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# Interaction between yeast mitochondrial and nuclear genomes: Null alleles of *RTG* genes affect resistance to the alkaloid lycorine in rho$^0$ petites of *Saccharomyces cerevisiae*

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**Abstract**

Some nuclear genes in *Saccharomyces cerevisiae* (*S. cerevisiae*) respond to signals from the mitochondria in a process called by Butow (Cell Death Differ. 9 (2002) 1043–1045) retrograde regulation. Expression of these genes is activated in cells lacking mitochondrial function by involvement of *RTG1*, *RTG2* and *RTG3* genes whose protein products bind to "R-boxes" in the promoter region; RTG2p is a cytoplasmic protein. Since *S. cerevisiae* rho$^0$ strains, lacking the entire mitochondrial genome, are resistant to lycorine, an alkaloid extracted from *Amaryllis* plants, it could be hypothesized that in rho$^0$ cells the dysfunctional mitochondrial status stimulates overexpression of nuclear genes very likely involved in both nuclear and mitochondrial DNA replication. In this report we show that the resistance of rho$^0$ cells to lycorine is affected by the deletion of *RTG* genes.


## 1. Introduction

Cells are able to monitor and respond to the functional state of their organelles. Yeast cells respond to mitochondrial dysfunction by altering the expression of a subset of nuclear genes (Parikh et al., 1987, 1989). This response, called retrograde regulation, functions to better adapt cells to mitochondrial defects. In derepressed, respiratory-deficient cells, such as those that have lost their mitochondrial DNA (mtDNA) (rho$^0$ petites), the expression of genes involved in anaplerotic pathways, transport of small molecules, peroxisomal activities, and stress responses are up-regulated (Liao et al., 1991; Liu and Butow, 1999; Hallstrom and Moye-Rowley, 2000; Traven et al., 2000; Epstein et al., 2001). In many cases, these changes in gene expression reflect activities that would compensate for the block in the tricarboxylic acid (TCA) cycle caused by the respiratory defect. Expression of a number of these retrograde responsive genes, such as CIT2, DLD3, and PDH1 encoding, respectively, a glyoxylate cycle isoform of citrate synthase, a cytosolic D-lactate dehydrogenase, and a protein involved in propionate metabolism, is controlled by *RTG1*, *RTG2* and *RTG3*. Rtg1p and Rtg3p are basic helix–loop–helix transcription factors (Jia et al., 1997) and Rtg2p is a cytoplasmic protein with an N-terminal ATP-binding domain similar to that of the actin/sugar kinase/hsp70 superfamily (Bork et al., 1992). Rtg2p plays a pivotal role in the retrograde pathway because it is both a sensor of the functional state of mitochondria and is required for the activation of RTG-dependent gene expression by promoting the cytoplasmic-to-nuclear translocation of Rtg1p and Rtg3p (Sekito et al., 2000). In addition, Rtg2p is required for the partial

*Abbreviations: S. cerevisiae*, *Saccharomyces cerevisiae*; TCA cycle, Tricarboxylic acid cycle; rho$^0$, Cells without mitochondrial DNA; rho$^-$, Cells with grossly altered mitochondrial DNA; rho$^+$, Cells with wild type mitochondrial DNA; mit$^-$, Cells with point mutations in the mitochondrial DNA.

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dephosphorylation of Rtg3p associated with its nuclear accumulation.

In a previous paper we have demonstrated that the alkaloid lycorine (Davey et al., 1998) is able to differentiate between cells devoid of mtDNA (rho$^0$) and cells with mtDNA, either rho$^+$ or rho$^-$. Wild-type rho$^+$ cells, neutral rho$^-$ cells, and moderately suppressive rho$^-$ cells are all sensitive to lycorine; rho$^0$ cells, however, are resistant to high concentrations of the drug (Del Giudice et al., 1984). Most hyper-suppressive rho$^-$ strains show an intermediate resistance to lycorine (Massardo et al., 1990). In addition, we have demonstrated the use of lycorine resistance as an effective means of detecting the mitotic stability of rho$^+$ and rho$^-$ mitochondrial genomes in the presence or absence of the CCE1 (cruciform cutting endonuclease; Schofield et al., 1998) gene product (Massardo et al., 2000).

In order to analyze a retrograde regulation process by lycorine resistance in rho$^0$ cells, we have controlled the growth of rho$^0$ Δ*rtg* strains, deleted in *RTG* genes, in presence of lycorine. We find that rho$^0$ Δ*rtg* mutants, lacking of the RTG retrograde regulator products, are sensitive to lycorine as rho$^+$ yeast strains.

## 2. Materials and methods

### 2.1. Strains

The *Saccharomyces cerevisiae* strains, their nuclear and mitochondrial genotypes, and their origins are listed in Table 1.

Derivatives of PSY142 (*MATa leu2*, *lys2*, and *ura3* rho$^+$) were constructed as follows: to construct the Δ*rtg1* derivative, a 674 bp *Hind*III–*Sst*I fragment of *RTG1* was replaced by a 1.2 kb *Xho*I–*Hind*III fragment of the *URA3* gene, as described in Liao and Butow (1993). Ura$^-$ derivatives were obtained by selection with 5-fluoro-orotic acid. A Δ*rtg2* derivative was constructed by replacing a *Sal*I–*Xba*I fragment of *RTG2* with a 2.2 kb fragment of the *LEU2* gene, thus deleting codons 23–573 of *RTG2* (Rothermel et al., 1995). To construct a Δ*rtg3* derivative, codons 175–340 of *RTG3* were replaced by a 1.6 kb fragment of the *LEU2* gene (Jia et al., 1997). Rho$^0$ derivatives of these strains were generated by several passages of rho$^+$ cells in YEPD medium supplemented with 25 μg/ml of ethidium bromide.

### 2.2. Media

Glucose-complete-medium (YEPD): 1% yeast extract, 1% Bacto peptone, and 2% glucose. Glycerol-complete-medium (YEPG): 1% yeast extract, 1% Bacto peptone, and 3% glycerol. Minimal medium (YNB): 0.67% yeast nitrogen base without amino acids, 2% galactose; supplementation when necessary.

To all solid media 2.5% Bacto agar was added.

### 2.3. Growth measurements

These were performed with a Klett–Summerson colorimeter.

### 2.4. Radioactive labelling

Cells were grown in YNB with 2% galactose and 30 μg/ml supplements at 30 °C under continuous shaking. Cells were collected by centrifugation and washed three times with YNB plus 1% galactose and supplements. The pellet was resuspended at $5–8 \times 10^8$ cells/ml in the latter medium and divided in four aliquots. After shaking for 20 min at 30 °C, antibiotics (cycloheximide: 500 μg/ml and lycorine: 100 μg/ml) were added 3 min (cycloheximide) or 15 min (lycorine) before the radioactive label. Labelling was stopped by chasing with 1% casamino acids (Difco) and a hundred-fold excess of unlabeled nutrients at 0 °C. Incorporation was measured in the material precipitated by 5% TCA and 95% ethanol. Radioactivity was counted in a liquid scintillation counter (Nuclear Chicago).

Protein synthesis was measured as incorporation of NEC-445 L-amino acid-$^{14}$C(U) mixture (100 μCi/ml, specific activity 3425 mCi/mM, obtained from NEN). The labelling period was 20 min.

DNA synthesis was measured as incorporation of $^{14}$C-adenine (1.5 μCi/ml; specific activity 276 mCi/mM, obtained from Amersham Bucks, England). The labelling

Table 1
List of strains, nuclear and mitochondrial genotypes and origins

| Strain | Nuclear genotype | Mitochondrial genotype | Origin |
|---|---|---|---|
| Sc57=YM654 | *a ura3-52 his3-Δ200 ade2-101 lys2-801 tyr1-501* | rho$^+$ | M. Johnston |
| Sc57-R3 | *a ura3-52 his3-Δ200 ade2-101 lys2-801 tyr1-501* | rho$^0$ | L. Del Giudice |
| RB4=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52* | rho$^+$ | R.A. Butow |
| RB5=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52* | rho$^0$ | R.A. Butow |
| RB6=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg1::URA3* | rho$^+$ | R.A. Butow |
| RB7=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg1::URA3* | rho$^0$ | R.A. Butow |
| RB8=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg2::LEU2* | rho$^+$ | R.A. Butow |
| RB9=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg2::LEU2* | rho$^0$ | R.A. Butow |
| RB10=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg3::LEU2* | rho$^+$ | R.A. Butow |
| RB11=PSY142 | *a leu2-2 leu2-112 lys2-801 ura3-52 rtg3::LEU2* | rho$^0$ | R.A. Butow |