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Telomere maintenance without telomerase

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Abstract. Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes that perform a number of vital functions. They allow a cell to distinguish between natural chromosome ends and chromosome breaks in order to delay the cell cycle and repair the broken end. Telomeres also compensate for the inability of DNA polymerase to replicate the chromosome completely. In most eukaryotes a special reverse transcriptase, telomerase, adds telomeric DNA repeats to the chromosome ends using an internal RNA template. However, evidence is accumulating for alternative elongation mechanisms in a variety of eukaryotes. In the yeast *Saccharomyces cerevisiae*, and possibly in humans, both of which normally use telomerase, a different mechanism can be used for chromosome length maintenance when telomerase is inactive or inactivated. Yeast apparently uses recombination for this purpose; the mechanism in humans is not known. Some insect and plant species, on the other hand, do not use telomerase as their primary mechanism for maintaining chromosome length. *Drosophila* makes use of specific retrotransposons for this purpose, while other dipterans use recombination. We summarize here the current knowledge of these alternative telomere elongation mechanisms.

Introduction

Telomeres were first defined some 60 years ago in the fruit fly *Drosophila melanogaster* based on the function of chromosome end protection (Muller 1938). This capping function appears to be universal, although the molecular components have not been identified in most species. Telomeres are essential chromosomal components whose functional integrity is required for cell cycle progression. One double-stand break in either a chromosome or a plasmid is sufficient to cause checkpoint-mediated cell cycle arrest in yeast (Bennett et al. 1993; Sandell and Zakian 1993), and damaged DNA causes p53-mediated G1 arrest in human cells (Kastan et al. 1991; Kuerbitz et al. 1992; Lane 1992; Di Leonardo et al. 1994). In addition, telomeres are important for terminal elongation to counterbalance chromosome shortening. Every eukaryotic organism must compensate for terminal loss of DNA from chromosome ends, because DNA polymerases cannot completely replicate the ends of linear chromosomes. Most eukaryotes possess long arrays of a short, tandemly repeated, DNA sequence motif on their chromosome ends (Kipling 1995). These tandem arrays are extended by a specific reverse transcriptase, telomerase, that carries an internal RNA template encoded by a single-copy gene in the genome (Blackburn 1992; Greider 1996). However, telomere elongation by telomerase is not universal. A very different telomere elongation mechanism has been demonstrated in the fruit fly *Drosophila melanogaster* that is based on attachment of specific retrotransposable elements, HeT-A and TART, to chromosome termini (Mason and Biessmann 1995), and in a few other species evidence is accumulating in favor of recombination mechanisms in telomere maintenance. Here, we summarize and discuss telomere maintenance mechanisms that apparently do not depend on the action of telomerase. While some species use such mechanisms as a primary pathway in telomere

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elongation, others use them as a secondary pathway. Recombination as an alternative backup elongation mechanism has been shown to exist in yeast, and recently telomerase-independent elongation has also been observed in immortal human cell lines.

Yeast

Yeast telomeres are mainly elongated by telomerase. Telomerase activity has been detected in the budding yeast *Saccharomyces cerevisiae* (Cohn and Blackburn 1995; Lin and Zakian 1995), and the telomerase RNA template gene has been identified (Singer and Gottschling 1994). There is evidence, however, that recombination may also be used to elongate telomeres under some circumstances. One form of evidence in favor of a recombinational mechanism for telomere elongation comes from a series of experiments on linear plasmids carrying terminal *Tetrahymena* $(C_4A_2)_n$ or *Oxytricha* $(C_4A_4)_n$ repeats introduced into yeast. When positioned in the natural orientation with respect to the terminus, the sequences at the ends of these plasmids are recognized as telomeric by the transformed cell, and yeast $C_{1-3}A$ telomeric sequences are added to the ends (Szostak and Blackburn 1982; Dani and Zakian 1983; Pluta et al. 1984). When the transforming plasmid has *Tetrahymena* repeats at one end and *Oxytricha* repeats at the other, telomeric repeats are transferred from one end to the other by recombination (Pluta and Zakian 1989) without loss of C_4A_2 repeats from their original position (Wang and Zakian 1990). Reciprocal recombination that would result in an exchange can be ruled out because the observed addition occurs without the loss of C_4A_4 repeats from the recipient end.

While telomere length is not constant, the average length of yeast telomeres is maintained at about 300 bp per tip. Several genes in *S. cerevisiae* have been identified that are required for maintenance of telomere length. For example, mutations in some genes, such as *RAP1* (Conrad et al. 1990; Lustig et al. 1990) or *TEL1* (Lustig and Petes 1986), cause a change in the equilibrium length, while strains that are mutant for other genes that are required for telomerase activity, gradually lose telomeric sequences (Lundblad and Szostak 1989; Cohn and Blackburn 1995; Singer and Gottschling 1994; Nugent et al. 1996). These mutant cells eventually suffer chromosome loss and die, possibly because the telomere repeat tracts fall below a minimum requirement. However, a small proportion of mutant cells survive by *RAD52*-dependent amplification, indicating involvement of recombination, and aquisition of Y′ subtelomeric repeats, and associated $C_{1-3}A$ sequences, on most or all their chromosomes (Lundblad and Blackburn 1993). This widespread rearrangement of Y′ repeats has been interpreted as a recombinational bypass pathway for maintaining chromosome end length. Whether this pathway is mechanistically related to the process of adding telomeric repeats onto newly transformed linear plasmids remains to be seen. A similar recombination pathway, probably not involving subterminal sequences, has recently been demonstrated in mutants of the related budding yeast *Kluyveromyces lactis* that are deleted for the telomerase RNA gene *TER1* (McEachern and Blackburn 1996). This bypass recombination pathway appears to be quite efficient, resulting in lengthened telomeres and supporting cell growth in the absence of a functional telomerase.

Insects

Telomeres have been studied in a few insect species of the order *Diptera*, *Lepidoptera* and *Hymenoptera*. The pentanucleotide (TTAGG)n, which lacks one guanine residue of the vertebrate telomeric repeat, is located at chromosome ends in the silkworm *Bombyx mori (Lepidoptera)*, suggesting that *B. mori*, may elongate its telomeres by telomerase (Okazaki et al. 1993). However, no enzymatic telomerase activity has yet been detected by *in vitro* assays (H. Fujiwara, pers. commun.). Southern blots of genomic DNA of representative species of most insect orders showed cross-hybridization with the TTAGG repeat (Okazaki et al. 1993), but no in situ hybridizations have been done to determine whether these cross-reacting sequences are telomeric, except in several ant species of the genus *Myrmecia*, where the repeat is located at the telomeres of all chromosomes (Meyne et al. 1995). None of the tested dipteran species cross-hybridized with this probe.

The chromosome ends of *Drosophila* are elongated very differently from those of most other eukaryotes (Mason and Biessmann 1995). *D. melanogaster* (*Diptera*, suborder *Cyclorrhapha)* lacks canonical telomeric repeats, as shown by the failure to cross-hybridize with various oligonucleotides chosen based on similarities of telomeric repeats in widely divergent eukaryotes (Richards and Ausubel 1988; Meyne et al. 1989; Levis 1993; Okazaki et al. 1993). Instead of telomeric repeats, two retrotransposable elements, HeT-A and TART, have been found at natural chromosome ends in *Drosophila* and can be acquired by broken ends (Traverse and Pardue 1988; Biessmann et al. 1990, 1992a,b, 1994; Danilevskaya et al. 1992, 1994; Karpen and Spradling 1992; Levis et al. 1993; Sheen and Levis 1994). These elements are members of a novel family of polyadenylated, non-LTR (long terminal repeat) retroposons and belong to the evolutionarily widespread family of long interspersed elements, LINEs. HeT-A and TART elements transpose to broken chromosome ends without specific sequences at the target site (Biessmann and Mason 1992; Biessmann et al. 1992a; Levis et al. 1993; Mason and Biessmann 1993). However, the presence of HeT-A elements does not protect the ends from gradual chromosome shortening (Biessmann et al. 1992b), and the length of the HeT-A and TART array, which may vary for a single chromosome tip within a population of flies, likely reflects the balance between transposition frequency and terminal nucleotide loss (Walter et al. 1995).

If telomere elongation by retrotransposition evolved in a dipteran ancestor, it may be possible to detect HeT-A and TART-like elements in other dipterans, or at least in other drosophilid flies. However, noncoding regions

of HeT-A do not cross-react well under normal hybridization conditions to other *Drosophila* species (Young et al. 1983). Yet some species in the *melanogaster* subgroup exhibit cross-reactivity, and in situ hybridization localizes these DNA fragments to some telomeres, suggesting that HeT-A-like elements may be present in these species and that they are also involved in telomere elongation (Young et al. 1983). An HeT-A like element was isolated from the close relative, *Drosophila simulans* (Pardue et al. 1996). More distantly related species such as *Drosophila virilis* do not show any HeT-A cross-reactivity (Young et al. 1983). Screening a library of *D. virilis* genomic DNA with an oligonucleotide from the highly conserved 3' noncoding region of HeT-A failed to yield any HeT-A-like elements (H. Biessmann, unpublished). In an independent study, a tandem repeat of 4.4 kb units has been isolated from *Drosophila miranda* that hybridizes to all chromosome tips in *D. miranda,* but does not cross-hybridize with *D. melanogaster* DNA (Steinemann 1984; Steinemann and Nauber 1986). These features are reminiscent of *D. melanogaster* HeT-A elements, which, in nontelomeric locations, can also occur in tandem arrays containing the 3 kb noncoding region of this element (Rubin 1978; Traverse and Pardue 1989). Thus, the presence of HeT-A-like elements with potential function in telomere elongation is suggested in *D. miranda,* but no sequence data are available as yet to confirm this notion (M. Steinemann, pers. commun.). An unusual situation exists in *Drosophila neohydei,* where ribosomal DNA sequences are located outside the nucleolus organizers and found at the tips of autosomes *2*, *3*, and *4* (Hennig et al. 1982). It is not known into which telomeric region these rDNA copies are integrated or whether they are involved in telomere elongation.

Although data on telomeric retroposons in the genus *Drosophila* are still very sketchy, it is possible that other drosophilid flies contain some form of transposable elements involved in telomere elongation. Why then is there so little sequence homology at the ends? One possibility is that different classes of transposable elements are being used in different species for telomere elongation function. This hypothesis needs to be tested by cloning DNA from chromosome ends from other *Drosophila* species without any bias for HeT-A and TART homologies. The closest relative of HeT-A and TART is the retroelement *jockey* in *D. melanogaster*, and it is conceivable that both have evolved from a *jockey*-like ancestor and acquired the capability, perhaps with the addition of an extended 3′ noncoding region, to transpose to chromosome ends. The novel capability of retrotransposons to attach to double-strand breaks and "heal" broken chromosomes has recently also been observed in yeast (Moore and Haber 1996; Teng et al. 1996), providing an example of how the *D. melanogaster* telomere-specific elements HeT-A and TART may have evolved. It is unclear whether HeT-A and TART have acquired telomere elongation function independently. Even though these two different LINE-like elements perform the same function in *D. melanogaster*, they exhibit very limited sequence homology outside the zinc finger region of the encoded gag-like protein. In addition, there seems to be a relatively low degree of sequence conservation among members of the HeT-A family of elements, suggesting a tolerance for divergence in these functionally important elements (Biessmann et al. 1994). Another possibility is suggested by the observation that telomere elongation by telomerase and by retrotransposition both require reverse transcriptase activity, but only the portion of the RNA template encoding the telomeric repeat is copied in the case of telomerase, while the entire RNA transposition intermediate is copied in the case of the retroposons (Pardue 1995). One can speculate that both may have evolved from a common, ancient RNA ligation mechanism, which after the evolution of DNA, required reverse transcriptase (Maizels and Weiner 1993).

Can these observations on telomere elongation in higher dipterans be applied to lower dipteran species? The genus *Chironomus* (suborder *Nematocera)* has been studied extensively with regard to telomeric DNA sequences. Chironomids carry large, 50–200 kb, blocks of complex, tandemly repeated sequences at seven of their eight chromosome tips, excepting only the kinetochore end of the telocentric IVth chromosome (Carmona et al. 1985; Saiga and Edström 1985). It has been proposed that these complex telomere-associated repeats may have evolved from sequences similar to simple telomeric repeats of other eukaryotes (Nielsen and Edström 1993). Different telomeres contain different subfamilies of repeats, but these subfamilies also show considerable variation in distribution at the same telomere among different individuals in the same stock (Cohn and Edström 1992a,b). The subfamily D3 is consistently located most distally as indicated by Bal31 digestion (Zhang et al. 1994), terminal tailing, and polymerase chain reaction (PCR) analyses (Lopez et al. 1996), and probably extends all the way to the ends of the chromosomes. Together with the apparent absence of simple telomeric repeats as judged from cross-hybridization experiments (Nielsen and Edström 1993; Zhang et al. 1994), these data support the proposal that telomeres in *Chironomus* are elongated by a gene conversion mechanism involving these long blocks of complex repeat units (Cohn and Edström 1992b; Lopez, et al. 1996).

Chromosome end regression and extension have also been studied in the mosquito *Anopheles gambiae* (suborder *Nematocera)*, which also does not contain sequences that cross-hybridize with the (TTAGG)n repeat of the silkworm *B. mori* (H. Biessmann, unpublished). A fortuitous transgenic insertion of the pUChsneo plasmid at the left end of chromosome 2 (Miller et al. 1987), conferring G418 resistance to the stock, provided a single-copy marker for measuring the dynamics of the 2L telomere over a period of about 10 years and for cloning terminal DNA fragments. The earliest molecular mapping of this transgene indicated that one and a half copies were integrated at or near the end of the 2L chromosome arm, and that shortly after the integration of the transgene the terminal DNA fragment shortened, until an unidentified elongation event occurred between generations 23 and 32 after the integration event (Graziosi et al. 1990). In 1993, about generation 100, the population was again sampled, and terminal length was found to be

relatively homogeneous (Roth et al. 1997). By cloning, no additional new sequence different from the pUChsneo sequence was observed at the end of chromosome 2L, arguing that mosquitoes apparently do not use retroposons to extend either their broken or their normal chromosome ends. Moreover, cloned terminal fragments did not end in short repeat sequences that could have been synthesized by telomerase. Two years later, some 2L chromosome ends had elongated by regeneration of part of the integrated pUChsneo plasmid, probably by recombination between homologous 2L chromosome ends (Roth et al. 1997). This mechanism may also be used in wild type telomere elongation in *A. gambiae*, although proof is still lacking. If normal mosquito telomeres are made of tandemly repeated minisatellite sequences (Biessmann et al. 1996), elongation by recombination between multiple repeats is expected to be much more efficient than that between the one and a half copies of the transgene at 2L. A telomere organization in *A. gambiae* consisting of a complex satellite structure, which extends to the chromosome end, would be comparable to the organization at the telomeres of *Chironomus*, a closely related dipteran.

It is tempting to speculate that telomerase may have been lost by mutation in a dipteran ancestor. As a consequence, alternative pathways for telomere elongation that may already have been present as "backup" mechanisms, may have evolved in this insect order, including terminal transpositions of specialized retroposons in *D. melanogaster*, and unequal recombination as suggested for *Chironomus* and demonstrated in *Anopheles*. As no data are available as to the efficiency of these mechanisms, the unusual telomere elongation strategies in dipteran insects may be less efficient than telomerase. If true, this telomere elongation mechanism may be the limiting factor in dipteran chromosome evolution, favoring the small chromosome numbers found throughout this insect order.

Humans

Long-term chromosome stability in humans depends on the addition of telomeric repeats. Telomerase is active in cells of the germ line, but telomerase activity has not been found in most somatic cells (Wright et al. 1996). As a consequence, somatic cells lose telomeric sequences as a function of population growth in vitro and in vivo (de Lange et al. 1990; Harley et al. 1990; Hastie et al. 1990; Lindsey et al. 1991). It has been proposed that the lack of telomerase activity and the gradual loss of telomeric material may provide a "mitotic clock" that serves to prevent the unlimited growth of somatic cells (Harley 1991). A two-stage model of cellular senescence in vitro has been proposed (Wright and Shay 1992). The first stage is a programmed cessation of mitotic growth, and is under the control of the *p53* and the *RB-1* genes (Shay et al. 1991; Rogan et al. 1995). Inactivation of these genes allows cells to resume growth for a limited period, after which they enter "crisis" and die. In most cases, during this limited period of growth telomeres continue to shorten, and very few cells escape crisis. Those that do can grow indefinitely and are considered immortalized. One of the changes required for cells to become immortalized is the ability to add telomeric repeats to chromosome ends. This is usually achieved by the reactivation of telomerase, and consequently, most of the immortal cell lines have stable, relatively short, telomere arrays and exhibit telomerase activity (Autexier and Greider 1996; Shay and Wright 1996). However, there are some notable exceptions, indicating that spontaneous immortalization can occur in human fibroblasts in the absence of telomerase activity (Rogan et al. 1995). In a survey of immortal cell lines, Kim et al. (1994) found that approximately 2% of cultured cells and 10% of tumors maintained long stretches of terminal repeats without detectable telomerase activity. In a smaller survey, Bryan et al. (1995) found that 40% of the immortal lines they tested showed no telomerase activity, yet these cell lines had very long and heterogeneous telomeres of up to 50 kb. To date, it is not clear what mechanism is responsible for the elongation of the terminal array in the apparent absence of telomerase. Intermittent bursts of telomerase activity have been discussed, but elongation by nonreciprocal recombination involving terminal and subterminal sequences appears more likely. A study of the dynamics of a single telomere that involved a single-copy transgene at chromosome 13 in a telomerase-negative immortal fibroblast cell line revealed dramatic bursts of telomere shortening and elongation, which may be caused by recombination events (Murnane et al. 1994). Telomerase-independent elongation in B and T cell lines, possibly caused by recombination, has recently been detected in experiments using inhibitors of retroviral reverse transcriptase to inhibit telomerase activity (Strahl and Blackburn 1996).

Other organisms

Canonical telomeric repeats of the *Arabidopsis* type (TTTAGGG)n have not been found in species of the plant genus *Allium* (Fuchs et al. 1995). In situ hybridization experiments reveal that these plants appear to have a more complex repeat array of about 375 bp repeat length at or near their chromosome ends (Pich et al. 1996a,b). The ciliate *Tetrahymena* has linear mitochondrial DNA molecules with terminal arrays encompassing repeat units of about 30–50 bp (Morin and Cech 1986, 1988). As in other organisms discussed above, recombination has been proposed as a possible elongation mechanism in these cases; however, direct evidence is still lacking.

Conclusions

Telomerase is the most commonly used way in eukaryotes for telomere elongation. However, telomere elongation in the absence of telomerase has been demonstrated in three very divergent groups: the budding yeasts, dipteran insects, and humans. Terminal attachment of retrotransposons elongate *D. melanogaster* telomeres, and

recombination rather than telomerase has been proposed in several other cases as summarized above. How can the occurrence of mechanisms that do not involve telomerase to enhance telomere length in such a wide variety of species be explained? Double-strand breaks occurring naturally during meiosis or induced by mutagens stimulate recombination, and thus the ends of DNA molecules in general may be recombinogenic. One might then speculate that, with the loss of telomerase activity and the resulting elimination of telomeric sequences and the proteins that bind to them, recombination might be stimulated by these naturally occurring double-stranded ends. Thus, eukaryotes may possess the dual capacity of telomere elongation by telomerase and recombination, but because of the possibly higher efficiency of the telomerase pathway, and/or restrictions conferred by chromatin structure, elongation by recombination occurs only in unusual situations. In order to survive in the absence of telomerase and grow, cells must somehow add new telomeric sequences to their chromosome ends, and recombination may be a ready alternative. Such backup elongation mechanisms have to be taken into consideration when telomerase is being targeted as a means of proliferation control in tumors.

The dipteran insects, and perhaps some Allium species, may have permanently lost their telomerase. Therefore, alternative and fairly efficient pathways for telomere elongation must have evolved in these genera. One of them is terminal transposition of specialized retroposons as in *D. melanogaster*; the other is unequal recombination, as suggested for the lower dipterans *Chironomus* and *Anopheles*, and for plants of *Allium* spp. The efficiency of such recombination mechanisms would be greatly enhanced by increasing the size of the homologous target sequences at the telomeres by long stretches of complex terminal satellites, as has indeed been found in these organisms.

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References

Autexier C, Greider CW (1996) Telomerase and cancer: revisiting the telomere hypothesis. Trends Biochem Sci 21:387–391

Bennett CB, Lewis AL, Baldwin KK, Resnick MA (1993) Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc Natl Acad Sci USA 90:5613–5617

Biessmann H, Champion LE, O'Hair M, Ikenaga K, Kasravi B, Mason JM (1992a) Frequent transpositions of *Drosophila melanogaster* HeT-A transposable elements to receding chromosome ends. EMBO J 11:4459–4469

Biessmann H, Donath J, Walter MF (1996) Molecular characterization of the *Anopheles gambiae* 2L telomeric region via an integrated transgene. Insect Mol Biol 5:11–20

Biessmann H, Kasravi B, Bui T, Fujiwara G, Champion LE, Mason JM (1994) Comparison of two active HeT-A retroposons of *Drosophila melanogaster*. Chromosoma 103:90–98

Biessmann H, Mason JM (1992) Genetics and molecular biology of telomeres. Adv Genet 30:185–249

Biessmann H, Mason JM, Ferry K, d'Hulst M, Valgeirsdottir K, Traverse KL, Pardue ML (1990) Addition of telomere-associated HeT DNA sequences "heals" broken chromosome ends in *Drosophila*. Cell 61:663–673

Biessmann H, Valgeirsdottir K, Lofsky A, Chin C, Ginther B, Levis RW, Pardue ML (1992b) HeT-A, a transposable element specifically involved in healing broken chromosome ends in *Drosophila melanogaster*. Mol Cell Biol 12:3910–3918

Blackburn EH (1992) Telomerases. Annu Rev Biochem 61:113–129

Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR (1995) Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 14:4240–4248

Carmona MJ, Morcillio G, Galler R, Martinez-Salas E, de la Campa AG, Edström JE (1985) Cloning and molecular characterization of a telomeric sequence from a temperature-induced Balbiani ring. Chromosoma 92:108–115

Cohn M, Blackburn EH (1995) Telomerase in yeast. Science 269:396–400

Cohn M, Edström JE (1992a) Chromosome ends in *Chironomus pallidivittatus* contain different subfamilies of telomere-associated repeats. Chromosoma 101:634–640

Cohn M, Edström JE (1992b) Telomere-associated repeats in *Chironomus* form discrete subfamilies generated by gene conversion. J Mol Evol 35:114–122

Conrad MN, Wright JH, Wolf AJ, Zakian VA (1990) RAP1 protein interacts with yeast telomeres in vivo: overproduction alters telomere structure and decreases chromosome stability. Cell 63:739–750

Dani GM, Zakian VA (1983) Mitotic and meiotic stability of linear plasmids in yeast. Proc Natl Acad Sci USA 80:3406–3410

Danilevskaya ON, Petrov DA, Pavlova MN, Koga A, Kurenova EV, Hartl DL (1992) A repetitive DNA element, associated with telomeric sequences in *Drosophila melanogaster*, contains open reading frames. Chromosoma 102:32–40

Danilevskaya O, Slot F, Pavlova M, Pardue ML (1994) Structure of the *Drosophila* HeT-A transposon: a retrotransposon-like element forming telomeres. Chromosoma 103:215–224

de Lange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM, Varmus HE (1990) Structure and variability of human chromosome ends. Mol Cell Biol 10:518–527

Di Leonardo A, Linke SP, Clarkin K, Wahl GM (1994) DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev 8:2540–2551

Fuchs J, Brandes A, Schubert I (1995) Telomere sequence localization and karyotype evolution in higher plants. Plant Syst Evol 196:227–241

Graziosi C, Sakai RK, Romans P, Miller LH, Wellems TE (1990) Method for in situ hybridization to polytene chromosomes from ovarian nurse cells of *Anopheles gambiae* (Diptera: Culicidae). J Med Entomol 27:905–912

Greider CW (1996) Telomere length regulation. Annu Rev Biochem 65:337–365

Harley CB (1991) Telomere loss: mitotic clock or genetic time bomb. Mutat Res 256:271–282

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345:458–460

Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC (1990) Telomere reduction in human colorectal carcinoma and with ageing. Nature 346:866–868

Hennig W, Vogt P, Jacob G, Siegmund I (1982) Nucleolus organizer regions in *Drosophila* species of the *repleta* group. Chromosoma 87:279–292

Karpen GH, Spradling AC (1992) Analysis of subtelomeric heterochromatin in the Drosophila minichromosome *Dp1187* by single-P element insertional mutagenesis. Genetics 132:737–753

Kastan MB, Onyewere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 in the cellular response to DNA damage. Cancer Res 51:6304–6311

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015

Kipling D 1995 The telomere. Oxford University Press, Oxford

Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB (1992) Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89:7491–7495

Lane DP (1992) Cancer. p53, guardian of the genome. Nature 358:15–16

Levis RW (1993) *Drosophila melanogaster* does not share the telomeric repeat sequence of another invertebrate, *Ascaris lumbricoides*. Mol Gen Genet 236:440–442

Levis RW, Ganesan R, Houtchens K, Tolar LA, Sheen FM (1993) Transposons in place of telomeric repeats at a *Drosophila* telomere. Cell 75:1083–1093

Lin JJ, Zakian VA (1995) An in vitro assay for *Saccharomyces* telomerase requires EST1. Cell 81:1127–1135

Lindsey J, Mcgill NI, Lindsey LA, Green DK, Cooke HJ (1991) In vivo loss of telomeric repeats with age in humans. Mutat Res 256:45–48

Lopez CC, Nielsen L, Edström JE (1996) Terminal long tandem repeats in chromosomes from *Chironomus pallidivittatus*. Mol Cell Biol 16:3285–3290

Lundblad V, Blackburn EH (1993) An alternative pathway for yeast telomere maintenance rescues est1- senescence. Cell 73:347–360

Lundblad V, Szostak JW (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. Cell 57:633–643

Lustig AJ, Petes TD (1986) Identification of yeast mutants with altered telomere structure. Proc Natl Acad Sci USA 83:1398–1402

Lustig AJ, Kurtz S, Shore D (1990) Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. Science 250:549–553

Maizels N, Weiner AM (1993) The genomic tag hypothesis: modern viruses as molecular fossils of ancient strategies for genomic replication. In: Gesteland RF, Atkins JF (eds) The RNA world. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 577–602

Mason JM, Biessmann HB (1993) Transposition as a mechanism for maintaining telomere length in *Drosophila*. In: Vig BK (ed) Chromosome segregation and aneuploidy. Springer-Verlag, Berlin, pp 143–149

Mason JM, Biessmann H (1995) The unusual telomeres of Drosophila. Trends Genet 11:58–62

McEachern MJ, Blackburn EH (1996) Cap-prevented recombination between terminal telomeric repeat arrays (telomere CPR) maintains telomeres in *Kluyveromyces lactis* lacking telomerase. Gene Dev 10:1822–1834

Meyne J, Ratliff RL, Moyzis RK (1989) Conservation of the human telomere sequence (TTAGGG)n among vertebrates. Proc Natl Acad Sci USA 86:7049–7053

Meyne J, Hirai H, Imai HT (1995) FISH analysis of the telomere sequences of bulldog ants (Myrmecia: Formicidae). Chromosoma 104:14–18

Miller LH, Sakai RK, Romans P, Gwadz RW, Kantoff P, Coon HG (1987) Stable integration and expression of a bacterial gene in the mosquito *Anopheles gambiae*. Science 237:779–781

Moore JK, Haber JE (1996) Capture of retrotransposon DNA at the sites of chromosomal double-strand breaks. Nature 383:644–646

Morin GB, Cech TR (1986) The telomeres of the linear mitochondrial DNA of *Tetrahymena thermophila* consist of 53 bp tandem repeats. Cell 46:873–883

Morin GB, Cech TR (1988) Mitochondrial telomeres: surprising diversity of repeated telomeric DNA sequences among six species of *Tetrahymena*. Cell 52:367–374

Muller HJ (1938) The remaking of chromosomes. The Collecting Net 8:182–195

Murnane JP, Sabatier L, Marder BA, Morgan WF (1994) Telomere dynamics in an immortal human cell line. EMBO J 13:4953–4962

Nielsen L, Edström JE (1993) Complex telomere-associated repeat units in members of the genus *Chironomus* evolve from sequences similar to simple telomeric repeats. Mol Cell Biol 13:1583–1589

Nugent CI, Hughes TR, Lue NF, Lundblad V (1996) Cdc13p: a single-strand telomeric DNA binding protein with a dual role in yeast telomere maintenance. Science 274:249–252

Okazaki S, Tsuchida K, Maekawa H, Ishikawa H, Fujiwara H (1993) Identification of a pentanucleotide telomeric sequence, (TTAGG)n, in the silkworm *Bombyx mori* and in other insects. Mol Cell Biol 13:1424–1432

Pardue ML (1995) *Drosophila* telomeres: another way to end it all. In: Blackburn EH, Greider CW (eds) Telomeres. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 339–370

Pardue ML, Danilevskaya ON, Lowenhaupt K, Wong J, Erby K (1996) The 'gag' coding region of the *Drosophila* telomeric retrotransposon, HeT-A, has an internal frameshift and a length polymorphic region. J Mol Evol 43:572–583

Pich U, Fritsch R, Schubert I (1996a) Closely related *Allium* species (Alliaceae) share a very similar satellite sequence. Plant Syst Evol 202:255–264

Pich U, Fuchs J, Schubert I (1996b) How do alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequences? Chromosome Res 4:207–213

Pluta AF, Dani GM, Spear BB, Zakian VA (1984) Elaboration of telomeres in yeast: recognition and modification of termini from *Oxytricha* macronuclear DNA. Proc Natl Acad Sci USA 81:1475–1479

Pluta AF, Zakian VA (1989) Recombination occurs during telomere formation in yeast. Nature 337:429–433

Richards EJ, Ausubel FM (1988) Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53:127–136

Rogan EM, Bryan TM, Hukku B, Maclean K, Chang AC, Moy EL, Englezou A, Warneford SG, Dalla Pozza L, Reddel RR (1995) Alterations in p53 and p16INK4 expression and telomere length during spontaneous immortalization of Li-Fraumeni syndrome fibroblasts. Mol Cell Biol 15:4745–4753

Roth CW, Kobeski F, Walter MF, Biessmann H (1997) Chromosome end elongation by recombination in the mosquito *Anopheles gambiae*. Mol Cell Biol (in press)

Rubin GM (1978) Isolation of a telomeric DNA sequence from *Drosophila melanogaster*. Cold Spring Harbor Symp Quant Biol 42:1041–1046

Saiga H, Edström JE (1985) Long tandem arrays of complex repeat units in *Chironomus* telomeres. EMBO J 4:799–804

Sandell LL, Zakian VA (1993) Loss of a yeast telomere: arrest, recovery, and chromosome loss. Cell 75:729–739

Shay JW, Wright WE (1996) The reactivation of telomerase activity in cancer progression. Trends Genet 12:129–131

Shay JW, Pereira-Smith O, Wright WE (1991) A role for both Rb and p53 in the regulation of cellular senescence. Exp Cell Res 196:33–39

Sheen FM, Levis RW (1994) Transposition of the LINE-like retrotransposon TART to *Drosophila* chromosome termini. Proc Natl Acad Sci USA 91:12510–12514

Singer MS, Gottschling DE (1994) *TLC1*: template RNA component of *Saccharomyces cerevisiae* telomerase. Science 266:404–409

Steinemann M (1984) Telomere repeats within the neo-Y-chromosome of *Drosophila miranda*. Chromosoma 90:1–5

Steinemann M, Nauber U (1986) Frequency of telomere repeat units in the *Drosophila miranda* genome. Genetica 69:47–57

Strahl C, Blackburn EH (1996) Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. Mol Cell Biol 16:53–65

Szostak JW, Blackburn EH (1982) Cloning yeast telomeres on linear plasmid vectors. Cell 29:245–255

Teng S-C, Kim B, Gabriel A (1996) Retrotransposon reverse transcriptase-mediated repair of chromosomal breaks. Nature 383:641–644

Traverse KL, Pardue ML (1988) A spontaneously opened ring chromosome of *Drosophila melanogaster* has acquired He-T DNA sequences at both new telomeres. Proc Natl Acad Sci USA 85:8116–8120

Traverse KL, Pardue ML (1989) Studies of He-T DNA sequences in the pericentric regions of *Drosophila* chromosomes. Chromosoma 97:261–271

Walter MF, Jang C, Kasravi B, Donath J, Mechler BM, Mason JM, Biessmann H (1995) DNA organization and polymorphism of a wild-type *Drosophila* telomere region. Chromosoma 104:229–241

Wang SS, Zakian VA (1990) Telomere-telomere recombination provides an express pathway for telomere acquisition. Nature 345:456–458

Wright WE, Shay JW (1992) The 2-stage mechanism controlling cellular senescence and immortalization. Exp Gerontol 27:383–389

Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW (1996) Telomerase activity in human germline and embryonic tissues and cells. Dev Genet 18:173–179

Young BS, Pession A, Traverse KL, French C, Pardue ML (1983) Telomere regions in *Drosophila* share complex DNA sequences with pericentric heterochromatin. Cell 34:85–94

Zhang YJ, Kamnert I, Lopez CC, Cohn M, Edström JE (1994) A family of complex tandem DNA repeats in the telomeres of *Chironomus pallidivittatus*. Mol Cell Biol 14:8028–8036