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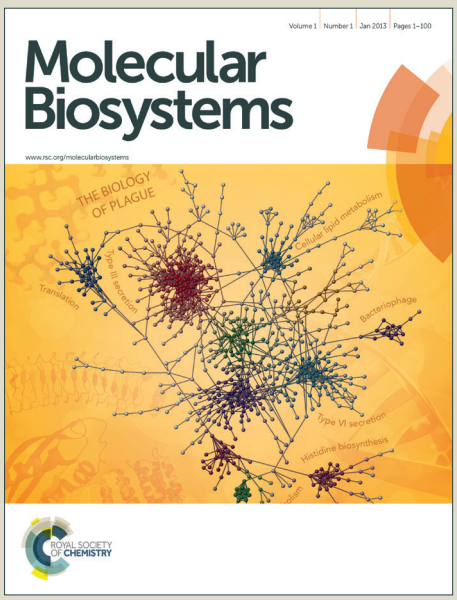

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Pleiotropic drug-resistance attenuated genomic library improves elucidation of drug mechanisms

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Identifying *Saccharomyces cerevisiae* genome-wide gene deletion mutants that confer hypersensitivity to a xenobiotic aids the elucidation of its mechanism of action (MoA). However, the biological activities of many xenobotics are masked by the pleiotropic drug resistance (PDR) network which effluxes xenobiotics that are PDR substrates. The PDR network in *S. cerevisiae* is almost entirely under the control of two functionally homologous transcription factors Pdr1p and Pdr3p. Herein we report the construction of a PDR-attenuated haploid non-essential DMA (PA-DMA), lacking *PDR1* and *PDR3*, which permits the MoA elucidation of xenobiotics that are PDR substrates at low concentrations. The functionality of four key cellular processes commonly activated in response to xenobiotic stress: oxidative stress response, general stress response, unfolded stress response and calcium signalling pathways were assessed in the absence of *PDR1* and *PDR3* genes and were found to unaltered, therefore, these key chemogenomic signatures are not lost when using the PA-DMA. Efficacy of the PA-DMA was demonstrated using cycloheximide and latrunculin A at low nanomolar concentrations to attain chemical genetic profiles that were more specific to their known main mechanisms. We also found a two-fold increase in the number of compounds that are bioactive in the *pdr1Δpdr3Δ* compared to the wild type strain in screening the commercially available LOPAC^{1280} library. The PA-DMA should be particularly applicable to mechanism determination of xenobiotics that have limited availability, such as natural products.

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Introduction

Use of *Saccharomyces cerevisiae* as a model system for xenobiotic-target identification permits the use of genome-wide studies that are highly informative and unbiased, obviating the need for prior information on the compound's mechanism of action (MoA) [1]. The most widely applied approach using *S. cerevisiae* as a tool to determine xenobiotic MoA utilises the yeast genome-wide deletion library to identify gene deletions that confer hypersensitivity to a xenobiotic; the resulting chemogenomic profile is then interpreted using GO term enrichment or cluster analysis approaches [2]. However, a serious limitation to this approach is the reduced drug sensitivity of yeast compared to mammalian cell lines [3,4]. Compounds that are highly active in cultured human cell lines and other non-human models with evolutionarily conserved targets often demonstrate reduced activity or no activity in yeast. This is partly attributed to the PDR network which comprises an array of highly inducible drug efflux transporters encoded by the ATP binding cassette (ABC) superfamily and major facilitator superfamily (MFS) genes [5].

This highly regulated PDR-network has an Achilles' heel. It is mainly controlled by two transcription factors: the binuclear $Zn(II)_2Cys_6$ zinc finger protein Pdr1p and its homolog Pdr3p. Pdr1p and Pdr3p can function either as homo- or heterodimers to regulate the expression of target genes [6]. To a lesser degree the PDR-network is also modulated by the bZIP family regulators Yrr1p and Yap1p [7]. Increased xenobiotic sensitivity resulting from loss of function mutations in either the drug efflux transporters or their transcriptional regulators is a well-studied phenomenon[8, 9]. Most of these studies focus solely on the function of a single ABC transporter: Pdr5p. Here we have constructed a yeast strain, *pdr1*Δ*pdr3*Δ, that lacks the two major transcription factors (Pdr1p and Pdr3p) that regulate the PDR-network in *S. cerevisiae*. This PDR-attenuated genetic background was then incorporated into the *MAT***a** genome-wide deletion mutant array (DMA) set to create a more drug-sensitive DMA library for screening hypersensitivity to xenobiotics of interest.

Results

The *pdr1*Δ*pdr3*Δ double deletion mutant is more sensitive to growth inhibitory xenobiotics

Liquid phase dose-dependent growth assays were performed using several xenobiotics to quantify the sensitivity of the *pdr1*Δ*pdr3*Δ double deletion strain relative to the laboratory wild type control strain, y7092, from which it was derived (Table 1 and Figure S1). The *pdr1*Δ*pdr3*Δ strain showed increased sensitivity to the known PDR-substrates: cycloheximide (CHX), quercetin, amphotericin B, fluconazole, and ketoconazole. With the exception of amphotericin B, these xenobiotics have been previously identified as more effective growth inhibitors in at least one of the single gene deletion strains of the major drug efflux transporters *PDR5*, *SNQ2* or *YOR1* [8-10]. The increased sensitivity of the *pdr1*Δ*pdr3*Δ strain to amphotericin B is likely a result of it being a substrate for several drug efflux transporters. The *pdr1*Δ*pdr3*Δ strain was also more sensitive to the natural products latrunculin A (LatA) and plakortolide X [11]. Deletion of *PDR5* caused increased sensitivity to LatA (Figure S2), suggesting that the basis of the increased sensitivity of the *pdr1*Δ*pdr3*Δ strain is due to reduced Pdr5p expression in this strain. In contrast, deletion of *PDR5*, *SNQ2* or *YOR1* or the minor drug efflux transporters did not alter plakortolide X sensitivity (Figure S3), suggesting that plakortolide X is a substrate for at least one other drug efflux pump or several drug efflux pumps under the control of Pdr1p/Pdrp3. In contrast, the sensitivity of the *pdr1*Δ*pdr3*Δ double mutant to rapamycin A and hygromycin B was comparable to wild type, suggesting that these two drugs are not substrates of the *PDR1*/*PDR3* regulated network pumps.

To gain an estimate of the number of compounds that the deletion of *PDR1* and *PDR3* would allow more efficient MoA determination for, the LOPAC^{1280} library was screened at a single concentration of 10 μM in Synthetic Complete (SC) medium (pH 4.5) or SC medium buffered to pH 8. Table S1 shows that it is possible to approximately double the number of growth inhibitory compounds identified in the LOPAC^{1280} library by using the *pdr1*Δ*pdr3*Δ double mutant. Thus, a yeast deletion library deficient in *PDR1* and *PDR3* would significantly increase the number of compounds that may otherwise not be able to be profiled using the chemogenomic approach in *S. cerevisiae*.

The improved sensitivity of *pdr1*Δ*pdr3*Δ is through an attenuated PDR response and not through an altered general stress response

In order for a sensitised gene deletion library to be utilised for MoA studies it is essential to ensure that the enhanced xenobiotic sensitivity is only due to a lack of efflux of the compound and not perturbation of other cellular processes. In order to assess whether the deletion of *PDR1* and *PDR3* induces constitutive stress pathway activation or compromises the ability of a cell to respond to stressful conditions, a small range of GFP reporter strains, representing activation of the general stress, oxidative stress, intracellular calcium, and the unfolded protein responses were used. Both *PDR1* and *PDR3* were deleted from strains expressing Msn4p-GFP, Yap1p-GFP, Crz1p-GFP or GFP under control of the unfolded protein response element (UPRE): 4xUPRE-GFP. The localisation of these transcription factors and expression from the UPRE were quantified using fluorescent confocal microscopy in the presence and absence of conditions that have been previously shown to cause re-localisation or enhanced expression, respectively.
Figure 1 and Tables S2 and S3 show that the deletion of *PDR1* and *PDR3* did not cause constitutive nuclear localisation of Msn4p-GFP, Crz1p-GFP or Yap1p-GFP or increased expression from the UPRE. Furthermore, deletion of *PDR1* and *PDR3* did not prevent nuclear localisation of Msn4p-GFP in response to sorbitol or NaCl exposure, Crz1p-GFP in response to exogenous $CaCl_2$ addition, or Yap1p-GFP in response to H_2O_2 or menadione exposure. Nor was the ability to increase UPRE expression in response to tunicamycin A or dithiothreitol (DTT) exposure compromised (Figure 1 and Tables S2 and S3). These results suggest that the deletion of *PDR1* and *PDR3* exclusively attenuates the PDR-response and does not cause general stress.

Deletion of *PDR1* and *PDR3* does not generate synthetic lethality with the majority of the deletion library

The *pdr1*Δ*pdr3*Δ double deletion mutant constructed above was used as a starting strain in Synthetic Genetic Array (SGA) methodology[12] to construct a triple mutant *MAT***a** haploid *pdr1*Δ*pdr3*Δ*xxx*Δ DMA library. A small number of apparent negative genetic interactions between *PDR1* and *PDR3* and other non-essential gene deletions were identified during the construction of the *pdr1*Δ*pdr3*Δ*xxx*Δ DMA library (Table S4). These apparent negative genetic interactions were further investigated by random spore analysis and were shown to be false positives (Table S5). The lack of negative genetic interactions observed with *pdr1*Δ*pdr3*Δ in the triple mutants reflects the dispensability of both Pdr1p and Pdr3p transcription factors in the absence of exogenous stress and provides further evidence that the deletion of *PDR1* and *PDR3* causes increased sensitivity to xenobiotics by exclusively attenuating the PDR-response. Importantly this means that the PA-DMA spans an array of biological processes comparable to the parental *MAT***a** DMA, making it applicable for use in the MoA determination of xenobiotics with a range of biological activities.

The PA-DMA library reveals cycloheximide interactions more specific to protein synthesis than the *MAT*a library

In order to establish whether the PA-DMA is more effective in determining the MoA of a PDR-substrate, parallel chemogenomic screens were performed with the PDR-substrate CHX, a translation elongation inhibitor that has been shown to be a substrate for at least one PDR pump [8], and the PA-DMA and parental *MAT***a** DMA. The CHX chemogenomic profile generated with the parental *MAT***a** DMA showed that very few translation-related single gene deletion strains displayed hypersensitivity to CHX (Table S6) and that there was no enrichment for gene deletions ascribed to translation-related GO terms (Table S7). The same GO analysis of the CHX hypersensitive single gene deletion mutants in Parsons *et al*. 2004 also did not show enrichment for translation related genes [2]. In contrast, the CHX chemogenomic profile generated using the PA-DMA was enriched for translation related GO terms (Table S9) and contained a greater proportion of translation-related mutants displaying hypersensitivity to CHX (Figure 2 and Table S8), despite identifying fewer CHX-hypersensitive triple mutants in total.
Gene deletions of the PDR network: *pdr1*Δ (0.22), *pdr3*Δ (0.15) *pdr5*Δ (0.21) and *snq2*Δ (0.15) are some of the most frequently identified drug hypersensitive deletion strains in chemogenomic screens [1]. Only 10 deletion strains were common between the PA-DMA and the parental *MAT***a** DMA chemogenomic profiles for CHX (Figure 2). Therefore, to establish whether the remaining CHX-hypersensitive deletion strains from the *MAT***a** DMA profile could be involved in the regulation of the PDR-network, the frequencies at which these deletion strains were previously identified as hypersensitive to PDR-substrates compared to non-PDR-substrates in the Parsons *et al*. 2006 dataset were determined. In this analysis a compound from the dataset was deemed a PDR-substrate if one of the following deletion strains was hypersensitive to it: *PDR1*, *PDR3*, *PDR5*, *PDR10*, *PDR12*, *PDR15*, *YOR1* or *SNQ2*. The proportion of PDR-substrate and non-PDR substrate profiles in which each CHX-hypersensitive gene deletion strain is present was then calculated and plotted (Figure S4). As expected the *pdr1*Δ and *pdr5*Δ deletion strains were exclusively hypersensitive to PDR-substrates, while others such as *bck1*Δ and *dbf2*Δ were equally hypersensitive to both PDR and

non-PDR substrates. This analysis showed that a significant majority of the CHX-hypersensitive deletions strains of the PDR-replete *MAT***a** background were more likely to be hypersensitive to xenobiotics that are substrates for the PDR-network, in particular the deletion of various components of the histone deacetylase complexes: HDA1 (*hda3*Δ), Set3C (*sif2*Δ), Rpd3S and Rpd3L (*sin3*Δ) or their regulation (*snt1*Δ). Gene deletions of histone deacetylase complexes: Rpd3L (*DEP1*, *PHO23*, *SAP30*) Rpd3S and Rpd3L (*SIN3*) were also identified to be hypertensive to CHX in Parsons *et al*. 2004 when using the *MAT***a** DMA and these were absent in the PA-DMA CHX screen. These results suggest that the CHX chemogenomic profiles generated with the PDR-replete DMAs are dominated by deletions strains that are involved in the regulation of the PDR-network and that the PA-DMA library is more effective at identifying drug-target-relevant hypersensitive deletions, at least in the case of CHX.

PA-DMA library used to elucidate the MoA of cycloheximide and latrunculin A using the yeast DNA barcode microarray

Agar-plate-based chemogenomic screening often requires significant amounts of xenobiotic. In order to assess whether the PA-DMA could be utilised in a more compound-conservative screening format, a pooled library method using a DNA barcode microarray [13] was performed with CHX and the actin destabilising agent LatA. A total of 31 CHX-hypersensitive deletion mutants were identified (Table 2), and consistent with the agar-based chemogenomic screen, this profile was enriched for translation-related deletion mutants.

A total of 51 LatA-hypersensitive strains were identified (Table S10), 25 of which were confirmed to be hypersensitive to LatA in an independent microtitre plate assay (Table 3). Consistent with the well-established effect of LatA on the actin microfilament network, deletion of the microfilament capping protein encoding genes *CAP1*, and *CAP2*, the formin *BNR1*, the actin cortical patch component *BBC1*, the major tropomyosin isoform *TPM1*, the dynamin-like GTPase *VPS1*, the septin component *SHS1*, and components of the prefoldin complex *PAC10* and *YKE2* which enhances folding of both actin and tubulin, cause hypersensitivity to LatA. In addition, a number of strains deficient in genes encoding proteins involved in mitosis, such as *ARP1*, *CIK1*, *KIP3* and *LDB18*, also caused LatA hypersensitivity, consistent with the role of the actin cytoskeleton in the positioning of the early mitotic spindle. The presence of these gene deletion strains in the PA-DMA chemogenomic profile for LatA causes enrichment of GO terms related to the regulation of actin cytoskeleton and mitosis.

The only other previously published LatA chemogenomic profile, using the wild type (PDR-replete) homozygous deletion library [14], failed to identify the level of mitosis-related GO term enrichment that was found in the current study using the PA-DMA. In fact only two of the dynactin components, *ARP1* and *JNM1*, were present in the top 120 genes (which were all $P < 0.001$). This result is proof that disabling the PDR-network in the DMA allows not only the identification of more specific drug target-related genes but also allows identification of genes that are functionally related to the drug target. This shows that the PA-DMA can be used in both agar-based and pooled barcode microarray-based chemogenomic screening approaches; the latter is particularly applicable to situations when compound availability is limited, such as in the case of novel natural products.

Discussion

Using PDR-attenuated yeast deletion mutants for chemogenomic profiling studies makes it possible to determine the MoA of PDR-substrates that were previously unfeasible for genome-wide studies, owing to high concentrations of xenobiotics needed to attain inhibitory activity towards *S. cerevisiae*. For instance, we have previously utilised the PA-DMA to successfully identify gene deletion strains that display hypersensitivity to the microtubule stabilising agents peloruside A and laulimalide [15]. In the case of peloruside A, this was not possible in an earlier study when a PDR-replete library was used [16].

In the present study we elected to generate the PA-DMA by only deleting the major drug efflux pump transcription factors *PDR1* and *PDR3*. While it would be possible to further enhance xenobiotic sensitivity by also deleting other genes (such as specific drug efflux pumps and the *YAP1* transcription factor) there is a trade-off between enhancing xenobiotic sensitivity and limiting the coverage of the deletion library by including too many multi-drug sensitising gene deletions. Deleting *PDR1* and *PDR3* represents an effective approach to sensitising the deletion library to a broad range of xenobiotics while retaining coverage of the majority of the non-essential deletion library. Previous studies have shown that the oxidative stress response transcription factor Yap1p is able to positively regulate Pdr1p/Pdr3p

target genes [17, 18]. In this study, we avoided further enhancing xenobiotic sensitivity by the deletion of *YAP1* so as to retain the potential for generating an oxidative stress specific chemogenomic profile with the PA-DMA, which consists of *YAP1* and numerous *YAP1* target genes.

The *pdr1*Δ*pdr3*Δ double deletion mutant has been shown to exhibit increased sensitivity to a number of substrates of the PDR-network [9]. The *pdr1*Δ*pdr3*Δ double mutant generated herein is hypersensitive to previously established compounds as well as known substrates of the major drug efflux transporters Pdr5p, Snq2p and Yor1p (Table 1). The increased sensitivity of the PA-DMA strains to xenobiotics is a consequence of reduction in the expression of the drug efflux transporter rather than the complete loss of expression, at least in the case of Pdr5p [8]. This was corroborated by the enhanced sensitivity of the *pdr1*Δ*pdr3*Δ*pdr5*Δ triple mutant to LatA compared to the *pdr1*Δ*pdr3*Δ double mutant. As testament to the appropriateness of deleting *PDR1* and *PDR3* rather than just the efflux pump encoding genes themselves, we found that the *pdr1*Δ*pdr3*Δ mutant is hypersensitive to compounds such as amphotericin B and plakortolide X, whereas the single deletion mutants of the major drug efflux transporters Pdr5p, Snq2p and Yor1p are not, suggesting that these compounds are likely to be substrates for several drug efflux transporters. By screening a commercially available, pharmacologically active chemical library we found that deleting *PDR1* and *PDR3* in the DMA library could increase the number of compounds that can be studied with the chemogenomic approach by approximately two-fold.

In order for the successful application of a PDR-attenuated deletion library in the determination of the MoA of a PDR-substrate, it is essential to establish whether the increased sensitivity of the *pdr1*Δ*pdr3*Δ double mutant is due to decreased drug efflux and not perturbation of some other cellular process**.** Therefore, the function of four key cellular responses, namely the general stress response (Msn2p/4p dependent), oxidative stress response (Yap1p dependent), unfolded protein response (Hac1p dependent) and calcium signalling (Crz1p dependent), that are activated in response to xenobiotics or environmental stresses, were assessed in the presence and the absence of *PDR1* and *PDR3*. Neither the basal nor the induced activation of these processes was altered by loss of Pdr1p and Pdr3p transcription factors. Furthermore, the absence of negative epistatic interactions between *pdr1*Δ*pdr3*Δ and the remaining gene deletions in the library, under optimal growth conditions, provides further evidence for dispensability of *PDR1* and *PDR3* genes, particularly in the absence of xenobiotic challenge or environmental stresses. Therefore, it is reasonable to assume that the increased sensitivity of the PA-DMA to PDR-substrates is a result of decreased efflux and hence should generate chemogenomic profiles that are specific to the MoA of the xenobiotic in question.

The validity of this final assertion is evident in the chemogenomic profiles of the Pdr5p substrates CHX and LatA. In both cases using the PA-DMA it was possible to generate a target-specific chemogenomic profile at a significantly lower concentration compared to the single deletion mutant pool while at the same time identifying more translation- and actin-specific interactions, respectively. In the case of CHX, this enhanced enrichment of translation-related GO terms occurred in both the solid phase library array and pooled DNA-barcode methods. The differences in triple mutants demonstrating sensitivity to CHX in the two chemical genetic profiling approaches are likely due to a combination of variability in high throughput screens, limitations in drug diffusion, and differences in growth of deletion mutants in agar and liquid, among others. The implication of these results is that, at least in the case of CHX, the chemogenomic profile generated using the PDR-replete DMA library is dominated by deletions involved in the regulation of drug efflux pump expression or function. For instance, the chemogenomic profile generated using the PDR-replete DMA library was enriched for histone deacetylase gene deletion strains, and previous studies have shown that Pdr5p expression is decreased in the absence of histone deacetylase activity [19].

Chemogenomic profiling is a powerful and increasingly common approach to determining the MoA of biologically active xenobiotics. While this approach was pioneered in the budding yeast *S. cerevisiae*, it has since been extended to the fission yeast *S. pombe* and the pathogenic yeast *C. albicans*. In common with *S. cerevisiae*, both *S. pombe* and *C. albicans* have extensive drug efflux networks under the control of the *PDR1* homologues *PRT1* [20] and *TAC1* [21], respectively, which will hinder their use for chemogenomic profiling of PDR-substrates. The approach we have taken in this study could be applied to *S. pombe*, owing to its haploid/diploid life-cycle and the availability of a modified SGA procedure for the mass generation of double mutants [22]. In contrast, *C. albicans* is an obligate diploid organism [23] which prevents the use of the same approach as we have taken to disable the PDR-network.

Conclusions

We describe here a novel modification of the yeast gene deletion mutant array (DMA), namely a conversion of the DMA to a *pdr1*Δ*pdr3*Δ genetic background. The simultaneous deletion of *PDR1* and *PDR3* increases sensitivity to known PDR-substrates, does not perturb the basal or induced activity of several key stress responses and does not genetically interact with other gene deletions in the library. We show that the PDR-attenuated deletion library allows enhanced sensitivity in chemogenomic interaction profiling and also increases specificity in defining hypersensitivity to well-known xenobiotics. This modified DMA should therefore have wide potential use in chemogenomic screening and drug target elucidation by increasing the number of compounds amenable to these methods. The PA-DMA library described in this manuscript will be available upon request from the Weizmann Institute of Science, Clone and Strain Repository.

Materials and methods

Chemicals and drugs

Latrunculin A (LatA) and plakortolide X, isolated from the marine sponges *Cacospongia mycofijiensis* [24] and genus *Plakortis* [11] from Tonga, were stored at -20°C at a concentration of 10 mM in dimethyl sulfoxide (DMSO). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA) unless stated otherwise.

S. cerevisiae strains

Y7092 'SGA ready' *MAT***α** haploid yeast strain [12] was used as wild type to construct the *pdr1*Δ*pdr3*Δ double deletion strain by PCR-mediated gene disruption with selectable markers as previously described [25] using the primers listed in Table S11. The *MAT***a** *pdr1*Δ*pdr3*Δ DMA library was created by mating the *MAT*α *pdr1*Δ*pdr3*Δ double deletion strain against the *MAT***a** single gene DMA library (gift from Charles Boone, University of Toronto), using SGA methodology [26] and was used in agar-plate based chemogenomic screens. Pools of the *pdr1*Δ*pdr3*Δ DMA library were created by removing the *his3*Δ border strains from all the plates, and the remaining colonies were scraped off the agar surface of each plate in 5 mL of YPD+G418 broth using a sterile glass rod. The *pdr1*Δ*pdr3*Δ*xxx*Δ pools were stored at -80°C at $1x10^9$ cells/mL (~1000x representatives of each strain) in YPD+G418 broth containing 20% glycerol. A pool of these mutant strains was used for the DNA barcode microarray experiments. The control strain used for the deletion mutant library was *pdr1*Δ*pdr3*Δ*his3*Δ which had undergone the same genetic manipulations as all the other strains in the PA-DMA library but is functionally the same as wild type with the exception of the *pdr1*Δ*pdr3*Δ deletions. Reporter strains Msn4p-GFP, Yap1p-GFP and Crz1p-GFP (Invitrogen [27]) and *4xUPRE-GFP* (constructed by Bircham [28]) expressing green fluorescent proteins fused to the gene of interest were used in the microscopy experiments.

GFP reporter screens

Each of the GFP reporter strains were grown to mid-log and 49.5 µL of $1x10^7$ cells/mL cell suspension was inoculated into each well of a 384-well microtiter plate (Perkin Elmer cell carrier) along with 0.5 µL of compound or carrier solvent. Each condition was performed in duplicate. The plates were vortexed at 2000 rpm for 30 s and were incubated at 30°C for 4 h unless indicated otherwise. The Yap1p-GFP reporter strain was screened at a higher starting cell density of $5x10^7$ cells/mL because the GFP localisation was assessed immediately after treatment with menadione, H_2O_2 or carrier solvent.

The images were acquired using an EvoTec OPERA (Perkin Elmer) high-throughput spinning disk confocal laser microscope using the 60x water NA 1.2 immersion lens as previously described [29]. The appropriate midsection out of 5 Z stacks was selected by identifying the midsection with the highest contrast difference between the cells and the background.

Agar-plate based chemogenomic profiling of DMA libraries

The *pdr1*Δ*pdr3*Δ*xxx*Δ DMA library and the parental *MAT***a** PDR-replete single DMA library were used as previously described [2, 30] for screening of CHX to determine the chemical genetic interactions involved in its MoA. CHX was tested

at 100 nM and 380 nM in the PA-DMA and the *MAT***a** DMA libraries, respectively. These concentrations gave approximately 20-30% growth inhibition compared to the carrier control, allowing assessment of strain sensitivity in the presence of each compound. The hypersensitive gene deletion strains were identified using a statistical algorithm known as 'SESA' (SGA Experiment Set Analyser) as previously described [29].

Chemogenomic profiling of the PA-DMA pools using DNA barcode microarrays

Pools of the haploid *pdr1*Δ*pdr3*Δ*xxx*Δ DMA library were used for the CHX and LatA barcode microarrays to determine the genetic networks involved in the MoA of these compounds. The method used was as previously described [31] except that the haploid *pdr1*Δ*pdr3*Δ*xxx*Δ DMA library was screened in the present study. CHX and LatA were tested at 30 nM and 28 nM, respectively. These concentrations gave approximately 20-30% growth inhibition compared to the carrier control, allowing assessment of strain sensitivity in the presence of each compound. A deletion strain was considered a hit if the Z-score calculated from normalised Cy5/Cy3 ratios was less than -3 in one or both UP and DN tags, [31] indicating the deletion strain was sensitive to the compound in the conditions of the microarray.

Validation of chemical-genetic interactions from the LatA barcode microarray

The chemical-genetic interactions identified in the primary screen from the pooled LatA microarray were independently validated to eliminate false positives. To this end, each strain was assayed in a 100 µL volume in a 96-well microtitre plate. The concentration of 70 nM LatA gave approximately 20-30% inhibition in the *pdr1*Δ*pdr3*Δ*his3*Δ deletion strain in these growth conditions. The method used was as previously described [15], except that the starting cell density for each strain in 100 µL was $5x10^5$ cells/mL, and plates were incubated for 10 h before reading the plates at 1 h intervals until an OD of 0.3 was reached.

Screening of the LOPAC^{1280} library

The commercial library of pharmacologically active compounds (LOPAC) (Sigma-Aldrich) comprising 1280 compounds were screened against *pdr1*Δ*pdr3*Δ and wild type (PDR-replete) strains at a single concentration of 100 nM. A volume of 99 µL containing $5x10^5$ cells/mL in SC broth (pH 4.5) or SC buffered with HEPES (pH 8) was inoculated with 1 µL of compound dissolved in DMSO or DMSO alone. The plates were incubated at 30°C for 17 h, and the OD_{600} was measured. The residual growth percentage (OD_{600}Exp/OD_{600}DMSOcontrol × 100) for each compound-treated condition was determined using the average of the carrier solvent (DMSO) control OD from each plate. Z-scores $(x-\mu)/\sigma$) were calculated for each experiment in each replicate, in which x is the residual growth % for each compound, µ is the average residual growth for one replicate screen including the 1280 compounds and DMSO controls on each plate and σ is the standard deviation for µ. Each condition was performed in biological triplicate.

Gene ontology term enrichment analysis

The Cytoscape 2.8.3 plugin BiNGO 2.44 [32] was used to group interactions from chemogenomic screens into categories according to biological processes using a reference set of the *pdr1*Δ*pdr3*Δ*xxx*Δ library genes. The *P*-value was further corrected for multiple testing using the Benjamini & Hochberg False Discovery Rate (FDR) correction. The most up-to-date gene names, GO categories, and annotations were used.

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Figures

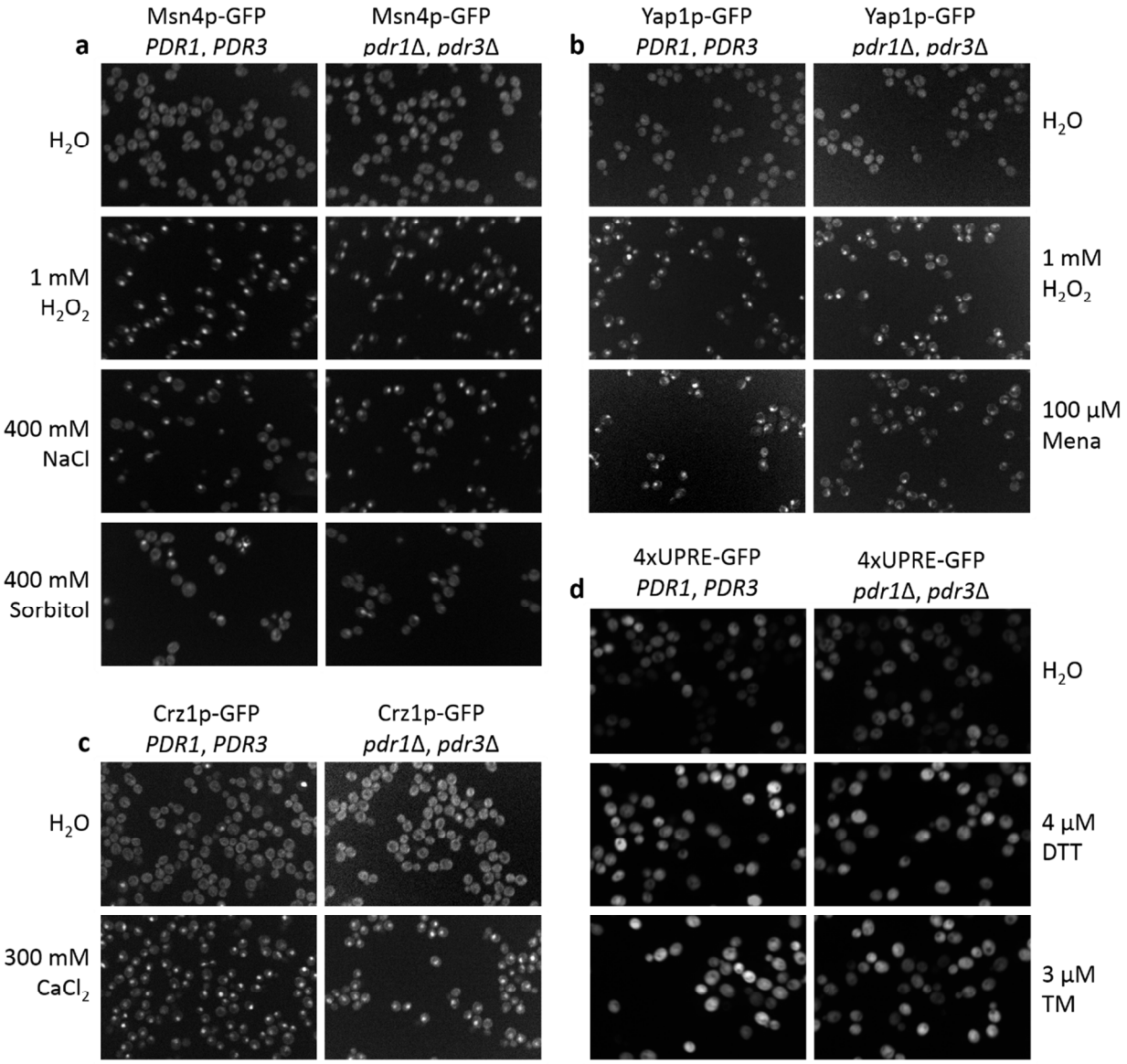

Figure 1 - Loss of Pdr1p and Pdr3p transcription factors neither induces nor impairs the general stress response, oxidative stress response, unfolded protein response or calcium signaling. *pdr1Δpdr3Δ* and wild type strains showing nuclear re-localisation of cytoplasmic GFP fusion proteins: (**a**) Msn4p-GFP in response to H_2O_2, NaCl and sorbitol treatment; (**b**) Yap1p-GFP in response to menadione and H_2O_2 treatment; (**c**) Crz1p-GFP in response to treatment with $CaCl_2$. (**d**) Induction of 4x*UPRE-GFP* (GFP fused to the unfolded response element repeats) in response to treatment with dithiothreitol (DTT) (Table S2) and tunicamycin (TM) (Table S3). Images presented are brightness/contrast adjusted and cropped using Image J [33].

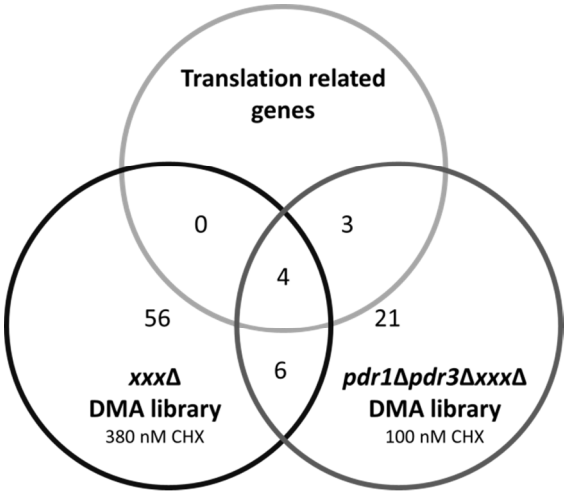

Figure 2 – Agar based cycloheximide (CHX) screen in the PA-DMA library identifies more protein translation relevant hits than the parental *MAT***a** DMA library. The PA-DMA and parental DMA libraries were screened at concentrations that gave approximately ~20-30% growth inhibition.

Compound	*pdr1*Δ*pdr3*Δ MIC (μM)	Wild type MIC (μM)	Relative MIC	Major drug efflux transporters
CHX	0.1	0.32	3.2	Pdr5p [34]
Fluconazole	3.2	100	32.0	Pdr5p [10]
Ketoconazole	3.2	100	31.0	Pdr5p and Snq2 [9]
Quercetin	100	> 100	ND	Yor1p [9]
Amphotericin B	32	> 100	ND	Other [9]
LatA	0.2	4.7	24.0	Pdr5p and Snq2p (Figure S2)
Plakortolide X	0.003	> 100	ND	Other (Figure S3)
Rapamycin A	0.03	0. 03	1.0	-
Hygromycin B	20	20	1.0	-

Table 1 – The *pdr1*Δ*pdr3*Δ double mutant strain demonstrates increased sensitivity to PDR substrates without altering sensitivity to non-PDR substrates. The *pdr1*Δ*pdr3*Δ double deletion strain showed increased sensitivity to the known PDR substrates cycloheximide (CHX), fluconazole, ketoconazole, quercetin, amphotericin B compared to wild type (PDR-replete) strain; while the sensitivity of *pdr1*Δ*pdr3*Δ to rapamycin and hygromycin B was comparable to wild type. The *pdr1*Δ*pdr3*Δ mutant also showed increased sensitivity to the previously uncharacterised PDR substrates latrunculin A (LatA) and plakortolide X. ND indicates where fold increase in MIC was not determined due to lack of growth inhibition in the wild type yeast strain.

ORF	Gene	GO: Biological process
YDL082W, *YBL087C*, *YMR194W*, *YLR185W*, *YBR031W*, *YGL147C*, *YDR385W*, *YDL130W*	*RPL13A*, *RPL23A*, *RPL36A*, *RPL37A*, *RPL4A*, *RPL9A*, *EFT2*, *RPP1B*	Translation (P value of 1.05E-04)
YKL007W, *YNL032W*	*CAP1*, *SWI14*	Cytoskeleton organisation
YKL017C, *YPL157W*	*HCS1*, *TGS1*	DNA replication
YOR298C-A,*YGL127C*	*MBF1*, *SOH1*	Transcription from RNA polymerase II promoter
YNL080C, *YML013W*, *YBR078W*, *YMR202W*, *YDR049W*, *YMR247C*	*EOS1*, *UBX2*, *ECM33*, *ERG2*, *VMS1*, *RKR1*	Multidrug resistance

YJR048W, *YLR377C*, *YFR009W*, *YMR132C*, *YNL323W*, *YOR231W*, *YPR189W*, *YGL211W*, *YGL082W*	*CYC1*, *FBP1*, *GCN20*, *JLP2*, *LEM3*, *MKK1*, *SKI3*, *NCS6*, *YGL082W*	Other
YIR042C, *YMR316C-A*	*YIR042C*, *YMR316C-A*	Uncharacterised

Table 2 – DNA-barcode microarray screen of PA-DMA against 30 nM cycloheximide showed enrichment for translation gene deletions. 31 CHX hypersensitive strains were identified from the DNA barcode microarray screening of the PA-DMA library. The BiNGO [32] plugin in Cytoscape [35] was used to calculate enrichments and P-values using the *MAT***a** PA-DMA library as a reference set and default settings. The remaining genes were assigned into GO terms determined by the GO slim mapper [36]. Genes that confer multidrug resistance in 20% of the chemical screens performed in Parsons *et al.* [1] were placed in their own category.

ORF	**Gene**	**GO: Biological process**
YJL020C, *YNL079C*	*BBC1*, *TPM1*	Cytoskeleton organisation
YIL159W, *YKL007W*, *YIL034C*	*BNR1*, *CAP1*, *CAP2*	Cytoskeleton organisation; actin filament capping (P value of 1.19E-05)
YHR129C, *YMR198W*, *YGL216W*, *YLL049W*	*ARP1*, *CIK1*, *KIP3*, *LDB18*	Cytoskeleton organisation; establishment of mitotic spindle location (P value of 8.20E-06)
YGR078C, *YLR200W*	*PAC10*, *YKE2*	Tubulin complex biogenesis (P value of 2.37E-03)
YAL026C, *YHL031C*	*DRS2*, *GOS1*	Golgi and vesicle transport
YMR202W, *YCR034W*	*ERG2*, *FEN1*	Lipid metabolism
YGL168W, *YOR153W*, *YDR011W*, *YIL073C*, *YDR074W*	*HUR1*, *PDR5*, *SNQ2*, *SPO22*, *TPS2*	Multidrug resistance (Parsons *et al*, 2004)
YER111C, *YBL054W*, *YDR247W*	*SWI4*, *TOD6*, *VHS1*	Other
YBR178W, *YFR016C*	*YBR178W*, *YFR016C*	Uncharacterised

Table 3 - DNA-barcode microarray screen of PA-DMA against 28 nM latrunculin A (LatA) showed enrichment of gene deletions involved in cytoskeleton organisation. 51 phenotypic enhancements were identified from a DNA barcode microarray screening of the PA-DMA library and 25 were confirmed to confer hypersentivity to LatA and are presented above. The BiNGO [32] plugin in Cytoscape [35] was used to calculate enrichments and P-values using the *MAT***a** PA-DMA library as a reference set and default settings. The remaining genes were assigned into GO terms determined by the GO slim mapper [36].