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The Pif1 family helicase Pfh1 facilitates telomere replication and has an RPA-dependent role during telomere lengthening

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Abstract

Pif1 family helicases are evolutionary conserved 5′ to 3′ DNA helicases. Pfh1, the sole *S. pombe* Pif1 family DNA helicase, is essential for maintenance of both nuclear and mitochondrial DNAs. Here we show that its nuclear functions include roles in telomere replication and telomerase action. Pfh1 promoted semi-conservative replication through telomeric DNA, as replication forks moved more slowly through telomeres when Pfh1 levels were reduced. Unlike other organisms, *S. pombe* cells overexpressing Pfh1 displayed markedly longer telomeres. Because this lengthening occurred in the absence of homologous recombination but not in a replication protein A mutant (*rad11-D223Y*) that has defects in telomerase function, it is probably telomerase-mediated. The effects of Pfh1 on telomere replication and telomere length are likely direct as Pfh1 exhibited high telomere binding in cells expressing endogenous levels of Pfh1. These findings argue that Pfh1 is a positive regulator of telomere length and telomere replication.

Keywords

DNA replication; Pif1 family helicase; Pfh1; Telomere; *Schizosaccharomyces pombe*

INTRODUCTION

Telomeres, the DNA-protein structures at the ends of eukaryotic chromosomes, are critical for genome stability. Telomeres in the fission yeast *Schizosaccharomyces pombe*, like their human counterparts, are assembled into a six membered protein complex called shelterin that protects them from degradation and end-to-end fusions [1]. The *S. pombe* shelterin

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CONFLICT OF INTERESTS
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AUTHOR CONTRIBUTIONS
KM, NS, CW, AND VZ designed the experiments. KM, NS, CW performed the experiments.
KM, NS, CW, AND VZ analyzed the data and wrote the paper.

consists of Pot1, the sequence specific telomere single-strand binding protein, Taz1, the sequence specific duplex DNA binding protein, Poz1, Ccq1, Rap1, and Tpz1 [1, 2].

Telomeres pose several problems for DNA replication. Conventional DNA polymerases cannot replicate the very ends of linear chromosomes. In virtually all eukaryotes, this problem is solved by telomerase, a telomere dedicated reverse transcriptase that uses its RNA component as a template to lengthen the G-strand of telomeric DNA. The *S. pombe* telomerase consists minimally of a catalytic subunit Trt1, the templating RNA subunit, TER1 and an accessory subunit, Est1 [3–6]. Although telomerase is critical for telomere maintenance, in *S. pombe*, telomerase deficient cells can survive by either chromosome circularization or Rhp51-dependent homologous recombination (ALT, alternative lengthening of telomeres) [7].

Conventional DNA polymerases also have problems during semi-conservative replication of telomeres. In both budding and fission yeast, replication forks move slowly through telomeric DNA positioned at the end or internally on the chromosome, even in wild type cells [8, 9]. In *S. pombe* and mouse, loss of the duplex telomere binding proteins Taz1 (*S. pombe*) or TRF1 (mouse) exacerbates problems in telomere replication [9, 10]. In multiple organisms, including humans, chromosomes end in t-loops, which are formed by invasion of the single-stranded G-rich tail of the telomere into duplex telomeric DNA [11]. Although t-loops have not been detected at *S. pombe* telomeres, incubation of 3′ tailed duplex *S. pombe* telomeric DNA with Taz1 generates t-loop structures *in vitro* [12]. T-loops are another challenge to the replication machinery. Taken together, these data suggest that telomeres are hard-to-replicate owing to both their non-nucleosomal protein structure and to the repetitive and G-rich nature of telomeric DNA.

Here we determine if the *S. pombe* Pfh1 DNA helicase, a member of the Pif1 family of 5′–3′ DNA helicases, affects telomeres [13, 14]. Unlike budding yeast, which encodes two Pif1 helicases, ScPif1 and ScRrm3 (Sc, *Saccharomyces cerevisiae*), most eukaryotes, including *S. pombe* and humans encode a single Pif1 family helicase, named, respectively, Pfh1 and hPIF1. The three yeast Pif1 family helicases are multifunctional, with critical roles in maintenance of both nuclear and mitochondrial DNA [14]. In *S. pombe*, Pfh1 is encoded by an essential gene, and the absence of either the nuclear or the mitochondrial isoform is lethal [15]. Pfh1 facilitates fork progression at many nuclear sites, including highly transcribed RNA Polymerase II and III genes, the mating type locus, the rDNA, and converged replication forks [16, 17]. Mutations in hPIF1 are found in families with high risk of breast cancer, and *S. pombe* cells with the corresponding mutation are not viable [18]. However, the effect of hPIF1 loss on telomere replication is not resolved [19].

So far, all tested eukaryotic Pif1 family helicases function at telomeres. ScPif1 is a negative regulator of telomere length and telomere addition at double-strand breaks that acts by displacing telomerase from DNA ends [20–23]. Its overexpression results in short telomeres [22], as does overexpression of hPIF1 in human tissue culture cells [24]. In addition, hPIF1 suppresses the long telomere phenotype of *pif1* budding yeast cells [25]. Although ScRrm3 does not inhibit telomerase, it promotes fork progression through telomeric DNA [8].

To understand the telomere functions of Pif1 helicases in an organism that expresses only one Pif1 helicase we examined the role of Pfh1 in *S. pombe* telomere replication. We find that Pfh1 was needed to facilitate fork progression at telomeric repeats, and that this effect is probably direct because telomeres had high Pfh1 association. To resolve conflicting results on the effects of Pfh1 on telomere length, we overexpressed Pfh1, which resulted in telomere lengthening, even in recombination deficient cells, but not in a RPA mutant that has telomerase defects. Thus, Pfh1 is a positive regulator of semi-conservative telomeric DNA replication and performs a unique PIF1 family function in telomerase-mediated telomere lengthening.

RESULTS AND DISCUSSION

Pfh1 facilitates replication fork progression through telomeres

Pfh1 promotes replication through multiple types of hard-to replicate sites [16, 17]. As *S. pombe* telomeric DNA impedes replication fork progression even in wild type (WT) cells [9], we asked if Pfh1 also affects semi-conservative replication at telomeres. To do so, we examined telomere replication intermediates in a strain (YSA60; Table S1) where Pfh1 was expressed as a GFP fusion under the control of the thiamine-repressible *nmt81* promoter (*nmt81-pfh1-GFP*). The Pfh1-GFP fusion was expressed from its endogenous locus (Fig S1).

To visualize replication intermediates, we used two-dimensional (2D) gel electrophoresis (Fig 1A–E). DNA from log phase cells was digested with EcoRV, which in our strain liberated three telomere fragments [9] (Fig 1B). As shown previously, in WT cells, replication forks slowed as they moved through telomeric DNA as reflected by the increased intensity of telomeric replication intermediates relative to other sequences in sub-telomeric DNA [9] (Fig 1C; see arrows). DNA was prepared from otherwise isogenic *nmt81-pfh1-GFP* expressing cells and examined by 2D gel analysis after 12 hr of growth in thiamine, when Pfh1 is no longer detected by western blot analysis [15, 16]. Replication fork pausing within telomeric DNA was three to four fold higher in Pfh1-depleted compared to isogenic Pfh1 expressing cells (Fig 1C–1E; see arrows). Thus, Pfh1 promotes fork progression through duplex telomeric DNA.

Pfh1 associates with telomeric DNA *in vivo*

Using Co-IP, mass spectrometry and ChIP-Seq we found that Pfh1 is a replisome component, which interacts with all nuclear sequences at their time of replication (Sabouri et al., in preparation). However, if Pfh1 has a direct effect on telomere replication, it is possible that its telomere association will be higher than at other genomic sites.

Pfh1 binding to telomeres was assessed using ChIP combined with quantitative PCR (qPCR). We used a strain expressing epitope-tagged Pfh1-13Myc expressed from the *leu1*$^+$ locus under control of the *pfh1*$^+$ promoter (the endogenous *pfh1*$^+$ locus was not modified) (YNS29; Table S1: Fig S1) [16]. As a control, we used an otherwise isogenic strain that expressed untagged Pfh1 from its endogenous locus (No tag; Fig S1 (wild-type)). In both strains, we compared Pfh1 binding to the sub-telomeric STE sequence (STE) to its binding to the *gal1*$^+$ gene (gal1). Although Pfh1 binding to *gal1*$^+$ was significantly higher than the

no tag control (as expected for a replisome component), Pfh1 association was ~25 fold higher at telomeres than at *gal1*$^+$ (Fig 1F). Thus, Pfh1 is telomere associated *in vivo* to a much greater extent than can be explained by its association with the replisome.

Pfh1 is a positive regulator of telomere length

To re-examine the effects of Pfh1 on telomere length, we overexpressed it using a multi-copy plasmid, pVS117 (*pfh1*$^+$ plasmid). Overexpression was verified by western blot analysis (Fig 2B and Fig S1), which showed that levels of Pfh1 were at least four 4 times higher in cells with the Pfh1 overexpressing plasmid compared to cells without the plasmid. The plasmid was introduced into a strain that expresses only Pfh1-mt* (YSP377; Table S1), a version of Pfh1 that is targeted almost exclusively to mitochondria, although it maintains enough nuclear Pfh1 to maintain viability at 30°C [15]. Telomere length was assessed by Southern blot analysis of ApaI-digested DNA (Fig 2A).

Cells overexpressing Pfh1 exhibited telomere lengthening. After 25 generations, telomeres in Pfh1 overexpressing cells were about 450 bps in length (i.e, ~150 bps longer than WT telomeres), and this length was maintained for multiple restreaks (Fig 2C, compare lane 1 to lane 2–10). Upon loss of the Pfh1 overexpression plasmid, telomeres returned to WT lengths (Fig 2C, lane 11–17). When the cells that had lost the plasmid were retransformed with the Pfh1 plasmid, telomeres again elongated (Fig 2C, lane 18–25). In contrast, cells retransformed with an empty vector had wild-type telomere length (Fig 2C, lane 26–34). Thus, overexpression of Pfh1 results in reversible telomere lengthening suggesting that Pfh1 is a positive regulator of telomere length.

Pfh1-induced telomere lengthening occurs in the absence of Rph51

Overexpression of Pfh1 could promote telomere lengthening by stimulating telomerase or by promoting recombination, as occurs in some cells lacking telomerase [7]. The ALT pathway is Rhp51-dependent [7], as is virtually all homologous recombination in *S. pombe*. When Pfh1 was overexpressed in *rhp51* Δ cells, telomeres still lengthened (Fig 3A, lane 4–6). However, lengthening did not occur in *rhp51* Δ cells carrying the empty vector (Fig 3A, lane 1–3) or no vector (Fig 3A, lane 10–12). The effect on telomere length required the helicase activity of Pfh1 as overexpressing a helicase dead variant, Pfh1-K388A, in which the invariant lysine in the Walker A box was mutated to alanine, did not result in longer telomeres (Fig 3A, lane 7–9). Thus, Pfh1-induced telomere lengthening is not due to homologous recombination but is helicase dependent. Consistent with this conclusion, Pfh1 overexpressing cells had stable STE1-hybridizing bands and did not produce novel banding patterns (Fig 2D–E), which are a characteristic of cells maintaining telomeres by recombination [26].

Pfh1 overexpression does not lengthen telomeres in *rad11-D223Y* cells

As Pfh1-induced telomere lengthening was not Rph51-dependent, it likely occurs by telomerase. Because we were unable to generate a telomerase null strain that overexpresses Pfh1, we used ChIP-qPCR to determine if Pfh1 overexpression increases telomere binding of Pot1, a telomere binding protein that is a positive regulator of telomeres or of telomerase itself [27]. However, ChIP-qPCR with Pot1, Trt1, and Est1 did not reveal significantly

increased telomere binding of any of the three proteins in Pfh1 overexpressing cells (Fig S2).

RPA is an essential protein that binds single-stranded DNA in a relatively sequence non-specific manner and, in all eukaryotes, is critical for replication, repair, and recombination. In both *S. cerevisiae* and *S. pombe*, RPA interacts with telomerase RNA [28]. In addition, *S. pombe rad11-D223Y* cells, which carry a mutation in the largest RPA subunit, have short telomeres [29], and the association of RPA with TER1 RNA is reduced in *rad11-D223Y* cells [28]. Moreover, telomeres in *rad11-D223Y* cells do not hyper-elongate in either a *poz1* Δ or *rap1* Δ background, presumably because telomerase function is compromised in the *rad11-D223Y* background.

Because a ChIP assay might not be sensitive enough to detect modest and/or transient increases in telomerase binding, we asked if telomeres lengthened in *rad11-D223Y* cells in response to Pfh1 overexpression (Fig 3B). As shown earlier [29], *rad11-D223Y* telomeres were ~120 bps shorter than WT telomeres (Fig 3B). This length was not affected, even after five restreaks, by overexpression of Pfh1 (Fig 3B). These data suggest that Pfh1 overexpression affects telomere length by acting as a positive regulator of telomerase. Consistent with this conclusion, mass spectrometry analysis of proteins that co-immunoprecipitate with Pfh1 reveals that RPA is a Pfh1-interacting protein (Sabouri et al. in preparation). In addition, we found that *pfh1* overexpression increased Rad11 binding at multiple loci, including telomeres (Fig S3). This result suggests that the high levels of Pfh1 at telomeres in normal cells may affect telomerase by increased RPA-telomerase interaction [28].

Thus by three criteria, Pfh1 affects telomeres. First, in cells depleted of Pfh1, the already slow movement of telomeric replication forks was reduced to the point that pausing was increased an additional three to four fold (Fig 1). We believe that this value likely underestimates Pfh1's role in telomere replication, as under the conditions of the experiment there was still enough Pfh1 to allow cells to cycle. Second, in WT cells, Pfh1 binding to telomeres was about 25-times higher than to the *gal1*$^{+}$ gene (Fig 1F), whose replication is not Pfh1-sensitive [16]. This result argues that Pfh1 action at telomeres is direct. Third, overexpression of Pfh1 results in reversible telomere lengthening that was not due to homologous recombination but rather is best explained by Pfh1's being a positive regulator of telomerase (Fig 2–3). Pfh1 probably promotes telomere lengthening by virtue of its helicase activity, as overexpression of a helicase dead allele did not cause lengthening in *rph51* Δ cells (Fig. 3A). However, this conclusion is tempered by results from overexpressing helicase dead Pfh1 in the Pfh1-mt* background (YSP377; Table S1), where lengthening was seen in one of three independent isolates. We suspect this variability is a result of the deleterious effects of overexpressing helicase dead Pfh1, which we propose can be overcome by secondary genetic events.

The effect of Pfh1 overexpression on telomere length resolves a discrepancy between two earlier experiments from our lab. In the first, depletion of Pfh1 in the same manner and strain that was used here to examine telomere replication intermediates (Fig 1) does not affect telomere length [15]. A negative result with this approach is not necessarily

meaningful as cells continue to divide under the depletion regime, demonstrating that some Pfh1 remains in the nucleus. In the second approach, *pfh1* Δ spore clones that divide only 1–3 times in the complete absence of Pfh1 have telomeres that are ~25 bps shorter than those in WT spores [30]. This approach has the disadvantage that while the effects on telomere length are reproducible, they are small. However, the results from the second approach are consistent with the finding reported here that overexpression of Pfh1 in telomerase proficient cells resulted in telomere lengthening (Fig 2–3). Indeed, the fact that a complete lack of Pfh1 results in telomere shortening after so few cell divisions, suggests it has a strong positive effect on telomerase. Also, our earlier data [15] combined with data presented here (Fig 1), suggest that semi-conservative replication of telomeres requires more Pfh1 than its role as a positive regulator of telomerase.

Our findings add *S. pombe* to the list of organisms where Pif1 family helicases bind preferentially to telomeres and affect their replication. ScPif1 is a negative regulator of telomerase [20–22]. ScRrm3 does not affect telomerase [8, 25] but promotes semi-conservative replication of both internal and terminal tracts of telomeric sequence [8]. Deletion of the sole mouse PIF1 does not affect telomere length (perhaps because mouse telomere are already very long), but both mouse and human Pif1 helicases are telomerase associated *in vivo* [31, 32], and several lines of evidence suggest that hPIF1 is a telomerase inhibitor [24, 25]. Thus, Pif1 family helicases do not necessarily affect telomeres in the same way, even in organisms that encode only a single family member. We speculate that Pfh1 promotes fork progression by unwinding G-quadruplex secondary DNA structures, structure that are unwound efficiently by ScPif1 and bacterial Pif1 helicases [25, 33] (Sabouri et al. submitted). It is possible that the removal of secondary structures during replication increases RPA access, which accounts for the increased RPA binding when *pfh1* is overexpressed (Fig S3). Alternatively, Pfh1 could help to remodel telomeric chromatin. Perhaps Pfh1 resolves t-loops, and this resolution increases telomerase access to the 3′-OH chromosome end. Pfh1 is the second DNA helicase shown to affect *S. pombe* telomeres, as the RecQ family helicase Rqh1 has a role in processing stalled replication intermediates within very long telomeres [26].

In conclusion, Pfh1 promotes both semi-conservation replication of telomeric DNA and telomerase action and/or access to telomeres. Thus, Pfh1 affects telomeres by both a mechanism previously identified in budding yeast, helping the fork move through telomeric DNA, a role it shares with ScRrm3, and by a novel mechanism not yet reported for Pif1 family helicases, promoting telomerase action.

MATERIAL AND METHODS

Growth conditions, strains and plasmids

All yeast strains used in this study are listed in Table S1. Cells were grown in either yeast extract medium (YES), synthetic minimal medium (EMM) in the presence or absence of 30 μM thiamine or in histidine drop-out EMM media and grown at 30°C. The mitochondrial only Pfh1 allele, called *pfh1-mt** was described previously [15]. Briefly, this allele contains mutations of the methionine codons M265 and M320 to alanine, and M170 to leucine, as well as, the addition of a carboxy-terminal nuclear export sequence. In overexpression

experiments, Pfh1 was expressed from plasmid pVS117 [30], which carries the *pfh1*$^+$ gene under the control of its own promoter in vector pBG2 [34, 35]. The pVS117 *his3*$^+$ marked plasmid was maintained in cells by growth in EMMS minus histidine, and lost by growth in fully supplemented EMMS media. Telomere length was assessed in ySP377 before and after the loss of pVS117.

Telomere blot analysis

S. pombe genomic DNA was prepared by phenol-chloroform extraction as previously described [35]. ApaI (NEB) digested DNA was resolved on 1.2% agarose gels in Tris-borate-EDTA. DNA was transferred to a nylon membrane (GE Healthcare). Southern blots were hybridized with a radiolabeled telomere oligonucleotide containing the *S. pombe* Taz1 binding sequence and imaged on a Typhoon Phosphorimager system (Moleulcar Dynamics).

The stable telomere length in the cells that lost the plasmid (Fig 2C) was not due to reversion of *pfh1-mt** to WT Pfh1, as established by sequencing *pfh1-mt** after restreaking the cells over several generations (data not shown).

ChIP analysis and real-time PCR

ChIP experiments were performed as described previously [4, 16]. Briefly, cells were cross-linked in 1% formaldehyde at 25°C for 5 min. The chromatin was sheared to an average of ~400 bps with a Covaris E220 system or with a probe sonicator and immunoprecipitated with anti-Myc antibody (Clontech Cat. nr 631206). Both input and immunoprecipitated DNA were purified and quantified by real-time PCR with either STE, *act1*$^+$, *gal1*$^+$, or *ade6*$^+$ primer pairs (Table S2) [36].

2D gel electrophoresis

2D gel electrophoresis was performed as described [16]. Briefly, DNA was prepared from 1000 ml of cells grown to 1×10^7 cells/ml in EMMS or EMMS supplemented with 30 μg/μl thiamine. Before harvesting, cells were treated with 1 mg/ml sodium azide for 10 min on ice. Genomic DNA was isolated by Qiagen Genomic-tip 500/G and 35 μg DNA digested with EcoRV was loaded for each gel. The gels were run as described [37]. The 2D gels were visualized by Southern blot analysis hybridized with STE1 probe [9]. The blot was exposed to a phosphorimager screen, scanned with Typhoon 9410, and quantified by Imagequant 5.2 software. Unsaturated 2D gel images were used for quantification of signal intensities. The intensity of each indicated site was quantified and divided by the signal in the 1N spot in the same gel. The obtained ratio in the thiamine-treated cells was divided by the corresponding ratio for the untreated cells, setting the untreated cells values as 1x.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Pfh1 binds telomeric DNA *in vivo*.
- Pfh1 facilitates telomere replication.
- Pfh1 is a positive regulator of telomere length.

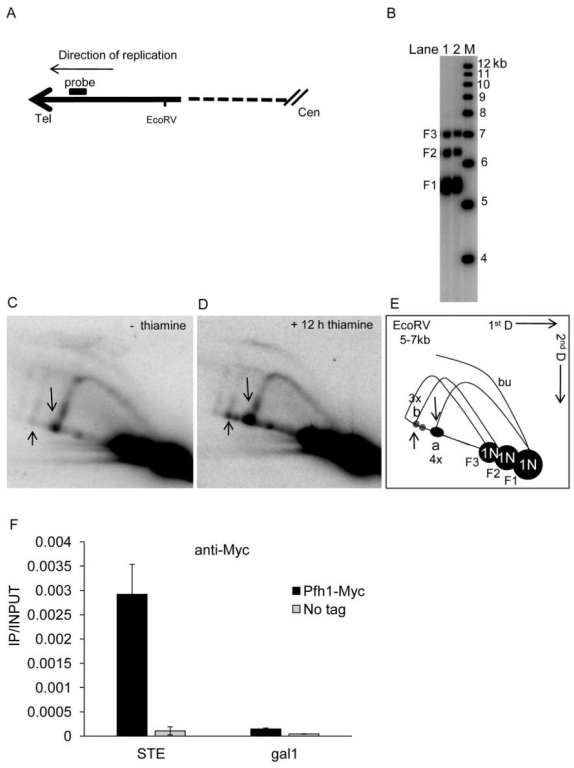

Figure 1. Pfh1 binds telomeres *in vivo* and its depletion causes fork slowing within telomeric repeats

(A) Schematic image of a telomeric and subtelomeric region showing position of restriction sites and the TELO hybridization probe, a 450-bps PstI-SacI telomeric fragment, used for Southern blot analyses [9]. (B) Genomic DNA was isolated from *nmt81-pfh1-GFP* cells grown in minimal media supplemented with thiamine for 0 h (Lane 1) and 12 h (Lane 2), digested with EcoRV, subjected to conventional gel electrophoresis and Southern blot analysis. Telomere blot was probed with TELO probe and the three liberated telomere fragments are marked as F1, F2, and F3. M indicates the DNA ladder marker. (C) 2D gel analysis of EcoRV digested DNA prepared from *nmt1-pfh1-GFP* cell cultures grown in minimal media. (D) 2D gel analysis of EcoRV digested DNA prepared from *nmt1-pfh1-GFP* cells grown in minimal media supplemented with thiamine for 12 hrs. The telomeric repeats comprise the terminal ~300 bp of the 5–7 kb fragments which are at about the position of the 2N spots. Arrows show increased replication pausing at telomeric repeats in Pfh1-depleted cells. (E) Cartoon showing 2D gels of EcoRV digested telomeric DNA. a and b mark paused forks, either 4-fold (a: 4x) or 3-fold (b: 3x) higher in cells grown in thiamine for 12 hrs compared to 0 hrs. 1 N represents non-replicating DNA fragments. Telomeric fragments (F1, F2 and F3) are marked as in b; bu, indicates bubble arc. Quantitation is an average of two independent DNA preparations and biological replicates. (F) Samples from an untagged control strain and otherwise isogenic cells expressing Pfh1-13Myc were chromatin immuno-precipitated using an anti-Myc antibody. The immuno-precipitated DNA was analyzed using quantitative PCR. Association is presented as immuno-precipitated DNA divided by input DNA. Primers are specific to STE; subtelomeric region and gal1; *gal1*$^{+}$ ORF. Data are the

mean of three independent cultures and error bars are standard deviation. $p<0.001$ was determined by two-tailed Student's t-test for both STE and gal1 regions.

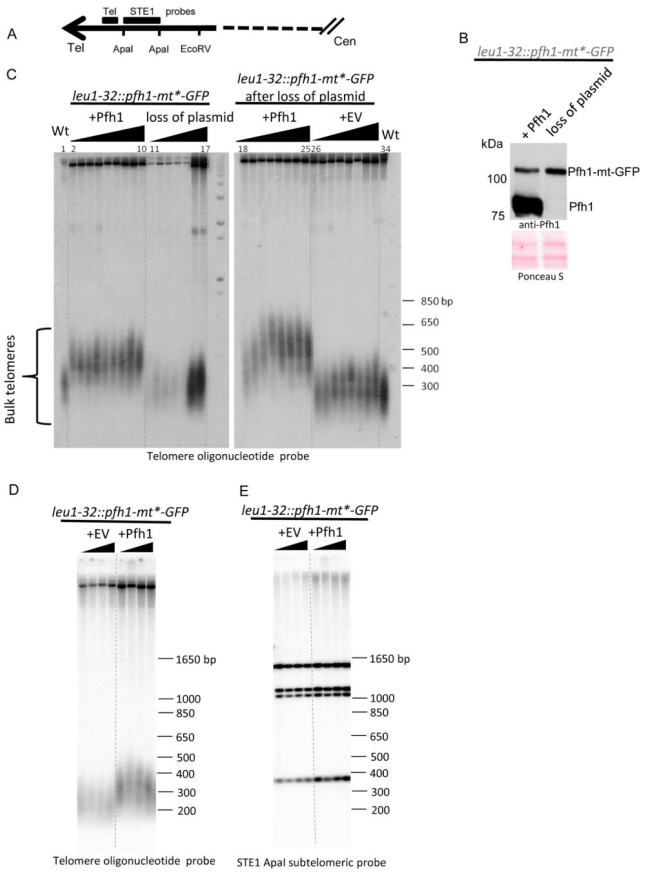

Figure 2. Overexpression of Pfh1 causes telomere lengthening

(A) Schematic illustration of telomeres showing positions of telomeric (Tel) and subtelomeric (STE1) probes and telomere restriction sites used in this study. (B) Western blot analysis using anti-Pfh1 antibodies on extracts from *pfh1-mt*-GFP* cells carrying pVS117 (Pfh1) or after loss of plasmid (top). Protein samples were separated on a 6% acrylamide gel. The membrane was Ponceau S stained after immunoblotting to control loading of samples (bottom). (C) Southern blot analysis of ApaI digested DNA from one of three independent isolates of WT (lane 1 and 34) or strain YSP377 (*leu1-32::PJK148-pfh1-mt*-GFP*) in the presence (lanes 2–10,18–25) or absence (lanes 11–17, 26–33) of the Pfh1 overexpressing plasmid pVS117. Each isolate was struck at least seven times (over 200 generations). In YSP377, in which only the mitochondrial isoform of Pfh1 is expressed (Pfh1-mt*), telomere length is observed to be longer than in WT cells in the presence of pVS117 (lanes 2–10). When pVS117 is lost, YSP377 telomeres return to WT length (lanes 11– 17). YSP377 cells that have lost the plasmid and have shorter telomeres were transformed with either pVS117 or EV (empty vector). Telomeres relengthened in cells transformed with pVS117 (lanes 18–25) while cells transformed with EV did not (lanes 26–33). (D) Southern blot of ApaI digested DNA over several generations in the presence of pVS117 or EV. (E) Southern blot of (D) was stripped and re-probed with STE1 subtelomeric probe. Abbreviations as in (D).

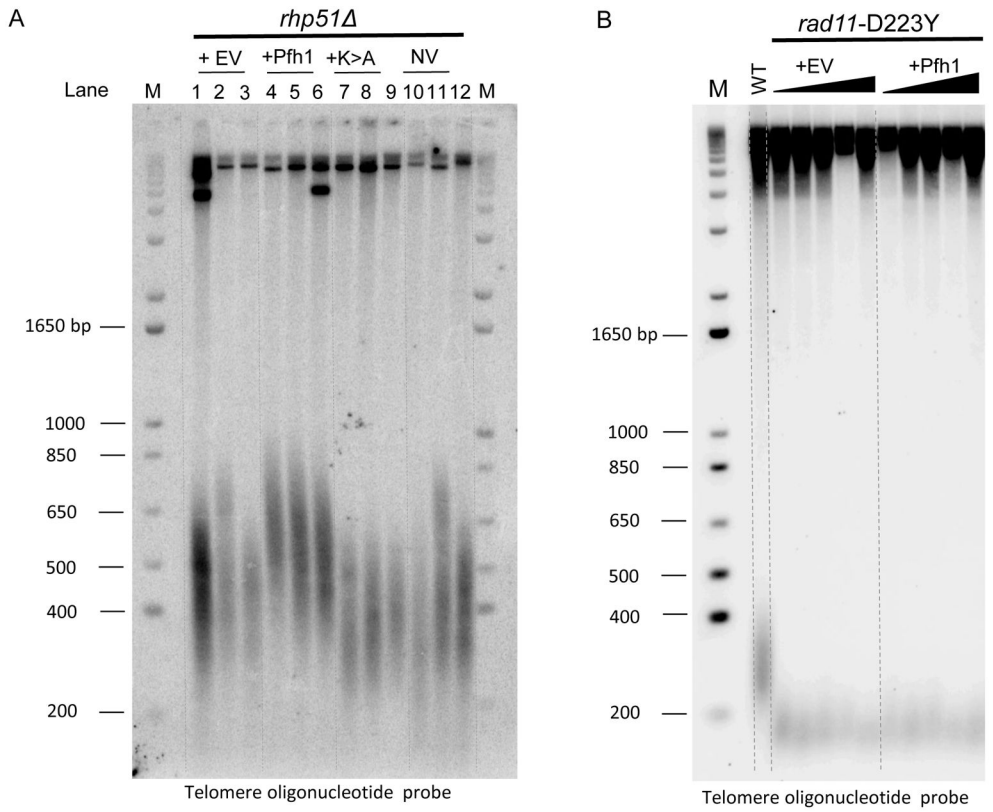

Figure 3. Overexpression of Pfh1 causes telomere lengthening in a recombination deficient strain, but lengthening does not occur in the RPA mutant *rad11-D223Y*

(A) Southern blot analysis of ApaI digested DNA from one of three independent isolates of *rhp5 1*Δ cells after three restreaks (approximately 75 generations) in the presence or absence of pVS117, a plasmid overexpressing Pfh1. In *rhp51* Δ cells, telomere length was longer in the presence of pVS117 (lanes 4–6) than in its absence (lanes 10–12). Cells transformed with EV (lanes 1–3) or vector expressing helicase dead Pfh1-KA (lanes 7–9) had telomeres of the same length as *rhp51* Δ cells. EV indicates empty vector; Pfh1, pVS117; K>A, pBG2-Pfh1-K338A vector; NV, no vector, M; DNA ladder marker. Southern blot was probed with telomere oligonucleotide probe. (B) Southern blot analysis of ApaI digested DNA from WT cells or *rad11-D223Y* cells after five restreaks (approximately 125 generations) in the presence of pVS117 plasmid (Pfh1), or empty vector (EV). Southern blot was probed with telomere oligonucleotide probe. Abbreviations as in (A).