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ARTICLE *in* CHROMOSOME RESEARCH · FEBRUARY 2005

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Telomeres in evolution and evolution of telomeres

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Key words: ALT, end-maintenance, evolution, plant, telomere, telomere cap

Abstract

This paper examines telomeres from an evolutionary perspective. In the monocot plant order Asparagales two evolutionary switch-points in telomere sequence are known. The first occurred when the *Arabidopsis*-type telomere was replaced by a telomere based on a repeat motif more typical of vertebrates. The replacement is associated with telomerase activity, but the telomerase has low fidelity and this may have implications for the binding of telomeric proteins. At the second evolutionary switch-point, the telomere and its mode of synthesis are replaced by an unknown mechanism. Elsewhere in plants (*Sessia*, *Vestia*, *Cestrum*) and in arthropods, the telomere "typical" of the group is lost. Probably many other groups with "unusual" telomeres will be found. We question whether telomerase is indeed the original end-maintenance system and point to other candidate processes involving t-loops, t-circles, rolling circle replication and recombination. Possible evolutionary outcomes arising from the loss of telomerase activity in alternative lengthening of telomere (ALT) systems are discussed. We propose that elongation of minisatellite repeats using recombination/replication processes initially substitutes for the loss of telomerase function. Then in more established ALT groups, subtelomeric satellite repeats may replace the telomeric minisatellite repeat whilst maintaining the recombination/replication mechanisms for telomere elongation. Thereafter a retrotransposition-based end-maintenance system may become established. The influence of changing sequence motifs on the properties of the telomere cap is discussed. The DNA and protein components of telomeres should be regarded – as with any other chromosome elements – as evolving and co-evolving over time and responding to changes in the genome and to environmental stresses. We describe how telomere dysfunction, resulting in end-to-end chromosome fusions, can have a profound effect on chromosome evolution and perhaps even speciation.

Introduction

The first realization that telomeres, the ends of eukaryotic chromosomes, had vitally important biological functions were the pioneering works of McClintock and Muller, who showed breakage–fusion–bridge cycles and chromosome healing in *Drosophila* (Muller 1938) and maize (McClintock 1938, 1941). However, telomere biology got established in its own right with the determination of the minisatellite telomere sequence and mechanism of its synthesis in the model ciliate *Tetrahymena* (Blackburn & Gall 1978, Greider & Blackburn 1985, 1987). Now, in the past decade, it has evolved from a peripheral, albeit interesting branch of cell biology studied by a few groups, to a major field involving hundreds of laboratories worldwide. Novel techniques have been developed and understanding of the structures, functions and roles of telomeres have evolved rapidly. In addition, the attention of the

wider public has been stimulated through proposed links between telomeres and aging and cancer.

All of these advances have been made using various model organisms (yeasts, protozoa, insects, vertebrates and plants), enabling comparisons between and within groups. But despite all this activity, one area, that of the response of telomeres to evolutionary change, has failed to be addressed in detail. This review concentrates on that deficiency. The area requires a global view of eukaryotes in general, and cannot concentrate on model organisms. We have utilized what is known of telomeric structures, functions and biochemistry and applied this with an evolutionary perspective.

We start the review, by necessity, on plant telomere biology where there is a phylogenetic context (Pich *et al*. 1996a, Sykorova *et al*. 2003b, 2003c). The only comparable perspective currently available is from insects (Sahara *et al*. 1999, Frydrychova & Marec 2002, Frydrychova *et al*. 2004, Vitkova *et al*. 2005). All data point towards the repeated change in telomeric sequence in the evolution of major phyla. In the second section we examine the evolution of end-maintenance systems, and place telomerase end-maintenance in an evolutionary context with ALT systems, including recombination and/or rolling circle replication and retrotransposition. Finally we review telomeric proteins at the telomere cap and discuss how there may be co-evolution of these proteins with changing telomere motif. We show how aberrant cap activity can lead to chromosome fusions, a process that can give novel karyotypes, and even perhaps new species.

The evolution of plant telomeres

In the past decade it has become apparent that the minisatellite repeat at the functional telomere may not define major phylogenetic groups. This is the case for both plants (Adams *et al*. 2001, Sykorova *et al*. 2003c) and invertebrates (Vitkova *et al*. 2005). Here we review what is known of the evolution of plant telomeres and add new data that shed light on possible evolutionary outcomes of altered telomere motifs.

The first indication that the minisatellite sequence (TTTAGGG)n first found in *Arabidopsis thaliana* (Richards & Ausubel 1988) was not ubiquitous to all flowering plants were from Alliacaeae, a group of monocots that includes the onions. Pich *et al*. (1996b) used fluorescent *in-situ* hybridization (FISH) and (TTTAGGG)n probes to species in *Allium*, *Nothoscordum* and *Tulbaghia* and failed to get any probe signal. They also failed to get signal using asymmetric PCR and Southern hybridization to *Allium fistulosum* and *Allium cepa* genomic DNA. In *A. cepa*, the sequence at the telomere may well have been replaced by one or more of a 375-bp satellite sequence (ACSAT), first described by Barnes *et al*. (1985), ribosomal DNA (rDNA), Ty1-copia group retrotransposons and En/Spm-like sequences (Pearce *et al*. 1996, Pich *et al*. 1996a, 1996b, Pich & Schubert 1998). It is also possible that the chromosome termini are not all the same in *Allium*, certainly the distribution of rDNA at, or near, the telomere can be highly variable between and within species, even at the level of an individual (Schubert & Wobus 1985). Nevertheless the nature of the repeats proposed at the telomere provides some clues as to the possible mechanism of telomere end maintenance. The telomeric satellite repeat ACSAT is reminiscent of the tandem repeats at the telomere in *Chironomus* (Rosen & Edstrom 2000) where amplification may involve homologous recombination, rolling circle replication and/or replication slippage. The retrotransposons associated with telomeric chromatin in *Allium* point to a possible mechanism of replenishment involving preferential retrotransposition of DNA to the telomeres, as occurs for HetA and TART in *Drosophila melanogaster*. But the organization and precise nature of the telomeres in *Allium* remain an open question.

Adams *et al*. reported that several species in the genus *Aloe* lack the *Arabidopsis*-type telomere (Adams *et al*. 2000). Since *Aloe* and *Allium* both belong to order Asparagales, these authors questioned whether other species in Asparagales also lacked the "typical" plant telomere. They took advantage of the phylogenetic scheme of Asparagales (Fay *et al*. 2000) to demonstrate that *Arabidopsis*-type telomeres had probably been lost at a single evolutionary switch-point and descendants now form a clade containing up to 6300 species and including many families and genera (Adams *et al*. 2001). The first evidence for replacement sequences was from FISH experiments using DNA and PNA (peptide nucleic acid) probes against the vertebrate-type telomere. The probes labelled the ends of chromosomes in *Aloe* (Weiss & Scherthan 2002),

an observation later extended to *Hyancinthella* (Puizina *et al*. 2003). Sykorova *et al*. (2003c) conducted a large-scale screen of Asparagales using dot-blot hybridization and probes against a range of variant minisatellite sequences thought to occur at the telomeres of *Arabidopsis*, vertebrates, *Bombyx* (insect), *Chlamydomonas* (green alga), *Oxytricha* (ciliate), *Tetrahymena* (ciliate), and *Ascaris* (nematode worm). They showed that Asparagales species derived from the evolutionary switch-point are unified by the occurrence of the vertebrate-type repeat as the predominant minisatellite at the telomeres (Figure 1). Species in Alliaceae, but outside of *Allium*, also show the same type of telomere structure (Sýkorová *et al*., manuscript in preparation). Thus in Asparagales there have been at least two switch-points in the evolution of telomeres. The first lead to a telomere motif based on TTAGGG-type repeat and a second, in the ancestor to *Allium*, leading to a telomere that has yet to be fully described.

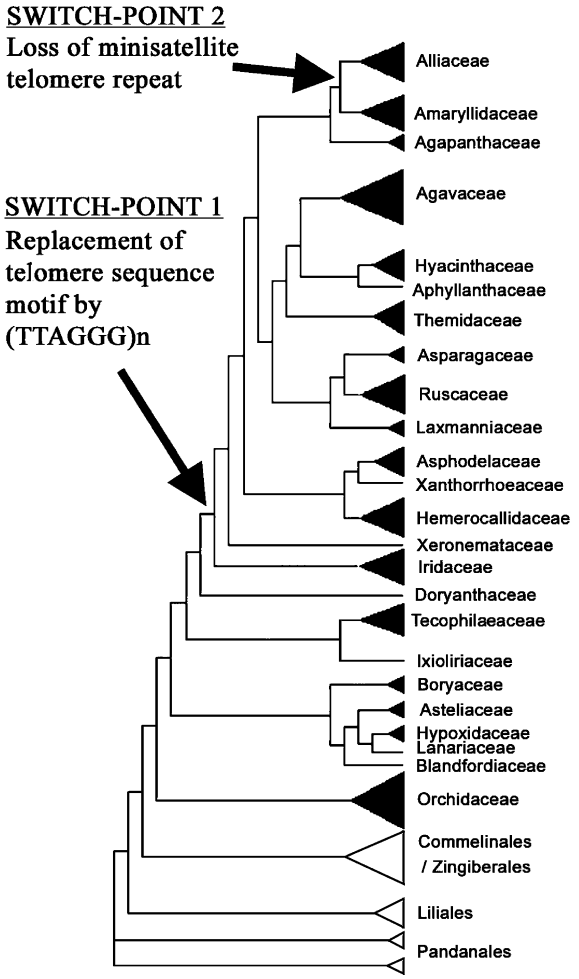

Figure 1. Phylogenetic tree of Asparagales (Fay *et al*. 2000). Arrows show the nodes where the two switch-points in the evolution of telomeres occurred. At switch-point 1 the *Arabidopsis*-type telomere was lost and replaced with the motif TTAGGG typical of vertebrates. At switch-point 2, minisatellite repeats were lost from the telomere. The number of species in each plant family is represented by the size of the triangles, the black shaded triangles represent families in Asparagales.

Many of the plants identified by Sykorova *et al*. (2003c) with substantial (TTAGGG)n sequences also had significant (although less) *Arabidopsis* and *Tetrahymena*-type repeats which co-localised with the telomeric array of TTAGGG repeats (Sykorova *et al*. 2003c, Rotkova *et al*. 2004, Fajkus *et al*. 2005). Nevertheless the terminal positions of the vertebrate-type repeat at the telomeres was confirmed by Bal31 exonuclease digestion and terminal restriction fragment analysis of *Othocallis siberica* genomic DNA (Weiss-Schneeweiss *et al*. 2004). An assay for telomerase activity (telomere repeat amplification protocol – TRAP) shows that telomerase synthesizes the vertebrate-type repeat, but that activity is of low fidelity (Sykorova *et al*. 2003c). This property of telomerase explains the mixed motifs at the telomere observed in some FISH experiments and dot-blot hybridizations. The telomerase "mistakes" could have had a role in the adaptation of telomere-binding proteins. Analysis of putative telomere-binding proteins in *Muscari armeniacum* and *Scilla peruviana* (both with vertebrate-type telomere motifs) has shown proteins with affinity to both *Arabidopsis*-type and vertebrate-type telomere motifs (Rotkova *et al*. 2004). It can be envisaged that at the potentially catastrophic event in the ancestor to Asparagales when telomerase mutated to generate an alternative telomere sequence, survival depended on either the rapid co-evolution of telomeric proteins, or sufficient similarity between the old and the new type of telomere for telomeric function to be maintained. A low-fidelity telomerase that generated variant minisatellite motifs would be consistent with the latter explanation.

In *Allium* a markedly different kind of telomeric DNA sequence occurs (possibly including e.g., ACSAT, rDNA) and it remains an open question as to how adaptation of telomeric proteins accommodated the change in sequence. To gain some insight into this question we used electrophoretic mobility shift assays

(EMSAs). Oligonucleotides resembling the *Arabidopsis*-type telomere were complexed with nuclear proteins from *Allium cepa* and *Scilla peruviana* in the presence of a non-specific competitor (primer TR1024, see (Rotkova *et al*. 2004, Schrumpfova *et al*. 2004) for details of methods). The results showed that *S. peruviana* (with vertebrate-type telomere motifs) and *A. cepa* (lacking both *Arabidopsis* and human-type of telomeres) form nucleoprotein complexes of similar stability, and these can be partially disrupted on adding a 100-fold excess oligonucleotide resembling the vertebrate-type telomere motif (Figure 2). We examined nuclear proteins complexed with different lengths of the *Arabidopsis*- (PL) or human (HU) type of oligonucleotides in the presence of competing non-specific oligonucleotides. The retarded fractions in EMSAs (Figure 3a) were UV cross-linked in the gel, excised (Figure 3b) and analysed using SDS-PAGE (Figure 3c). The results show that trimers of the *Arabidopsis*-type repeats do not complex significantly with nuclear proteins of *Allium cepa* while trimers of the vertebrate-type motif do form complexes. Further analysis revealed proteins of molecular weight 30 and 38 kDa in complexes with tetramers and hexamers of *Arabidopsis*- and vertebrate-type telomeric oligonucleotides. A protein of about 18 kDa is specific to complexes with *Arabidopsis*-type telomeric sequence and a protein of 24 kDa is specific for complexes with a vertebrate-type telomeric sequence. These data indicate conservation of putative telomere-binding proteins, as previously observed (Rotkova *et al*. 2004), even though the telomere sequence itself is much diverged (especially in *A. cepa*) from the ancestral motif.

The Asparagales are not the only group of plants without *Arabidopsis*-type telomeres. Three dicot genera, *Vestia*, *Sessia* and *Cestrum* (all Solanaceae), also appear to have lost these minisatellite repeats at the telomere (Sykorova *et al*. 2003b). Variability in Solanaceae is particularly surprising because the family contains model plants for the study of "typical" plant telomeres, e.g., species in *Nicotiana*

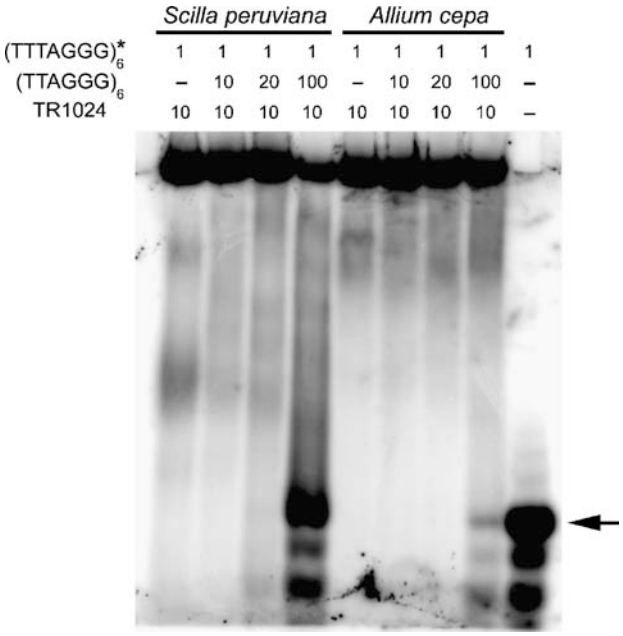

Figure 2. Nucleoprotein complexes of putative telomere-binding proteins from *Scilla peruviana* (possessing vertebrate-type telomeres) and *Allium cepa* (lacking both vertebrate and *Arabidopsis*-type telomeres). Reactions consisted of 25 pmol of non-specific competitor oligonucleotide (TR1024), 2.5 pmol of radioactively labelled oligonucleotide (*) and 10 μg of total proteins from either *Scilla* or *Allium*, except in the negative control (right-most lane). The position of the free oligonucleotide probe is marked with an arrow. The result obtained for *Scilla* demonstrates a relatively high affinity of its nuclear proteins to the *Arabidopsis*-type telomeres (the ancestral type) since the complex is disrupted only at a 100-fold excess of the vertebrate-type telomeric oligonucleotide. Interestingly, the *Arabidopsis*-type telomeric oligonucleotide forms highly specific complexes in *Allium* extracts since even a 100-fold excess of the human-type telomeric oligonucleotide only partially disrupts the complexes.

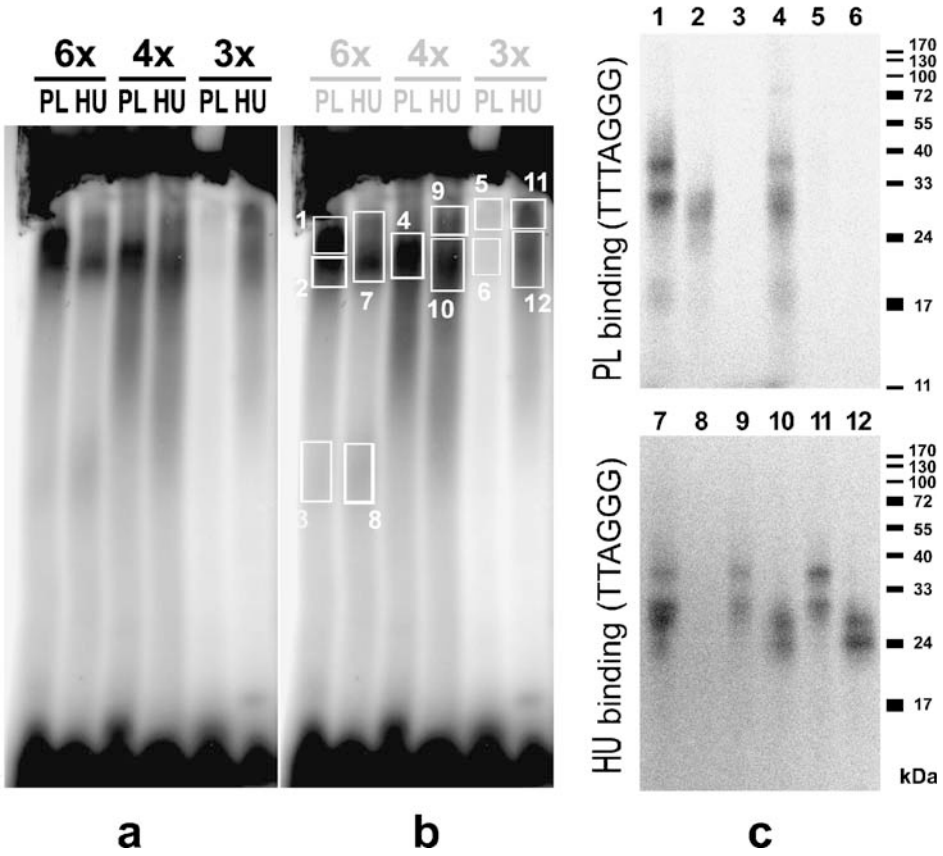

Figure 3. EMSA using protein extracts from *Allium cepa* and oligonucleotides of different lengths of telomeric repeats (6×, 4×, 3×) typical of *Arabidopsis* (PL) or human (HU) in the presence of competing non-specific oligonucleotide TR1024. The retarded fractions (**a**) were UV-cross-linked in a gel, complexes excised (**b**) and analysed using SDS-PAGE (**c**). Trimers of PL repeats result in little or no complex formation while the HU trimer does form nucleoprotein complexes. Cross-linked complexes show that proteins of molecular weight 30 and 38 kDa are present in complexes of both PL and HU oligonucleotides. A protein of about 18 kDa is present in complexes of PL sequence, and of 24 kDa with HU sequence.

and *Solanum* (Ganal *et al*. 1991, Broun *et al*. 1992, Fajkus *et al*. 1995, Fransz *et al*. 1996, Kovařík *et al*. 1996, Fajkus *et al*. 2002). It is noteworthy also that *Vestia*, *Sessia* and *Cestrum* have a distinctive A/T rich minisatellite sequence which distinguishes them from sister genera in Solanaceae and it is possible that the sequence evolved as an evolutionary response to the loss of the (TTTAGGG)n type telomere (Sykorova *et al*. 2003a). The chromosome size of species in these three genera is particularly large for angiosperms, an observation that led Sykorova et al. (2003a) to speculate that there may have been a skewed balance between synthesis of alternative telomeres and their loss through incomplete 5′ replication. Perhaps a transient telomere crisis caused by the loss of telomerase activity and attempts to repair the DNA could have initiated chromosome fusions and large-scale duplications mediated by recombination. This would have resulted in larger chromosomes. Alternatively, activation of ALT, which probably involves recombination and rolling circle replication, is able to amplify repeats of virtually any kind (not only those at chromosome ends) and could have contributed to an increased genome size.

The evolution of telomere end maintenance systems

Telomerase-based end maintenance is likely to be very old since it is found in widely diverged eukaryotes that represent many of the major eukaryote

lineages (ciliates, animals, fungi, green plants). The loss of telomerase is a catastrophic event unless there is immediate (within a few generations) replacement by an alternative system. This is insufficient time for the evolution of a complex novel system; instead it is probably necessary to hijack existing processes such as retrotransposition, recombination and/or rolling circle replication. Thus the replacement systems are derived conditions and telomerase-independent end-maintenance function should be considered as advanced stages in the evolution of telomere activity, as pointed out in a recent review (Louis 2002).

But ALT mechanisms may be a normal part of development in plants. In TERT knockout mutants of *Arabidopsis*, plants can survive up to ten generations (Ríha *et al*. 2001) with severe cytological and chromosomal abnormalities occurring after about eight generations (Siroky *et al*. 2003). The number of generations before deleterious mutations is surprisingly many. This is because there is no cell mobility in plant development and cell lines are not sequestrated for later use (as in the germ line of mammals). In plants an apical meristem consists of a small group of stem cells that cuts off a linear series of cells behind; these differentiate into an array of cell types that make a shoot and root, and flowers initiate from the L2 layer of the meristem in mature plants. The consequence of this mode of development is that meristem cells, which give rise to all tissues including germ-line cells, undergo many divisions, calculated at approximately 1000 divisions from seed to seed (Friml, personal communication). Yet it has been shown that telomere shortening in telomerase-deficient *Arabidopsis* mutants leads to a discrete spectrum of terminal restriction fragments, which are very homogeneous in lengths and telomeres declined by only 250–500 bp per plant generation (Fitzgerald *et al*. 1999). Consider now that between 50 and 100 bp of DNA loss at telomeres occurs per cell division (observed in mammalian cells), then this loss allows for only 5–10 cell divisions per plant generation. It is of course possible that the loss of telomeres per cell division is smaller in plants than in mammals. When considering only 10 nucleotide loss per cell division (the average length of RNA primer for synthesis of Okazaki fragment) as the minimum plausible loss of telomere (under the very improbable scenario that the primer sits exactly to the end of the 3′ end of the parental DNA strand), then the number of cell divisions would still be only 25–50. Thus we propose that an ALT system occurs in normal plant development. If so, then it is straightforward to envisage that the loss of telomerase activity requires only changes in regulation of an end-maintenance system already functioning at the telomere in development.

There now exists a tantalizing hint as to the processes that may occur in the evolution of ALT mechanisms. We know the immediate consequences of telomerase loss, as exemplified by most human ALT cell lines, yeast Type II survivors or cells of silkworm (*Bombyx mori*) (Bryan *et al*. 1997, Teng & Zakian 1999, Sasaki & Fujiwara 2000, Chen *et al*. 2001). In these cells the minisatellite telomere repeats may still be present, but their amplification becomes dependent on recombination and/or replication (unequal chromatid exchange, break-induced replication, rolling-circle replication). Rolling-circle replication may use extrachromosomal t-circles, as has been shown in human ALT cells, and in a wide variety of organisms including yeasts, higher plants, and *Xenopus laevis* (reviewed by Tomaska *et al*. 2004). Interestingly, linear mitochondrial telomeres of *Candida parapsilosis*, which are composed of long tandem repeats, also form t-circle intermediates (Tomaska *et al*. 2000, Nosek *et al*. 2005). The widespread occurrence of t-circles across eukaryote lineages suggests that t-circles (replicating via rolling-circle replication) may not only represent a backup in the event of telomerase dysfunction, but may perhaps be the primordial systems of telomere maintenance.

Divergence in the ALT mechanism could involve the replacement of the minisatellite telomere repeats by other tandem repeats, probably by those repeats that were the originally subtelomeric repeats. Examples of this stage in the evolution of ALT systems are seen in insect telomeres (*Anopheles* or *Chironomus*), and may also be forming the telomeres in *Allium* (using ACSAT), *Cestrum*, *Vestia* and *Sessea*. Interestingly satellite repeats are frequently associated with retroelements and possibly recombination/replication-based ALT systems give way to one based on retroelement retrotransposition. Increased retroelement activity could result in the telomere-maintenance system being completely dependent on targeted retrotransposition, as is observed in *Drosophila*. Evidence in favour of this hypothesis comes from *Chironomus* polytene chromosomes. Here immunohistochemistry against a reverse transcriptase-related protein

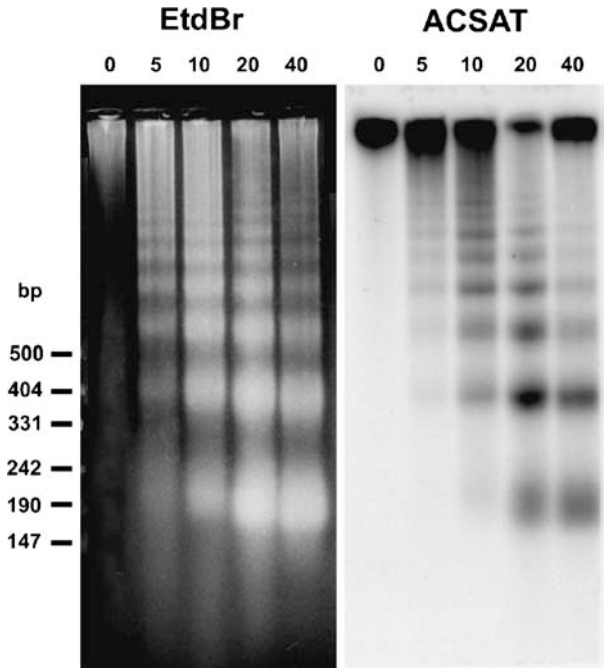

Figure 4. Micrococcal nuclease (MNase) digestion of *Allium cepa* leaf nuclei. The nuclei were digested for 0, 5, 10, 20 and 40 min with 0.1 U of MNase per μg DNA at 37°C. Extracted DNA was electrophoretically separated in 3% NuSieve agarose (FMC). The bulk nucleosomes were visualised by ethidium bromide staining (EtdBr). The pattern of ACSAT-nucleosomes was detected by Southern hybridisation with radioactively labelled ACSAT-probe. Periodicity of ACSAT-nucleosomes (185 ± 6 bp) does not differ significantly from the bulk of chromatin (198 ± 10 bp).

revealed signal at the telomeres, a localization dependent on gene transcription. Furthermore signal increased dramatically when telomeric heat shock puffs were induced (Lopez *et al*. 1999).

Telomere capping and chromosome evolution

Telomere capping involves protein–nucleic acid complexes that stabilize chromosome ends, prevent

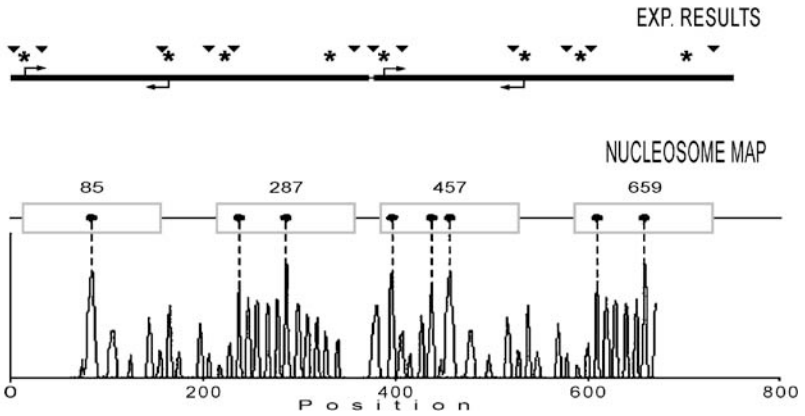

Figure 5. Comparison of experimental and computer-predicted positions of nucleosomes of ACSAT chromatin. Dominant and alternative positions of nucleosome boundaries are given. They were obtained either by using restriction endonuclease/Southern hybridisation mapping (triangles) or by primer-extension mapping (asterisks). Positions of primers used for primer-extension mapping are depicted by arrows.

end-to-end chromosome fusion, inhibit inappropriate DNA repair and regulate telomere elongation. It is widely recognized that telomeres have specific terminal nucleoprotein structures and it is worthwhile addressing those features conserved in telomeric chromatin to better understand capping functions. Telomeric nucleosomes in both animals (Makarov *et al*. 1993, Tommerup *et al*. 1994, Lejnine *et al*. 1995, Bedoyan *et al*. 1996) and plants (Fajkus *et al*. 1995, Vershinin & Heslop-Harrison 1998) are usually 30–40 bp shorter than nucleosomes from the bulk of chromatin of the same organism. In micrococcal nuclease digestions, telomeric chromatin falls into two categories: that which is digested to subnucleosomal fragments of mono- and dinucleosome-sized particles, and a larger fraction, comprising long arrays with regular spacing of telomeric nucleosomes. The short, subnucleosomal fragments probably arise by nucleosomes sliding on the DNA fibre in the absence of nucleosome positioning signals (Fajkus *et al*. 1995, Rossetti *et al*. 1998). The longer arrays of telomeric chromatin with regularly spaced nucleosomes are best explained by the columnar model of telomeric chromatin structure (Fajkus & Trifonov 2001). In this model telomeric DNA is continuously wound, in a parallel manner, around columns of stacked histone octamers. The model predicts that linker DNA is deformed in the same manner as the deformable part of typical nucleosome DNA and octamer-to-octamer stacking contacts cooperatively maintain the structure preventing the whole nucleosome array from sliding.

The Fajkus and Trifonov (2001) model is valid for telomeres composed of relatively homogeneous tracts of minisatellite repeats (no matter whether of human- or *Arabidopsis*-type) and even for telomere-associated satellite repeats (Vershinin & Heslop-Harrison 1998, Sykorova *et al*. 2001). But in plants possessing mixed arrays of human-type and *Arabidopsis*-type telomeric repeats (*Iris tectorum*, *Muscari armeniacum*, order Asparagales), telomeric nucleosomes coordinate similar periodicities of DNA (ca. 180 bp) as the bulk of chromatin (Rotkova *et al*. 2004). When exploring the chromatin structure in the putative *Allium* telomeric sequence ACSAT, we found that it shares nucleosome periodicity with the bulk of chromatin and reveals no significant subnucleosomal fragmentation (Figure 4). It also has highly preferred nucleosomal positions as shown by restriction enzyme digestion and primer extension assays to isolated nucleosomal monomers and dimers. The preferred sites correspond well with nucleosome positions generated from computer predictions using AA and TT dinucleotide distributions (Ioshikhes *et al*. 1992, 1996). Each monomer of ACSAT can bind two nucleosomes and the dominant positions of the nucleosome centres are at positions 85 and 287 or shifted about 55 bp towards the 5′-end of the sequence (Figure 5). Thus ACSAT has a regular chromatin structure typically observed in subtelomeric repeats (Fajkus *et al*. 1992, Gazdova *et al*. 1995, Kralovics *et al*. 1995, Vershinin & Heslop-Harrison 1998, Sykorova *et al*. 2001). These data point to different folding properties of telomeres in plants with "unusual" telomere sequences.

In species lacking canonical telomere repeats, telomere capping must require sequence-independent binding of proteins. Evidence in support of this supposition comes from *S. pombe* where the telomeric repeat binding protein, Taz1, and the heterochromatin protein, Swi6, remain associated at subtelomeres even in the absence of minisatellite telomeric repeats (Sadaie *et al*. 2003). There is also some evidence that epigenetic factors are important in telomere capping, as for centromeric heterochromatin. Mouse telomeres are enriched in di- and trimethylated H3-Lys9 and disruption of this methylation pattern in telomeric heterochromatin deregulates telomere lengths (Sadaie *et al*. 2003, Garcia-Cao *et al*. 2004). Therefore the connection between DNA and protein components at telomeres may be less specific than previously expected and may be influenced by epigenetic modifications.

An increasing number of proteins appear to bind telomeric DNA indirectly, via association with pre-existing chromatin complexes. For example hPot1 is a protein that participates in chromosome end-protection and telomere length regulation. This protein binds telomeric single-stranded DNA via its oligosaccharide-binding (OB) folds (Lei *et al*. 2004) or it can be recruited to telomeres via PTOP protein, a component of the TRF1 telomeric complex (Liu *et al*. 2004). The ortholog of this protein in *Arabidopsis thaliana*, AtPot1, seems to show a similar behaviour (Kuchar & Fajkus 2004). In addition, proteins that are normally associated with other processes are increasingly being shown to have telomeric function. The proteins that comprise the 3 Rs of DNA replication, repair and recombination are intimately associated (West *et al*. 2004) and some also have a telomeric

role. The proteins Ku70 and Ku80, involved in non-homologous end-joining (NHEJ), and which together function to hold the DNA ends at a double-strand break (Dudasova *et al*. 2004), also have a role in maintaining telomere length (Boulton & Jackson 1996, Porter *et al*. 1996, Featherstone & Jackson 1998). End-to-end chromosome fusions are typical products of telomere dysfunction and are consequences of malfunction in telomere cap activity. Mutations of Mre11 in *Arabidopsis* result in anaphase bridges arising from chromosome fusions (Puizina *et al*. 2004). Similarly, a dominant negative allele of TRF2 (involved in telomere folding) induces end-to-end fusions (van Steensel *et al*. 1998). Recently de Lange reported in a mouse cell line deficient in TRF2 tandem fusions of chromosomes into mega-chromosomes with rows of embedded telomeres along their length (personal communication). A very similar chromosome morphology is observed naturally in the Indian Muntjac (*Muntiacus muntjak vaginalis*) which has an extreme tandem fusion karyotype and chromosomes have many embedded telomeres along their length (Lee *et al*. 1993, Yang *et al*. 1995, Hartmann & Scherthan 2004).

Chromosome fusions are associated with the divergence of many plant and animal species, e.g., in plant genus *Cymbispatha* (formerly *Tradescantia*) (Levin 2002) and numerous species in Orthoptera and rodents where many population genetic studies have been carried out (Barton & Hewitt 1981, Hewitt *et al*. 1987, Hauffe *et al*. 2004). However it remains a matter of debate whether chromosome fusions are a cause or consequence of genetic isolation (Butlin 1993). Those in favour of fusions being a cause of speciation argue that the fusion establishes isolation barriers through reduced fertility in heterozygotes and the fused karyotype becomes fixed through inbreeding (self-fertilization is common in plants) or meiotic drive (the selection of particular rearrangements) (White 1973, King 1993).

Conclusion

Understanding the role of telomeres in the evolution of genomes, chromosomes, terminal sequences, genes and proteins remains in its infancy. Here we attempt to assimilate those aspects of protein/nucleic acid associations at the telomere (e.g., in the formation of the telomere cap and in mechanisms of chromosome elongation) that enable us to propose patterns of evolutionary change in eukaryote divergence. Now that telomere biology is becoming sophisticated, more work is required beyond model organisms to give a broader understanding of telomeres in eukaryotes as a whole. An eukaryote-based perspective is important to enable those features of telomeres which are organism specific to be distinguished from those that are conserved. The perspective will lead to a much deeper understanding of the origin, nature and evolution of telomere capping and maintenance systems.

Acknowledgements

The research is supported by the grants from GACR (projects 521/05/0055, 204/04/P104), GAASCR (IAA600040505) and the institutional support (MSM0021622415, AVOZ50040507) and by the Leverhulme Trust. We thank Jiri Friml (Tubingen, Germany) for helpful comments.

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