

Telomere regulation and function during meiosis

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Abstract

Telomeres are essential for genomic stability and their dysfunction has been implicated in cancer and ageing. The most prominent function of the telomeres is to protect chromosome ends against degradation and fusion, which, in turn, requires maintenance of telomere DNA to a critical length that allows assembly of end-capping structures. During early meiosis, telomeres play the distinctive function of anchoring chromosomes to the inner nuclear membrane. Subsequently, as a consequence of the nuclear membrane polarization, telomeres cluster together into a bouquet configuration, which facilitates pairing and recombination of the homologous chromosomes. Here we review how the two fundamental aspects of telomere maintenance, elongation and protection, contribute to the essential functions performed by telomeres during meiosis.

Telomere integrity during meiosis: elongation and capping

Telomeres are complex structures at chromosome termini, consisting of arrays of repetitive DNA sequences and associated proteins (Blackburn 2001, de Lange 2005). They protect chromosome ends from degradation and fusion, both of which stimulate checkpoint activation, ultimately leading cells to apoptosis or irreversible growth arrest known as replicative senescence (Maser & DePinho 2002). However, in cells lacking checkpoint responses, short or uncapped telomeres elicit rampant genomic instability, similar to that caused by unrepaired DNA double-strand breaks (DSBs). This type of instability stems from chromosomal fusion-breakage cycles known to generate and amplify chromosomal aberrations, one of the factors promoting the development of cancer. Thus, telomere dysfunction, exhibited either as telomere shortening or loss of telomeric capping structures, is associated with

genetic instability and onset of tumorigenesis or premature ageing in mitotically dividing cells.

In contrast with the considerable attention received by somatic telomeres connected with cancer development and progression, as well as organismal ageing, our understanding of the mechanisms of telomere maintenance in meiosis is still at a very early stage. Traditionally, research in this field has focused on the cytological aspects of telomere dynamics in meiosis due to the undoubted aesthetic attraction of the floral arrangement of chromosomes and the dramatic changes in positioning, with relevance to the meiotic chromosome recombination and segregation. However, a complete understanding of meiotic telomeres functions can only be achieved by integrating the cytology with the two features central to telomere maintenance in all types of cells: elongation and protection (capping). Here, we explore the significant contribution of telomere homeostasis and protection to the integrity and function of meiotic chromosomes, ultimately ensuring fertility. The existing evidence

mainly originates from the meiotic phenotype of the *Terc*^{-/-} mouse carrying a null mutation in the telomerase RNA component (Blasco *et al.* 1997, Lee *et al.* 1998), the only *bona fide* mouse model for telomere dysfunction generated so far.

Telomere length regulation

Mammalian telomeres consist of tandem arrays of duplex TTAGGG repeats which vary in length from approximately 10 kb in humans to >40 kb in mouse (de Lange *et al.* 1990, Zijlmans *et al.* 1997). The double-stranded telomeric DNA track ends with a 150-nucleotide single-stranded 3' protrusion of the G-rich strand. This so-called 3' overhang represents the substrate for telomerase, which provides the most versatile and widely used activity of telomere maintenance (McEachern *et al.* 2000).

Telomerase is a ribonucleoprotein with reverse transcriptase activity (Greider & Blackburn 1985, Blasco *et al.* 1995), which extends the G-rich strand of the telomere, thus preventing gradual loss of DNA sequences from the chromosome ends with each round of replication (Harley *et al.* 1990, Bodnar *et al.* 1998). This 'end replication problem' stems from the inability of conventional polymerases to complete synthesis of chromosome ends (Watson 1972). In the absence of telomerase, continuous cell division results in reduction of telomeres to a short critical length, which elicits a checkpoint response similar to the one provoked by DNA damage (Wahl & Carr 2001, de Lange 2002). For example, in telomerase-deficient mice, telomere attrition leads to end-to-end fusions and cell death (Blasco *et al.* 1997, Lee *et al.* 1998, Herrera *et al.* 1999). Cell growth and telomere homeostasis are restored upon re-introduction of telomerase both in cultured cells and in the *Terc*^{-/-} mouse (Bodnar *et al.* 1998, Hemann *et al.* 2001, Samper *et al.* 2001). Also, telomerase activation in somatic cells normally lacking detectable levels of this activity is thought to maintain the immortal growth capacity of more than 90% of human tumours (Hiyama & Hiyama 2002).

Most mammalian somatic cells, including adult stem cells, do not have sufficient telomerase activity to support telomere replenishment at each cell division, which leads to progressive shortening of telomeres throughout adult life (Harley *et al.* 1990, Flores *et al.* 2005). However, certain types of cells in

the adult organism retain high telomerase activity essential for their function and viability; a well-studied example is the germ line (reviewed in Blasco 2005). Consistent with this, in telomerase-negative mice the progressive loss of telomeric sequences leads gradually to infertility, as evidenced by reduced litter size and compromised embryo development (Lee *et al.* 1998, Herrera *et al.* 1999), indicating that maintenance of telomere length is essential in the germ line.

Meiosis is an evolutionarily conserved process consisting of two successive divisions leading to generation of haploid cells (gametes). Upon fertilization, these can reconstruct the diploid organism. In mammals, meiosis occurs in testes and ovaries, both consisting of highly heterogeneous cell populations. Telomerase activity was found in fetal, newborn and mature ovaries and testes, but mature oocytes and adult sperm samples lack telomerase activity (Wright *et al.* 1996).

The ovary

In females, the oocytes initiate meiosis very early during embryonic development and undergo a prolonged arrest at prophase I, which lasts for weeks in mice and years in humans until puberty. Gonadotrophin stimulation induces resumption of meiosis and progression of competent oocytes through the remaining stages of meiosis I, until metaphase II where they arrest again until fertilization. In the ovary, oocytes are surrounded by somatic cells, which communicate hormone-mediated signals and nourish and protect germ cells.

In contrast to the male germ line, all germ cells in the ovary initiate meiosis within a period of days in the mouse and weeks in humans; therefore, isolation of semi-synchronous oocyte populations has been possible. Measurements of telomerase activity in an *in vitro* TRAP assay (Kim *et al.* 1994) have shown a peak in the pre-ovulation oocytes (Eisenhauer *et al.* 1997) and subsequent decrease in mature oocytes (Wright *et al.* 2001) and cleavage-stage embryos, until the 8-cell stage. At this point telomerase is reactivated and significant increase in activity occurs during progression to morula and blastocyst stages (Wright *et al.* 1996, Betts & King 1999, Xu & Yang 2000, Wright *et al.* 2001). The very high levels of telomerase activity detected in the immature oocytes raises the possibility that telomere elongation occurs

in these cells during their passage through meiosis. The equally high activity detected at the blastocyst stage of embryonic development suggests that telomere elongation also occurs in the differentiating germ line. The fact that telomerase activity showed high variability among embryonic cells (Wright *et al.* 2001) indicates that from very early developmental stages some cells may be more proficient than others in telomere elongation. It is still to be established whether the germ line cells are among those with high telomerase activity.

The fact that oocytes at fertilization stage are characterized by low telomerase activity may provide a selection mechanism for cells that have already acquired sufficient telomere length to be fertilized successfully. Consistent with this is the observation by Liu *et al.* (2002b) that oocytes with short telomeres produced by telomerase-null mice fail fertilization and early cleavage embryonic stages.

The testis

In contrast with the female germ line, from which oocytes can easily be isolated and analysed individually or in homogenous populations, the testis contains a mixture of cells with various functions, undergoing different processes in an asynchronous manner. For example, spermatogonia undergo mitotic divisions during germ line development, then reach maturation followed by entry into meiosis. Most testis cells are engaged in meiotic divisions (spermatogenesis) to produce haploid gametes, which then undergo a differentiation process (spermiogenesis) characterized by dramatic changes in cell morphology and size to generate mature sperm.

De Lange and colleagues (de Lange *et al.* 1990) established that sperm telomeres are significantly longer than those of somatic cells; however, telomerase activity has not been detected in mature sperm (Wright *et al.* 1996, Eisenhauer *et al.* 1997). Thus, one important aspect of meiotic telomere maintenance that remains to be elucidated is which of the several cell types in the testis are proficient in telomerase-mediated telomere elongation, or indeed whether telomerase is the only mechanism of telomere maintenance in the germ line. The major challenge in this direction has been fractionation of synchronous cell populations from testis, which is practically difficult owing to the lack of preferential positioning of various cell types within the seminiferous tubules

and also to the similarity in their sizes. Methods for isolation of fractions enriched in specific meiotic stages have been reported, including centrifugal elutriation (Grabske *et al.* 1975, Meistrich *et al.* 1977), which can allow enrichment of up to 90% in pachytene cells, as determined by immunofluorescence staining with markers specific for this stage such as the synaptic protein SYCP1 (Tarsounas *et al.* 1999).

Eisenhauer and colleagues (Eisenhauer *et al.* 1997) showed that extracts from fractionated pachytene spermatocytes and round spermatids have high telomerase activity in an *in vitro* TRAP assay (Kim *et al.* 1994), while spermatozoa had no detectable activity. However, the composition of these fractions has not been directly determined in these experiments, and the authors acknowledge the possibility of contamination with other types of cells. Other studies have used detection of telomerase expression by *in-situ* hybridization to establish the timing of telomere elongation in the testis. According to these experiments, expression of the telomerase RNA component peaks in meiosis I spermatocytes and Sertoli cells of adult human testis (Yashima *et al.* 1998, reviewed in Bekaert *et al.* 2004), and it is relatively high in all cells of human pre-pubertal testis. However, telomerase expression does not necessarily imply active telomere elongation, therefore, a direct method for *in-situ* detection of telomerase activity (*in-situ* TRAP assay) has recently been developed by Tanemura and colleagues (Tanemura *et al.* 2005). The classical TRAP assay for telomerase activity is based on PCR amplification of telomerase products containing the sequence (TTAGGG)_n (Kim *et al.* 1994). The *in situ* version of the assay allows detection and quantification of telomere extension in tissue sections, thus directly identifying telomerase-proficient cells. In this assay, telomerase activity peaked in replicating pre-leptotene cells and round spermatids of adult mouse testis, which suggests that telomere elongation occurs before and after meiosis. The *in-situ* TRAP assay has a great potential for accurate identification of telomerase-proficient cells, particularly if used in combination with molecular markers for various meiotic stages. The downside is that it has not yet been validated in telomerase-negative mice. Tanemura *et al.* (2005) have also employed telomere restriction fragment analysis (TRF; van Steensel & de Lange 1997) to demonstrate that telomeric DNA tracts are significantly longer in the testis of sexually mature mice (12 weeks old)

than in those of pre-pubertal mice (4-weeks old). This is an interesting finding, as it indicates that entry into meiosis triggers telomere lengthening.

Does telomere elongation occur during meiosis?

The overall image that has emerged from various studies is that telomerase acts in the germ line, and its timing is governed by a sexual dimorphism, consistent with clear differences between meiosis in the two sexes (Figure 1). In oocytes, telomere elongation probably occurs during the first meiotic division, similarly to prophase I spermatocytes. In addition, in the testis, elongation may also occur before cell commitment to meiosis and after completion of meiosis. A reliable method for accurately defining the stage of telomere elongation in the testis or ovary is currently lacking, mainly owing to inefficient methods for isolation of homogeneous populations of cells synchronously undergoing meiosis. This complexity is partly overcome in the ovary where the meiotic arrest facilitates study of certain meiotic stages. The development of methods for *in-situ* telomerase activity detection in testis holds a

promise for future studies, once its specificity is confirmed in the telomerase-negative mice. Molecular markers are nowadays available for accurate identification of individual stages of meiotic progression (e.g. SCP1, SCP3, REC8), which should significantly aid these studies, in conjunction with the widely used morphological criteria implemented by Leblond & Clermont (1952).

An important mechanism of telomere length regulation in all cells is the assembly of the active form of telomerase at the telomere (Blackburn 2001), which is dependent upon the availability of an open DNA end. Thus, exposure of the telomeric G overhang is a major requirement for successful elongation, and a poorly understood aspect of meiotic telomere biology. Some clues to availability of the G overhang during meiosis may come from the distribution of the telomeric factor TRF2, crucial for the formation of the t-loop structure with a role in protecting the telomeric G-rich tail (Figure 1). Scherthan and colleagues (Scherthan *et al.* 2000) have demonstrated that TRF2 is detected at the telomeres of mouse spermatocytes during prophase I. Here we present evidence that TRF2 association

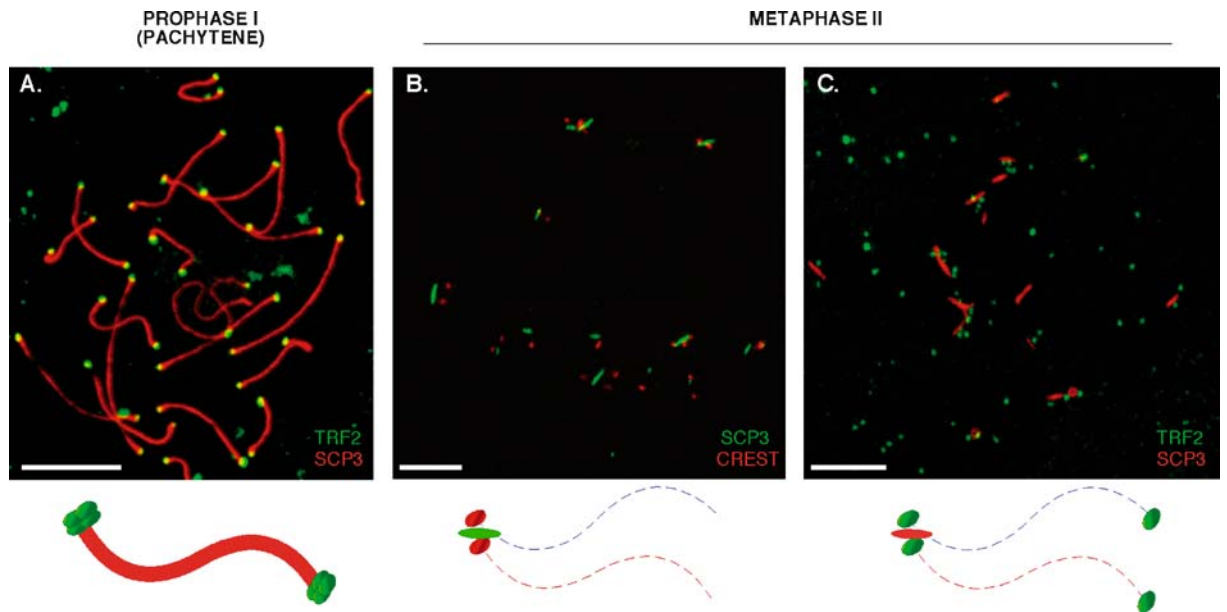


Figure 1. Association of TRF2 with mouse telomeres in meiosis I and meiosis II spermatocytes. (A) TRF2 (green) stains the telomeres of synapsed chromosomes during the pachytene stage of meiosis I. (B) Metaphase II spermatocytes are identified by the characteristic residual SCP3 staining (green) between the separating kinetochores (CREST serum; red). (C) At metaphase II, half of the TRF2-labelled mouse telomeres (green), coinciding with centromeres, associate with residual SCP3 staining (red). Scale bars = 10 μ m.

with meiotic telomeres can be detected at metaphase II (Figure 1). While the entire length of the meiotic chromosomes can be visualized during first meiotic prophase using antibodies against the SCP3 component of the axial elements (Figure 1A), metaphase II is identifiable by the characteristic residual SCP3 staining between sister centromeres (Figure 1B). As mouse chromosomes are telocentric, half of their telomeres coincide cytologically with the centromeres; therefore, SCP3 is detectable between two sister telomeres (Figure 1C). Presence of TRF2 at the telomeres during both meiotic divisions may suggest that t-loops are assembled and telomeres are inaccessible for elongation throughout meiosis. Alternatively, some TRF2 may remain at telomeres even when the t-loop opens and telomeres are elongated. This is one aspect of telomere maintenance that is not fully understood at the moment. In mitotically diving cells, telomerase extends chromosome ends during S phase, coincidentally with telomere replication (Marcand *et al.* 2000). If meiotic cells were to follow the mitotic paradigm, then telomere elongation should occur in the precursor cells undergoing pre-meiotic replication.

Telomere protection

A fundamental role of the telomeres is to protect chromosome termini from being sensed by the cell as broken DNA (the capping function; Takai *et al.* 2003), which can then trigger a DNA damage checkpoint response to elicit cell-cycle arrest that prevents further telomere erosion (diFagagna *et al.* 2004). Cells can escape this block in cell growth and attempt to repair their uncapped telomeres by engaging the DNA repair machinery, which leads to chromosomal end-to-end fusions. Thus, to preserve chromosome integrity, cells need to discriminate between broken DNA ends and the natural ends of the chromosomes. The mechanism that enables cells to distinguish between the two remains elusive. One possibility is that telomere-binding proteins impose a unique chromatin structure at the telomeres, which prevents their recognition as DSBs. Three proteins that bind telomeric repeats with sequence specificity *in vitro* have been identified in mammalian cells: TRF1 (Chong *et al.* 1995) and TRF2 (van Steensel *et al.* 1998), which bind to double-stranded repetitive

telomeric DNA and localize to the telomeres in mammalian cells, and POT1 (Baumann & Cech 2001), which binds to the 3' telomeric overhang. These proteins further recruit other factors to the telomere (e.g. TRF1 recruits among others tankyrase and, similarly, TRF2 recruits RAP1 and its modulator, TIN2), ultimately generating a higher-order complex with end-capping and length-control functions (reviewed in Blasco 2005, de Lange 2005).

Recently, homologous recombination, a major pathway for DSB repair within all cells and of special significance for cells undergoing meiotic divisions, has emerged as an important mechanism for telomere protection (Henson *et al.* 2002). Homologous recombination is thought to remodel telomeric DNA into a structure called the t-loop (Griffith *et al.* 1999, Figure 2), thus protecting it from DNA-damage sensors. The t-loop may form by invasion of the 3' telomeric overhang into the duplex DNA of the same telomere, thereby generating a Holliday junction (HJ), the hallmark recombination intermediate (Liu & West 2004). Telomeric HJs will sequester the invading terminus away from the DNA processing activities and DNA damage sensors present within the cell.

T-loops appear to be evolutionarily conserved structures at chromosome ends (de Lange *et al.* 2006). The existence of t-loops in meiotic cells has not been formally demonstrated, but they are expected to exist at least in some species from extrapolation of data available for the telomeres of mitotically diving cells. Many aspects related to their assembly, stabilization and dissolution have not yet been elucidated, although the requirement for DNA repair activities has been clearly demonstrated (Verdun & Karlseder 2006). It has been postulated that some homologous recombination activities, mainly provided by the RAD51 paralog family, may act in protecting or stabilizing the telomeric HJ (Tarsounas & West 2005). For example, RAD51D is present at meiotic telomeres throughout prophase I, potentially acting in protecting HJ and telomere integrity (Tarsounas *et al.* 2004, Figure 3A), consistent with the HJ binding activity demonstrated *in vitro* for a RAD51D-containing complex (Yokoyama *et al.* 2004). Additionally, the RAD51C/XRCC3 complex associated with HJ resolution activities in HeLa extracts (Liu *et al.* 2004b), and with a role in HJ binding and targeting the resolution activity to HJs, is detected in

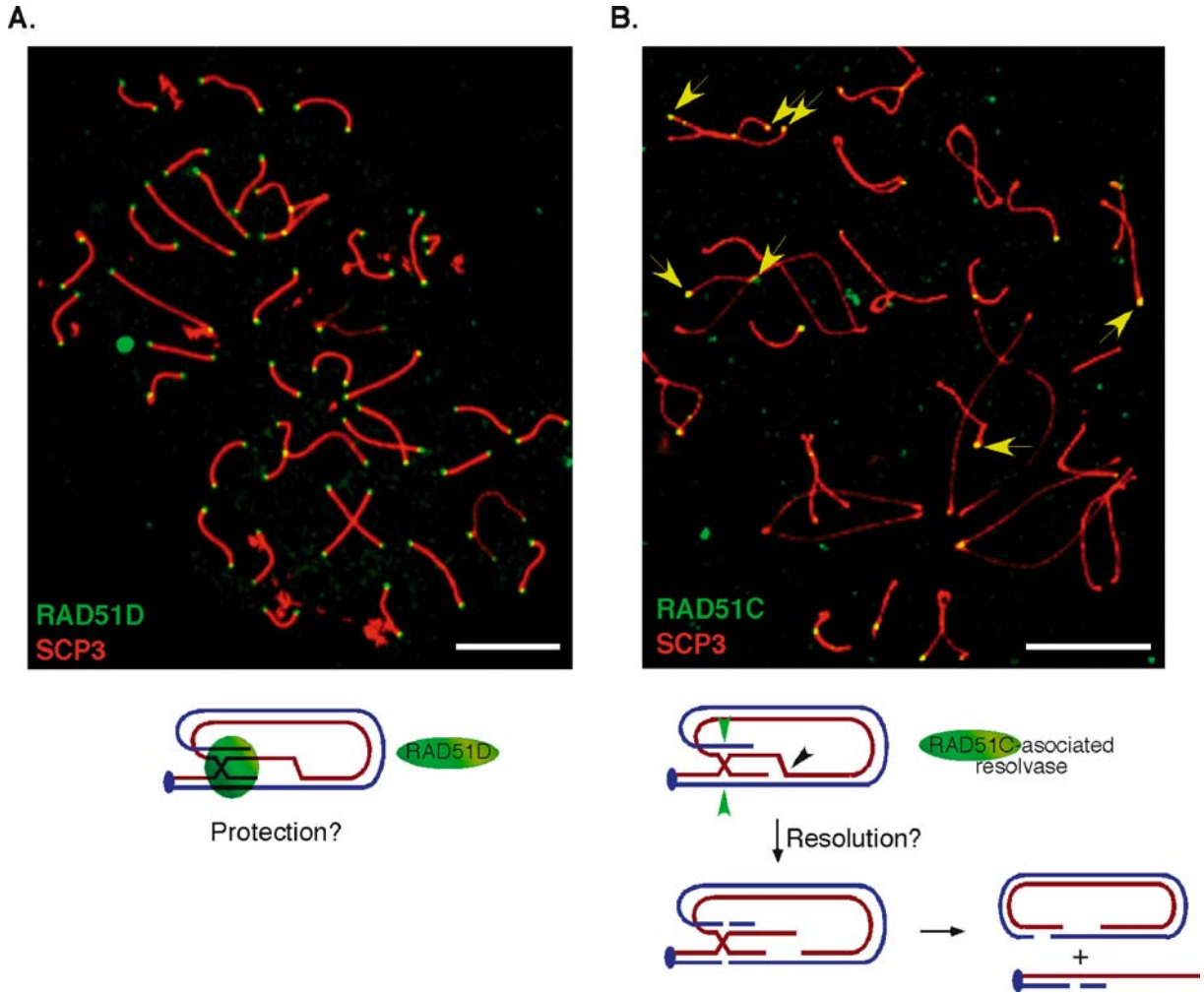


Figure 3. Association of RAD51 paralogs with meiotic telomeres and possible roles in telomere regulation. (A) RAD51D (green) associates with mouse telomeres during meiosis I, where it may stabilize telomeric t-loops. (B) RAD51C (green) binds to a subset of the telomeres (yellow arrows) in mouse spermatocytes, where the RAD51C-associated resolvase activity may be required for telomere processing reactions similar to mitotic TRD. (Reproduced with permission from *J Biol Chem* (2007) **282**: 1973). SCP3, a structural component of meiotic chromosomes, is in red. Scale bars = 10 μ m.

Do telomerase-deficient mice with short telomeres undergo meiosis?

In 1997, Blasco and colleagues generated the first mice lacking the telomerase RNA gene (*TR*^{-/-} or *Terc*^{-/-} mice; Blasco *et al.* 1997). Telomerase activity was completely abolished in these mice, which led to progressive telomere shortening and loss of telomeric capping function with successive generations. While early-generation *Terc*^{-/-} mice showed normal fertility in spite of shorter than wild-type telomeres, in the late generations the telomere-exhausted chromosomes and associated end-to-end

fusions resulted in genomic instability and sterility (Lee *et al.* 1998, Herrera *et al.* 1999). Consistent with differences between males and females in meiosis progression, late generation *Terc*^{-/-} mice exhibited sex-specific meiotic differences: in males, the high rate of apoptosis induces a striking depletion of spermatocytes from the seminiferous tubules (Franco *et al.* 2002, Liu *et al.* 2004a), while the female meiosis seems less compromised and is still able to generate oocytes. The tolerance of short telomeres in females can be explained by less stringent checkpoint control of meiotic progression in oocytes, leading to an increased proportion of mature oocytes carrying

chromosomal aberrations (Hunt & Hassold 2002). This apparent meiotic competence however, comes at a price, as telomerase-deficient oocytes engage in aberrant fertilization leading to abnormal cleavage of embryos (Liu *et al.* 2002b).

In late-generation *Terc*^{-/-} males, meiotic progression is impaired at the synaptic pachytene stage owing to compromised inter-homolog recombination (Liu *et al.* 2004a). Short telomeres are inefficient in tethering chromosome ends to the nuclear envelope (Figure 3), which in turn interferes with homologous chromosome alignment and recombination. Similar defects in females impact negatively upon the integrity of meiotic spindles (Liu *et al.* 2002a), but the rate of elimination in oocytes is far lower, and many reach maturity (Liu *et al.* 2002b). TRF1 levels in the germ cells of late generation *Terc*^{-/-} mice are significantly reduced compared to wild type (Franco *et al.* 2002), consistent with the idea that the amount of TRF1 at each telomere reflects the length of the telomeric tract, similarly to the telomere repeat counting role of Rap1 in budding yeast (Marcand *et al.* 1997). It still remains to be elucidated whether TRF2, an indicator of the protection status of the telomere, remains associated with short meiotic telomeres in the late-generation telomerase-deficient mice. On the basis of previous results (van Steensel *et al.* 1998), it is conceivable that TRF2 association with short dysfunctional telomeres is significantly reduced. It is worth emphasizing that no end-to-end fusions have been reported so far in the meiotic cells of the late-generation *Terc*^{-/-} mice, in contrast with the high incidence of such fusions in somatic cells of the same genotype. This could be due to a yet unknown protection mechanism for very short telomeres upon entry into meiosis, or to the down-regulation of the non-homologous end-joining machinery, commonly required for generating such fusions, during early meiosis (Goedecke *et al.* 1999).

In mitotically dividing cells, telomere attrition leads to rampant genomic instability, which triggers senescence or apoptosis through activation of the p53 pathway (Maser & DePinho 2002). Consistent with this, genetic inactivation of p53 partially abrogates several of the phenotypes caused by telomere shortening in *Terc*^{-/-} mice, including the striking germ cell depletion (Chin *et al.* 1999). Although the *p53*^{-/-}*Terc*^{-/-} mice can be bred for two additional generations compared to the single *Terc*^{-/-} mutant mice, they also eventually become sterile. It is

conceivable that lack of p53 compromises efficient detection of homologous recombination defects caused by short telomeres in these mice, and allows meiotic progression. However, an in-depth analysis of meiotic progression in these mice might unravel an origin of meiotic failure different from merely short telomeres. Interestingly, erratic telomere elongation has been reported in some seminiferous tubules of the *p53*^{-/-}*Terc*^{-/-} mice (Chin *et al.* 1999), which may be due to activation of alternative pathways of telomere lengthening.

ALT—Is it active during meiosis?

Telomerase provides the major cellular enzymatic activity for direct addition of telomeric DNA repeats to chromosome ends. However, most cells in adult organism lack telomerase activity, which limits their life span. A pathway for telomere elongation alternative to telomerase has been defined in cells lacking telomerase or the ability to activate it, named alternative lengthening of telomeres (ALT), and unlimited proliferation can emerge (Henson *et al.* 2002). The mechanism of ALT-mediated telomere maintenance is not well understood, but it is thought to engage the homologous recombination machinery in reactions involving recombination between a short telomere and a donor DNA molecule containing telomeric repeats—a longer telomere, an extra-chromosomal circle, etc. (reviewed by Henson *et al.* 2002). These reactions often generate exceedingly long telomeres, which contribute to the heterogeneity in telomere length within the same cell, one of the hallmarks of ALT activation.

Late-generation *Terc*^{-/-} mice with telomere-exhausted chromosome ends and high apoptotic rate are sterile and often succumb to severe malfunction of various organs (reviewed in Blasco 2005). It is surprising that only very modest activation of telomere-rescue mechanisms has been detected in these mice and this is limited to the germinal centres of the spleen: proliferating splenocytes show abnormally long telomeres upon antigen stimulation (Herrera *et al.* 2000). More recently, Maria Blasco's group has demonstrated that ALT is activated in *Terc*-deficient keratinocytes when TRF2 is overexpressed (Blanco *et al.* 2007). The only indication so far of ALT activation in the germ line comes from the study of late-generation *Terc*^{-/-} mice that also lack *p53* (Chin *et al.* 1999). In these mice, most

seminiferous tubules were depleted of spermatocytes; however, some appeared meiotically active, and their cells had very long telomeres. This suggests that germ cells have the capacity to activate ALT in the absence of telomerase. Although it is not clear where this activation occurred, it can be anticipated that cells at the early stages of meiosis where recombinational DSBs repair occurs could have the ability to engage their already active recombination machinery in elongating their own eroded telomeres.

Lusting and colleagues have recently reported an intriguing role for homologous recombination in telomere maintenance during meiosis in budding yeast (Joseph *et al.* 2005). The authors postulate the existence of a mechanism for shortening exceedingly long telomeres in meiosis through a mechanism involving inter-chromatid recombination similar to telomere rapid deletion (TRD) in mitotic cells (Lustig 2003). TRD is thought to occur by t-loop resolution and excision of telomeric DNA fragments (Bucholc *et al.* 2001), and ensures that the length of all telomeres is reset within a narrow distribution at the time cells commit to meiosis. This observation strengthens the possibility that occasional processing of telomeric tract by homologous recombination activities on certain telomeres occurs during meiosis. Whether this reflects active telomere length alteration or just the repair of DSBs accidentally introduced in the telomeric DNA tract is hard to distinguish. Perhaps such occasional processing could explain the sporadic association of homologous recombination proteins with some telomeres but not others on meiotic chromosome spreads (Figure 3B).

Telomere contribution to meiotic chromosome architecture and meiotic spindle integrity

Nuclear membrane attachment and bouquet formation

During the early stages of the meiotic prophase I, chromosomes undergo a series of morphological changes including compaction, formation of axial elements that structurally define each homolog and establishment of inter-homolog interactions which facilitate their recombination at DNA level (reviewed in Zickler & Kleckner 1998). This period also includes the bouquet stage, when telomeres embedded in the inner nuclear membrane move into a polarized

configuration and cluster together surrounding the spindle pole body (SPB) in fission yeast or the centrosome in mammals (reviewed in Harper *et al.* 2004). Attachment of the telomeres to the nuclear envelope and bouquet formation are two distinct aspects of telomere behaviour during meiosis (Zickler & Kleckner 1998): telomeres remain anchored to the inner surface of the nuclear envelope from leptotene to late pachytene, while the bouquet assembly is a far more transient phenomenon occurring at the leptotene–zygotene transition.

Although bouquet configuration is a universal feature of meiosis, its precise function remains obscure. The severity of its absence for cell viability cannot simply be equated with the subsequent defect in homologous recombination, which has been reported in yeast mutants with defective bouquet formation. It is expected that future studies will unravel some novel essential function for this structure in meiosis. Equally, the molecular basis for telomere clustering remains largely unknown in most eukaryotes. In budding yeast, the telomeric protein Ndj1p seems to be required for both bouquet formation and telomere attachment (Trelles-Sticken *et al.* 2000). More recently, lack of bouquet formation has also been reported in cells lacking the Set1 histone H3 methyltransferase (Trelles-Sticken *et al.* 2005). In maize, the *pam1* mutant shows a clustering defect similar to that of *ndj1*; however, nuclear envelope attachment is normal (Golubovskaya *et al.* 2002). A meiosis-specific protein similar to Ndj1p has not been identified so far in mammalian cells.

The most detailed insights into the molecular mechanism of bouquet formation come from studies in fission yeast. During meiotic prophase in this organism, the nucleus becomes dramatically elongated and engaged in a robust back-and-forth movement between the cell poles. This movement, driven by the SPB and associated telomeres, promotes homolog pairing and keeps homologous chromosomes aligned as they undergo recombination. Mutations in the Taz1 telomere-binding protein reduce telomere clustering and their association with the SPB (Cooper *et al.* 1998, Nimmo *et al.* 1998). In addition to the telomeric Taz1, two other factors required for bouquet formation, Bqt1 and Bqt2, have been recently identified in *S. pombe* (Chikashige *et al.* 2006). Genetic and molecular analyses led to characterization of a complex in which Bqt1 and Bqt2 interact with each other and mediate the

interaction of the SBP component Sad1 with telomeric factor Rap1, thus allowing bouquet formation. Mammalian SUN-domain protein Sun2, related to *S. pombe* Sad1, has been shown to localize to the telomeric attachment sites on the nuclear envelope during meiosis (Schmitt *et al.* 2007). The homologs of Bqt1 and Bqt2 in mammalian cells have not yet been identified. Screening for proteins interacting with RAP1 specifically during meiosis may lead to the identification of these bridging factors and could help define the molecular mechanism of telomere tethering in mammalian cells.

Is the telomeric DNA tract important for attachment and bouquet formation?

Analysis of chromosome derivatives in various organisms demonstrated that the presence of telomeric DNA repeats is essential for bouquet formation. Human circular chromosomes lacking telomeric sequences do not participate in the bouquet (Voet *et al.* 2003). Similarly, maize and fission yeast ring chromosomes associate with the SPB as part of the bouquet provided that telomeric sequences are present on these chromosomes (Carlton & Cande 2002; Sadaie *et al.* 2003).

It seems that not only the presence but also the length of the telomere repeat is essential for nuclear envelope attachment: short telomeres in late-generation telomerase-deficient mice lack the perinuclear distribution characteristic of mid-prophase I stages (Liu *et al.* 2004a); however, no effect on bouquet formation has been reported. Thus, the direct link between telomere length and bouquet assembly is still to be established. In these mice, telomere attrition also triggers defects in meiotic homologous recombination and chromosome segregation leading to meiosis failure. This is illustrated by the high rate of apoptosis in the testis and frequent arrest of meiotic progression in females.

Late-generation *Terc*^{-/-} mice exhibit short axial elements (Liu *et al.* 2002a). These, however, may not contribute the meiotic defect in these animals, as correctly assembled axial elements do not appear to be a prerequisite for telomere attachment and clustering in mouse (Liebe *et al.* 2004). Importantly, correct nuclear envelope attachment plaques and bouquet assembly seem to require components of the cohesin complex in maize and mice (Revenkova

et al. 2004, Golubovskaya *et al.* 2006). Therefore it is possible that cohesin may mediate the interaction between chromosome ends and the SPB/centrosome components during meiosis.

Does homologous recombination control bouquet dissolution?

An intriguing feature of the bouquet stage is its dissolution, which seems to be dependent upon successful completion of recombination events and therefore could be checkpoint regulated. Correct telomere positioning at the nuclear envelope and into the bouquet configuration facilitates (but does not initiate; Wu & Burgess 2006) proper alignment of the homologous chromosomes, which is in turn a prerequisite for their recombination (Scherthan 2001, Harper *et al.* 2004). Telomeres cluster normally in mice lacking various homologous recombination activities, but exit from this stage is delayed (Liebe *et al.* 2006). This is likely due to activation of an intra-prophase I checkpoint that monitors correct progression of recombination reactions during meiosis. Most likely, this checkpoint response involves ATM (Di Giacomo *et al.* 2005), a serine-threonine kinase that phosphorylates several DNA repair and checkpoint proteins involved in the repair of the DNA double-strand breaks that initiate meiotic recombination (Shiloh 2003, Jeggo & Lobrich 2006).

Spindle integrity

One of the most striking differences between meiosis in males and females in humans is the rate of meiotic chromosomal errors. Between 10% and 25% of fetuses have the wrong chromosome complement (Hassold & Hunt 2001). The most common errors are trisomies and monosomies, the majority of which are due to non-disjunction at maternal meiosis I, a process in which the spindle integrity and correct kinetochore attachment to the spindle are critical factors. This extraordinarily high rate of erroneous meiosis I in females is thought to be due to less stringent checkpoint control of the first meiotic division, thus allowing alignment and segregation of cells carrying chromosomal abnormalities (Hunt & Hassold 2002).

An interesting connection between meiotic progression in oocytes and telomere length has recently been established (Liu *et al.* 2002a). This work showed that short telomeres in late-generation telomerase-negative mice lead to dramatic defects in chromosome alignment and spindle integrity at metaphase I. However, the vulnerability of metaphase I spindle in these mice may be indirectly affected by telomere length: it is possible that the mechanism underlying misalignment and dysfunctional spindle originates in defects in chromosome pairing and recombination during prophase I, which could have been directly caused by short telomeres. Consistent with a pairing defect, prophase I oocytes show high incidence of unpaired/discontinuous axial elements (Liu *et al.* 2004a) and detection of bivalents with few or no chiasmata, as determined by lack of MHL1 foci, suggests defects in recombinational DSB repair.

Conclusions

Although telomeres have been viewed as structures with very specialized function during meiosis, the picture emerging from their molecular characterization suggests that they might share more similarities with telomeres of somatic cells than originally anticipated. Some major questions related to the function of mammalian meiotic telomeres remain to be answered, and one key issue is how telomere capping impacts on meiosis. Availability of conditional mouse models for components of the telomeric complex, allowing specific deletion in ovaries and testis, are expected to provide some insight into how fundamental telomere maintenance mechanisms contribute to meiotic progression. Additionally, the meiotic telomeric complex still awaits a detailed biochemical characterization. Understanding its molecular composition might help elucidate the mysterious function of the telomeric bouquet in meiosis. Alternatively, the more conservative approach of visualizing telomere-associated factors identified in somatic cells at meiotic telomeres would allow formulation of hypotheses on their potential roles during meiosis based on spatiotemporal localization patterns. Ultimately, this type of approach will hopefully enable progress towards a better understanding of how telomeres contribute to human fertility.

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