Molecular Biology of the Cell
Vol. 12, 2023–2030, July 2001

Telomere Dysfunction Triggers Developmentally Regulated Germ Cell Apoptosis

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Submitted January 19, 2001; Revised April 9, 2001; Accepted April 20, 2001
Monitoring Editor: Elizabeth H. Blackburn

Telomere dysfunction results in fertility defects in a number of organisms. Although data from fission yeast and *Caenorhabditis elegans* suggests that telomere dysfunction manifests itself primarily as defects in proper meiotic chromosome segregation, it is unclear how mammalian telomere dysfunction results in germ cell death. To investigate the specific effects of telomere dysfunction on mammalian germ cell development, we examined the meiotic progression and germ cell apoptosis in late generation telomerase null mice. Our results indicate that chromosome asynapsis and missegregation are not the cause of infertility in mice with shortened telomeres. Rather, telomere dysfunction is recognized at the onset of meiosis, and cells with telomeric defects are removed from the germ cell precursor pool. This germ cell telomere surveillance may be an important mechanism to protect against the transmission of dysfunctional telomeres and chromosomal abnormalities.

INTRODUCTION

Telomeres protect chromosome ends from degradation and rearrangement and from being recognized as DNA breaks. Telomere DNA consists of tandem repeats of simple sequences, although the exact number of repeats varies from chromosome to chromosome (reviewed in Greider, 1996). Telomere length is maintained by the enzyme telomerase, which adds telomere repeats onto chromosome ends de novo (Greider and Blackburn, 1985). In the absence telomerase, telomeres shorten progressively, and after a lag period, telomere function is lost. The heterogeneity of the initial telomere length leads to heterogeneity in the loss of telomere function in a population of cells (Lundblad and Szostak, 1989; Singer and Gottschling, 1994; Lee *et al.*, 1998a). Short telomeres, the presence of an abnormal chromosome end structure, or chromosome rearrangements can trigger a checkpoint resulting in cell cycle arrest and apoptosis (Lee *et al.*, 1998a; Chin *et al.*, 1999; Karlseder *et al.*, 1999). Bypass of this checkpoint can be achieved by removing mediators of the damage signaling pathway such as p53 (Chin *et al.*, 1999; Karlseder *et al.*, 1999). In mammals, telomerase is active in germ cells, preventing telomere shortening (Prowse and Greider, 1995; Wright *et al.*, 1996). Although mice lacking the RNA component of telomerase (mTR−/−) are initially fertile, progressive mating of these mice leads to a decline in fecundity and, eventually, sterility (Lee *et al.*, 1998a). The first mouse generation lacking telomerase is designated mTR−/− G1, and subsequent generations derived through interbreeding are designated mTR−/− G2 through mTR−/− G7 (Lee *et al.*, 1998a). Testicular atrophy and germ cell depletion accompany infertility in young mTR−/− G6 males, such that the generation of G7 litters is extremely rare (Rudolph *et al.*, 1999), whereas the reproductive systems of young adult males are structurally and functionally normal up to mTR−/− G3 (Lee *et al.*, 1998a).

Recent experiments have shown that telomeres play an important role in meiosis (Dernburg *et al.*, 1995; Cooper *et al.*, 1998; Nimmo *et al.*, 1998; Rockmill and Roeder, 1998). Specifically, telomere interaction with spindle pole bodies in the horsetail stage of meiosis in *Schizosaccharomyces pombe* facilitates homologue recognition and recombination. Mutations affecting telomere function in yeast lead to meiotic asynapsis, improper chromosome segregation, and sporulation defects (Cooper *et al.*, 1998; Nimmo *et al.*, 1998). Although mammalian telomeres show specific associations in meiotic cells (Dernburg *et al.*, 1995; Scherthan *et al.*, 2000), it is unclear what effect telomere dysfunction has in mouse meiosis. The germ cell apoptosis in late generation (G4-G7) mTR−/− mice might be due to the high number of mitotic cell divisions in this tissue (Lee *et al.*, 1998a); alternatively, it may be due to a specific meiotic defect. To address this

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question, we examined the proportion of cells at various stages of meiosis, the cell type, and the developmental onset of germ cell apoptosis in mTR−/− mice.

MATERIALS AND METHODS

Meiotic Analysis

Air-dried slides of fixed testicular cell suspensions were prepared as described (Evans *et al.*, 1964). Slides were stained with toluidine blue and scored with the use of a low-power objective. More careful analysis of diakinesis and metaphase I configurations, as well as photography of cells in meiotic prophase, was performed with the use of a high-power objective (630× magnification). Diakinesis and metaphase I stages were classified based on the level of chromosome condensation and separation of the bivalents (Welshons *et al.*, 1962; Evans *et al.*, 1964; Odorisio *et al.*, 1998). Statistical analysis was performed with the use of two-sample *t* tests.

Histology

Histological analysis was carried out on either 10% formalin or Bouin's-fixed, paraffin-embedded testis sections stained with hematoxylin and eosin. Sections were examined and photographed under light microscopy (400× magnification).

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL) Analysis and Germ Cell Nuclear Antigen 1 (GCNA) Staining

Bouin's-fixed, paraffin-embedded testis sections were deparaffinized in two changes of xylene and hydrated to water by successive 5-min washes in 100% ethanol, 90% ethanol, 80% ethanol, 70% ethanol, and distilled H_2O. Sections were then permeabilized by incubating slides in 1% Triton X-100/1% sodium citrate for 2 min at 4°C. After permeabilization, slides were rinsed in phosphate-buffered saline (PBS) and individual testis sections were circled with a PAP pen (Zymed Laboratories, San Francisco, CA). TUNEL mixture (50 μl) (Roche Molecular Biochemicals In Situ Cell Death Detection kit, Fluorescein; Roche Molecular Biochemicals, Indianapolis, IN) was then added to each section. Slides were incubated with the TUNEL mixture for 60 min at 37°C. After incubation, slides were rinsed three times in PBS. Slides prepared for quantifying the extent of apoptosis in individual testis were then mounted with coverslips with the use of Vectashield mounting media (Vector Laboratories, Burlingame, CA). Sections prepared to examine localization of apoptotic cells were then incubated with an undiluted monoclonal anti-GCNA1 antibody (Enders and May, 1994) for 1 h at 32°C. After incubation, slides were rinsed three times in PBS and incubated with a 1:600 dilution of goat anti-rat IgM-Cy3 secondary antibody (Jackson Immunochemicals, West Grove, PA) for 20 min at room temperature. Slides were then rinsed in PBS and mounted with coverslips with the use of Vectashield mounting media (Vector Laboratories). Calculation of p values was performed with the use of a two-sample *t* test.

Ku70 Staining

Bouin's-fixed, paraffin-embedded testis sections were deparaffinized in two changes of xylene and hydrated to water by successive 5-min washes in 100% ethanol, 90% ethanol, 80% ethanol, 70% ethanol, and distilled H_2O. Antigen unmasking was performed by microwaving slides three times for 5 min in 1 mM EDTA. After unmasking, slides were rinsed twice in PBS. Slides were then incubated with 10% normal donkey serum for 20 min at room temperature. After incubation, slides were washed three times in PBS and incubated with a 1:400 dilution of goat anti-Ku70 (Santa Cruz Biotechnology, Santa Cruz, CA) for 60 min at room temperature. After antibody incubation, slides were washed three times in PBS and incubated with a 1:600 dilution of donkey anti-goat Cy3 (Jackson Immunoresearch) for 30 min at room temperature. Slides were then washed three times in PBS and mounted with coverslips with the use of Vectashield mounting media. In this case the mounting media contained 4′,6-diamidino-2-phenylindole (Sigma, St. Louis, MO), for use as a DNA counterstain.

Fluorescent In Situ Hybridization (FISH) Analysis

Eight postnatal day 10 mTR−/− G5 and G6 and 10 postnatal day 22–24 mTR−/− G5 and G6 mice were injected peritoneally with 1 μg/g colcemid. After 3 h, testes were harvested and air-dried slides of fixed testicular cell suspensions were prepared as described (Evans *et al.*, 1964). Spleens from the same mice were removed, and metaphases were prepared by swelling splenocytes in 0.075 M KCl, fixing the cells in 3:1 methanol/acetic acid, and dropping cells onto glass slides. Telomere FISH was performed on testis and spleen slides as described (Blasco *et al.*, 1997). Chromosome fusions with no detectable telomere repeats at the fusion junction were scored as two chromosome ends without detectable repeats.

RESULTS

Late Generation mTR−/− Mice Show No Specific Meiotic Defects

In mutant mice with known synaptic or chromosomal defects, the inability of chromosomes to synapse properly can result in an arrest with an accumulation of cells at a specific stage of meiosis (Xu *et al.*, 1996; Pittman *et al.*, 1998; Yoshida *et al.*, 1998; Edelmann *et al.*, 1999). In some mice with cytogenetic abnormalities, including those with single asynaptic sex chromosomes or X-autosome translocations (Odorisio *et al.*, 1998), the ratio of cells in meiotic metaphase I to those in diakinesis is elevated. Chromosomal rearrangements have been documented in several cell types in the mTR−/− mice, including lymphocytes, splenocytes, thymocytes, embryonic fibroblasts, and tumors (Blasco *et al.*, 1997; Greenberg *et al.*, 1999; Rudolph *et al.*, 1999). To address whether chromosome rearrangements might lead to a specific meiotic arrest in late generation mTR−/− mice, air-dried preparations of meiotic cells were examined to determine the proportion of cells in specific stages of meiotic prophase. Some seminiferous tubules examined in late generation mTR−/− mice were atrophic, and thus there was a reduction in overall number of meiotic cells. However, no accumulation of any specific meiotic stage was found in mTR−/− G5 or mTR−/− G6 mice compared with wild type (Figure 1 and Table 1). The metaphase I/diakenesis ratio was similar in wild-type, mTR−/− G4, mTR−/− G5, and mTR−/− G6 mice ($p = 0.58$, 0.30, and 0.30 comparing wild-type to mTR−/− G4, mTR−/− G5, and mTR−/− G6 mice, respectively). Furthermore, the ratio of cells in diakinesis and metaphase I to the total number of meiotic cells did not increase in late generation mTR−/− cells ($p = 0.83$, 0.85, and 0.28 comparing wild-type to mTR−/− G4, mTR−/− G5, and mTR−/− G6 mice, respectively). These data suggest that there is no specific arrest point in late meiotic prophase in late generation mTR−/− mice. In *S. pombe* mutants defective in telomere binding proteins, loss of telomere function leads to abnormal telomere length regulation, decreased meiotic recombination, and high rates of chromosome loss (Cooper *et al.*, 1998; Nimmo *et al.*, 1998). This chromosome loss during meiotic metaphase results in spore aneuploidy and the loss of spore viability. In mTR−/− G4, G5, or G6 testes, how-

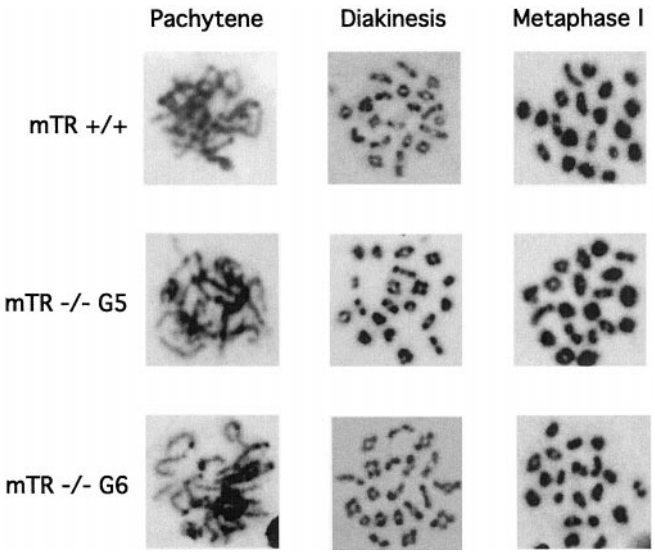

Figure 1. Representative pachytene, diakinesis, and metaphase I figures from mTR+/+, mTR−/− G5, and mTR−/− G6 air-dried testis spreads. Quantitative data are shown in Table 1. No visible cytogenetic abnormalities were seen in mTR−/− testes (630× magnification).

ever, neither metaphase I arrest nor the presence of aneuploid meiosis II metaphases was seen (Table 1; our unpublished results). In addition, no fusions were found in >250 meiotic metaphases examined, although chromosomal fusions are found at a frequency of ~0.5 per metaphase in somatic tissues in late generation mTR−/− mice (Lee *et al.*, 1998a; Herrera *et al.*, 1999; Rudolph *et al.*, 1999) (Figure 1; our unpublished results). These data suggest that the loss of telomerase does not lead to aneuploidy or chromosome fusions in mammalian meiotic cells.

Germ Cell Apoptosis Occurs at Onset of Meiosis in Late Generation mTR−/− Mice

The lack of a specific arrest point in meiotic prophase, combined with a decreased number of cells in meiosis, suggested that cell death might occur early during germ cell development. To examine which meiotic cells were undergoing apoptosis, we performed TUNEL labeling, a marker for apoptosis, in conjunction with immunohistochemical staining for GCNA1. GCNA1 is a marker of spermatogonia and early spermatocytes and is abundant in premeiotic germ cells (Enders and May, 1994). In late generation mTR−/− testis, 86.2% (n = 500) of TUNEL-positive cells were found to reside within the GCNA1-positive zone located near the periphery of nondystrophic seminiferous tubules (Figure 2; our unpublished results). Apoptosis occurs normally during germ cell development (Allan *et al.*, 1987); however, the number of TUNEL-positive cells in late generation mTR−/− testes was increased four- to fivefold relative to the number of TUNEL-positive cells in testes from mTR+/+ mice (Figure 2; our unpublished results; Lee *et al.*, 1998a). The location of these TUNEL-positive cells in the GCNA-positive zone of late generation mTR−/− seminiferous tubules indicates that premeiotic cells, or cells just entering meiotic prophase, are undergoing apoptosis.

To further identify which subset of cells in the GCNA1-positive zone was undergoing apoptosis, we performed TUNEL labeling in conjunction with immunohistochemical staining for Ku70. Ku70, a protein essential for nonhomologous DNA end-joining, is present early in mitotic germ cell precursors, undetectable in cells in the very early stages of meiotic prophase, and then is present again in cells at later stages of meiosis (Goedecke *et al.*, 1999). This absence of Ku70 in cells at the onset of meiotic prophase is thought to

Table 1. Meiotic staging in wild-type and mTR −/− testis

Knock-out generation	No.	Age (mo)	Testis weight (mg)	Metaphase I/diakinesis (ratio)	Metaphase + diakinesis/total meiotic cells (%)
Wild type	1	3.5	114	17/33 (0.52)	2.8
	2	8	100	11/42 (0.26)	3.2
	3	8	104	8/22 (0.36)	2.4
G4	1	5	80	16/34 (0.47)	3.0
	2	7	60	7/25 (0.28)	2.8
	3	8	39	13/37 (0.35)	2.4
	4	8	20	0	
G5	1	5.5	24	11/20 (0.56)	2.8
	2	5.5	23	11/25 (0.44)	2.7
	3	5.5	20	0	
	4	5.5	15	0	
	5	8	14	0	
	6	8	12	0	
G6	1	3.5	30	15/34 (0.44)	3.0
	2	3.5	28	18/32 (0.56)	3.4
	3	3.5	18	0	
	4	4.5	10	0	

WT, wild type.

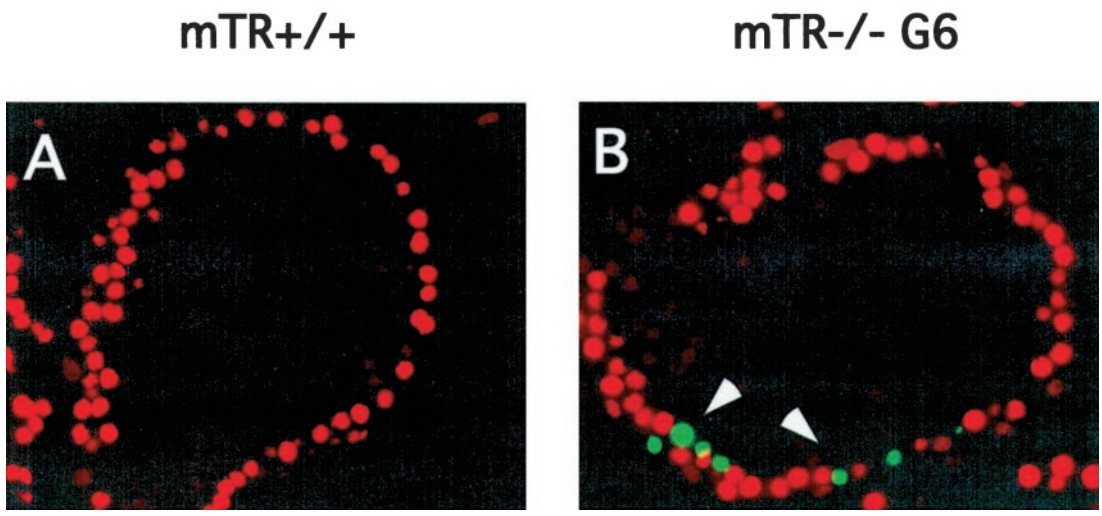

Figure 2. GCNA1 and TUNEL staining colocalize near the periphery of seminiferous tubules in late generation mTR−/− testis. (A) GCNA1 (red) and TUNEL (green) staining of a seminiferous tubule from a 10-wk-old mTR+/+ mouse testis (630× magnification). (B) GCNA1 (red) and TUNEL (green) staining of a seminiferous tubule from a 10-wk-old mTR−/− G6 mouse testis. White arrows indicate clusters of TUNEL-positive cells.

prevent premature processing of DNA breaks during meiotic recombination (Goedecke *et al*., 1999). Thus, seminiferous tubules at different stages of development show a characteristic pattern of Ku70 staining (Oakberg, 1971; Critchlow and Jackson, 1998; Goedecke *et al*., 1999). Serial sections of late generation mTR−/− testis were stained for Ku70 and TUNEL. In all stages of spermatogenesis, TUNEL-positive cells were localized to the Ku70-negative zone of the seminiferous tubule. In stage II-IV seminiferous tubules, for example, early meiotic cells are localized two to three cell layers from the basement membrane. Ku70-negative and TUNEL-positive cells were both found in this layer of cells in stage II-IV tubules (Figure 3, A and B). At stages VIII-IV, early meiotic cells are localized on the basement membrane of the seminiferous tubule. TUNEL-positive cells were again found in the Ku70 negative zone in stage VIII-IV tubules in mTR−/− G5 and G6 testes (Figure 3, C and D). As mentioned above, ~15% of the apoptotic cells in mTR−/− testes do not localize to the periphery of the tubule and may represent normal ongoing apoptosis unrelated to the short telomeres. In these cells we did find costaining for TUNEL and Ku70. Thus, the absence of Ku70 in TUNEL-positive cells was not due to the specific removal of Ku70 from apoptotic cells. The localization of TUNEL-positive cells to the Ku70-negative zone of the mTR−/− G5 and G6 seminiferous tubules indicates that cells are undergoing apoptosis at the onset of meiotic prophase.

Only a subset of the early meiotic GCNA1-positive and Ku70-negative cells was TUNEL-positive. This is likely due to the heterogeneous nature of telomere lengths. Telomere length on any given chromosomes end is regulated about a mean, thus within a cell and within an organism, only a subset of chromosomes may have critically short telomeres. Our data suggest it is this subset that will undergo apoptosis (see below).

Developmental Analysis of Germ Cell Phenotypes in Late Generation mTR−/− Mice

The appearance of atrophic seminiferous tubules in late generation mTR−/− mice could be due to the death of germ cell precursors early in development, or could be due to death of committed germ cells in the testes. To distinguish between these two possibilities, we examined the developmental timing of apoptosis and loss of cellularity in the testis. The developmental onset of meiosis begins between postpartum days 11 and 13 in male mice (Nebel *et al*., 1961; Bellve, 1979). In late generation mTR−/− mice at postpartum day 11, the number of TUNEL-positive cells was similar to the number seen in age-matched, wild-type testes. In contrast, at postpartum day 13, testes from mTR−/− G6 and G7 mice exhibited a significant ($p < 0.0005$) increase in apoptosis relative to wild-type testes (Figure 4A). This suggests that apoptosis is triggered after the developmental onset of meiosis.

Consistent with this hypothesis, histological examination showed a correlation of the loss of cellularity with the developmental onset of meiosis. Testes from mice before the onset of meiosis did not show a loss of cellularity (our unpublished results); however, beginning at postpartum day 12–13 some loss of cellularity was seen (Figure 4C). Consistent with this histological observation, testes weights were similar between wild-type and late generation mTR−/− mice at day 10–12, before entry into meiotic prophase. At postnatal day 17–20, after entry into meiotic prophase, there was a significant decrease in testis weight in late generation mTR−/− mice compared with wild type (Figure 4B). Although it is unclear how apoptosis of cells at the onset of meiotic prophase leads to the significant seminiferous tubule degeneration seen in late generation mTR−/− mice, severe vacuolization of the seminiferous tubules, including the absence of spermatogonia and support cells, is often associated with spermatocyte apoptosis

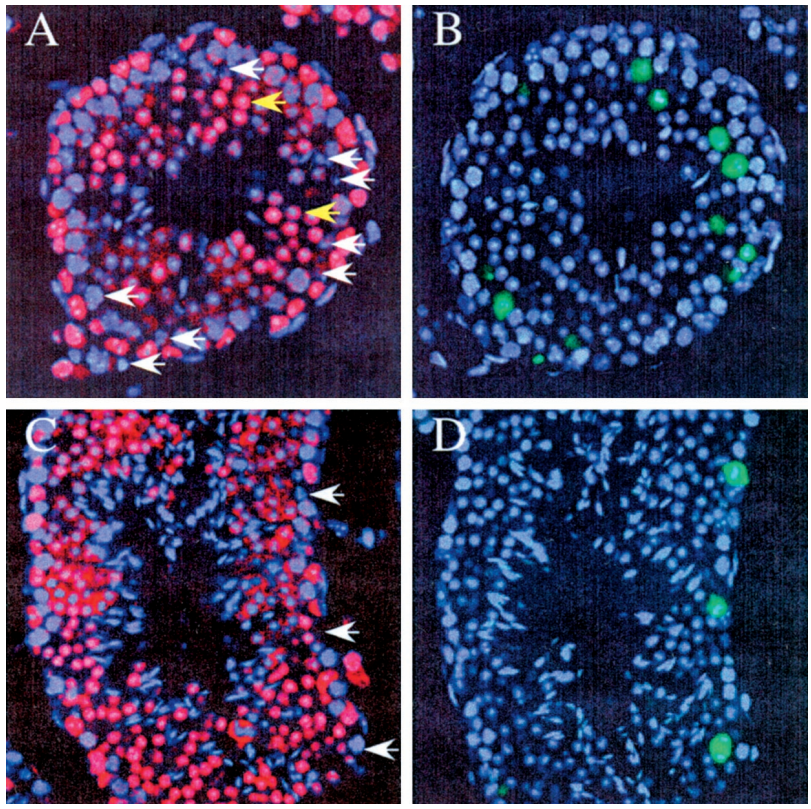

Figure 3. TUNEL-positive cells localize to the Ku70-negative zone in late generation mTR−/− testes. (A) Ku70 (red) and (B) TUNEL (green) staining of serial sections of a stage II-IV seminiferous tubule. Early meiotic cells at this stage are localized two to three cell layers from the basement membrane of the seminiferous tubule. Ku70-negative and TUNEL-positive cells colocalize to this layer of cells in early meiotic prophase. (C) Ku70 (red) and (D) TUNEL (green) staining of serial sections of a stage VIII-IV seminiferous tubule. Early meiotic cells are localized at the basement membrane of the seminiferous tubule. Ku70-negative and TUNEL-positive cells colocalize to this layer of cells in early meiotic prophase. White arrows indicate TUNEL-positive cells without detectable Ku70 staining. Yellow arrows indicate the few TUNEL-positive cells with detectable Ku70 staining, likely from normal apoptotic processes. Cell nuclei in these tissue sections are counterstained with 4,6-diamidino-2-phenylindole.

(Kodaira *et al.*, 1996). Thus, germ cell apoptosis in telomerase-deficient mice coincides developmentally with the entry of precursor germ cells into meiosis.

Apoptosis in Late Generation mTR−/− Mice Removes Cells Containing Dysfunctional Telomeres from Germline

The initiation of apoptosis in a subset of germ cells near the onset of meiosis could be due to a subset of meiotic cells that have short dysfunctional telomeres. To determine whether cells with short telomeres are specifically eliminated during meiosis, we assayed telomere length before and after the developmental onset of meiosis in germ cells and in cells from a somatic lineage (splenocytes). To determine telomere length, we used the quantitative-FISH technique developed by Lansdorp *et al.* (1996). In somatic cells of the mTR−/− mice, progressive telomere sequence loss leads to loss of FISH signal from some chromosome ends and subsequently to chromosome fusions (Blasco *et al.*, 1997). Because we did not find chromosome fusions in late generation mTR−/− germ cells, we used the absence of a fluorescent signal on a chromosome end as an indicator of potential telomere dysfunction (Blasco *et al.*, 1997; Hande *et al.*, 1999; our unpublished results). Before the onset of meiosis, at postnatal day 10, splenocytes and germ cells from mTR−/− G5 and G6 mice showed similar numbers of signal-free ends per metaphase. However after the onset of meiosis, at postnatal day 22–24, splenocytes from mTR−/− G5 and G6 mice showed significantly more signal-free ends per metaphase than germ cells from the same mice ($p < .0001$; Figure 5). These results suggest the apoptosis at the onset of meiotic prophase removes cells with dysfunctional telomeres from the germ cell population.

DISCUSSION

Severe telomere shortening and associated chromosomal instability triggers both p53-dependent and -independent cell death in late generation mTR−/− testis (Chin *et al.*, 1999). The lack of a specific arrest point in meiotic prophase, the identification of apoptotic cells as very early meiotic cells, and the onset of apoptosis coincident with developmental initiation of meiosis, suggest that telomere dysfunction leads to apoptosis upon entry into meiotic prophase. The decrease in chromosome ends without detectable telomere repeats after the onset of meiosis is also consistent with the specific removal of germ cells with dysfunctional telomeres. These data suggest that there is a surveillance mechanism in germ cells that specifically targets cells with dysfunctional telomeres for apoptosis.

Apoptosis in cells with dysfunctional telomeres could be due to a direct effect of telomere dysfunction or an indirect affect of secondary chromosome breaks induced in cells that divided with a fused chromosome. Previous work showed that in the absence of the telomere binding protein TRF2, apoptosis is mediated directly by dysfunctional telomeres. Cells expressing dominant negative TRF2 underwent apoptosis before going although mitosis (Karlseder *et al.*, 1999).

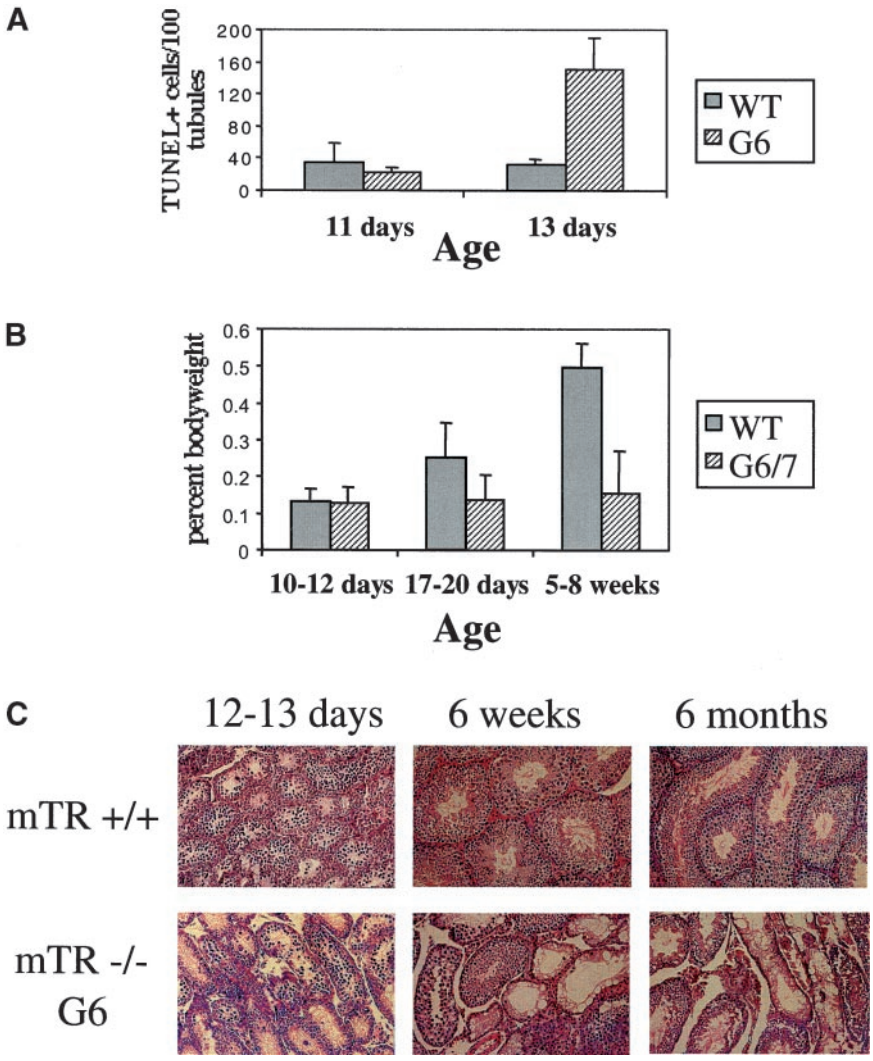

Figure 4. Seminiferous tubule atrophy, germ cell apoptosis, and decreased testicular weight are seen at specific developmental stages in late generation mTR−/− mice. (A) Number of TUNEL-positive cells per 100 seminiferous tubule cross sections from mTR+/+ and mTR−/− G6 mice, compared at day 11 and day 13 postpartum. (B) Relative testis weights of mTR+/+ and mTR−/− G6/G7 mice compared at different postpartum developmental ages. (C) Hematoxylin and eosin-stained testis sections from 12–13 d, 6 wk, and 6-mo-old mTR+/+ and mTR−/− G6 mice (400× magnification).

In the case presented here, we did not detect any chromosome fusions in germ cells. Thus, apoptosis cannot be the secondary consequence of breaking a fused chromosome at mitosis. In late generation mTR−/− mice, apoptosis occurred specifically in cells lacking detectable Ku70 protein. Ku70 is known to bind telomeres in vivo and is thought to play a role in normal telomere function (Laroche *et al.*, 1998; Lee *et al.*, 1998b; Nugent *et al.*, 1998; Bailey *et al.*, 1999; Hsu *et al.*, 1999). The fusion of chromosomes in somatic cells of late generation mTR−/− mice may be mediated by a Ku70-dependant nonhomologous end-joining. In germ cells, the absence of Ku70, and the resulting decrease in nonhomologous end-joining, may preclude chromosome fusion as a mechanism to repair dysfunctional telomeres. In the absence of chromosome fusions, dysfunctional telomeres may be recognized as a DNA double-strand breaks and elicit an apoptotic response. This kind of tissue-specific surveillance of DNA breaks has recently been shown to exist in mouse neural development (Gao *et al.*, 1998). In the absence of nonhomologous end-joining proteins, including Ku70, unrepaired double-strand breaks specifically elicit neuronal apoptosis.

Although telomeres and telomere binding proteins are thought to play a critical role in chromosome movement and segregation in mammalian meiosis (Dernburg *et al.*, 1995; Scherthan *et al.*, 2000), the removal of dysfunctional telomeres at the onset of meiotic prophase results in the absence of any significant chromosome asynapsis or missegregation in late generation mTR−/− mice. Thus, there is a cellular assessment of telomere function before the point at which meiosis-specific telomere function is required. This added level of telomere surveillance may be unique to mammalian systems. In *S. pombe*, telomere shortening, as well as mutations in telomere attachment to the spindle pole body, yield similar germ cell defects (Cooper *et al.*, 1998; Naito *et al.*, 1998; Nimmo *et al.*, 1998). In *C. elegans*, telomere shortening results in defects in meiotic chromosome segregation (Ahmed and Hodgkin, 2000). In mouse, however, defects related to telomere dysfunction likely manifest themselves before defects in chromosome synapsis or other meiotic-specific telomere functions occur.

Our results suggest that telomere dysfunction resulting from telomere shortening may be monitored and processed

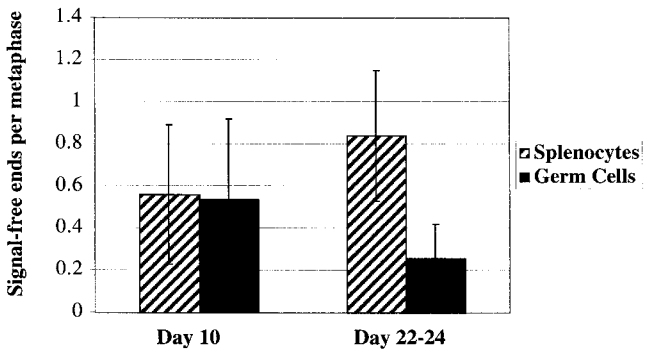

Figure 5. Underrepresentation of germ cells compared with somatic cells that lack telomere signal in postnatal day 22–24 mTR−/− G5 and G6 mice. Metaphases from postnatal day 10 mTR−/− G5 and G6 and postnatal day 22–24 mTR−/− G5 and G6 somatic cells (splenocytes) and germ cells were hybridized with a $(TTAGGG)_3$ quantitative-FISH probe and scored for the number of chromosome ends without detectable telomere repeats per metaphase. The average from 20 metaphases from each of eight independent postnatal day 10 mTR−/− G5 and G6 or10 independent postnatal day 22–24 mTR−/− G5 and G6 mice is shown.

in a cell type and developmental stage-specific manner. Although germ cells are highly proliferative, loss of telomeres during proliferation cannot account for the sudden developmental onset of germ cell apoptosis between days 11 and 13 after birth. The failure to detect chromosome fusions in germ cells is consistent with the failure to find inheritance of stable chromosome fusions in these mice (Blasco *et al.*, 1997; Lee *et al.*, 1998a; our unpublished results). Chromosome fusion may represent a mechanism by which somatic cells limit the damage induced by dysfunctional telomeres. Loss of telomere function may look to the cell like a DNA break. Elimination of the apparent break by chromosome fusion may allow the cell to progress in the cell cycle. In the germline, however, transmission of cytogenetic abnormalities could have catastrophic developmental effects on future generations. Thus, a specific germ cell telomere surveillance mechanism may protect the germline from chromosomal abnormalities.

ACKNOWLEDGMENTS

M.T.H. was supported by the Predoctoral Training Program in Human Genetics National Institutes of Health Grant GM-07814 and work in the Greider lab was supported by National Cancer Institute Grant CA-16519. Work in the DePinho lab was supported in part by grants from the National Institutes of Health (HD-348880 and HD-28317) to RAD. R.A.D. is an American Cancer Society Research Professor. K.L.R. is supported by the Deutsche Forschungsgemeinschaft (Ru 745/1-1). L.C. is supported by a National Institutes of Health Mentored Clinician Scientist Award K08-AR02104. Support from the Dana Farber/Harvard Cancer Center Core Grant to R.A.D. and L.C. is acknowledged. We thank Dr. George Enders for providing GCNA1 antibody, Dr. Patricia Hunt for providing the protocol for air-dried meiotic preparations, and Drs. Geraldine Seydoux and Patricia Hunt for critical reading of the manuscript.

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