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## NOTES

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

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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  $\beta$ -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  $\mu$ M 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 non-repressed 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  $\beta$ -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  $\beta$ -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'-CCCGGATCCATGTTTCTTTTCGATCAAAA-3' and 5'-CCCGGATCCATTATTAATTTAGTGTGTGT-3'; *CYB2*, 5'-CCC GAATTCACGCATACATCGGAAGGATC-3' and 5'-CCCGGATCCATTGACTACTTTTGTGTTGCT-3'; *CYC3*, 5'-CCCGAATTCTGCGACAAAAGTGTGACCGA-3' and 5'-CCCGGATCCATTTTTGTAGTTTCTGTTG-3'; *RPO41*, 5'-CCCGAATTCCAAAGAAAGAAGATTACAAC-3' and 5'-CCCGGATCCATATTGAGTGAATAT

TABLE 1. Yeast strains used in this study

| Name    | Genotype  | Source and/or reference |
|---------|---|-------------------------|
| YPH500  | $\alpha$ <i>ade2-101 his3<math>\Delta</math>200 leu2<math>\Delta</math>1 lys2-801 trp1<math>\Delta</math>63 ura3-52</i> | P. Hieter (15a)         |
| YPH499  | <b>a</b> <i>ade2-101 his3<math>\Delta</math>200 leu2<math>\Delta</math>1 lys2-801 trp1<math>\Delta</math>63 ura3-52</i> | P. Hieter (15a)         |
| PSY142  | $\alpha$ <i>lys2-801 leu2-112 ura3-52</i>   | M. Rosencrantz (7a)     |
| BWG1-7A | $\alpha$ <i>his4-519 leu2-3,112 ade1-100 ura3-52</i>  | M. Rosencrantz (7a)     |
| W303-1A | <b>a</b> <i>ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1 can1-100</i>   | R. Rothstein            |

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

| Strain                    | Oxygen uptake <sup>a</sup> | β-Gal expression <sup>b</sup> |             |              |             |              |
|---------------------------|----------------------------|-------------------------------|-------------|--------------|-------------|--------------|
|                           |                            | <i>CYB2</i>                   | <i>CYC1</i> | <i>RPO41</i> | <i>CYC3</i> | <i>MnSOD</i> |
| Repressed <sup>c</sup>    |                            |                               |             |              |             |              |
| 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 <sup>d</sup> |                            |                               |             |              |             |              |
| 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        |

<sup>a</sup> Oxygen consumption is in nanomoles of oxygen per 100 mg of cells per minute. Values are the average of three separate assays.

<sup>b</sup> 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.

<sup>c</sup> Repressed expression indicates after growth in 10% glucose.

<sup>d</sup> 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 ATTCGACGCC-3' and 5'-CCCGGATCCATCCTGGTAC GTTTTATAGGA-3'. The resulting amplicons were ligated into *EcoRI*-*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 aspar-

tate codon between this initial methionine and the proline at codon 8 of the bacterial *lacZ* gene.

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<sub>1</sub>-F<sub>0</sub> 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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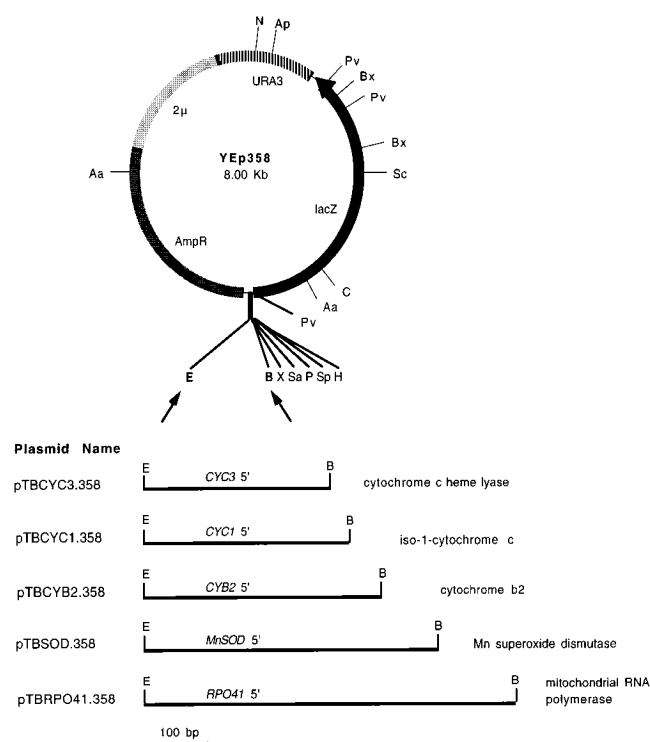


FIG. 1. Reporter plasmids for measuring transcription of nucleus-encoded mitochondrial proteins through expression of β-galactosidase. YEp358 and the five PCR amplicons were digested with *EcoRI* and *Bam*HI and ligated to form this set of β-galactosidase fusion plasmids. An additional set was also made 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, *EcoRI*; B, *Bam*HI; X, *Xba*I; Sa, *Sal*I; P, *Pst*I; Sp, *Sph*I; H, *Hind*III; Pv, *Pvu*II; Sc, *Sac*I; Bx, *Bst*XI; Ap, *Apa*I; N, *Nco*I; Aa, *Aat*II.

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