Genome-wide studies on the nuclear PDR3-controlled response to mitochondrial dysfunction in yeast

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Abstract Gain-of-function mutations in the transcription factors Pdr1p and Pdr3p lead to the up-regulation of genes controlling plasma membrane properties. Pdr3p is involved in a retrograde response in which mitochondrial dysfunctions activate *PDR5*, a gene encoding an ABC membrane transporter. We carried out genome-wide analyses of the *PDR3*-controlled genes activated by the deletion of the mitochondrial DNA. We present evidence showing that *PDR1* does not interfere with this *PDR3* response. We also showed that the mitochondrially activated *PDR3* response is highly sensitive to both yeast strain variations and carbon sources. These observations explain the apparent discrepancies in published studies and better describe the connections between the mitochondrial state and plasma membrane properties.

Key words: Yeast; Microarray; Drug resistance; Mitochondrion

1. Introduction

Mitochondria play a fundamental role in cell physiology, producing cellular energy, essential metabolites and reactive oxygen species. Mitochondrial activity is tightly controlled by the nucleus, yet numerous signals originate from mitochondria and influence nuclear gene expression and thus biochemical pathways in the whole cell [1]. In fungi, the mitochondrial control of nuclear gene expression is called the retrograde response. It has been characterized in yeast cells lacking the mitochondrial genome (rho0 cells) and it was shown that a respiratory deficiency induces a suite of genes associated with both peroxisomal activities and metabolite-restoration pathways [2]. This transcriptional retrograde response is controlled by two transcription factors: Rtg1p and Rtg3p [3]. Epstein et al. [2] used genome-wide microarrays to show that the rho0 phenotype induces extensive changes in gene expression, only a small number of which are dependent on RTG genes. This suggests that other transcription regulation networks are connected to mitochondria signalling pathways [2]. For example, a large number of genes involved in pleiotropic drug resistance (PDR) are activated in rho0 cells [4]. Two major regulators of the PDR phenomenon have been identified in *Saccharomyces cerevisiae*, Pdr1p and Pdr3p [5,6]. Pdr1p and Pdr3p are close homologues and recent genome-wide studies have shown that their activities largely overlap. About 20 target genes have been identified. These mainly encode ABC transporters (e.g. *PDR5*), permeases and proteins involved in membrane biogenesis [7,8]. *PDR3* controls its own transcription and can also be regulated by Pdr1p [9]. Hallstrom et al. [10] screened for negative regulators of *PDR5* expression and described a *PDR3*-specific activation of *PDR5* transcription in cells lacking the mitochondrial genome and in cells deleted for components of the electron transport chain [11]. These effects were correlated with the resistance of rho0 cells to a large number of drugs. We used whole-genome microarrays to complete the list of genes up-regulated in rho0 cells. We showed that all of the mitochondria-dependent PDR effects are dependent on an increase in *PDR3* expression and independent of *PDR1* activity. We suggest that, for some of these genes, *PDR3* requires specific factors other than the rho0 status of the cells to mediate transcriptional activation. Finally, we observed that PDR activation in rho0 cells is influenced by both the carbon source and the background of the strains. These results imply that there is a tight link between *PDR3* activity and mitochondrial activity, independently of any drug treatment. This genome-wide study provides an overview of the control that mitochondria status exerts on membrane properties.

2. Materials and methods

2.1. Yeast strains and growth conditions

The strains FY1679-28C (Matα *ura3-52 trp1Δ63 leu2Δ1 his3Δ200*) and SEY6210 (Matα *ura3-52 trp1Δ901 leu2-3 his3Δ200 suc2Δ9 lys2-801*) and their derivatives FYΔ*pdr3* (FY1679 Δ*pdr3::HIS3*), FYΔ*pdr1* (FY1679 Δ*pdr1::TRP1*), FYΔ*pdr3*Δ*pdr1* (FY1679 Δ*pdr3::HIS3* Δ*pdr1::TRP1*) and SEYΔ*pdr3* (SEY6210 Δ*pdr3::HisG*) were used for genome-wide analyses of the role of PDR1 and PDR3 in rho0 and rho+ cells. Strain EC70 (FY1679 Δ*pdr3::HIS3::PDR3-7* Δ*pdr1::TRP1*) was constructed in this study. Strain PSY142 (Matα *ura3 leu2 lys2*) and its isogenic rho0 derivative were kindly provided by Ronald Butow. Strains W303-1B (Matα *ura3-1 ade2-1 trp1 leu2-3 his3-11 suc2Δ9 lys2-801 can1-100*) and BY4742 (MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) were used for Northern blot analyses of *PDR3* and *PDR5* expression. Strains were grown in a rich medium containing 1% yeast extract, 1% bactopeptone and 2% of the corresponding carbon source. Cells were grown at 30°C to an OD_{600nm} of 0.6–0.8. They were collected by centrifugation and frozen at −80°C prior to RNA extraction.

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Abbreviations: PDR, pleiotropic drug resistance

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2.2. Ethidium bromide treatment

The rho0 derivatives of the strains described above were obtained by plating the cells twice on agar plates containing 40 μg/ml ethidium bromide (2% glucose, 1% yeast extract, 1% bactopeptone, 20 mg/l adenine, 0.05 M phosphate buffer pH 6.2, 2% agar, 40 μg/ml ethidium bromide).

2.3. RNA extraction and microarray analyses

mRNA was purified using the Micro-Fasttrack 2.0 system from Invitrogen. Yeast microarrays were obtained from Hitachi Software and DNAChip Research. 2 μg of mRNA was used for reverse transcription reactions. Detailed protocols are available at www.biologie.ens.fr/fr/genetiqu/puces/protocoles puces.html. The arrays were analyzed with the Genepix 3.0 software. We excluded artefactual spots, saturated spots and low signal spots. Assuming that the expression of most genes was unchanged, the Cy3/Cy5 ratios were normalized by use of the median of all the ratios for each experiment using Arrayplot (Marc et al., submitted). The cluster images shown in Figs. 1 and 2 were generated by Treeview [20].

2.4. Northern blot analyses

Total RNA (15 μg) was denatured in formamide at 65°C and separated on a 1.5% agarose gel. Northern analyses were performed as described previously [21]. Probes were radioactively labelled using the Klenow enzyme and the Nonaprimer labelling kit (Q-Biogene).

3. Results

3.1. Genome-wide analysis of the rho0-mediated activation of Pdr3p

We used five strains throughout this study: SEY 6210, FY 1679-18B, W303-1B, BY4742 and PSY142. For the sake of simplicity, they will be called SEY, FY, W303, BY and PSY, respectively. The form of Pdr3p that is activated by mitochondrial dysfunction will be called Pdr3p*.

We carried out a genome-wide study on the transcriptome modifications induced by the production of Pdr3p* in SEY and FY. SEY was chosen because it is the first strain in which the role of Pdr3p* was demonstrated [10]. Rho0 derivatives of the appropriate strains were constructed by classical methods. Comparative transcriptome analyses, using cDNA-based microarrays containing most of the yeast open reading frames, were conducted in triplicate on wild-type and rho0 strains, and on rho0 strains in the presence or absence of *PDR3*. The first type of experiments reveals the global effects of the rho0 phenotype in these two strains (Fig. 1, first and third columns), and the second experiment points out the nuclear genes which are specifically up- or down-regulated by Pdr3p* (Fig. 1, second and fourth columns). The genes induced in both cases (Fig. 1A) probably respond to mitochondrial dysfunction via *PDR3*. The fact that all of these genes contain at least one PDR-responsive element in their promoter region suggests that Pdr3p* directly interacts with these promoters. The six genes in group B (Fig. 1) might also be direct targets of Pdr3p*, but they behaved differently in the two strains. Finally, many genes were up-regulated in a *PDR3*-independent manner in the rho0 context (Fig. 1C). Most of these genes have previously been characterized and depend on other pathways of cross-talk between mitochondria and nucleus [4]. The RTG network is the best characterized. The RTG genes activated by mitochondrial dysfunction include several genes involved in peroxisome biogenesis [2]. The genes that respond

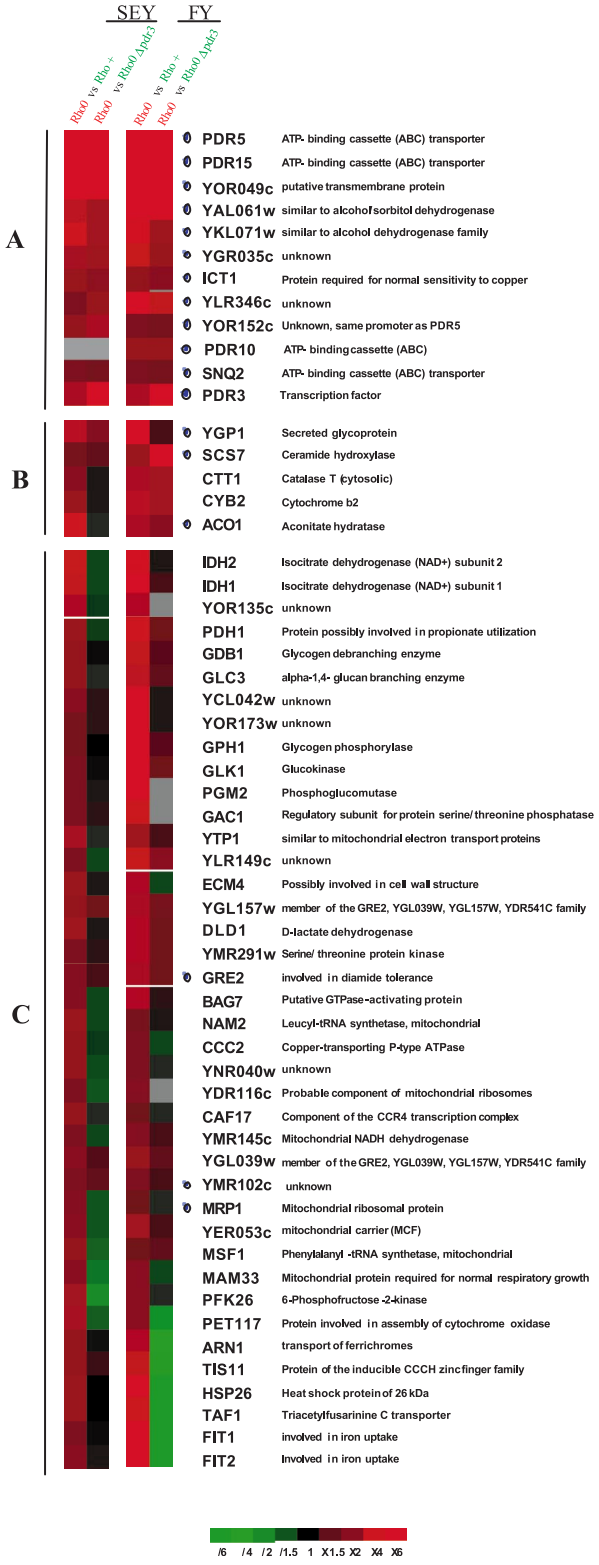

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Fig. 1. Genes activated by Pdr3p* in rho0 cells. The transcription profiles of 54 genes, the expression of which changed significantly in both SEY (cluster on the left) and FY (cluster on the right) following the loss of mtDNA (first column), were arranged according to their *PDR3* dependence (second column). The blue dots indicate PDR-responsive elements in the corresponding promoters. A: Genes whose activation is dependent on PDR3. B: Genes whose activation is dependent on PDR3 in either FY or SEY background. C: Genes whose activation is independent of PDR3. All of the information for YPD [22] is mentioned. Changes in expression are indicated by a colored bar in the bottom right corner. The corresponding values are given in Table 1 at www.biologie.ens.fr/yeast-publi.html.

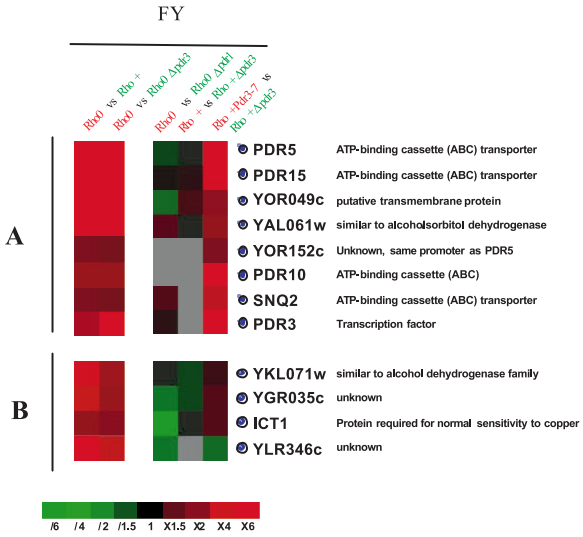

Fig. 2. Effect of *PDR1* and the gain-of-function allele, *PDR3-7*, on the genes activated by Pdr3p* in rho0 cells. The transcription profiles of 23 genes, the expression of which significantly changed in FY background following the loss of mtDNA (first cluster on the left), were arranged according to their sensitivity to PDR1 activity in rho0 cells (second cluster, first column) and to PDR3-7 dependence (second cluster, third column) in rho+ cells. A: Pdr3p*-dependent genes whose activation is independent of PDR1 in rho0 cells and dependent on PDR3-7 in rho+ cells. B: Pdr3p*-dependent genes whose activation is independent of PDR1 on rho0 cells and of PDR3-7 in rho+ cells. The corresponding values are given in Table 1 at www.biologie.ens.fr/yeast-publi.html.

to RTG were poorly activated in our experiments. Both strain difference and growth in glucose rather than in raffinose explain this difference (see below). Additionally, a group of genes involved in iron uptake and homeostasis was activated as a result of the mtDNA defect. This group includes two siderophore transporters (Taf1p and Arn1p), two unknown membrane proteins (Fit1p and Fit2p) thought to be iron uptake facilitators [12], and the regulator, Tis11p [13].

3.2. *Variations in strains and growth conditions modulate the activation of Pdr3p**

3.2.1. Pdr3p activity is not dependent on PDR1 in rho0 cells. Pdr3p and Pdr1p have essentially the same targets in wild-type cells [8]. Moreover, when Pdr1p is overexpressed it can positively regulate the *PDR3* promoter [9]. However, the role of Pdr1p in the activation of Pdr3p* targets is ambiguous. As shown by β-galactosidase assays, the activation of *PDR5* in cells deficient for mitochondrial function is not dependent on *PDR1* [10]. On the other hand, the activation of a *PDR5-LacZ* reporter in rho0 cells has been found to be slightly modulated by *PDR1* [14]. Given these data, we attempted to determine the role of *PDR1* in the rho0 activation of PDR targets. Remarkably, the deletion of *PDR1* from rho0 cells had no effect on the activation of the Pdr3p*-dependent genes, as shown by microarray (Fig. 2A,B) and Northern blot (data not shown) analyses. According to these results, Pdr3p* activity is independent of *PDR1*.

3.2.2. The insertion of the PDR3-7 gain-of-function allele into the genome of rho+ cells partially mimics the effects of mtDNA loss. As shown by Hallstrom et al. [10], the activation of *PDR3* transcription in rho0 cells is dependent on the post-transcriptional activation of Pdr3p itself. Thus, we decided to determine whether a Pdr3p gain-of-function mutant in a rho+ background would be able to reproduce the PDR activation observed in rho0 cells. Therefore, we compared the transcriptomes of cells deleted for *PDR3* and *PDR1* with the same cells carrying a genomic insertion of the gain-of-function allele *PDR3-7* at the *PDR3* locus. Pdr3-7p activated the transcription of *PDR5*, *PDR15*, *YOR049c*, *YAL061w*, *YOR152c*, *SNQ2*, *PDR10* and *PDR3-7* itself (Fig. 2A). Interestingly, *PDR3-7* did not activate *YKL071w*, *YGR035c*, *ICT1* or *YLR346c* (Fig. 2B). All of these genes except *YLR346c* had previously been reported to be independent of *PDR3-7* carried on a centromeric plasmid [8]. This implies that Pdr3p alone cannot activate these genes and that some other factors activated in rho0 but not in rho+ cells are required.

3.2.3. The rho0-mediated PDR activation depends on the carbon source and is sensitive to strain variations. The above results, together with previous ones, demonstrated the intricate relation between rho0 phenotype and the PDR network. However, similar microarray experiments carried out by Epstein et al. [2] on rho0 cells of PSY background grown in raffinose gave quite different results. In these conditions, none of the PDR targets were activated. This raised the question of whether the PSY strain can activate *PDR3* following the loss of mtDNA and whether the carbon source affects

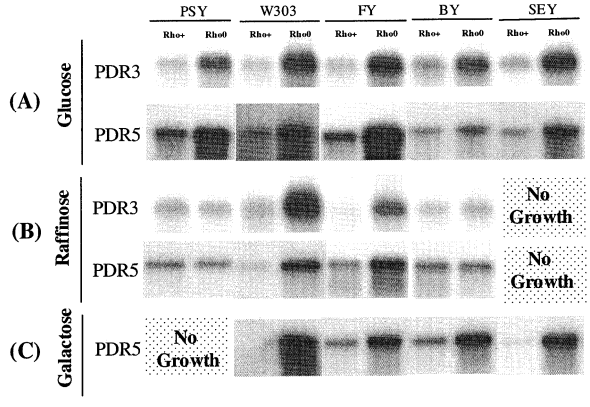

Fig. 3. Northern blot analyses of the amount of *PDR3* and *PDR5* mRNA in rho0 and rho+ cells from different backgrounds grown on different carbon sources. A: Glucose. B: Raffinose. C: Galactose. No Growth: The invertase gene (*SUC2*) of SEY contains a mutation and thus SEY fails to grow on raffinose. The PSY rho0 strain did not grow on galactose.

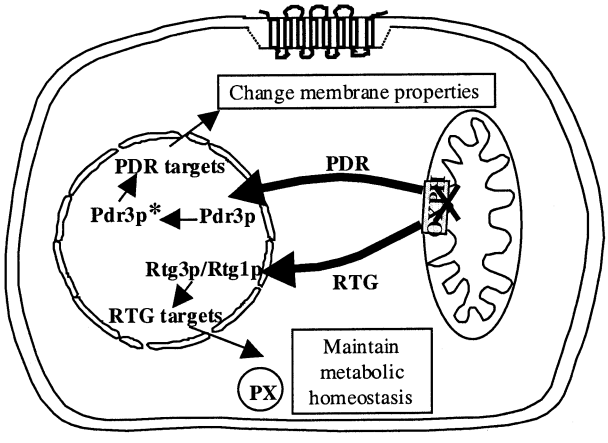

Fig. 4. Multiple signals regulating nuclear gene expression originate from mitochondria dysfunction. PX: peroxisome. OXPH: oxidative phosphorylation.

Pdr3p activity. To address these points, we investigated gene expression in rho0 cells in five different backgrounds (PSY, W303, FY, BY, SEY), grown on three different carbon sources (glucose, raffinose, galactose). Northern blot analyses using *PDR3* and *PDR5* probes as controls for PDR activation showed that PDR activation occurs in all rho0 cells grown in glucose (Fig. 3A). However, PSY and BY strains could not activate *PDR3* or *PDR5* when grown in raffinose, whereas FY and W303 exhibited the same PDR activation as in glucose (Fig. 3B). To determine whether this effect was due to the low fermentative rate of raffinose compared with glucose we investigated the rho0-mediated activation of *PDR5* in galactose, which like raffinose is a poor substrate for fermentation. Surprisingly, all of the strains tested, including BY, were able to activate *PDR5* in a rho0 background (Fig. 3C). Therefore, the differences observed in raffinose and in glucose were not caused by a difference in the metabolic rates, and the PDR response of rho0 cells is not specific to glucose repressive conditions.

4. Discussion

4.1. Pdr3p is activated by mitochondrial dysfunction

Alterations in mitochondrial function modify the expression of a large number of genes [2] [4]. Each of these changes has to be analyzed individually to distinguish direct effects from indirect effects. We confirmed, at the genome level, that mitochondrial dysfunction activates the transcription factor Pdr3p in a form (Pdr3p*) which remains to be understood (Fig. 4). We nevertheless offer several clues on the properties of this Pdr3p* form. We show that 11 (or possibly 14) genes are targets of Pdr3p* (Fig. 1A,B). Interestingly, a genomic version of PDR3, carrying the gain-of-function allele, PDR3-7, had fewer target genes. At least three genes (*ICT1*, *YGR035C* and *YKL071W*) are up-regulated by Pdr3p* but not regulated by the gain-of-function mutant. This suggests that other transcription factors act in concert with Pdr3p*. Although Pdr1p and Pdr3p are very similar, Pdr1p is not involved in this process. This shows that although *PDR1* and *PDR3* control similar sets of target genes, their own regulation is very different. This is true at the transcription level (only *PDR3* is autoregulated [9]), at the nucleus import level (the β-karyopherins differ [15]) and at the post-translational level (mitochondria dysfunction only activates Pdr3p [10] and this study). Interestingly, the overexpression of *PDR3* is connected with the suppression of mitochondrial import defects [16]. However, the genes involved in this process, like *TOM72*, are probably not activated directly by Pdr3p*.

4.2. Different yeast strains elicit different PDR3-controlled responses

One important aspect of this work is the clear demonstration that the activation of Pdr3p* is dependent on both the yeast strains and the carbon sources used. This clarifies the conflicting data in the literature, which reported differences in the nuclear responses to mitochondrial dysfunction [2,4]. We did not find an active form of Pdr3p* in PSY or BY strains when they were grown on raffinose, whereas the *PDR3* autoactivation process was clearly observed in W303 or FY strains grown in the same conditions. The carbon source influences this phenotype, as shown by the fact that when PSY or BY were grown in glucose the *PDR3* autoactivation process occurred as in W303 and FY. It is difficult to known whether carbon metabolism affects the mitochondrial response in a strain-dependent manner because we do not know the nature of the molecular process that controls the activation of Pdr3p. It has been suggested that glutamate levels are a key signalling component in the retrograde response pathway [17]. Alternatively, the mitochondrial NADH has been proposed to be a signalling molecule in mammalian cells [18]. The NADH/NAD level, which reflects the electron transport chain activity, might activate Pdr3p in rho0 cells. Finally, the specific features of the PSY and BY strains provide a convenient model to screen for regulators that could restore *PDR3* activity on raffinose. The identification of such regulators will greatly improve our understanding of the physiological regulation of Pdr3p activity and may help us to identify the signalling pathways that connect mitochondria activity to membrane properties in yeast. Such a connection between mitochondrial DNA status and membrane damage has been shown to contribute to tumor progression and metastasis in human cells [19].

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