Cell-cycle-dependent telomere elongation by telomerase in budding yeast

Shang LI

Program in Cancer and Stem Cell Biology, Duke-NUS Graduate Medical School Singapore, 8 College Road, Singapore 169857, Singapore

Synopsis

Telomeres are essential for the stability and complete replication of linear chromosomes. Telomere elongation by telomerase counteracts the telomere shortening due to the incomplete replication of chromosome ends by DNA polymerase. Telomere elongation is cell-cycle-regulated and coupled to DNA replication during S-phase. However, the molecular mechanisms that underlie such cell-cycle-dependent telomere elongation by telomerase remain largely unknown. Several aspects of telomere replication in budding yeast, including the modulation of telomere chromatin structure, telomere end processing, recruitment of telomere-binding proteins and telomerase complex to telomere as well as the coupling of DNA replication to telomere elongation during cell cycle progression will be discussed, and the potential roles of Cdk (cyclin-dependent kinase) in these processes will be illustrated.

Key words: ataxia telangiectasia mutated (ATM), cyclin-dependent kinase (Cdk), DNA polymerase, nucleoprotein structure, replication protein A (RPA), telomere, telomerase

INTRODUCTION

Telomeres are specialized nucleoprotein structures at the chromosome ends that consist of tandem DNA repeats bound by a large array of telomeric-specific binding proteins. Telomeres are essential for both the stability of linear chromosomes and the complete replication of genomic information [1]. In the majority of eukaryotes, the telomeres are synthesized by telomerase. Telomere elongation by telomerase counteracts the loss of telomeric DNA repeats due to the inability of DNA polymerase to replicate the extreme end of the lagging-strand DNA template. In the absence of telomere maintenance mechanisms, the telomeres progressively shorten with every round of DNA replication. This eventually leads to uncapping of the chromosome ends and induction of DNA damage responses, resulting in cellular senescence. Therefore telomere length limits the replicative potential of cells [2]. Both telomere-binding proteins and telomerase are essential for the regulation of telomere length homeostasis.

In humans, telomerase activity is barely detectable in most of the adult tissues with the exception of germ cells and stem cells [3–6]. Robust telomerase expression prevents rapid telomere shortening and supports the continued proliferation of germ and stem cells, which is necessary for tissue regeneration throughout our lifetime. Patients with insufficient telomerase activity, such as those with dyskeratosis congenita, display progressive bone marrow failure and premature aging, along with increased cancer susceptibility [7,8]. Reduced telomerase function has also been implicated in idiopathic pulmonary fibrosis and aplastic anaemia [9,10]. Consistent with the observations in humans, loss of telomerase in mouse models results in multiple organ defects, including decreased adult stem cell functionality, premature development of age-associated pathologies and reduced life-span [11–14]. In contrast, up-regulation of telomerase has been observed in more than 85–90% of human cancers, and increased expression of telomerase in mouse models leads to heightened tumour susceptibility [15–17]. These results highlight the crucial role of balanced telomere maintenance by telomerase, as altered telomerase function can lead to both cancer and early onset of aging. Thus a better understanding of telomere maintenance by telomerase will allow the development of novel therapeutic approaches to limit cancer growth and prevent the early onset of aging. In this review, I will focus on the cell-cycle-dependent regulation of telomere maintenance by Cdk (cyclin-dependent kinase) in budding yeast. Such cell-cycle-dependent regulatory mechanisms are likely conserved and will provide critical insight into the regulation of telomere maintenance in human cells.

In budding yeast, the telomeric DNA consists of 250–350 bp of double-stranded C1–3A/TG1–3 repeats with a short single-stranded TG1–3 3′ overhang [18,19]. There are two major

Abbreviations used: ATM, ataxia telangiectasia mutated; Cdc13, cell division cycle 13; Cdk, cyclin-dependent kinase; CST, Cdc13–Stn1–Ten1; MRX, Mre11–Rad50–Xrs2; Polα, polymerase α; POT1, protection of telomeres 1; RPA, replication protein A; TRF, telomere repeat-binding factor.

1 email shang.li@duke-nus.edu.sg
telomeric DNA-binding proteins in budding yeast: Rap1 and Cdc13 (cell division cycle 13). Rap1 binds duplex C_{1-3}A/TG_{1-3} DNA repeats [20–22], and Cdc13 binds the single-stranded TG_{1-3} 3’ overhang [23]. Rap1 interacts with Rif1–Rif2 and plays a major role in the regulation of telomere length homeostasis [24,25]. Cdc13 positively and negatively regulates telomere elongation through its interaction with two distinct protein complexes. Association of Cdc13 with the telomerase complex, which contains the protein catalytic subunit Est2, the integral RNA template TLC1, Est1 and Est3 [26], promotes telomere elongation, while the association of Cdc13 with the Stn1–Ten1 complex results in telomere protection and inhibition of telomere elongation by telomerase [26–33]. These sequence-specific double- and single-stranded telomeric DNA-binding proteins and their associated factors form a dynamic structure at the telomeres [34,35]. This dynamic nucleoprotein structure is essential for telomere replication, telomere silencing, telomere end protection and telomere length regulation.

In budding yeast, telomere elongation by telomerase is cell-cycle-dependent. The remodelling of telomere ends and the generation of the G-rich single-stranded overhangs, which serve as the substrate for the binding of telomerase, are cell-cycle-regulated and depend on the activity of Cdk1 [36,37]. The timing of telomere elongation in late S/G2-phase correlates with the binding of protein factors involved in telomere elongation to the telomeres, including Est1, Est2 and Cdc13 [38,39]. These results have suggested that the assembly of functional telomerase complexes at the telomeres is restricted to late S/G2-phase of the cell cycle. However, recent results also indicate that the assembly of the telomere protecting complex CST (Cdc13–Stn1–Ten1) [40,41], the telomere-specific RPA (replication protein A)-like complex [42–44], is also cell-cycle-regulated and peaks around late S/G2-phase. Since the Stn1–Ten1 complex competes with the telomerase complex for binding to the telomeres, the recruitment of the telomerase complex and Stn1–Ten1 complex by Cdc13 must be co-ordinated to ensure proper and efficient telomere elongation during cell cycle progression. The processes leading to the complete replication of telomeres, including telomere remodelling, telomere end processing, telomerase recruitment and telomere protection, are tightly regulated and coordinated, and Cdk1 probably plays a key role in co-ordinating these processes in budding yeast (Figure 1).

In budding yeast, the regulation of cell cycle progression depends on a single Cdk, namely Cdk1 (or Cdc28). Cdk1 forms complexes with various periodically expressed cyclins at different stages of the cell cycle and regulates cell cycle progression by phosphorylating specific protein substrates [45]. The consensus Cdk phosphorylation motif is (S/T)-P-X-(K/R), and the minimal Cdk phosphorylation motif is (S/T)-P; although the cell-cycle-restricted nature of telomere elongation by telomerase has been known for more than a decade [46,47], the targets of Cdk1 phosphorylation in telomere- and telomerase-associated factors remain largely unknown. Many potential Cdk1 phosphorylation motifs can be identified in telomere- and telomerase-associated proteins. However, only the Cdk1-dependent phosphorylation of the single-stranded telomere-specific binding protein Cdc13 has been well characterized in vivo [40]. Further identification and characterization of potential Cdk1 substrates in telomere- and telomerase-associated proteins in budding yeast will provide important insights into the signalling cascades that regulate cell-cycle-dependent telomere elongation by telomerase.

**DNA REPLICATION AND TELOMERE ELONGATION**

Using a telomere seed sequence at the end of an HO endonuclease-induced chromosome break, it was shown that lengthening of the seed sequence by telomerase is restricted to late S/G2-phase in vivo [46,47]. The same telomere seed sequence failed to elongate in G1, despite a similar level of telomerase activity in both G1- and M-phase cells as measured using a standard in vitro assay [46]. These results suggest that telomerase activity in vivo is regulated during the cell cycle and that telomere elongation is co-ordinated with the DNA replication machinery, which is necessary for synthesis of the opposite C_{1-3}A strand of telomeric DNA.

Several additional lines of evidence indicate direct co-ordination between telomere elongation by telomerase and DNA replication. First, DNA Polα (polymerase α), one of the three replicative DNA polymerases identified in budding yeast, is directly involved in telomeric DNA replication, since telomerase is unable to extend telomeres in the absence of DNA Polα in vivo [46]. Polδ, but not Polε, is also required for telomere elongation [46]. Secondly, the presence of a single ARS (autonomous replication sequence) is necessary for the telomerase-dependent elongation of the telomeres present on a yeast episome [47]. Finally, the replication of most of the subtelomeric chromatin regions in budding yeast happens in late S-phase due to the late firing of DNA replication origins in subtelomeric regions, which correlates with the elongation of telomeres by telomerase in late S/G2-phase. However, short telomeres can trigger the firing of nearby DNA replication origins early in S-phase. Such early replication events may contribute to an increased telomere elongation rate by telomerase, resulting in enhanced elongation of short telomeres [48]. Although the underlying mechanisms of such regulation remain unclear, it is very likely that chromatin structure may play a major role. The less condensed chromatin of short telomeres may contribute to an increased telomere elongation rate by telomerase, resulting in enhanced elongation of short telomeres [48]. Although the underlying mechanisms of such regulation remain unclear, it is very likely that chromatin structure may play a major role. The less condensed chromatin of short telomeres may contribute to an increased telomere elongation rate by telomerase, resulting in enhanced elongation of short telomeres [48].
In budding yeast, the telomere-binding proteins form non-nucleosomal chromatin at chromosome ends. Sir-mediated interactions between Rap1/Rif1/Rif2 proteins at the telomeres and histones in internal nucleosomal chromatin may create folded-back higher-order structures that protect the chromosome ends from degradation, end-to-end fusion and recombination. Passage of a replication fork may transiently open up the telomere, allowing its elongation by telomerase. In budding yeast, the short telomeres are preferentially elongated. Tel1 plays an essential role in the recruitment of telomerase to short telomeres, leading to preferential elongation of short telomeres during normal cell cycle progression. At the long telomere, the higher number of Rap1–Rif1–Rif2 complexes inhibit the MRX complex-dependent recruitment of Tel1 to the long telomere. While Rif1 and Rif2 are still present at short telomeres, the number of bound proteins is reduced, which may in turn result in reduced inhibition of Tel1 recruitment by the MRX complex. Cdc13, a single-stranded telomeric-specific DNA-binding protein, co-ordinates the recruitment of either the telomerase complex (Est1–Est2–Est3–TLC), leading to telomere elongation, or the repressive Stn1–Ten1 complex, leading to telomeric end protection and inhibition of telomere elongation. Phosphorylation of Cdc13 by Cdk1 (as indicated by the solid arrow) leads to preferential binding of the telomerase complex, thereby inducing telomere elongation. Replication of the telomere C strand requires DNA Polα. Further studies are necessary to address whether Cdk1-dependent phosphorylation events (as indicated by the broken arrow) play a regulatory role in the cell-cycle-dependent end processing of the telomere G-rich 3' overhang by the MRX complex and other nucleases, as well as the coupling of DNA replication and telomere elongation in vivo. The question marks indicate potential phosphorylation targets of Cdk1 that may regulate cell-cycle-dependent telomere replication and elongation.

Interestingly, the timing of telomere replication differs between yeast and human cells. Although telomere DNA replication in yeast occurs in late S-phase, telomeric replication occurs throughout S-phase in human cells [53]. One possible explanation is that, while telomere elongation by telomerase only occurs at a few telomeres in budding yeast [54], recent evidence suggests that telomere elongation in human cells occurs at most chromosome ends [55]. The extension of telomeric DNA replication timing in human cells may be necessary to accommodate the replication of many more telomeres.
How is telomere elongation coupled to telomeric DNA replication during S-phase in vivo? In budding yeast, DNA Polα has four subunits: Pol1, Pol12, Pri1 and Pri2. Both genetic and biochemical data have shown that Pol1 can interact with the single-stranded telomeric DNA-binding protein Cdc13 [56]. Furthermore, Pol12 was found to interact with Stn1 in a yeast two-hybrid screen [41] and by affinity-capture Western blotting [57]. How these interactions influence cell-cycle-dependent telomere elongation remains to be addressed. Previous studies using chromatin immunoprecipitation have shown that the recruitment of both Cdc13 and Stn1 is cell-cycle-dependent and peaks at late S/G2-phase [30]. These observations suggest that DNA replication in yeast remains unclear.

Finally, how do Cdk1 signalling cascades regulate the coupling of DNA replication and telomere elongation in budding yeast? Our previous results indicated that Cdk1 is directly involved in the regulation of telomere elongation by telomerase through phosphorylation of Cdc13 [40]. Interestingly, previous analysis by MS identified several potential Cdk1 phosphorylation sites on the Pol1 and Pol12 subunits of DNA Polα. Many of these phosphorylation sites contain the signature motif of Cdk1, (S/T)-P. However, these phosphorylation sites have not been characterized and their role in coupling telomere elongation and telomeric DNA replication in yeast remains unclear.

MODULATION OF TELOMERE COMPOSITION DURING CELL CYCLE PROGRESSION

In most eukaryotes, the telomere length of telomerase-positive cells is maintained at a constant mean value. The typical mean yeast telomere length is ~250–350 bp with a short single-stranded G-rich overhang. In contrast with other chromatin regions, yeast telomeric DNA repeats are bound by non-nucleosomal protein complexes. The sequence-specific binding protein Rap1 binds the double-stranded telomeric DNA repeats. Rap1 interacts with Rifa1, Rifa2 and Sir proteins, creating a repressive structure that protects the chromosome ends from degradation, recombination and end-to-end fusion [50,51]. The binding of Rap1 to TG13 repeats at the telomere and at subtelomeric Y′ repeats can extend as far as 3–15 kb from the chromosome terminus.

One major function of Rap1 is the maintenance of yeast telomere length at a mean of ~250–350 bp. The cellular mechanism determining the mean telomere length has been described by the ‘protein counting model’ [24,25]: the higher number of proteins bound by a long telomere repeat tract represses telomerase activity at that particular telomere. This creates a negative feedback loop that maintains telomere length homeostasis. In budding yeast, the Rif1 and Rif2 proteins, which interact with the major double-stranded telomere-binding protein Rap1, are the critical proteins mediating this negative feedback. The longer the telomere, the higher the number of Rap1−Rifa1−Rifa2 complexes present on that telomere. Recent results suggest that Rifa1 and Rifa2 inhibit the MRX (Mre11−Rad50−Xrs2) complex-dependent recruitment of Tel1 [the homologue of the human ATM (ataxia telangiectasia mutated)] to the telomeres [60]. Tel1 is necessary for the recruitment of telomerase complexes to telomeres [61]. In addition, it has been shown that Tel1 plays an essential role in the recruitment of telomerase to short telomeres, leading to preferential elongation of short telomeres during normal cell cycle progression [62–65]. While Rifa1 and Rifa2 are still present at short telomeres, the number of bound proteins is reduced, which may in turn result in reduced inhibition of Tel1 recruitment by the MRX complex.

The association of Rap1−Rifa1−Rifa2 with telomeres also shows dynamic changes during cell cycle progression [50,51]. Rap1 and Rifa1 are maximally associated with telomeres in late S/G2-phase, while telomeric Rifa2 decreases steadily into the S-phase and then increases in M-phase. These results suggest a dynamic modulation of telomere chromatin structure during cell cycle progression. However, the exact telomere-binding factors present at individual telomeres at each stage of the cell cycle remains to be determined. Such detailed mapping of telomere composition may provide a better understanding of how telomere chromatin structure controls telomere elongation and telomere protection in vivo during cell cycle progression. The role of Cdk1 in modulating cell-cycle-dependent telomeric chromatin changes also remains to be determined. Potential Cdk1 phosphorylation sites have been identified in many telomere-associated proteins [45], but their significance is unknown.

Common mechanisms for telomere protection and telomere length regulation are conserved from yeast to human, but there are also distinct differences. While budding yeast telomeric DNA is bound by non-nucleosomal protein complexes [21], human telomeric DNA is bound by both nucleosomal proteins and telomere-specific binding proteins [66]. More than 200 telomere-associated proteins have been identified in human cells [67]. This large array of telomere-associated proteins indicates a far greater degree of complexity at human telomeres. The mammalian shelterin complex, which includes TRF1 (telomere repeat-binding factor 1), TRF2, RAPA1, Tin2 (TRF1-interacting nuclear factor 2), TP1 and POT1 (protection of telomeres 1), plays essential roles in telomere end protection and regulation of telomere length homeostasis [68]. TRF1 and TRF2 bind independently to the double-stranded telomeric DNA and serve as negative regulators of telomere shortening by telomerase. The recruitment of human telomerase depends on the single-stranded telomere-binding protein POT1 and its partner TP1 [69,70]. Although human RAPA1 is largely dispensable for telomere capping, it is required to inhibit telomeric sister chromatid exchange [71,72]. In addition, mammalian RAPA1 has recently been shown to play a role in genome-wide transcriptional regulation through direct binding at non-telomeric ‘TTAGGG’ consensus motifs and through cytoplasmic activation of NF-κB (nuclear factor κB) signalling.

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cascades [72,73]. This newly recognized function of mammalian Rap1 closely parallels the function of yeast Rap1, which also regulates genome-wide transcription.

Like yeast telomere-binding proteins, human TRF1, TRF2 and POT1 associate with telomeric DNA in a cell-cycle-regulated fashion [74]. This cell-cycle-dependent recruitment is likely regulated by Cdk phosphorylation, but the potential Cdk substrates remain largely unknown. The recently identified CST-like complex in human cells [58,59] may be important Cdk substrates, given the important role of Cdk1-dependent Cdc13 phosphorylation in yeast telomere regulation [40]. How nucleosominal proteins, the shelterin complex, and the CST complex interact with telomeric DNA during cell cycle progression remains to be addressed.

CELL-CYCLE-DEPENDENT GENERATION OF THE SINGLE-STRANDED TELOMERE G TAIL

While most internal chromosomal regions are replicated by forks moving in either direction, replication forks at the telomeres originate in subtelomeric regions, resulting in unidirectional fork movement towards the telomeric terminus. Therefore the replication of the G-rich telomeric strand is expected to generate a 3' overhang due to the degradation of the RNA primer on the terminal Okazaki fragment, and the replication of the C-rich telomeric strand by leading strand synthesis will result in the generation a blunt end (Figure 2). However, G-rich single-stranded overhangs are detected on both daughter strands in vivo, suggesting that nucleolytic processing of the C strand occurs just after the completion of telomeric DNA replication. In budding yeast, the single-stranded G-rich overhangs are 12–14 bp long during most of the cell cycle but become longer in late S-phase during telomere replication [75]. The passage of the DNA replication fork is required for the formation of G tails and their elongation during S-phase [76]. G-rich single-stranded overhangs play an essential role in the regulation of telomere length in vivo, since they serve as the binding substrate for both telomerase and the single-stranded telomeric DNA-specific binding protein Cdc13. In cycling cells, Cdk1 activity is necessary to generate cell-cycle-dependent G-rich overhangs, and the inhibition of Cdk1 activity blocks the resection of the C strand [36].

Recent results have shown that the MRX/NBS1 (Nijmegen breakage syndrome 1) (MRX) complex is preferentially recruited to leading-strand telomeres and that this recruitment is necessary for the binding of both the CST complex and the telomerase complex to the leading-strand telomeres during S-phase. In contrast, binding of the CST and telomerase complexes to lagging-strand telomeres is not dependent on the MRX complex [77]. These results are consistent with the idea that leading-strand telomeric ends require nucleolytic processing for G tail formation, while lagging-strand telomeres acquire G tails due to incomplete replication of the telomeric terminus (Figure 2).

The MRX complex, together with other helicases and nucleases, is thought to co-ordinate nucleolytic resection of double-stranded DNA ends to generate 3' overhangs. Generation of these 3' single-stranded overhangs is a critical step in both DNA recombinational repair and telomeric G tail formation. Although the multiple mechanisms mediating nucleolytic end resection are not fully understood, recent work in budding yeast suggests that the MRX complex co-ordinates with Sae2 to generate short 3' terminal overhangs. More extensive end resection is then mediated by several pathways dependent on Exo1 or Sgs1/Dna2 [78,79].

How telomere end processing is coupled to telomeric DNA replication during cell cycle progression remains to be addressed. Cell-cycle-dependent recruitment of the MRX complex to telomeres has been well documented in budding yeast [80]. In addition, the MRX complex associates with the human double-stranded telomeric DNA-binding protein TRF2 in a cell-cycle-dependent manner. Thus MRX complex recruitment likely underlies the cell-cycle-dependent resection of leading strand telomeres. Since Cdk1 kinase activity is necessary for G tail formation in yeast, further identification of Cdk1 substrates in the MRX complex and other relevant nucleases and helicases will shed light on the cell-cycle-regulated G tail formation in both yeast and humans.

CELL-CYCLE-DEPENDENT RECRUITMENT OF TELOMERASE COMPLEX TO TELOMERES

The recruitment of the telomerase complex to telomeres in budding yeast depends on Cdc13, which binds the single-stranded TG1−3 3' telomere overhang [23]. Cdc13 plays a major role in the recruitment of telomerase complexes to telomeres [26,28,29,31−33,81]. Association of Cdc13 with Est1 is essential for recruitment of the telomerase holoenzyme, which contains the protein catalytic subunit Est2 and the integral RNA template TLC1, as well as Est1 and Est3 [26]. Est1 binds TLC1 directly via a bulged stem RNA structure [82], and it also has weak binding affinity for telomeric single-stranded G-rich overhangs [33]. Est3 has been shown to associate with Est2 and TLC1, although its function remains unclear [81]. While in vitro addition of telomeric DNA only requires Est2 and TLC1, deletion of EST1, EST2, EST3 or TLC1 results in telomere shortening and senescence in vivo indicating that Est1 and Est3 are essential for proper telomerase function.

Telomerase does not appear to act on every telomere in each cell cycle in budding yeast, and the frequency of elongation of a given telomere is inversely related to its length [54]. Cdc13 also interacts with the Sln1—Ten1 protein complex, which plays an essential role in telomere end protection and negatively regulates telomere elongation [83]. Recent results have shown that the CST complex functions as a telomeric-specific RPA-like complex [42−44]. Unlike RPA, which is a non-specific
Figure 2 Telomere end processing during telomere DNA replication

In budding yeast, the replication of telomeric DNA initiates from a sub-telomeric DNA replication origin and proceeds toward the tip of the chromosome. The replication of the G-rich strand (G strand) is therefore accomplished by lagging strand synthesis, resulting in a 3′ single-stranded DNA overhang after the removal of the RNA primer from the terminal Okazaki fragment. In contrast, the C-rich strand (C strand) is replicated by leading strand synthesis, resulting in the generation of a blunt end. The 3′ G-rich ssDNA tail is essential for binding Cdc13, the sequence-specific single-stranded telomere DNA-binding protein, which is necessary for telomere protection by the CST complex as well as telomere elongation by the telomerase complex. Therefore the blunt end generated by leading strand synthesis requires further processing to generate a 3′ single-stranded overhang. This process requires the MRX/Nbs1 (MRX) complex, along with Sgs1/Dna2 and/or Exo1.

Single-stranded-DNA-binding complex, the CST complex is specific for single-stranded telomere 3′ overhangs. Hence, Cdc13 tightly regulates telomere elongation through its interactions with both the Est1−Est2−Est3−TLC1 complex and the competing Stn1−Ten1 complex [27]. Since these two complexes counteract each other in terms of telomere length regulation, their recruitment by Cdc13 must be co-ordinated during cell cycle progression to achieve efficient telomere elongation.

Previous results suggested that the assembly of active telomerase complex at telomeres is cell-cycle-regulated [39], but the factors mediating this process were unclear. Data from the Friedman laboratory [84] suggested that Est1 and Est3 only associate with Est2 and TLC1 during late S/G2-phase, which coincides with the timing of telomere elongation by telomerase in vivo. Est1 is unstable during G1 due to proteasome-dependent degradation. Lack of Est1 in G1 also prevents the stable association of Est3 with Est2. These results provide a mechanism for functional assembly of telomerase complex during cell cycle progression. However, overexpression of Est1 in G1−phase to force the assembly of catalytic active telomerase complex fails to elongate telomeres in G1, indicating that other regulatory events associated with S-phase are required for the activation of telomerase at telomeres.

Our recent work has shown that Thr308 of Cdc13 is phosphorylated by Cdk1 in vivo during late S/G2-phase of the cell cycle, the period during the cell cycle that the telomerase complex is recruited to telomeres [38−40]. The Cdk1-dependent phosphorylation of Cdc13 is necessary for efficient recruitment of the
that was independent of the phosphorylation status of Cdc13 [40]. Acids of Stn1 resulted in over-elongation of telomeres, a result consistent with this proposed model, blocking the interaction of Cdc13 with Stn1 inhibiting telomerase-dependent telomere addition [27,85]. Consequently, recruitment of the telomerase complex by Cdc13, thereby interaction of the Stn1 complex with Cdc13 could compete with recruitment of the telomerase complex by Cdc13, thereby inhibiting telomerase-dependent telomere addition [27,85]. Consistent with this proposed model, blocking the interaction of Cdc13 with Stn1—Ten1 complex and the inhibition of telomere elongation. Hence, Cdk1-dependent phosphorylation of Cdc13 co-ordinates cell cycle progression with telomere length homeostasis. Our model is consistent with previous results suggesting that the interaction of the Stn1—Ten1 complex with Cdc13 could compete with recruitment of the telomerase complex by Cdc13, thereby inhibiting telomerase-dependent telomere elongation. These results suggest that when Cdc13 can no longer interact with Stn1, phosphorylation of Cdc13 Thr308 is no longer necessary to promote the interaction between Cdc13 and Est1.

In addition to Cdk1, the Tel1 and Mec1 kinases [homologues of mammalian ATM and ATR (ATM- and Rad3-related)] also play important roles in telomere length maintenance. Tel1 and Mec1 control the DNA damage response in budding yeast through phosphorylation of proteins involved in checkpoint control (such as Rad53) and DNA replication (such as RPA) [86–88]. In budding yeast, Tel1 plays a minor role in the cellular response to DNA damage, but a major role in telomere length maintenance. In contrast, Mec1 is the key mediator of the DNA damage response, but plays a lesser role at telomeres. Loss of Tel1 results in telomere shortening [89], whereas tel1 mec1 double mutants have a senescence phenotype [90]. Consistent with this observation, Tel1 and Mec1 are required for the normal association of Est1 and Est2 with telomeres, suggesting a potential role in promoting the recruitment of telomerase complex to telomeres [61]. Recent studies have also indicated that Tel1 plays a direct role in the elongation of short telomeres during cell cycle progression. Tel1 kinase activity is crucial for preferential recruitment of telomerase to short telomeres [62–65]. Recent work has shown that Tel1 and Mec1 can phosphorylate Cdc13 at multiple sites in vitro, and mutation of some of these phosphorylation sites also resulted in telomere shortening in vivo [91]. However, whether these potential Tel1/Mec1 phosphorylation sites on Cdc13 are phosphorylated by Tel1/Mec1 in vivo remains to be confirmed. It is conceivable that phosphorylation of Cdc13 by Cdk1 and by Tel1 and/or Mec1 may act synergistically to determine which telomere is elongated by preferential recruitment of a functional telomerase complex during late S/G2-phase of the cell cycle.

Unlike the preferential elongation of short telomeres in budding yeast, recent results from human cancer cells suggested that most of the telomeres are replicated during a single cell cycle [55]. Nevertheless, there is clear evidence of cell-cycle-dependent regulation of telomere maintenance in mammalian cells, although the signalling cascades involved remain largely unknown. Cell-cycle-dependent recruitment of shelterin complexes to telomeres has been well documented [74]. Recruitment of the MRX complex and ATM to telomeres is also cell-cycle-regulated and is required for the processing and formation of telomere end protecting complexes [74]. The Cdk and ATM substrates in human telomere-associated proteins remain largely unknown. Their functions in cell-cycle-dependent telomere elongation and replication in human cells await further characterization.

CONCLUSIONS

Understanding the cell-cycle-dependent regulation of telomere maintenance by telomerase in budding yeast will also provide key insights into the regulation of telomere maintenance in human cells. Such regulatory mechanisms may provide new targets for anti-cancer therapy and for prevention of aging-related diseases.

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