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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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 breakage induced replication. We performed a synthetic screen to identify nonessential 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 *Saccharomyces 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 the 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 suppresses DNA damage at G4 motifs (Ribeyre *et al.* 2009; Paeschke *et al.* 2011, 2013; Piazza *et al.* 2012). 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 nonoverlapping, except at G4 motifs (Paeschke *et al.* 2013). Rrm3 does not

Strain Name	Mutation, Strain Background, Previous Name/Previous Study if Applicable
Query-pif1m2::NATMX	pif1m2 mutation and NATMX cassette added to: DBY11087; S288C, MATα, his3Δ leu2Δ ura3Δ lyp1Δ met15Δcyh2Δ LYS2 can1::Pste2-S.P. his5
Control-hoΔ::NATMX	hoΔ::natMX deletion added to: DBY11087; MATα, his3Δ leu2Δ ura3Δ lyp1Δ met15Δcyh2Δ LYS2 can1::Pste2-S.P. his5
Prototrophic Deletion Mutation Array	DBY15001 W303 derived, MATa. Prototrophic deletion collection; created by Drs. Amy Caudy and David Hess. (Klosinska et al. 2011)

appear to be a backup for Pif1 at many of its genomic targets (Ivessa et al. 2000, 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 (C. F. Chen, S. Pott, and V. A. Zakian, 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 (J. L. Stundon and V. A. Zakian, unpublished results). 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 difficulty 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 Table 1 and Table 2. The prototroph deletion collection, which contains 4783 strains with a single deletion of a nonessential 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 polymerase chain reaction 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 yeast extract peptone dextrose media at 30°, and then mated, and diploids were selected using yeast extract peptone dextrose 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 pif1-m2. Surprisingly, this screen did not identify any of the over 100 genes that have been shown or proposed to encode a helicase as having a synthetic relationship with pif1-m2, including sgs1Δ and rad3Δ, which were shown previously to be synthetic sick with pif1Δ (Wagner et al. 2006; Moriel-Carretero and Aguilera 2010). The fact that pif1-m2 is not a null allele and retains residual nuclear function (Schulz and Zakian 1994) most likely explains why we did not observe synthetic relationships between pif1-m2 and other helicases and/or genes previously reported to have a synthetic phenotype with pif1Δ. Alternatively, synthetic phenotypes reported earlier may be due to the respiratory deficiencies, rather than the nuclear defects, of pif1Δ cells (Supporting Information, Table S1). 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 (Pan et al. 2004, 2006), others used S288c (Zhang and Durocher 2010), and some used a combination of strain backgrounds (Osman et al. 2009; Moriel-Carretero and Aguilera 2010).

Synthetic lethal genes

Eleven genes were identified whose deletion from a pif1-m2 cell generated inviable cells. Here, we list those genes and provide some information on their functions and potential interactions with PIF1.

APM1 encodes a protein that is a subunit of the clathrin-associated protein complex. It is involved in the vesicular transport process (Nakayama et al. 1991; Stepp et al. 1995). apm1Δ cells have abnormal vacuolar transportation and abnormal Golgi protein sorting (Phelan et al. 2006).

Plasmid Name	Description of Plasmid Use
pAG25	Insertion of the NATMX cassette (Goldstein and McCusker 1999)
pvs31	Insertion of the *pif1m2* mutant via pop-in/pop-out (Schulz and Zakian 1994)

ARG80 encodes a transcription factor that is involved in the regulation of arginine responsive genes (Dubois *et al.* 1987). *arg80Δ* cells have abnormal vacuolar morphology and decreased fitness (Michaillat and 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 copurify 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.
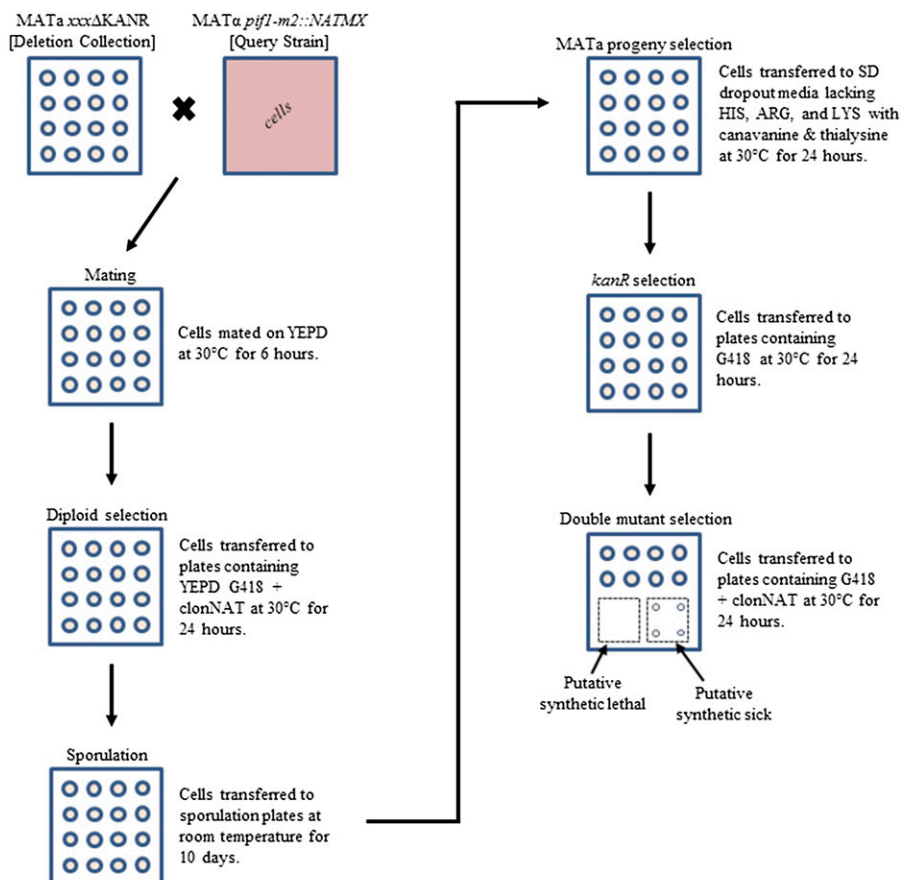

Figure 1 Schematic of steps for synthetic genetic analysis. All cells grown in quadruplicate as shown. Use of the *hoΔNATMX* control strain in parallel is not pictured. YEPD, yeast extract peptone dextrose.

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 data from our lab shows that *pif1-m2* cells are more sensitive to proteasomal inhibition (J. L. Stundon and V. A. Zakian, unpublished results). *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 result from impaired Pif1 proteolysis.

YCK1 encodes a palmitoylated 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 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 the maintenance of telomeres, as *def1Δ* telomeres are 200 bp shorter than the wild-type length of ∼300 bp (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 multifunctional 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.

LITERATURE CITED

Askree, S. H., T. Yehuda, S. Smolikov, R. Gurevich, J. Hawk *et al.*, 2004 A genome-wide screen for Saccharomyces cerevisiae deletion mutants that affect telomere length. Proc. Natl. Acad. Sci. USA 101: 8658–8663.

Becker, D. M., and V. Lundblad, 2001 Introduction of DNA into Yeast Cells in Current Protocols in Molecular Biology, John Wiley & Sons, Inc., Hoboken, New Jersey.

Bochman, M. L., N. Sabouri, and V. A. Zakian, 2010 Unwinding the functions of the Pif1 family helicases. DNA Repair (Amst.) 9: 237–249.

Bockler, S., and B. Westermann, 2014 Mitochondrial ER contacts are crucial for mitophagy in yeast. Dev. Cell 28: 450–458.

Boule, J. B., and V. A. Zakian, 2007 The yeast Pif1p DNA helicase preferentially unwinds RNA DNA substrates. Nucleic Acids Res. 35: 5809–5818.

Boule, J. B., L. R. Vega, and V. A. Zakian, 2005 The yeast Pif1p helicase removes telomerase from telomeric DNA. Nature 438: 57–61.

Byrne, K. P., and K. H. Wolfe, 2005 The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species. Genome Res. 15: 1456–1461.

Chen, Y. B., C. P. Yang, R. X. Li, R. Zeng, and J. Q. Zhou, 2005 Def1p is involved in telomere maintenance in budding yeast. J. Biol. Chem. 280: 24784–24791.

Clifton, D., S. B. Weinstock, and D. G. Fraenkel, 1978 Glycolysis mutants in Saccharomyces cerevisiae. Genetics 88: 1–11.

de Laat, W. L., N. G. Jaspers, and J. H. Hoeijmakers, 1999 Molecular mechanism of nucleotide excision repair. Genes Dev. 13: 768–785.

Dubois, E., J. Bercy, and F. Messenguy, 1987 Characterization of two genes, ARGRI and ARGRIII required for specific regulation of arginine metabolism in yeast. Mol. Gen. Genet. 207: 142–148.

Ferguson, J. L., W. C. Chao, E. Lee, and K. L. Friedman, 2013 The anaphase promoting complex contributes to the degradation of the S. cerevisiae telomerase recruitment subunit Est1p. PLoS One 8: e55055.

Garcera, A., L. Barreto, L. Piedrafita, J. Tamarit, and E. Herrero, 2006 Saccharomyces cerevisiae cells have three Omega class glutathione S-transferases acting as 1-Cys thiol transferases. Biochem. J. 398: 187–196.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast 15: 1541–1553.

Harper, J. W., J. L. Burton, and M. J. Solomon, 2002 The anaphase-promoting complex: it's not just for mitosis any more. Genes Dev. 16: 2179–2206.

Henry, K. R., K. D'Hondt, J. S. Chang, D. A. Nix, M. J. Cope *et al.*, 2003 The actin-regulating kinase Prk1p negatively regulates Scd5p, a suppressor of clathrin deficiency, in actin organization and endocytosis. Curr. Biol. 13: 1564–1569.

Herrero, E., 2005 Evolutionary relationships between Saccharomyces cerevisiae and other fungal species as determined from genome comparisons. Rev. Iberoam. Micol. 22: 217–222.

Holland, M. J., T. Yokoi, J. P. Holland, K. Myambo, and M. A. Innis, 1987 The GCR1 gene encodes a positive transcriptional regulator of the

enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in Saccharomyces cerevisiae. Mol. Cell. Biol. 7: 813–820.

Hwang, J. Y., S. Smith, and K. Myung, 2005 The Rad1-Rad10 complex promotes the production of gross chromosomal rearrangements from spontaneous DNA damage in Saccharomyces cerevisiae. Genetics 169: 1927–1937.

Inadome, H., Y. Noda, Y. Kamimura, H. Adachi, and K. Yoda, 2007 Tvp38, Tvp23, Tvp18 and Tvp15: novel membrane proteins in the Tlg2-containing Golgi/endosome compartments of Saccharomyces cerevisiae. Exp. Cell Res. 313: 688–697.

Ishikawa, T., K. Unno, G. Nonaka, H. Nakajima, and K. Kitamoto, 2005 Isolation of Saccharomyces cerevisiae RNase T1 hypersensitive (rns) mutants and genetic analysis of the RNS1/DSL1 gene. J. Gen. Appl. Microbiol. 51: 73–82.

Ivanov, E. L., and J. E. Haber, 1995 RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in Saccharomyces cerevisiae. Mol. Cell. Biol. 15: 2245–2251.

Ivessa, A. S., J. Q. Zhou, and V. A. Zakian, 2000 The Saccharomyces Pif1p DNA helicase and the highly related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. Cell 100: 479–489.

Ivessa, A. S., J. Q. Zhou, V. P. Schulz, E. K. Monson, and V. A. Zakian, 2002 Saccharomyces Rrm3p, a 5′ to 3′ DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. Genes Dev. 16: 1383–1396.

Iwamoto, M. A., S. R. Fairclough, S. A. Rudge, and J. Engebrecht, 2005 Saccharomyces cerevisiae Sps1p regulates trafficking of enzymes required for spore wall synthesis. Eukaryot. Cell 4: 536–544.

Jordan, P. W., F. Klein, and D. R. Leach, 2007 Novel roles for selected genes in meiotic DNA processing. PLoS Genet. 3: e222.

Klosinska, M. M., C. A. Crutchfield, P. H. Bradley, J. D. Rabinowitz, and J. R. Broach, 2011 Yeast cells can access distinct quiescent states. Genes Dev. 25: 336–349.

Kozubowski, L., H. Panek, A. Rosenthal, A. Bloecher, D. J. DeMarini et al., 2003 A Bni4-Glc7 phosphatase complex that recruits chitin synthase to the site of bud emergence. Mol. Biol. Cell 14: 26–39.

Lichten, M., 2014 Tetrad, random spore, and molecular analysis of meiotic segregation and recombination. Methods Mol. Biol. 1205: 13–28.

Lin, K. W., K. R. McDonald, A. J. Guise, A. Chan, I. M. Cristea et al., 2015 Proteomics of yeast telomerase identified Cdc48-Npl4-Ufd1 and Ufd4 as regulators of Est1 and telomere length. Nat. Commun. 6: 8290.

Luo, W., and A. Chang, 1997 Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. J. Cell Biol. 138: 731–746.

Luo, W. J., X. H. Gong, and A. Chang, 2002 An ER membrane protein, Sop4, facilitates ER export of the yeast plasma membrane [H+]ATPase, Pma1. Traffic 3: 730–739.

Mateyak, M. K., and V. A. Zakian, 2006 Human PIF helicase is cell cycle regulated and associates with telomerase. Cell Cycle 5: 2796–2804.

Michaillat, L., and A. Mayer, 2013 Identification of genes affecting vacuole membrane fragmentation in Saccharomyces cerevisiae. PLoS One 8: e54160.

Moriel-Carretero, M., and A. Aguilera, 2010 A postincision-deficient TFIIH causes replication fork breakage and uncovers alternative Rad51- or Pol32-mediated restart mechanisms. Mol. Cell 37: 690–701.

Mountain, H. A., A. S. Bystrom, J. T. Larsen, and C. Korch, 1991 Four major transcriptional responses in the methionine/threonine biosynthetic pathway of Saccharomyces cerevisiae. Yeast 7: 781–803.

Myung, K., C. Chen, and R. D. Kolodner, 2001 Multiple pathways cooperate in the suppression of genome instability in Saccharomyces cerevisiae. Nature 411: 1073–1076.

Nakayama, Y., M. Goebl, B. O'Brine Greco, S. Lemmon, E. Pingchang Chow et al., 1991 The medium chains of the mammalian clathrin-associated proteins have a homolog in yeast. Eur. J. Biochem. 202: 569–574.

O'Rourke, T. W., N. A. Doudican, H. Zhang, J. S. Eaton, P. W. Doetsch et al., 2005 Differential involvement of the related DNA helicases Pif1p and Rrm3p in mtDNA point mutagenesis and stability. Gene 354: 86–92.

Osman, C., M. Haag, C. Potting, J. Rodenfels, P. V. Dip et al., 2009 The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. J. Cell Biol. 184: 583–596.

Osterhage, J. L., J. M. Talley, and K. L. Friedman, 2006 Proteasome-dependent degradation of Est1p regulates the cell cycle-restricted assembly of telomerase in Saccharomyces cerevisiae. Nat. Struct. Mol. Biol. 13: 720–728.

Paeschke, K., J. A. Capra, and V. A. Zakian, 2011 DNA replication through G-quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase. Cell 145: 678–691.

Paeschke, K., M. L. Bochman, P. D. Garcia, P. Cejka, K. L. Friedman et al., 2013 Pif1 family helicases suppress genome instability at G-quadruplex motifs. Nature 497: 458.

Pan, X., D. S. Yuan, D. Xiang, X. Wang, S. Sookhai-Mahadeo et al., 2004 A robust toolkit for functional profiling of the yeast genome. Mol. Cell 16: 487–496.

Pan, X., P. Ye, D. S. Yuan, X. Wang, J. S. Bader et al., 2006 A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell 124: 1069–1081.

Phelan, J. P., S. H. Millson, P. J. Parker, P. W. Piper, and F. T. Cooke, 2006 Fab1p and AP-1 are required for trafficking of endogenously ubiquitylated cargoes to the vacuole lumen in S. cerevisiae. J. Cell Sci. 119: 4225–4234.

Phillips, J. A., A. Chan, K. Paeschke, and V. A. Zakian, 2015 The Pif1 Helicase, a Negative Regulator of Telomerase, Acts Preferentially at Long Telomeres. PLoS Genet. 11: e1005186.

Piazza, A., A. Serero, J. B. Boule, P. Legoix-Ne, J. Lopes et al., 2012 Stimulation of Gross Chromosomal Rearrangements by the Human CEB1 and CEB25 Minisatellites in Saccharomyces cerevisiae Depends on G-Quadruplexes or Cdc13. PLoS Genet. 8: 17.

Pike, J. E., P. M. Burgers, J. L. Campbell, and R. A. Bambara, 2009 Pif1 helicase lengthens some Okazaki fragment flaps necessitating Dna2 nuclease/helicase action in the two-nuclease processing pathway. J. Biol. Chem. 284: 25170–25180.

Ramos, P. C., J. Hockendorff, E. S. Johnson, A. Varshavsky, and R. J. Dohmen, 1998 Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly. Cell 92: 489–499.

Reddi, A. R., and V. C. Culotta, 2013 SOD1 integrates signals from oxygen and glucose to repress respiration. Cell 152: 224–235.

Ribeyre, C., J. Lopes, J. B. Boule, A. Piazza, A. Guedin et al., 2009 The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences in vivo. PLoS Genet. 5: e1000475.

Robinson, L. C., E. J. Hubbard, P. R. Graves, A. A. DePaoli-Roach, P. J. Roach et al., 1992 Yeast casein kinase I homologues: an essential gene pair. Proc. Natl. Acad. Sci. USA 89: 28–32.

Saini, N., S. Ramakrishnan, R. Elango, S. Ayyar, Y. Zhang et al., 2013 Migrating bubble during break-induced replication drives conservative DNA synthesis. Nature 502: 389–392.

Samanta, M. P., and S. Liang, 2003 Predicting protein functions from redundancies in large-scale protein interaction networks. Proc. Natl. Acad. Sci. USA 100: 12579–12583.

Schulz, V. P., and V. A. Zakian, 1994 The saccharomyces PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. Cell 76: 145–155.

Somesh, B. P., J. Reid, W. F. Liu, T. M. Sogaard, H. Erdjument-Bromage et al., 2005 Multiple mechanisms confining RNA polymerase II ubiquitylation to polymerases undergoing transcriptional arrest. Cell 121: 913–923.

Stepp, J. D., A. Pellicena-Palle, S. Hamilton, T. Kirchhausen, and S. K. Lemmon, 1995 A late Golgi sorting function for Saccharomyces cerevisiae Apm1p, but not for Apm2p, a second yeast clathrin AP medium chain-related protein. Mol. Biol. Cell 6: 41–58.

Symington, L. S., 2002 Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol. Mol. Biol. Rev. 66: 630–670

Taggart, A. K., S. C. Teng, and V. A. Zakian, 2002 Est1p as a cell cycle-regulated activator of telomere-bound telomerase. Science 297: 1023–1026.

Tong, A. H., and C. Boone, 2006 Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol. Biol. 313: 171–192.

Visintin, R., S. Prinz, and A. Amon, 1997   CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science 278: 460–463.

Wagner, M., G. Price, and R. Rothstein, 2006   The absence of Top3 reveals an interaction between the Sgs1 and Pif1 DNA helicases in Saccharomyces cerevisiae. Genetics 174: 555–573.

Wilson, M. A., Y. Kwon, Y. Xu, W. H. Chung, P. Chi *et al.*, 2013   Pif1 helicase and Poldelta promote recombination-coupled DNA synthesis via bubble migration. Nature 502: 393–396.

Woodbury, E. L., and D. O. Morgan, 2007   Cdk and APC activities limit the spindle-stabilizing function of Fin1 to anaphase. Nat. Cell Biol. 9: 106–112.

Woudstra, E. C., C. Gilbert, J. Fellows, L. Jansen, J. Brouwer *et al.*, 2002   A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature 415: 929–933.

Zeng, X., and T. J. Kinsella, 2010   BNIP3 is essential for mediating 6-thioguanine- and 5-fluorouracil-induced autophagy following DNA mismatch repair processing. Cell Res. 20: 665–675.

Zhang, W., and D. Durocher, 2010   De novo telomere formation is suppressed by the Mec1-dependent inhibition of Cdc13 accumulation at DNA breaks. Genes Dev. 24: 502–515.

Zhou, J., E. K. Monson, S. C. Teng, V. P. Schulz, and V. A. Zakian, 2000   Pif1p helicase, a catalytic inhibitor of telomerase in yeast. Science 289: 771–774.

Zhou, R., J. Zhang, M. L. Bochman, V. A. Zakian, and T. Ha, 2014   Periodic DNA patrolling underlies diverse functions of Pif1 on R-loops and G-rich DNA. eLife 3: e02190.

Communicating Editor: S. L. Jaspersen