

The controversial telomeres of lily plants

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Abstract. The molecular structure of the exceptional telomeres of six plant species belonging to the order Asparagales and two species of the order Liliales was analyzed using Southern blot and fluorescence in situ hybridization. Three different situations were found, namely: i) In the two Liliales species, *Tulipa australis* (Liliaceae) and *Merendera montana* (Colchicaceae), the chromosome ends display hybridization signals with oligonucleotides resembling telomere repeats of both plants (TTTAGGG)_n and vertebrates (TTAGGG)_n. ii) Asparagales species such as *Phormium tenax* (Hemerocallidaceae), *Muscari*

comosum (Hyacinthaceae), *Narcissus jonquilla* (Amaryllidaceae) and *Allium sativum* (Alliaceae) lack both the plant telomere repeats and the vertebrate telomere repeats. iii) Two other Asparagales species, *Aloe vera* (Asphodelaceae) and an *Iris* hybrid (Iridaceae), display positive hybridization with the vertebrate telomere repeats but not with the plant telomere repeats. Southern blot hybridization revealed concurring results. On this basis, the composition of the telomere structure in this plant group is discussed.

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It is well known that the DNA located at chromosome termini of many organisms is normally composed of highly characteristic tandem repeat arrays of a short sequence. This structure is remarkably conserved in eukaryotic organisms although with slight differences among groups. For example, the consensus telomere sequence (TTAGGG)_n is conserved in all vertebrates, (TTAGG)_n in most arthropods and (TTTAGGG)_n in plants, whereas the sequences (TTGGGG)_n or (TTTTGGGG)_n are predominant in ciliates (Blackburn and Greider, 1995; Kipling, 1995).

Nevertheless, there are some exceptions to this generalization, the most remarkable being the telomeres of some dipteran species, in the case of animals, and some Asparagales species, in the case of plants. The best-known case of a different mechanism to resolve a possible lack of telomere sequences is *Drosophila melanogaster*. This species has overcome the lack of telomere repeats by the recruitment of non-LTR retrotransposons

(*HeT-A* and *TART*) to perform the cell function of capping the ends (Mason and Biessmann, 1995; Pardue et al., 1996; Eickbush, 1997). However, this does not appear to be a universal solution for the absence of conventional telomeres because related species such as those of the *D. virilis* group (Biessmann et al., 2000) and *Chironomus* (López et al., 1996; Martínez et al., 2001) present satellite DNA sequences at their chromosome ends instead of retrotransposons. In these cases, satellite DNA sequences could be involved in telomere elongation by homologous recombination (Biessmann et al., 2000).

Richards and Ausubel (1988) used cloning techniques to determine that the telomeres of *Arabidopsis thaliana* were composed of the repetitive sequence (TTTAGGG)_n. Later, based on in situ hybridization approaches, it was reported that many plant species share this, or a similar, sequence (Richards, 1995). Exceptions to this rule have been reported in families of the order Asparagales such as Alliaceae, Hyacinthaceae or Asphodelaceae (Pich et al., 1996a, b; Pich and Schubert, 1998; Adams et al., 2000) but not in others, such as Doryanthaceae, Tecophilaeaceae or Orchidaceae (Adams et al., 2001). It should be mentioned that the taxonomy and phylogeny of lily plants has recently changed. Lily plants were traditionally classified within a single order, Liliales, including different families such as Liliaceae, Amaryllidaceae, Iridaceae (Thorne, 1976; Cronquist, 1988). However, morphological (Huber, 1969; Dahlgren

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Table 1. List of plant species analyzed in this study, including the hybridization results (+ positive; – negative) of the vertebrate and plant telomere repeats to the genomic DNA of each species

Order	Species	Family	Vertebrate-like telomere	<i>Arabidopsis</i> -like telomere	Provenance
Asparagales	<i>Iris × hybrida</i> Retz.	Iridaceae	+	–	Cultured
	<i>Phormium tenax</i> J.R. & G. Forsters	Hemerocallidaceae	–	–	Cultured
	<i>Muscari comosum</i> (L.) Miller	Hyacinthaceae	–	–	Wild (Padul-Granada, Spain)
	<i>Narcissus jonquilla</i> L.	Amaryllidaceae	–	–	Wild (Cazorla, Spain)
	<i>Allium sativum</i> L.	Alliaceae	–	–	Cultured
	<i>Aloe vera</i> L.	Asphodelaceae	+	–	Cultured
Liliales	<i>Tulipa australis</i> Link	Liliaceae	+	+	Wild (Padul-Granada, Spain)
	<i>Merendera montana</i> Lange	Colchicaceae	+	+	Wild (Padul-Granada, Spain)
Capparales	<i>Arabidopsis thaliana</i> Heynh	Brassicaceae	+	+	Cultured

et al., 1985) and molecular evidence (Duvall et al., 1993; Chase et al., 1995a, b, 1999) support a classification with two orders: 1) The order Liliales including only some genera of the old Liliaceae family such as *Tulipa*, *Lilium*, *Fritillaria* and other families such as Colchicaceae or Smilacaceae; and 2) the order Asparagales including families such as Alliaceae, Asphodelaceae, Hyacinthaceae (from the old Liliaceae) but also Amaryllidaceae, Iridaceae and even Orchidaceae and related families.

Some hypotheses have been proposed for alternative sequences ending the chromosomes in Asparagales species lacking the *Arabidopsis* telomere repeats. For example, Pich et al. (1996a, b), Pich and Schubert (1998) and Adams et al. (2000, 2001) have proposed that satellite DNA sequences, and possibly rDNA or transposable element-like sequences, are the most likely to have acquired telomere functions in these species. However, Cuñado et al. (2001), analyzing the structure of the terminal regions of synaptonemal complex spreads in two *Allium* species, concluded that none of these sequences displayed the characteristic organization of telomere sequences at pachytene. We have reported a similar situation in *Muscari comosum*, a species in which telomeres lack sequences of the most abundant satellite DNA family (De la Herrán et al., 2001), rDNA sequences (Cuñado et al., 2000) or retrotransposons (unpublished data).

It has also been reported that, although the plant telomere repeats did not hybridize to the DNA of *Aloe* species (Asphodelaceae), there were positive hybridization signals at the ends of the chromosomes when probes containing vertebrate telomere repeats were used (Weiss and Scherthan, 2002). Indeed, in some species of Asparagales the ends of the chromosomes are composed of vertebrate telomere repeats, although plant telomere repeats could also be found (Sýkorová et al., 2003; Weiss-Schneeweiss et al., 2004). On this basis, a possible explanation for the absence of *Arabidopsis*-type telomere repeats at the chromosome ends of some species of Asparagales could be that telomere repeats represent variations on the primitive tandem repeat array produced by mutations either in the telomerase RNA template or in the telomerase active site. To test this hypothesis, we analyzed the presence of the vertebrate telomere repeat (TTAGGG)_n and the plant telomere repeat (TTTAGGG)_n in species of six genera belonging to this order (see Table 1).

Materials and methods

The plant materials employed in this study included two species of Liliales: *Tulipa* (Liliaceae) and *Merendera* (Colchicaceae), and six species of Asparagales: *Muscari* (Hyacinthaceae), *Narcissus* (Amaryllidaceae), *Iris × hybrida* Retz. (*Iris variegata* L. × *I. pallida* Lam. [Iridaceae]), *Phormium* (Hemerocallidaceae), *Allium* (Alliaceae) and *Aloe* (Asphodelaceae) (see Table 1). *Arabidopsis thaliana* and human DNA were used as controls.

DNA of the plant species was isolated from leaves or bulbs following the procedure of Dellaporta et al. (1983). Human DNA was isolated from blood using the Ultraclean DNA Blood Isolation kit (MO BIO, U.S.A.).

For Southern blot, DNAs were digested with *Bcl*II and *Hae*III, resolved in a 0.9% agarose gel, and transferred to a Hybond N⁺ (Amersham) nylon filter (Sambrook et al., 1989). The filter was hybridized with different probes: i) two different oligonucleotides resembling telomere repeats of vertebrates and plants, (TTAGGG)₇ and (TTTAGGG)₇, respectively; ii) a ribosomal DNA probe, the insert of the pTa71 recombinant plasmid containing the wheat ribosomal DNA unit (Gerlach and Bedbrook, 1979); and iii) a satellite DNA probe, the insert of the recombinant plasmid pMc8, containing a monomer unit of a satellite DNA family from the genome of *Muscari comosum* (De la Herrán et al., 2001). Labeling, hybridization and detection of the hybridization sites were performed using the DIG oligonucleotide 3' end labeling kit and the DIG-luminescent detection kit (Roche) following the manufacturer's instructions. The filters were consecutively hybridized with the four probes.

Anthers from young flowers were fixed without pretreatment in 1:3 acetic acid:ethanol. The fixed material was squashed in a drop of 45% acetic acid and coverslips removed at –80°C. The procedure of fluorescence in situ hybridization (FISH) described in Cuñado et al. (2000) was then applied to detect telomere repeats in the chromosomes of meiotic cells.

Results and discussion

Figure 1 shows the results of Southern blot hybridization using the two telomere repeats, (TTTAGGG)₇ and (TTAGGG)₇ as probes. Both probes coincided in showing a positive hybridization in *Arabidopsis*, humans, *Tulipa australis* and *Merendera montana* DNAs, and negative results in *Phormium tenax*, *Muscari comosum*, *Narcissus jonquilla* and *Allium sativum* DNAs. However, the probes differed in the hybridization pattern to the *Iris* hybrid and *Aloe vera* DNAs. Thus, the vertebrate telomere repeats hybridized to the DNA of both species but no hybridization was detected with the plant telomere repeats (see also Table 1). On the other hand, the fact that the ribosomal probe pTa71 cross-hybridized with all

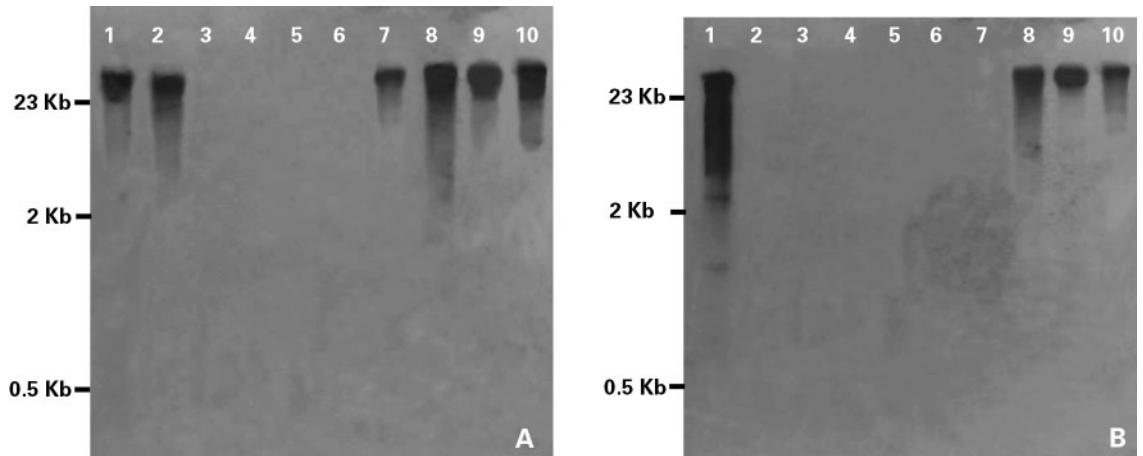


Fig. 1. Southern blot hybridization. A nylon filter containing DNA of nine different plant species and human DNA digested with *Bcl*II was hybridized with the oligonucleotides (TTAGGG)₇ (A) and (TTTAGGG)₇ (B). 1: *Arabidopsis thaliana*; 2: *Iris* hybrid; 3: *Phormium tenax*; 4: *Muscari comosum*; 5: *Narcissus jonquilla*; 6: *Allium sativum*; 7: *Aloe vera*; 8: *Tulipa australis*; 9: *Merendera montana*; 10: Human. Molecular size markers are depicted.

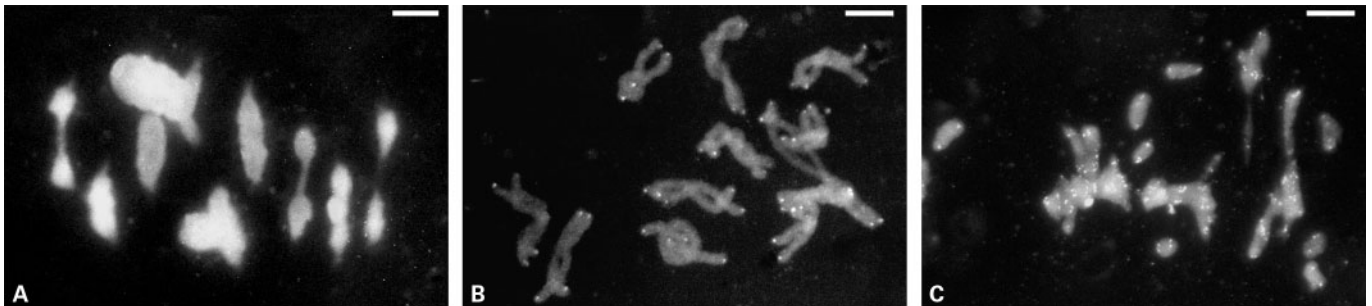


Fig. 2. Meiocytes of different species analyzed after in situ hybridization with the plant and the vertebrate telomere repeats, (TTTAGGG)_n and (TTAGGG)_n, respectively. (A) Metaphase I of *Muscari comosum* after in situ hybridization with the (TTTAGGG)_n probe. Note the total absence of hybridization signals. The result with the vertebrate probe was identical. (B, C) Location of telomeric sequences after in situ hybridization with the vertebrate telomeric repeat (TTAGGG)_n. (B) Diplotene of *Tulipa australis*. (C) Metaphase I of an *Iris* hybrid. Bars represent 10 µm.

DNAs and that the satellite DNA probe, isolated from *Muscari comosum*, only hybridized to the DNA of this species demonstrates the appropriate DNA transfer to the nylon filter.

Similar results were obtained when the plant and the vertebrate telomere DNA probes were hybridized to the chromosomes of the species analyzed (Fig. 2). Thus, *Muscari comosum* revealed telomeres lacking sequences able to cross-hybridize with the (TTTAGGG)_n and the (TTAGGG)_n probes (Fig. 2A), whereas both probes produced hybridization signals at the ends of all chromosomes in *Tulipa australis* (Fig. 2B). In the *Iris* hybrid, only the probe containing vertebrate telomere repeats hybridized to the ends of the chromosomes. It also revealed telomere loci at interstitial regions (Fig. 2C). This is a common phenomenon in invertebrate, vertebrate and plant species (see review by Kipling, 1995).

These findings add species of the genera *Phormium*, *Narcissus* and *Muscari* to the list of Asparagales species which lack the

standard plant telomere repeats and suggest that non-standard telomere constitution is widespread in this order. However, in genera such as *Ornithogalum* or *Scilla*, there are some species with the consensus plant telomere repeats (Adams et al., 2001). Additionally, in the genera *Aloe* and *Iris* vertebrate telomere repeats are localized at the chromosome ends. A possible explanation for the absence of hybridization signals in the telomeres of some species of Asparagales when the plant telomere repeats are used as a probe could be that the telomere repeats in these species represent variations on the primitive tandem repeat array produced by mutations either in the telomerase RNA template (Kipling, 1995) or in the telomerase active site (Fitzgerald et al., 2001). Indeed, it has been postulated that the telomerase active site can evolve rapidly in plants (Fitzgerald et al., 2001).

It is frequently assumed that telomere repeats of one representative species are conserved in an entire phylum or king-

dom. In many cases, this conclusion is based on data obtained by means of hybridization techniques, Southern and FISH, with a few exceptions in which direct evidence by cloning was found (Fanning, 1987; Adegoke et al., 1993; Garrido-Ramos et al., 1998). Cross-hybridization approaches are also further complicated by the unusual annealing properties of telomere oligonucleotides (Kipling, 1995) and only direct data based on the determination of the DNA sequences are reliable for the detection of new variants. On the other hand, there are examples of species having telomeres that share the common structure composed of tandem repeat arrays of short sequences but with a sequence markedly different from the consensus sequences, T_xG_y or T_xAG_y , expected in eukaryotes (Henderson, 1995). Variations in repeat arrays such as those found in budding and fission yeasts or in *Dictyostelium discoideum* (Kipling, 1995) could also account for the results found in Asparagales.

In fact, the constitution of the telomere DNA of *Aloe* (Weiss and Scherthan, 2002; this paper) and *Iris* (this paper) shows that in some Asparagales species the consensus telomere sequence of plants is replaced by the vertebrate variant repeat. In addition, both the vertebrate tandem repeat and the consensus plant telomere repeat have been found in the chromosome ends of wheat (Tsujiimoto et al., 1999), *Nicotiana tabacum* (Weiss and Scherthan, 2002), several species of Asparagales (Sýkorova et al., 2003; Weiss-Schneeweiss et al., 2004), and *Tulipa australis* and *Merendera montana* (this paper).

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