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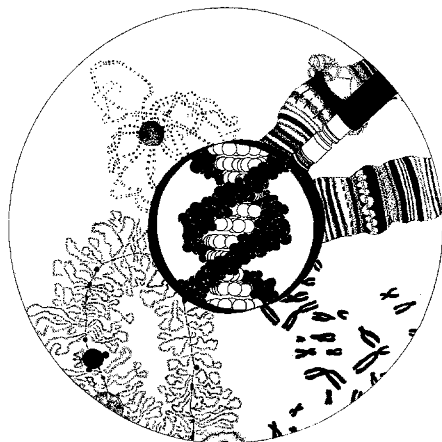

Telomeric repeat sequences

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Abstract. Chromosomes not only carry transcribed genes and their regulatory DNA sequences, but also contain regions that are required for the stability and maintenace of the chromosome as a unit. These include centromeres, telomeres and origins of replication. It is clear for replication origins and centromeres that the positions of these chromosomal organelles are determined by sites of the appropriate DNA sequences, but also that functional performance requires one or more contributing proteins. Telomeres are also structurally complex, with one or more DNA components, including simple telomeric repeats and more complex telomere-associated sequences, as well as one or more specific proteins that recognize these sequences. Accumulating evidence suggests that the simple telomeric repeats are required in most, but not all species, although they are not sufficient to determine the chromosomal position of a telomere.

Two essential telomere functions

Telomeres were recognized more than 50 years ago as necessary components of stable eukaryotic linear chromosomes (Muller 1938). The original observation leading to the proposal of telomeres was that, in radiation experiments, recovered broken chromosomes in *Drosophila* virtually always carried at their ends the distal region of the original treated chromosomes. Simple, one-break deletions were not recovered, although it was assumed that they were intermediates in the formation of the recovered rearrangements. Thus it was proposed that the tip regions are required for the stability of linear chromosomes and act as "caps" to protect chromosome ends. At the same time, McClintock (1938) showed that a single broken chromosome end in maize fuses with its sister after replication to form a dicentric chromosome, which forms a bridge at the next mitotic anaphase and breaks to repeat the cycle. This telomeric capping function eventually led to the suggestion that, in yeast (Weinert and Hartwell 1988), *Drosophila* (Baker et al. 1987), and humans (Lock and Ross 1990), the presence of a broken chromosome end arrests the cell cycle at defined check points, until the broken end becomes repaired or "capped." A second essential function of telomeres is to maintain the length of the chromosomes in the absence of complete DNA replication of the termini of linear eukaryotic chromosomes (Watson 1972; Olovnikov 1973).

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Investigations into the DNA structure of telomeres in a number of widely divergent species have revealed a strong conservation of telomeric DNA sequences. These sequences consist of short, usually 5–8 bp, tandemly repeated units with a G-rich strand that extends past the C-rich strand as a 3' overhang. An unusual, non-Watson-Crick paired, G-quartet structure, perhaps stabilized by specific proteins (Fang and Cech 1993a, b), may be formed by the G-rich overhang. Its significance is currently not understood (Biessmann and Mason 1992; Williamson 1993).

There has been some confusion as to the definition of "telomere," with some authors defining the term according to function and others according to structure. Still others use the two definitions interchangeably. This is due, in part, to the simplifying view that the telomeric DNA repeats found at the ends of many eukaryotic organisms are the telomere and, therefore, are both necessary and sufficient for telomere function. It is clear, however,

that the telomere is structurally complex, containing several DNA and protein components. For purposes of the present discussion, we define a telomere as whatever structure(s) perform(s) the capping and/or replication functions. It is not assumed that a single structure must be responsible for both. In fact, we have argued in the past that the recovery of heritable broken chromosome ends suggested the separation of these two functions (Biessmann et al. 1990a), at least in *Drosophila*. Further, a requirement for a specific telomere component by one function, e.g., maintenance of chromosome length, does not imply a requirement for that component by another function, e.g., capping.

We summarize here recent data supporting the argument that the short, canonical, telomeric DNA repeats may not be sufficient for telomere function, or even necessary, depending on the species.

Ectopic telomeric DNA repeats and the generation of a new telomere

If telomeric repeats and the proteins that bind to them were sufficient for telomere function, one would expect that addition of these sequences anywhere in the genome would result in the creation of a telomere with high frequency at the site of insertion, similar to the situation in which centromeric DNA inserted into yeast chromosomes or plasmids forms a centromere at the site of insertion (reviewed by Hegemann and Fleig 1993). That is usually not observed. In contrast, interstitial telomeric repeats, at least in some species and in certain cytological locations, appear to be quite stable. In a survey of 100 vertebrate species (Meyne et al. 1990), more than half carried detectable telomeric repeats at interstitial chromosomal sites. Interstitial telomeric repeats also occur in plant (Richards et al. 1991), ciliate (Cherry and Blackburn 1985; Herrick et al. 1985; Stoll et al. 1993), nematode (Cangiano and Lavolpe 1993), and human (Weber et al. 1990; Wells et al. 1990) genomes. Most of these sites are in or at the edges of blocks of constitutive heterochromatin, usually in centric regions.

How might interstitial repeats have arisen? In many eukaryotes, insterstitial telomeric sequences may be remnants of chromosome rearrangements that occurred during genome evolution (Meyne et al. 1990). The interstitial telomere blocks resulting from ancestral chromosome fusion are sometimes but not always detectable by in situ hybridization (Scherthan 1990; Schubert et al. 1992; Lee et al. 1993). Interstitial telomeric sequences might also arise from a random short sequence array with fortuitous homology to telomeric repeats, which may become extend by slippage during replication (Henderson and Petes 1992; Schlötterer and Tautz 1992) and further modified by unequal recombination or deletion (Wiley et al. 1992). Another possibility is that telomeric repeats are attached by telomerase or recombination to extrachromosomal linear DNA fragments, which may then integrate into the genome. Such activity has been shown in ciliates to add telomeric repeats onto microinjected linear DNA molecules (Gilley et al. 1988; Bourgain and Katinka 1991) and has recently also been proposed in human cells (Murnane and Yu 1993). Other evidence comes from the analysis of putative transposable elements in the micronucleus of ciliates (Cherry and Blackburn 1985; Herrick et al. 1985; Stoll et al. 1993). These elements are flanked by short arrays of telomeric repeats that may have been added to a putative linear intermediate (Hunter et al. 1989) and account for most of the interstitial telomeric repeats. This mechanism may be peculiar to ciliates, which possess a very efficient developmentally programmed chromosome breakage mechanism that requires the de novo formation of a large number of telomeres.

On the other hand, interstitial telomeric repeats may be associated with genomic instability (Hastie and Allshire 1989). Recent data obtained from situations where interstitial telomeric repeats have been generated artificially suggest that they may indeed have a destabilizing effect. It is difficult to determine, however, whether they are directly involved in initiating chromosome breaks, or function indirectly by stabilizing spontaneous or induced broken chromosome ends.

Circular plasmids containing telomeric sequences introduced into yeast cells can be transmitted stably for many cell cycles (Murray et al. 1988). However, if the introduced sequence has a head-to-head arrangement of the repeating units, this arrangement is resolved at a low rate (1% per cycle) to give a linear plasmid with telomeres at either end. Similarly, when linear DNA fragments containing about 500 bp of human telomeric repeats, as well as some subtelomeric sequences were introduced into hamster/human hybrid cells, stable integration of the fragment occurred mostly at interstitial sites, with about 20% of the transformed lines carrying the integration near a chromosome end (Farr et al. 1991). Interestingly, these newly generated interstitial telomeric repeats exhibit differences in stability depending on their site of integration, but it has not been investigated whether length of the integrated telomeric repeat also affects its stability. In a more extensive experiment using a variety of human and mouse cell lines as recipients, 2–63% of the integration events were able to generate a new telomere at the site of insertion depending on the cell line (Barnett et al. 1993). Transformed cells were much more efficient than primary cultures in generating ectopic telomeres, probably due to the presence of an active telomerase. Most of the integration sites giving rise to a new telomere had occurred close to a natural chromosome end, so that the resulting terminal deletions were not detectable by cytological methods (see also Murnane and Yu 1993). It was, however, evident that the newly integrated telomeric repeats had become terminal and had been elongated by telomerase.

If interstitial telomeric repeats alone could initiate the formation of a new telomere, they should do so independently of the site of integration. Because the cell lines used were mostly aneuploid, and no essential genetic material would necessarily be lost, initiating a telomere at a more internal site would simply result in a longer terminal deficiency. Then why do the more internal integrations appear more stable than the ones near the chromosome ends? A possible answer may be that sponta-

neous chromosome breaks occur more frequently in the telomere-adjacent region than in more internal regions. To date, there is no evidence for such a preference. It is more likely that terminal deletions with breaks in the subtelomeric region may be stabilized and recovered more frequently because telomere binding proteins and telomerase, interacting with each other and with DNA, are already in close proximity to the break. In many species telomeres are sequestered to a restricted region of the nucleus, where there may be a relatively high concentration of such proteins (reviewed by Biessmann and Mason 1992). Distal double-strand breaks may then expose the integrated telomeric repeats to telomerase. The fact that larger terminal deletions, i.e., those with proximal breaks, were not easily recovered, argues against the notion that interstitial telomeric repeats alone can efficiently initiate chromosomal breaks. With proper selection methods, however, such rare chromosome breaks occurring in targeted euchromatic regions can be recovered (Itzhaki et al. 1992), and by rigorously applying positive and negative selection methods that force chromosome breaking, non-targeted integrations of telomere repeats can even be used to generate nested terminal deficiencies (Farr et al. 1992). It therefore appears that interstitial stretches of telomeric repeats per se are not inherently unstable, but that their presence increases the probability of stabilizing a double strand break by functioning as elongation primers for telomerase, especially when they are located near an existing telomere.

Interstitial telomeric repeats also create hotspots for recombination in *Paramecium* (Katinka and Bourgain 1992) and rodents (Ashley et al. 1993; Ashley and Ward 1993). However, this feature may be independent of their telomeric elongation function and may reflect the similarity of such guanine-rich tandem arrays with hypervariable minisatellites and bacterial *chi* recombination sequences (Krowczynska et al. 1990). Hypervariability and increased recombination frequency near telomeric repeats have been observed in yeast (Pluta and Zakian 1989) and the human pseudoautosomal region (Rouyer et al. 1986; Petit et al. 1988). Recently, interstitial telomere sequences have been associated with general genomic instability as one of the factors leading to neoplasia (Hastie and Allshire 1989; Park et al. 1992; Bouffler et al. 1993a). Although only a few cases of interstitial telomere repeat-mediated rearrangements in human tumors have yet been reported (Shippey et al. 1990), recent studies on murine radiation-induced, myeloid leukemia provide evidence for an involvement of interstitial head-to-head arrays of telomeric repeats in the generation of frequently occurring breaks on the second chromosome (Bouffler et al. 1993b; Silver and Cox 1993). However, a major interstitial telomere repeat array at 2q13 on the human chromosome 2 that probably results from an ancestral chromosome fusion event does not appear to coincide with the fragile site FRA2B in the same cytological location (Ijdo et al. 1992).

In summary, it appears that interstitial telomeric repeats are not sufficient to initiate telomere formation. However, they may promote two activities, which may then increase chromosome breakage and subsequent healing. First, their structural similarity to hypervariable minisatellites and to bacterial *chi* recombination sequences may cause general genomic instability that is controlled by other, yet unknown factors. Perhaps gene products of the DNA mismatch repair system, such as those recently characterized in yeast (Strand et al. 1993) and human (Fishel et al. 1993; Leach et al. 1993; Parsons et al. 1993), may affect their stability in a manner similar to that of minisatellites. Second, once a break has occurred, interstitial telomeric repeats are exposed at the ends and may become elongated by telomerase, provided the enzyme happens to be active in the particular cell.

Are telomeric repeats required for telomere functions?

Maintenance of chromosome length

Slimpe telomeric repeats certainly play an important role in the elongation reaction in organisms in which such repeats occur. In these organisms it is well established that telomere elongation is achieved by the addition of more of such repeats to the ends. This reaction is catalyzed by an enzyme, telomerase, that contains an RNA molecule encoding the species-specific template sequence for the telomeric repeat and has a reverse transcriptase activity (reviewed by Blackburn 1992).

At least two other chromosome elongation mechanisms exist that do not require telomerase or simple tandem telomeric repeats. Recombination has been demonstrated in *Saccharomyces* as a means of adding telomere sequences to transforming linear plasmids that already have short stretches of telomeric repeats (Wang and Zakian 1990). A similar mechanism could account for most telomere elongation in yeast, although the mechanism of chromosome end maintenance is not known. Many yeast and slime mold species do not have the simple telomeric repeats that would be predicted by the telomerase model (Zakian 1989). This, however, does not argue strongly against the use of telomerase in these species (Yu and Blackburn 1991). Transposition of a specific retroposon, the HeT-A element, has been proposed as the primary mechanism for maintaining chromosome length in *Drosophila* (Biessmann et al. 1990b, 1992; Mason and Biessmann 1993), and recently, another *Drosophila* telomere-specific retroposon, TART, has been identified (Levis et al. 1993). TART elements very probably have similar functions in telomere elongation, and other related LINE-like elements with similar properties may be found in *Drosophila* and perhaps other dipterans.

Chromosomes capping

Work from a number of species suggests that telomeric repeats at the ends of chromosomes are necessary for survival. In *Tetrahymena*, for example, alteration of the repeat sequence added by telomerase causes cell senescence (Yu et al. 1990). Loss of telomeric sequences from yeast chromosomes in the *est1* strain due to an inability to extend these sequences also leads to senescence (Lundblad and Szostak 1989). However, rare survivors occur in these dying cultures that have acquired additio-

nal telomeric DNA, apparently via recombination between subtelomeric Y' elements (Lundblad and Blackburn 1993). These Y' sequences resemble mobile elements (Louis and Haber 1992), although there is no evidence that they are currently able to transpose. In any case, the results show that when the predominant mechanism for maintaining chromosomes ends is lost, alternative mechanisms may be available.

Recent evidence from *Saccharomyces* indicates that telomeric sequences are required for capping function. In other words, broken chromosome ends without telomeric repeats are recognized at the cell cycle checkpoint as "uncapped." A single, site-specific, unrepaired double strand break in a dispensable, single-copy plasmid is enough to cause cell death after a few cell divisions (Bennett et al. 1993). Virtually all of the survivors have lost the plasmid. Similarly, a yeast chromosome that has lost a telomere causes a transient cell cycle arrest and loss of the broken chromosome (Sandell and Zakian 1993), although stabilized broken chromosomes can be recovered after the addition of new telomeric repeats. Although the mechanism(s) of this addition are not fully understood, the majority of the events are *RAD*52 dependent and involve homologous recombination, whereas other events appear to involve de novo telomere addition. The terminal telomeric repeats apparently restore fully functional chromosome cap. (V.A. Zakian, personal communication). Addition of new telomere repeats onto a broken chromosome end in yeast is facilitated by repeat sequences within 128 base pairs from the site of the addition, as well as a short (1–9 base pair) G or G, T sequence at the terminus (Kramer and Haber 1993). A terminal deletion for human chromosome 16 has been described (Wilkie et al. 1990) that also shows terminal repeats added to the broken end at a site with only a short region of homology with the telomere sequence (Morin 1991).

In other organisms in which telomeric repeats have been identified, when a chromosome is broken artificially, or in the process of developmentally programmed DNA elimination, or when a linear DNA molecule is introduced into the nucleus, a healing reaction adds appropriate telomeric repeats to the broken end. This is the case in several species of ciliates (Yokoyama and Yao 1986; Baroin et al. 1987; Herrick et al. 1985; Forney and Blackburn 1988; Gilley et al. 1988; Baird and Klobutcher 1989; Yu and Blackburn 1991), *Plasmodium* (Pologe and Ravetch 1988; Cappai et al. 1989; Scherf and Mattei 1992), *Schizosaccharomyces* (Matsumoto et al. 1987), and *Ascaris* (Müller et al. 1991).

It is not known whether capping involving telomeric repeats depends on the formation of a non-Watson-Crick G-quartet structure (Williamson 1993) or the association with specific proteins, although these possibilities are not mutually exclusive.

Chromosomes without telomeric repeats

In contrast to these results, evidence is accumulating that the simple telomeric repeats found in a variety of eukaryotes are not required for any of the several telomeric functions in *Drosophila melanogaster*. Broken chromosomes have been recovered in *Drosophila* that do not terminate in telomeric DNA sequences (Biessmann et al. 1990a) and continuously lose DNA sequences from the end (Biessmann and Mason 1988; Levis 1989; Biessmann et al. 1992). Yet these chromosomes are stably transmitted over many generations, behave as if they are capped, and do not cause cell cycle arrest. Since telomeric sequences are absent, and no defined DNA sequence is permanently exposed at the chromosome ends, capping of *Drosophila* chromosome ends must be achieved by a sequence-independent mechanism, perhaps by a protein (Biessmann et al. 1990a). Such a telomeric capping protein might bind to the ends of any double-stranded DNA. When present at a double-stranded end, it would protect the end from nucleolytic attack or ligation. It is not clear how a newly broken end might acquire such a protein, but the mutator *mu*2 (Mason et al. 1984) may play a role in such an acquisition.

Several attempts have been made to find evidence for telomeric repeats in the order *Diptera*. Various oligonucleotides, chosen based on similarities of telomeric repeats in a widely divergent set of eukaryotes (see Zakian 1989), have been used to probe *Drosophila* DNA. Although the probes usually exhibit interspecies cross-hybridization, all results were negative, indicating the apparent absence of such repeats in *Drosophila* (Richards and Ausubel 1988; Meyne et al. 1989; Levis 1993). Even the pentameric $(AATGG)_n$ sequence that appears to be the telomeric repeat in *Lepidoptera* and some other insects fails to cross-hybridize with dipteran DNA (Okazaki et al. 1993). Other than by hybridization with heterologous probes, DNA sequences that are involved in telomere capping and elongation can be identified by examination of healed chromosome ends. If *Drosophila* had telomeric repeats, one would expect to find them attached to the ends of broken chromosomes; this has never been observed (Biessmann et al. 1990a).

Direct addition of telomeric repeats in yeast and humans and transposition of HeT-A elements in *Drosophila* to terminally deleted chromosomes seem to be steps in pathways for rebuilding a new telomere at a broken chromosome end (Fig. 1). Other possible steps are not understood, although stabilization of the broken end in *Drosophila* may be an early step that is performed by a non-DNA component of the telomere (Biessmann and Mason 1988). While it may be comforting to use the programmed addition of telomeric repeats to chromosomal fragments during development of a macronucleus in ciliates as a model (reviewed by Biessmann and Mason 1992), it is not clear how these developmentally regulated events relate to the occasional, rare healing of a broken chromosome end. Indeed, linear DNA molecules acquire telomeric repeats when injected into the macronucleus of *Paramecium*, but not when transformed into yeast, where, if they are maintained, they circularize or integrate into a chromosome.

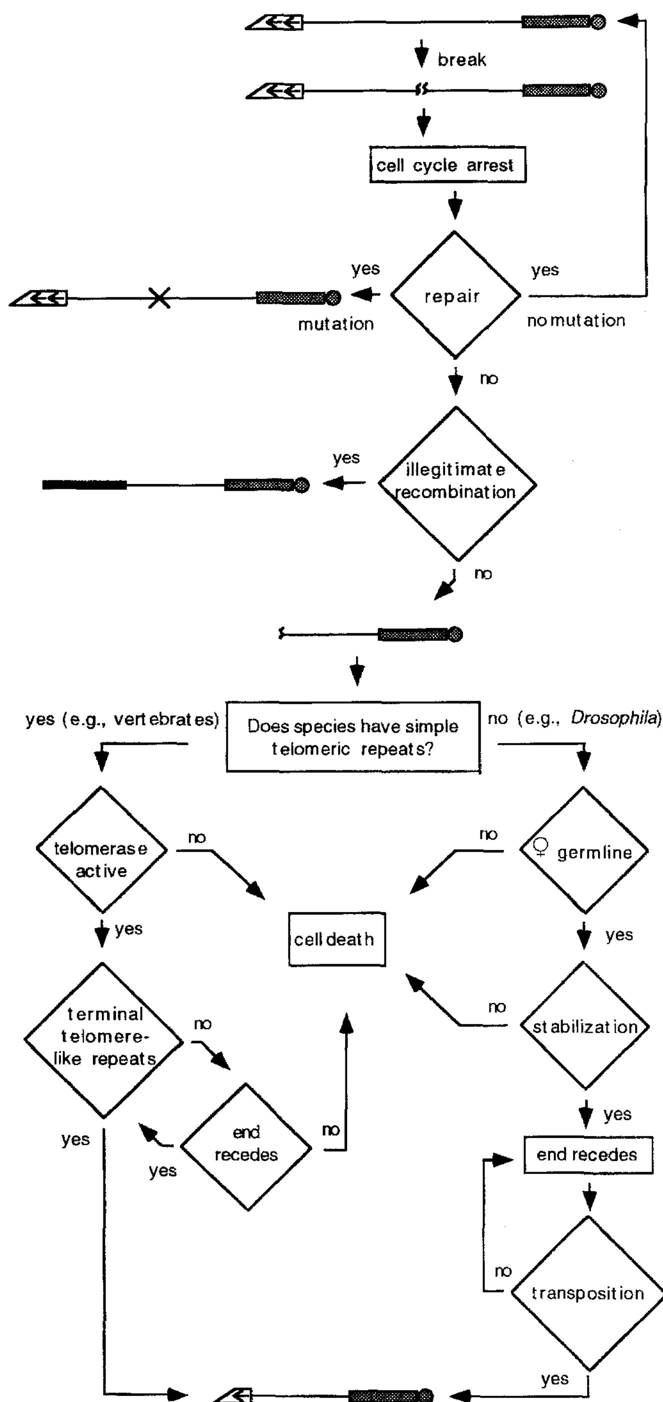

Fig. 1. Breakage and healing of chromosomes. The flow chart is a simplified diagram of the steps between damage inflicted on a chromosome and the healing of a broken chromosome end. The nature of the initial damage is not specified, but may lead to a chromosome break. Either the initial lesion or the subsequent break induces cell cycle arrest, which allows time for DNA repair. Three types of repair are shown as "repair" with and without mutation at the site of the damage, and "illegitimate recombination." A description of the mechanisms of these repair processes is not within the scope of this review. In the bottom half of the diagram, a broken chromosome is the substrate for the addition of telomeric DNA, either simple repeats by the activity of telomerase, or a complex sequence, by transposition. Telomerase is likely required for the addition of simple telomeric repeats, as well as a short elomerase primer (see text). If an appropriate sequence is not available at the site of the lesion, exonuclease activity may remove material until such a primer sequence is found. In *Drosophila*, "stabilization" of a broken end to allow it to be recovered in a viable cell seems to require that the initial lesion be in an oocyte chromosome, although the "stabilization" event does not occur until fertilization (Mason, Champion and Hook, in preparation). Recombination between sequences immediately proximal to the broken end and an unbroken homolog has been shown to add telomeric repeats to a break in yeast (V.A. Zakian, personal communication)

Evolutionary perspective

Weiner (1988) hypothesized that telomerase, with its intrinsic RNA template and reverse transcriptase activity, may have evolved very early from an ancient RNP that added nucleotides to the termini of RNA molecules and later acquired its reverse transcriptase activity when DNA became the molecule for storage of genetic information. Consistent with this notion, telomerase has been identified in species as diverse as protozoa and vertebrates. The simple telomeric repeats that are the substrate/product of telomerase are found in many animal and plant species and may have evolved from a simple CCA RNA "tag" originally required for replication (Maizels and Weiner 1993). Whereas replication by telomerase seems to be an efficient mechanism for maintenance of telomeres, other mechanisms have arisen. A few genera have been identified that have lost the simple telomeric repeats from their telomeres, including the yeast *Candida* (McEachern and Hicks 1993), *Drosophila melanogaster*, and potentially other dipterans. In these organisms without telomeric repeats, the lengths of the repeating telomeric DNA unit ranges from 23 bp in *Candida* to 6 kb in *Drosophila*. Other organisms, e.g., the yeast *Saccharomyces*, while maintaining the G-strand motif, have substituted a more complex repeat of uneven length for the simple repeating unit predicted by the telomerase model of telomere replication. These exceptions indicate that there are other mechanisms for maintaining telomere length that do not require telomerase. Transposition has been proposed as the primary mechanism for maintaining chromosome ends in *Drosophila* (Biessmann et al. 1992; Mason and Biessmann 1993; Levis et al. 1993). Recombination has been demonstrated in *Saccharomyces* as a means of adding telomere sequences to transforming linear plasmids (Wang and Zakian 1990), although the mechanism for chromosome end maintenance in *S. cerevisiae* is not known.

It seems likely that telomerase arose very early as a mechanism for maintaining chromosome length, and that organisms, such as *Drosophila*, without this activity lost it sometime during their evolution. In order to survive, an organism without telomerase must substitute an alternative mechanism. A clue to how telomeres in *Drosophila* might have arisen can be seen in the appearance of survivors among *est1* cells of yeast that are unable to replicate telomeric sequences (Lundblad and Blackburn 1993). These survivors have elongated their chromosome ends by *RAD52*-mediated recombination that is distinct from the normal *RAD52*-independent pathway. In other words, when the primary pathway of telomere

elongation was lost, an alternative pathway rescued some cells. An analogous situation may have arisen in a *Drosophila* ancestor; when the primary pathway for telomere elongation (telomerase) was lost, an alternative pathway (transposition) rescued the survivors. Since the closest relative of the HeT-A and TART retroposons is the *Drosophila* transposable element jockey (Biessmann et al. 1992; Danilevskaya et al. 1992; Levis et al. 1993), it is conceivable that both have evolved from a jockey-like ancestor and acquired the capability to transpose to chromosome ends specifically. It is tempting to speculate that of the two elements that have been found at chromosome ends in *Drosophila*, HeT-A and TART, in evolutionary terms the former may have served to elongate chromosomes earlier than the latter. HeT-A is more widely distributed among chromosome ends in *D. melanogaster* and the members of their family analyzed lack a reverse transcriptase. Thus, they cannot mediate their own transposition and may be controlled by the organism. TART elements carry a reverse transcriptase gene and may, therefore, transpose independently of *Drosophila* control.

Incremental evolution of telomeres might be slowed by the complex interactions among DNA sequences and protein components of the telomere (Maizels and Weiner 1993). Thus saltatory evolution of telomere structures might, therefore, be more likely than a slow divergence. A very slow divergence of telomeric repeats has been noted (Zakian 1989). The similarities of TTGGGG in holotrichous ciliates, TTAGG in many insects, TTAGGG in vertebrates, and TTTAGGG in flowering plants is striking. Maizels and Weiner (1993) have argued that the G-strand motif, with its possibilities for G-residue stacking, may have been a requirement for RNA replication and that this motif may be conserved because of close protein interactions and thus the need for co-evolution of the repeat sequence and the proteins that bind to it. If true, this suggests that the non-Watson-Crick G-quartet structure, per se, may not be an absolute requirement for modern telomere function (see also Williamson 1993). This is supported by the observation that at least *Drosophila* and *Candida* appear to have telomeres that do not use this motif.

In some respects, the wide distribution of telomeric repeats and the presumed use of telomerase to erxtend them resemble another mechanism for maintaining chromosomes that appears to have arisen very early in the evolution of eukaryotes. Meiosis as a mechanism for reducing chromosome number after the union of germ cells is wide spread, but not universal. Features of meiosis found in most eukaryotic organisms include synaptonemal complex during synapsis, high levels of recombination between homologues required for proper segregation, and two nuclear divisions without an intervening S phase. Some organisms have diverged slightly from this mechanism. In *Drosophila* males, for example, homologous chromosomes pair and two nuclear divisions ensue, but without the benefit of synaptonemal complex or meiotic recombination. Other organisms have diverged greatly from the canonical meiosis. In some species of scale insects, only a single maturation division occurs during gametogenesis. Chromosome reduction is achieved by removing specific chromosomes from the nucleus, during either spermatogenesis or the early cleavage divisions in the embryo (White 1973).

Conclusions

Simple telomeric repeats are widely distributed and conserved among eukaryotic organisms and are likely to be as old as eukaryotes themselves. Indeed, it is possible that the telomere motif of G residues seggregated to one strand may be descended from a much older RNA replication mechanism. Today, however, the telomeric repeats play important roles in both replication and capping functions associated with telomeres. However, a few genera have been identified that have lost the G-strand motif from their telomeres, including the yeast *Candida*, *Drosophila melanogaster*, and potentially other dipterans. Other organisms, e.g., the yeast *Saccharomyces*, while maintaining the G-strand motif, have substituted a more complex repeat of uneven length for the simple repeating unit predicted by the telomerase model of telomere replication. An exploration of these exceptions may increase our understanding of the basic requirements of chromosome ends.

Whether the ability of the 'G-strand' to form a quadruplex structure is important for telomere function, or whether the G strand is inhibited from rapid evolution by proteins that bind to it specifically and perform telomere functions, is an open question. In either case, the telomeric repeats themselves, located at an interstitial site on a chromosome arm, are not sufficient to nucleate a functional telomere with high frequency. The genomes of many eukaryotes have accumulated sites of interstitial telomere repeats, probably through chromosomal rearrangements. While the positions of the interstitial repeats seem to be associated with the locations of regions of frequent chromosome breakage or recombination, the mechanisms of these genomic instabilities are currently not understood.

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