Telomere Regeneration in Spermatogenesis and During Early Embryogenesis

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Summary

Telomeres, replicated by telomerase, protect the ends of chromosomes from degradation and fusions that normal DNA replication cannot avoid. The regeneration of telomeres in mammals occurs in several phases throughout spermatogenesis and embryogenesis. During sperm formation, telomerase activity resides primarily in the α6+SP stem cells, and is most active during this early undifferentiated state of development, yet reach a maximum length during the elongated spermatid phase. The drop in telomerase activity during spermatogenesis is achieved by a decrease in TERT expression. Telomeres undergo further lengthening during embryogenesis, especially between the morula and blastocyst stages of development, where telomeres reach a predefined length regardless of initial length.

Introduction

Telomeres are made up of a repeating hexanucleotide (TTAGGG in mammals) sequence that has been found to have an important role in protecting DNA during its replication cycle. Specifically, telomeres prevent DNA terminal fusions and degradation (Tanemura et al., 2005). When DNA polymerase synthesizes DNA, it does so in chunks going from the 5′ to 3′ end of the chromosome, but cannot replicate the very end of the molecule where the telomeres are. Thus the telomerase ribonucleoprotein complex is needed to maintain telomere length (Coussens et al., 2006). Telomerase is made up of a nine nucleotide RNA sequence (the length can vary depending on the species) that serves as a template for telomere repair (Blackburn et al., 2006) and the telomerase reverse transcriptase (TERT) enzyme (Coussens et al., 2006). Most human cells do not feature telomerase, and thus their chromosomes lose telomere length with each cell replication. As telomeres become too short to function, the cell will undergo the process of replicative senescence, where cell growth ceases. The limited number of times a cell can divide before this occurs is determined by the telomere length of the cell that started the population (Baird et al., 2006), indicating that the length of the telomeres in the early embryo is extremely important in determining the number of times subsequent cells can divide. It is important to understand how telomeres are kept at a functional length during the processes of spermatogenesis and embryogenesis, as these are the cells that are performing the most divisions and would be prone to rapid telomere degradation.

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Spermatogenesis

The activity of telomerase in the development of germ cells is vital to developing the telomeres to a length that will allow an embryo created with the germ cell to develop properly and have its cells divide a sufficient number of times. Germ cells in both genders are developed from embryonic stem cells that are known as primordial germ cells (PGCs). In males, the PGCs lie dormant after an initial period of proliferation until just after birth, when they begin to differentiate into spermatogonia (Coussens et al., 2006).

The experiments performed by Coussens et al. demonstrated the role of TERT in telomerase activity during spermatogenesis. They showed that the already well-established drop in telomerase activity during the quiescent period of developing PGCs is a result of lowered TERT expression (2006). They tested 1500 purified primordial germ cells using Pou5f1-GFP transgenic mouse embryos that had been engineered to have fluorescent proteins in the PGCs to test for the presence of Tert mRNA, measured in comparison to Hprt1 levels, which indicates the level of TERT expression. During days 10.5 and 12.5 of embryo development, while the PGCs are still developing,

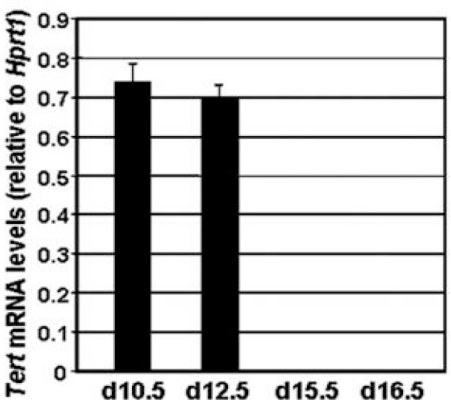

Figure 1. The level of TERT expression in 1500 primordial germ cells relative to the *Hpert1*mRNA internal control on days 10.5, 12.5, 15.5, and 16.5. No significant difference existed between days 10.5 and 12.5 ($P > 0.1$) (Figure reproduced from Coussens et al., 2006).

similar levels of Tert mRNA were found, but on days 15.5 and 16.5, when the PGCs are in a quiescent state, no Tert mRNA was found (fig. 1). Further experimentation was performed to determine that TERT activity was not halted after translation from DNA. A transgenic mouse strain using the chicken gene to ensure TERT expression (CAG-Tert strain) was created. This strain shows a significantly greater amount of telomerase activity in comparison to non-transgenic embryos (fig. 2A). The homozygous CAG-Tert mice were then crossed with homozygous Pou5f1-GFP mice, using homozygous Pou5f1-GFP embryos as a control. Coussens et al. found that not only did the transgenic embryos have about a 2.3 fold increase in

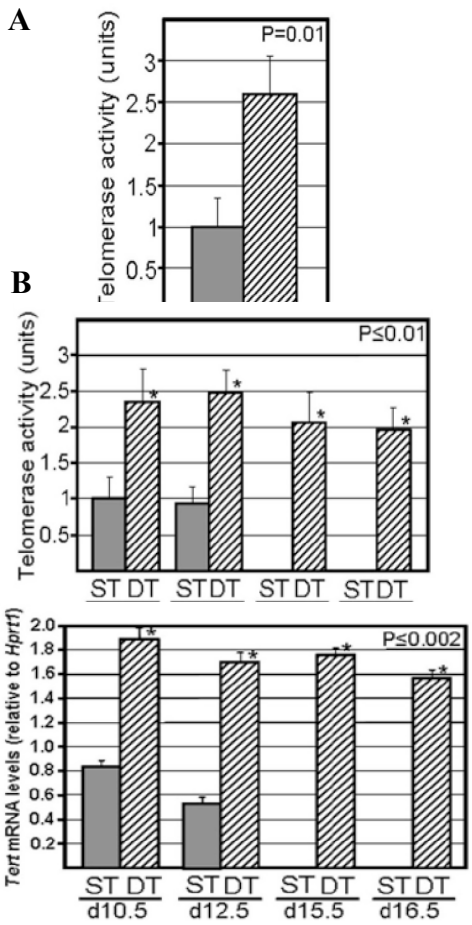

Figure 2. A) Comparison of telomerase activity in 12.5 day old transgenic mice with chicken TERT gene and nontransgenic, wild type mice using a TRAP assay. A significant difference was found (P = 0.01). **B)** Comparison of telomerase activity in primordial germ cells taken from double transgenic (DT) mice with both the TERT knockout and the chicken TERT allele, and single transgenic (ST) mice with only the TERT knockout allele. A significant difference was found between the ST and DT groups ($P \leq 0.01$). **C)** Comparison of Tert mRNA levels relative to *Hprt1* showing TERT expression in ST and DT mice on various days. The DT Tert mRNA levels were significantly greater than the ST Tert mRNA levels ($P \leq 0.002$) (Reproduced from Coussens et al., 2006).
* indicates a significant difference between ST and DT groups.

telomerase activity when compared with control embryos (P ≤ 0.01), but also that when the controls are quiescent and have no telomerase activity (days 15.5 and 16.5) the CAG-Tert transgenic PGCs still had telomerase activity (figs. 2B, 2C) (2006). Therefore, because separate expression of TERT restores telomerase activity, the primary inhibition process cannot be post-translational. A further experiment by Coussens et al. shows that though TERT is normally expressed before but not during quiescence; a TERT knockout strain that would have no TERT expression at any stage, or a CAG-Tert strain that would over-express TERT at all stages, all show no significant variation from the wild-type strain when measuring the frequency of cycling in PGCs (fig. 3). These studies demonstrate that despite telomerase activity being controlled by the expression of TERT, the presence of extra telomerase or its complete absence will have no effect on the development and division of primordial germ cells (Coussens et al., 2006). Even after the quiescent phase of the PGCs TERT expression has no effect on whether or not cells continue to differentiate into spermatozoa (Tanemura et al., 2005). These mutated mouse strains will most likely have debilitating effects on future generations if they were allowed to breed, or if the mutation occurred in nature. Future generations with the knockout gene for TERT will most likely face DNA degradation and chromosomal fusing. In fact, it has been shown that mice mutant for the RNA

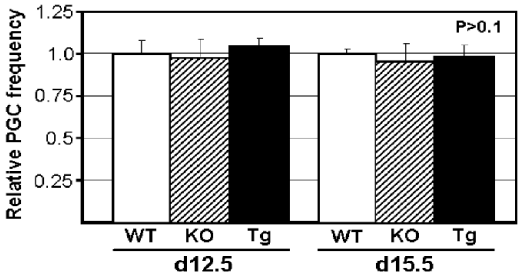

Figure 3. Comparison of primordial germ cell replicative frequency between wild type mice, mice with the Tert knockout allele, and transgenic mice with the chicken TERT allele that over express TERT mRNA. No significant difference was found between these three groups (P > 0.1) ((Reproduced from Coussens et al., 2006).

component in telomerase (Terc-/-) become infertile after the sixth generation (Tanemura et al., 2005).

Riou et al. performed a series of experiments to determine which specific cells feature telomerase activity in relation to spermatogenesis in adult mice; they attempt to discover when and where in spermatogenesis the telomeres are restored (2005). The researchers isolated the SP fraction from the testis. This population of cells contains spermatogonia and germinal stem cells. Magnetic-activated cell sorting was used to select cells containing the α6-integrin protein. The α6-integrin-positive Side Population (α6+SP) cells were then tested to see if they contained Ep-CAM, an indicator of spermatogonia cells. Ninety-five percent of the cells were seen to contain Ep-CAM (fig. 4A). The cells were then tested for the presence of CD9, another indicator of germinal stem cells and spermatogonia. Ninety-seven percent of the α6+SP stem cells displayed the CD9 marker (fig. 4B). The experimenters then performed assays that revealed telomerase activity in the α6+SP stem cells to be greater than in the total extracts from the rest of the testis, indicating that telomerase activity is primarily in the α6+SP stem cells of adult testis (Riou et al., 2005). Further assays were performed to compare α6+SP telomerase activity with telomerase activity in spermatocytes I, round and elongated spermatids, and epididymal spermatozoa (fig. 5B). Telomerase activity in the α6+SP population was shown to be significantly greater than the activity in all the other tested groups,

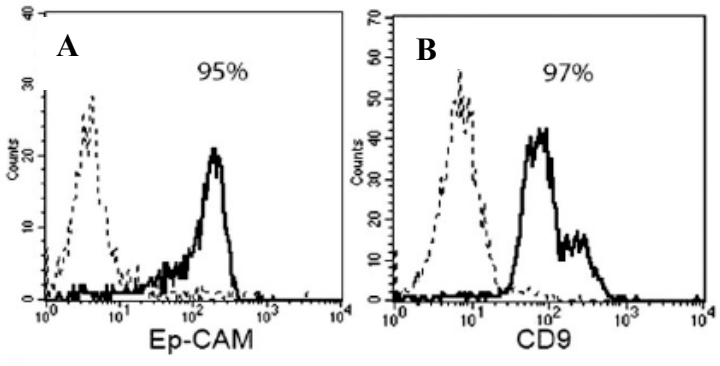

Figure 4. A) Presence of Ep-CAM marker in α6$^+$SP cells. **B)** Presence of CD9 in α6$^+$SP cells (Modified from Riou et al., 2005).

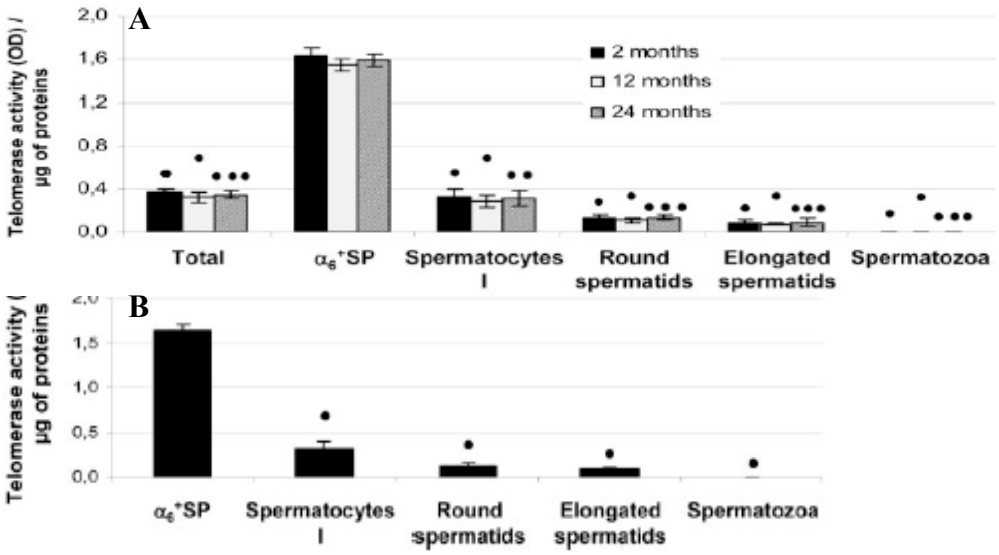

Figure 5. A) Comparison of telomerase activity as measured by µg of proteins in various cells of mice at differing ages. **B)** Comparison of telomerase activity as measured by µg of proteins in various cells of 2 month old mice (Modified from Riou et al., 2005) ●, P <0.01; ●●, P < 0.001; ●●●, P < 0.0001.

and that epididymal spermatozoa contain no telomerase activity at all. These groups were tested in mice testes that were 2, 12, and 24 months old (fig. 5A). The experiment revealed no significant change in the telomerase activity in relation to age. These experiments serve to show that telomere repair to create spermatozoa with sufficient length to produce healthy offspring occurs primarily in the α6+SP cells, and that telomerase activity sharply drops as the sperm cells become more developed, and stops altogether in mature spermatozoa (Riou et al., 2005).

Tanemura et al. also performed experiments to measure telomere length in different stages of spermatogenesis in mouse testes. Fluorescence in situ hybridization (FISH) showed that telomeres shorten during the pachytene stage of prophase in spermatocytes undergoing meiosis. The telomeres remain short until the round spermatid stage of spermatogenesis (Tanemura et al., 2005). Despite decreasing telomerase activity (Riou et al., 2005), the telomeres grow longer as the spermatid itself elongates because of the increased length of the sperm development stages. The telomeres longest during the elongated spermatid phase (fig. 6). Interestingly, when measuring TERT activity in these cells, Tanemura et al.found that TERT is indeed active in early spermatocytes before the pachytene phase, and again in elongating spermatids; however, not all elongating spermatids showed TERT activity. This, along with evidence of telomere extension during Terc-/- strain spermatogenesis, suggests that these cells make use of an alternative pathway for telomere extension (Tanemura et al., 2005) that is not yet understood.

Embryogenesis

Telomere regeneration does not necessarily end once the germ cells have completed maturation. Schaetzlein et al. have shown that telomere lengthening occurs during embryogenesis in mammals (2004). Initial experimentation compared telomere length in bovine embryos that were developed by in vivo fertilization, in

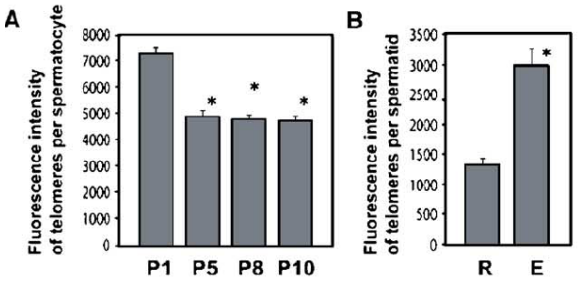

Figure 6. A) Comparison of pachytene spermatocytes' telomere lengths as seen by fluorescence intensity in progressing stages I, V, VIII, and X. **B)** Comparison of telomere lengths of round spermatids and elongated spermatids as seen by fluorescence intensity (Reproduced from Tanemura et al., 2005).
*, $P < 0.01$

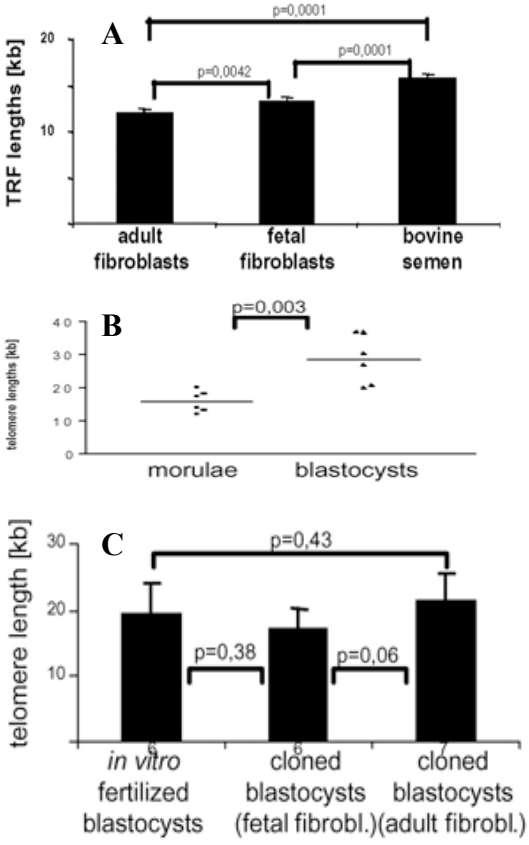

Figure 7. A) Comparison of telomere restriction fragment (TRF) lengths found in adult fibroblasts, fetal fibroblasts, and bovine semen. **B)** Comparison of individual and average values found for telomere length (kb) between the morula and blastocyst stages. This graph implies a jump in telomerase activity in the transition between stages. **C)** Comparison of mean telomere lengths from blastocysts derived from *in vitro* fertilization, cloning from a fetal fibroblast, and cloning from an adult fibroblast ((Modified from Schaetzlein et al., 2004).

vitro fertilization, and animals created from cloning an adult fibroblast cell or a fetal fibroblast. In the morula stage of embryo development there was a significant difference (P = .0001) between the telomere lengths in the fertilized embryos and those in cloned embryos; however, the difference within the groups did not prove to be significant (fig. 7A). When the telomere lengths were measured in bovine blastocysts there was a significant increase (P = .003, fig. 7B) in all embryo groups. Also, the difference in telomere length between the clone embryos and the fertilized embryos is no longer significant (P = .43, fig. 7C). To test if this is a universal process in mammals, mouse embryos also underwent telomere length measurement using mTERC-/- and mTERC+/+ strains to determine if the process is telomerase dependent. Significant telomere length increase was found in the mTERC+/+ strain

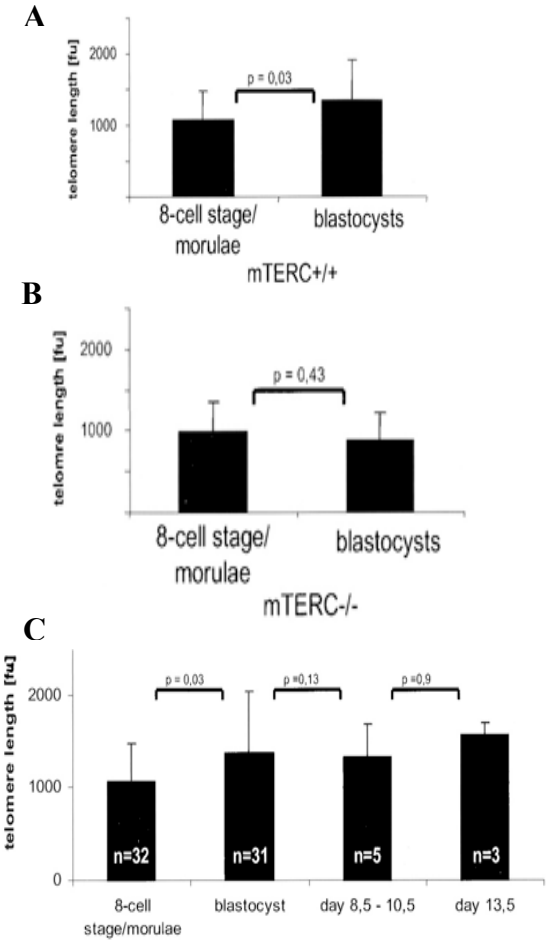

Figure 8. A) Comparison of telomere length measured in mean fluorescent units (FU) in murale/8-cell stage embryos and blastocysts that are homogenous for the mTERC allele. **B)** Comparison of telomere length in muralae/8-cell stage embryos and blastocysts featuring the mTERC knockout allele. **C)** Comparison of mean telomere length between morulae/8-cell stage, blastocysts, embryos at days 8.5-10.5 of development, and embryos at day 13.5 of development (Modified from Schaetzlein et al., 2004).

between the morula and blastocyst stages (P = .03, fig. 8A), but no significant difference was found to exist in the mTERC-/- strain (P = .43, fig. 8B). Also, the mTERC+/+ strain did not have significant telomerase activity after the blastocyst stage was reached (fig. 8C) (Schaetzlein et al., 2004). These experiments show that a mechanism exists in mammalian embryos to lengthen telomeres to an appropriate level regardless of the original length.

Discussion

The importance of telomeres to a healthy lifespan in mammals is undeniable: telomeres that are too short will cause DNA degradation. The evolution of mammalian reproduction has included several mechanisms in an attempt to ensure that telomeres are at a proper, healthy, length when an embryo is developing. Some of the necessary telomere repair occurs in the germ line, ensuring that embryogenesis can reach a stage where telomerase is once again reactivated and telomeres are rebuilt to a preordained length regardless of their length at fertilization. The functioning of telomerase during early development ensures that mammals do not suffer from early senescence caused by chromosomal damage, and that the degradation that occurs throughout a normal life is not passed onto the offspring. Further study is necessary to determine how telomeres are extended in Terc-/- mouse strain spermatogenesis, as well as how the extension of telomeres in mammalian embryos is maintained at an appropriate length.

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