Identification of *Saccharomyces cerevisiae* Genes Whose Deletion Causes Synthetic Effects in Cells with Reduced Levels of the Nuclear Pif1 DNA Helicase

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Yeast Genes that are Synthetic with *pif1-m2*

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ABSTRACT

The multifunctional *Saccharomyces cerevisiae* Pif1 DNA helicase affects the maintenance of telomeric, ribosomal, and mitochondrial DNAs, suppresses DNA damage at G-quadruplex motifs, influences the processing of Okazaki fragments and promotes breakage induced replication. All of these functions require the ATPase/helicase activity of the protein. Owing to Pif1’s critical role in the maintenance of mitochondrial DNA, *pif1Δ* strains quickly generate respiratory deficient cells and hence grow very slowly. This slow growth makes it difficult to carry out genome-wide synthetic genetic analysis in this background. Here, we used a partial loss of function allele of *PIF1*, *pif1-m2*, which is mitochondrial proficient but has reduced abundance of nuclear Pif1. Although *pif1-m2* is not a null allele, *pif1-m2* cells exhibit defects in telomere maintenance, reduced suppression of damage at G-quadruplex motifs and defects in break-induced replication. We performed a synthetic screen to identify non-essential genes with a synthetic sick or lethal relationship in cells with low abundance of nuclear Pif1. This study identified eleven genes that were synthetic lethal (*APM1, ARG80, CDH1, GCR1, GTO3, PRK1, RAD10, SKT5, SOP4, UMP1* and *YCK1*) and three genes that were synthetic sick (*DEF1, YIP4* and *HOM3*) with *pif1-m2*.

INTRODUCTION

Pif1 family DNA helicases are found in all three kingdoms (reviewed in Bochman et al. 2010). The best studied of these helicases is the founding member of the family, the *S. cerevisiae* Pif1. There are two isoforms of Pif1 that depend on whether the first or second methionine is used to initiate translation of the mRNA (Schulz and Zakian 1994; Zhou et al. 2000). One isoform is targeted to the mitochondria (mt) where it is critical for maintenance of mtDNA and for
respiratory competence. The second isoform is localized to the nucleus and functions in multiple pathways that affect genome integrity. Pif1 is a negative regulator of telomere lengthening and de novo telomere addition by virtue of its ability to displace telomerase from DNA ends (SCHULZ and ZAKIAN 1994; BOULE et al. 2005; PHILLIPS et al. 2015). It is required to generate long flap Okazaki fragments (PIKE et al. 2009) and to promote breakage induced replication (SAINI et al. 2013; WILSON et al. 2013). Pif1 promotes DNA replication through G-quadruplex (G4) motifs, which are sequences that form G4 structures in vitro and Pif1 suppresses DNA damage at G4 motifs (RIBEYRE et al. 2009; PAESCHKE et al. 2011; PIAZZA et al. 2012; PAESCHKE et al. 2013). Additionally, Pif1 helps maintain the replication fork barrier (RFB) in the ribosomal DNA (rDNA) (IVESSA et al. 2002). Although Pif1 has weak unwinding activity on conventional 5’ tailed duplex DNA substrates, it robustly unwinds G4 structures and RNA/DNA hybrids in vitro (BOULE and ZAKIAN 2007; RIBEYRE et al. 2009; PAESCHKE et al. 2011; ZHOU et al. 2014).

Despite its multiple and diverse functions, PIF1 is not an essential gene. We anticipated that other genes might act in parallel with Pif1 to carry out its various cellular functions. S. cerevisiae encodes a second Pif1 family helicase, Rrm3, whose helicase domain is 40% identical to that of Pif1. However, the functions of Rrm3 and Pif1 are largely non-overlapping, except at G4 motifs (PAESCHKE et al. 2013). Rrm3 does not appear to be a backup for Pif1 at many of its genomic targets (IVESSA et al. 2000; IVESSA et al. 2002; O’ROURKE et al. 2005). We predicted that PIF1 might have synthetic interactions with genes involved in regulating telomere length, Okazaki fragment maturation, breakage induced replication, and G-quadruplex unwinding. Additionally, because Pif1 binds in vivo to the promoters of ~130 genes (Chi Fu Chen, Sebastian Pott and VAZ, unpublished results), Pif1 might have as yet undescribed roles in transcriptional regulation, which could result in interactions with transcription factors. We anticipated that we might detect indirect
synthetic lethal relationships owing to Pif1’s effect on gene expression and/or genome integrity. In addition, as pif1-m2 cells are more sensitive to proteasomal inhibition and have a higher basal level of autophagy, pif1-m2 cells may be more dependent on the proteasome for cellular maintenance and survival (JLS and VAZ, in preparation). Thus, pif1-m2 might have synthetic interactions with other genes with roles in autophagy and proteasomal function.

**Rationale For Screen:** As we are particularly interested in the nuclear functions of Pif1, we sought to identify genes whose deletion affected the viability of or reduced the growth rate of pif1-m2 cells, which are deficient in the nuclear form of Pif1 (SCHULZ and ZAKIAN 1994; ZHOU et al. 2000). This strategy avoided the difficult of using pif1Δ cells, which are very slow growing, behavior that might obscure synthetic interactions.

**MATERIALS AND METHODS**

**Screen Design:** Strains and plasmids used in this study are listed in Tables 1 and 2. The prototroph deletion collection, which contains 4,783 strains with a single deletion of a non-essential gene, tagged with the kanMX antibiotic resistance marker, was used. The pif1-m2 query strain was created using the pvs31 plasmid, an integrating plasmid with a URA3 selectable marker (SCHULZ and ZAKIAN 1994). The pvs31 plasmid was linearized with HindIII (NEB) and transformed into DBY11087 using lithium acetate transformation (BECKER and LUNDBLAD 2001). After introduction of the pif1-m2 mutation, the natMX resistance cassette was added proximal to the pif1-m2 gene (GOLDSTEIN and MCCUSKER 1999). The pif1-m2 mutation was confirmed by PCR and sequencing and shown to segregate 2:2 with the natMX marker. Mating, sporulation and selection were performed as described (TONG and BOONE 2006). Synthetic genetic analysis was performed as described (TONG and BOONE 2006) as outlined in Figure 1. Briefly, the mutant and
query strains were grown on YEPD media at 30°, and then mated, and diploids were selected using YEPD with G418 + clonNAT. The strains were transferred to sporulation media, then MATa haploids were selected using drop out media lacking HIS, ARG and LYS with canavanine and thialysine, followed by selection with drop out media with G418 and finally by selection of double mutant haploids on drop out media with G418 + clonNAT.

**Phenotypes:** Each strain was mated in quadruplicate with the control (hoΔ::NATMX) and query (pif1-m2::NATMX) strains to generate double mutant diploids, which were then sporulated. Double mutant haploid clones (i.e., pif1-m2 geneXΔ) were derived from these spores. Strains that failed to form viable double mutant haploids when mated with the pif1-m2 strain but successfully formed viable double mutant haploids when mated with the control strain were considered putative synthetic lethal interactors. Strains that formed slow growing double mutant haploids when mated with the pif1-m2 strain (determined by visual inspection as being <50% of the size of either single mutant) were considered candidates for putative synthetic sick interactors. The use of the robotic pins, and the mixing steps utilized in the RoTOR robot (Singer, RoTOR-HDA), prevented the visualization of less severe synthetic growth differences. The synthetic sick mutants were not tested for mitochondrial proficiency.

**Verification of Mutants:** Each putative synthetic relationship was re-examined by mating the appropriate strains by hand, selecting for diploids, which were sporulated and the resulting tetrads dissected. In some cases, random spore analysis was used as described (LICHTEN 2014).

**RESULTS**

The genetic screen identified eleven genes that were synthetic lethal and three genes that were synthetic sick with the pif1-m2. Surprisingly, this screen did not identify any of the over 100 genes
that have been shown or inferred to encode a helicase as having a synthetic relationship with \textit{pif1-m2}, including \textit{sgs1Δ} and \textit{rad3Δ}, which were shown previously to be synthetic sick with \textit{pif1Δ} (\textsc{wagner} et al. 2006; \textsc{moriel-carretero} and \textsc{aguilera} 2010). The fact that \textit{pif1-m2} is not a null allele and retains residual nuclear function (\textsc{schulz} and \textsc{zakian} 1994) most likely explains why we did not observe synthetic relationships between \textit{pif1-m2} and other helicases and/or genes previously reported to have a synthetic phenotype with \textit{pif1Δ}. Alternatively, synthetic phenotypes reported earlier may be due to the respiratory deficiencies, rather than the nuclear defects, of \textit{pif1Δ} cells (Supplemental Table 1). It is also possible that the W303 based prototrophic deletion collection used here may contribute to the differences between this study and earlier analyses, as several earlier studies were completed in the BY4741 background (\textsc{pan} et al. 2004; \textsc{pan} et al. 2006), others used S288c (\textsc{zhang} and \textsc{durocher} 2010), and some used a combination of strain backgrounds (\textsc{osman} et al. 2009; \textsc{moriel-carretero} and \textsc{aguilera} 2010).

**Synthetic Lethal Genes:** Eleven genes were identified whose deletion from a \textit{pif1-m2} cell generated inviable cells. Here we list those genes and provide some information on their functions and potential interactions with \textit{PIF1}.

\textit{APM1} encodes a protein that is a subunit of the clathrin associated protein complex. It is involved in the vesicular transport process (\textsc{nakayama} et al. 1991; \textsc{stepp} et al. 1995). \textit{apm1Δ} cells have abnormal vacuolar transportation and abnormal golgi protein sorting (\textsc{phelan} et al. 2006).

\textit{ARG80} encodes a transcription factor that is involved in the regulation of arginine responsive genes (\textsc{dubois} et al. 1987). \textit{arg80Δ} cells have abnormal vacuolar morphology and decreased fitness (\textsc{michaillat} and \textsc{mayer} 2013). Given that Pif1 binds promoters of many genes (see introduction), this synthetic phenotype may reflect a transcriptional problem in the double mutant.
CDH1 encodes a protein that activates the anaphase promoting complex/cyclosome (Visintin et al. 1997; Harper et al. 2002; Woodbury and Morgan 2007). Cdh1 is a cell-cycle regulated protein that directs the ubiquitination of cyclins and helps to orchestrate the mitotic exit from the cell cycle. cdh1Δ cells have increased telomere length and abnormal cell cycle progression (Visintin et al. 1997; Askree et al. 2004). Pif1 abundance is also cell-cycle regulated in a proteasome dependent manner, suggesting a potential relationship between Cdh1 and Pif1 (Mateyak and Zakian 2006). In addition, the essential telomerase subunit Est1 is cell cycle regulated (Taggart et al. 2002) in a proteasome and Cdh1 dependent manner (Osterhage et al. 2006; Ferguson et al. 2013). Moreover, many of the proteins that co-purify with yeast telomerase, as determined by mass spectrometry analysis, affect ubiquitin and proteolysis (Lin et al. 2015). Thus, the lethality of pif1-m2 cdh1Δ cells may be due to impaired proteolysis that affects telomere length or other Pif1 functions.

GCR1 encodes a DNA binding protein that interacts with the transcriptional activator Gcr2 to promote transcriptional activation of genes involved in glycolysis (Clifton et al. 1978; Holland et al. 1987). As with ARG80, this interaction may be due to a transcriptional function of Pif1.

GTO3 encodes a glutathione transferase with a poorly defined function. It is putatively localized to the cytosol and gto3Δ cells have abnormal vacuolar morphology (Herrero 2005; Garcera et al. 2006).

PRK1 encodes a serine/threonine protein kinase that is involved in cytoskeletal organization and actin function (Byrne and Wolfe 2005; Zeng and KinSELLA 2010). Endocytosis is reduced in prk1Δ cells (Henry et al. 2003).
RAD10 encodes a single-stranded DNA endonuclease with roles in both nucleotide excision repair and single-strand annealing-mediated recombination (Ivanov and Haber 1995; de Laat et al. 1999; Symington 2002). Pif1 inhibits telomerase-mediated double-strand break repair (Schulz and Zakian 1994). Rad10 promotes the creation of gross chromosomal rearrangements (GCR), which are increased in both pif1-m2 and pif1Δ cells (Myung et al. 2001; Hwang et al. 2005; Piazza et al. 2012; Paeschke et al. 2013). We speculate that pif1-m2 rad10Δ cells may be inviable due to combined defects in two different DNA repair pathways. Surprisingly, even though Rad1 and Rad10 act together in nucleotide excision repair and single strand annealing, this screen did not identify a synthetic relationship between pif1-m2 and RAD1. This result might indicate that RAD10 has a function that is distinct from RAD1, which is responsible for the synthetic relationship between RAD10 and pif1-m2. For example, a telomere-dedicated single strand annealing pathway that is RAD10- but not RAD1-dependent might act on the highly repetitive telomeric DNA.

SOP4 encodes an endoplasmic reticulum membrane protein that is involved in the export of Pma1 and Pma1-7, proteins that regulate cytoplasmic pH and help to maintain the plasma membrane potential from the endoplasmic reticulum (Luo and Chang 1997; Luo et al. 2002). sop4Δ cells have abnormal vacuolar morphology (Michaillat and Mayer 2013).

SKT5 encodes a protein that activates the chitin synthetase Chs3 that helps form spore walls (Iwamoto et al. 2005). skt5Δ cells have decreased vegetative growth rate and decreased viability (Kozubowski et al. 2003; Byrne and Wolfe 2005).

UMP1 encodes a protein that is a chaperone required for the maturation of the 20S proteasome (Ramos et al. 1998; Ishikawa et al. 2005). In ump1Δ cells, the proteasome is functionally
impaired (RAMOS et al. 1998) and unpublished data from our lab shows that pif1-m2 cells are more sensitive to proteasomal inhibition (JLS and VAZ). ump1Δ cells with decreased nuclear Pif1 may be inviable due to strain on the proteasomal machinery. As with CDH1, the synthetic effects of pif1-m2 and ump1Δ may reflect synthetic effect resulting from impaired Pif1 proteolysis.

YCK1 encodes a palmitolylated membrane bound casein kinase that is involved in endocytic trafficking and glucose sensing (ROBINSON et al. 1992; REDDI and CULOTTA 2013). yck1Δ cells have abnormal vacuolar morphology (MICHAILLAT and MAYER 2013).

**Synthetic Sick Genes:** Our screen also identified genes whose deletion in a pif1-m2 cell resulted in a slow growth phenotype where double mutants grew to <50% of the size of the either single mutant strain. This analysis identified three such genes, **DEF1, YIP4,** and **HOM3.**

The identification of a synthetic relationship between PIF1 and DEF1 was particularly exciting, as both genes function in genome maintenance, telomere length and maintenance of mtDNA. Def1 forms a complex with Rad26, a protein that functions in transcription-coupled repair (WOUDSTRA et al. 2002; SOMESH et al. 2005; JORDAN et al. 2007). Def1 also plays a role in maintenance of telomeres as def1Δ telomeres are 200 bp shorter than the wild-type length of ~300 bps (CHEN et al. 2005). Like pif1Δ cells, def1Δ cells display increased mitophagy and abnormal vacuolar morphology (MICHAILLAT and MAYER 2013; BOCKLER and WESTERMANN 2014). We speculate that the reduced growth rate of the pif1-m2 def1Δ strain is due to their shared roles in DNA repair and telomere length.

**YIP4** encodes a protein that interacts with Rab GTPases and is involved in vesicle-mediated transport (SAMANTA and LIANG 2003; INADOME et al. 2007).
HOM3 encodes an aspartate kinase that is localized to the cytoplasm and catalyzes methionine and threonine biosynthesis (Mountain et al. 1991).

**Discussion:** The identified genes whose deletion had synthetic effects with pif1-m2 support the known multi-functional nature of Pif1. In addition to its multiple described functions, our data suggest potential new roles for Pif1 in proteasome function, transcription coupled repair, endocytosis and vacuolar morphology, although some of these effects may be indirect if Pif1 has a transcriptional function. Future work will focus on elucidating the connections between PIF1 and the genes identified in this study, with a particular interest in examining the relationship between PIF1, CDH1, RAD10, and DEF1, as these genes all affect telomeres and/or DNA repair (Schulz and Zakian 1994; Woudstra et al. 2002; Askree et al. 2004; Chen et al. 2005). A second major focus will be on the role of Pif1 in proteasomal function as the synthetic relationships between pif1-m2 and CDH1 and UMP1 reported here, coupled with unpublished data from our lab, suggest a role for Pif1 in proteasomal function, which may be important for cells to tolerate stress. The synthetic effects of CDH1 and pif1-m2 and UMP1 might additionally help elucidate a role for PIF1 in autophagy. Future work will also examine the role of PIF1 on transcriptional regulation, which may help identify indirect interactions that are responsible for some of the synthetic genetic relationships reported here.
Table 1: Strains used in this study

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Mutation, Strain Background, Previous Name/Previous Study if Applicable</th>
</tr>
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<tbody>
<tr>
<td>Query- <em>pif1m2::NATMX</em></td>
<td><em>pif1m2</em> mutation and NATMX cassette added to: DBY11087; S288C, MATα, <em>his3Δ leu2Δ ura3Δ lyp1Δ met15Δcyh2Δ LYS2 can1::Pste2-S.P. his5</em></td>
</tr>
<tr>
<td>Control- <em>hoΔ::NATMX</em></td>
<td><em>hoΔ::natMX</em> deletion added to: DBY11087; MATα, <em>his3Δ leu2Δ ura3Δ lyp1Δ met15Δcyh2Δ LYS2 can1::Pste2-S.P. his5</em></td>
</tr>
<tr>
<td>Prototrophic Deletion Mutation Array</td>
<td>DBY15001 W303 derived, MATα. Prototrophic deletion collection: created by Drs. Amy Caudy &amp; David Hess. (KLOSINSKA et al. 2011)</td>
</tr>
</tbody>
</table>
Table 2: Plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid Name</th>
<th>Description of plasmid use</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAG25</td>
<td>Insertion of the NATMX cassette (GOLDSTEIN and McCUSKER 1999)</td>
</tr>
<tr>
<td>pvs31</td>
<td>Insertion of the pif1m2 mutant via pop-in/pop-out (SCHULZ and ZAKIAN 1994)</td>
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Figure 1:

Legend: Schematic of steps for synthetic genetic analysis. All cells grown in quadruplicate as shown. Not pictured is the use of *hoΔNATMX* control strain in parallel.
**MATa xxxΔKANR**
[Deletion Collection]

**MATa pif1-m2::NatMX**
[Query Strain]

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**Mating**
Cells mated on YEPD at 30°C for 6 hours.

**Diploid selection**
Cells transferred to plates containing YEPD G418 + clonNAT at 30°C for 24 hours.

**Sporulation**
Cells transferred to sporulation plates at room temperature for 10 days.

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**MATa progeny selection**
Cells transferred to SD dropout media lacking HIS, ARG, and LYS with canavanine & thiostrepton at 30°C for 24 hours.

**kanR selection**
Cells transferred to plates containing G418 at 30°C for 24 hours.

**Double mutant selection**
Cells transferred to plates containing G418 + clonNAT at 30°C for 24 hours.

**Putative synthetic lethal**

**Putative synthetic sick**
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