Tel1\textsuperscript{ATM} dictates the replication timing of short yeast telomeres

Carol Cooley, Anoushka Davé, Mansi Garg & Alessandro Bianchi*

Abstract

Telomerase action is temporally linked to DNA replication. Although yeast telomeres are normally late replicating, telomere shortening leads to early firing of subtelomeric DNA replication origins. We show that double-strand breaks flanked by short telomeric arrays cause origin firing early in S phase at late-replicating loci and that this effect on origin firing time is dependent on the Tel1\textsuperscript{ATM} checkpoint kinase. The effect of Tel1\textsuperscript{ATM} on telomere replication timing extends to endogenous telomeres and is stronger than that elicited by Rif1 loss. These results establish that Tel1\textsuperscript{ATM} specifies not only the extent but also the timing of telomerase recruitment.

Keywords DNA replication; origin firing; replication timing; Tel1; telomeres

Subject Categories DNA Replication, Repair & Recombination

DOI 10.15252/embr.201439242 | Received 30 June 2014 | Revised 17 July 2014 | Accepted 23 July 2014 | Published online 13 August 2014

EMBO Reports (2014) 15: 1093–1101

Introduction

The preservation of genome integrity in eukaryotes requires protective nucleoprotein structures at chromosome ends, the telomeres, which are maintained by telomerase, a reverse transcriptase-like ribonucleoprotein responsible for the synthesis of the telomeric DNA repeats. Telomerase acts preferentially at the shortest telomeres, which in yeast are marked for elongation by association with the Tel1\textsuperscript{ATM} kinase [1–3]. Telomerase action is coordinated with conventional DNA replication of the bulk of the telomere [4] taking place from replication forks originating from subtelomeric origins of DNA replication [5,6]. Initiation of DNA replication from a DNA-bound pre-replicative complex made of the origin recognition complex (ORC) and the origin-unwinding hexameric MCM helicase requires CDK and DDK kinase action to promote MCM activation and recruitment of additional factors for replication fork assembly, including the DNA polymerases. In eukaryotic genomes, these events do not take place simultaneously at all origins but follow a controlled programme. In budding yeast, origins (or autonomously replicating sequences—ARSs) have defined DNA sequence requirements, but their activity and timing is affected by chromatin context and histone modifications [7–9].

Although yeast telomeres are among the latest-replicating regions in the genome, this replication pattern is dependent on telomere length and telomeres in the shorter length range are replicated by early-firing subtelomeric origins [10,11]. Even though it has been shown that both Rif1 and the yeast Ku protein (Yku) are required for the late replication of yeast telomeres [11,12], it remains unclear how telomere length acts as a determinant of the timing of origin firing.

Results and Discussion

Induction of a DSB flanked by short telomeric tracts leads to a change in the timing of origin firing at the broken locus

To test whether the replication timing of short telomeres might be related to their transient uncapping [1–3,13], we investigated the behaviour of a double-strand break (DSB) generated in G1 at a late-replicating locus. The DSB was flanked by a short array of telomeric repeats (short-TG hereafter; bearing about 80 bp of yeast telomeric sequences). A long TG-tract was present at the distal end of the break, since a large number of telomeric repeats were required to make the locus late replicating in the absence of cleavage (Supplementary Fig S1A and B). Each array was arranged in the telomere-like orientation towards the free end. We used chromatin immunoprecipitation (ChIP) to assess association of the DSB with the leading-strand DNA polymerase Pol\textit{e} (coded by the POL2 gene), which binds to activated origins and travels with the replication fork. Whereas in the uncleaved locus Pol\textit{e} association at the short TG-tract peaked at 60 min after release (Fig 1A, left), cells that received a DSB displayed a peak at 40 min (Fig 1A, right), indicating that the DSB had caused a shift in the timing of the association of the polymerase with the locus (the 40-min time point, highlighted with a blue bar in all ChIP figures, is indicative of early S phase and coincides with peak binding of the early-firing origin ARS607). When we inserted the TG80-HO-CA250 cassette at a second subtelomeric site, on chromosome V-R, this locus too displayed late S-phase association with Pol\textit{e}; when uncleaved (Fig 1B, left), and a peak of Pol\textit{e} association in early S phase upon DSB formation (Fig 1B, right). Importantly, the shift in Pol\textit{e} binding at the DSB was not observed with the long array (Fig 1C and Supplementary Fig S1B), or at the distal end, which bears the long TG-tract array (Fig 1A and B, right).
These results demonstrate that the introduction of a DSB near short, but not long, arrays of telomeric repeats changes the timing of association of the leading DNA polymerase from late to early S phase.

To demonstrate directly that Pol\(\varepsilon\) association with the DSB was related to origin activation, we analysed replication intermediates by two-dimensional gel electrophoresis. To address the activity of ARS700.5, an origin located in the vicinity of the ADH4 locus [10,14], we analysed this region by 2D gels before and after short-TG DSB formation. In agreement with the ChIP data, in the absence of DSB, we detected a ‘bubble arc’, consistent with origin firing at ARS700.5, late in S phase (Fig 1D, left, top panels), similar to late origin ARS522 (Fig 1D, right). Strikingly, DSB induction led to the appearance of the arc 20 min earlier (Fig 1D, left, bottom panels) and coincident with its appearance at the early origin ARS607 (Fig 1D, middle). The DSB did not affect the timing of the two control origins. These results indicate that recruitment of Pol\(\varepsilon\) to the DSB is related to origin firing and that break formation specifically affects the replication programme of the affected locus. The conclusion is further supported by analysis of Cdc45 binding to the break site (Supplementary Fig S1C). Thus, similarly to short telomeres, DSBs flanked by short TG-tracts led to a change in the activation time of origins of DNA replication from late to early S phase.

In the distal fragment severed by the DSB at VII-I, a (likely unique) replication origin is located in the subtelomeric X element. Interestingly, a construct with a TG250-HO-CA80 cassette did not lead to early replication at either DSB end (Supplementary Fig S1D), suggesting that the endogenous telomere in this fragment (which is much closer to the origin than the DSB end is) might exert a repressive effect.

The shift in replication timing at a DSB flanked by short telomeric arrays requires Tel1\(^{ATM}\)

Because short-TG DSBs, like short telomeres and unlike long-TG DSBs, recruit high levels of Tel1 [1,2,10,15], we sought to determine whether the kinase might be required for the change in the origin
firing programme at the short-TG DSB. When we monitored PolE recruitment at this locus in a strain lacking Tel1, we failed to observe the early S phase peak of association with the proximal end of the break (Fig 2A, top). We similarly could not detect a significant level of PolE association in early S at this locus in cells lacking the C-terminal domain of Xrs2 (Fig 2A, middle), which is required for Tel1 localisation to DSBs and telomeres [1]. Finally, the kinase activity of Tel1 was required for the shift in timing of PolE binding (Fig 2A, bottom).

Consistent with the above results, alkaline gel analysis of the replication intermediates obtained from cells released into hydroxyurea (HU), which specifically suppresses late origins, detected firing
at ARS700.5 in the presence of a DSB, but not in its absence (Fig 2B, top left). Early and late origins ARS305 and ARS522, respectively, served as controls (Fig 2B, middle and right). A strain lacking Tel1, on the other hand, failed to produce replication intermediates at ARS700.5 even in the presence of cleavage (Fig 2B, bottom left). Taken together, these results reveal that the kinase activity of Tel1 is required for the early S-phase activation of an origin of DNA replication at a DSB flanked by short arrays of telomeric repeats.

The early replication of short yeast telomeres requires Tel1ATM

To generate yeast cells with short unmodified telomeres, we used a catalytically inactive telomerase (Est2) allele. Telomeres in cells bearing this allele will progressively shorten and therefore catalytically inactive telomerase (Est2) allele. Telomeres in cells

Telomere-bound Rif1 determines the replication timing of budding and fission yeast telomeres [11,21] by recruiting protein phosphatase 1 to reverse the action of DDK on the MCM helicase [22–24]. Because Rif1 is an in vivo substrate of the ATM/ATR kinases, in principle Tel1 could act by repressing the origin-suppressing activity of Rif1 at telomeres. However, this view is not supported by a comparison of the replication timing of short and rif1 telomeres: whereas short telomeres and short-TG DSBs displayed early S Polc recruitment at the uncut ade4 locus was not prominent in early S and, as expected, became so upon DSB formation (Fig 2D, middle). These results indicate that early replication timing is a general feature of short yeast telomeres and that the early recruitment of Polc is independent of TG-strand synthesis, both at endogenous telomeres and the short-TG DSB.

Notably, although the MRX complex and Tel1 (which act in a single pathway to regulate telomerase action) have very short telomeres [16–18], cells lacking any of these components do not replicate their short telomeres early (Fig 2A) [19], consistent with our data that Tel1 activity is required for their early replication. To further address this role of Tel1 at endogenous telomeres, we used an inducible form of Tel1, which is repressed in glucose medium and induced by galactose [13]: under conditions of Tel1 repression, Polc telomere association took place in late S (Fig 2E, left), whereas exposure to galactose medium during the G1 arrest dramatically changed the profile of Polc association with the XV-L telomere, shifting it to early S (Fig 2E, right). Interestingly, telomere VI-R was largely refractory to early S-phase association after galactose induction within this first cell cycle, suggesting that telomere-specific effects are also at play.

The short telomeres of cells lacking Yku replicate in early S [11,12,20], raising the possibility that this might be due to their uncapped state and documented increased Tel1 recruitment [2]. In agreement with this idea, while Polc, as expected, peaked in early S at telomeres in yku cells (Fig 2F, left; compare to wild-type in Fig 3A), it associated later in S phase at the VI-R telomere if Tel1 was also absent (Fig 2F, right). The suppression of the early replication timing of telomere XV-L, which is one of the earliest replicating telomeres, was only minor in the double mutant, again pointing to telomere-specific effects. Taken together, these results suggest that Tel1 is required for the early replication of short budding yeast telomeres.

Tel1 acts independently of Rif1 in affecting the replication timing of telomeres

These findings were further confirmed by an analysis of replication intermediates in cultures arrested in HU. Consistent with the ChIP data, no replication intermediates were observed at ARS700.5 at the short-TG DSB in rif1 tel1 cells (Fig 3C, top centre). In addition, in a rif1 strain where a new telomere had been allowed to form and stabilise at the DSB, no replication intermediates were detected (Fig 3C, top right), in agreement with the idea that loss of Rif1 only
lends a relatively minor reprieve to the late replication programme of yeast telomeres.

Taken together, these results do not support a simple model for a role of Tel1 upstream of Rif1 in regulating replication timing. To directly test the hypothesis that Rif1 might be a target of Tel1 in this pathway, we created strains bearing an allele of Rif1 with all 14 serines or threonines in the Mec1ATR/Tel1ATM consensus sites mutated to alanines (rif1-14A). In this mutant strain, the pattern of Polc recruitment to telomeres and to short-TG DSBs was unaltered compared to wild type (Fig 3D), suggesting that putative phosphorylation of Rif1 by Tel1 is not sufficient to relieve the origin-delaying action of Rif1.

Tel1 is needed for the G1 phase association of Cdc45 with an origin located near the short TG-tract DSB

Because the DDK-dependent binding of Cdc45 and Sld3 in G1 is a characteristic of early origins [25], we decided to test whether Tel1...
plays a role in assisting the loading of Cdc45 at an origin flanking the short-TG DSB. For this purpose, we arrested cells in the M phase with nocodazole and then released them into medium containing α-factor and galactose, to prevent exit from G1 while inducing a short-TG DSB in the proximity of a the short-TG DSB. For this purpose, we arrested cells in the M phase. As expected, we detected robust enrichment of Cdc45 in G1, compared to M phase, at the early ARS607, but not at the late ARS1412 (Fig 4, centre and left, respectively). We also failed to observe G1 enrichment for Cdc45 at ARS700.5 in a strain lacking an HO site at this locus (Fig 4, right, green bar); in contrast, introduction of the short-TG DSB at adh4 gave rise to an increase in Cdc45 G1 phase binding at this origin (Fig 4, right, yellow bar). Strikingly, the G1 enrichment in Cdc45 binding at the DSB-flanking origin was lost in the absence of Tel1 (Fig 4, right, grey bar). These results suggest that Tel1 acts to set the firing time of origins by affecting the loading of Cdc45 in the G1 phase. One possibility is that Tel1 acts directly on the MCM helicase to promote Cdc45 recruitment, in a manner analogous to DDK. Interestingly, the association of DDK with MCMs requires priming phosphorylation events, some of which are the result of Mec1/ATR action [26]. A similar mode of action for Tel1 at short telomeres is not currently supported by our analysis of mcm4 and mcm6 mutants lacking the Mec1 sites at their N-termini (Supplementary Fig S2). It is, however, possible that other, possibly redundant, targets of Tel1 exist within the MCM complex.

Requirement of telomeric repeats for replication timing shift at a DSB

The observation that DSBs flanked by short (but not long) arrays of telomeric repeats caused a local change in the timing of origin firing raised the question of whether DSBs lacking telomeric repeats altogether might also lead to such a change. In striking contrast to what was seen at the adh4 locus with the short-TG DSB, in the absence of telomeric repeats we failed to detect early S phase association with the DSB (Fig 5A). This was confirmed at a second TG-less DSB, at the late-replicating ARS1412 locus, about 200 kb from the left telomere of chromosome XIV (Fig 5B). Importantly, the insertion of a short array of telomeric repeats was able to induce the early recruitment of Pol6 also at this internal locus (Fig 5C). Analysis of replication intermediates for these strains confirmed that origin firing in presence of HU took place at the DSB only in the strain with TG-repeats at the break site, at adh4 (Fig 5B, top left) or ARS1412 (Fig 5F, left), but not in strains where the DSB was devoid of TG-repeats, either at adh4 (Fig 5D, left) or ARS1412 (Fig 5E, left). The failure to observe replication intermediates in the TG-less DSBs was not caused by loss of DNA to unchecked resection (Supplementary Fig S3). These results indicate that the shift in the timing of origin firing at DNA breaks is not a general phenomenon but is instead specific for those breaks that are flanked by telomeric repeats, suggesting that this is a unique characteristic of telomeric loci. Conceivably, this might reflect a specific role of Tel1 over Mec1 in the process [15,27,28] or be due to specific characteristics of the chromatin environment at telomeres. We have failed to observe an effect of enzymatically inactive Sir2 histone deacetylase and Tel1/Mec1-dependent phosphorylation of histone H2A on the replication timing of a short-TG DSB (Supplementary Fig S4).

Conclusions

Our work identifies a novel function for Tel1 in modulating the timing of origin firing specifically at telomeres. Recent evidence has revealed that some negative regulators of telomerase (Taz1 in fission yeast; Rif1 and Rif2 in budding yeast) act at least in part by restricting the action of telomerase within the cell cycle [29–31]. We suggest that regulation of replication timing by Tel1 adds another layer to the regulation of telomerase at endogenous telomeres. These findings extend the recognised role of Tel1 in enhancing the action of telomerase at telomeres and demonstrate that this kinase controls not only the extent but also the timing of telomerase telomere association. It will be interesting to determine whether this function of Tel1 in controlling origin activity might be related to the role of ATR in promoting the activation of dormant origins at sites of replication stress in higher eukaryotes. Because the effect of Tel1 is confined to telomeres, it is tempting to speculate that whereas the DDK kinase appears primarily to act directly on replicative factors, Tel1 might act to modify the chromatin context at telomeric origins.

Materials and Methods

Strains and plasmids

All strains were generated in the W303 background (MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 RAD5). A list of the strains used, including those in Supplementary Figures, is reported in Supplementary Table S1. The plasmids used to modify the ADH4, NARI and YER188W loci, at chromosomes VII, XIV and V, respectively, are listed in Supplementary Table S2. Standard budding yeast handling and growth conditions were used. Rich medium was...
YPAD, and drop-out media were made using pre-made mixes from USB.

Induction of HO endonuclease and synchronisation of yeast cultures

To analyse cells in S phase, cells were grown in overnight cultures in the appropriate drop-out SC medium containing raffinose at 30°C. The cultures were diluted into YPA-raffinose and arrested in G1 phase of the cell cycle with 0.025 μM α-factor. Cells were then switched to YPA-galactose for 4 h at 30°C, while maintaining the arrest with 0.025 μM α-factor. Cells were released into S phase in YPAD containing 0.125 mg/ml pronase at 18°C. For alkaline smear analysis, cells were instead released from G1 arrest into S phase in the presence of 200 mM hydroxyurea.

To analyse Cdc45 recruitment in G1, overnight cultures in the appropriate drop-out SC medium containing raffinose were diluted into YPA-raffinose and grown at 30°C until in log phase. Cells were then arrested in G2/M phase of the cell cycle with the addition of 20 μg/ml nocodazole to the media and incubating the cells for 90 min at 30°C. Cells were then washed and released into YPA-galactose and incubated with 0.025 μM α-factor for 4 h at 30°C.

Analysis of replication intermediates

Analysis of DNA replication intermediates was performed by 2D gel electrophoresis. DNA was prepared using Qiagen genomic prep columns and the DNA was digested with either XmnI (to analyse 5.2 kb ARS700.5 fragment and 6.5 kb ARS522 fragment) or PstI (to analyse 7.0 kb ARS607 fragment). Probes were prepared by PCR using oligos DO958/959 (ARS700.5), DO1272/1279 (ARS607), and DO1275/1276 (ARS522) (see Supplementary Table S3). For analysis of DNA replication intermediates by alkaline agarose gel electrophoresis probes used were the same as for 2D gels, except that ARS305

Figure 5. Telomeric repeats are required for early S-phase origin firing at a DSB.

A CHIP analysis of Polɛ binding to a DSB at the adh4 locus, devoid of TG-repeat sequences. The same strain was either induced (right) or not (left) for HO cleavage by using glucose or galactose during G1 arrest, respectively.

B Similar analysis to (A) but in a strain where the HO cut was made at an internal, and late replicating, locus near ARS1412. This DSB was also lacking adjacent TG-repeats.

C Similar analysis to (A) but in a strain where the HO cut was made at an internal, and late replicating, locus near ARS1412.

D–F Analysis of replication intermediates by alkaline gel electrophoresis as in Fig 2B. Strain in (D) is same as in (A), (E) same as in (B) and (F) same as in (C).
was probed with a PCR product obtained with oligos DO1787/DO1788.

ChiP

ChiP was performed as described in Supplementary Methods. Immunoprecipitations were carried out with anti-Myc 9E10 (supernatant from a 9E10 hybridoma cell-line) against C-terminally Myc-tagged proteins or with anti-Flag antibody (Sigma M2 antibody, F3165) against C-terminally Flag-tagged proteins and ProteinG Dynabeads (Invitrogen). Quantitation of immunoprecipitated DNA was obtained by real-time PCR using SYBR Green detection on a Roche Light Cycler 480 II instrument and expressed as per cent of starting (input) material. Primers used are listed in Supplementary Table S3.

Supplementary information for this article is available online: http://emboj.embopress.org

Acknowledgements

We wish to thank members of the Bianchi lab, Eva Hoffmann and David Shore for critical reading of the manuscript. We thank David Shore, Maria Pia Longhese, Carol Greider, Jessica Downs and Danesh Moazed for the gift of plasmids and strains. We are grateful to Anne Donaldson for the exchange of results prior to publication. This work was supported by grants from the Medical Research Council (GO701428) and Cancer Research UK (C28567/A12720) to AB.

Author contributions

CC, AD and MG performed experiments. CC, AD and AB analysed data. CC and AB designed experiments. AB designed the study and wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Sabourin M, Tuzon CT, Zakian VA (2007) Telomerase and Tel1p preferentially associate with short telomeres in S. cerevisiae. Mol Cell 27: 550 – 561
2. Hector RE, Shtofman RL, Ray A, Chen BR, Nyun T, Berkner KL, Runge KW (2007) Tel1p preferentially associates with short telomeres to stimulate their elongation. Mol Cell 27: 851 – 858
3. Bianchi A, Shore D (2007) Increased association of telomerase with short telomeres in yeast. Genes Dev 21: 1726 – 1730
4. Dionne I, Wellinger RJ (1998) Processing of telomeric DNA ends requires the passage of a replication fork. Nucleic Acids Res 26: 5365 – 5371
5. McCarroll RM, Fangman WL (1988) Time of replication of yeast centromeres and telomeres. Cell 50: 505 – 513
6. Makovets S, Herskovitz I, Blackburn EH (2004) Anatomy and dynamics of DNA replication fork movement in yeast telomeric regions. Mol Cell Biol 24: 4019 – 4031
7. Friedman KL, Diller JD, Ferguson BM, Nyland SV, Brewer BJ, Fangman WL (1996) Multiple determinants controlling activation of yeast replication origins late in S phase. Genes Dev 10: 1595 – 1607
8. Stevenson JB, Gottschling DE (1999) Telomeric chromatin modulates replication timing near chromosome ends. Genes Dev 13: 146 – 151
9. Vogelauer M, Rubbi L, Lucas I, Brewer BJ, Grunstein M (2002) Histone acetylation regulates the time of replication origin firing. Mol Cell 10: 1223 – 1233
10. Bianchi A, Shore D (2007) Early replication of short telomeres in budding yeast. Cell 128: 1051 – 1062
11. Lian HY, Robertson ED, Hirage S, Alvino GM, Collingwood D, McCune HJ, Sridhar A, Brewer BJ, Rughuraman MK, Donaldson AD (2011) The effect of Ku on telomere replication time is mediated by telomere length but is independent of histone tail acetylation. Mol Biol Cell 22: 1753 – 1765
12. Cosgrove AJ, Nieduszynski CA, Donaldson AD (2002) Ku complex controls the replication time of DNA in telomere regions. Genes Deu 16: 2485 – 2490
13. Viscardi V, Baroni E, Romano M, Lucchini G, Longhese MP (2003) Sudden telomere lengthening triggers a Rad53-dependent checkpoint in Saccharomyces cerevisiae. Mol Biol Cell 14: 3126 – 3143
14. Siow CC, Nieduszynska SR, Muller CA, Nieduszynski CA (2012) OniDB, the DNA replication origin database updated and extended. Nucleic Acids Res 40: D682 – D686
15. Hirano Y, Fukunaga K, Sugimoto K (2009) Rif1 and Rif2 inhibit localization of tel1 to DNA ends. Mol Cell 33: 312 – 322
16. Lustig AJ, Petes TD (1986) Identification of yeast mutants with altered telomere structure. Proc Natl Acad Sci USA 83: 1398 – 1402
17. Nugent Cl, Bosco G, Ross LO, Evans SK, Salinger AP, Moore JK, Haber JE, Lundblad V (1998) Telomere maintenance is dependent on activities required for end repair of double-strand breaks. Curr Biol 8: 657 – 660
18. Ritchie KB, Petes TD (2000) The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. Genetics 155: 475 – 479
19. Goursouzian LK, Tuzon CT, Zakian VA (2006) S. cerevisiae Tel1p and Mre11p are required for normal levels of Est1p and Est2p telomere association. Mol Cell 24: 603 – 610
20. Fisher TS, Taggart AK, Zakian VA (2004) Cell cycle-dependent regulation of yeast telomerase by Ku. Nat Struct Mol Biol 11: 1198 – 1205
21. Hayano M, Kanoh Y, Matsumoto S, Renard-Guillet C, Shirahige K, Masai H (2012) Rif1 is a global regulator of timing of replication origin firing in fission yeast. Genes Deu 26: 137 – 150
22. Davé A, Cooley C, Garg M, Bianchi A (2014) Protein phosphatase 1 recruitment by Rif1 regulates DNA replication origin firing by counteracting DDR activity. Cell Rep 7: 53 – 61
23. Mattarocci S, Shiyian M, Lemmens L, Damay P, Altintas DM, Shi T, Bartholomew CR, Thoma NH, Hardy CF, Shore D (2014) Rif1 controls DNA replication timing in yeast through the PP1 phosphatase Gic7. Cell Rep 7: 62 – 69
24. Hiraga S-I, Alvino M, Chang F, Lian H-Y, Sridhar A, Kubota T, Brewer J, Weinreich M, Rughuraman K, Donaldson D (2014) Rif1 controls DNA replication by directing Protein Phosphatase 1 to reverse Cdc7-mediated phosphorylation of the MCM complex. Genes Deu 28: 372 – 383
25. Aparicio OM, Stout AM, Bell SP (1999) Differential assembly of Cdc45p and DNA polymerases at early and late origins of DNA replication. Proc Natl Acad Sci USA 96: 9130 – 9135
26. Randell JC, Fan A, Chan C, Francis Li, Heller RC, Galani K, Bell SP (2010) Mec1 is one of multiple kinases that prime the Mcm2-7 helicase for phosphorylation by Cdc7. Mol Cell 40: 353 – 363
27. McGee JS, Phillips JA, Chan A, Sabourin M, Paeschke K, Zakian VA (2010) Reduced Rif2 and lack of Mec1 target short telomeres for elongation rather than double-strand break repair. Nat Struct Mol Biol 17: 1438 – 1445
28. Clerici M, Trovesi C, Galbiati A, Lucchini G, Longhese MP (2014) Mec1/ATR regulates the generation of single-stranded DNA that attenuates Tel1/ATM signaling at DNA ends. EMBO J 33: 198 – 216
29. Chang Y-T, Moser BA, Nakamura TM (2013) Fission yeast shelterin regulates DNA polymerases and Rad3\textsuperscript{ATR} kinase to limit telomere extension. PLoS Genet 9: e1003936

30. Dehé PM, Rog O, Ferreira MG, Greenwood J, Cooper JP (2012) Taz1 enforces cell-cycle regulation of telomere synthesis. Mol Cell 46: 797–808

31. Gallardo F, Laterreur N, Cusanelli E, Ouenzar F, Querido E, Wellinger RJ, Chartrand P (2011) Live cell imaging of telomerase RNA dynamics reveals cell cycle-dependent clustering of telomerase at elongating telomeres. Mol Cell 44: 819–827

License: This is an open access article under the terms of the Creative Commons Attribution 4.0 License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.