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Review

Retrograde regulation of multidrug resistance in *Saccharomyces cerevisiae*

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Abstract

Communication between the mitochondria and the nucleus is essential to ensure correct metabolic coordination of the cell. Signaling pathways leading from the mitochondria to the nucleus are referred to as retrograde signaling and were first discovered in the yeast *Saccharomyces cerevisiae*. Cells that lack their mitochondrial genome (ρ^0 cells) trigger expression of the nuclear *CIT2* gene in order to ensure adequate amino acid biosynthesis. More recently, it has been found that a different set of genes involved in multidrug resistance in *S. cerevisiae* is strongly induced in ρ^0 cells. During a search for negative regulators of the ATP-binding cassette (ABC) transporter-encoding gene *PDR5*, it was observed that ρ^0 mutants exhibited dramatic up-regulation of the transcript of this gene. This induction was due to the post-translational activation of a direct upstream regulator of *PDR5* that was designated Pdr3p. Loss of the *LGE1* gene led to a block in ρ^0-mediated induction of *PDR5* expression. Lge1p has been observed by others to be involved in histone H2B ubiquitination along with the ubiquitin-conjugating enzyme Rad6p and the ubiquitin ligase Bre1p. Our studies provide evidence that Lge1p has another function unique from H2B ubiquitination that is required for retrograde regulation of *PDR5* transcription. We have also found that the Pdr pathway regulates expression of several genes involved in sphingolipid biosynthesis. These findings suggest that the physiological role of the *PDR* genes might be to regulate membrane homeostasis and ρ^0-triggered changes in this parameter may be the signal controlling *PDR* gene expression.

Keywords: Mitochondria; Transcription; *PDR3*; *PDR5*; ATP-binding cassette transporter

1. Introduction

A defining characteristic of eukaryotic cells is the presence of multiple genomes within a common cytoplasm. These organelle-associated genomes include the DNA within the mitochondria and the chloroplasts. While it is believed that these organellar genomes have evolved from free-living organisms, their DNA content has been adjusted over time to encode only a small number of the total proteins that will ultimately make up the functional organelle. While several hundred different proteins are required to synthesize a fully operational mitochondrion, only 13 of these are encoded by the mitochondrial genome (see (Reichert and Neupert, 2004) for a recent review). Coordinating the expression of the nuclear and mitochondrial genomes is critical for proper metabolism and physiology. The regulatory connection from the mitochondria to the nucleus in cells is referred to as retrograde regulation (reviewed in (Butow and Avadhani, 2004)).

Retrograde regulation was first genetically defined in the yeast *Saccharomyces cerevisiae* in studies of nuclear gene expression of cells that lack their mitochondrial DNA (ρ^0 cells) (Parikh et al., 1987). Expression of the nuclear *CIT2* gene was found to be strongly induced at the transcriptional level in order to maintain levels of the tricarboxylic acid cycle intermediate α-ketoglutarate, the crucial precursor for the amino acid glutamate (Small et al., 1995). Extensive genetic analyses of the retrograde regulation of *CIT2* identified two basic region-helix-loop-helix transcription

Abbreviations: PDRE, Pdr1p/Pdr3p response element; ABC, ATP-binding cassette; Pdr, Pleiotropic drug resistance; IPC, Inositol-phosphoceramide; MIPC, Mannose–inositol-phosphoceramide; $M(IP)_2C$, Mannose–(inositol-P)$_2$-phosphoceramide; LCB, Long chain base; LCBP, Long chain base phosphate; DHS, Dihydrosphingosine; PHS, Phytosphingosine.

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factors designated Rtg1p and Rtg3p as the key transcriptional activators of *CIT2* expression in response to loss of the mitochondrial genome (Liao and Butow, 1993; Jia et al., 1997). More recent experiments have focused on elaborating the signaling pathway connecting the mitochondria with activation of Rtg1p/Rtg3p function and this impressive progress was recently reviewed (Butow and Avadhani, 2004).

Since many different metabolic processes interface with the mitochondria, ρ^0 cells require transcriptional reprogramming beyond activation of *CIT2* expression in order to remain viable. Several years ago (Hallstrom and Moye-Rowley, 2000), our laboratory found that ρ^0 cells strongly induced expression of multidrug resistance genes along with the previously described activation of *CIT2* transcription. Recent progress in the understanding of this new retrograde regulatory pathway will be discussed here.

2. Multidrug resistance in *S. cerevisiae*

Early genetic studies on drug resistant mutants led to the unexpected finding that a large number of mutant alleles in a locus designated *PDR1* led to the simultaneous acquisition of resistance to a number of different, functionally unrelated drugs (Rank and Bech-Hansen, 1973). These different drugs included a number of mitochondrial poisons as well as the cytoplasmic translation elongation inhibitor cycloheximide. Genetic analyses of these drug hyper-resistant *PDR1* alleles indicated that the mutant forms of this gene were likely gain-of-function hypermorphs as elevated drug resistance could be seen even in the presence of a wild-type copy of the gene (Rank and Bech-Hansen, 1973). Cloning and characterization of the *PDR1* locus demonstrated that this gene encoded a Zn_2Cys_6 zinc cluster-containing transcriptional regulatory protein (Balzi et al., 1987). A combination of genetic analyses and DNA sequencing led to the discovery that a protein sharing a high degree of sequence similarity with Pdr1p was present near the centromere of chromosome II and that similar drug hyper-resistant forms of this protein could be selected (Subik et al., 1986; Delaveau et al., 1992). This homologue was designated Pdr3p.

The knowledge that both *PDR1* and *PDR3* encoded transcription factors argued that neither of these genes was directly responsible for the observed effects on drug resistance and that target genes under the influence of these proteins were likely the effectors of multidrug resistance. A screen of a high-copy-number plasmid library for genes that conferred multidrug resistance when present in elevated copy number identified the *PDR5* locus (Leppert et al., 1990). Molecular characterization of *PDR5* determined that this gene encodes an ATP-binding cassette (ABC) transporter protein that was transcriptionally induced in cells carrying hyperactive gain-of-function alleles of *PDR1* (Meyers et al., 1992) and *PDR3* (Nourani et al., 1997). Mutational analyses and DNA binding experiments established that both Pdr1p and Pdr3p recognized three elements in the *PDR5* promoter that have been designated Pdr1p/Pdr3p response elements (PDREs) (Katzmann et al., 1994, 1996). These PDREs are typically found in the promoters of all genes known to be regulated by Pdr1p and/or Pdr3p.

3. Retrograde control of *PDR5* expression

Analyses of the gain-of-function forms of Pdr1p and Pdr3p led to the finding that these mutant alleles represented single amino acid substitution mutations clustered in discrete regions of each factor (Carvajal et al., 1997; Nourani et al., 1997; Simonics et al., 2000). Coupled with the known dominant or semi-dominant behavior of these mutants, these findings supported the hypothesis that these factors might normally be maintained in a state of repressed activity that is relieved by these mutant alleles. To test this hypothesis, we carried out a search for negatively acting regulatory genes using a transposon mutagenesis strategy (Burns et al., 1994). Transposons were inserted at random into the genome of a wild-type *S. cerevisiae* strain containing an integrated *PDR5-lacZ* reporter gene. Cycloheximide hyper-resistant colonies were selected and clones that also exhibited high level expression of *PDR5-lacZ* were retained. Genetic analysis and cloning of the genes inactivated by transposon insertion led to the finding that disruption of the *FZO1* or *OXA1* genes elicited overproduction of *PDR5* (Hallstrom and Moye-Rowley, 2000). Fzo1p is required for normal mitochondrial fusion and loss of this protein causes cells to become ρ^0 (Hermann et al., 1998; Rapaport et al., 1998). Oxa1p is an important assembly factor that is required for faithful production of the mitochondrial inner membrane complexes present in the cytochrome *c* oxidase and Fo ATPase subcomplex (Altamura et al., 1996). Mutants lacking *OXA1* are nuclear petites but segregate ρ^0 cells at a high frequency (unpublished data). Further analyses demonstrated generation of ρ^0 cells by ethidium bromide treatment of a wild-type strain was sufficient to lead to both a robust multidrug resistant phenotype and *PDR5* overexpression.

To examine the molecular basis for this observed induction of *PDR5* in ρ^0 cells, genetic studies were performed in which either the *PDR1* or *PDR3* gene was removed individually from ρ^0 cells. Loss of *PDR3* was sufficient to block the increased *PDR5* transcription that would normally occur in ρ^0 cells while *PDR1* could be eliminated without decreasing *PDR5* induction. Importantly, loss of *RTG1* only slightly diminished *PDR5* activation in ρ^0 cells, demonstrating that the retrograde pathway connecting multidrug resistance to the mitochondria is distinct from that linking *CIT2* expression to mitochondrial status.

A feature found in the *PDR3* gene but not *PDR1* is the presence of an autoregulatory loop controlling *PDR3* transcription (Delahodde et al., 1995). Two PDREs are