

Telomere and Telomerase Modulation by the Mammalian Rad9/Rad1/Hus1 DNA-Damage-Checkpoint Complex

Sofia Francia,^{1,5} Robert S. Weiss,^{2,5,*}
M. Prakash Hande,³ Raimundo Freire,⁴
and Fabrizio d'Adda di Fagnana^{1,*}

¹Italian Foundation for Cancer Research
Institute of Molecular Oncology Foundation (IFOM)
20139 Milan
Italy

²Department of Biomedical Sciences
Cornell University
Ithaca, New York 14853

³Department of Physiology
Yong Loo Lin School of Medicine
National University of Singapore
Singapore 117597
Singapore

⁴Unidad de Investigacion
Hospital Universitario de Canarias
38320 Tenerife
Spain

Summary

Telomeres, the termini of linear chromosomes, are exceptional in that they are DNA ends that do not normally trigger a DNA-damage response (DDR) and are compatible with normal cellular proliferation. Mammalian telomeres are nevertheless a physiological substrate of the DDR apparatus, as shown by the fact that the inactivation of genes encoding certain DDR factors results in telomere dysfunction [1–3]. However, how DDR factors are integrated with telomere physiology, including telomere length regulation by the specialized reverse transcriptase telomerase, is still largely unclear. Here we report that the mammalian Rad9/Rad1/Hus1 (911) checkpoint complex, which localizes to sites of genome damage and promotes DDR signaling [4], is an integral component of the telomere in human and mouse cells. By the use of quantitative telomere-length measurements, we demonstrate severe telomeric shortening in both *Hus1*-deficient mouse embryonic fibroblasts and thymocytes from conditional *Hus1*-knockout mice. We also show that 911 is found in association with catalytically competent telomerase in cell lysates and is a positive regulator of its DNA polymerase activity. These findings identify an unanticipated function for the 911 checkpoint complex at telomeres in mammals and provide a mechanistic link between the activity of DNA-damage-checkpoint proteins and the telomere-maintenance machinery.

Results and Discussion

911 Is a Novel Component of the Mammalian Telomere

To test whether components of the 911 complex are associated with the human telomere, we performed chromatin immunoprecipitation (ChIP) assays, a technique that allows detection of the association of proteins with chromatin at specific genomic sites [5]. Figure 1A shows that a panel of antibodies raised against different portions of the individual subunits of this complex [6, 7] all immunoprecipitated consistent amounts of telomeric DNA as determined by dot-blot hybridization, whereas the corresponding preimmune sera did not. By contrast, when the same membrane was stripped and hybridized with an Alu DNA probe, as a measure of nonspecific DNA binding, no difference was detected between the signal generated by the immune versus preimmune sera. Quantification of the percentage of telomeric DNA immunoprecipitated and the incremental ratio between the signal generated by the telomeric and Alu probes demonstrated that the 911 complex was specifically associated with telomeric DNA. The association of 911 with telomeres required crosslinking prior to cell harvesting (Figure 1A and data not shown), indicating that this interaction probably occurred *in vivo* and was not the consequence of *in vitro* manipulation. In addition, increasing amounts of antibodies against the 911 components brought down increasing amounts of telomeric DNA, up to the saturation point (Figure S1A in the Supplemental Data available with this article online). An additional set of experiments performed with immunopurified antibodies confirmed the results obtained with crude sera (Figure S1B). The robustness and specificity of our approach was validated in two additional ways: precipitation of telomeric DNA by anti-Hus1 antibodies was eliminated when the ChIP assays were done with chromatin extracted from *Hus1*-deficient cells (Figure S1C), and antibodies raised against HUS1 and TRF1, a telomeric DNA binding protein, or CENP-A, a specific centromeric DNA-associated factor, preferentially immunoprecipitated telomeric DNA or centromeric DNA, respectively, but not the reciprocal or Alu DNA sequences (Figure 1B).

Telomeric DNA is generally a relatively poor replication template, causing DNA replication forks to stall more frequently at these than at other regions of the genome [8, 9]. Because the 911 complex has been shown to respond to DNA damage during S phase [10], we tested whether the association of 911 with telomeric DNA was dependent on ongoing DNA replication by carrying out a set of ChIP assays with chromatin prepared from human diploid fibroblasts (HDFs) that were either proliferating or in a quiescent, noncycling state induced by contact inhibition (Figure 1C). We observed that 911 also associated with telomeres in nondividing cells, indicating that its association is independent of telomeric-DNA replication. In addition, we observed that 911

*Correspondence: rsw26@cornell.edu (R.S.W.); fabrizio.dadda@ifom-ieo-campus.it (F.d'A.d.F.)

⁵These authors contributed equally to this work.

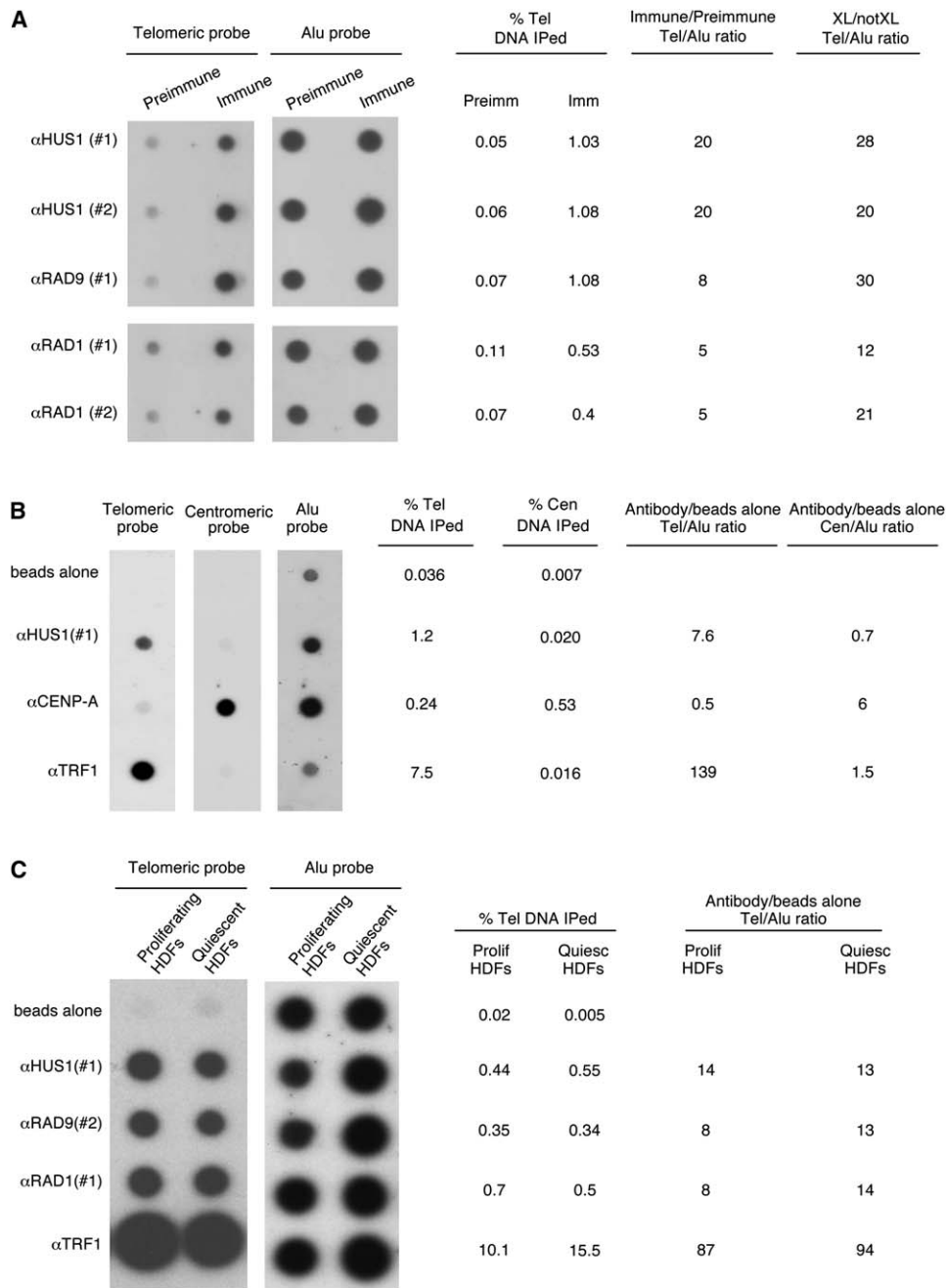


Figure 1. The 911 Complex Is a Novel Component of Human Telomeres

(A) Chromatin immunoprecipitations were performed in HeLa cells by means of a panel of matched preimmune and immune sera raised against the individual factors HUS1, RAD9, or RAD1 (numbers in parentheses indicate individual immunized rabbits). DNA was spotted onto a membrane and sequentially hybridized with telomeric or Alu DNA probes. Values for percent of telomeric DNA immunoprecipitated (first two columns) were calculated by dividing the ChIP signal by the signal for a known amount of input chromatin spotted directly onto the membrane. Values shown in the third column represent the calculated fold increase of the telomeric DNA signal of the immune over the preimmune sera immunoprecipitations upon normalization with the Alu DNA probe. Values shown in the far right column represent the ratio between values shown in the third column and those obtained in an identical set of immunoprecipitations with chromatin that was not crosslinked (data not shown).

(B) In HeLa cells, antibodies against HUS1 and TRF1, a telomeric DNA binding protein, or against CENP-A, a centromeric-associated protein, preferentially immunoprecipitate telomeric or centromeric DNA, respectively.

(C) 911 complex is associated with telomeric DNA independently from DNA replication and proliferation in HDFs. HDFs were synchronized by contact inhibition and subjected to ChIP as described above. Lack of DNA replication was monitored by BrdU incorporation (data not shown).

association with telomeres was also detectable in other cell types, including transformed and primary cells, as well as in cells that maintain their telomeres through ALT (alternative lengthening of telomeres) mechanisms,

consistent with a previous report [11] (data not shown). From these results, we therefore conclude that 911 is a novel component of human and mouse telomeres in different cell types.

Hus1 Regulates Telomere Length

Deletion of mouse *Hus1* results in embryonic lethality, and *Hus1* null fibroblasts grow extremely poorly in culture [10]. However, inactivation of *p21* allows the growth in culture of *Hus1*-deficient mouse embryonic fibroblasts (MEFs) and thereby permits us to determine the function of *Hus1* in telomere homeostasis. Notably, we observed that immortalized *Hus1*^{Δ1n/Δ1n}*p21*^{-/-} MEFs, but not *Hus1*^{+/+}*p21*^{-/-} MEFs, progressively accumulated chromosomal aberrations, including chromosome fusions (Figure S2A). Analysis of 20 metaphases from two independent *Hus1*^{Δ1n/Δ1n}*p21*^{-/-} MEF cultures revealed an average 0.4 and 0.6 chromosome fusions per cell, whereas no chromosomal fusions were identified in matched *Hus1*^{+/+}*p21*^{-/-} MEF cultures. Fluorescence in situ hybridization (FISH) was performed with a telomere-specific probe to determine whether the junction between the fused chromosomes retained telomeric sequences. Two out of seven junction sites from chromosomal fusions identified in *Hus1*^{Δ1n/Δ1n}*p21*^{-/-} MEFs contained telomeric sequences (Figure S2B). Consistent with previous results from analysis of primary *Hus1*^{Δ1n/Δ1n} MEFs [10], chromatid gaps and breaks were also much more common in *Hus1*^{Δ1n/Δ1n}*p21*^{-/-} MEFs (0.9 or 0.6 gaps and breaks per cell in two independent cultures) than in *Hus1*^{+/+}*p21*^{-/-} MEFs (0.25 or 0.19 gaps and breaks per cell).

Telomere fusions can result from telomere shortening. We therefore tested whether *Hus1* inactivation impacts telomere length regulation by using quantitative fluorescence in situ hybridization (qFISH), a technique that detects and quantifies the signal generated by hybridization of a fluorescent probe complementary to TTAGGG telomeric repeats at individual chromosome ends at the single-cell level [12, 13]. Strikingly, when we examined telomere length in primary (passage 0) *Hus1*^{+/+}, *Hus1*^{+/Δ1n}, or *Hus1*^{Δ1n/Δ1n} MEFs (Figure 2A), we observed that *Hus1*-deficient cells exhibited a dramatic loss of nearly half of their telomere length (48 kb telomere length in *Hus1*^{+/+} cells and 27 kb in *Hus1*^{Δ1n/Δ1n} cells). Heterozygous *Hus1*^{+/Δ1n} cells also displayed a significant reduction (~7 kb) of telomeric repeats when compared to wild-type cells, demonstrating a haploinsufficiency of *Hus1* in telomere length control. Without exception, *Hus1*^{Δ1n/Δ1n} cells had shorter telomeres than *Hus1*^{+/+} cells, and *Hus1*^{+/Δ1n} cells had telomeres of intermediate length. Further analysis of the distribution of telomere fluorescence demonstrated that telomere shortening involved the bulk of telomeres rather than a subset of them. Additional analyses of these primary cells revealed no end-to-end fusions in 26 *Hus1*^{+/+} metaphase spreads and only one in 59 spreads from heterozygotes, but 5 end-to-end fusions were identified in 30 *Hus1*^{Δ1n/Δ1n} metaphase spreads. Two of these lacked telomeric sequences at the junction, while the remaining three junctions had an average length of 41 kb of telomeric DNA.

To ascertain that the telomere maintenance defect observed in *Hus1*-deficient fibroblasts was not exclusive to cultured cells or restricted to a specific genetic background or technique, we performed additional telomere length measurements on primary thymocytes derived from a conditional *Hus1* knockout model in which mice carrying the conditional *Hus1* allele *Hus1*^{lox} were

bred to *Lck-cre* transgenic mice in order to achieve T cell-specific inactivation of *Hus1* [14]. Southern blot analyses confirmed the efficient T cell-specific deletion of *Hus1* in this system (Figure S3). Thymocytes from the same mice were also analyzed by flow-FISH, a technique that measures the total amount of telomeric DNA in large numbers of permeabilized intact cells in interphase [15] (Figure 2B). Results from the analysis of mice from four independent litters demonstrated that complete *Hus1* inactivation was associated with severe telomere shortening, with an average loss of 45% of total telomeric signal. We therefore conclude that both constitutive and inducible inactivation of *Hus1* leads to dramatic telomere shortening that is detectable in different tissues by two independent techniques.

Since yeast orthologs of 911 components have been shown to affect telomere resection [16], we also tested whether telomere structure was compromised in early-passage *Hus1*^{Δ1n/Δ1n}*p21*^{-/-} MEFs or in unpassaged thymocytes derived from the conditional *Hus1* knockout model [14]. However, we found no significant differences in the presence of the single-strand telomeric overhang between wild-type and *Hus1*-deleted cells (Figure S4A). Furthermore, we found no evidence that the lack of *Hus1* leads to telomere uncapping. Uncapping triggers a DDR at telomeres that can be detected by ChIP assay with antibodies directed against phosphorylated histone H2AX (γ-H2AX) or against the consensus phosphorylation site of the ATM and ATR kinases (pS/TQ) [17–19]. There was no significant association of these DDR markers with telomeric DNA in *Hus1*-deficient MEFs (Figure S4B). In a parallel control experiment performed in human cells, telomere uncapping induced by the expression of a dominant-negative allele of TRF2 [20] triggered a DDR at the telomere (Figure S4C). These data suggest that telomeres remained capped in the absence of *Hus1*, although we cannot rule out the possibility that 911 itself may be necessary for DNA-damage signaling from uncapped telomeres. We conclude that lack of *Hus1* does not lead to detectable alterations to telomere structure but does have a profound effect on telomere length.

911 Interacts with Telomerase and Affects Its Activity

The 911 complex shows structural similarities with PCNA, a ring-shaped factor that associates with proteins involved in DNA replication and repair [4]. We therefore looked for evidence of a biochemical interaction between the 911 complex and telomerase, the enzyme that mediates telomere replication in mammals [12]. A Flag-tagged version of the 911 complex was transiently expressed in cultured cells, and the ability of anti-Flag antibodies to immunoprecipitate telomerase activity was monitored by TRAP assay, a method that measures the *in vitro* catalytic activity of telomerase in cell extracts. Figure 3A (left) shows that an anti-Flag immunoprecipitation brought down high levels of telomerase activity from extracts of cells expressing Flag-tagged 911. Importantly, the interaction between 911 and telomerase was specific, as a Flag peptide, but not an unrelated peptide, significantly reduced the amount of telomerase activity brought down. Furthermore, when we tested whether endogenous 911 was associated with active telomerase, we found that affinity-purified

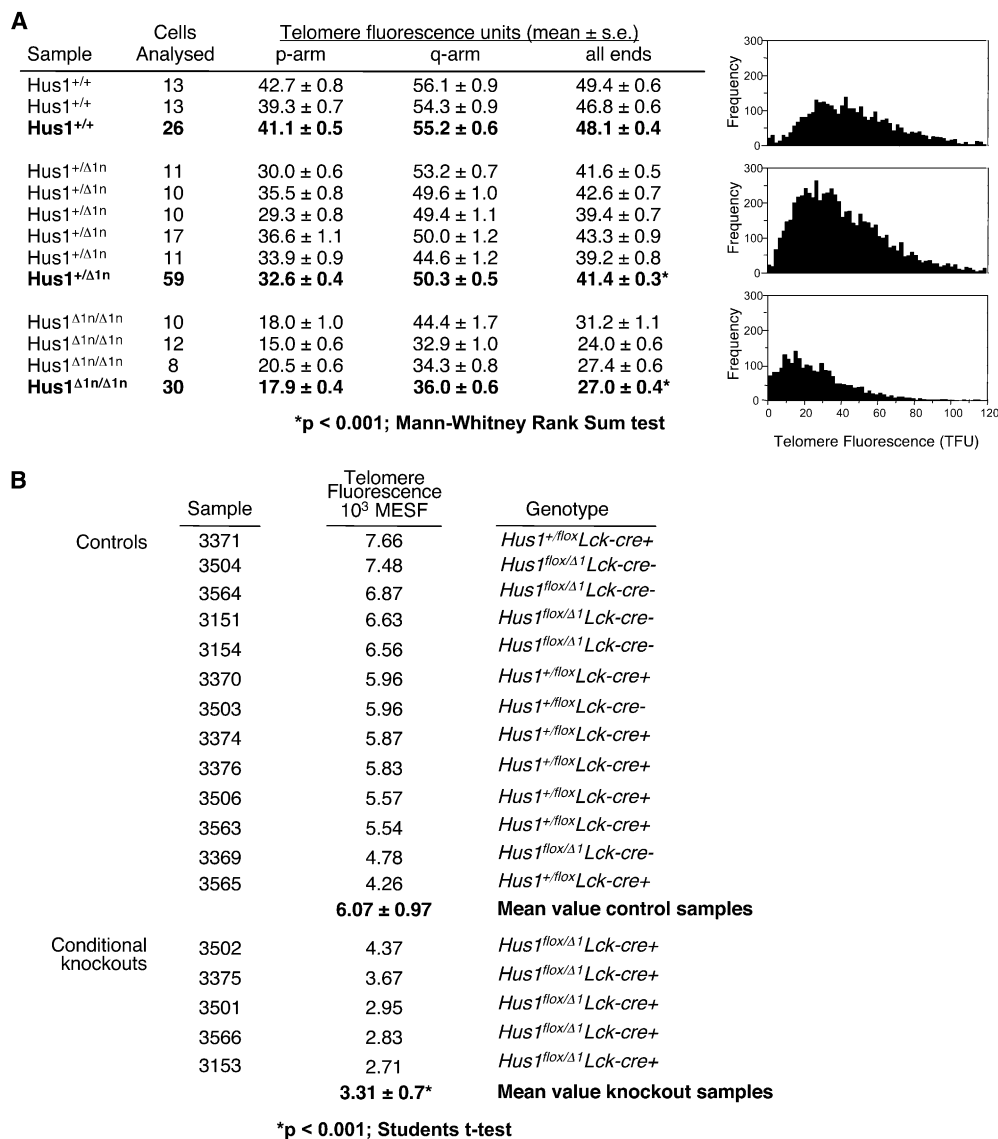


Figure 2. Loss of *Hus1* Leads to Telomere Shortening

(A) Results from qFISH analysis of telomere length in primary MEFs obtained from two independent litters generated from heterozygous crosses are shown. One TFU (telomere fluorescence unit) corresponds to 1 kb of TTAGGG repeats. Results are shown for p-arm telomeres, q-arm telomeres, and all ends. Values are expressed as mean \pm standard error. The mean value for each genotype is indicated in bold font. Histograms describe the frequency distribution of qFISH values for the three different genotypes. The horizontal axis shows the intensity of each signal, and the vertical axis shows the frequency of telomeres of a given length.

(B) Results from telomere length analysis by flow-FISH of uncultured thymocytes are shown. Values are expressed in thousands of molecules of equivalent soluble fluorochrome (MESF). Values are reported next to their genotype. Thymocytes from *Hus1^{flox/ Δ 1}Lck-cre⁺* (conditional knockout), *Hus1^{flox/ Δ 1}Lck-cre⁻* (control), *Hus1^{+/flox}Lck-cre⁻* (control), and *Hus1^{+/flox}Lck-cre⁺* (control) experimental animals were analyzed. The mean value \pm standard error for conditional *Hus1* knockout and control samples is indicated in bold font. The statistical significance of telomere differences between genotypes is described by the calculated p values.

antibodies raised against HUS1, RAD1, or RAD9 consistently immunoprecipitated significant amounts of telomerase activity (Figure 3B, top). Preincubation of the anti-RAD1 antibody with a RAD1 peptide eliminated the coimmunoprecipitation of telomerase activity, suggesting that the interaction was specific. Additional experiments with crude preimmune and immune sera raised against the individual subunits of 911 confirmed the interaction of 911 with telomerase (Figure S5). Importantly, ATR and the p34 and p70 subunits of RPA, chromatin-associated members of the same DDR

signaling pathway as 911 [21], were not found in association with telomerase activity (Figure 3C), an observation that strongly suggests that the association between 911 and telomerase activity was not merely an indirect association between two DNA bound factors. Taken together, these data reveal that 911 is uniquely found in association with catalytically active telomerase.

We then tested the biological significance of the observed 911-telomerase interaction by studying telomerase catalytic activity in cell extracts generated from *Hus1^{+/+}p21^{-/-}* and *Hus1 ^{Δ 1n/ Δ 1n}p21^{-/-}* MEFs by TRAP

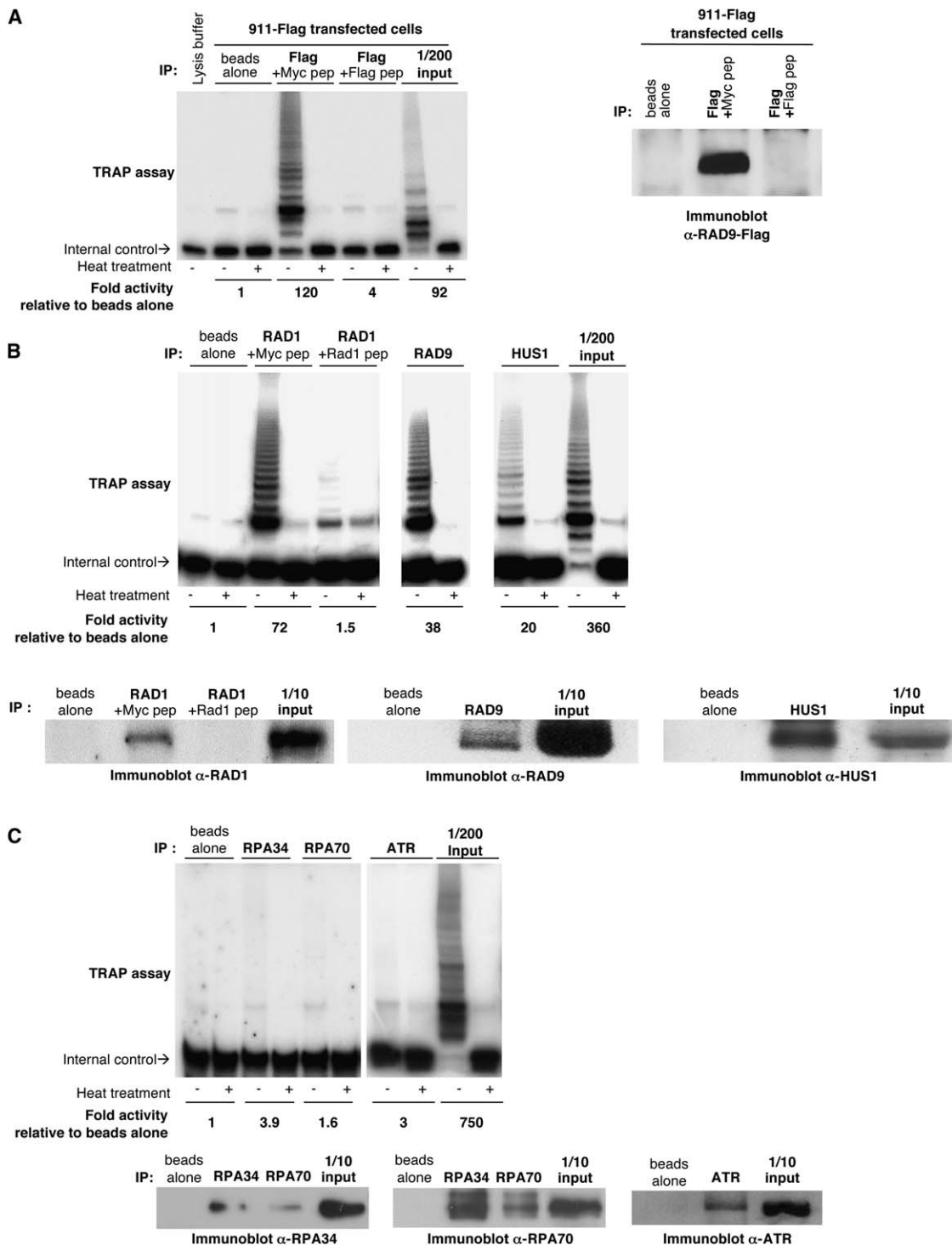


Figure 3. The 911 Complex Is Associated with Catalytically Active Telomerase

(A) FLAG antibody coimmunoprecipitates telomerase activity with 911 from 293T cells transiently transfected with FLAG-tagged Rad9, Rad1, and Hus1, as detected by TRAP assays. A specific competitor peptide, but not an equal amount of an unrelated one, abolished 911 binding to the beads as determined by immunoblotting (right) and strongly reduced immunoprecipitated TRAP activity. TRAP assays were performed on untreated or heat-treated samples. Each lane includes an internal control for PCR amplification. Fold increase relative to activity for beads alone is shown at the bottom.

(B) Affinity-purified antibodies raised against the individual subunits of the 911 complex immunoprecipitate TRAP activity from HeLa cells. A RAD1 peptide against which the antibody was raised, but not an equal amount of an unrelated peptide, specifically competes for RAD1 binding as determined by immunoblotting (bottom) and reduces the amount of immunoprecipitated TRAP activity (top).

(C) Antibodies raised against RPA34, RPA70, and ATR do not coimmunoprecipitate telomerase activity from HeLa cells. Successful immunoprecipitation of RPA34, RPA70, and ATR is demonstrated by immunoblotting (bottom).

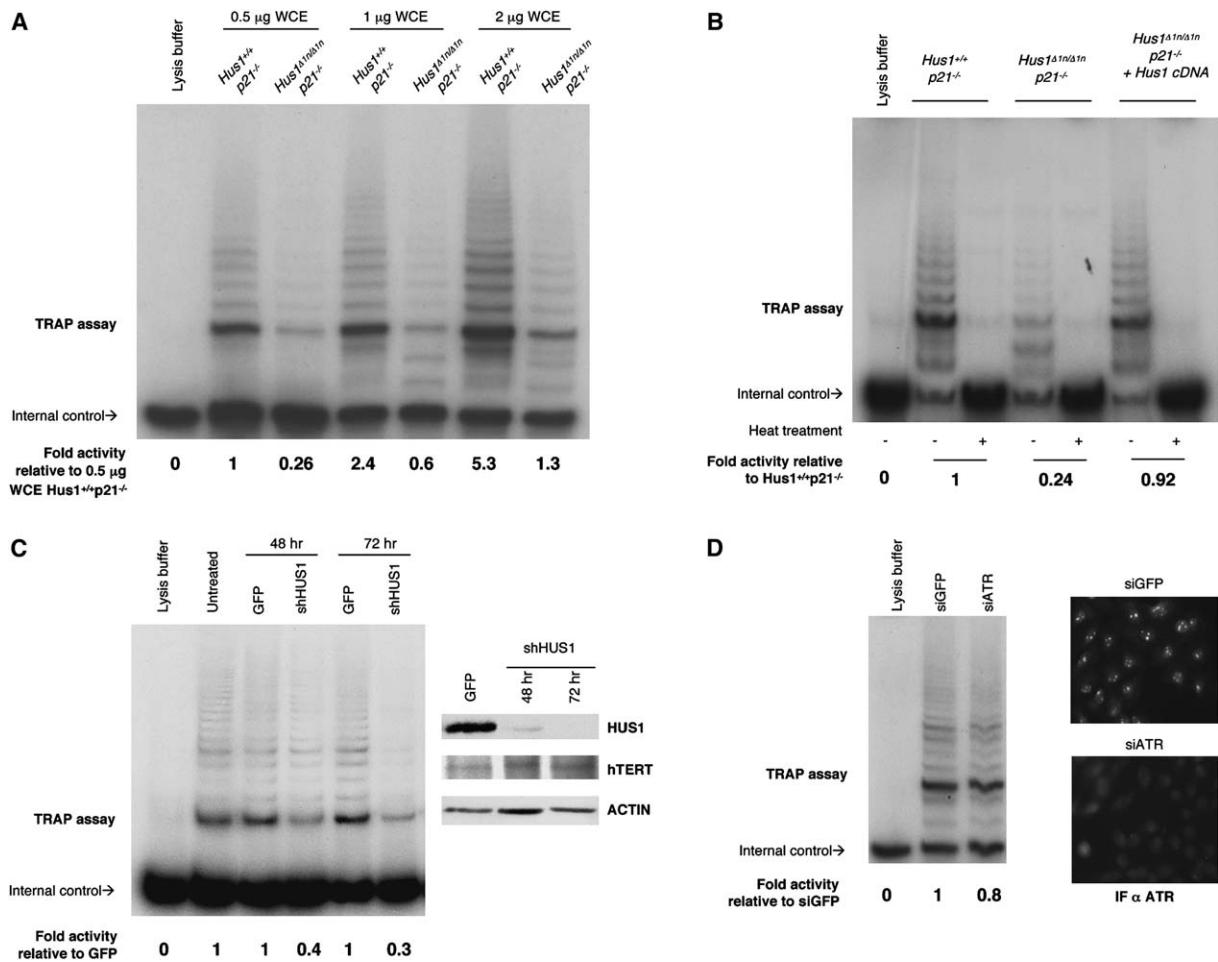


Figure 4. Lack of *Hus1* Impairs In Vitro Telomerase Activity

(A) *Hus1* null cells have reduced telomerase activity compared to *Hus1*-expressing cells over a range of concentrations. Extracts were prepared from cells of the indicated genotype and subjected to analysis by TRAP assay. (B) Expression of a *Hus1* cDNA in *Hus1*-deficient cells rescues telomerase activity defect. *Hus1*^{+/+}p21^{-/-} and *Hus1* $\Delta^{1n/\Delta^{1n}}$ p21^{-/-} MEFs, stably expressing *GFP* or *Hus1* from a retroviral vector, were subjected to TRAP analysis. (C) Expression of short interfering RNAs against *HUS1* in human cells reduces telomerase activity. HeLa cells were infected with lentiviruses encoding *GFP* or shRNAs against *HUS1*, and the resulting extracts were subjected to TRAP analysis at the indicated times post selection. (D) Expression of short interfering RNAs against *ATR* in human cells does not affect telomerase activity. HeLa cells were transfected with siRNAs against *GFP* or *ATR*, and the resulting extracts were subjected to TRAP analysis. Reduced *ATR* expression was confirmed by indirect immunofluorescence analysis in which cells were stained with anti-*ATR* antiserum and DAPI (right).

assay. This analysis revealed a reproducible 3- to 5-fold decrease in telomerase activity in cells lacking *Hus1* (Figure 4A). Importantly, the TRAP assays were carried out within the linear range, and these observations could be reproduced in multiple cell lines generated from independent embryos (data not shown). *Hus1* deficiency did not lead to significant alterations in the expression of core components of telomerase complex as evidenced by similar amounts of TERT protein and TERC RNA in *Hus1*^{+/+}p21^{-/-} and *Hus1* $\Delta^{1n/\Delta^{1n}}$ p21^{-/-} MEFs (Figure S6). The telomerase activity defect in *Hus1* $\Delta^{1n/\Delta^{1n}}$ p21^{-/-} MEFs was specifically due to the absence of *Hus1*, as it was rescued by expression of a *Hus1* cDNA (Figure 4B).

We further extended our observations to human cells by expressing a short hairpin RNA against human *HUS1* and monitoring its effect on *HUS1* protein accumulation and telomerase activity (Figure 4C). *HUS1* knockdown

caused progressive reduction of the ability of telomerase to synthesize telomeric repeats, to the same extent observed in mouse cells. Likewise, reducing expression of 911 component RAD9, or the clamp loader RAD17, by RNA interference also impaired telomerase activity (Figure S7A). These results were specific, as shown by the fact that knockdown of *ATR*, or overexpression of dominant-negative (kinase-dead) *ATR*, did not significantly affect TRAP activity (Figure 4D; also Figure S7B). We therefore conclude that 911 is a positive regulator of in vitro telomerase activity.

In summary, these results show that the 911 complex is a novel constitutive component of the mammalian telomere. One potential mechanism that could lead to the recruitment of 911 to mammalian telomeres is via RPA, because RPA-coated ssDNA at sites of DNA damage promotes loading of 911 by a Rad17-containing clamp-loading complex [22, 23]. However, although

telomeres end with a protruding ssDNA overhang that, at least in budding yeast, is bound by RPA [24], we did not robustly detect RPA70 or RPA34 at human telomeres by ChIP (data not shown). Furthermore, when we used different anti-RAD17 antibodies in ChIP assays, we found them to generate a relatively weak telomere signal (data not shown), suggesting either that RAD17 is not localized to telomeres or that it associates with them in a transient, and therefore hard-to-detect, manner. Consistent with the latter possibility, knockdown of RAD17 expression resulted in reduced telomerase activity, suggesting that it may be required for 911 loading at telomeres. Nevertheless, binding of 911 to telomeres also might involve distinct mechanisms relative to how it associates with sites of DNA damage and could require interactions with other telomeric DNA binding factors, although we did not detect coimmunoprecipitation of 911 with TRF1, TRF2, or hPot1 (data not shown).

Our findings, together with a recent report that *Rad9* inactivation is associated with telomere loss and chromosomal fusions [25], indicate that mammalian 911 regulates telomeres. This appears to be an evolutionarily conserved function for 911, because orthologs of this complex in *Schizosaccharomyces pombe* have been reported to associate with telomeric repeats and to be required for telomere-length maintenance [26]. Furthermore, progressive telomere shortening has been observed in *Caenorhabditis elegans* lacking HUS-1 [27] or MRT-2, a worm ortholog of Rad1 [28]. Interestingly, mutation of *mrt-2* is epistatic with telomerase inactivation [29], a genetic observation that is also in line with our biochemical results of 911 interaction with telomerase in human cell extracts. Notably, the 911 complex was previously shown to interact with and stimulate other DNA polymerases involved in DNA lesion bypass and DNA repair [6, 30, 31], suggesting that 911 may have the more general ability to facilitate the activity of atypical DNA polymerases dealing with discontinuous DNA templates.

911 is the first DDR factor reported to associate with telomerase. Other components of the same DNA-damage-signaling pathway such as RPA70, RPA34, and ATR were not observed to coimmunoprecipitate with telomerase activity. This intriguing observation suggests that 911, although integrated in a DNA-damage-signaling pathway, also has a unique and independent role in telomere maintenance. The observation that *in vitro* telomerase activity is impaired in the absence of 911 suggests that telomere shortening in *Hus1*-deficient cells could be the result of inefficient maintenance of telomeres by telomerase. Notably, the rate of telomere shortening that we observed in *Hus1* null cells is higher than that reported for mice lacking TERC, the RNA component of telomerase, suggesting that additional mechanisms of telomere shortening might be at play in the absence of *Hus1*. It is worth considering that the presence of a poorly processive telomerase on DNA could be more detrimental to telomere stability than not having telomerase at all: a poorly processive polymerase on DNA can cause DNA replication fork stalling, which can lead to fork reversal and hyperrecombination [32]. Therefore, it is possible that in cells lacking *Hus1*, a crucial component of the S-phase checkpoint mechanism, a poorly processive telomerase complex leads to

generation of aberrant DNA structures that can result in the rapid loss of telomeric repeats through recombination [33]. Indeed, both *Hus1* [34] and *Rad9* [25] function in homologous recombinational repair, and defects in this process may further promote telomere instability. Thus, telomere shortening in *Hus1*-deficient cells may be the consequence of a combination of inefficient telomerase activity and DNA-damage-response deficiencies.

Supplemental Data

Supplemental Data include seven figures and Supplemental Experimental Procedures and can be found with this article online at <http://www.current-biology.com/cgi/content/full/16/15/1551/DC1/>.

Acknowledgments

We thank the Q-PCR unit at IFOM for support; R. Abraham, T. de Lange, P. Gasparini, W.C. Hahn, J. Lingner, and A. Musacchio and his lab for sharing reagents; L. Spath for help with some experiments; M. Foiani and members of the d'Adda di Fagagna and Weiss labs for discussions; and Steve Jackson for support in the early phases of this project and advice. F.d'A.d.F. is supported by AIRC, S.F. is a SEMM student (European School of Molecular Medicine), and R.S.W. is supported by National Institutes of Health grant R01 CA108773-01. M.P.H. acknowledges the support from the National University of Singapore and National Medical Research Council, Ministry of Health, Singapore. Dr. Anuradha Poonepalli and Mr. Aik Kia Khaw are thanked for their help during the study.

Received: February 15, 2006

Revised: June 6, 2006

Accepted: June 8, 2006

Published: August 7, 2006

References

1. d'Adda di Fagagna, F., Teo, S.-H., and Jackson, S.P. (2004). Functional links between telomeres and proteins of the DNA-damage response. *Genes Dev.* 18, 1781–1799.
2. Crabbe, L., Verdun, R.E., Haggblom, C.I., and Karlseder, J. (2004). Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306, 1951–1953.
3. Tarsounas, M., Munoz, P., Claas, A., Smiraldi, P.G., Pittman, D.L., Blasco, M.A., and West, S.C. (2004). Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* 117, 337–347.
4. Parrilla-Castellar, E.R., Arlander, S.J.H., and Karnitz, L. (2004). Dial 9-1-1 for DNA damage: the Rad9-Hus1-Rad1 (9-1-1) clamp complex. *DNA Repair (Amst.)* 3, 1009–1014.
5. Orlando, V., and Paro, R. (1993). Mapping Polycomb-repressed domains in the bithorax complex using *in vivo* formaldehyde cross-linked chromatin. *Cell* 75, 1187–1198.
6. Toueille, M., El-Andaloussi, N., Frouin, I., Freire, R., Funk, D., Shevelev, I., Friedrich-Heineken, E., Villani, G., Hottiger, M.O., and Hubscher, U. (2004). The human Rad9/Rad1/Hus1 damage sensor clamp interacts with DNA polymerase {beta} and increases its DNA substrate utilisation efficiency: implications for DNA repair. *Nucleic Acids Res.* 32, 3316–3324.
7. Xiang, S.L., Kumano, T., Iwasaki, S.I., Sun, X., Yoshioka, K., and Yamamoto, K.C. (2001). The J domain of Tpr2 regulates its interaction with the proapoptotic and cell-cycle checkpoint protein, Rad9. *Biochem. Biophys. Res. Commun.* 287, 932–940.
8. Ohki, R., and Ishikawa, F. (2004). Telomere-bound TRF1 and TRF2 stall the replication fork at telomeric repeats. *Nucleic Acids Res.* 32, 1627–1637.
9. Ivesa, A.S., Zhou, J.-Q., Schulz, V.P., Monson, E.K., and Zakian, V.A. (2002). *Saccharomyces* Rrm3p, a 5' to 3' DNA helicase that promotes replication fork progression through telomeric and subtelomeric DNA. *Genes Dev.* 16, 1383–1396.
10. Weiss, R.S., Enoch, T., and Leder, P. (2000). Inactivation of mouse *Hus1* results in genomic instability and impaired responses to genotoxic stress. *Genes Dev.* 14, 1886–1898.

11. Nabetani, A., Yokoyama, O., and Ishikawa, F. (2004). Localization of hRad9, hHus1, hRad1, and hRad17 and caffeine-sensitive DNA replication at the alternative lengthening of telomeres-associated promyelocytic leukemia body. *J. Biol. Chem.* 279, 25849–25857.
12. Blasco, M.A., Lee, H.W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A., and Greider, C.W. (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91, 25–34.
13. Hande, M.P., Samper, E., Lansdorp, P., and Blasco, M.A. (1999). Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J. Cell Biol.* 144, 589–601.
14. Levitt, P.S., Liu, H., Manning, C., and Weiss, R.S. (2005). Conditional inactivation of the mouse *Hus1* cell cycle checkpoint gene. *Genomics* 86, 212–224.
15. Baerlocher, G.M., Mak, J., Tien, T., and Lansdorp, P.M. (2002). Telomere length measurement by fluorescence in situ hybridization and flow cytometry: tips and pitfalls. *Cytometry* 47, 89–99.
16. Jia, X., Weinert, T., and Lydall, D. (2004). Mec1 and Rad53 inhibit formation of single-stranded DNA at telomeres of *Saccharomyces cerevisiae cdc13-1* mutants. *Genetics* 166, 753–764.
17. d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P., and Jackson, S.P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426, 194–198.
18. Herbig, U., Jobling, W.A., Chen, B.P., Chen, D.J., and Sedivy, J.M. (2004). Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol. Cell* 14, 501–513.
19. Takai, H., Smogorzewska, A., and de Lange, T. (2003). DNA damage foci at dysfunctional telomeres. *Curr. Biol.* 13, 1549–1556.
20. van Steensel, B., Smogorzewska, A., and de Lange, T. (1998). TRF2 protects human telomeres from end-to-end fusions. *Cell* 92, 401–413.
21. Cortez, D. (2005). Unwind and slow down: checkpoint activation by helicase and polymerase uncoupling. *Genes Dev.* 19, 1007–1012.
22. Zou, L., Liu, D., and Elledge, S.J. (2003). Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc. Natl. Acad. Sci. USA* 100, 13827–13832.
23. Ellison, V., and Stillman, B. (2003). Biochemical characterization of DNA damage checkpoint complexes: clamp loader and clamp complexes with specificity for 5' recessed DNA. *PLoS Biol.* 1, e33.
24. Schramke, V., Luciano, P., Brevet, V., Guillot, S., Corda, Y., Longhese, M.P., Gilson, E., and Geli, V. (2004). RPA regulates telomerase action by providing Est1p access to chromosome ends. *Nat. Genet.* 36, 46–54.
25. Pandita, R.K., Sharma, G.G., Laszlo, A., Hopkins, K.M., Davey, S., Chakhparonian, M., Gupta, A., Wellinger, R.J., Zhang, J., Powell, S.N., et al. (2006). Mammalian Rad9 plays a role in telomere stability, S- and G2-phase-specific cell survival, and homologous recombinational repair. *Mol. Cell Biol.* 26, 1850–1864.
26. Nakamura, T.M., Moser, B.A., and Russell, P. (2002). Telomere binding of checkpoint sensor and DNA repair proteins contributes to maintenance of functional fission yeast telomeres. *Genetics* 161, 1437–1452.
27. Hofmann, E.R., Milstein, S., Boulton, S.J., Ye, M., Hofmann, J.J., Stergiou, L., Gartner, A., Vidal, M., and Hengartner, M.O. (2002). *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis. *Curr. Biol.* 12, 1908–1918.
28. Ahmed, S., and Hodgkin, J. (2000). MRT-2 checkpoint protein is required for germline immortality and telomere replication in *C. elegans*. *Nature* 403, 159–164.
29. Meier, B., Clejan, I., Liu, Y., Lowden, M., Gartner, A., Hodgkin, J., and Ahmed, S. (2006). trt-1 is the *Caenorhabditis elegans* catalytic subunit of telomerase. *PLoS Genetics* 2, e18.
30. Kai, M., and Wang, T.S. (2003). Checkpoint activation regulates mutagenic translesion synthesis. *Genes Dev.* 17, 64–76.
31. Sabbioneda, S., Minesinger, B.K., Giannattasio, M., Plevani, P., Muzi-Falconi, M., and Jinks-Robertson, S. (2005). The 9-1-1 checkpoint clamp physically interacts with Pol{zeta} and is partially required for spontaneous Pol{zeta}-dependent mutagenesis in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280, 38657–38665.
32. Sogo, J.M., Lopes, M., and Foiani, M. (2002). Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. *Science* 297, 599–602.
33. Wang, R.C., Smogorzewska, A., and de Lange, T. (2004). Homologous recombination generates T-loop-sized deletions at human telomeres. *Cell* 119, 355–368.
34. Wang, X., Hu, B., Weiss, R.S., and Wang, Y. (2006). The effect of Hus1 on ionizing radiation sensitivity is associated with homologous recombination repair but is independent of nonhomologous end-joining. *Oncogene* 25, 1980–1983.