Yeast Genome Maintenance by the Multifunctional PIF1 DNA Helicase Family

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Abstract: The two PIF1 family helicases in Saccharomyces cerevisiae, Rrm3, and ScPif1, associate with thousands of sites throughout the genome where they perform overlapping and distinct roles in telomere length maintenance, replication through non-histone proteins and G4 structures, lagging strand replication, replication fork convergence, the repair of DNA double-strand break ends, and transposable element mobility. ScPif1 and its fission yeast homolog Pfh1 also localize to mitochondria where they protect mitochondrial genome integrity. In addition to yeast serving as a model system for the rapid functional evaluation of human Pif1 variants, yeast cells lacking Rrm3 have proven useful for elucidating the cellular response to replication fork pausing at endogenous sites. Here, we review the increasingly important cellular functions of the yeast PIF1 helicases in maintaining genome integrity, and highlight recent advances in our understanding of their roles in facilitating fork progression through replisome barriers, their functional interactions with DNA repair, and replication stress response pathways.

Keywords: Rrm3; Pif1; Pfh1; G4 structures; DNA replication; rDNA; replication fork pausing; genome instability

1. History of the PIF1 DNA Helicase Family

The PIF1 DNA helicase family is conserved from bacteria to mammals [1,2]. While the yeast Schizosaccharomyces pombe and more complex multicellular eukaryotes, including humans, only encode one PIFI family helicase, Saccharomyces cerevisiae expresses two: Rrm3 and ScPif1. The ScPIF1 gene was originally identified in a screen to determine mutations that change the recombination frequency of tandemly arrayed repeats within mitochondria, and was therefore named after that defect, petite integration frequency (ScPIF1) [3]. In a quest to determine genes that suppress recombination of tandem repeats of the ribosomal DNA (rDNA) and copper chelatin (CUP1) genes in S. cerevisiae, the ribosomal DNA recombination mutation 3 (RRM3) gene was identified and later classified as a member of the PIFI DNA helicase family based on sequence similarity [4,5]. Both Rrm3 and ScPif1 belong to the superfamily 1B and have 5′-3′ translocase activity that is encoded in helicase domains that share 40% identical residues [4,6–8]. An overview of the domain structure and functional motifs of PIF1 helicase family members in yeasts and humans is provided in Figure 1. This leaves the intrinsically disordered N-terminal extensions of Rrm3 and ScPif1 to regulate their enzymatic activity and their recruitment to specific sites within the yeast genome, where they perform the many distinct cellular functions discussed in this review.
Figure 1. Structure and functional motifs of the yeast and human PIF1 family helicases. *Saccharomyces cerevisiae* expresses two members of the PIF1 family, Rrm3, and ScPif1, whereas *Schizosaccharomyces pombe* and higher eukaryotes express one. PIF1 helicases share the conserved ATPase/helicase domain and an intrinsically disordered N-terminal tail of variable sequence. Post-translational modification sites, proliferating cell nuclear antigen (PCNA)-interacting protein (PIP) box and alternative start sites, which give rise to mitochondrial isoforms, are marked [9–14].

2. Replication Through the rDNA Replication Fork Barrier

After initiation of replication of the highly repetitive ribosomal DNA (rDNA) locus that spans approximately 1.5 Mb on chromosome XII, the leftward-moving replication fork encounters a cis-acting sequence near the 3’ end, called the replication fork barrier (RFB). RFB is located in a non-transcribed spacer and contains two termination sites, Ter1 and Ter2, that are bound by Fob1 to ensure that replication of the rDNA locus occurs in a unidirectional manner [15]. The PIF1 helicase family members in yeasts whose functions are fairly well-understood (Rrm3, ScPif1, SpPfh1) all accumulate at the RFB and 35S regions of the rDNA, indicating an intimate involvement in the regulation of rDNA replication [16,17]. However, they appear to have opposite effects; Rrm3 and Pfh1 promote replication through the RFB whereas ScPif1 maintains it, although the molecular mechanisms by which they modulate the RFB remain unclear [16,17]. These distinct roles in rDNA replication are evidenced by the increased number of chromosomal rDNA repeats, converged forks and fork pausing in *rrm3* and *pfh1* mutants when compared to *pif1* mutants or wildtype cells [16,18]. It is thought that Rrm3’s helicase activity removes DNA-bound proteins, including Fob1, ahead of the replication forks to prevent pausing [18,19]. However, deletion of *FOB1* only partially restores replication fork movement through the rDNA locus in *rrm3* mutants, indicating the presence of Fob1-independent barriers, such as the 35S and 5S rRNA genes and the inactive ARS [18,20]. Additionally, removal of RFBs by deletion of *FOB1* cannot rescue lethal interactions of *rrm3* with deletions of the RecQ helicase gene *SGS1*, or the fork protection complex gene *MRC1*, indicating that forks stalled at RFB and their intermediates are not toxic to *sgs1Δ* and *mrc1Δ* mutants, and that Rrm3 performs crucial functions at genomic loci besides RFB [18,20]. Two other subunits of the fork protection complex, Tof1 and Csm3, actually inhibit the “sweepase” activity of Rrm3 to remove DNA-bound proteins at termination sites of chromosomal rDNA and RNA polymerase III transcription [20], either directly by inhibiting the helicase activity of Rrm3 or indirectly by causing a conformational change of the replisome leading to restriction of Rrm3 activity [20]. Although disruption of the fork protection
complex abolishes pausing at termination sites, further deletion of RRM3 leads to a partial re-establishment of the termination site similar to the observation in rrm3 fob1 mutants [18,20]. This is consistent with the observation that replication forks in rrm3 mutants also stall at Fob1-independent sites. Other DNA helicases, such as Sgs1 and Srs2, which are also capable of removing DNA-bound proteins [21,22], were dispensable for replication through RFB [20]. This raises the possibility that the factors that promote fork escape in rrm3 tof1 and rrm3 csm3 mutants may not be another DNA helicase, but could involve DNA motor proteins that remodel DNA or chromatin.

Notably, unlike hydroxyurea (HU)-induced fork pausing, pausing at RFB is independent of the DNA-damage checkpoint kinases Mec1 and Rad53 and DNA synthesis resumes without breakage or recombination at RFBs [23], suggesting that naturally occurring replication pause sites are processed differently than those formed during DNA replication stress. This likely explains why deletion of MRC1 does not affect replication fork pausing at Fob1-RFBs and why the rrm3Δ mrc1-AQ mutant, which is defective in the checkpoint function but not the replication function of Mrc1, is viable [23–26]. A better understanding of the chromatin environment in which natural barriers of DNA replication reside, compared to the environment established at genotoxin-induced paused forks will help to elucidate the mechanisms by which the mechanistically poorly understood Rrm3 and other PIF1 helicases contribute to genome maintenance and stability. An overview of the function of the yeast PIF1 helicases and their genetic and physical interactions is provided in Table 1 and Figures 2 to 4.

Table 1. Yeast PIF1 helicase function in the maintenance of genomic integrity. Rrm3 and ScPif1 localize mainly to the same genomic sites in the yeast genome and have several overlapping functions. Green boxes indicate the ability to perform the function, red boxes mark the absence of the function, and yellow indicates a backup role. Pfh1 localizes to similar sites as ScPif1 and Rrm3, but several functions and associations with genomic loci known for Rrm3 and ScPif1 have not been tested for Pfh1. *Pif1 was not found to affect replication through telomeres when assayed by 2D gels [27], but the more recently discovered role of Pif1 in G4 unwinding might suggest such a role. Numbers indicate references to relevant studies. N.D. indicates not determined.

| Function | S. cerevisiae | S. pombe |
|----------|---------------|----------|
|          | Rrm3 | Pif1 |
| Essential | [28] | [28] |
| Nucleus | No | No |
| Mitochondria | Yes | [28] |
| Telomeres | [27] | [30,31] |
| mtDNA | N.D. | [33] |
| rDNA | [16] | [16] |
| tRNA genes | [34,35] | [35,36] |
| Centromeres | [34,37] | [37] |
| Highly transcribed genes | [38] | [39] |
| Active/Inactive DNA replication forks | [10] | [10] |
| Transcription-replication conflicts | [38] | [38] |
| Rad52 DNA-damage foci | [28] | [28] |
| Origins of replication | [16,42] | [16] |
| Replication through telomeres | [27] | N.D.* |
| Telomere anchoring | [43] | [44] |
| Telomerase inhibition | [27] | [13] |
| G4 structures | [45] | [39,46] |
| Okazaki fragment maturation | [36] | [48] |
| Centromere Replication and Segregation | [37] | [37] |
| Sister chromatid cohesion | [50] | [37] |
| Sister chromatid exchange | [51] | [51] |
| Break-induced replication | [11,52] | [11,52] |
| DNA synthesis restriction (HU) | [42] | N.D. |
| Fork convergence | [16,53] | [53] |
| Daughter-strand gap repair | [51] | [55] |
| Silent mating type locus | [34] | N.D. |
| Fork progression through tRNA genes | [35,36] | [35,36] |
Fork progression through rDNA
Repression of Ty1 mobility
Maintenance of mtDNA
H2AX/H2A phosphorylation

|          | [16] | [16] | [17] |
|----------|------|------|------|
|          | [56,57] | N.D. | N.D. |
|          | [36] | [33,36] | [28] |
|          | N.D. | N.D. | [17] |

Figure 2. Synthetic lethal interactions of RRM3 and ScPIF1 [25,26,34,49,58–79].

Figure 3. Functional interactions of RRM3, ScPIF1, and PFH1. (a) Synthetic growth defects of rrm3, pif1, and pfh1 (pfh1-mt* and pfh1-R20) mutants. *Overexpression of CSE4 leads to a growth defect in the rrm3 mutant. [51,54,55,62,69,75,77,80–92]. (b) Effects of pif1, rrm3, and pfh1-m21 mutations on telomere length [93–96]. Telomeres are depicted in pink and genes are in blue stripes (c) Genes that promote or suppress gross-chromosomal rearrangement formation in pif1 (left) and rrm3 (right) mutants. [25,34,48,91,94,97–105].
Figure 4. Physical interactions of Rrm3, ScPif1, and Pfh1. Interactions of Rrm3, ScPif1, and Pfh1 with proteins in lighter colored boxes have been verified by yeast-two-hybrid and/or co-immunoprecipitation. Binding sites on Rrm3, ScPif1, and Pfh1 and the method of detection are indicated. Putative binding proteins shown in darker colored boxes were identified in various high-throughput affinity capture experiments [9,11,19,42,52,93,106–130].

3. Termination of DNA Replication

With the focus of the past decades on the role of the PIF1 helicase family in the elongation step of DNA replication, a role in DNA replication termination only emerged recently. Fork convergence and dissolution during replication termination is primarily the responsibility of the essential type II topoisomerase Top2. ScPif1 and Rrm3 are now recognized to contribute to fork convergence by unwinding the final stretch of parental DNA in a pathway independent of Top2 [53]. Consistent with this finding, Top2 was upregulated in the chromatin fraction of $rrm3$Δ cells [19]. Similarly, in $S. pombe$, deletion of $pfh1$ leads to short stretches of unreplicated DNA, which cause the accumulation of anaphase bridges, signaling problems with chromosome segregation [54,131].

4. Replication Through tRNA Genes

In the absence of Rrm3, increased replication fork pausing is not only observed in the highly transcribed rDNA locus, but also in tRNA genes [18,20,34,35,38,132]. Pausing at tRNA genes is even more severe in the absence of ScPif1 [35,36], indicating that, in contrast to being dispensable for negotiating the Rbf1-Rfh1 pathway, both ScPif1 and Rrm3 possess higher affinity for tRNA substrates than for duplex DNA [35,36]. Since transcription continues during genome duplication in S-phase, Rrm3 and ScPif1 may also facilitate DNA replication by unwinding RNA-DNA hybrids formed at highly transcribed genes. Indeed, ScPif1 and Rrm3 accumulate at higher rates at tRNA genes in the absence of the RNA helicase H1 (Rnh1) when RNA-DNA hybrids, such as R-loops, accumulate in rDNA genes [35,36].

In another model, head-on collisions between replication forks and transcription machinery, rather than R-loops, are responsible for fork arrest in tRNA genes in the absence of Rrm3 and ScPif1 [34,36,134]. Figure 4. Physical interactions of Rrm3, ScPif1, and Pfh1. Interactions of Rrm3, ScPif1, and Pfh1 with proteins in lighter colored boxes have been verified by yeast-two-hybrid and/or co-immunoprecipitation. Binding sites on Rrm3, ScPif1, and Pfh1 and the method of detection are indicated. Putative binding proteins shown in darker colored boxes were identified in various high-throughput affinity capture experiments [9,11,19,42,52,93,106–130].
(FACT) complex, Rrm3 binding to longer and highly expressed RNAPII-transcribed genes increased, especially at the 3’ end of open reading frames where R-loops are more likely due to increased fork impediments, suggesting that Rrm3 is directly involved in resolving R-loops in a common pathway with the FACT complex [135].

Similar to Rrm3, Pfh1 binds to rDNA and tRNA genes, the RFB of rDNA, and mating type loci in *S. pombe* [17], suggesting that Pfh1 also facilitates fork progression through those sites. Indeed, in the absence of Pfh1, fork pausing at tRNA genes increased significantly, especially in those tRNA genes where replication and transcription are at risk of meeting head-on, or where converged forks accumulate [17]. Unlike Rrm3, however, Pfh1 not only associates with highly expressed RNAPII-transcribed genes, but also facilitates their replication [17]. The proposal that Pfh1, like Rrm3, travels with the replication fork is supported by physical interactions of Pfh1 with several replisome components [40].

5. Telomere Length Maintenance

ScPif1 was identified in a screen for mutations that inhibit *de novo* telomere formation and telomere elongation [30]. Indeed, ScPif1 associates with telomeres *in vivo*, and inhibits telomerase by removing it through its helicase activity, leading to the characteristically elongated telomeres of *pif1* mutants [13,31]. ScPif1 removes telomerase by interacting directly with the finger domain of the telomerase subunit Est2 [136]. Recently, ScPif1 was found to regulate telomere elongation by affecting the spatial distribution of telomerase components by segregating the limiting component of telomerase, the RNA TLC1, to the nucleolus [44]. While it is known how ScPif1 removes telomerase from telomere DNA, the mechanism of sequestering it to the nucleolus remains to be identified. While *rrm3* mutants also have slightly elongated telomeres, they do not appear to be the result of increased telomerase activity, but most likely stem from replication problems [27]. Notably, Rrm3 physically interacts with Def1, a protein of unknown biochemical activity that is required for normal telomere length maintenance [93]. However, the functional significance of this interaction for the replication of telomeres or other genomic sites is unknown. The effect of *pif1*, *rrm3*, and *pfh1* mutations on telomere length is summarized in Figure 3b.

During telomeric replication, ScPif1 generates single-stranded DNA (ssDNA) of the 3’ end by regulating a nuclease activity that functions in parallel to the 5’-3’ exonuclease Exo1 [75]. This nuclease remains to be identified, but a potential candidate is Dna2, which is also needed for Okazaki fragment maturation, and acts with the Sgs1 helicase in the long-range resection of DNA double-strand breaks to produce 3’ overhangs for homologous recombination [75,137]. The possibility that ScPif1 possesses nuclease activity was recently tested as it contains the conserved residues for 3’-5’ exonuclease activity in its helicase core domain; however, cleavage was negligible even at extremely high concentrations [138]. These residues are not conserved in Rrm3 or hPif1, indicating that other members of the PIF1 family are also unlikely to possess exonuclease activity.

Following senescence, two types of survivors with altered telomere structures can be identified in yeast cells. Type I survivors have short telomeric TG1-3 tracts with highly amplified subtelomeric Y-elements, and Type II survivors have long heterogeneous telomeric TG1-3 tracts, which are generated by homologous recombination (HR) and by break-induced replication (BIR), respectively [75,139,140]. Interestingly, *pif1* mutants emerging from senescence do not clearly fall into either category, suggesting that ScPif1 is involved in both survival pathways [75]. Another study showed a stronger requirement of ScPif1 for the formation of recombinant-dependent Type I survivors [141].

Just recently, Hrq1, a DNA helicase that has similarity to the human RecQ helicase RecQL4 [142], was shown to functionally interact with ScPif1 to synergistically modulate telomere length homeostasis by regulating telomerase activity [143]. Depending on whether the ScPif1 helicase or the Hrq1 helicase was active, telomerase was either inhibited or stimulated, and the authors propose that post-translational modifications that modulate the helicase activity of either one, such as already shown for ScPif1 [144,145], could thus regulate telomerase activity [143].
Broken chromosomes can be repaired by homologous recombination and non-homologous end-joining, or be healed by de novo telomere addition [146]. While de novo telomere additions at DNA-double-strand breaks (DSBs) are rare, their frequency and distribution are increased in the absence of ScPif1, but not in the absence of Rrm3 [27,30,105,146]. The drastic increase of de novo telomere additions in the pif1 mutant is significantly reduced by RRM3 deletion, emphasizing their differing modes of action at telomeres and in DSB repair pathways [27].

Depending on where in the genome a DSB forms, long-range resection can lead to the exposure of single-stranded DNA sequences that resemble G-rich telomere-like repeats [147]. Based on its mechanism at telomeres, ScPif1 most likely inhibits de novo telomere additions at DSBs by preventing telomerase from binding to these repeats. Indeed, ScPif1 localizes to DNA damage foci at DSBs, indicating a direct role in their DNA repair. The Mec1/Rad53 signaling pathway has been identified to regulate de novo telomere addition at DSBs, with phosphorylated ScPif1 ensuring telomerase removal and phosphorylated Cdc13 inhibiting its recruitment [91,144]. However, how ScPif1 is recruited to DSBs is unknown. In addition to phosphorylation specifically in response to DSBs, a basal level of ScPif1 phosphorylation is observed that is independent of the DNA damage signaling pathway [144], but its source and functional significance are unclear.

ScPif1 overexpression causes accumulation of ssDNA and activates the Rad53-dependent DNA-damage checkpoint. Excessive removal of telomerase, such as by ScPif1, leads to C-strand degradation in an Exo1-dependent manner [78,148]. Thus, in addition to regulating activity by post-translational modification, expression levels of ScPif1 are tightly regulated, with low levels in G1/S phase and high levels in late S/G2 [78]. In contrast, overexpression of Pfh1 in S. pombe leads to longer telomeres and therefore is considered a positive regulator of telomere length and replication [32].

6. Okazaki Fragment Maturation

In S. cerevisiae, Okazaki fragments are initiated by DNA polymerase α/primase, which generates 10-nucleotide long RNA primers followed by 10-20 nucleotides of DNA [149]. DNA polymerase δ extends this substrate until it reaches the 5' end of the previous Okazaki fragment. A flap is generated during the removal of the RNA primer, which is ultimately removed by the flap endonuclease Fen1/Rad27, leading to a gap that is resynthesized and sealed by DNA ligase I [150,151]. However, in vitro longer flaps of 20-30 nucleotides also form, which cannot be processed by Fen1/Rad27 [152]. These longer flaps are coated by replication protein A, which inhibits Fen1/Rad27 nuclease activity but promotes Dna2 nuclease activity, which shortens the flaps [153–155]. After shortening, Fen1/Rad27 processes the final step to generate a nick, which is sealed by DNA ligase I [156]. A role for ScPif1 in Okazaki maturation has been proposed, as disruption of ScPif1 nuclear function, but not RRM3 deletion, suppresses defects caused by dna2-1 and dna2-2 mutations as well as negative genetic interactions of DNA2, but not those with genes that have roles in Okazaki fragment processing [48]. Based on the genetic interaction between PIF1 and DNA2, Budd et al. [48] propose that ScPif1 stimulates flap formation and thereby enforces the two-nuclease pathway of Okazaki fragment maturation that requires Dna2 and Fen1/Rad27. Thus, ScPif1 functions as a backup in Okazaki fragment maturation to shorten flaps when they escape from Fen1/Rad27. Besides ScPif1, Pol32 can also generate the long flaps for processing by Dna2 [157].

ScPif1 is also able to process excessive flaps that have folded back on themselves and cannot be processed by endonucleases [158]. These fold-back flaps possess a 5’ end that can be captured by the 5’-3’ helicase ScPif1 and unwound, including the DNA:RNA hybrid at the downstream primer [158]. This ScPif1 function is further supported by biochemical analysis of ScPif1’s helicase activity, which unwinds duplex DNA as efficiently as ssRNA-DNA flaps and is stimulated if a 5’ ssDNA substrate of at least 10 nucleotides is available [49]. It is also conceivable that flaps contain more complex secondary DNA structures, such as G-quadruplexes (G4 structures), that can be resolved by ScPif1. S. pombe Pfh1 and Rrm3 have also been implicated in Okazaki fragment maturation. Pfh1 functionally interacts with several Okazaki fragment maturation factors and, like ScPif1, has been implicated in flap processing, whereas Rrm3 appears to promote Pol δ processivity [36,49].
7. Resolution of G4 structures

A decade ago, ScPif1 was first shown to actively resolve G4 structures and prevent genome instability at repeats capable of forming G4 structures [159]. Besides association with DSBs and highly transcribed genes, ScPif1 is found at approximately 25% of G4 structures within the yeast genome, probably due to its preference for unwinding the rarer antiparallel G4s [39,78,160,161]. Depending on their stability, ScPif1 can unwind G4 structures in an ATP-dependent or ATP-independent manner. These preferences of ScPif1 suggest that other DNA helicases, such as those of the RecQ family, typically unwind the other, more common types of G4 structures [161–164].

Sub1, a co-transcriptional activator and suppressor of genome instability caused by transcription-induced negative helical torsion, and Mms1, a ubiquitin ligase component, are found at G4 structures where they may affect ScPif1 activity [119,165]. In particular Mms1, which preferentially binds lagging strand G4 structures, was shown to enhance ScPif1 binding at some G4s [165]. ScPif1’s role in resolving G4 structures likely extends to the mitochondrial genome, which has greater density of G4-motifs than the nuclear genome [39,166,167].

S. pombe Pfh1 is also found at G4-motifs and its depletion causes fork pausing, DNA damage, phosphorylation of histone H2A, and genome rearrangements [41,47]. Interestingly, most of the bound G4-motifs were found on the transcribed strand of highly transcribed RNAPII-transcribed genes, which could act not only as a recruiting platform for Pfh1 to unwind G4 structures, but also to resolve RNA-DNA hybrids to maintain replication-transcription stability [47].

ScPif1 resolves G4 structures by translocating on ssDNA as a monomer in an ATP-dependent manner to unwind the G-quadruplex one strand at the time, then waits at the ss/ds DNA junction where it dimerizes, transforming the translocase into a potent DNA helicase capable of unwinding dsDNA [163,168–171]. Monomeric ScPif1 appears to “patrol” sequences at risk of forming secondary structures by remaining in their proximity to repeatedly resolve G4 structures and RNA:DNA hybrids [171]. Constant unwinding of G4 structures could prevent dimerization of ScPif1 and thereby, acting as a regulatory substrate, inhibit ScPif1’s helicase activity.

Notably, ScPif1 also possesses an annealing activity, which might anneal unwound G4 structures with their complementary strand to restore dsDNA and, thereby, inhibit G4 structure re-formation [160]. Though unresolved G4 structures act as barriers to DNA replication and can cause genome instability, sequences capable of adopting G4 structures are enriched at human origins of replication, gene promoters, telomeres, as well as in rDNA and the mitochondrial genome, suggestive of a functional role. Could ScPif1 regulate the formation of such functional G4 structures, using its annealing activity? Such regulation of G4 formation and G4 resolution could regulate expression of highly transcribed genes to avoid collisions with replication or repair machineries, establish replication fork barriers as an alternative to those formed by proteins, or modulate telomerase activity at telomeres.

Dahan et al. [12] have shown that ScPif1 is essential for replication through G4 structures on the lagging strand where its processivity is increased by its interaction with PCNA via its canonical PCNA-interacting protein (PIP) box. This interaction acts independently of the ScPif1-PCNA interaction during break-induced replication (BIR), which enhances DNA polymerase δ strand displacement synthesis via a non-canonical PIP sequence [12,52]. Thus, during unperturbed replication ScPif1 mostly resolves G4 structures on the lagging strand to suppress DNA breakage and maintain replication fork progression [39]. Resolving or repairing G4 structures in pif1 mutants is error-prone, leading to G4-motifs that can no longer form G-quadruplexes [39,172].

The recently published crystal structure of hPif1 shows a conserved ssDNA binding channel that is important for DNA unwinding, but not for G4-DNA binding or DNA annealing [45]. In fact, mutations within the ssDNA binding channel enhance annealing activity as they reduce helicase activity [45]. Although such mutagenesis studies are beginning to shed light on the relationship between the annealing and unwinding activities, the biological significance of the annealing activity of PIF1 family helicases, and that of other DNA helicases with this activity, such as the RecQ family helicases Sgs1 and BLM, remains enigmatic.
8. Cellular Response to Replication Fork Stalling in the Absence of Rrm3

Populations of rrm3Δ cells exhibit a cell cycle defect with a DNA content intermediate between 1N and 2N, indicative of problems with timely progression through S-phase [83]. Deletion of SRS2 or SGS1 enhances this S-phase progression defect and causes a severe fitness defect that can be rescued by disrupting HR genes RAD51 or RAD55, suggesting a role for Rrm3 either in preventing the formation of replication-dependent HR substrates or contributing to their repair [62,83,173]. In contrast, the synthetic lethality between deletions of RRM3 and MRE11 or RAD50, which code for subunits of the Mre11-Rad50-Xrs2 (MRX) DSB repair complex, is not due to illegitimate HR since it could not be rescued by RAD54 or RAD55 deletions [83]. This suggests that, in addition to HR, Rrm3 functions in another MRX-mediated pathway, such as non-homologous end joining, telomere maintenance or S-phase checkpoint activation.

How cells deal with HR substrates and other replication problems that arise from replication forks paused at thousands of protein-bound sites in Rrm3-deficient cells is poorly understood. Syed et al. [19] used Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC), coupled to mass spectrometry, to determine that topoisomerase Top2 and the SWI/SNF ATPases Rad5 and Rdh54 were significantly upregulated in cells lacking Rrm3, implicating their role in replication stress tolerance. Increased Top2 most likely compensates for the loss of Rrm3’s contribution to resolving converging replication forks [53]. Rdh54 is important for HR in diploid cells, but roles in haploid cells are also emerging. Notably, the recently identified role for Rdh54 in regulating D-loop formation [174], and thereby HR levels, could become increasingly important in the absence of Rrm3 when greater numbers of HR substrates are likely to form at replication pause sites. That Sgs1-Top3-Rmi1 and Srs2 define two independent pathways of D-loop reversal could contribute to the synthetic lethality of the rrm3Δ mutation with sgs1Δ and srs2Δ mutations as well as its suppression by RAD51 deletion [62,83,173,174]. Upregulation of Mph1, which also functions in D-loop disassembly [175], was also observed in the rrm3Δ mutant, albeit to a lesser extent than Top2, Rdh54 and Rad5, and further supports the increased requirement for tight regulation of D-loop formation in the absence of Rrm3 [19,175].

Rad5 has replication fork reversal activity [176,177] and its upregulation in the rrm3Δ mutant may indicate that fork reversal is a major mechanism to restart forks that are stalled at protein barriers [19]. Considering the association of Rrm3 with replisome components Polε and PCNA [9,106], one could also speculate that Rrm3 itself can facilitate fork reversal to allow the forks to pass through protein-bound sites in a pathway that functions in parallel to replication stress-induced Rad5-mediated fork reversal.

In the absence of Rrm3, the Rad53-dependent DNA-damage checkpoint is activated in a Rad9-dependent manner and remains active even after preventing the formation of HR intermediates by deleting RAD51 [62,83,178]. During replication stress, Rrm3 itself is phosphorylated in a Rad53-dependent manner; however, phosphorylation is not required for replication across natural pause sites [10]. The biological function of Rrm3 phosphorylation remains unknown, but has been suggested to inhibit Rrm3 activity to prevent genome instability during replication stress [10].

9. A Helicase-Independent Function of Rrm3 During Replication Stress

During replication stress, cells lacking Rrm3 continue to progress into S-phase. The ability of Rrm3 to restrict DNA synthesis depends on the integrity of the 230-amino-acid long disordered N-terminal tail of Rrm3, but not its ATPase/helicase activity [19]. Increased nucleotide levels are not sufficient for S-phase progression as helicase-dead rrm3 mutants also have increased dNTP levels, but do not progress into S-phase in hydroxyurea [19]. Notably, Rrm3 interacts with the origin recognition complex (ORC) subunit Orc5 and the region of the Rrm3 N-terminus required for inhibiting DNA synthesis during replication stress is required for the Rrm3-Orc5 interaction [19,110]. Moreover, the N-terminal tail of Rrm3 is required for its association with origins of replication during replication stress, but not during the unperturbed cell cycle, raising the possibility that Rrm3 acts at replication origins to restrict DNA synthesis during replication stress.
10. Protection of Mitochondrial DNA

The *ScPIF1* gene encodes two *ScPif1* isoforms that are translated from two different in-frame start codons. Mutating the second start codon leads to expression of the longer isoform, *ScPif1*-m2, which contains a mitochondrial signal sequence and localizes to the mitochondria [13,30]. *ScPif1* and its homologue in *S. pombe*, *Pfh1*, move with the replisome during mitochondrial (mt)DNA replication and require their catalytic activity for efficient mtDNA replication [33,179]. Replication fork blockages in mtDNA cannot be resolved by increased dNTPs, but rather require a direct involvement of *ScPif1* in fork progression [179]. Thus, while Rrm3 acts as a “sweepase” during nuclear replication, *ScPif1* might perform the same function on mitochondrial DNA.

Notably, *ScPIF1* deletion leads to thermosensitivity and loss of mtDNA, which led to the suggestion that lack of *ScPif1* association with the whole mitochondria genome leaves the DNA “naked” and unprotected [179]. However, the underlying mechanism for the temperature sensitivity remains unknown. Cellular levels of *ScPif1* drastically decrease in the absence of mtDNA, leading the authors to propose that either *ScPif1* is degraded in the mitochondria or the *ScPIF1* gene is strongly down-regulated by the mitochondrial retrograde signaling pathway [180,181]. Moreover, *ScPif1* and *Pfh1* appear to protect the mitochondrial genome from DSBs or facilitate their repair [33]. *ScPif1* prevents mtDNA mutagenesis from oxidative damage in cooperation with Ntg1 in a manner that is independent of recombination [182,183]. However, it remains to be determined how *ScPif1* prevents oxidative base damage. Finally, the main cause for the petite phenotype of *PIF1* deletion mutants remains ambiguous, but probably involves gross deletions and rearrangements of the mitochondrial genome.

While the importance of *ScPif1* for mitochondrial health in *S. cerevisiae* is firmly established, a mitochondrial role of Rrm3 is debated. A putative mitochondrial localization sequence has been proposed in Rrm3, and Rrm3 was identified in a screen for mitochondrially localized proteins in yeast, but not individually verified [29]. *RRM3* deletion causes an increase in mutagenesis of mtDNA [184] and suppresses mtDNA instability in the *pif1Δ* mutant by elevating the nucleotide pool through *Rad53* activation [184,185]. Thus, even though *ScPif1* and Rrm3 impact mtDNA integrity, they do so through different mechanisms and to a greatly different extent. This distinction is further highlighted by the finding that Rrm3 can alleviate the nuclear defects of a *PFH1* deletion, but not its mitochondrial defects [28].

11. Localization to Centromeres

Besides associating with tRNA genes, telomeres and origins of replication, Rrm3 and *ScPif1* were recently identified at centromeres [37]. This association is cell cycle regulated, with Rrm3 locating at centromeres from early to mid-S-phase and *ScPif1* in late S/G2 phase, indicative of different roles for each helicase at centromeres, and requires the helicase activity of Rrm3, but not that of *ScPif1* [37]. Curiously, in the absence of either Rrm3 or *ScPif1*, the remaining helicase appears to compensate by adopting the binding profile of the other. Furthermore, Rrm3’s centromere association, but not *ScPif1*’s, was stronger in *tof1* mutants, indicating a competitive relationship of Rrm3 and Tof1 for centromere affiliation [37]. Thus, even though both helicases are found at centromeres where they facilitate replication, their actions differ, with *ScPif1* playing a backup role in the absence of Rrm3 as was previously observed at tRNA genes [36].

12. DNA Break Repair

Potential roles for *ScPif1* in DNA repair, replication and recombination were indicated by its localization to DNA damage foci and preference for binding 3’ss/dsDNA junctions, which are DNA intermediates generated during these processes [171,186]. *ScPif1*’s function in BIR, which cells use for the repair of one-ended DSBs that can arise at eroded telomeres or when a replication fork encounters a single-strand nick in the template [187], is indicated by the increase in half cross-over products in the absence of *ScPif1* [52]. During BIR, *ScPif1* is found at and downstream of the site of strand invasion, which led to the suggestion that *ScPif1* is directly involved in D-loop migration [52,188].
Binding of ScPif1 to PCNA, which stimulates strand displacement DNA synthesis by DNA polymerase δ, also stimulates BIR [11]. Furthermore, ScPif1’s processivity is regulated by force on ssDNA [189]. During BIR, the movement of the D-loop generates a greater force on the ssDNA and thereby increases the unwinding activity of ScPif1 [189]. ScPif1’s role in BIR is regulated by Rad53-mediated phosphorylation [145]. Recruitment of ScPif1 to DNA damage sites is poorly understood, but seems to depend on its physical interaction with PCNA [55]. There, ScPif1 facilitates the formation of ssDNA behind the replication fork to initiate template switching [55].

Muñoz-Galván et al. [51] recently determined that Rrm3 contributes to the repair of replication-dependent DSBs. The repair of these DSBs by sister-chromatid-recombination was significantly reduced in Rrm3-deficient cells whereas repair of replication-independent DSBs, such as those induced by homothallic switching endonuclease HO, were not affected by Rrm3. In contrast to ScPif1, DSB repair by Rrm3 occurred by a sister chromatid exchange mechanism, not BIR [51,52]. Rrm3 also localizes to replication-dependent DSBs and, similar to ScPif1, this association could be facilitated by its PIP-box-mediated physical interaction with PCNA [9,51].

13. Regulation of Ty1 Transposition

Rrm3 was originally identified as a suppressor of Ty1 transposition [5,56]. Potential sources for increased frequency of retro-mobility are higher levels of transcription of Ty1 or enhanced complementary DNA (cDNA) synthesis. In rrm3 mutants multimeric Ty1 cDNA arrays form, possibly due to fork stalling at transposition-related RNA:DNA hybrids that cannot be resolved in the absence of Rrm3 [57]. Indeed, RNase H1 overexpression prevented Ty1 multimers. Increased replication stress caused by simultaneous depletion of Rrm3 and Tof1, a component of the fork protection complex, caused a dramatic increase in Ty1 transposition [190]. In addition to increased transposition, rrm3Δ mutants show increased integration at tRNA genes, highlighting Rrm3’s role not only in the suppression of Ty1 mobility and multimerization, but also in integration site selection [190].

Despite its major role in suppressing Ty1 and rDNA rearrangements, deletion of RRM3 does not have a major effect on the accumulation of spontaneous gross-chromosomal rearrangements (GCRs) [25,191]. Upon induction of replication stress by HU, however, the GCR rate of the rrm3Δ mutant increased 7-fold, indicative of a role at stalled or paused forks [19]. Rrm3 also strongly suppresses homologous-recombination-dependent GCRs in cells lacking the RecQ family helicase Sgs1, consistent with a recent report of a role of Rrm3 in DSB repair [25,51,191]. An overview of suppressors and promoters of GCRs in pif1 and rrm3 mutants is provided in Figure 3c.

14. Fragile Site Expression

Naturally occurring fragile sites, such as the replication slow zone (RSZ) in the yeast genome, can cause DSBs during unperturbed replication [192]. While deletion of RRM3 leads to fragility as a result of replication fork stalling at certain non-nucleosomal DNA-bound proteins, such as Fob1, it suppressed the expression of RSZ [18][178]. Rrm3 also reduced chromosome breakage at RSZs in a mec1 mutant by preventing DSB formation at RSZs [178]. In contrast to rrm3Δ-sensitive fragile sites that are caused by impeded replication forks encountering non-nucleosomal DNA-bound proteins, fragile site expression in RSZs is the result of depletion of the dNTP pool [178], suggesting that two types of fragile sites exist within the yeast genome: dNTP-sensitive (RSZ) and rrm3-sensitive (tRNA genes, RFB, rDNA, telomeres) [178].

15. Yeast as a Model System for the Functional Evaluation of hPif1 Mutations

Human Pif1 has been suggested to act as a tumor suppressor [193]. Multiple PIF1 variants that code for single amino changes of uncertain significance have been identified in cancer patients and L319P functionally evaluated in yeast. The completely conserved L319 is located in the helicase domain of hPif1 and mutation of its corresponding residue in Pfh1 was lethal, suggesting that it disrupts both nuclear and mitochondrial functions of Pfh1 and that L319P likely inactivates hPif1
[193]. Other hPif1 mutations from cancer genomes map near conserved helicase motifs (S223T) or affect other relatively conserved residues in the helicase domain (P357L, R592C), suggesting that they could also impair hPif1 function [193]. P109S, although located far upstream of the conserved helicase domain, also affects a completely conserved residue, but of unknown function [193]. The physical interaction between hPif1 and Brca1 in the resolution of G4 structures also supports a potential role for hPif1 in cancer suppression [194].

16. Concluding Remarks

Even though we know that PIF1 DNA helicases associate with over a thousand discrete sites in the yeast genome, including 274 tRNA genes and ~900 sites in the rDNA array, as well as replication origins, boundary elements, and sites of replication fork convergence, some of the underlying mechanisms by which helicases of the PIF1 family prevent fork stalling at these sites remain unknown [34–36,38,195]. Despite Rrm3’s and ScPif1’s common binding regions in the yeast genome, such as tRNA genes, rDNA locus, centromeres, and telomeres, their mode of action at those sites appears distinct. Their highly disordered N-terminal tails, which are not conserved at the amino acid level, may be responsible for this difference by recruiting distinct sets of genome maintenance factors and being subject to distinct post-translational modification. Indeed, the recent identification of Rrm3 functions that map to the N-terminal tail rather than the helicase domain, N-terminal phosphorylation sites that regulate the helicase activity, and distinct as well as shared N-terminal binding partners keep adding to the ever-expanding properties of the PIF1 helicase family and their roles in maintaining genome integrity in unperturbed and stressed cells [10,19,59]. In addition to further elucidating the regulatory function of the N-terminal tails of PIF1 family helicases, other puzzling observations still await explanations. What is so special about genome maintenance in some yeasts that it requires two PIF1 helicases when eukaryotes with more complex genomes, including humans, cope with one? What extra functions make S. pombe Pfh1 essential for survival? Are these functions performed by other proteins in other eukaryotes or are these functions not required? Besides providing detailed insights into the growing number of cellular functions and biochemical characteristics of the PIF1 helicase family, yeast can also serve as a powerful model system for the functional evaluation of hPif1, and potentially disease-associated hPif1 mutations.

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References

1. Boule, J.B.; Zakian, V.A. Roles of Pif1-Like Helicases in the Maintenance of Genomic Stability. *Nucleic Acids Res.* **2006**, *34*, 4147–4153.
2. Bochman, M.L.; Judge, C.P.; Zakian, V.A. The Pif1 Family in Prokaryotes: What Are Our Helicases Doing in Your Bacteria? *Mol. Biol. Cell* **2011**, *22*, 1955–1959.
3. Foury, F.; Kolodynski, J. Pif Mutation Blocks Recombination between Mitochondrial Rho+ and Rho- Genomes Having Tandemly Arrayed Repeat Units in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 5345–5349.
4. Bessler, J.B.; Zakian, V.A. The Pif1p Subfamily of Helicases: Region-Specific DNA Helicases? *Trends Cell Biol.* **2001**, *11*, 60–65.
5. Keil, R.L.; McWilliams, A.D. A Gene with Specific and Global Effects on Recombination of Sequences from Tandemly Repeated Genes in *Saccharomyces cerevisiae*. *Genetics* **1993**, *135*, 711–718.
6. Enemark, E.J.; Joshua-Tor, L. On Helicases and Other Motor Proteins. *Curr. Opin. Struct. Biol.* **2008**, *18*, 243–257.
7. Lohman, T.M.; Tomko, E.J.; Wu, C.G. Non-Hexameric DNA Helicases and Translocases: Mechanisms and Regulation. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 391–401.
8. Singleton, M.R.; Dillingham, M.S.; Wigley, D.B. Structure and Mechanism of Helicases and Nucleic Acid Translocases. *Annu. Rev. Biochem.* 2007, 76, 23-50.

9. Schmidt, K.H.; Derry, K.L.; Kolodner. *Saccharomyces cerevisiae* Rrm3, R.D.; a 5' to 3' DNA Helicase, Physically Interacts with Proliferating Cell Nuclear Antigen. *J. Biol. Chem.* 2002, 277, 45331-45337.

10. Rossi, S.E.; Ajazi, A.; Carotenuto, W.; Foiani, M.; Giannattasio, M. Rad53-Mediated Regulation of Rrm3 and Pif1 DNA Helicases Contributes to Prevention of Aberrant Fork Transitions under Replication Stress. *Cell Rep.* 2015, 13, 80-92.

11. Buzovetsky, O.; Kwon, Y.; Pham, T.N.; Kim, C.; Ira, G.; Sung, P.; Xiong, Y. Role of the Pif1-Pcn1 Complex in Pol α-Dependent Strand Displacement DNA Synthesis and Break-Induced Replication. *Cell Rep.* 2017, 21, 1707-1714.

12. Dahan, D.; Tsirkas, I.; Dvorat, D.; Sparks, M.A.; Singh, S.P.; Galletto, R.; Aharoni, A. Pif1 Is Essential for Efficient Replisome Progression Through Lagging Strand G-Quadruplex DNA Secondary Structures. *Nucleic Acids Res.* 2018, 46, 11847-11857.

13. Zhou, J.; Monson, E.K.; Teng, S.C.; Schulz, V.P.; Zakian, A.V. Pif1p Helicase, a Catalytic Inhibitor of Telomerase in Yeast. *Science* 2000, 289, 771-774.

14. Kazak, L.; Reyes, A.; Duncan, A.L.; Rorbach, J.; Wood, S.R.; Brea-Calvo, G.; Gammage, P.A.; Robinson, A.J.; Minczuk, M.; Holt, I.J. Alternative Translation Initiation Augments the Human Mitochondrial Proteome. *Nucleic Acids Res.* 2013, 41, 2354-2369.

15. Kobayashi, T.; and T. Horiuchi. A Yeast Gene Product, Fob1 Protein, Required for Both Replication Fork Blocking and Recombinational Hotspot Activities. *Genes Cells* 1996, 1, 465-474.

16. Ivesa, A.S.; Zhou, J.Q.; Zakian, A.V. The *Saccharomyces* Pif1p DNA Helicase and the Highly Related Rrm3p Have Opposite Effects on Replication Fork Progression in Ribosomal DNA. *Cell* 2000, 100, 479-489.

17. Sabouri, N.; McDonald, K.R.; Webb, C.J.; Cristea, I.M.; Zakian, A.V. DNA Replication through Hard-to-Replicate Sites, Including Both Highly Transcribed Rna Pol Ii and Pol Iii Genes, Requires the *S. Pombe* Pfh1 Helicase. *Genes Dev.* 2012, 26, 581-593.

18. Torres, J.Z.; Bessler, J.B.; Zakian, V.A. Local Chromatin Structure at the Ribosomal DNA Causes Replication Fork Pausing and Genome Instability in the Absence of the *S. cerevisiae* DNA Helicase Rrm3p. *Genes Dev.* 2004, 18, 498-503.

19. Syed, S.; Desler, C.; Rasmussen, J.L.; Schmidt, H.K. A Novel Rrm3 Function in Restricting DNA Replication Via an Orc5-Binding Domain Is Genetically Separable from Rrm3 Function as an ATPase/Helicase in Facilitating Fork Progression. *PLoS Genet.* 2016, 12, e1006451.

20. Mohanty, B.K.; Bairwa, N.K.; Bastia, D. The Tof1p-Csm3p Protein Complex Counteracts the Rrm3p Helicase to Control Replication Termination of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 2006, 103, 897-902.

21. Crickard, J.B.; Xue, C.; Wang, W.; Kwon, Y.; Sung, P.; Greene, E.C. The Recq Helicase Sgs1 Drives ATP-Dependent Disruption of Rad51 Filaments. *Nucleic Acids Res.* 2019, 47, 4694-4706.

22. Kreici, L.; Van Komen, S.; Li, Y.; Villemaim, J.; Reddy, M.S.; Klein, H.; Ellenberger, T.; Sung, P. DNA Helicase Srs2 Disrupts the Rad51 Presynaptic Filament. *Nature* 2003, 423, 305-309.

23. Calzada, A.; Hodgson, B.; Kanemaki, M.; Bueno, A.; Labib, K. Molecular Anatomy and Regulation of a Stable Replisome Eukaryotic DNA at a Paused Replication Fork. *Genes Dev.* 2005, 19, 1905-1919.

24. Osborn, A.J.; and J. Elledge. Mrc1 Is a Replication Fork Component Whose Phosphorylation in Response to DNA Replication Stress Activates Rad53. *Genes Dev.* 2003, 17, 1755-1767.

25. Schmidt, K.H.; and R. D. Kolodner. Suppression of Spontaneous Genome Rearrangements in Yeast DNA Helicase Mutants. *Proc. Natl. Acad. Sci. USA* 2006, 103, 18196-18201.

26. Szyjka, S.J.; Viggiani, C.J.; Aparicio, O.M. Mrc1 Is Required for Normal Progression of Replication Forks Throughout Chromatin in *S. cerevisiae*. *Mol. Cell* 2005, 19, 691-697.

27. Ivesa, A.S.; Zhou, J.Q.; Schulz, V.P.; Monson, E.K.; Zakian, A.V. *Saccharomyces* Rrm3p, a 5' to 3' DNA Helicase That Promotes Replication Fork Progression through Telomeric and Subtelomeric DNA. *Genes Dev.* 2002, 16, 1383-1396.

28. Pinter, S.F.; Aubert, S.D.; Zakian, A.V. The *Schizosaccharomyces* Pombe Pfh1p DNA Helicase Is Essential for the Maintenance of Nuclear and Mitochondrial DNA. *Mol. Cell. Biol.* 2008, 28, 6594-6608.

29. Prokisch, H.; Scharfe, C.; Camp, D.G. Integrative Analysis of the Mitochondrial Proteome in Yeast. *PLoS Biol.* 2004, 2, e160.
30. Schulz, V.P.; and V. A. Zakian. The Saccharomyces Pif1 DNA Helicase Inhibits Telomere Elongation and De Novo Telomere Formation. *Cell* 1994, 76, 145–155.

31. Boule, J.B.; Vega, L.R.; Zakian, A.V. The Yeast Pif1p Helicase Removes Telomerase from Telomeric DNA. *Nature* 2005, 438, 57–61.

32. McDonald, K.R.; Sabouri, N.; Webb, J.C.; Zakian, A.V. The Pif1 Family Helicase Pfh1 Facilitates Telomere Replication and Has an Rpa-Dependent Role During Telomere Lengthening. *DNA Repair* 2014, 24, 80–86.

33. Cheng, X.; Dunaway, S.; Ivessa, S.A. The Role of Pif1p, a DNA Helicase in *Saccharomyces cerevisiae*, in Maintaining Mitochondrial DNA. *Mitochondrion* 2007, 7, 211–222.

34. Ivessa, A.S.; Lenzmeier, B.A.; Bessler, J.B.; Goudsouzian, L.K.; Schnakenberg, S.L.; Zakian, A.V. The *Saccharomyces cerevisiae* Helicase Rrm3p Facilitates Replication Past Nonhistone Protein-DNA Complexes. *Mol. Cell* 2003, 12, 1525–1536.

35. Tran, P.L.T.; Pohl, T.J.; Chen, C.F.; Chan, A.; Pott, S.; Zakian, A.V. Pif1 Family DNA Helicases Suppress R-Loop Mediated Genome Instability at Trna Genes. *Nat. Commun.* 2017, 8, 15025.

36. Osmundson, J.S.; Kumar, J.; Yeung, R.; Smith, J.D. Pif1-Family Helicases Cooperatively Suppress Widespread Replication-Fork Arrest at tRNA Genes. *Nat. Struct. Mol. Biol.* 2017, 24, 162–U06.

37. Chen, C.F.; Pohl, T.J.; Pott, S.; Zakian, A.V. Two Pif1 Family DNA Helicases Cooperate in Centromere Replication and Segregation in *Saccharomyces cerevisiae*. *Genetics* 2019, 211, 105–119.

38. Azvolinsky, A.; Giresi, P.G.; Lieb, J.D.; Zakian, A.V. Highly Transcribed Rna Polymerase II Genes Are Impediments to Replication Fork Progression in *Saccharomyces cerevisiae*. *Mol. Cell* 2009, 34, 722–734.

39. Paeschke, K.; Capra, J.A.; Zakian, A.V. DNA Replication through G-Quadruplex Motifs Is Promoted by the *Saccharomyces cerevisiae* Pif1 DNA Helicase. *Cell* 2011, 145, 678–691.

40. McDonald, K.R.; Amanda, J.; Guise, Pourbozorgi-Langrouri, P.; Cristea, I.M.; Zakian, V.A.; Capra, J.A.; Sabouri, N. Pfh1 Is an Accessory Replicative Helicase That Interacts with the Replisome to Facilitate Fork Progression and Preserve Genome Integrity. *PLoS Genet.* 2016, 12, e1006238.

41. Wallgren, M.; Mohammad, J.B.; Yan, K.P.; Pourbozorgi-Langrouri, P.; Ebrahimi, M.; Sabouri, N. G-Rich Telomeric and Ribosomal DNA Sequences from the Fission Yeast Genome Form Stable G-Quadruplex DNA Structures in Vitro and Are Unwound by the Pfh1 DNA Helicase. *Nucleic Acids Res.* 2016, 44, 6213–6231.

42. Bedard, L.G.; Dronamraju, R.; Kerschner, L.J.; Hunter, O.G.; Axley, D.E.; Boyd, K.A.; Strahl, D.B.; Mosley, L.A. Quantitative Analysis of Dynamic Protein Interactions During Transcription Reveals a Role for Casein Kinase II in Polymerase-Associated Factor (Paf) Complex Phosphorylation and Regulation of Histone H2b Monoubiquitylation. *J. Biol. Chem.* 2016, 291, 13410–13420.

43. Ohyu, T.; Arai, H.; Kubota, Y.; Shinagawa, H.; Hishida, T. A Sumo-Like Domain Protein, Esc2, Is Required for Genome Integrity and Sister Chromatid Cohesion in *Saccharomyces cerevisiae*. *Genetics* 2008, 180, 41–50.

44. Ouenzar, F.; Lalonde, M.; Laprade, H.; Morin, G.; Gallardo, F.; Tremblay-Belzile, S.; Chartrand, P. Cell Cycle-Dependent Spatial Segregation of Telomerase from Sites of DNA Damage. *J. Cell Biol.* 2017, 216, 2355–2371.

45. Dehghani-Tafti, S.; Levdiyov, V.; Antson, A.A.; Bax, B.; Sanders, C.M. Structural and Functional Analysis of the Nucleotide and DNA Binding Activities of the Human Pif1 Helicase. *Nucleic Acids Res.* 2019, 47, 3208–3222.

46. Lopes, J.; Piazza, A.; Bermejo, R.; Kriegsman, B.; Colosio, A.; Teulade-Fichou, M.P.; Foiani, M.; Nicolas, A. G-Quadruplex-Induced Instability During Leading-Strand Replication. *EMBO J.* 2011, 30, 4033–4046.

47. Sabouri, N.; Capra, J.A.; Zakian, A.V. The Essential Schizosaccharomyces Pombe Pfh1 DNA Helicase Promotes Fork Movement Past G-Quadruplex Motifs to Prevent DNA Damage. *BMC Biol.* 2014, 12, 101.

48. Budd, M.E.; Reis, C.C.; Smith, S.; Myung, K.; Campbell, L.J. Evidence Suggesting That Pif1 Helicase Functions in DNA Replication with the Dna2 Helicase/Nuclease and DNA Polymerase δ. *Mol. Cell Biol.* 2006, 26, 2490–2500.

49. Ryu, G.H.; Tanaka, H.; Kim, H.D.; Kim, H.J.; Bae, H.S.; Kwon, N.Y.; Rhee, S.J.; MacNeill, A.S.; Seo, S.Y. Genetic and Biochemical Analyses of Pfh1 DNA Helicase Function in Fission Yeast. *Nucleic Acids Res.* 2004, 32, 4205–4216.

50. Hiraga, S.; Botsios, S.; Donaldson, D.A. Histone H3 Lysine 56 Acetylation by Rtt109 Is Crucial for Chromosome Positioning. *J. Cell Biol.* 2008, 183, 641–651.
51. Munoz-Galvan, S.; Garcia-Rubio, M.; Ortega, P.; Ruiz, F.J.; Jimeno, S.; Pardo, B.; Gomez-Gonzalez, B.; Aguilera, A. A New Role for Rrm3 in Repair of Replication-Born DNA Breakage by Sister Chromatid Recombination. *PLoS Genet.* 2017, 13, e1006781.
52. Wilson, M.A.; Kwon, Y.; Xu, Y.; Chung, H.W.; Chi, P.; Niu, H.; Mayle, R.; Chen, X.; Malkova, A.; Sung, P.; et al. Pif1 Helicase and Pol δ Promote Recombination-Coupled DNA Synthesis Via Bubble Migration. *Nature* 2013, 502, 393–396.
53. Deegan, T.D.; Baxter, J.; Bazán, M.A.O.; Yeeles, J.T.; Labib, K.P. Pif1-Family Helicases Support Fork Convergence During DNA Replication Termination in Eukaryotes. *Mol. Cell* 2019, 74, 231–244.
54. Steinacher, R.; Osman, F.; Dalgaard, J.Z.; Lorenz, A.; Whitby, M.C. The DNA Helicase Pfh1 Promotes Fork Merging at Replication Termination Sites to Ensure Genome Stability. *Genes Dev.* 2012, 26, 594–602.
55. Garcia-Rodriguez, N.; Wong, R.P.; Ulrich, D.H. The Helicase Pif1 Functions in the Template Switching Pathway of DNA Damage Bypass. *Nucleic Acids Res.* 2018, 46, 8347–8356.
56. Scholes, D.T.; Banerjee, M.; Bowen, B.; Curcio, J.M. Multiple Regulators of Ty1 Transposition in *Saccharomyces cerevisiae* Have Conserved Roles in Genome Maintenance. *Genetics* 2001, 159, 1449–1465.
57. Stamenova, R.; Maxwell, P.H.; Kenny, A.E.; Curcio, J.M. Rrm3 Protects the *Saccharomyces cerevisiae* Genome from Instability at Nascent Sites of Retrotransposition. *Genetics* 2009, 182, 711–723.
58. Branzei, D.; Sollier, J.; Liberi, G.; Zhao, X.; Maeda, D.; Seki, M.; Enomoto, T.; Ohta, K.; Foiani, M. Ubc9- and Mms21-Mediated Sumoylation Counteracts Recombinogenic Events at Damaged Replication Forks. *Cell* 2006, 127, 509–522.
59. Bessler, J.B.; and V. A. Zakian. The Amino Terminus of the *Saccharomyces cerevisiae* DNA Helicase Rrm3p Modulates Protein Function Altering Replication and Checkpoint Activity. *Genetics* 2004, 168, 1205–1218.
60. Xu, H.; Boone, C.; Klein, L.H. Mrcl1 Is Required for Sister Chromatid Cohesion to Aid in Recombination Repair of Spontaneous Damage. *Mol. Cell Biol.* 2004, 24, 7082–7090.
61. Wei Tao, T.; Budd, M.; Hoopes, L.L.; Campbell, L.J. Dna2 Helicase/Nuclease Causes Replicative Fork Stalling and Double-Strand Breaks in the Ribosomal DNA of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 2003, 278, 22513–22522.
62. Torres, J.Z.; Schnakenberg, S.L.; Zakian, V.A. *Saccharomyces cerevisiae* Rrm3p DNA Helicase Promotes Genome Integrity by Preventing Replication Fork Stalling: Viability of Rrm3 Cells Requires the Intra-S-Phase Checkpoint and Fork Restart Activities. *Mol. Cell Biol.* 2004, 24, 3198–3212.
63. Tong, A.H.; Evangelista, M.; Parsons, B.A.; Xu, H.; Bader, G.D.; Page, N.; Robinson, M.; Raghibizadeh, S.; Hogue, C.W.; Bussey, H.; et al. Systematic Genetic Analysis with Ordered Arrays of Yeast Deletion Mutants. *Science* 2001, 294, 2364–2368.
64. Chang, M.; Bellaoui, M.; Zhang, C.; Desai, R.; Morozov, P.; Delgado-Cruzata, L.; Rothstein, R.; Freyer, G.A.; Boone, C.; Brown, G.W. Rmi1/Nce4, a Suppressor of Genome Instability, Encodes a Member of the Recq Helicase/Topo Ii Complex. *EMBO J.* 2005, 24, 2024–2033.
65. Keogh, M.C.; Kim, J.A.; Downey, M.; Fillingham, J.; Chowdhury, D.; Harrison, J.C.; Onishi, M.; Datta, N.; Galicia, S.; Emili, A.; et al. A Phosphatase Complex That Dephosphorylates GammaH2ax Regulates DNA Damage Checkpoint Recovery. *Nature* 2006, 439, 497–501.
66. Luciano, P.; Dehe, P.M.; Audebert, S.; Géli, V.; Corda, Y. Replisome Function During Replicative Stress Is Modulated by Histone H3 Lysine 56 Acetylation through Ctf4. *Genetics* 2015, 199, 1047–1063.
67. Mirzaei, H.; Syed, S.; Kennedy, J.; Schmidt, H.K. Sgs1 Truncations Induce Genome Rearrangements but Suppress Detrimental Effects of Blm Overexpression in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 2011, 405, 877–891.
68. Morohashi, H.; Maculinis, T.; Labib, K. The Amino-Terminal Tpr Domain of Dia2 Tethers Scf(Dia2) to the Replisome Progression Complex. *Curr. Biol.* 2009, 19, 1943–1949.
69. Pan, X.; Ye, P.; Yuan, S.D.; Wang, X.; Bader, J.S.; Boeke, J.D. A DNA Integrity Network in the Yeast *Saccharomyces cerevisiae*. *Cell* 2006, 124, 1069–1081.
70. Putnam, C.D.; Hayes, T.K.; Kolodner, D.R. Post-Replication Repair Suppresses Duplication-Mediated Genome Instability. *PLoS Genet.* 2010, 6, e1000933.
71. Sacher, M.; Pfänder, B.; Hoege, C.; Jentsch, S. Control of Rad52 Recombination Activity by Double-Strand Break-Induced Sumo Modification. *Nat. Cell Biol.* 2006, 8, 1284–1290.
72. Arora, S.; Deshpande, R.A.; Budd, M.; Campbell, J.; Revere, A.; Zhang, X.; Schmidt, K.H.; Paull, T.T. Genetic Separation of Sae2 Nuclease Activity from Mre11 Nuclease Functions in Budding Yeast. *Mol. Cell Biol.* 2017, 37.
73. Banerjee, S.; Smith, S.; Myung, K. Suppression of Gross Chromosomal Rearrangements by Yku70-Yku80 Heterodimer through DNA Damage Checkpoints. *Proc. Natl. Acad. Sci. USA* 2006, 103, 1816–1821.

74. Bonetti, D.; Villa, M.; Gobbin, E.; Cassani, C.; Tedeschi, G.; Longhese, M.P. Escape of Sgs1 from Rad9 Inhibition Reduces the Requirement for Sac2 and Functional Mrx in DNA End Resection. *EMBO Rep.* 2015, 16, 351–361.

75. Dewar, J.M.; and D. Lydall. Pif1- and Exo1-Dependent Nucleases Coordinate Checkpoint Activation Following Telomere Uncapping. *EMBO J.* 2010, 29, 4020–4034.

76. Garbacz, M.A.; Lujan, S.A.; Burkholder, A.B.; Cox, P.B.; Wu, Q.; Zhou, X.Z.; Haber, E.J.; Kunkel, A.T. Evidence That DNA Polymerase δ Contributes to Initiating Leading Strand DNA Replication in *Saccharomyces cerevisiae*. *Nat. Commun.* 2018, 9, 858.

77. Stundon, J.L.; and V. A. Zakian. Identification of *Saccharomyces cerevisiae* Genes Whose Deletion Causes Synthetic Effects in Cells with Reduced Levels of the Nuclear Pif1 DNA Helicase. *G3 Genes Genomes Genet.* 2015, 5, 2913–2918.

78. Vega, L.R.; Phillips, J.A.; Thornton, B.R.; Benanti, J.A.; Toczyski, D.P.; Zakian, A.V. Sensitivity of Yeast Strains with Long G-Tail s to Levels of Telomere-Bound Telomerase. *PLoS Genet.* 2007, 3, e105.

79. Wagner, M.; Price, G.; Rothstein, R. The Absence of Top3 Reveals an Interaction between the Sgs1 and Pif1 DNA Helicases in *Saccharomyces cerevisiae*. *Genetics* 2006, 174, 555–573.

80. Gibson, D.G.; Aparicio, J.G.; Hu, F.; Aparicio, M.O. Diminished S-Phase Cyclin-Dependent Kinase Function Elicits Vital Rad53-Dependent Checkpoint Responses in *Saccharomyces cerevisiae*. *EMBO J.* 2013, 32, 3213–3226.

81. Menolfi, D.; Delamarre, A.; Lengronne, A.; Pasero, P.; Branzei, D. Essential Roles of the Smc5/6 Complex in Replication through Natural Pausing Sites and Endogenous DNA Damage Tolerance. *Mol. Cell* 2015, 60, 835–846.

82. Ciftci-Yilmaz, S.; Au, W.C.; Mishra, P.K.; Eisenstatt, J.R.; Chang, J.; Dawson, R.A.; Zhu, I.; Rahman, M.; Bilke, S.; Costanzo, M.; et al. A Genome-Wide Screen Reveals a Role for the Hir Histone Chaperone Complex in Preventing Mislocalization of Budding Yeast Cenp-A. *Genetics* 2018, 210, 203–218.

83. Schmidt, K.H.; and R. D. Kolodner. Requirement of Rrm3 Helicase for Repair of Spontaneous DNA Lesions in Cells Lacking Srs2 or Sgs1 Helicase. *Mol. Cell Biol.* 2004, 24, 3213–3226.

84. Reid, R.J.; Gonzalez-Barrera, S.; Sunjenvicar, I.; Alvaro, D.; Ciccone, S.; Wagner, M.; Rothstein, R. Selective Ploidy Ablation, a High-Throughput Plasmid Transfer Protocol, Identifies New Genes Affecting Topoisomerase I-Induced DNA Damage. *Genome Res.* 2011, 21, 477–486.

85. Srikumar, T.; Lewicki, M.C.; Costanzo, M.; Tkach, M.J.; H. van Bakel, K.; Tsui, E.S.; Johnson, G.W.; Brown, B.J.; Andrews, C.; Boone, G.; et al. Global Analysis of Sumo Chain Function Reveals Multiple Roles in Chromatin Regulation. *J. Cell Biol.* 2013, 201, 145–163.

86. Addinall, S.G.; Holstein, E.M.; Lawless, C.; Yu, M.; Chapman, K.; Banks, A.P.; Ngo, H.P.; Maringele, L.; Taschuk, M.; Young, A.; et al. Quantitative Fitness Analysis Shows That Nmd Proteins and Many Other Protein Complexes Suppress or Enhance Distinct Telomere Cap Defects. *PLoS Genet.* 2011, 7, e1001362.

87. Lin, Y.Y.; Qi, Y.; Lu, Y.J.; Pan, X.; Yuan, D.S.; Zhao, Y.; Bader, J.S.; Boeke, J.D. A Comprehensive Synthetic Interaction Network Governing Yeast Histone Acetylation and Deacetylation. *Genes Dev.* 2008, 22, 2062–2074.

88. Moriel-Carretero, M.; and A. Aguilera. A Postincision-Deficient Tfiih Causes Replication Fork Breakage and Uncovers Alternative Rad51- or Pol32-Mediated Restart Mechanisms. *Mol. Cell* 2010, 37, 690–701.

89. Osman, C.; Haag, M.; Potting, C.; Rodenfels, J.; Dip, P.V.; Wieland, F.T.; Brugger, B.; Westermann, B.; Langer, T. The Genetic Interactome of Prohibitins: Coordinated Control of Cardiolipin and Phosphatidylethanolamine by Conserved Regulators in Mitochondria. *J. Cell Biol.* 2009, 184, 583–596.

90. Ye, P.; Peyser, B.D.; Pan, X.; Boeke, D.J.; Spencer, A.F.; Bader, S.J. Gene Function Prediction from Congruent Synthetic Lethal Interactions in Yeast. *Mol. Syst. Biol.* 2005, 1, 2005.0026.

91. Zhang, W.; and D. Durocher. De Novo Telomere Formation Is Suppressed by the Mec1-Dependent Inhibition of Dccl3 Accumulation at DNA Breaks. *Genes Dev.* 2010, 24, 502–515.

92. Audry, J.; Maestroni, L.; Delagoutte, E.; Gauthier, T.; Nakamura, T.M.; Gachet, Y.; Saintome, C.; Geli, V.; Coulon, S. Rpa Prevents G-Rich Structure Formation at Lagging-Strand Telomeres to Allow Maintenance of Chromosome Ends. *EMBO J.* 2015, 34, 1942–1958.
93. Chen, Y.B.; Yang, C.P.; Li, R.X.; Zeng, R.; Zhou, Q.J. Def1p Is Involved in Telomere Maintenance in Budding Yeast. *J. Biol. Chem.* 2005, 280, 24784–24791.

94. Meng, F.L.; Hu, Y.; Shen, N.; Tong, J.X.; Wang, J.; Ding, J.; Zhou, J.Q. Sua5p a Single-Stranded Telomeric DNA-Binding Protein Facilitates Telomere Replication. *EMBO J.* 2009, 28, 1466–1478.

95. Bohman, M.L.; Paeschke, K.; Chan, A.; Zakian, A.V. Hrq1, a Homolog of the Human Recq4 Helicase, Acts Catalytically and Structurally to Promote Genome Integrity. *Cell Rep.* 2014, 6, 346–356.

96. Ji, H.; Adkins, C.J.; Cartwright, B.R.; Friedman, L.K. Yeast Est2p Affects Telomere Length by Influencing Association of Rap1p with Telomeric Chromatin. *Mol. Cell Biol.* 2008, 28, 2380–2390.

97. Hwang, J.Y.; Smith, S.; Myung, K. The Rad1-Rad10 Complex Promotes the Production of Gross Chromosomal Rearrangements from Spontaneous DNA Damage in *Saccharomyces cerevisiae*. *Genetics* 2005, 169, 1927–1937.

98. Myung, K.; Smith, S.; Kolodner, D.R. Mitotic Checkpoint Function in the Formation of Gross Chromosomal Rearrangements in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 2004, 101, 15980–15985.

99. Nene, R.V.; Putnam, C.D.; Li, B.Z.; Nguyen, K.G.; Srivatsan, A.; Campbell, S.C.; Desai, A.; Kolodner, R.D. Cdc73 Suppresses Genome Instability by Mediating Telomere Homeostasis. *PLoS Genet.* 2018, 14, e1007170.

100. Banerjee, S.; Smith, S.; Oum, H.J.; Liaw, J.H.; Hwang, Y.J.; Sikdar, N.; Motegi, A.; Lee, S.E.; Myung, K. Mph1p Promotes Gross Chromosomal Rearrangement through Partial Inhibition of Homologous Recombination. *J. Cell Biol.* 2008, 181, 1083–1093.

101. Liang, J.; Li, B.Z.; Tan, A.P.; Kolodner, C.D.; Zhou, H. Sumo E3 Ligase Mms21 Prevents Spontaneous DNA Damage Induced Genome Rearrangements. *PLoS Genet.* 2018, 14, e1007250.

102. Putnam, C.D.; Pallis, K.; Hayes, K.T.; Kolodner, D.R. DNA Repair Pathway Selection Caused by Defects in Tel1, Sae2, De Novo Telomere Addition Generates Specific Chromosomal Rearrangement Signatures. *PLoS Genet.* 2014, 10, e1004277.

103. Sakofsky, C.J.; Ayyar, S.; Deem, K.A.; Chung, H.W.; Ira, G.; Malkova, A. Translesion Polymerases Drive Microhomology-Mediated Break-Induced Replication Leading to Complex Chromosomal Rearrangements. *Mol. Cell* 2015, 60, 860–872.

104. Smith, S.; Gupta, A.; Kolodner, D.R.; Myung, K. Suppression of Gross Chromosomal Rearrangements by the Multiple Functions of the Mre11-Rad50-Xrs2 Complex in *Saccharomyces cerevisiae*. *DNA Repair* 2005, 4, 606–617.

105. Myung, K.; Chen, C.; Kolodner, D.R. Multiple Pathways Cooperate in the Suppression of Genome Instability in *Saccharomyces cerevisiae*. *Nature* 2001, 411, 1073–1076.

106. Azvolinsky, A.; Dunaway, S.; Torres, Z.J.; Bessler, B.J.; Zakian, A.V. The *S. cerevisiae* Rrm3p DNA Helicase Moves with the Replication Fork and Affects Replication of All Yeast Chromosomes. *Genes Dev.* 2006, 20, 3104–3116.

107. Balakirev, M.Y.; Mullally, J.E.; Favier, A.; Assard, N.; Sulpice, E.; Lindsey, D.F.; Rulina, A.V.; Girola, X.; Wilkinson, K.D. Wss1 Metalloprotease Partners with Cdc48/Doa1 in Processing Genotoxic Sumo Conjugates. *Elife* 2015, 4, e06763.

108. Buser, R.; Kellner, V.; Melnik, A.; Wilson-Zbinden, C.; Schellhaas, R.; Kastner, L.; Piwko, W.; Dees, M.; Picotti, P.; Maric, M.; et al. The Replisome-Coupled E3 Ubiquitin Ligase Rtt101mms22 Counteracts Mrc1 Function to Tolerate Genotoxic Stress. *PLoS Genet.* 2016, 12, e1005843.

109. Kurat, C.F.; Lambert, J.P.; Petschnigg, J.; Friesen, H.; Kawson, T.; Rosebrock, A.; Gingras, A.C.; Fillingham, J.; Andrews, B. Cell Cycle-Regulated Oscillator Coordinates Core Histone Gene Transcription through Histone Acetylation. *Proc. Natl. Acad. Sci. USA* 2014, 111, 14124–14129.

110. Matsuda, K.; Makise, M.; Sueyasu, Y.; Takehara, M.; Asano, T.; Mizushima, T. Yeast Two-Hybrid Analysis of the Origin Recognition Complex of *Saccharomyces cerevisiae* Interaction between Subunits and Identification of Binding Proteins. *FEBS Yeast Res.* 2007, 7, 1263–1269.

111. Miller, J.E.; Zhang, L.; Jiang, H.; Li, Y.; Pugh, B.F.; Reese, J.C. Genome-Wide Mapping of Decay Factor-mRNA Interactions in Yeast Identifies Nutrient-Responsive Transcripts as Targets of the Deadenylase Ccr4. *G3 Genes Genomes Genet.* 2018, 8, 315–330.

112. Oliete-Calvo, P.; Serrano-Quilez, J.; Nuno-Cabanes, C.; Perez-Martinez, E.M.; Soares, M.L.; Dichtl, B.; Buratowski, S.; Perez-Ortin, J.E.; Rodriguez-Navarro, S. A Role for Mog1 in H2bub1 and H3k4me3 Regulation Affecting RNA Pol II Transcription and mRNA Export. *EMBO Rep.* 2018, 19, e45992.

113. Ptacek, J.; Devgan, G.; Michaud, G.; Zhu, H.; Zhu, X.; Fasolo, J.; Guo, H.; Jona, G.; Breitkreutz, A.; Sopko, R.; et al. Global Analysis of Protein Phosphorylation in Yeast. *Nature* 2005, 438, 679–684.
114. Willmund, F.; M. del Alamo, S.; Pechmann, T.; Chen, V.; Albanese, E.B.; Dammer, J.; Peng, J. Frydman. The Cotranslational Function of Ribosome-Associated Hsp70 in Eukaryotic Protein Homeostasis. Cell 2013, 152, 196–209.

115. Telekawa, C.; Boisvert, F.M.; Bachand, F. Proteomic Profiling and Functional Characterization of Post-Translational Modifications of the Fission Yeast Rna Exosome. Nucleic Acids Res. 2018, 46, 11169–11183.

116. Zamir, L.; Zaretsky, M.; Fridman, Y.; Ner-Gaon, H.; Rubin, E.; Aharoni, A. Tight Coevolution of Proliferating Cell Nuclear Antigen (Pcna)-Partner Interaction Networks in Fungi Leads to Inseparable Network Incompatibility. Proc. Natl. Acad. Sci. USA 2012, 109, E406–E414.

117. She, R.; Chakravarty, A.K.; Layton, C.J.; Chircus, L.M.; Andreason, J.O.; Damara, N.; McMahon, L.P.; Buenrostro, D.J.; Jarosz, F.D.; Greenleaf, J.W. Comprehensive and Quantitative Mapping of RNA-Protein Interactions across a Transcribed Eukaryotic Genome. Proc. Natl. Acad. Sci. USA 2017, 114, 3619–3624.

118. Ramanagoudr-Bhojappa, R.; Blair, L.P.; Tackett, A.J.; Raney, D.K. Physical and Functional Interaction between Yeast Pif1 Helicase and Rim1 Single-Stranded DNA Binding Protein. Nucleic Acids Res. 2013, 41, 1029–1046.

119. Lopez, C.R.; Singh, S.; Hambarde, S.; Griffin, C.W.; Gao, J.; Chib, S.; Yu, Y.; Ira, G.; Raney, K.D.; Kim, N. Yeast Sub1 and Human Pcd Are G-Quadruplex Binding Proteins That Suppress Genome Instability at Cotranscriptionally Formed G4 DNA. Nucleic Acids Res. 2017, 45, 5850–5862.

120. Lapointe, C.P.; Wilinski, D.; Saunders, A.H.; Wickens, M. Protein-Rna Networks Revealed through Covalent Rna Marks. Nat. Methods 2015, 12, 1163–1170.

121. Lakshminarasimhan, M.; Boanca, G.; Banks, A.C.; Hattem, L.G.; Gabriel, E.A.; Grouppe, D.B.; Smoyer, C.; Malanowski, K.E.; Peak, A.; Flores, L.; et al. Proteomic and Genomic Analyses of the Rvb1 and Rvb2 Processivity Than DNA:DNA Duplexes. Mol. Cell Proteom. 2016, 15, 960–974.

122. Kershaw, C.J.; Costello, J.L.; Talavera, D.; Rowe, W.; Castelli, M.L.; Sims, F.P.; Grant, M.C.; Ashe, P.M.; Hubbard, J.S.; Pavitt, D.G. Integrated Multi-Omics Analyses Reveal the Pleiotropic Nature of the Control of Gene Expression by Pu3p. Sci. Rep. 2015, 5, 15518.

123. Henry, R.A.; Balakrishnan, L.; Ying-Lin, T.S.; Campbell, L.J.; Bambara, A.R. Components of the Secondary Pathway Stimulate the Primary Pathway of Eukaryotic Okazaki Fragment Processing. J. Biol. Chem. 2010, 285, 28496–28505.

124. Gilmore, J.M.; Sardi, M.E.; Venkatesh, S.; Stutzman, B.; Peak, A.; Seidel, C.W.; Workman, J.L.; Flores, L.; Washburn, M.P. Characterization of a Highly Conserved Histone Related Protein, Ydl156w, Its Functional Associations Using Quantitative Proteomic Analyses. Mol. Cell Proteom. 2012, 11, M111.01544.

125. Dekker, C.; Stirling, P.C.; McCormack, E.A.; Filmore, H.; Paul, A.; Brost, L.R.; Costanzo, M.; Boone, C.; Leroux, M.R.; Willison, K.R. The Interaction Network of the Chaperonin Cct. EMBO J. 2008, 27, 1827–1839.

126. Elbaz-Alon, Y.; Rosenfeld-Gur, E.; Shinder, V.; Futerman, H.A.; Geiger, T.; Schuldiner, M. A Dynamic Interface between Vacuoles and Mitochondria in Yeast. Dev. Cell 2014, 30, 95–102.

127. Batisse, J.; Batisse, C.; Budd, A.; Bottcher, B.; Hurt, E. Purification of Nuclear Poly(A)-Binding Protein Nab2 Reveals Association with the Yeast Transcriptome and a Messenger Ribonucleoprotein Core Structure. J. Biol. Chem. 2009, 284, 34911–34917.

128. Barranco-Medina, S.; and R. Galletto. DNA Binding Induces Dimerization of Saccharomyces cerevisiae Pif1. Biochemistry 2010, 49, 8445–8454.

129. Babour, A.; Shen, Q.; Dos-Santos, J.; Murray, S.; Gay, A.; Challal, D.; Fasken, M.; Palanca, B.; Corbett, A.; Libri, D.; et al. The Chromatin Remodeler Isw1 Is a Quality Control Factor That Surveys Nuclear Mrnp Biogenesis. Cell 2016, 167, 1201–1214.

130. Akiyoshi, B.; Sarangapani, K.K.; Powers, A.F.; Nelson, C.R.; Reichow, S.L.; Arellano-Santooyo, H.; Gonen, T.; Ranish, A.J.; Asbury, L.C.; Biggins, S. Tension Directly Stabilizes Reconstituted Kinetochore-Microtubule Attachments. Nature 2010, 468, 576–579.

131. Sofueva, S.; Osman, F.; Lorenz, A.; Steinacher, R.; Castagnetti, S.; Ledesma, J.; Whitby, M.C. Ultrafine Anaphase Bridges, Broken DNA and Illegitimate Recombination Induced by a Replication Fork Barrier. Nucleic Acids Res. 2011, 39, 6568–6584.

132. Gomez-Gonzalez, B.; Garcia-Rubio, M.; Bermejo, R.; Gaillard, H.; Shirahige, K.; Marin, A.; Foiani, M.; Aguilera, A. Genome-Wide Function of Tho/Trex in Active Genes Prevents R-Loop-Dependent Replication Obstacles. EMBO J. 2011, 30, 3106–3119.

133. Chib, S.; Byrd, A.K.; Raney, D.K. Yeast Helicase Pif1 Unwinds RNA:DNA Hybrids with Higher Processivity Than DNA:DNA Duplexes. J. Biol. Chem. 2016, 291, 5889–5901.
134. Prado, F.; and A. Aguilera. Impairment of Replication Fork Progression Mediates RNA PolIII Transcription-Associated Replication. *EMBO J.* 2005, 24, 1267–1276.

135. Herrera-Moyano, E.; Mergui, X.; Garcia-Rubio, L.M.; Barroso, S.; Aguilera, A. The Yeast and Human Fact Chromatin-Reorganizing Complexes Solve R-Loop-Mediated Transcription-Replication Conflicts. *Genes Dev.* 2014, 28, 735–748.

136. Eugster, A.; Lanzuolo, C.; Bonneton, M.; Luciano, P.; Pollice, A.; Pulitzer, J.F.; Stegberg, E.; Berthiau, A.S.; Forstemann, K.; Corda, Y.; et al. The Finger Subdomain of Yeast Telomerase Cooperates with Pif1p to Limit Telomere Elongation. *Nat. Struct. Mol. Biol.* 2006, 13, 734–739.

137. Mimitou, E.P.; and L. S. Symington. Sae2, Exo1 and Sgs1 Collaborate in DNA Double-Strand Break Processing. *Nature* 2008, 455, 770–774.

138. Wei, X.B.; Zhang, B.; Bazelle, N.; Yu, Y.; Liu, N.N.; Rene, B.; Mauffret, O.; Xi, X.G. A 3'-5' Exonuclease Activity Embedded in the Helicase Core Domain of Candida Albicans Pif1 Helicase. *Sci. Rep.* 2017, 7, 42865.

139. Le, S.; Moore, J.K.; Haber, J.E.; Greider, W.C. Rad50 and Rad51 Define Two Pathways That Collaborate to Maintain Telomeres in the Absence of Telomerase. *Genetics* 1999, 152, 143–152.

140. McEachern, M.J.; and J. E. Haber. Break-Induced Replication and Recombinational Telomere Elongation in Yeast. *Annu. Rev. Biochem.* 2006, 75, 111–135.

141. Hu, Y.; Tang, H.B.; Liu, N.N.; Tong, X.J.; Dang, W.; Duan, M.Y.; Fu, H.X.; Zhang, Y.; Peng, J.; Meng, F.L.; et al. Telomerase-Null Survivor Screening Identifies Novel Telomere Recombination Regulators. *PLoS Genet.* 2013, 9, e1003208.

142. Rogers, C.M.; Wang, J.C.; Noguchi, H.; Imasaki, T.; Takagi, Y.; Bochman, M.L. Yeast Hrq1 Shares Structural and Functional Homology with the Disease-Linked Human Recq4 Helicase. *Nucleic Acids Res.* 2017, 45, 5217–5230.

143. Nickens, D.G.; Rogers, C.M.; Bochman, L.M. The *Saccharomyces cerevisiae* Hrq1 and Pif1 DNA Helicases Synergistically Modulate Telomerase Activity in Vitro. *J. Biol. Chem.* 2018, 293, 14481–14496.

144. Makovets, S.; and E. H. Blackburn. DNA Damage Signalling Prevents Deleterious Telomere Addition at DNA Breaks. *Nat. Cell Biol.* 2009, 11, 1383–1386.

145. Vasiyanovich, Y.; Harrington, L.A.; Makovets, S. Break-Induced Replication Requires DNA Damage-Induced Phosphorylation of Pif1p and Leads to Telomere Lengthening. *PLoS Genet.* 2014, 10, e1004679.

146. Mangahas, J.L.; Alexander, M.K.; Sandell, L.L.; Zakian, A.V. Repair of Chromosome Ends after Telomere Loss in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 2001, 12, 4078–4089.

147. White, C.I.; and J. E. Haber. Intermediates of Recombination During Mating Type Switching in *Saccharomyces cerevisiae*. *EMBO J.* 1990, 9, 663–673.

148. Chang, M.; Luke, B.; Kraft, C.; Li, J.Z.; Peter, M.; Lingner, J.; Rothstein, R. Telomerase Is Essential to Alleviate Pif1-Induced Replication Stress at Telomeres. *Genetics* 2009, 183, 779–791.

149. Bambara, R.A.; Murante, R.S.; Henricksen, A.L. Enzymes and Reactions at the Eukaryotic DNA Replication Fork. *J. Biol. Chem.* 1997, 272, 4647–4650.

150. Harrington, J.J.; and M. R. Lieber. The Characterization of a Mammalian DNA Structure-Specific Endonuclease. *EMBO J.* 1994, 13, 1235–1246.

151. Jin, Y.H.; Ayyagari, R.; Resnick, A.M.; Gordenin, A.D.; Burgers, M.P. Okazaki Fragment Maturation in Yeast. II. Cooperation between the Polymerase and 3'-5' Exonuclease Activities of Pol δ in the Creation of a Ligatable Nick. *J. Biol. Chem.* 2003, 278, 1626–1633.

152. Rossi, M.L.; and R. A. Bambara. Reconstituted Okazaki Fragment Processing Indicates Two Pathways of Primer Removal. *J. Biol. Chem.* 2006, 281, 26051–26061.

153. Bae, S.H.; Bae, K.H.; Kim, J.A.; Seo, S.Y. Rpa Governs Endonuclease Switching During Processing of Okazaki Fragments in Eukaryotes. *Nature* 2001, 412, 456–461.

154. Kao, H.I.; Campbell, J.L.; Bambara, A.R. Dna2p Helicase/Nuclease Is a Tracking Protein, Like Fen1, for Flap Cleavage During Okazaki Fragment Maturation. *J. Biol. Chem.* 2004, 279, 50840–50849.

155. Kao, H.I.; Veeraraghavan, J.; Polaczek, P.; Campbell, L.J.; Bambara, A.R. On the Roles of *Saccharomyces cerevisiae* Dna2p and Flap Endonuclease 1 in Okazaki Fragment Processing. *J. Biol. Chem.* 2004, 279, 15014–15024.

156. Zaher, M.S.; Rashid, F.; Song, B.; Joudeh, I.L.; Sobhy, A.M.; Tehseen, M.; Hingorani, M.M.; Hamdan, S.M. Missed Cleavage Opportunities by Fen1 Lead to Okazaki Fragment Maturation Via the Long-Flap Pathway. *Nucleic Acids Res.* 2018, 46, 2956–2974.
Genes 2020, 11, 224

157. Stith, C.M.; Sterling, J.; Resnick, A.M.; Gordenin, A.D.; Burgers, M.P. Flexibility of Eukaryotic Okazaki Fragment Maturation through Regulated Strand Displacement Synthesis. *J. Biol. Chem.* 2008, 283, 34129–34140.

158. Pike, J.E.; Henry, R.A.; Burgers, P.M.; Campbell, J.L.; Bambara, A.R. An Alternative Pathway for Okazaki Fragment Processing: Resolution of Fold-Back Flaps by Pif1 Helicase. *J. Biol. Chem.* 2010, 285, 41712–41723.

159. Ribeyre, C.; Lopes, J.; Boule, B.J.; Piazza, A.; Guedin, A.; Zakian, V.A.; Mergny, J.L.; Nicolas, A. The Yeast Pif1 Helicase Prevents Genomic Instability Caused by G-Quadruplex-Forming Ceb1 Sequences in Vivo. *PloS Genet.* 2009, 5, e1000475.

160. Byrd, A.K.; and K. D. Raney. A Parallel Quadruplex DNA Is Bound Tightly but Unfolded Slowly by Pif1 Helicase. *J. Biol. Chem.* 2015, 290, 6482–6494.

161. Wang, L.; Wang, Q.M.; Wang, Y.R.; Xi, X.G.; Hou, M.X. DNA-Unwinding Activity of *Saccharomyces cerevisiae* Pif1 Is Modulated by Thermal Stability, Folding Conformation, Loop Lengths of G-Quadruplex DNA. *J. Biol. Chem.* 2018, 293, 18504–18513.

162. Byrd, A.K.; Bell, M.R.; Raney, D.K. Pif1 Helicase Unfolding of G-Quadruplex DNA Is Highly Dependent on Sequence and Reaction Conditions. *J. Biol. Chem.* 2018, 293, 17792–17802.

163. Galletto, R.; and E. J. Tomko. Translocation of *Saccharomyces cerevisiae* Pif1 Helicase Monomers on Single-Stranded DNA. *Nucleic Acids Res.* 2013, 41, 4613–4627.

164. George, T.; Wen, Q.; Griffiths, R.; Ganesh, A.; Meuth, M.; Sanders, C.M. Human Pif1 Helicase Unwinds Synthetic DNA Structures Resembling Stalled DNA Replication Forks. *Nucleic Acids Res.* 2009, 37, 6491–6502.

165. Wanzek, K.; Schwindt, E.; Capra, A.J.; Paeschke, K. Mms1 Binds to G-Rich Regions in *Saccharomyces cerevisiae* and Influences Replication and Genome Stability. *Nucleic Acids Res.* 2017, 45, 7796–7806.

166. Capra, J.A.; Paeschke, K.; Singh, M.; Zakian, A.V. G-Quadruplex DNA Sequences Are Evolutionarily Conserved and Associated with Distinct Genomic Features in *Saccharomyces cerevisiae*. *PLoS Comput Biol.* 2010, 6, e1000861.

167. Wanrooij, P.H.; Uhler, J.P.; Shi, Y.H.; Westerlund, F.; Falkenberg, M.; Gustafsson, M.C. A Hybrid G-Quadruplex Structure Formed between Rna and DNA Explains the Extraordinary Stability of the Mitochondrial R-Loop. *Nucleic Acids Res.* 2012, 40, 10334–10344.

168. Zhang, B.; Wu, W.Q.; Liu, N.N.; Duan, X.L.; Li, M.; Dou, X.S.; Hou, M.X.; Xi, G.X. G-Quadruplex and G-Rich Sequence Stimulate Pif1p-Catalyzed Downstream Duplex DNA Unwinding through Reducing Waiting Time at Ss/dsDNA Junction. *Nucleic Acids Res.* 2016, 44, 8385–8394.

169. Duan, X.L.; Liu, N.N.; Yang, Y.T.; Li, H.H.; Li, M.; Dou, X.S.; Xi, G.X. G-Quadruplexes Significantly Stimulate Pif1 Helicase-Catalyzed Duplex DNA Unwinding. *J. Biol. Chem.* 2015, 290, 7722–7735.

170. Li, J.R.; Lu, C.Y.; Lin, J.J.; Li, W.H. Multiple Pif1 Helicases Are Required to Sequentially Disrupt G-Quadruplex Structure and Unwind Duplex DNA. *Biochem. Biophys. Res. Commun.* 2016, 473, 1235–1239.

171. Zhou, R.; Zhang, J.; Bochman, L.M.; Zakian, A.V.; Ha, T. Periodic DNA Patrolling Underlies Diverse Functions of Pif1 on R-Loops and G-Rich DNA. *Elife* 2014, 3, e02190.

172. Paeschke, K.; Bochman, M.L.; Garcia, P.D.; Ceka, P.; Friedman, L.K.; Kowalczewski, C.S.; Zakian, A.V. Pif1 Family Helicases Suppress Genome Instability at G-Quadruplex Motifs. *Nature* 2013, 497, 458–462.

173. Ooi, S.L.; Shoemaker, D.D.; Boeke, J.D. DNA Helicase Gene Interaction Network Defined Using Synthetic Lethality Analyzed by Microarray. *Nature Genet.* 2003, 35, 277–286.

174. Piazza, A.; Shah, S.S.; Wright, W.D.; Gore, S.K.; Koszul, R.; Heyer, D.W. Dynamic Processing of Displacement Loops During Recombinational DNA Repair. *Mol. Cell* 2019, 73, 1255–1266.

175. Prakash, R.; Satory, D.; Dray, E.; Papusha, A.; Scheller, J.; Kramer, W.; Krejci, L.; Klein, H.; Haber, J.E.; Sung, P.; et al. Yeast Mph1 Helicase Dissociates Rad51-Made D-Loops: Implications for Crossover Control in Mitotic Recombination. *Genes Dev.* 2009, 23, 67–79.

176. Quinet, A.; Lemacon, D.; Vindigni, A. Replication Fork Reversal: Players and Guardians. *Mol. Cell* 2017, 68, 830–833.

177. Shin, S.; Hyun, K.; Kim, J.; Hohng, S. ATP Binding to Rad5 Initiates Replication Fork Reversal by Inducing the Unwinding of the Leading Arm and the Formation of the Holliday Junction. *Cell Rep.* 2018, 23, 1831–1839.

178. Hashash, N.; Johnson, A.L.; Cha, S.R. Regulation of Fragile Sites Expression in Budding Yeast by Mec1, Rrm3 and Hydroxyurea. *J. Cell Sci.* 2011, 124, 181–185.
179. Cheng, X.; Qin, Y.; Ivessa, S.A. Loss of Mitochondrial DNA under Genotoxic Stress Conditions in the Absence of the Yeast DNA Helicase Pif1p Occurs Independently of the DNA Helicase Rrm3p. Mol. Genet. Genom. 2009, 281, 635–645.

180. Cheng, X.; Ivessa, A.S. Association of the Yeast DNA Helicase Pif1p with Mitochondrial Membranes and Mitochondrial DNA. Eur J. Cell Biol. 2010, 89, 742–747.

181. Liu, Z.; and R. A. Butow. Mitochondrial Retrograde Signaling. Annu. Rev. Genet. 2006, 40, 159–185.

182. Doudican, N.A.; Song, B.; Shadel, S.G.; Doetsch, W.P. Oxidative DNA Damage Causes Mitochondrial Genomic Instability in Saccharomyces cerevisiae. Mol. Cell Biol. 2005, 25, 5196–5204.

183. O’Rourke, T.W.; Doudican, N.A.; Mackereth, M.D.; Doetsch, P.W.; Shadel, S.G. Mitochondrial Dysfunction Due to Oxidative Mitochondrial DNA Damage Is Reduced through Cooperative Actions of Diverse Proteins. Mol. Cell. Biol. 2002, 22, 4086–4093.

184. O’Rourke, T.W.; Doudican, N.A.; Zhang, H.; Eaton, S.J.; Doetsch, W.P.; Shadel, S.G. Differential Involvement of the Related DNA Helicases Pif1p and Rrm3p in mtDNA Point Mutagenesis and Stability. Genes 2005, 354, 86–92.

185. Taylor, S.D.; Zhang, H.; Eaton, S.J.; Rodeheffer, S.M.; Lebedeva, A.M.; O’Rourke, T.W.; Siede, W.; Shadel, G.S. The Conserved Mec1/Rad53 Nuclear Checkpoint Pathway Regulates Mitochondrial DNA Copy Number in Saccharomyces cerevisiae. Mol. Biol. Cell 2005, 16, 3010–3018.

186. Hou, X.M.; Wu, W.Q.; Duan, X.L.; Liu, N.N.; Li, H.H.; Fu, J.; Dou, X.S.; Li, M.; Xi, X.G. Molecular Mechanism of G-Quadruplex Unwinding Helicase: Sequential and Repetitive Unfolding of G-Quadruplex by Pif1 Helicase. Biochem. J. 2015, 466, 189–199.

187. Llorente, B.; Smith, C.E.; Symington, S.L. Break-Induced Replication: What Is It and What Is It For? Cell Cycle 2008, 7, 859–864.

188. Saini, N.; Ramakrishnan, S.; Elango, R.; Ayyar, S.; Zhang, Y.; Deem, A.; Haber, J.E.; Lobachev, K.S.; Malkova, A. Migrating Bubble During Break-Induced Replication Drives Conservative DNA Synthesis. Nature 2013, 502, 389–392.

189. Li, J.H.; Lin, W.X.; Zhang, B.; Nong, G.D.; Ju, P.H.; Ma, B.J.; Xu, H.C.; Ye, F.F.; Xi, G.X.; Li, M.; et al. Pif1 Is a Force-Regulated Helicase. Nucleic Acids Res. 2016, 44, 4330–4339.

190. Bairwa, N.K.; Mohanty, B.K.; Stamenova, R.; Curcio, J.M.; Bastia, D. The Intra-S Phase Checkpoint Protein Tof1 Collaborates with the Helicase Rrm3 and the F-Box Protein Dia2 to Maintain Genome Stability in Saccharomyces cerevisiae. J. Biol. Chem. 2011, 286, 2445–2454.

191. Schmidt, K.H.; Wu, J.; Kolodner, D.R. Control of Translocations between Highly Diverged Genes by Sgs1, the Saccharomyces cerevisiae Homolog of the Bloom’s Syndrome Protein. Mol. Cell Biol. 2006, 26, 5406–5420.

192. Cha, R.S.; and N. Kleckner. Atr Homolog Mec1 Promotes Fork Progression, Thus Averting Breaks in Replication Slow Zones. Science 2002, 297, 602–606.

193. Chisholm, K.M.; Aubert, S.D.; Freese, K.P.; Zakian, V.A.; King, M.C.; Welsh, L.P. A Genomewide Screen for Suppressors of Alu-Mediated Rearrangements Reveals a Role for Pif1. PLoS ONE 2012, 7, e30748.

194. Jimeno, S.; Camarillo, R.; Mejias-Navarro, F.; Fernandez-Avila, J.M.; Soria-Bretones, I.; Prados-Carvajal, R.; Huertas, P. The Helicase Pif1 Facilitates Resection over Sequences Prone to Forming G4 Structures. Cell Rep. 2018, 25, 3543.

195. Chung, W.H. To Peep into Pif1 Helicase: Multifaceted All the Way from Genome Stability to Repair-Associated DNA Synthesis. J. Microbiol. 2014, 52, 89–98.

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