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Roles of the Checkpoint Sensor Clamp Rad9-Rad1-Hus1 (911)-Complex and the Clamp Loaders Rad17-RFC and Ctf18-RFC in *Schizosaccharomyces pombe* Telomere Maintenance

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Abstract

While telomeres must provide mechanisms to prevent DNA repair and DNA damage checkpoint factors from fusing chromosome ends and causing permanent cell cycle arrest, these factors associate with functional telomeres and play critical roles in the maintenance of telomeres. Previous studies have established that Tel1 (ATM) and Rad3 (ATR) kinases play redundant but essential roles for telomere maintenance in fission yeast. In addition, the Rad9-Rad1-Hus1 (911) and Rad17-RFC complexes work downstream of Rad3 (ATR) in fission yeast telomere maintenance. Here, we investigated how 911, Rad17-RFC and another RFC-like complex Ctf18-RFC contribute to telomere maintenance in fission yeast cells lacking Tel1 and carrying a novel hypomorphic allele of *rad3* (*DBD-rad3*), generated by the fusion between the DNA binding domain (DBD) of the fission yeast telomere capping protein Pot1 and Rad3. Our investigations have uncovered a surprising redundancy for Rad9 and Hus1 in allowing Rad1 to contribute to telomere maintenance in *DBD-rad3 tel1Δ* cells. In addition, we found that Rad17-RFC and Ctf18-RFC carry out redundant telomere maintenance functions in *DBD-rad3 tel1Δ* cells. Since checkpoint sensor proteins are highly conserved, genetic redundancies uncovered here may be relevant to telomere maintenance and detection of DNA damage in other eukaryotes.

Keywords

telomere; checkpoint; Rad9-Rad1-Hus1; Rad17; Ctf18

Introduction

Telomeres, the ends of eukaryotic chromosomes, are essential for protection of chromosome ends against rearrangements and fusions. In most eukaryotes, telomeres also allow telomerase to counteract the gradual erosion of chromosome ends, caused by the inability of replicative DNA polymerases to fully replicate ends of linear DNA, by extending the telomeric GT-rich repeat sequences.[1] GT-rich telomeric repeats consist of both double-stranded regions and 3′ single-stranded overhangs, known as G-tails. G-tails are essential for both telomeric repeat extension by telomerase and protection of telomeres by the telomere-capping complex.[2]

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In mammalian cells, telomeres are capped and protected by the "shelterin" complex, composed of TRF1, TRF2, RAP1, TIN2, TPP1 and POT1.[2] A corresponding shelterin-like complex, composed of Taz1, Rap1, Poz1, Ccq1, Tpz1 and Pot1, was recently identified in fission yeast.[3] TRF1 and TRF2 specifically bind to the double-stranded telomeric repeats in mammalian cells, while Taz1 specifically binds the double-stranded telomeric repeats in fission yeast cells.[2] In both mammals and fission yeast, Pot1, in collaboration with TPP1/Tpz1, is thought to protect telomeres by binding to the G-tails,[3-5] and to contribute to the recruitment and/or activation of telomerase.[3, 6, 7] In contrast, budding yeast cells lack TRF1/TRF2/Taz1 and Pot1 orthologs, and instead utilize Rap1 and Cdc13 to recognize the double-stranded DNA (dsDNA) and G-tail portions of telomeric repeats, respectively.[2]

The catalytic subunit of telomerase, known as telomerase reverse transcriptase (TERT), stably associates with the telomerase RNA subunit, which functions both as a platform for the assembly of telomerase subunits and as a template for telomeric repeat extension by telomerase.[8, 9] In fission yeast, the *trt1*$^{+}$ gene encodes the TERT subunit and the *ter1*$^{+}$ gene encodes the telomerase RNA.[10-12] Recent studies have shown that the fission yeast shelterin subunit Ccq1 is required to promote the interaction between Tpz1 and telomerase as well as the recruitment of telomerase to telomeres.[3, 13, 14] Ccq1 is also required to protect telomeres against checkpoint activation and recombination,[3, 13] and regulates heterochromatin formation at telomeres by interacting with the Snf2/Hdac-containing repressor complex (SHREC).[15] However, it is currently unclear if the mammalian shelterin complex also associates with a Ccq1-like molecule to regulate telomere protection and/or telomerase recruitment.

Unlike other types of DNA double-strand breaks (DSBs), telomeres should not be repaired (i.e. fused) and should not fully activate DNA damage checkpoint responses. Thus, one might expect that functional telomeres would exclude DNA repair and DNA damage checkpoint proteins. However, studies have found that proteins involved in repair of DSBs, such as the Ku and Mre11-Rad50-Nbs1 (MRN) complexes, are recruited to telomeres, and they are in fact required for proper maintenance of telomeres.[16, 17] Furthermore, proteins responsible for the detection of DSBs in DNA damage checkpoint responses, such as the PIKK (phosphatidyl inositol 3-kinase-like kinase) family kinases ATM and ATR, are required for stable maintenance of telomeres in a wide variety of organisms,[18-23] and they are recruited to functional telomeres.[24-26] In fission yeast, we have recently shown that Tel1 (ATM) and Rad3 (ATR) contribute redundantly to telomere protection and telomerase recruitment by promoting the efficient accumulation of Ccq1 at telomeres.[14] In addition, other checkpoint "sensor" complexes, such as the proliferating cell nuclear antigen (PCNA)-like ring shaped Rad9-Rad1-Hus1 (911) complex as well as the alternative replication factor C (RFC)-like complexes Rad17-RFC and Ctf18-RFC, have been shown to contribute to telomere maintenance.[19, 20, 22, 23, 27, 28] However it is still not understood how the 911, Rad17-RFC and Ctf18-RFC complexes contribute to the stable maintenance of telomeres.

In this study, we investigated how 911, Rad17-RFC and Ctf18-RFC complexes contribute to fission yeast telomere maintenance in the context of a novel hypomorphic allele of rad3, *DBD-rad3*, generated by the fusion between the Pot1 DNA binding domain and Rad3. While we expected to find that *rad1Δ*, *rad9Δ*, *hus1Δ* and *rad17Δ* cells would exhibit identical telomere phenotypes, our investigations uncovered a surprising redundancy for the 911-complex subunits Rad9 and Hus1 in allowing Rad1 to contribute to telomere maintenance in *DBD-rad3 tel1Δ* cells. Furthermore, we show that the Rad17-RFC and Ctf18-RFC complexes contribute redundantly to telomere maintenance in *DBD-rad3 tel1Δ* cells.

Results

Creation of fusion proteins between the DNA-binding domain (DBD) of Pot1 and checkpoint proteins

In budding yeast, studies utilizing a series of fusion proteins between the DNA binding domain of Cdc13 and various telomerase and telomere binding proteins have yielded valuable insights on how telomere maintenance is regulated.[29-31] Motivated by such studies, we explored the possibility of creating fusion proteins between the DNA binding domain of Pot1 and various factors that might play a role in telomere function, in order to test if the DNA binding domain of Pot1 could be utilized to ectopically target the resulting fused proteins to the G-tails of fission yeast telomeres.

The DNA binding domain of Pot1 has been shown to reside within the N-terminal 185 amino acids and to form an oligonucleotide/oligosaccharide-binding (OB) fold,[32] much like the DNA binding domain of budding yeast Cdc13.[33] We chose to clone a cDNA version of the Pot1 DNA N-terminal fragment corresponding to amino acids 1-261 of Pot1 (this particular fragment will be hereafter referred to as DBD) into a plasmid, and utilized it further in our fusion protein studies. In addition to the wild-type Pot1 DBD, we also created mutant versions of DBD, which carry either Threonine 62 to Valine (T62V) or Phenylalanine 88 to Alanine (F88A) mutations that have previously been shown to abolish the G-tail binding activity of Pot1 DBD[32] (Fig. 1A).

We were interested in understanding how checkpoint signaling is modulated at telomeres, and wondered if the forced targeting of DNA damage checkpoint proteins to telomeres might result in permanent cell cycle arrest. Thus, we decided to N-terminally fuse Pot1 DBD to either the checkpoint kinase $Rad3^{ATR}$ or the checkpoint adaptor protein Crb2/Rhp9. These two checkpoint proteins were chosen due to the contrasting telomere phenotypes exhibited by their deletions. Previous studies have established that the $Rad3^{ATR}$-$Rad26^{ATRIP}$ complex is very important for maintenance of wild-type length telomeres, and it is recruited to functional telomeres during telomere replication.[22, 24, 34] By contrast, *crb2Δ* cells were found to carry essentially wild-type length telomeres,[22] and thus Crb2 might be normally excluded from telomeres to prevent permanent activation of the checkpoint by telomeric DNA ends. For $Rad3^{ATR}$ fusion, the wild-type $rad3^{+}$ gene was replaced with the *DBD-rad3* allele. For Crb2 fusion, the *DBD-crb2* allele was integrated at the $leu1^{+}$ locus in a *crb2Δ* strain. Therefore, the engineered Pot1 DBD fusion proteins were the only copies of $Rad3^{ATR}$ or Crb2 expressed in both cases (see **Materials and Methods** for details).

We first examined if the cells showed any sign of elongation or slow growth, possibly indicating an ectopic activation of checkpoint signaling at telomeres. We found that both *DBD-rad3* and *DBD-crb2* cells grew normally and did not generate highly elongated cells, expected of cells with activated DNA damage checkpoint response (data not shown). Thus, we concluded that these fusion proteins did not cause ectopic activation of DNA damage checkpoint responses.

Since we did not have the means to directly examine if the fusion proteins were expressed properly or indeed localized to telomeres, it was possible that these particular fusion constructs simply generated inactive $Rad3^{ATR}$ or Crb2 proteins. On the other hand, we examined DNA damage sensitivities of *DBD-rad3* and *DBD-crb2* strains, and found that they were more sensitive to both hydroxyurea (HU) and UV than wild-type cells, but less sensitive than *rad3Δ* or *crb2Δ* cells, respectively (Fig. 1B and data not shown). Thus, it is likely that both DBD-$Rad3^{ATR}$ and DBD-Crb2 are expressed, and that these fusion proteins retain at least some function. In addition, *DBD-rad3* cells were found to carry intermediate telomere length that is shorter than $rad3^{+}$ but longer than *rad3Δ* cells (Fig. 2), suggesting

that the DBD-Rad3^{ATR} protein retains at least partial functions of Rad3^{ATR} in telomere maintenance. As for *DBD-crb2* cells, they showed normal telomere length, much like *crb2Δ* cells (data not shown). Since we were more interested in the telomere length regulation mechanism and DBD-Crb2 was unable to induce DNA damage checkpoint activation, we decided to focus our attention solely on characterizing the *DBD-rad3* mutant allele.

Characterization of the *DBD-Rad3* hypomorphic allele

We wished to test if the UV hypersensitivity of *DBD-rad3* cells (Fig. 1B) might be caused by the ability of Pot1 DBD to bind to the telomeric G-tail and potentially sequester Rad3^{ATR} to telomeres and therefore prevent it from recognizing DNA damage occurring elsewhere in the genome. Thus, we examined if we might be able to rescue the UV hypersensitivity of *DBD-rad3* cells by introducing mutations in Pot1 DBD to abolish its G-tail binding activity.[35] Since we observed similar UV hypersensitivities for *DBD-rad3*, *DBD^{T62V}-rad3* and *DBD^{F88A}-rad3* cells (Fig. 1C), we concluded that the UV hypersensitivity of *DBD-rad3* cells is not necessarily caused by the G-tail binding activity of Pot1 DBD. Instead, it is more likely that addition of Pot1 DBD to the N-terminus of Rad3^{ATR} is hindering its proper function and causing various hypomorphic phenotypes in *DBD-rad3* cells.

It has been previously established that Rad3^{ATR}-Rad26^{ATRIP} and Tel1^{ATM}-MRN represent two redundant pathways essential for telomere maintenance in fission yeast.[22, 36] Thus, cells simultaneously lacking these two pathways, such as *rad3Δ tel1Δ* and *rad26Δ tel1Δ*, lose their telomeres and circularize all three chromosomes.[22, 34, 36] Since we observed that *DBD-rad3* cells already exhibit telomere shortening (Fig. 2), we next examined if *DBD-rad3 tel1Δ* cells could still maintain telomeres or circularize their chromosomes. When average telomere-repeat length was analyzed by Southern blot hybridized to a telomeric repeat DNA probe (Fig. 2), we found that *DBD-rad3 tel1Δ* and *rad3Δ* cells carry identical telomere length. Chromosome circularization in fission yeast cells can be conveniently monitored by separating NotI-digested chromosomal DNA by pulsed-field gel electrophoresis (PFGE), and then performing Southern blot analysis with telomeric NotI fragment-specific probes C, I, L and M (Fig. 3A). In this assay, cells carrying circular chromosomes lose individual telomeric NotI fragments and show I+L and C+M bands, corresponding to the fused telomeric fragments from chromosomes I and II. As shown in Fig. 3B, we found that *DBD-rad3 tel1Δ* cells can stably maintain linear chromosomes. Thus, it appeared that the *DBD-rad3* allele retained critical telomere function of Rad3^{ATR}, essential for telomere maintenance in cells lacking the Tel1^{ATM} kinase.

We next tested if the potential G-tail binding activity provided by Pot1 DBD might be critical for telomere maintenance in *DBD-rad3 tel1Δ* cells. Mutations in Pot1 DBD (T62V and F88A) of *DBD-Rad3* did cause a slight but reproducible telomere shortening in *DBD^{T62V}-rad3 tel1Δ* and *DBD^{F88A}-rad3 tel1Δ* cells when compared to *DBD-rad3 tel1Δ* cells (Fig. 2). However, both *DBD^{T62V}-rad3 tel1Δ* and *DBD^{F88A}-rad3 tel1Δ* cells stably maintained linear chromosomes (Fig. 2 and 3B). Thus, while the G-tail binding activity of Pot1 DBD might provide a slight advantage for the DBD-Rad3 fusion protein to function more effectively at telomeres, the G-tail binding activity of Pot1 DBD is not essential for telomere maintenance in *DBD-rad3 tel1Δ* cells.

Rad26^{ATRIP} is still necessary for telomere function(s) provided by DBD-Rad3^{ATR}

The Rad3^{ATR} kinase forms a complex with its regulatory subunit Rad26^{ATRIP}, and previous studies have shown that the ATRIP subunit plays a critical role in the recruitment of ATR kinase to sites of DNA damage and telomeres.[37-39] Thus, we hypothesized that we might be able to bypass the requirement for Rad26^{ATRIP} in telomere maintenance if Pot1 DBD was indeed able to target the DBD-Rad3^{ATR} to telomeres, independently of Rad26^{ATRIP}. We

have previously established that *rad3Δ, rad26Δ* and *rad3Δ rad26Δ* cells show identical telomere length by Southern blot analysis, leading to the conclusion that $Rad3^{ATR}$ and $Rad26^{ATRIP}$ work in the same epistasis group for telomere maintenance.[22] Moreover, we have shown that *rad26Δ tel1Δ* cells carry circular chromosomes, much like *rad3Δ tel1Δ* cells.[22]

Thus, if DBD-$Rad3^{ATR}$ is able to bypass the requirement for $Rad26^{ATRIP}$ for $Rad3^{ATR}$ function, we expect *DBD-rad3 rad26Δ* cells to show longer telomere length than *rad3Δ* or *rad26Δ* cells, and *DBD-rad3 rad26Δ tel1Δ* cells to still maintain telomeres. However, we found that *DBD-rad3 rad26Δ* cells carry the same telomere length as *rad3Δ* cells (Fig. 2), and *DBD-rad3 rad26Δ tel1Δ* cells lose telomeres and circularize their chromosomes (Fig. 2A and 3B). Recent studies have provided evidence that the ATRIP subunit is not only important for the recruitment of ATR to sites of DNA damage, but also for the regulation of ATR kinase activity.[40, 41] Therefore, a failure of DBD-$Rad3^{ATR}$ to bypass the telomere function of $Rad26^{ATRIP}$ might simply reflect the fact that $Rad3^{ATR}$ kinase cannot function without $Rad26^{ATRIP}$. In any case, based on our current data, we were unable to establish whether the Pot1 DBD module could indeed target $Rad3^{ATR}$ to telomeres in the absence of $Rad26^{ATRIP}$.

Telomere maintenance in *DBD-rad3 tel1Δ* cells depends on the telomerase catalytic subunit $Trt1^{TERT}$

We have recently shown that *rad3Δ tel1Δ* cells are defective in both telomerase recruitment to telomeres and telomere protection.[14] Thus, we next examined if *DBD-rad3 tel1Δ* cells could still recruit telomerase to telomeres, and if telomere maintenance in *DBD-rad3 tel1Δ* is dependent on telomerase. When the telomerase catalytic subunit $Trt1^{TERT}$ was eliminated, we found that the resulting *DBD-rad3 tel1Δ trt1Δ* cells were no longer able to maintain linear chromosomes (Fig. 3C). Therefore, telomere maintenance in *DBD-rad3 tel1Δ* cells depends on telomerase. Based on quantitative chromatin immunoprecipitation (ChIP) assays, we found that $Trt1^{TERT}$ recruitment to telomeres was reduced in *DBD-rad3 tel1Δ* cells compared to $rad3^{+}$, *DBD-rad3* or *tel1Δ* cells (Fig. 4A); however, $Trt1^{TERT}$ was still detected at telomeres significantly above the untagged control in *DBD-rad3 tel1Δ* cells. We thus concluded that the residual telomere function provided by DBD-$Rad3^{ATR}$ was sufficient to allow the recruitment of telomerase to telomeres and maintain telomeres in *DBD-rad3 tel1Δ* cells.

Rad1, but not Rad9 or Hus1, is essential for telomere maintenance in *DBD-rad3 tel1Δ* cells

Even though we could not establish if the Pot1 DBD can target $Rad3^{ATR}$ to the telomeric G-tail, we were still intrigued by the telomere phenotypes exhibited by *DBD-rad3* mutant cells. We also reasoned that the ability to stably maintain telomeres in *DBD-rad3 tel1Δ* cells might provide us with a convenient functional assay to characterize factors that collaborate with the $Rad3^{ATR}$-$Rad26^{ATRIP}$ complex in telomere maintenance. Therefore, we next investigated what additional factors besides $Trt1^{TERT}$ and $Rad26^{ATRIP}$ might be required for telomere maintenance in *DBD-rad3 tel1Δ* cells.

We focused our attention on the 911 checkpoint sensor complex, which forms a stable ring shaped complex resembling PCNA.[42, 43] Deletions of individual components of this complex (*rad1Δ, rad9Δ* or *hus1Δ*) result in identical telomere shortening phenotypes,[22, 44] and previous epistasis analyses have allowed us to establish that the 911-complex represents one of multiple redundant pathways that are regulated by the $Rad3^{ATR}$-$Rad26^{ATRIP}$ complex in telomere maintenance.[22] The fission yeast 911-complex has also been shown to associate with telomeres by ChIP assays.[22]

Based on our previous characterization of the 911-complex deletion mutants, we expected identical telomere phenotypes for *DBD-rad3 tel1Δ rad1Δ*, *DBD-rad3 tel1Δ rad9Δ*, and *DBD-rad3 tel1Δ hus1Δ* cells. Surprisingly, we found that while *DBD-rad3 tel1Δ rad1Δ* cells are unable to maintain telomeres and circularize their chromosomes, *DBD-rad3 tel1Δ rad9Δ* and *DBD-rad3 tel1Δ hus1Δ* cells stably maintain telomeres (Fig. 3D). Additionally, we found that *DBD-rad3 tel1Δ rad9Δ hus1Δ* cells are unable to maintain telomeres (Fig. 3D). Thus, Rad1 appears to provide the most critical telomere maintenance function, while Rad9 and Hus1 play redundant roles that allow Rad1 to carry out its essential telomere function in *DBD-rad3 tel1Δ* cells.

To better understand why Rad9 and Hus1 are redundantly required for telomere maintenance in *DBD-rad3 tel1Δ* cells, we monitored how telomere recruitment of Rad1 is affected in *rad9Δ*, *hus1Δ* or *rad9Δ hus1Δ* cells, compared to wild-type cells. Our working hypothesis was that Rad9 and Hus1 would provide redundant functions for the recruitment of Rad1 to telomeres, and thus cells would completely lose the ability to maintain telomeres in the *rad9Δ hus1Δ* background, much like in *rad1Δ* cells. Accordingly, we hoped to observe a significant association of Rad1 with telomeres in *rad9Δ* or *hus1Δ* cells, but not in *rad9Δ hus1Δ* cells. However, we found that *rad9Δ* and *hus1Δ* cells already show reduced levels of telomeric DNA pulled down by Rad1, and we could not detect any differences among *rad9Δ*, *hus1Δ* and *rad9Δ hus1Δ* cells in terms of Rad1 recruitment efficiency to telomeres (Fig. 4B). Thus, while we still presume that Rad1 would probably need to associate with telomeres to perform its telomere function, we were unable to correlate the efficiency of Rad1 association with the observed differences in telomere maintenance phenotypes among *DBD-rad3 tel1Δ rad9Δ*, *DBD-rad3 tel1Δ hus1Δ* and *DBD-rad3 tel1Δ rad9Δ hus1Δ* cells.

Studies in mammalian cells have shown that the 911-complex interacts with telomerase and positively regulates telomerase activity.[19] In addition, studies in *Caenorhabditis elegans* have found that the 911-complex is essential for telomerase-dependent telomere maintenance.[20, 45] Since we observed that *DBD-rad3 tel1Δ* cells show residual $Trt1^{TERT}$ recruitment to telomeres (Fig. 4A) while *rad3Δ tel1Δ* cells fail to recruit telomerase to telomeres,[14] we next examined if the deletion of *rad1* or *rad9* differentially affects the ability of *DBD-rad3 tel1Δ* cells to recruit $Trt1^{TERT}$ to telomeres by ChIP assay. However, we did not observe significant differences in $Trt1^{TERT}$ recruitment between *DBD-rad3 tel1Δ rad1Δ* and *DBD-rad3 tel1Δ rad9Δ* cells (Fig. 4A). Furthermore, we have thus far been unsuccessful in detecting an interaction between Rad1 and $Trt1^{TERT}$ or between Rad1 and *TER1* telomerase RNA by co-immunoprecipitation assays (data not shown). Taken together, it thus appears that the 911-complex does not significantly contribute to telomerase recruitment to telomeres in *DBD-rad3 tel1Δ* cells. Furthermore, it is unlikely that differences in telomerase recruitment efficiency could account for the difference in the ability of *DBD-rad3 tel1Δ rad1Δ* and *DBD-rad3 tel1Δ rad9Δ* cells to maintain telomeres.

Next, we tested if Rad1 might contribute to telomere protection against chromosome fusion in *DBD-rad3 tel1Δ* cells. Since we have previously observed that *rad3Δ tel1Δ* cells are defective in preventing the association of the homologous recombination (HR) repair protein $Rhp51^{Rad51}$ with telomeres,[14] we next monitored the recruitment of $Rhp51^{Rad51}$ in various mutant backgrounds. As shown in Fig. 4C, deletion of *rad1* as well as the simultaneous deletion of *rad9* and *hus1* in *DBD-rad3 tel1Δ* cells led to a significant increase in $Rhp51^{Rad51}$ recruitment to telomeres, compared to *DBD-rad3 tel1Δ*, *DBD-rad3 tel1Δ rad9Δ* or *DBD-rad3 tel1Δ hus1Δ* cells. Thus, reduced protection against telomere fusions appears to account for the chromosome circularization phenotype observed in *DBD-rad3 tel1Δ rad1Δ* and *DBD-rad3 tel1Δ rad9Δ hus1Δ* cells.

Rad17-RFC and Ctf18-RFC are redundantly required for telomere maintenance in *DBD-rad3 tel1Δ* cells

Previous studies have established that the replication factor C (RFC)-like checkpoint complex Rad17 (Rad17-RFC) is important for the loading of the 911-complex to sites of DNA damage.[46-48] Additionally, Rad17 is essential for telomere maintenance in the same pathway as the 911-complex in *C. elegans*,[23] and Rad17 and the 911-complex work in the same pathway for telomere length regulation in fission yeast.[22] Thus, we investigated if Rad17 is also required for telomere maintenance in *DBD-rad3 tel1Δ* cells. Since we believed Rad17 might be essential for recruitment of Rad1 to telomeres, we expected *DBD-rad3 tel1Δ rad17Δ* cells to circularize their chromosomes, much like *DBD-rad3 tel1Δ rad1Δ* cells. However, contrary to our prediction, we found that *DBD-rad3 tel1Δ rad17Δ* cells can stably maintain telomeres (Fig. 5A). Therefore, it appears that *DBD-rad3 tel1Δ* cells have the ability to allow Rad1 to function at telomeres even in the absence of the Rad17-RFC complex.

We wondered if another RFC-like complex containing Ctf18 (Ctf18-RFC) might have redundant telomere functions with Rad17-RFC and allow Rad1 to provide telomere maintenance functions in *DBD-rad3 tel1Δ* cells. We suspected the involvement of the fission yeast Ctf18-RFC complex in telomere maintenance since the budding yeast Ctf18-RFC complex functions redundantly with the Rad24-RFC complex (ortholog of fission yeast Rad17-RFC) for the regulation of the 911-complex in DNA replication checkpoint.[49] When we monitored telomere status by PFGE, we found that *DBD-rad3 tel1Δ rad17Δ ctf18Δ* cells circularized their chromosomes while *DBD-rad3 tel1Δ ctf18Δ* cells still maintained telomeres (Fig. 5A). Thus, Rad17-RFC and Ctf18-RFC are indeed redundantly required for telomere maintenance in *DBD-rad3 tel1Δ* cells. Southern blot analysis revealed that *ctf18Δ* cells carry short telomeres, although the extent of telomere shortening was less than *rad17Δ* cells (Fig. 5B, C). On the other hand, since *rad17Δ* and *rad17Δ ctf18Δ* cells showed identical telomere length distribution, it appears that Rad17-RFC and Ctf18-RFC contribute to telomere length maintenance through a single pathway.

We have previously established that Rad1 and Rad17 work in the same pathway to regulate telomere length, based on the observation that *rad1Δ*, *rad17Δ* and *rad1Δ rad17Δ* cells show identical telomere length distribution.[22] Additionally, we monitored the association of Ctf18 with telomeres by ChIP assay, and found that Ctf18 can efficiently pull down telomeric DNA, even better than Rad17 (Fig. 5D). Taken together, we concluded that both Rad17-RFC and Ctf18-RFC are recruited to normal telomeres, and that they are likely to play redundant roles in allowing the 911-complex to contribute positively to the maintenance of telomeres in fission yeast.

Based on these observations, we hypothesized that Rad17-RFC and Ctf18-RFC might contribute redundantly to telomere recruitment of Rad1. Therefore, we next examined how loss of Rad17 and/or Ctf18 would affect the recruitment of Rad1 to telomeres by ChIP assay. We observed that Rad1 recruitment to telomeres was greatly reduced in *rad17Δ* cells compared to wild-type cells, and that the level of Rad1 recruitment in *rad17Δ* cells became essentially background (Fig. 5E). Moreover, the level of Rad1 recruitment in *rad17Δ ctf18Δ* cells was indistinguishable from *rad17Δ* cells. On the other hand, *ctf18Δ* cells showed Rad1 recruitment level similar to wild-type cells (Fig. 5E). Thus, our data did not enable us to provide support for the notion that Ctf18-RFC contributes to telomere length regulation by promoting the recruitment of the 911-complex to telomeres.

Discussion

Can Pot1 DBD be used to target proteins to telomere G-tails?

In this study, we described our initial attempts to ectopically target proteins to the G-tail of fission yeast telomeres by creating fusion proteins between the target protein and the G-tail binding domain of Pot1. We chose to fuse amino acids 1-261 of Pot1 to either Rad3^{ATR} or Crb2, with the hope that such fusion constructs will be targeted to the G-tails of fission yeast telomeres. Our choice to use the N-terminal 261 amino acids was based on previous studies that have mapped the G-tail binding activity of Pot1 to the region within the N-terminal 185 amino acid fragment of Pot1.[5, 32] Unfortunately, based on our characterization of these fusion constructs, we were unable to obtain evidence that our constructs were indeed targeted to the G-tail by the Pot1 DBD.

It's possible that we would need to use longer N-terminal fragments of Pot1 to target fusion proteins to the G-tail, since recent studies have found that the N-terminal 389 amino acid fragment of Pot1 binds much tighter to single-stranded G-rich telomere primers than the Pot1 187 amino acid N-terminal fragment.[50, 51] Of course, it is also possible that the addition of the Pot1 DBD at the N-terminus might have interfered with the normal functions of Rad3^{ATR} and Crb2, and thus our 261 amino acid Pot1 DBD construct might be more successful in targeting other fused proteins to the G-tails of fission yeast telomeres. In any case, further optimization is clearly necessary to ensure successful targeting of the fused proteins to the telomeric G-tails by the G-tail binding domain of Pot1.

DBD-rad3 as an unusual but useful new hypomorphic allele of *rad3* to dissect factors involved in telomere maintenance

Despite the fact that we could not be sure if DBD-Rad3^{ATR} is indeed targeted to G-tails by the Pot1 DBD more than wild-type Rad3^{ATR}, we utilized the *DBD-rad3* mutant allele as a new interesting hypomorphic allele in order to investigate how the 911, Rad17-RFC and Ctf18-RFC complexes contribute to telomere maintenance in fission yeast. Previously, epistasis analyses based on measurements of steady state average telomere lengths for single and multiple combinations of deletion mutants among DNA repair and checkpoint factors established that Rad3^{ATR}-Rad26^{ATRIP} and Tel1^{ATM}-MRN represent two redundant pathways that are essential for telomere maintenance in fission yeast[22, 36] (Fig. 6).

In addition, we have previously determined that the 911, Rad17-RFC, and Rad3^{ATR}-Rad26^{ATRIP} complexes contribute to telomere maintenance in a single pathway. This conclusion was reached because double mutants, that carried one mutation from Rad1, Rad9, Hus1 or Rad17 and another mutation from either Rad3^{ATR} or Rad26^{ATRIP}, behaved like single deletion mutants of the Rad3^{ATR}-Rad26^{ATRIP} complex. However, since only *rad3Δ* and *rad26Δ* mutants, but not *rad1Δ, rad9Δ, hus1Δ*, and *rad17Δ* mutants, showed synergistic chromosome circularization phenotypes when they were combined with deletion of Tel1^{ATM} or MRN subunits, the Rad3^{ATR}-Rad26^{ATRIP} complex was hypothesized to have additional target(s) other than the 911 and Rad17-RFC complexes that are important for telomere maintenance. Further epistasis analyses revealed that the Ku70-Ku80 non-homologous end-joining (NHEJ) DNA repair complex represents an additional epistasis group which, in addition to the 911 and Rad17-RFC complexes, acts in the Rad3^{ATR}-Rad26^{ATRIP} pathway because telomere shortening phenotypes exhibited by *rad3Δ* and *rad26Δ* mutants were epistatic to that of *pku70Δ* mutation. However, since cells simultaneously lacking the Tel1^{ATM}-MRN, 911/Rad17-RFC and Ku70-Ku80 pathways maintained short but stable telomeres, we concluded that the Rad3^{ATR}-Rad26^{ATRIP} complex must have an additional unidentified telomere maintenance pathway besides the 911/Rad17-RFC and Ku70-Ku80 pathways[22] (Fig. 6).

We have not been successful in determining what factor(s) represent the unknown pathway(s) regulated by the $Rad3^{ATR}$-$Rad26^{ATRIP}$ complex. However, *DBD-rad3* mutant cells must be simultaneously defective in both the Ku-dependent mechanism of telomere maintenance/protection and at least one additional pathway that is regulated by the $Rad3^{ATR}$-$Rad26^{ATRIP}$ complex, so that the Rad1-dependent pathway now represents the only remaining mechanism that allows telomere maintenance in *DBD-rad3 tel1Δ* cells (Fig. 6). Alternatively, it is possible that DBD-Rad3 (but not wild-type Rad3) strictly depends on Rad1 for the phosphorylation of critical telomere target(s), since the 911-complex, in collaboration with TopBP1/Dpb11 (ortholog of fission yeast Cut5/Rad4), is required to activate ATR/Mec1 kinase in mammals and budding yeast.[40, 41, 52] We have previously generated triple mutant cells simultaneously carrying *tel1Δ, rad1Δ* and various hypomorphic alleles of *rad3*, hoping to see that such triple mutant cells would show chromosome circularization. However, to date, *DBD-rad3* has been the only allele of *rad3* found to cause chromosome circularization in a *tel1Δ rad1Δ* background. In any case, future screens for suppressors of chromosome circularization in *DBD-rad3 tel1Δ rad1Δ* might finally allow us to identify the factor(s) regulated by $Rad3^{ATR}$-$Rad26^{ATRIP}$ that work redundantly with the 911/Rad17-RFC and Ku70-Ku80 for telomere maintenance in fission yeast.

The 911-complex subunits have non-equivalent roles in telomere maintenance

The 911-complex forms a stable heterotrimeric complex resembling PCNA,[42, 43] and we and others have previously found that *rad1Δ, rad9Δ* and *hus1Δ* cells exhibit a similar extent of telomere shortening.[22, 44] Thus, we were surprised to find that deletions of different subunits of the 911-complex resulted in completely different telomere phenotypes for *rad1Δ* versus *rad9Δ* or *hus1Δ* in *DBD-rad3 tel1Δ* cells (Fig. 3D). In fact, our ChIP data indicated that Rad1 recruitment to telomeres is greatly reduced in *rad9Δ* or *hus1Δ* cells (Fig. 4B), consistent with the earlier finding that all three proteins must exist for the 911-complex to maintain wild-type telomere length.[22, 44]

On the other hand, there has been a report that observed shorter telomere lengths for various alleles of *rad1* mutant cells (*rad1-1, rad1-S3, rad1-S4* and *rad1Δ*), but wild-type telomere lengths for *rad9-192* and *hus1Δ* cells.[27] Thus, unknown genetic variations, which somehow allow *rad9* and *hus1* mutant cells to retain wild-type length telomeres, might exist in fission yeast. Importantly, this earlier report is in agreement with the notion that Rad1 plays more critical roles in telomere maintenance than Rad9 or Hus1.[27]

Since previous studies have found direct interactions between Rad1 and Rad17 in fission yeast and humans,[53-55] Rad1 alone, in the absence of both Rad9 and Hus1, could potentially collaborate with Rad17-RFC to provide telomere function. However, based on our observations that Rad9 and Hus1 provide redundant essential telomere maintenance functions in *DBD-rad3 te1lΔ* (Fig. 3D), we favor the notion that two redundant sub-complexes of the 911-complex, Rad1-Rad9 and Rad1-Hus1, represent minimal functional units that would allow telomere maintenance in *DBD-rad3 tel1Δ* cells (Fig. 6). Furthermore, previous biochemical studies of the 911-complex in budding yeast and humans support the notion that two subunits of the 911-complex can indeed form a stable complex in the absence of the third subunit.[56, 57]

There have been other reports where mutations in different subunits of the 911-complex caused different phenotypes. For example, a study in fission yeast has found that *rad1Δ* but not *hus1Δ* cells are hypersensitive to the microtubule-depolymerizing drug TBZ.[58] Previous budding yeast studies also reported that *rad17Δ* (fission yeast *rad1* ortholog) cells show telomere shortening[59] but *mec3Δ* (fission yeast *hus1* ortholog) cells carry either longer[59, 60] or wild-type[61] length telomeres. Thus, the budding yeast Rad1 ortholog also appears to play a more critical role in telomere maintenance than the other subunits of the 911-complex.

Budding yeast Rad17^{Rad1} has been reported to show DNA damage-induced self-interaction.[62] Thus, a Rad17^{Rad1} dimer could be loaded to telomeres by Rad24^{Rad17}-RFC and provide telomere function in the absence of both Ddc1^{Rad9} and Mec3^{Hus1} subunits. Alternatively, analogous to our findings in fission yeast, Ddc1^{Rad9} and Mec3^{Hus1} subunits could function redundantly in assisting Rad17^{Rad1} to fulfill its telomere function in budding yeast.

Two RFC-like clamp loaders, Rad17-RFC and Ctf18-RFC, play redundant roles in telomere maintenance

Our analyses of factors critical for telomere maintenance in *DBD-rad3 tel1Δ* cells also revealed that Rad17-RFC and Ctf18-RFC play redundant roles in telomere maintenance (Fig. 6). Since only *rad17Δ*, but not *ctf18Δ*, affects the efficiency of Rad1 recruitment to telomeres (Fig. 5D), it is currently unclear if both Rad17-RFC and Ctf18-RFC are involved in the regulation of the 911-complex in fission yeast telomere maintenance. In fact, since mammalian Ctf18-RFC has been reported to interact with PCNA but not with the 911-complex,[63] it's possible that Ctf18-RFC could collaborate with PCNA, rather than the 911-complex to regulate telomere length. In that case, the observed redundant requirement for Rad17-RFC and Ctf18-RFC in the 911-complex-dependent telomere maintenance mechanism might be caused by the indirect role of the Ctf18-RFC in regulating DNA replication in general.[64, 65]

On the other hand, budding yeast studies have found that *rad17Δ* (fission yeast *rad1* ortholog) and *ctf18Δ* cells show telomere shortening while *rad24Δ* (fission yeast *rad17* ortholog) cells maintain wild type telomere length.[59, 66] Moreover, it has been shown that budding yeast Rad24^{Rad17}-RFC and Ctf118-RFC contribute redundantly to the Rad17^{Rad1}-dependent activation of the DNA replication checkpoint.[49] Thus, evidence from budding yeast support the notion that Rad24^{Rad17}-RFC and Ctf118-RFC are redundantly required to recruit/regulate the 911-complex for its functions in the DNA replication checkpoint and telomere maintenance. In any case, further investigation is necessary to fully understand how Rad17-RFC and Ctf18-RFC redundantly provide essential telomere maintenance functions in *DBD-rad3 tel1Δ* cells.

Perspectives

By utilizing an unusual hypomorphic allele of *rad3*, which artificially joins the Pot1 DBD to the N-terminus of Rad3^{ATR}, we have uncovered unsuspected non-equivalent roles for the subunits of the 911-complex and redundant roles of two RFC-like complexes in fission yeast telomere maintenance. While our current study has only focused on the regulation of telomere maintenance by these factors in fission yeast, similar redundancies appear to exist in budding yeast telomere length regulation as well. Furthermore, since studies in recent years have uncovered many similarities in the way DNA repair factors and DNA damage/replication checkpoint sensor proteins treat telomeres and accidental DNA double-strand breaks, our current findings might be more generally applicable to help us understand how these factors collaborate in DNA damage responses.

Materials and Methods

Fission yeast strains and plasmids

The fission yeast strains used in this study were constructed by standard techniques,[67] and they are listed in supplementary Table S1. Original sources for *trt1Δ*, *rad3Δ*, *rad26Δ*, *tel1Δ*, *rad1Δ*, *rad9Δ*, *hus1Δ*, *rad17Δ*, *ctf18Δ*, *rhp51Δ*, *rad1-myc*, *rad17-myc*, *ctf18-myc* and *trt1-myc* were described previously.[10, 11, 14, 22, 65] DNA primers used for construction of

plasmids are listed in supplementary Table S2. Plasmids used in this study are listed in supplementary Table S3.

The cDNA version of the Pot1 DNA binding domain (DBD), corresponding to amino acids 1-261 of Pot1, was amplified by PCR using DNA primers pot1-DB1 and pot1-DB2, and cloned as a SacI-KpnI fragment into pBluescript II SK+, generating the pBS-pot1-DBD plasmid. The SacI-BamHI fragment from pBS-pot1-DBD was exchanged with the T62V mutant version, generated by PCR using pot1-DB1 and pot1-T62V-rev primers, to construct the pBS-pot1-DBD-T62V plasmid. The BamHI-ApaI fragment from pBS-pot1-DBD was exchanged with the F88A mutant version, generated by PCR using pot1-F88A-fwd and Pot1-DB2 primers, to construct the pBS-pot1-DBD-F88A plasmid.

These wild-type or mutant Pot1 DBD fragments were then cloned as a NdeI-NdeI fragment into the pBF150 plasmid, which carries genomic DNA corresponding to the promoter and the N-terminal ~3.6 kb of the Rad3 ORF with an engineered NdeI site just prior to the start codon of the Rad3 ORF. The resulting DBD-rad3 constructs (wild-type, T62V or F88A) were then excised as PstI-SmaI fragments, and then used to transform fission yeast strains carrying the *ura4*$^+$ marker inserted within the promoter region of the *rad3*$^+$ gene (TN2101; see Table S1), and then selected for the loss of the *ura4*$^+$ gene on 5-FOA-containing agar plates. Correct integration of the DBD-Rad3 constructs was then confirmed by sequencing.

For *DBD-crb2*, Pot1 DBD was excised from the pBS-pot1-DBD plasmid as a BglII-BglII fragment, and cloned into the BamHI site of pJK148-Crb2(BamHI), which carries the *crb2*$^+$ genomic locus with an engineered BamHI site at the N-terminus of the Crb2 ORF and the fission yeast *leu1*$^+$ gene. The resulting pJK148-DBD-Crb2 plasmid was then digested within the *leu1*$^+$ ORF with NruI, and used to integrate the DBD-crb2 construct at the *leu1-32* locus of the *crb2Δ::ura4*$^+$ strain (TN862; see Table S1). Correct integration of the DBD-Crb2 construct was then confirmed by sequencing.

UV viability

Exponentially growing cells were counted and plated in triplicates on YES plates. The plates were then irradiated in a UV cross-linker (Stratagene) at the indicated doses. Following three days of incubation at 32 °C, colonies were counted and the percent viabilities, compared to non-irradiated controls were calculated.

Pulsed-Field gel electrophoresis (PFGE)

Chromosomal DNA samples were prepared in agarose plugs from fission yeast strains as previously described.[22] NotI-digested DNA samples were fractionated in a 1% agarose gel with 0.5X TAE buffer (20 mM Tris acetate and 0.5 mM EDTA) at 14 °C, using the CHEF-DR III system (Bio-Rad) at 6 V/cm (200 V) and a pulse time of 60 to 120 seconds for 24 hours. The telomeric repeat C, I, L, and M probes were prepared as previously described.[68]

Southern blot analysis

ApaI-digested DNA was prepared from the indicated fission yeast strains and separated in a 2% agarose gel at 100 V for 5 hours. DNA was then transferred to a Hybond XL membrane (GE Amersham Biosciences) overnight in transfer buffer (1.5M NaCl, 0.02M NaOH). The membrane was then hybridized with a telomeric repeat DNA probe.[22]

Chromatin immunoprecipitation (ChIP) analysis

Exponentially growing cells were processed for ChIP as previously described.[24] For Trt1-myc, Rad1-myc, Rad17-myc and Ctf18-myc ChIP, monoclonal anti-myc antibody (9B11, Cell Signaling) was used. For Rad51 (Rhp51) ChIP, polyclonal anti-rad51 (A-92, Santa

Cruz) was used. While Rad1-myc ChIP data were quantified using dot blot hybridization,[69] Trt1-myc, Rad17-myc, Ctf18-myc and Rad51 ChIP data were analyzed by several independent triplicate SYBR Green-based real-time PCR (Bio-Rad) using TAS1 primers jk380 and jk381.[24] Western blot analysis was performed to monitor the expression levels of the proteins tested by ChIP as previously described.[69] Anti-Cdc2 (y100.4, Abcam) antibody was used as loading control. Two-tailed Student's t-tests were performed, and P values ≤0.05 were considered as statistically significant differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

911	Rad9-Rad1-Hus1
ChIP	Chromatin immunoprecipitation
DBD	DNA binding domain
DSBs	double-strand breaks
dsDNA	double-stranded DNA
HU	hydroxyurea
MRN	Mre11-Rad50-Nbs1
NHEJ	non-homologous end-joining
OB fold	oligonucleotide/oligosaccharide-binding fold

PCNA	proliferating cell nuclear antigen
PFGE	Pulsed-Field gel electrophoresis
PIKK	phosphatidyl inositol 3-kinase-like kinase
RFC	replication factor C
SHREC	Snf2/Hdac-containing repressor complex
TERT	telomerase reverse transcriptase
UV	ultraviolet

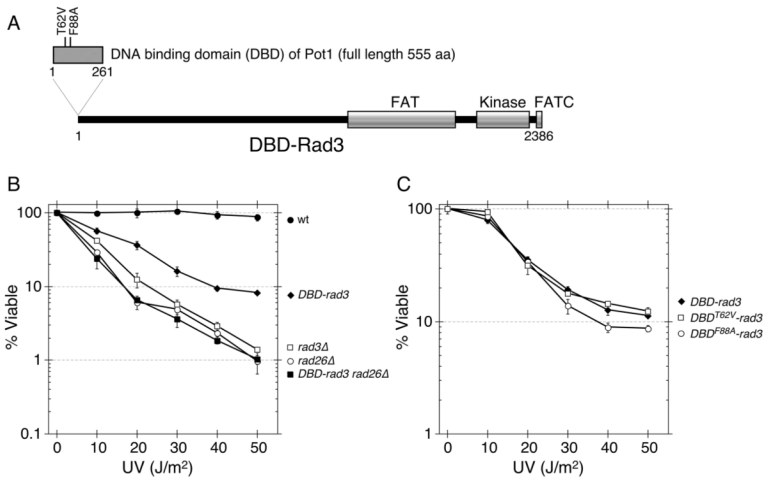

Figure 1.

Construction and initial characterization of *DBD-rad3* related strains. (A) A schematic diagram representing the *DBD-rad3* construct, integrated at the *rad3*$^{+}$ locus. The conserved functional domain of Rad3 and the two point mutations (T62V and F88A) in the Pot1 DNA binding domain used in this study are also indicated. (B, C) UV survival experiments involving *DBD-rad3* strains. Experiments were repeated at least three times, and error bars correspond to standard deviations.

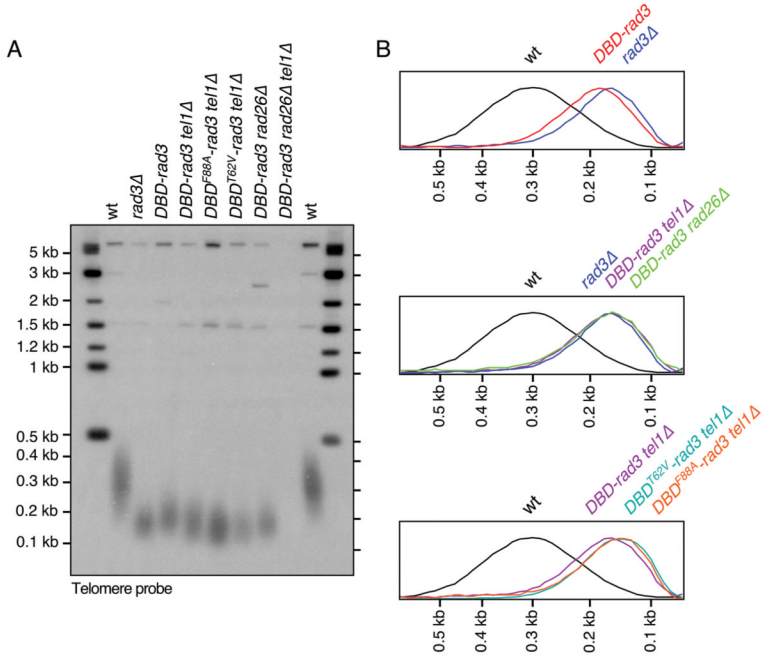

Figure 2.

Telomeric repeat length analysis of *DBD-rad3* strains. (A) Southern blot hybridization analysis of ApaI digested genomic DNA, probed with telomeric repeat sequences. All strains were extensively streaked on agar plates prior to preparation of genomic DNA to ensure terminal telomere phenotype, and all lanes contained comparable genomic DNA based on ethidium bromide (EtBr) staining (data not shown). (B) Quantification of hybridization signal shown in (A) by ImageQuant software (Molecular Dynamics). Peaks of telomeric repeat hybridization signal were normalized and plotted against DNA size.

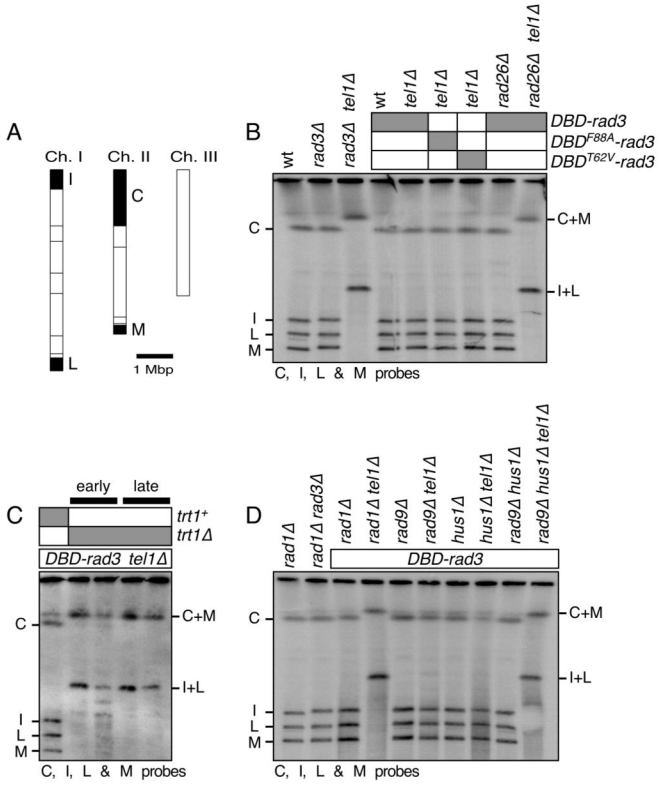

Figure 3.

PFGE analysis of NotI digested chromosomal DNA for the indicated *DBD-rad3* related strains. (A) NotI restriction enzyme map of fission yeast chromosomes (horizontal lines). The telomeric probes C, I, L and M, used in Southern blot hybridization are filled black and marked. Chromosome circularization is indicated by the appearance of C+M and I+L fusion bands. Chromosome III was not monitored since it lacks NotI site. (B-D) Chromosomal DNA was prepared in agarose plugs, digested with NotI, and then used in PFGE for the indicated strains, which were extensively restreaked on agar plates to ensure terminal telomere phenotype. DNA was then transferred to a Nylon membrane and hybridized to C, I, L and M specific probes. For (C), “early” samples are prepared from confirmed *trt1Δ* strains immediately after transformation with the *trt1Δ* construct, while “late” samples are prepared from strains restreaked four times on agar plates after transformation with the *trt1Δ* construct.

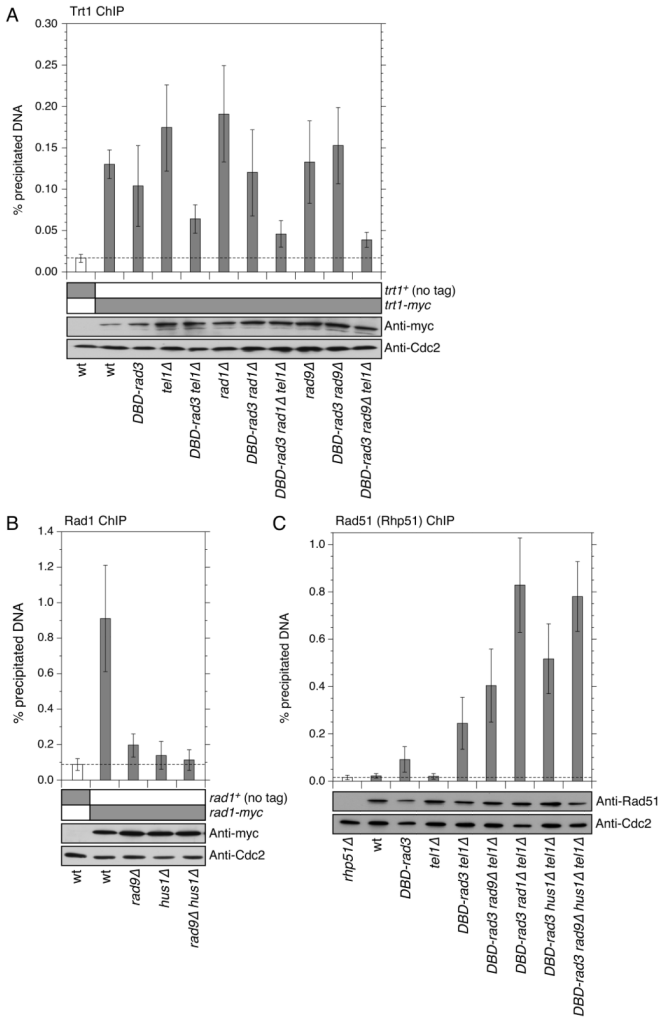

Figure 4.

Quantitative ChIP assays to monitor the recruitment of Trt1 (A), Rad1 (B) and Rad51 (Rhp51) (C) to telomeres for the indicated strains. Error bars represent standard deviation among at least three independent experiments. Expression levels for the indicated proteins were monitored by Western blot analysis, and Western blots with anti-Cdc2 served as loading controls. For (A), Trt1-myc showed statistically significant enrichment of telomeric DNA over no tag control for all strains tested ($P < 0.015$), while the difference in % precipitated (ppt.) telomeric DNA values between *DBD-rad3 rad1Δ tel1Δ* and *DBD-rad3 rad9Δ tel1Δ* was not statistically significant ($P = 0.47$). For (B), Rad1-myc showed statistically significant enrichment of telomeric DNA over no tag control for wild-type (wt) ($P = 0.0010$) and *rad9Δ* ($P = 0.020$) but not for *hus1Δ* ($P = 0.30$) and *rad9Δ hus1Δ* ($P = 0.51$). On the other hand, Rad1-myc ChIP analyses found no statistically significant differences among *rad9Δ*, *hus1Δ* and *rad9Δ hus1Δ* strains ($P = 0.067 \sim 0.60$). For (C), Rhp51 showed statistically significant enrichment of telomeric DNA over *rhp51Δ* control for all strains tested ($P < 0.0089$), except wt ($P = 0.54$) and *tel1Δ* ($P = 0.50$) strains. In addition, % ppt. telomeric DNA values for *DBD-rad3 tel1Δ rad1Δ* cells showed statistically significant difference against *DBD-rad3 tel1Δ* ($P = 0.00075$), *DBD-rad3 tel1Δ rad9Δ* ($P = 0.0044$), *DBD-rad3 tel1Δ hus1Δ* ($P = 0.013$), but not against *DBD-rad3 tel1Δ rad9Δ hus1Δ* cells ($P = 0.57$) for Rhp51 ChIP.

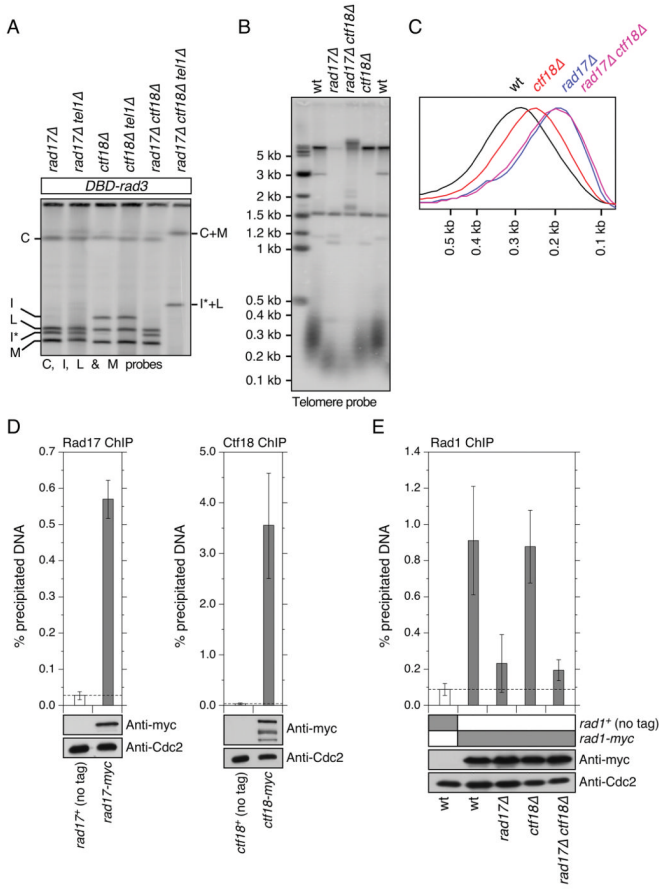

Figure 5.

Characterization of telomere phenotypes involving Rad17 and Ctf18. (A) PFGE analysis of NotI digested chromosomal DNA for the indicated strains. DNA was then transferred to a Nylon membrane and hybridized to C, I, L and M specific probes. Since *rad17*$^{+}$ gene resides on the "I" fragment and *rad17Δ* introduced an additional NotI site, the "I" fragment migrates faster (marked "I*") in *rad17Δ* background. (B) Southern blot hybridization analysis of ApaI digested genomic DNA, probed with telomeric repeat sequences. All strains were extensively streaked on agar plates prior to preparation of genomic DNA to ensure terminal telomere phenotype, and all lanes contained comparable genomic DNA based on EtBr staining (data not shown). (C) Quantification of hybridization signal shown in (B) by ImageQuant software (Molecular Dynamics). Peaks of telomeric repeat hybridization signal were normalized and plotted against DNA size. (D, E) Quantitative ChIP assays to monitor the recruitment of Rad17, Ctf18 and Rad1 to telomeres for the indicated strains. Error bars represent standard deviation among at least three independent experiments. Expression levels for the indicated proteins were monitored by Western blot analysis, and Western blots with anti-Cdc2 served as loading controls. For (D), both Rad17-myc ($P = 0.000067$) and Ctf18-myc ($P = 0.0044$) showed statistically significant enrichment of telomeric DNA over no tag control. For (E), Rad1-myc ChIP showed statistically significant changes against wild-type (wt) cells for *rad17Δ* ($P = 0.0021$) and *rad17Δ ctf18Δ* ($P = 0.00075$), but not for *ctf18Δ* ($P = 0.83$) cells.

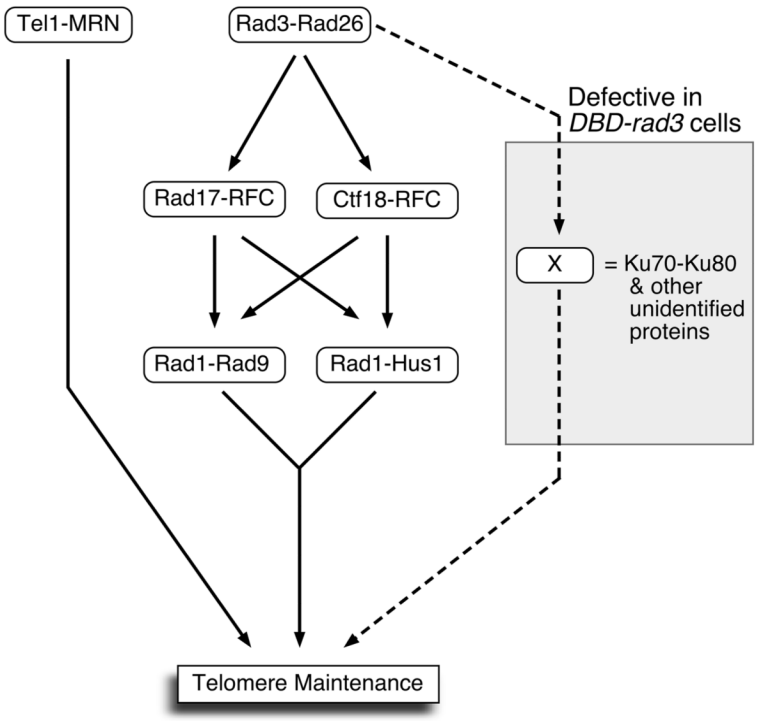

Figure 6.

A possible genetic interaction model for telomere maintenance in fission yeast. The model incorporates the observed redundant telomere maintenance functions of Rad1-Rad9 and Rad1-Hus1 as well as redundant telomere functions for Rad17-RFC and Ctf18-RFC in *DBD-rad3 tel1Δ* cells. It should be noted that the current study does not provide direct molecular evidence for the existence of Rad1-Rad9 and Rad1-Hus1 sub-complexes. In addition, observed redundancy for the Rad17-RFC and Ctf18-RFC complexes in telomere maintenance could involve downstream target(s) other than the 911-complex.