNAs in C149 cells

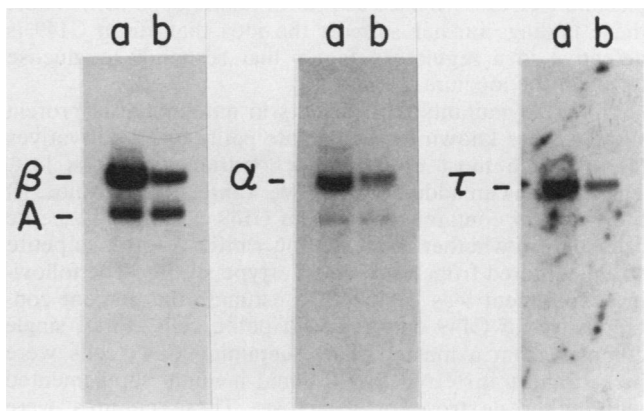

FIG. 7. α, β, and τ mRNA levels in strain C149 and the wild-type parental strain. A Northern blot, containing ~10 μg of RNA extracted from D273-10B (a) and C149 (b) cells growing in 2% galactose, was hybridized to radioactively labeled pYactI and β, α, and τ plasmid DNA probes, in succession. (Before the blot was rehybridized to the α and τ plasmid probes, the previously hybridized probes were first removed by the procedure described in the text.) The autoradiograms that were obtained from these hybridizations are shown, and the bands corresponding to the actin (A), β, α, and τ mRNAs are indicated. Densiotometric quantitation of the bands on these autoradiograms indicated that the α, β, and τ mRNA levels were higher in strain D273-10B than in strain C149 by 2.5, 3.5, and 3 times, respectively.

TABLE 3. Rates of synthesis of α, β, and τ mRNAs in strain C149 and the wild-type parental strain

DNA filter and strain	Labeling time (min)	Cpm added (10^5)	Cpm hybridized[a]	Fraction cpm hybridized[b] (10^{-4})
pBR12-5 (α)				
D273-10B	5	3.7	655	17.7
	10	6.1	988	16.2
C149	5	1.6	56	3.5
	10	2.5	120	4.8
pBR14-2 (β)				
D273-10B	5	3.7	466	12.6
	10	6.1	82	13.4
C149	5	1.6	66	4.1
	10	2.5	95	3.8
pBR6-19-28 (τ)				
D273-10B	5	3.7	551	14.9
	10	6.1	982	16.1
C149	5	1.6	66	4.1
	10	2.5	132	5.3

[a] The values for counts per minute hybridized were calculated by subtracting the counts per minute bound to a control filter containing pBR322 DNA from those bound to the pBR12-5, pBR14-2, or pBR6-19-28 DNA filter.

[b] These values were obtained by dividing the counts per minute hybridized by the counts per minute added.

grown under derepressing and repressing conditions by Northern hybridization. (Our expectation was that if strain C149 was defective in a transcription factor not involved in glucose control, then the α, β, and τ mRNA levels would be even further reduced in C149 cells grown under repressing conditions.) The results of the Northern hybridization experiment with the β plasmid DNA probe (Fig. 8, panel I) indicated that the levels of β mRNA are approximately the same in C149 cells grown in repressing and derepressing conditions. Similar results were obtained when the α and τ plasmid DNAs were used as probes (data not shown). Thus, these findings further support the idea that strain C149 is defective in a regulatory factor that responds to glucose levels in the medium.

Many *pet* mutants with defects in mitochondrial protein synthesis are known to accumulate petite or ρ^- derivatives (4) in which most of the mitochondrial genome is lost. Therefore, as an added control, we wanted to determine: (i) the extent of contamination of our C149 cultures with petite cells and (ii) whether the α, β, and τ mRNA levels in petite strains differed from levels in wild-type strains. The following experiment was designed to estimate the percent contamination of C149 cultures with petite cells. First, single colonies from a master plate containing C149 cells were picked and transferred to YP liquid medium supplemented with either dextrose or galactose. These cultures were passaged in liquid medium for 5 days and then spread onto YP agar plates supplemented with dextrose. Finally, single colonies were picked and crossed with the strain CB11, a ρ^0 *PET*$^+$ tester strain of the opposite mating type. The respiratory abilities of the resulting diploids were determined by their ability to grow on a nonfermentable carbon source (only ρ^+ *pet*/ρ^0 *PET*$^+$ diploids will grow). From this analysis, it was determined that C149 cultures maintained in liquid medium for 5 days are contaminated with between 10 and 30% petite derivatives. (RNA was routinely prepared from C149 cultures that were picked from individual colonies on the master plate and then maintained in liquid medium for not more than 5 days.) To determine whether α, β, and τ mRNA synthesis was affected by the ρ^- mutation, Northern hybridizations were performed with RNA extracted from strain D273-10B and strain D273-10B-1, a ρ^- derivative of D273-10B, grown under both repressing and derepressing conditions. The results of the hybridization experiment with the β plasmid DNA probe (Fig. 8, panel II) indicated that the β mRNA levels were approximately the same in the wild-type strain and its ρ^- derivative. Similar results were obtained when the α and τ plasmid DNAs were used as probes (data not shown). The results of these control experiments, therefore, demonstrate that the reduction in α, β, and τ mRNA levels must be due to the *pet* mutation in strain C149 and not due to the absence of an intact mitochondrial genome in contaminating petite derivatives.

Finally, we wanted to know whether the mRNA levels specifying any other glucose-repressed mitochondrial components were also reduced in strain C149. RNA blots, identical to those shown in Fig. 7, were probed with radioactively labeled pYeCYC1(0.6), a pBR322 recombinant plasmid containing the iso-1-cytochrome *c* gene (22). The results of this hybridization (not shown) indicated that the level of cytochrome *c* mRNA is not reduced in strain C149 compared with strain D273-10B.

DISCUSSION

The molecular mechanism for glucose control of mitochondrial functions in *S. cerevisiae* is not well understood.

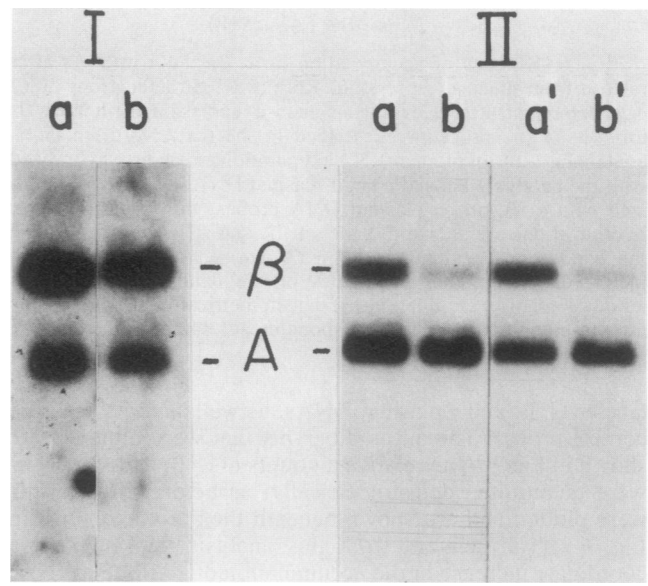

FIG. 8. Effect of carbon source on β mRNA levels in strain C149 and in a ρ^- derivative of the wild-type parental strain. (I) A Northern blot, containing ~10 μg of RNA extracted from C149 cells grown in 2% galactose (a) and 5% dextrose (b), was hybridized to radioactively labeled pYactI and β plasmid DNA probes. Densitometric quantitation of the bands on the autoradiogram (shown) indicated that the β/actin (A) band intensity ratios were 1.9 and 2.2 in lanes a and b, respectively. (II) A Northern blot was prepared from RNAs extracted from the ρ^- strain D273-10B-1, grown in 2% galactose (a) and 5% dextrose (b), and from the wild-type parental strain D273-10B, grown in 2% galactose (a') and 5% dextrose (b'). Radioactively labeled pYactI and β plasmid DNA probes were hybridized to the blot, and the autoradiogram that was obtained is shown. The β and actin (A) band intensities were quantitated densitometrically, and the values obtained for the relative β/A ratios are as follows: (a) 0.8; (b) 0.25; (a') 0.95; (b') 0.3.

The level at which the vast majority of the nuclear-coded mitochondrial components are regulated by glucose remains unknown, and the specific regulatory factors that mediate glucose control have yet to be identified. In two of the cases that have been studied, cytochrome *c* (28) and the ubiquinol-cytochrome *c* reductase complex (27), glucose has been shown to diminish the levels of the mRNAs specifying these mitochondrial components. In the case of cytochrome *c*, it has been shown that it is the rate of transcription of the cytochrome *c* gene that is repressed.

The results presented here clearly demonstrate that glucose also represses the rates of transcription of three other nuclear genes encoding the following mitochondrial components: the α and β subunits of ATPase and the ATP/ADP translocator. The reduction in rates corresponds quite well to the reduction in steady-state levels: the reduction in the rate of β mRNA synthesis in dRZ1 cells is ca. 5.5-fold, whereas the reduction in the steady-state levels of β mRNA in the same strain is ca. sixfold (see Tables 1 and 2). This correspondence suggests that differential degradation of α, β, and τ mRNAs can at most be a minor factor in the regulatory scheme. In addition, it was found that the rates of synthesis of α, β, and τ mRNAs were diminished coordinately within the first 2.5 min after the addition of glucose to derepressed cells. These same kinetics were observed when the time course for the repression of transcription of the cytochrome *c* gene was investigated (28). A coordinate increase in the levels of α, β, and τ mRNAs after release from glucose repression was also observed; the levels of all three transcripts reached steady state within 3 h. Together, these results support the notion that common regulatory factors may control transcription of genes encoding the α and β subunits of ATPase and the ATP/ADP translocator (and perhaps other mitochondrial components) in the presence and absence of glucose.

In an effort to identify regulatory elements that may control mitochondrial functions in response to glucose, we searched for mutants that may be defective in transcriptional control of known glucose-repressed genes. We hoped to find mutants that had repressed levels of α, β, and τ mRNAs under derepressing conditions of growth. We screened a collection of *pet* mutants (obtained from A. Tzagoloff) that were known to be deficient in a number of mitochondrial functions (see above) and thus were good candidates for the types of regulatory mutants we were looking for. One mutant, C149, was found to have lowered levels of α, β, and τ mRNAs when it was grown in medium containing a derepressing carbon source. All three transcripts were reduced by ca. threefold in the mutant when compared with the wild-type parental strain, D273-10B. These reduced levels closely corresponded to the lowered levels of α, β, and τ mRNAs measured in the glucose-repressed state of strain D273-10B. Furthermore, hybridization experiments with an excess of DNA demonstrated that the rates of synthesis of α, β, and τ mRNAs were lower in strain C149 than in strain D273-10B by about the same amount. Finally, the levels of α, β, and τ mRNAs in C149 cells were found to be approximately the same under derepressing and repressing conditions. The results of these experiments, therefore, suggest that the C149 mutant may be defective in a specific glucose-responsive regulatory function required for derepression of transcription of the genes encoding the α and β subunits of the mitochondrial ATPase and the ATP/ADP translocator.

The fact that transcription of the actin and the cytochrome *c* genes is not reduced in strain C149 demonstrates that this mutant does not have a general defect in transcription of nuclear genes. Furthermore, if strain C149 is, in fact, defective in a glucose-responsive regulatory function, then the finding that cytochrome *c* gene expression is not affected argues that the glucose-repressible biogenesis of mitochondria is controlled by a number of different regulatory elements. Although one set of factors may control transcription of the genes encoding the α and β ATPase subunits, the ATP/ADP translocator, and perhaps other mitochondrial components, another set of factors may control expression of the cytochrome *c* gene, along with other nuclear genes perhaps encoding heme proteins. The idea that the glucose control network that regulates numerous metabolic functions in *S. cerevisiae* is a branched pathway involving multiple regulatory factors has been suggested previously by others (2, 5). Experiments are in progress to test whether or not the function that is deficient in strain C149 is, in fact, a regulatory protein that operates in this network to modulate transcription rates of genes encoding certain mitochondrial components.

ACKNOWLEDGMENTS

We gratefully acknowledge M. Douglas for providing us with the α, β, and τ plasmid DNAs, R. Ng for pYactI, A. Tzagoloff for the collection of 10 pleiotropic *pet* mutants, and R. Zitomer for the yeast strain dRZ1.

This work was supported by grants from the National Science Foundation (PCM-8110892) and the Robert A. Welch Foundation (AQ-965). E.S. was supported by an American Cancer Society Fellowship.

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