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On the organization of the nucleosomes associated with telomeric sequences

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ABSTRACT

The functions of telomeres and, probably, of interstitial telomeric sequences (ITSs) are influenced by their chromatin structure and organization. Telomeres in higher eukaryotes fold into nucleosomes that are about 20–40 bp shorter than the nucleosomes associated with bulk chromatin. Although the functional relevance of this short nucleosomal organization remains unknown, it is believed that short nucleosomes should contribute to telomere function. Whereas telomeric nucleosomes have been widely studied in different organisms, very little is known about the nucleosomal organization of ITSs. Chinese hamster ITSs have been found to associate with short nucleosomes. However, we have found that *Arabidopsis thaliana* ITSs fold into nucleosomes that have a repeat length similar to bulk chromatin. We discuss how the primary sequence of telomeres and ITSs could influence their nucleosomal organization.

Telomeres play an essential role in preventing chromosome fusions and degradation by exonucleases [1–3]. In addition, telomeres can silence the expression of subtelomeric genes and are implicated in DNA repair, homologous recombination, chromosome pairing and segregation. A wide variety of studies have revealed that telomeric sequences are also present at interstitial chromosomal loci in different eukaryotes [4–6]. The analysis of available genome sequences at http://www.ncbi.nlm.nih.gov/mapview reveals that ITSs have a widespread distribution in different model systems including zebrafish, chicken, opossum, mouse, dog, cattle, horse, human, rice, poplar or *Arabidopsis*. These ITSs have been related to chromosomal aberrations, fragile sites, hot spots for recombination and diseases caused by genomic instability [5].

In *Arabidopsis*, telomeres consist of telomeric repeat arrays that are 2.5–5 kbp in length [7,8]. Previous studies have revealed the presence of these repeats at interstitial chromosomal loci. Most of these repeats localize in the pericentromeric region of chromosome I as long arrays that can spread several kbp (Fig. 1a) [6,9,10]. To evaluate how ITSs might affect the analysis of telomeres and vice versa, we estimated the contribution of telomeres and ITSs to the signal detected after hybridization of *Arabidopsis* genomic DNA with a telomeric probe. We found that the major part of the signal detected after hybridization corresponded to ITSs (Fig. 1).

Fig. 1b shows the ethidium bromide stain of wild-type genomic DNA digested with *Cfo*I, with *Tru*9I or with *Tru*9I plus a cocktail of enzymes. Whereas *Cfo*I is sensitive to methylation and slices the *Arabidopsis* genome to an average size >1 kbp, *Tru*9I is methylation insensitive and generates DNA fragments of an average size <1 kbp. These fragments are even smaller when the digestion is performed with *Tru*9I plus the cocktail of enzymes (Fig. 1b). When wild-type genomic DNA digested with *Cfo*I is hybridized with the telomeric probe, a smeared band of high molecular weight is detected (Fig. 1c). This band corresponds to both, telomeres and ITSs. By contrast, the smeared band detected after digestion of wild-type DNA with *Tru*9I corresponds only to telomeres (Fig. 1c). This band is exclusively telomeric because it shifts upwards in the gel in a *ku*70 mutant, which has longer telomeres than the wild-type (Fig. 1c) [11]. Long exposures of wild-type genomic DNA digested with *Tru*9I reveal the presence of three low molecular weight bands (Fig. 1d). These bands correspond to ITSs because they are also present in the *ku*70 mutant (Fig. 1d).

The intensity of the main hybridization band detected after digesting wild-type genomic DNA with *Tru*9I is lower than the intensity of the band observed after digesting the same amount of wild-type DNA with *Cfo*I (Fig. 1c). By contrast, similar hybridization signals are detected when equal amounts of wild-type genomic DNA are digested with *Tru*9I, with *Tru*9I plus the cocktail of enzymes (Fig. 1c) or with the cocktail of enzymes alone (not shown). These results indicate that the terminal telomeric repeats are not digested by the cocktail of enzymes or by *Tru*9I, and argue that *Arabidopsis* telomeres are essentially made of perfect telomeric repeats. Therefore, the loss of hybridization signal detected with *Tru*9I relative to *Cfo*I is due to the digestion of ITSs, which are degenerated and contain many *Tru*9I sites (TTAA). In fact, it is very uncommon to find tandem arrays with more than six perfect telomeric repeats at ITSs. To estimate the contribution of telomeres and ITSs to the signal detected after hybridization of wild-type *Arabidopsis* DNA with the telomeric probe, we compared the intensity of the band generated by *Cfo*I (corresponding to telomeres and ITSs) with the intensity of the main band generated by *Tru*9I (which only corresponds to telomeres) (Fig. 1c). Since the

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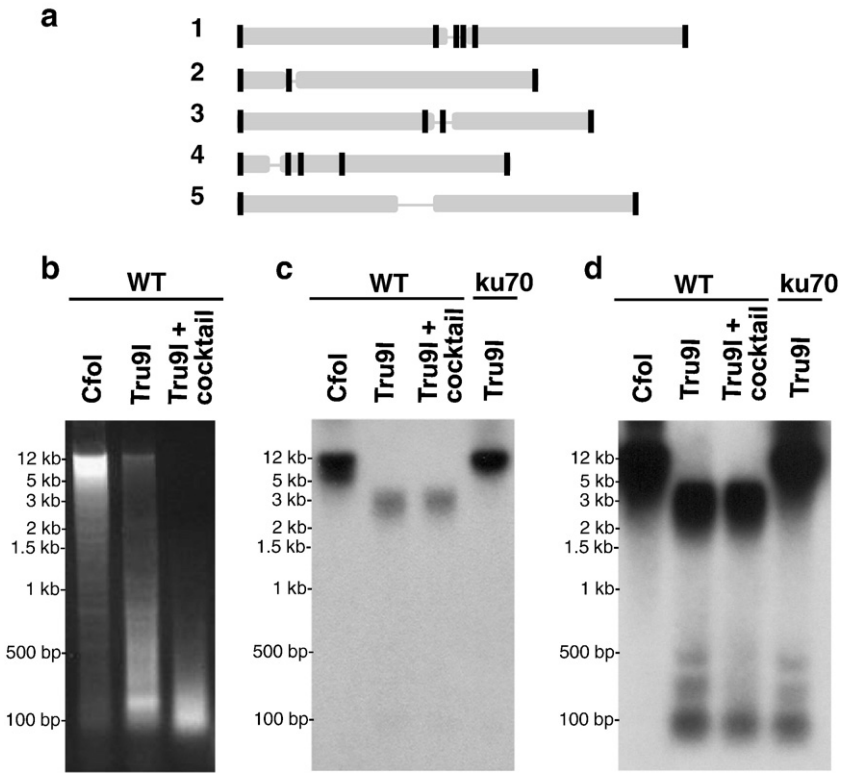

Fig. 1. Detection of telomeric repeats in *Arabidopsis*. (a) Distribution of the main telomeric repeat arrays in the five *Arabidopsis* chromosomes. This representation has been performed with information obtained from NCBI (http://www.ncbi.nlm.nih.gov/mapview) using the blast program. Telomeric repeat arrays are represented as black vertical rectangles. (b) Equal amounts of wild-type genomic DNA were digested with *Cfo*I (GCGC), with *Tru*9I (TTAA) or with *Tru*9I plus a cocktail of enzymes containing *Alu*I (AGCT), *Dra*I (TTTAAA), *Nar*I (GGCGCC), *Sau*3A (GATC) and *Msp*I (CCGG), resolved on an agarose gel and stained with ethidium bromide. (c) Southern blot displaying *Arabidopsis* telomeres and ITSs. Equal amounts of genomic DNA from the wild-type (WT) and from an isogenic *ku70* mutant (*ku70*) were digested with different restriction endonucleases and hybridized with the telomeric probe. The restriction endonucleases used are indicated at the top of each lane. The *ku70* mutant (line SALK_105816) was obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus, USA) and segregated to obtain a homozygous line that was verified by PCR. (d) This panel displays a longer exposure of the hybridization shown in panel b. The migration distances of molecular weight markers are shown in the left.

intensity of the *Cfo*I band was 3.5 times higher than the intensity of the *Tru*9I band, we concluded that the major part of the signal detected after hybridizing *Arabidopsis* genomic DNA with the telomeric probe corresponded to ITSs (about 70%).

We have used micrococcal nuclease to analyze the distribution of nucleosomes at *Arabidopsis* ITSs (Fig. 2). Micrococcal nuclease digests the linker DNA between nucleosomes, producing a nucleosome ladder when DNA from nuclei digested with it is resolved on an agarose gel. Hybridization of the nucleosome ladder with a specific DNA sequence provides an indication of whether this sequence is associated with nucleosomes in chromatin. Several nucleosomal bands of bulk genomic DNA were observed after digesting *Arabidopsis* nuclei preparations with increasing concentrations of micrococcal nuclease and staining with ethidium bromide (Fig. 2a). These bands revealed the packaging of the *Arabidopsis* genome into nucleosomes that contain an average repeat length of about 185 bp. Since nucleosomal bands of similar size were observed when these DNA samples were hybridized with the telomeric probe, we concluded that ITSs associate with nucleosomes that have a similar repeat length than bulk chromatin (Fig. 2b).

The repeat length of the nucleosomes associated with a specific repetitive sequence *in vivo* is a statistical average because each nucleosome is flanked by linkers of variable length [12]. This statistical average might differ from that of bulk chromatin, which should be determined by the sequence *per se*, by some chromatin determinants or by both. For telomeric sequences, *in vitro* experiments support a sequence-driven short nucleosomal organization. Nucleosome positioning and stability are determined by DNA properties like curvature and flexibility [13–16]. DNA molecules containing perfect arrays of mammal or plant type telomeric sequences (TTAGGG and TTTAGGG, respectively) are straight and do not contain nucleosome positioning information [17,18]. Therefore, it is not surprising that perfect telomeric sequences have the lowest affinities for the histone octamer among the sequences so far tested [19–21]. The lack of positional information also increases the mobility of telomeric nucleosomes and affects their repeat length, as shown by *in vitro* experiments. When nucleosomes reconstitute *in vitro* on an array of perfect telomeric repeats, the nucleosome positioning observed is random. Interestingly, at increasing saturation of nucleosomes the internucleosomal distance shortens to 157 bp. This short internucleosomal length is not observed for other repetitive sequences at increasing saturation of nucleosomes, which supports a sequence dependent short nucleosomal organization of perfect telomeric sequences *in vivo* [18,22,23].

As mentioned above, short telomeric nucleosomes have been described in different model organisms, including plant and animals [15,17,24–26]. Since telomeric DNA in these organisms is poorly cut by frequently cutting enzymes, it is believed that telomeres are essentially composed of perfect telomeric repeats (Fig. 1) [9,27–30]. Therefore, the presence of degenerated repeats at telomeres should have been counter-selected through evolution. They might interfere with telomeric functions. We speculate that mutations that introduce degenerated repeats at telomeres could lead to the generation of positional information and to lower nucleosomal mobility, which might affect short nucleosomes formation.

Chinese hamster ITSs are not frequently cut by restriction enzymes, reflecting that they are mainly composed of perfect

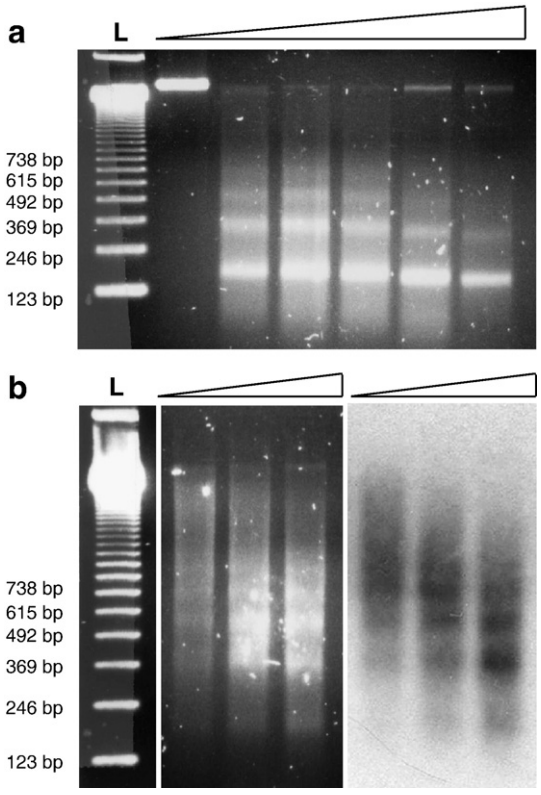

Fig. 2. *Arabidopsis* ITSs fold into nucleosomes that have a similar repeat length than bulk chromatin. (a) Micrococcal nuclease sensitivity analysis of *Arabidopsis* chromatin. Nuclear preparations from *Arabidopsis* were digested with increasing concentrations of micrococcal nuclease (from 0.0 up to 0.45 units of micrococcal nuclease per mL), as previously described [26]. After digestion, the DNA samples were purified, resolved on an agarose gel and stained with ethidium bromide. A 123 bp ladder is shown in the left (indicated as L). The triangle on the top of the figure indicates increasing concentrations of nuclease. (b) Micrococcal nuclease sensitivity analysis of *Arabidopsis* ITSs. Nuclear preparations from *Arabidopsis* were digested with 0.08, 0.12 and 0.15 units of micrococcal nuclease per mL. After digestion, the DNA samples were purified, resolved on an agarose gel and hybridized with a telomeric probe. The left panel shows a 123 bp ladder (indicated as L). The digestion profiles displayed after staining with ethidium bromide are shown in the middle panel. The right panel shows the digestion profiles obtained after hybridization. Triangles on the top of the panels indicate increasing concentrations of nuclease. The telomeric template used for hybridization was constructed by annealing and ligation of telomeric sequence oligos containing specific restriction sites at their ends. These sites were used for cloning in pUC18 [34]. The sequence of the template is as follows: *Hind*III site–$(TTTAGGG)_{12}$–*Sph*I site–$(TTTAGGG)_{10}$–*Sal*I site–$(TTTAGGG)_{15}$–*Eco*RI site. Prior to hybridization, the template was excised from pUC18 using the *Hind*III and *Eco*RI sites, purified and labeled using a Ready-To-Go™ kit from GE Healthcare. Hybridization was performed overnight at 65°C in the presence of 5× SSPE, 5× Denhardt's solution, 0.5% SDS and 40 μg/mL of denatured salmon sperm DNA. After hybridization, the membrane was washed 3 times at room temperature during 10 minutes with 2× SSPE plus 0.1% SDS and once at 65°C during 45 minutes with 1× SSPE and 0.1% SDS.

telomeric repeats [31]. These ITSs localize at pericentromeric regions and associate with nucleosomes that have a shorter repeat length than bulk chromatin [32]. Based on these results, a sequence-driven telomeric chromatin organization has been proposed [32]. We have found that *Arabidopsis* ITSs associate with nucleosomes that have a similar repeat length than bulk chromatin. Since *Arabidopsis* ITSs are composed of very short arrays of perfect repeats interspersed with degenerated repeats, our results are compatible and support a sequence-driven short nucleosomal organization of perfect telomeric repeats [6,9,10]. The degenerated repeats present at *Arabidopsis* ITSs could provide nucleosomal positional information, which might help to fold nucleosomes *in vivo* and limit their mobility and compaction. The reasons why *Arabidopsis* ITSs are degenerated whereas hamster ITSs are essentially perfect remain unknown.

In vivo experiments support that the short nucleosomal organization of perfect telomeric sequences is influenced by some chromatin determinants. In mice, the overexpression of the telomeric repeat binding factor TRF2 causes increased nucleosomal spacing at telomeres [25,33]. This result supports that TRF2 might be involved in the establishment of the short repeat length found at mice telomeric nucleosomes. We believe that a mixed model could explain the short nucleosomal organization of higher eukaryotes telomeres and hamster ITSs: specific proteins or chromatin features could lead to the compaction of nucleosomes, which might require the presence of perfect telomeric repeat arrays.

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References

[1] E. Blackburn, Telomeres and telomerase: their mechanisms of action and the effects of altering their functions, FEBS Lett. 579 (2005) 859–862.
[2] T. Cech, Beginning to understand the end of the chromosome, Cell 43 (2004) 405–413.
[3] C. Greider, Telomerase RNA levels limit the telomere length equilibrium, Cold Spring Harbor Symp. Quant. Biol. 71 (2006) 225–229.
[4] J. Meyne, R. Baker, H. Hobart, T. Hsu, O. Ryder, O. Ward, J. Wiley, D. Wurster-Hill, T. Yates, R. Moyzis, Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes, Chromosoma 99 (1990) 3–10.
[5] K. Lin, J. Yan, Endings in the middle: current knowledge of interstitial telomeric sequences, Mutat. Res. 658 (2008) 95–110.
[6] E. Richards, H. Goodman, F. Ausubel, The centromere region of *Arabidopsis thaliana* chromosome 1 contains telomere-similar sequences, Nucleic Acids Res. 19 (1991) 3351–3357.
[7] E. Richards, F. Ausubel, Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*, Cell 53 (1988) 127–136.
[8] E. Shakirov, D. Shippen, Length regulation and dynamics of individual telomere tracts in wild-type *Arabidopsis*, Plant Cell 16 (2004) 1959–1967.
[9] F. Regad, M. Lebas, B. Lescure, Interstitial telomeric repeats within the *Arabidopsis thaliana* genome, J. Mol. Biol. 239 (1994) 163–169.
[10] W. Uchida, S. Matsunaga, R. Sugiyama, S. Kawano, Interstitial telomere-like repeats in the *Arabidopsis thaliana* genome, Genes Genet. Syst. 77 (2002) 63–67.
[11] K. Riha, J. Watson, J. Parkey, D. Shippen, Telomere length deregulation and enhanced sensitivity to genotoxic stress in *Arabidopsis* mutants deficient in Ku70, EMBO J. 21 (2002) 2819–2826.
[12] H. Drew, C. Calladine, Sequence-specific positioning of core histones on an 860 base-pair DNA. Experiment and theory, J. Mol. Biol. 195 (1987) 143–173.
[13] C. Anselmi, G. Bocchinfuso, P. De Santis, M. Savino, A. Scipioni, Dual role of DNA intrinsic curvature and flexibility in determining nucleosome stability, J. Mol. Biol. 286 (1999) 1293–1301.
[14] G. Mengeritsky, E. Trifonov, Nucleotide sequence-directed mapping of the nucleosomes, Nucleic Acids Res. 11 (1983) 3833–3851.
[15] S. Pisano, A. Galati, S. Cacchione, Telomeric nucleosomes: Forgotten players at chromosome ends, Cell Mol. Life Sci. 65 (2008) 3553–3563.
[16] A. Travers, H. Drew, DNA recognition and nucleosome organization, Biopolymers 44 (1997) 423–433.
[17] J. Fajkus, A. KovariK, R. Kralovics, M. Bezdek, Organization of telomeric and subtelomeric chromatin in the higher plant *Nicotiana tabacum*, Mol. Gen. Genet. 247 (1995) 633–638.
[18] S. Pisano, E. Marchioni, A. Galati, R. Mechelli, M. Savino, S. Cacchione, Telomeric nucleosomes are intrinsically mobile, J. Mol. Biol. 369 (2007) 1153–1162.
[19] S. Cacchione, M. Cerone, M. Savino, In vitro low propensity to form nucleosomes of four telomeric sequences, FEBS Lett. 400 (1997) 37–41.
[20] I. Filesi, S. Cacchione, P. De Santis, L. Rossetti, M. Savino, The main role of the sequence-dependent DNA elasticity in determining the free energy of nucleosome formation on telomeric DNAs, Biophys. Chem. 83 (2000) 223–237.
[21] L. Rossetti, S. Cacchione, M. Fuà, M. Savino, Nucleosome assembly on telomeric sequences, Biochemistry 37 (1998) 6727–6737.
[22] R. Mechelli, C. Anselmi, S. Cacchione, P. De Santis, M. Savino, Organization of telomeric nucleosomes: atomic force microscopy imaging and theoretical modeling, FEBS Lett. 566 (2004) 131–135.
[23] S. Pisano, E. Pascucci, S. Cacchione, P. De Santis, M. Savino, AFM imaging and theoretical modeling studies of sequence-dependent nucleosome positioning, Biophys. Chem. 124 (2006) 81–89.
[24] V. Makarov, S. Lejnine, J. Bedoyan, J. Langmore, Nucleosomal organization of telomere-specific chromatin in rat, Cell 73 (1993) 775–787.
[25] H. Tommerup, A. Dousmanis, T. de Lange, Unusual chromatin in human telomeres, Mol. Cell. Biol. 14 (1994) 5777–5785.
[26] A. Vershinin, J. Heslop-Harrison, Comparative analysis of the nucleosomal structure of rye, wheat and their relatives, Plant Mol Biol. 36 (1998) 149–161.

[27] R. Allshire, J. Gosden, S. Cross, G. Cranston, D. Rout, N. Sugawara, J. Szostak, P. Fantes, N. Hastie, Telomeric repeat from *T. thermophila* cross hybridizes with human telomeres, Nature 332 (1988) 656–659.
[28] D. Kipling, H. Cooke, Hypervariable ultra-long telomeres in mice, Nature 347 (1990) 400–402.
[29] R. Moyzis, J. Buckingham, L. Cram, M. Dani, L. Deaven, M. Jones, J. Meyne, R. Ratliff, J. Wu, A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes, Proc. Natl. Acad. Sci. USA 85 (1988) 6622–6626.
[30] J. Starling, J. Maule, N. Hastie, R. Allshire, Extensive telomere repeat arrays in mouse are hypervariable, Nucleic Acids Res. 18 (1990) 6881–6888.
[31] M. Faravelli, C. Azzalin, L. Bertoni, O. Chernova, C. Attolini, C. Mondello, E. Giulotto, Molecular organization of internal telomeric sequences in Chinese hamster chromosomes, Gene 283 (2002) 11–16.
[32] D. Revaud, J. Mozziconacci, L. Sabatier, C. Desmaze, C. Lavelle, Sequence-driven telomeric chromatin structure, Cell Cycle 8 (2009) 1099–1100.
[33] R. Benetti, S. Schoeftner, P. Muñoz, M. Blasco, Role of TRF2 in the assembly of telomeric chromatin, Cell Cycle 7 (2008) 3461–3468.
[34] C. Yanisch-Perron, J. Vieira, J. Messing, Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors, Gene 33 (1985) 103–119.