Responses of Pathogenic and Nonpathogenic Yeast Species to Steroids Reveal the Functioning and Evolution of Multidrug Resistance Transcriptional Networks

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Steroids are known to induce pleiotropic drug resistance states in hemiascomycetes, with tremendous potential consequences for human fungal infections. Our analysis of gene expression in Saccharomyces cerevisiae and Candida albicans cells subjected to three different concentrations of progesterone revealed that their pleiotropic drug resistance (PDR) networks were strikingly sensitive to steroids. In S. cerevisiae, 20 of the PDR1p/Pdr3p target genes, including PDR3 itself, were rapidly induced by progesterone, which mimics the effects of PDR1 gain-of-function alleles. This unique property allowed us to decipher the respective roles of Pdr1p and Pdr3p in PDR induction and to define functional modules among their target genes. Although the expression profiles of the major PDR transporters encoding genes ScPDR5 and CaCDR1 were similar, the S. cerevisiae global PDR response to progesterone was only partly conserved in C. albicans. In particular, the role of Tac1p, the main C. albicans PDR regulator, in the progesterone response was apparently restricted to five genes. These results suggest that the C. albicans and S. cerevisiae PDR networks, although sharing a conserved core regarding the regulation of membrane properties, have different structures and properties. Additionally, our data indicate that other as yet undiscovered regulators may second Tac1p in the C. albicans drug response.

Candida albicans is a commensal organism that colonizes a large proportion of the population on the mucosal surfaces of the gastrointestinal and urogenital tracts without clinical symptoms. But in immunocompromised patients, C. albicans causes a wide spectrum of diseases ranging from mucocutaneous infections like oral thrush to disseminated candidiasis, with a mortality rate of ~40% despite the use of available antifungal agents (19). The various sites of Candida infection in the human body present different challenges, and the conditions within the microenvironments that change the relationship of C. albicans with the host from commensal to pathogenic need to be studied in more detail. Recent efforts have revealed that yeast cells can respond to human steroids (15, 27). Some investigators have determined the presence in yeast of steroid binding proteins, such as estradiol binding protein (32), corticosteroid binding protein (33), and progesterone binding protein (12), but their exact role in the steroid response is not known. Early and more recent studies have shown that steroids can induce a pleiotropic drug resistance (PDR) state in both C. albicans and Saccharomyces cerevisiae. This PDR state was mediated by the overexpression of genes encoding PDR determinants, such as the ABC transporter-encoding genes CaCDR1, CaCDR2, and ScPDR5, which are known to be regulated by the CaTac1p, ScPdr1p, and ScPdr3p transcription factors, respectively (11, 34). It is noteworthy that the CaTac1p gene was itself found responsive to estradiol (9) and progesterone (this study). de Micheli et al. (15) investigated the transcriptional basis of the steroid responsiveness of Candida genes by analyzing the CDR1 promoter and identified a nucleotide stretch spanning −462 to −213 (with respect to ATG) which mediates both drug and steroid responses (15). Although belonging to the same family of Gal4-like zinc finger proteins, Tac1p is not orthologous to Pdr1p and Pdr3p and has less than 20% sequence identity with these proteins. As a consequence, the DNA binding sites of Pdr1p and Pdr3p (named PDRE [5′-TCCGYGGR-3′]) are significantly different from the Tac1p recognition motif (named DRE [5′-CGG AA/TATCGGATA-3′]) (15). Our own analysis of the CDR1 promoter led to the identification of two hitherto-unknown cis-regulatory regions responding specifically to progesterone and β-estradiol and showed that other steroid-responsive genes in the C. albicans genome also contain these sequences, indicating a conserved role in mediating the steroid response in C. albicans (5, 26). More recently it has been shown by transcriptome analysis that the yeast responses to high doses of progesterone involved a large number of both C. albicans and S. cerevisiae genes (5, 6, 9).

In the present study, we aimed at deciphering a more accurate evolutionary significance of the steroid response in yeasts.

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by submitting *S. cerevisiae* and *C. albicans* cells to several doses of progesterone for different time periods. The diploid *C. albicans* evolved and diverged from the haploid *S. cerevisiae* several million years ago. The question of the commonalities and differences between the responses in the two species was particularly intriguing. Indeed, *C. albicans* is a pathogenic fungus which has to adapt to host defense and the relatively stable yet location-specific human-body environments, whereas *S. cerevisiae*, a free-living yeast, has to deal with the very variable chemical and physical features of its environment. These yeast species are also distinct in basal metabolism, which has important implications for the structure and evolution of their transcriptional networks (24).

We show here that the PDR pathway is the most sensitive to progesterone in both species. Our data indicate that progesterone is very close to the optimal chemical signal that triggers transcriptional networks (24). We used this property to decipher the respective roles of the wild-type versions of Pdr1p and Pdr3p in the foundation of PDR in pathogenic yeasts. In the present work, we used this property to decipher the respective roles of the wild-type versions of Pdr1p and Pdr3p in the foundation of PDR in *C. albicans* and *S. cerevisiae*. We showed that in comparison to that of the Pdr1p/Pdr3p pair, the activity of Tac1p in *C. albicans* is much more focused and probably involves other yet undiscovered transcription factor(s).

**MATERIALS AND METHODS**

Strains, media, and growth conditions. All *C. albicans* and *S. cerevisiae* strains used in this study were derived from SC5314 and BY4741, respectively. The pdrΔ and pdrΔΔ strains were obtained from Euroscarf. The pdrΔ pdrΔΔ strain is described in reference 20. Cells were grown in yeast extract-peptone-dextrose at 30°C till the mid-log phase was reached (optical density at 600 nm [OD600] of 0.8 to 1.0) and then treated with progesterone (stock solution in ethanol; Sigma-Aldrich) for two time points (30 min and 90 min) at three different concentrations (10^-7 M, 10^-6 M, and 10^-5 M). For studying the long-term effects of steroids, progesterone (10^-7 M, 10^-6 M, or 10^-5 M) was added to the medium at the time of inoculation (inoculum OD600 0.125) and cells were collected after 7 h of treatment (with the final OD600 remaining the same). For mock-treated cells, the growth conditions were the same except that only ethanol was added in the medium. The cells were harvested at room temperature in 50 ml centrifuge tubes at 3,000 rpm for 3 min. Thereafter, the cells were washed once with sterile milliQ water and snap-frozen in liquid nitrogen. The cell pellets were stored at -80°C.

RNA isolation, cDNA preparation, and microarray hybridization. Total RNAs were isolated by breaking the cells with glass beads in a Fast-Prep FP120 cell disruptor (Bio 101) at 4°C, followed by RNA purification on columns according to the manufacturer’s protocol (RNAeasy minikit; Qiagen). The *C. albicans* microarrays (batch C050G) were obtained from Eurogentec (Seraing, Belgium) and contained cDNA probes, deposited in duplicates, for 98% of the Candida Genomic Open Reading Frames (assembly 19). The *S. cerevisiae* microarrays were homemade from the Openon collection of yeast open reading frame (ORF) oligonucleotide probes (version 1.1.2), deposited in duplicate on Corning ultragrap slides, using an Omnimgrid II spotter from Biorobotics (Genomic Solutions). Each experiment was done at least three times, with independent biological replicates and using a dye swap. Fifteen micrograms of total RNA was resuspended in 20 μl of RNase-free H2O along with 1 μl of RNasin RNase inhibitor (Promega) and then used for labeled cDNA synthesis. The direct-labeling protocol provided by Eurogentec was used for *C. albicans* experiments. The *S. cerevisiae* cDNA labeling-and-hybridization protocol can be found at www.transcriptome.ens.fr/sdb

**RESULTS**

Quantitative features of the steroid response in *C. albicans* and *S. cerevisiae*. We analyzed the transcriptomes of wild-type cells of both species treated with three different doses of progesterone (two supraphysiological doses, 10^-4 and 10^-6 M, and one physiological dose, 10^-9 M) for three different time periods (30 min, 90 min, or 7 h). None of these doses affected cell growth (data not shown). The corresponding cDNAs were competitively hybridized on DNA microarrays versus cDNAs obtained from mock-treated cells. We observed that for both species, the number of genes whose expression changed significantly and reproducibly was maximal at the 10^-5 M dose, with only a few genes being induced by a 10^-6 M treatment (Fig. 1). Few significant and reproducible gene expression changes could be detected for the physiological dose (see Table S1 in the supplemental material). The steroid response was fast and transient, peaking at 30 min for both species (Fig. 1). Only a few genes remained induced or repressed up to 7 h of treatment. The numbers of genes in *C. albicans* and *S. cerevisiae* that changed expression in the presence of progesterone were similar except after 30 min of the 10^-4 M treatment, when the *C. albicans* response involved about 10 times more genes than that in *S. cerevisiae* (Fig. 1B). A gene ontology analysis indicated that this was mainly due to the induction of genes involved in the oxidative stress response, sulfur and glucose metabolism, and proteasome activity and the repression of genes involved in ribosome biogenesis and in translation (see Table S1 in the supplemental material). This oxidative stress-like
response in *C. albicans* was fast and transient and was not observed at longer times or lower doses. To get a biologically relevant view of the pathways which are preferentially sensitive to progesterone, we looked for gene groups which were significantly enriched in the lists of significant gene expression variations (see Table S1 in the supplemental material). Note that due to the high number of replicate measurements (six per gene on average) and their high reproducibility, we were able to point out as statistically significant gene expression variations as low as 0.3 (log2 value). Also, it is important to mention that all the genes discussed in the rest of this article satisfied three criteria: their expression variations were significantly different from the bulk of yeast genes (*Mi* cutoff), this *Mi* value was reproducible over the six measurements which were performed (*S* value criteria), and they belong to a gene category which is significantly enriched in the lists of genes induced by progesterone (see Methods). Moreover, the biological relevance of many of the genes discussed below was supported by Northern blot (see Fig. S2 in the supplemental material) and/or further microarray analyses investigating the transcriptional mechanisms underlying the progesterone response (see Fig. 3 and Table 2). Finally, several of these genes showed changed expression at several time points and doses. For all these reasons, we have a very high level of confidence in the biological and statistical relevance of the results discussed below, even when *Mi* values were unusually low.

**Progesterone activates PDR1/3 and ergosterol pathways in *S. cerevisiae*.** In *S. cerevisiae*, two pathways are mainly responsible for pleiotropic drug resistance phenotypes: the PDR pathway, controlled by the Pdr1p and Pdr3p transcription factors, and the ergosterol biosynthesis pathway (2). Gene ontology and DNA regulatory motif mining of our microarray data indicated that progesterone specifically and extensively activated these two pathways (see Table S1 in the supplemental material) (Table 1). Four types of PDRE bound by Pdr1 and Pdr3 have been described (16), named A (TCCGCGGA), B (TCCGTGGA), C (TCCGCGCA), and D (TCCGCGGG). PDRE A, B, and D were found to be significantly correlated with progesterone induction at all time points of the supra-physiological-dose treatments except at 7h with a 10^-6 M concentration (Table 1). Half of the genes induced at 30 min by 10^-4 M progesterone are referenced as targets of Pdr1p and Pdr3p (Fig. 1) (see Table S1 in the supplemental material). These genes represent about 80% of the Pdr1p/Pdr3p targets defined previously (1, 16, 17, 18, 20, 28), including genes encoding Pdr3p itself, the ABC transporters Pdr5p, Pdr15p, Pdr10p, Yor1p, and Snq2p, the membrane phospholipid metabolism and transport proteins Rsb1p, Pdr16p, and Rta1p, the oxidoreductase Gre2p, etc.

At 90 min of the treatment with a 10^-4 M concentration, the PDR response was followed by the induction of genes involved in ergosterol biosynthesis, which represent about 20% of the genes induced at this time point (Fig. 1 and 2B) (see Table S1 in the supplemental material). As a consequence, ergosterol metabolism was one of the main gene ontology categories to be significantly enriched in the list of genes induced at 90 min and 7 h of the 10^-4 M concentration treatment (see Table S1 in the supplemental material). These genes (*ERG25, ERG11, ERG5*,
Table 1. Known motifs overrepresented in genes up-regulated by progesterone and identified by using t-profiler with default parameters (

<table>
<thead>
<tr>
<th>Organism</th>
<th>Time point, dose, and motif</th>
<th>Name of motif (types)</th>
<th>t value</th>
<th>No. of ORFs</th>
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<td>TWCCCM</td>
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<td>PDRE (A, B, D)</td>
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<td>Leu3</td>
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<tr>
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<td>7 h, 10^{-4} M</td>
<td>TGACTCA</td>
<td>GCN4</td>
<td>5.83</td>
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ERG4, ERG3, and ERG28) encode enzymes from the ERG pathway. Interestingly, the UPC2 gene, encoding a transcriptional activator of the ERG genes, although not reaching our confidence limit, exhibited an expression profile similar to that of the ERG genes (Fig. 2B). Moreover, the Upc2p DNA binding motif was significantly correlated with progesterone induction at 90 min of the 10^{-4} M treatment (Table 1). The PDR and ERG pathways were very stably induced by progesterone, since some of these genes were still induced after 7 h of treatment, although their levels of induction tended to decrease with time (Fig. 2A and B; Table 1).

Transcriptional regulation of the progesterone-induced PDR response in S. cerevisiae. Considering the large set of PDR genes which were activated by progesterone, we used microarrays to analyze the effects of deletion of PDR1 and PDR3 on the steroid-induced expression of genes belonging to the PDR network (Fig. 3). The progesterone induction experiments were done with the pdr1Δ, pdr3Δ, or pdr1Δ pdr3Δ strain at time 30 min and with a dose of progesterone of 10^{-4} M, which corresponded to the maximum PDR response in the wild-type cells (Fig. 2A). We observed three different types of gene expression profiles (Fig. 3A). Some genes (e.g., GRE2, ICT1, YOR1, YPL088w, YLL056c, and PDR10) were exclusively Pdr1p dependent (group I), whereas for other genes (PDR16, RSB1, YGR035c, and YLR346c), the absence of PDR1 could be partially complemented by Pdr3p (group II). Finally, group III included genes (PDR5, PDR15, SNQ2, TPO1, and YAL061w) which are sensitive only to the double deletion and for which the absence of Pdr1p is fully complemented by Pdr3p. Interestingly, we observed that as far as steroid stimulation is concerned, the PDR3 induction is insensitive to the PDR1 deletion (Fig. 3A). Even though the number of genes in each group was low, we observed biases in the type and number of PDRE present in each group, which may reflect some functional differences in the binding affinities of Pdr1p and Pdr3p (Fig. 3B). Indeed, the genes depending exclusively on Pdr1p for steroid regulation contained a lower number of PDRE (only one in most cases), which are only of the B type, in their promoters. On the contrary, the promoters at which Pdr3p can fully complement Pdr1p are among the richest in PDRE (2.4 per promoter on average) and almost always contain both the A and B types (Fig. 3B). We verified some of these results by using Northern blotting. The control genes have been chosen so as to include genes from all three groups in Fig. 3.
example, ICT1 from group I, PDR16 from group II, and PDR5 and YAL061w from group III (see Fig. S2A and B in the supplemental material).

**The PDR response to steroids is only partly conserved in C. albicans.** Except for the 30-min time point with the $10^{-4}$ M dose (see above), the C. albicans response to progesterone was restricted to fewer than 60 different genes involved in arginine metabolism, drug export, lipid and ergosterol metabolism, biofilm formation (up-regulated), and iron and glucose transport (down-regulated) see Table S1 in the supplemental material). As observed for S. cerevisiae, PDR transporter-encoding genes (namely, CDR1 and CDR2) were among the most highly and
stably steroid-induced genes (Fig. 4) (see Fig. S1 in the supplemental material). The CaCDR1 gene expression profile was remarkably similar to the ScPDR5 expression profile, indicating that the mechanisms involved in progesterone induction of these genes may be conserved from S. cerevisiae to C. albicans. This is in spite of the fact that the promoters of the two genes harbor different regulatory elements (discussed below). To get a global qualitative and quantitative estimation of the conservation of the PDR and ERG responses to progesterone in both a nonpathogenic and pathogenic yeast species, we made two independent hierarchical clusterings of the PDR and ERG genes induced by progesterone in S. cerevisiae and of their closest homologues in C. albicans. We then directly compared the gene expression profiles of the homologous gene pairs using Pearson correlation distances (Fig. 5) (see Table S2 in the supplemental material). We observed various situations depending on the gene categories. We found no conservation of the ERG response, identified in S. cerevisiae and in C. albicans. No conservation was observed for the expression profiles of most of the ERG genes (UPC2, ERG1, ERG5, ERG10, ERG11, ERG12, ERG25, and ERG16), which were even repressed at 30 min of a 10^{-4} M progesterone treatment (see Table S1 in the supplemental material) (Fig. 5). In contrast, we found full conservation of the induction of the oxidoreductases (Gre2p-like)-encoding and putative flippase (Rsb1p-like)-encoding genes involved in the PDR response, with two homologous genes being similarly induced in both species (Fig. 5). In the case of the ABC transporter family, we found a partial conservation, with only two Candida genes (CDR1 and CDR2) being sensitive to progesterone when four of their homologues (PDR5, PDR15, PDR10, and SNQ2) were induced in S. cerevisiae.

Although about 20 Pdr1/Pdr3 target genes were induced in S. cerevisiae, we could find only 9 homologues of these genes being similarly regulated in C. albicans (see Table S1 in the supplemental material) (Fig. 5). One simple explanation would be that the PDR pathways in C. albicans involve genes which are not clear homologues of the S. cerevisiae PDR genes but which play similar functional roles. We found that this is the case for the C. albicans PDR transcriptional regulator Tac1p, which is not strictly homologous to S. cerevisiae Pdr3p but which belongs to the same family of Gal4p-like transcription factors and exhibited a similar expression profile in response to progesterone (Fig. 2A and 4). Almost all known putative Tac1p targets were induced by progesterone (Fig. 4). This includes CDR1, CDR2 (encoding homologues of Pdr5p), RTA3 (encoding an orthologue of the putative S. cerevisiae flippase Rsb1p) and IFU5 (Fig. 4). To get a more complete view of the Tac1p role and of the C. albicans PDR network involved in the steroid response, we compared the transcriptome of either parental cells or the revertant of the tac1 knockout (11) with the same treatment. We observed a very limited effect of TAC1 deletion on the progesterone response. Only CDR1, CDR2, RTA3, HSP70, and MET15 exhibited significantly reduced expression in the mutant strain (Table 2). The involvement of Tac1p in the steroid-induced expression of CDR1 and RTA3 was confirmed by Northern blotting experiments (see Fig. S2C in the supplemental material).

**DISCUSSION**

Progesterone specifically induces a long-term PDR response in pathogenic and nonpathogenic yeasts. Although it has been established for a long time that human steroids affect yeast growth, morphogenesis, and drug resistance, their molecular action and signaling in fungi remain unresolved. Previous microarray experiments showed that an elevated dose (10^{-3} M) of progesterone rapidly (30 min) and transiently induced a large stress response in both S. cerevisiae and C. albicans (5, 6).
The response included an overexpression of genes involved in the PDR phenomenon. In the present study, we analyzed the *C. albicans* and *S. cerevisiae* responses to lower doses of progesterone (10^{-4} M to 10^{-9} M) for extended time periods (30 min to 7 h) and compared the transcriptional regulation of PDR responses in these two species. We found that the steroid response is more prominent at supraphysiological concentrations and at shorter time points. In other words, the steroid response is transient in nature, the yeast cells probably adapt to the presence of steroids after continuous exposure at low doses, and gene expression probably reaches an equilibrium that is difficult to measure with physiological doses for relatively short time periods. We observed that the PDR pathways and especially the genes encoding the multidrug ABC transporters Pdr5p in *S. cerevisiae* and Cdr1p in *C. albicans* were the most sensitive to and stably induced by progesterone. This was especially impressive with *S. cerevisiae*, in which almost 100% of the genes induced by a 1 μM, 7-h progesterone treatment belong to the PDR pathway. The *S. cerevisiae* PDR network is composed of at least 25 genes regulated by the Pdr1p and Pdr3p transcription factors (34). Our experiments showed that more than 80% of these genes responded to progesterone. For

![Comparative functional genomics of the PDR and ERG responses to progesterone.](image)

**FIG. 5.** Comparative functional genomics of the PDR and ERG responses to progesterone. The ERG and PDR genes of *S. cerevisiae* were clustered according to their expression profiles following progesterone treatment. A similar clustering was done for the *C. albicans* orthologues or closest homologues of these genes. The homology links can be found in Table S2 in the supplemental material. Color coding indicates the main PDR gene families in both species: blue, ABC transporters; red, oxidoreductases from the Gre2 family; brown, putative flipases from the RTA family; gray, ICT1; green, putative aryl alcohol dehydrogenases; black, others. An arrow connects two homologues when the Pearson correlation distance between their expression profiles is significantly low (d < 0.4).
comparison, only 40% of these genes are up-regulated by flu-
phenazine, which is known to induce PDR in both S. cerevisiae and C. albicans (20, 23). This makes progesterone the most
efficient inducer of the PDR pathway in S. cerevisiae (see Fig.
S1 in the supplemental material).

For C. albicans, our knowledge of PDR regulation is more
limited than for S. cerevisiae. A major mechanism of PDR is the up-regulation of multidrug transporters belonging to the
ABC (ATP-binding cassette) transporter family (e.g., CDR1 and CDR2), which is the equivalent of the PDR pathway of budding yeast, or of the major facilitator family (e.g., MDRI),
which is the equivalent of the YAP1/FLR1 pathway in budding yeast (11). Remarkably, progesterone induced almost all the
genes known to be coregulated with CDR1/CDR2 in C. albi-
cans. This included IFU5, RTA3, HSP70, HSP90, and TAC1. This striking sensitivity of the PDR network to progesterone in both species suggests that steroids act directly on the physio-
logical parameters which trigger the PDR response. In S. cer-
erviae, the progesterone treatment strikingly mimicked the
gene expression profiles obtained from cells expressing "mini-
Pdr1p" or gain-of-function versions of Pdr1p in which the
whole Gal4-like inhibitory domain has been deleted or mut-
tated (17). One reasonable hypothesis would be that progester-
one indirectly triggers an efficient deregulation of Pdr1p
through its inhibitory domains. Progesterone could act on the
PDR networks by directly modifying the activity and properties
of some PDR ABC transporters. Although it was shown that
progesterone is a good substrate of Pdr5p and Cdr1p (27), it is
unlikely that progesterone acts just by competing "natural"
Pdr5p or Cdr1p substrates for transport, for the following reasons: (i) other good substrates of Pdr5p/Cdr1p, like flucon-
zole, are poor inducers of the PDR response (27), and (ii) low
(1 μM) doses of progesterone are enough to trigger an efficient
PDR3/CDR1 induction. Recently estradiol derivatives were
shown to efficiently inhibit the drug transport and ATPase
activities of Pdr5p and Cdr1p (10). This suggests that human
steroids act directly on the activity of the PDR transporters
through a mechanism which is yet to be determined but which
has important and long-term implications for the activity of the
PDR transcriptional networks and thus the PDR status of yeast
strains. Noteworthy here is our finding that the steroid re-
sponse is more prominent at supraphysiological concentrations
and at shorter time periods. In other words, the steroid re-
sponse is transient in nature, the yeast cells probably adapt to
the presence of steroids after continuous exposure at low
doses, and gene expression probably reaches an equilibrium
that is hard to measure at low doses for short time periods.

It should be pointed out that in addition to PDR genes, we
found that the ergosterol biosynthetic pathway genes (ERG
genes) were also induced by progesterone. Of note, ERG
genes have often been found to be coregulated along with
CDR1 and CDR2 in both experimentally induced and azole-
resistant clinical isolates of C. albicans, and overexpression of
ERG genes, especially ERG11, is one of the predominant
mechanisms of multidrug resistance (MDR) in Candida (7, 22,
30, 39). Hence, the observed up-regulation of ERG genes
along with MDR genes in the presence of progesterone is
perhaps an indication of the adaptation of the cells to a drug
resistance phenotype. UPC2 is a transcription factor involved
in regulation of ergosterol biosynthesis genes and a regulator
of sterol uptake across the plasma membrane (37), which was
also upregulated by progesterone (present results). Addition-
ally, it is pertinent to mention that the efflux pump proteins,
such as Cdr1p, are selectively localized in ergosterol-rich mem-
brane microdomains (36), and an overexpression of Cdr1p may
necessitate up-regulation of ERG genes as a compensatory
sterol homeostasis mechanism (35).

New insights into functioning of yeast PDR transcriptional
networks. In S. cerevisiae, Pdr1p and Pdr3p share a very similar
set of target genes and recognize the same DNA consensus
sequence (PDRE) in their promoters (14, 16). The PDR3 gene
itself has been shown to be regulated by Pdr3p and Pdr1p (13)
and was sensitive to progesterone in our experiments. In this
study, we found that most of the PDR genes were insensitive
to progesterone in a pdr1Δ pdr3Δ strain. Remarkably, Pdr1p was
dispensable for the regulation of PDR3 under these conditions,
although it is constitutively bound to its promoter (20), sug-
gest that the progesterone induction of PDR3 occurs through
autoregulation. We propose that progesterone can be
used as a tool to investigate the respective roles of the wild-
type versions of Pdr1p and Pdr3p in the PDR response. We
confirmed that PDR3 has a dispensable role in the response to
drugs. Based on their PDR3 dependency in a pdr1Δ back-
ground, we were able to distinguish between three different
groups of PDR targets. We found a correlation between these
groups and the nature and number of PDRE present in the
promoters of the corresponding genes, suggesting that the
DNA binding affinities of Pdr1p and Pdr3p may be different.
Interestingly, these groups also present biases in the function
of the proteins encoded by the corresponding genes. Pdr1p and
Pdr3p have identical activities on the genes encoding most of
the main transporters involved in pleiotropic drug export
(PDR5, PDR15, SNQ2, and TPO1). They have an overlapping
effect, with a dominant effect of Pdr1p on genes encoding
actors of lipid metabolism and some coregulated proteins of
unknown function (RSB1, PDR16, YGR035c, and YLR346c
genesis), and Pdr1p specifically regulates genes which, in most
cases, are sensitive to other stress response pathways (GRE2,
YPL088w, YLL056c, ICT1, YOR1, and PDR10 genes). The
differential regulation of the PDR1/3 genes may reflect differ-
ences in their physiological roles and the functional associa-
tions of the corresponding proteins.

### Table 2. Effect of TAC1 knockout on progesterone response in C. albicans

<table>
<thead>
<tr>
<th>ORF</th>
<th>Name</th>
<th>Ratio of responses to progesterone&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Progesterone/ mock, WT</td>
</tr>
<tr>
<td>CA6066</td>
<td>CDR1</td>
<td>3.85</td>
</tr>
<tr>
<td>CA6099</td>
<td>CDR2</td>
<td>3.31</td>
</tr>
<tr>
<td>CA2565</td>
<td>MDR15</td>
<td>2.45</td>
</tr>
<tr>
<td>CA1230</td>
<td>HSP70</td>
<td>2.17</td>
</tr>
<tr>
<td>CA3606</td>
<td>RTA3</td>
<td>1.53</td>
</tr>
</tbody>
</table>

<sup>a</sup> The response of tac1Δ (Tac1KO) cells to a 30-min treatment with 0.1 mM progesterone was compared to the responses of the parental (WT) (second column) and Tac1-revertant (Tac1Rev) (third column) strains by using microarray. The numbers are log<sub>2</sub> values of expression ratios. Gene induction by progesterone in the parental strain is indicated (first column). Only genes which are involved in the progesterone response and which were significantly and reproducibly differentially expressed in the tac1Δ strain are represented here.

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In *C. albicans*, Tac1p is the only clear regulator of the PDR response identified to date (11). Tac1p belongs to the same family of Gal4p-like transcription factors as Pdr1p and Pdr3p, and we found that the progesterone induction profile of *Tac1* was very similar to that of *PDR3*, although no evidence exists for autoregulation of *TAC1*. Only six Tac1p target genes (*CDR1*, *CDR2*, *RTA3*, *IFU5*, *PDR16*, and *HSP12*) have been identified, and Tac1p has been shown to recognize the DRE present in their promoters, which is different from the PDRE of *S. cerevisiae* (11, 15). A deletion of *TAC1* is enough to decrease the basal level of expression of *CDR1* and *CDR2*, which are different from the situation for *S. cerevisiae*, in which the double deletion of *PDR1* and *PDR3* is needed to observe the same effect (11, 14). All these observations suggested that progesterone would be a perfect model for exploring the Tac1p-related transcriptional pathway. Surprisingly, only five progesterone would be a perfect model for exploring the Tac1p, only 8 of which displayed Tac1p dependence for their activity, which identified about 37 genomic loci bound by Tac1p target genes, CaRTA3/CaScRBS1, CaCDR1/CaScPDR5, and CaCDR2/CaScPDR15), we observed a remarkable similarity of expression profiles between the two yeast species. The most impressive case was that of CaCDR1/ScPDR5, which exhibited almost identical patterns. Of note, the cis elements known to regulate the drug response of *CDR1* (DRE) and *PDR5* (PDRE) are quite different (11, 15, 16). It is also worth mentioning here that within the steroid-responsive region found by Karnani et al. (26) in the *CDR1* promoter, which responds to both progesterone and β-estradiol, three consensus 5-bp stretches of nucleotides (AAGAA, CCGAA, and ATTTG) exist, which has also been found in the *PDR5* promoter (5, 26). This may account for the commonality in expression patterns of *CDR1* and *PDR5*.

On closer inspection, several differences also emerged between the responses of the two yeasts to progesterone. For instance, the expression profiles of four ABC transporter genes, *PDR5*, *SNQ2*, *PDR10*, and *PDR15*, in *S. cerevisiae* correlate with the pattern of only two homologous genes, *CDR1* and *CDR2*, in *C. albicans*, while *CDR3* and *CDR4* are apparently insensitive to progesterone stimulation. In other cases, the *S. cerevisiae* PDR genes just do not have clear homologues in *C. albicans*. This is the case, for instance, for *YGR035c* and *YLR346c*. Similarly, *WWM1*, the *S. cerevisiae* homologue of CaFU5 (one of the Tac1p targets responding to steroids), is insensitive to progesterone and has no role in PDR.

Comparative genomics has been used in the past to identify meaningful, conserved DNA or protein motifs from sequence comparisons, and the interspecies comparison of transcriptome data can be very informative in identifying conserved transcriptional modules of genes likely to have common essential functions (25). In the case of yeast PDR, our comparison of the Pdr1p/Pdr3p and Tac1p transcriptional networks suggests that although the DNA binding motifs and the protein sequences of these regulators have significantly diverged, there must have been a strong selection to conserve the pattern of at least two ABC transporters (CaCDR1/ScPDR5 and CaCDR2/ScPDR15) and one putative flippase (CaRTA3/ScRBS1) (Fig. 6). This conserved module, which functions in pleiotropic drug export and is also related to membrane phospholipid translocation, may thus constitute a central, original core of MDR in pathological and nonpathological hemiascomycetes species.

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REFERENCES


