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Timothy A. Brown
Dartmouth College

Bernard L. Trumpower
Dartmouth College

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NOTES

Strain-Dependent Variation in Carbon Source Regulation of Nucleus-Encoded Mitochondrial Proteins of *Saccharomyces cerevisiae*

TIMOTHY A. BROWN AND BERNARD L. TRUMPOWER*

Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755-3844

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Nuclear genes encoding mitochondrial proteins are regulated by carbon source with significant heterogeneity among four *Saccharomyces cerevisiae* strains. This strain-dependent variation is seen both in respiratory capacity of the cells and in the expression of β-galactosidase reporter fusions to the promoters of *CYB2*, *CYC1*, *CYC3*, *MnSOD*, and *RPO41*.

Mitochondrial biogenesis is largely controlled at the transcriptional level by carbon source in the yeast *Saccharomyces cerevisiae*. When grown on fermentable carbons, *S. cerevisiae* undergoes glucose or catabolite repression, in which the steady-state mRNA levels of hundreds of genes are lowered, primarily by decreasing transcriptional initiation rates (17, 22).

Genetic approaches to the question of how carbon sources regulate gene expression have revealed numerous regulatory genes which are involved in repression and derepression, some of which control mitochondrial biogenesis. Mutations in the *HXK2*, *CYC8* (*SSN6*), *REG1*, *CAT4*, and *CYC9* (*TUP1*/*FLK1*/*AER2*/*SFL2*/*AER2*/*UMR7*/*AMM1*) genes have identified these as regulators of at least some mitochondrial enzymes (1, 4, 14, 15, 20, 21). It is clear from the diversity of mutants, the genes they affect, and their epistatic relationships with known positive effectors that this transcriptional regulation is not a linear event resulting from a single signal transduction pathway to a common effector. Rather, it appears to be a highly branched regulatory network that involves the interaction of both positive and negative regulators (3).

We have examined the respiratory capacity and expression of five nucleus-encoded mitochondrial proteins in four laboratory strains of *S. cerevisiae* (Table 1). Respiratory rates were obtained from cells grown in 8% dextrose or 4% ethanol–3% glycerol and are shown in Table 2. By using ethanol as a respiration-dependent substrate and 6 μM carbonyl cyanide *m*-chlorophenylhydrazone to uncouple oxidative phosphorylation, strain differences in catabolite repression of respiration are apparent, as reflected in the ratios of repressed and nonrepressed respiration rates. By this criterion, YPH500 is the most responsive, having a repressed respiration rate which is only 19% that of the nonrepressed rate. PSY142 follows with a repressed rate of 34% that of maximum, and then BWG1-7A follows at 45%. W303-1A, which has a repressed rate which is 61% that of the nonrepressed expression level, is the least responsive.

Since the respiratory measurements indicated that not all strains respond equally to external carbon sources, we were interested in determining if the strain difference is from a single rate-limiting enzymatic step in one of the respiratory complexes, or if the difference is apparent at the transcriptional level. The β-galactosidase reporter plasmids which we used to assay transcriptional repression of five different promoters are shown in Fig. 1. Plasmid copy number was quantified in each of the four strains during growth in each carbon source. All four yeast strains maintain four plasmid copies, regardless of the carbon source (results not shown). Therefore, the β-galactosidase activities reflect transcriptional differences and not plasmid copy number differences. Five glucose-repressible nuclear genes were chosen as diverse reporters representing several mitochondrial systems. These include *CYC1*, encoding iso-1-cytochrome *c* (6, 16, 22); *CYB2*, encoding the L-(+)-lactate cytochrome *c* oxidoreductase (7, 8); *CYC3*, encoding cytochrome *c* heme lyase (2); *RPO41*, encoding the large subunit of the mitochondrial RNA polymerase (10, 19); and *MnSOD*, encoding the manganese superoxide dismutase (9, 18). PCRs were performed with the GeneAmp system (Perkin-Elmer) with W303-1A genomic DNA as the template. PCR primers were synthesized on a Biosearch Cyclone DNA synthesizer: *CYC1*, 5′-CCCGGATCCATGTTTTCTTTCGATCAAAA-3′ and 5′-CCCGGATCCATTATTAATTTAGTGTGTGT-3′; *CYB2*, 5′-CCCGAATTCACGCATACATCGGAAGGATC-3′ and 5′-CCCGGATCCATTGACTACTTTTGTTTGCT-3′; *CYC3*, 5′-CCCGAATTCTGCGACAAAAGTGTGACCGA-3′ and 5′-CCCGGATCCATTTTTTGTAGTTTCTGTTG-3′; *RPO41*, 5′-CCCGAATTCCAAAGAAAGAAGATTACAAC-3′ and 5′-CCCGGATCCATATTGAGTGAATAT

TABLE 1. Yeast strains used in this study

Name	Genotype	Source and/or reference
YPH500	α *ade2-101 his3Δ200 leu2Δ1 lys2-801 trp1Δ63 ura3-52*	P. Hieter (15a)
YPH499	**a** *ade2-101 his3Δ200 leu2Δ lys2-801 trp1Δ63 ura3-52*	P. Hieter (15a)
PSY142	α *lys2-801 leu2-112 ura3-52*	M. Rosencrantz (7a)
BWG1-7A	α *his4-519 leu2-3,112 ade1-100 ura3-52*	M. Rosencrantz (7a)
W303-1A	**a** *ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1 can1-100*	R. Rothstein

* Corresponding author. Phone: (603) 650-1621. Fax: (603) 650-1389.

TABLE 2. Repression and derepression of oxygen consumption and nucleus-encoded mitochondrial gene expression in four yeast strains

Strain	Oxygen uptake[a]	β-Gal expression[b]				
		CYB2	*CYC1*	*RPO41*	*CYC3*	*MnSOD*
Repressed[c]						
YPH500	120	200	7,600	1,700	900	500
BWG1-7A	265	225	8,300	2,800	3,100	3,000
PSY142	360	50	3,000	1,900	2,200	1,200
W303-1A	330	300	8,300	2,200	3,900	1,800
Nonrepressed[d]						
YPH500	630	73,500	67,600	12,600	48,700	13,300
BWG1-7A	590	10,400	15,500	4,100	5,600	5,200
PSY142	1,045	22,100	23,900	1,300	14,900	7,600
W303-1A	540	10,000	22,900	No growth	6,500	4,500

[a] Oxygen consumption is in nanomoles of oxygen per 100 mg of cells per minute. Values are the average of three separate assays.
[b] β-Galactosidase expression from each of the five reporter plasmids shown in Fig. 1 was measured for the four yeast strains listed. β-Galactosidase activities were calculated as (optical density at 420 nm × 1,000)/(milliliters per minute per milligrams of protein). The values are means of four independent transformants. Standard errors were 10 to 15% of the mean value.
[c] Repressed expression indicates after growth in 10% glucose.
[d] Nonrepressed expression indicates after growth on 4% ethanol–3% glycerol for the oxygen uptake rates and after growth on 4% lactate for the β-galactosidase assays.

CAAAG-3′; and *MnSOD*, 5′-CCCGAATTCCAAAGCCAT ATTTCGACGCC-3′ and 5′-CCCGGATCCATCCTGGTAC GTTTTTAGGA-3′. The resulting amplicons were ligated into *Eco*RI-*Bam*HI-linearized YEp358 and YEp368 provided to us by Carol Lusty (12). The fusions placed the 5′ noncoding region adjacent to the initial ATG codon and placed an aspartate codon between this initial methionine and the proline at codon 8 of the bacterial *lacZ* gene.

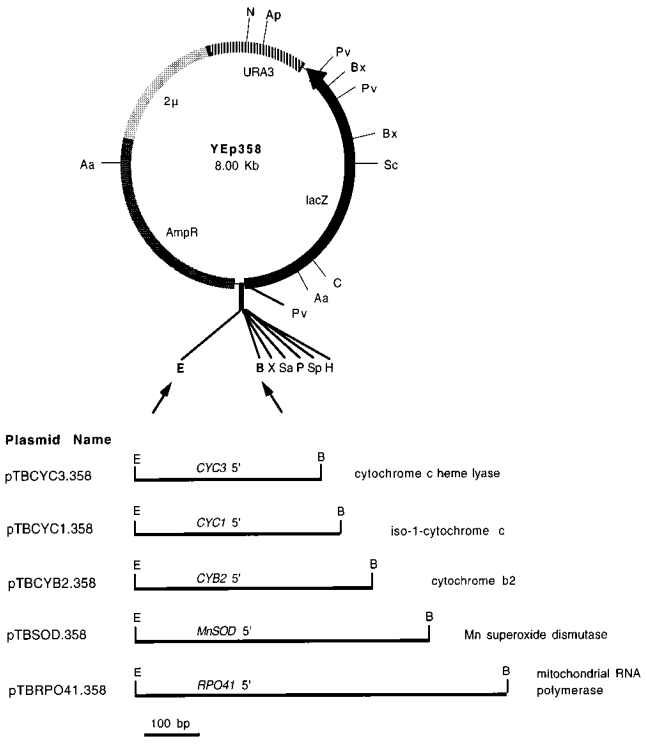

FIG. 1. Reporter plasmids for measuring transcription of nucleus-encoded mitochondrial proteins through expression of β-galactosidase. YEp358 and the five PCR amplicons were digested with *Eco*RI and *Bam*HI and ligated to form this set of β-galactosidase fusion plasmids. An additional set was also made with the *LEU2*-carrying plasmid YEp368, which is not shown. The *Bam*HI site at the 3′ end of each amplicon was used in regenerating the initiation codon of the *lacZ* gene. **E**, *Eco*RI; **B**, *Bam*HI; X, *Xba*I; Sa, *Sal*I; P, *Pst*I; Sp, *Sph*I; H, *Hin*dIII; Pv, *Pvu*II; Sc, *Sac*I; Bx, *Bst*XI; Ap, *Apa*I; N, *Nco*I; Aa, *Aat*II.

Repressed and nonrepressed expression from the *CYC1*, *CYC3*, *CYB2*, *RPO41*, and *MnSOD* promoters in each of these four strains are summarized in Table 2. YPH500 displays a slightly enhanced ability to repress expression in 10% glucose, and the other three strains have similar repressed expression from each of the promoters tested. The differences among the strains, however, become more apparent in the nonrepressing carbon, lactate. Expression from the *CYB2*, *RPO41*, *CYC3*, and *MnSOD* promoters was the highest in YPH500. PSY142 and BWG1-7A are less able in this regard. Of the four strains, W303-1A always yields the lowest expression of the five genes tested. Similar results were obtained after growth in galactose (data not shown).

A plausible explanation for the strain-dependent variations in regulation by carbon source is that a component of the mitochondrial genome is involved. We assayed expression of *CYB2*, *CYC1*, and *RPO41* in diploids which maintained the mitochondrial genome of only one parent. In all three cases, the mitochondrial genotype did not influence gene expression. This experiment does not rule out the possibility that the mitochondrial genome might contribute to carbon regulation of these genes but does indicate that the differences among these strains are not due solely to the mitochondrial genome. Examination of *CYB2* expression in the meiotic progeny of a cross between the carbon-responsive strain YPH500 and the relatively unresponsive strain W303-1A also revealed that the difference in regulation of derepression is a result of multiple genetic loci (data not shown).

Perlman and Mahler (13) first documented a strain variability in the carbon regulation of several mitochondrial enzyme activities. Later, a direct measurement of steady-state mRNA levels of the β-subunit of the mitochondrial F_1-F_0 ATP synthase established that there is a strain-dependent difference at the transcriptional level (17). Our findings extend these results and emphasize the importance of this variation for the design and interpretation of experiments involving carbon source regulatory effects on the expression of nucleus-encoded mitochondrial proteins in *S. cerevisiae*.

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REFERENCES

1. **Borralho, L. M., D. R. Malamud, A. D. Panek, M. N. Tenan, D. E. Oliveira, and J. R. Mattoon.** 1989. Parallel changes in catabolite repression of haem biosynthesis and cytochromes in repression-resistant mutants of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **135:**1217–1227.
2. **Dumont, M. E., J. F. Ernst, D. M. Hampsey, and F. Sherman.** 1987. Identification and sequence of the gene encoding cytochrome c heme lyase in the yeast *Saccharomyces cerevisiae*. EMBO J. **6:**235–241.
3. **Entian, K.-D.** 1986. Glucose repression: a complex regulatory system in yeast. Microbiol. Sci. **3:**366–371.
4. **Frohlich, K.-U., K.-D. Entian, and D. Mecke.** 1985. The primary structure of yeast hexokinase PII gene (HXK2) which is responsible for glucose repression. Gene **36:**105–111.
5. **Guarente, L.** 1983. Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. Methods Enzymol. **101:**181–191.
6. **Guarente, L., B. LaLonde, P. Gifford, and E. Alani.** 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. Cell **36:**503–511.
7. **Guiard, B.** 1985. Structure, expression and regulation of a nuclear gene encoding a mitochondrial protein: the yeast L(+)-lactate cytochrome c oxidoreductase (cytochrome b2). EMBO J. **4:**3265–3272.

7a. **Kim, K.-S., M. S. Rosenkrantz, and L. Guarente.** 1986. *Saccharomyces cerevisiae* contains two functional citrate synthase genes. Mol. Cell. Biol. **6:**1936–1942.

8. **Lodi, T., and B. Guiard.** 1991. Complex transcriptional regulation of the *Saccharomyces cerevisiae* CYB2 gene encoding cytochrome b2: CYP1(HAP1) activator binds to the CYB2 upstream activation site UAS1-B2. Mol. Cell. Biol. **11:**3762–3772.
9. **Maares, C. M., A. P. G. M. VanLoon, P. Oudshoorn, H. VanSteeg, and L. A. Grivell.** 1985. Nucleotide sequence analysis of the nuclear gene coding for manganese superoxide dismutase of yeast mitochondria, a gene previously assumed to code for the Rieske iron-sulphur protein. Eur. J. Biochem. **147:**153–161.
10. **Masters, B. S., L. L. Stohl, and D. A. Clayton.** 1987. Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. Cell **51:**89–99.
11. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Myers, A. M., A. Tzagoloff, D. M. Kinney, and C. J. Lusty.** 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene **45:**299–310.
13. **Perlman, P. S., and H. R. Mahler.** 1974. Derepression of mitochondria and their enzymes in yeast: regulatory aspects. Arch. Biochem. Biophys. **162:**248–271.
14. **Rothstein, R. J., and F. Sherman.** 1980. Genes affecting the expression of cytochrome c in yeast: genetic mapping and genetic interactions. Genetics **94:**871–889.
15. **Schuller, H.-J., and K.-D. Entian.** 1991. Extragenic suppressors of yeast glucose derepression mutants leading to constitutive synthesis of several glucose-repressible enzymes. J. Bacteriol. **173:**2045–2052.

15a. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.

16. **Smith, M., D. W. Leung, S. Gillam, C. R. Astell, D. L. Montgomery, and B. D. Hall.** 1979. Sequence of the gene for iso-1-cytochrome c in *Saccharomyces cerevisiae*. Cell **16:**753–761.
17. **Szekely, E., and D. L. Montgomery.** 1984. Glucose represses transcription of *Saccharomyces cerevisiae* nuclear genes that encode mitochondrial components. Mol. Cell. Biol. **4:**939–946.
18. **Westerbeek-Marres, C. A. M., M. M. Moore, and A. P. Autor.** 1988. Regulation of manganese superoxide dismutase in *Saccharomyces cerevisiae*: the role of respiratory chain activity. Eur. J. Biochem. **174:**611–620.
19. **Wilcoxen, S. E., C. R. Peterson, C. S. Winkley, M. J. Keller, and J. A. Jaehning.** 1988. Two forms of RPO41-dependent RNA polymerase. J. Biol. Chem. **263:**12346–12351.
20. **Wright, R. M., and R. O. Poyton.** 1990. Release of two *Saccharomyces cerevisiae* cytochrome genes, COX6 and CYC1, from glucose repression requires the SNF1 and SSN6 gene products. Mol. Cell. Biol. **10:**1297–1300.
21. **Zimmermann, F. K., and I. Scheel.** 1977. Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. Mol. Gen. Genet. **154:**75–82.
22. **Zitomer, R. S., D. Montgomery, D. L. Nichols, and B. Hall.** 1979. Transcriptional regulation of the yeast cytochrome c gene. Proc. Natl. Acad. Sci. USA **76:**3627–3631.