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Characterization of telomere-subtelomere junctions in *Silene latifolia*

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Abstract Telomere-associated regions represent boundaries between the relatively homogeneous telomeres and the subtelomeres, which show much greater heterogeneity in chromatin structure and DNA composition. Although a major fraction of subtelomeres is usually formed by a limited number of highly repeated DNA sequence families, their mutual arrangement, attachment to telomeres and the presence of interspersed unique or low-copy-number sequences make these terminal domains chromosome specific. In this study, we describe the structures of junctions between telomeres and a major subtelomeric repeat of the plant *Silene latifolia*, X43.1. Our results show that on individual chromosome arms, X43.1 is attached to the telomere either directly at sites corresponding to nucleosome boundaries previously mapped in this sequence, or via other spacer sequences, both previously characterized and newly described ones. Sites of telomere junctions are non-random in all the telomere-associated sequences analysed. These data obtained at the molecular level have been verified using in situ hybridization to metaphase chromosomes and extended DNA fibres.

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Introduction

Telomeres are nucleoprotein structures that form and protect the ends of eukaryotic chromosomes. Their DNA component usually consists of tandemly repeated, simple oligonucleotide sequences. These telomeric repeats (TRs) occur in only a few variants in eukaryotes, e.g. TTAGGG in *Homo sapiens*, TTTAGGG in *Arabidopsis thaliana*, and TTAGG in *Bombyx mori* (reviewed by Wellinger and Sen 1997). The lengths of telomeres can, however, vary widely, not only among species [from hundreds of bp in protists and green algae (Blackburn and Chiou 1981; Douglas et al. 2001) to several kb in *A. thaliana* or *Silene latifolia* (The Arabidopsis Genome Initiative 2000; Riha et al. 1998; Richards and Ausubel 1988) and up to hundreds of kb in *Nicotiana tabacum* and *N. sylvestris* (Fajkus et al. 1995a; Suzuki et al. 1994)] but also among chromosome ends in a single nucleus (Burr et al. 1992).

In the centromeric direction, the telomere is followed by the subtelomere. The DNA sequences that make up subtelomeres are able to substitute, at least to some extent, for the protective role of telomeric DNA sequences in case of telomere loss (Palladino and Gasser 1994). Subtelomeres also influence the so-called telomeric position effect (Gottschling et al. 1990; Pryde and Louis 1999) and thus function as a buffer zone between the telomere and internal chromosome domains (Wellinger and Sen 1997). In subtelomeric regions in plants, both tandem and dispersed repeated sequences are found in large excess over single-copy or low-copy-number sequences, and are frequently used as markers for identifying genomic components of hybrid organisms in plant breeding and in taxonomic studies (Gebhardt et al. 1995; Vershinin et al. 1996).

The subtelomere is attached to the telomere by means of so-called telomere-associated sequences (TASs). The particular arrangement of this region has been studied in only a few cases. For example, the telomere-subtelomere junction has been analysed in tomato using fluorescence in situ hybridization to extended DNA fibres (EDF-FISH) (Zhong et al. 1998). A modified vectorette approach (Arnold and Hodgson 1991) has been used to characterize variants of TASs in barley (Kilian and Kleinhofs 1992) and *Nicotiana* species (Horakova and Fajkus 2000; Chen et al. 1997). The attachment of a highly repetitive subtelomeric sequence, HRS60, to telomeres in tobacco (Fajkus et al. 1995b) and of an rDNA cluster in *A. thaliana* (Copenhaver and Pikaard 1996) has been analysed using direct PCR, which is the convenient method in cases where information on the macrostructure of the chromosome end is available. In *A. thaliana*, rDNA genes are located on the ends of chromosomes 2 and 4, separated from the telomeres by a spacer of about 500 bp. In other cases of subtelomeric repeats where the exact sequence of the junction with the telomere has been described (HvRT in barley, TAS49 in *N. tomentosiformis* and HRS60 in *N. tabacum*), the linker is formed by a short oligonucleotide sequence.

It has also been shown that different kinds of chromatin structures are juxtaposed in the TAS region. While the telomeric nucleosomes are characterized by short periodicity, low stability and a lack of strict positioning (Fajkus et al. 1995a; Makarov et al. 1993; Rossetti et al. 1998) – features consistent with a recently proposed columnar model of telomeric chromatin (Fajkus and Trifonov 2001) – subtelomeric nucleosomes usually show a periodicity similar to that of bulk chromatin, and occupy preferential positions on the DNA sequence (Fajkus et al. 1992, Vershinin and Heslop-Harrison 1998). Moreover, it has been shown recently that the transition between the telomeric and subtelomeric modes of nucleosome arrangement is not necessarily found at the telomere-subtelomere sequence junction itself, but may occur farther into the subtelomere (Sykorova et al. 2001), depending probably on the length of the telomere, the strength of nucleosome positioning signals in a given subtelomeric sequence, and the presence of a chromatin boundary element (e.g., a matrix-attachment region-MAR). From the point of view of possible effects of chromatin structure on gene expression, the TAS region may thus be an interesting target for study, especially with respect to the regulation of the activity of subtelomeric genes. TAS regions may well be considered as the point at which the sequentially and structurally conservative and homogeneous telomere meets the subtelomere, which is heterogeneous in these respects. It has even been suggested in tomato that all chromosome ends could possess their own unique arrangement (Zhong et al. 1998).

In the present study we have analysed a subset of TAS variants in a model dioecious plant, *Silene latifolia*. This plant possesses relatively short telomeres (Riha et al. 1998) and a number of subtelomeric repeats (Bůžek et al. 1997). One of the subtelomeric repeats, the X43.1 sequence, is a high-copy-number, tandem repeat observed in situ at all chromosome ends. This sequence has therefore been chosen in this work as a suitable candidate for a TAS, and has been used for PCR-based isolation and cloning of other sequences that participate in forming the link between the telomere and X43.1 on different chromosome arms.

Materials and methods

Plant material, DNA/RNA isolation

Leaves of *Silene latifolia* ssp. *alba* were used for the isolation of genomic DNA or total RNA. Genomic DNA was prepared from cell nuclei as described previously (Fajkus et al. 1995a). Total RNA was isolated using an RNeasy Mini Kit (Qiagen). Plasmid DNAs were purified using a Qiagen Plasmid Mini Kit.

Primers and probes

The following primers were used for PCR: TELPR (5′-CCGAATTCAACCCTAAACCCTAAACCCTAAACCC-3′, (Kilian and Kleinhofs 1992), X43F (5′-TCGTACCGGAACCTGTTTCT-3′) and X43R (5′-GACCTTCCGAACGGTTGAAA-3′, provided by K. Riha, Institute of Biophysics, Brno), 19L270 (5′-TGCTCCTTATTGCTGGTCACG-3′), 19L2988 (5′-TGCAACACAACCCAAGATTCAC-3′) and 19U266 (5′-CGCTCCCGAGCTGATTCACC-3′). To probe the genomic organization of the 19Bst sequence, the following fragments of the 43FtC19 clone were prepared: p19/2.7K (2.7 kb) was obtained by PCR using the primers 19U266 and 19L2988; p19Bst320 (320 bp) was recovered by digestion with *Bst*BI, and p19HL13 (1680 bp) by cleavage with *Hae*III (see Fig. 1).

18S rDNA was detected using as a probe the *Eco*RI fragment of the tomato 18S-rDNA gene (kindly provided by Prof. T. Kiss, Institute of Plant Physiology, Szeged, Hungary).

PCR and cloning

PCR on genomic DNA (50 ng) was performed using the protocol recommended for the Expand High Fidelity PCR system (Roche) with the telomere C-strand primer TELPR (Kilian and Kleinhofs 1992) and either X43F or X43R primers specific for both strands of the X43.1 sequence. Initial denaturation (94°C, 4 min) was followed by 10 cycles of denaturation (94°C, 30 s), annealing (56°C, 30 s) and extension (68°C, 4 min). A further 25 cycles of PCR were performed under the same conditions, increasing the extension time by 20 s in each successive cycle. The 19Bst sequence was analysed by asymmetric PCR using the 19L270 primer alone for the first 10 cycles. Then the second primer (TELPR) was added, and the reaction was continued for 25 cycles under the above conditions. PCR products were either purified using a Qiagen PCR Purification kit, or separated by agarose electrophoresis and extracted from the gel using a Qiagen Gel Extraction kit. PCR products were then ligated into the *Eco*RV site of the plasmid pZErO-1 (Invitrogen) and ligation mixtures were used for transformation of XL1-Blue cells (Stratagene). The cloned products were sequenced either directly or, in the case of the clone 43FtC19, after physical mapping; restriction fragments of 43FtC19 obtained by digestion with *Hae*III, *Rsa*I, *Sma*I and *Nci*I (NEB) were isolated, cloned and sequenced using an AmpliCycle sequencing kit (Perkin Elmer). To obtain the complete 43FtC19 sequence further primers were designed as required (not shown).

Fig. 1 A schematic diagram of the clone 43FtC19 (~4.0 kb) from the telomeric end (*left*) towards the X43.1 sequence (in *grey*, see Fig. 2 for details). The *black boxes* depict regions present in DNA probes prepared using PCR (p19/2.7K) or restriction enzyme digestion (pBst320, p19HL13). The *arrows* indicate primer locations, and the restriction sites used are marked with *triangles*

Analysis of copy numbers and detection of transcripts

For determination of copy numbers, genomic DNA (0.5 and 5 μg) and the probes p19/2.7K, p19Bst320, p19HL13 (0.1 to 500 pg of DNA) were electrophoresed on 0.7% agarose gels, blotted onto nylon membrane and hybridized with the corresponding radioactively labelled probe. A value of 5.6×10^9 bp for the size of the *S. latifolia* haploid genome (Vagera et al. 1994) was used to calculate copy numbers. Total RNA was fractionated on a 1% agarose gel, blotted and hybridized with the probe p19/2.7K. Ready-To-Go ([α-^{32}P]dCTP) DNA Labelling Beads (AP Biotech) were used to label DNA probes. Hybridization signals were visualized by autoradiography or using a Phosphoimager STORM860, and evaluated with ImageQuant software (Molecular Dynamics). All standard DNA and RNA manipulations were performed as described in Sambrook et al. (1989).

Computer analysis

Sequence analyses were performed using the BLAST (GenBank, http://www.ncbi.nlm.nih.gov/), Genescan (http://genome.dkfz-heidelberg.de/cgi-bin/GENSCAN/genscan.cgi), MARFinder (http://www.futuresoft.org/MAR-Wiz/), SSEARCH (http://www.ddbj.nig.ac.jp/E-mail/homology.html), DNASIS (Hitachi Software Engineering) and DNASTAR (DNASTAR Inc.) programs.

Fluorescence in situ hybridization (FISH) on metaphase chromosomes and extended DNA fibres (EDFs)

DNA probes for X43.1, 15Ssp and 19Bst (see above) were amplified and labelled by PCR with biotin-16-dUTP (Roche, Germany) or digoxigenin-11-dUTP (Roche) as described by Ohmido and Fukui (1997). A telomere-specific DNA probe was generated according to Ijdo et al. (1991). Metaphase chromosome preparations from root tips of germinated *S. latifolia* seedlings were made using the enzymatic maceration and air-drying (EMA) method (Fukui 1996).

FISH was carried out according to the procedures of Ohmido and Fukui (1997). Biotin-labelled probes were detected using Fluorescein Avidin DCS (Vector Laboratories) and the signals were amplified using biotinylated anti-avidin D (Vector Laboratories). For digoxigenin-labelled probes, detection was done using Anti-Dig Rhodamine and signal amplification with sheep anti-Texas Red (Vector Laboratories). Counterstaining was performed using 4′,6-diamidino-2-phenylindole (DAPI). Fluorescent signals were observed using a fluorescence microscope (Axioplan, Zeiss) and images were captured with a cooled CCD (charge-coupled device) camera and analysed using the IPLab Spectrum software.

For preparation of extended DNA fibres (EDFs), cell nuclei were prepared from fresh *S. latifolia* leaves according to the method described by Fransz et al. (1996). Nuclei were resuspended in 100–150 µl of EDF-PBS (10 mM sodium phosphate pH 7.0, 140 mM NaCl). Two microliters of the suspension was pipetted onto one end of an APTES (3-aminopropyltriethoxysilane)-coated slide (Matsunami Glass Industries) and dried. The nuclear membrane was digested by adding 25 µl of STE2 lysis buffer [0.5% (w/v) SDS, 5 mM EDTA, 100 mM TRIS-HCl pH 7.0] to the nuclei for 5 min. Then the slide was tilted at an angle of approximately 90° to allow the DNA fibers to be stretched along the surface by fluid flow. The slides were then dried completely, fixed in ethanol:glacial acetic acid (3:1 v/v) for 2 min and then baked at 60°C for 2 h. The slides were stained with 1 μg/ml YOYO-1 (Molecular Probes) and the quality of extended DNA fibres was checked under the fluorescence microscope. Fixed EDF slides were kept at room temperature for up to several weeks. For EDF-FISH, denatured probes were directly placed onto EDF slides and the preparation was denatured for 4 min at 80°C on a hotplate. Hybridization was carried out for 16–18 h at 37°C. Detection of EDF-FISH signals was performed as for chromosome FISH, but without the blocking steps.

Results and discussion

Isolation and analysis of variants of X43.1-telomere junctions

Analysis of the telomere-subtelomere junction region was performed by PCR with the telomere primer TELPR and the primers X43F and X43R derived from both strands of the subtelomeric X43.1 sequence (GenBank Accession No. AF251508). This repetitive sequence was previously shown to be localized in the terminal regions of all *S. latifolia* chromosomes (Bůžek et al. 1997; Matsunaga et al. 1999), but its distance from the telomere and its orientation remained unknown. Reaction conditions were chosen to obtain PCR products of up to 6 kb in length. PCR using the primer pair TELPR/X43F yielded a number of discrete bands after electrophoresis on an agarose gel. This mixture of PCR products was cloned into a plasmid vector, and a set of clones with 0.2- to 4-kb inserts was obtained. Sixteen clones containing an *A. thaliana* -like TR (3–27 units) at one end and the X43.1 sequence at the opposite end were analysed further. Comparison of the clones, and subsequent analyses, revealed the presence of nine variants of the TR-X43.1 junction which could be sorted into two basic classes (Fig. 2): variants in which X43.1 was directly attached to a TR and variants in which the connection was indirect. In individual variants, the sequence at the TR-X43.1 or TR-spacer junction and the spacer-X43.1 junction is conserved.

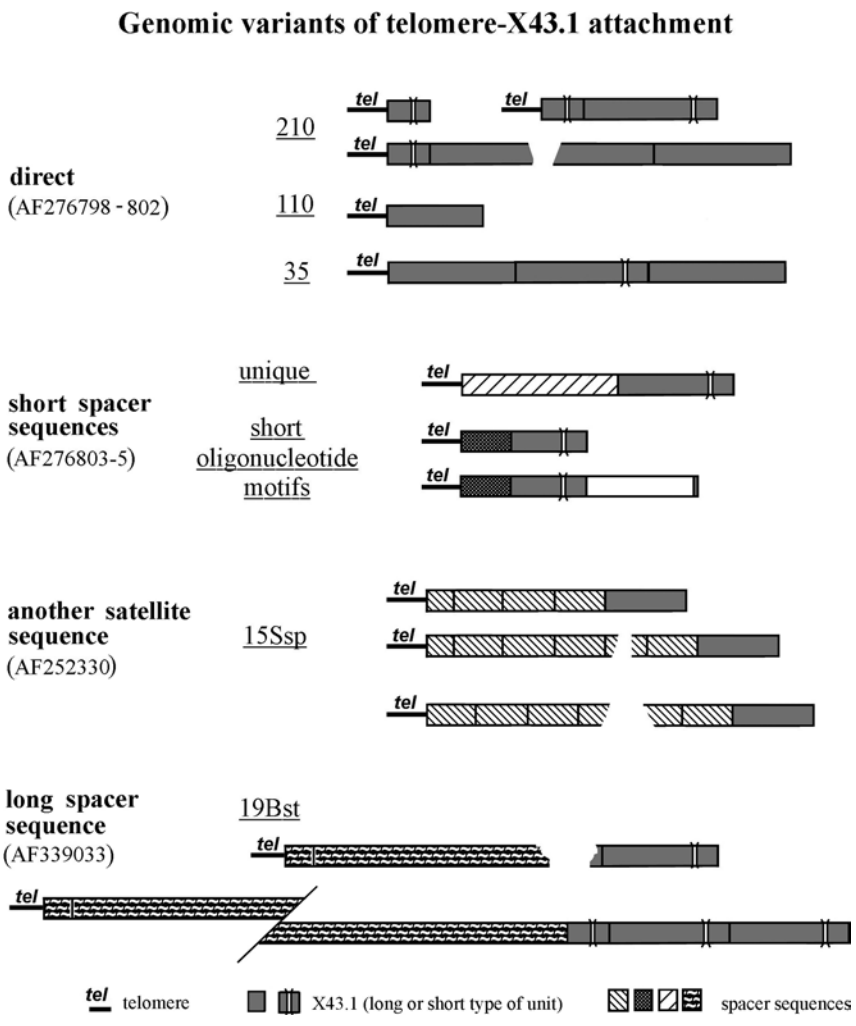

Fig. 2 Variations in the attachment of the X43.1 sequence to the telomere. Individual variants are classified according to the type of attachment. Accession numbers of the attachment sequences in GenBank are given in *parentheses*. Subgroups (*underlined*) are characterized either by type of spacer sequence, or, in the case of direct attachment, by the junction site in the consensus X43.1 sequence. The X43.1 sequence occurs in the clones in two variants that differ by the presence or absence of a 22-bp subrepeat at the marked site in the sequence (315–337 bp)

Direct TR-X43.1 attachment

Three different sites of the *direct* TR-X43.1 attachment were identified in six clones analysed (GenBank AF276798–AF276802, see also Fig. 2). It is known that two length variants of the monomeric X43.1 sequence unit occur in the *S. latifolia* genome, which differ by the presence of a 22-bp subrepeat (Bůžek et al. 1997; Garrido-Ramos et al. 1999; Sykorova et al. 2001). Both types of monomers are present in diverse arrangements in the clones analysed here (Fig. 2). Features of the X43.1 chromatin have been described in detail in our previous study (Sykorova et al. 2001) including its double nucleosome periodicity, which is similar to the distribution of nucleosomes on pSC200 and pSC250 sequences in rye (Vershinin and Heslop-Harrison 1998). The shorter periodicity resembles the spacing of telomeric nucleosomes, while the longer is similar to that of bulk chromatin. Interestingly, in the clones analysed here (Fig. 2), X43.1 is attached to the telomere at sites corresponding to previously characterized borders of preferred nucleosome positions on X43.1 (Sykorova et al. 2001). It is therefore plausible that the telomere-specific chromatin structure (Fajkus and Trifonov 2001) is not interrupted at the TR-X43.1 boundary (see below).

TR-spacer-X43.1 attachment

In the variants in which a spacer intervenes between TR and X43.1, the spacer sequences are unrelated to either TR, X43.1 or other sequences in GenBank. These variants (Fig. 2) can be further subdivided according to the type of spacer sequence into: (1) short spacer sequence, (2) satellite sequence (other than X43.1), and (3) long spacer sequence variants.

The first group comprises three clones (GenBank AF276803–5), one of which contains a 400-bp spacer sequence that is attached to the telomere by degenerate telomere motifs. The other two clones comprise a short spacer sequence (142 bp) with repeated degenerate minisatellite motifs (<25 bp). In all three clones the spacer is attached to the shorter variant of the X43.1 sequence unit.

The second group is represented by a spacer sequence composed of the high-copy-number tandem repeat 15Ssp (GenBank AF252330). Three clones analysed have a variable number of 15Ssp units in a head-to-tail

arrangement, which are linked to the telomere at two different sites separated by 73 bp (Fig. 2). All these clones are characterized by the presence of the longer X43.1 sequence unit. No other spacer sequences are present between 15Ssp and TR. This highly conserved, AT-rich sequence (obtained in the initial stages of this study) and its chromatin structure have been characterized by our group recently (Sykorova et al. 2001). The short nucleosome periodicity and low stability of 15Ssp mononucleosomes are reminiscent of telomeric chromatin. Moreover, in contrast to all other subtelomeric sequences described so far, 15Ssp nucleosomes show no preference for particular positions, another typical feature of telomeric chromatin.

The last group is represented by four clones with a long spacer sequence. In this case, the spacer is formed by the sequence 19Bst, which is linked to the TR by a sequence containing degenerate telomeric motifs (see Fig. 3). The sequence 19Bst occurs in a low number of copies per genome (4–6 per diploid genome), as determined using p19/2.7K (2740 bp) and p19HL13 (1670 bp) fragments as probes. However, some parts of the 19Bst sequence can also be found in an arrangement that differs from that found in the clone 43FtC19. Thus, the p19Bst320 fragment containing the 19Bst region proximal to the TR (see Fig. 1) occurs in 22 copies per diploid genome. The complete sequence of the 43FtC19 clone (GenBank AF339033) is 4023 bp long and includes one incomplete and two complete units of the short variant of the X43.1 sequence.

Characterization and analysis of the 19Bst sequence

To further characterize the last mentioned type of junction, the primer 19L270 was designed to amplify from the 43FtC19 sequence towards the telomere. PCR using this primer in combination with TELPR results in a product of about 500 bp. Using these primers on genomic DNA as a template, a PCR product was obtained, cloned into the plasmid vector pZErO-1 and the clones obtained were sequenced (Fig. 3). The resulting 14 clones represent two major variants with respect to the mode of attachment of the 19Bst sequence to the telomere, **19I** (seven clones, GenBank AF339034) and **19II** (five clones, GenBank AF339035). The clones within each group show high sequence homology.

The attachment region between TR and 19Bst in the **19I** variant is about 100 bp long, comprises mainly various degenerate telomere motifs, and is homologous to the corresponding region of the 43FtC19 clone. Most of the 140-bp attachment region of the **19II** variant is completely different from the attachment region found in the **19I** group. At the boundary between each of these attachment regions and the 19Bst sequence, an internally symmetrical sequence occurs which is rich in short repeats and has the potential for to form hairpin structures. Furthermore, a sequence motif (TAAAAAAAT) is present which has previously been found to form a direct attachment between the subtelomeric sequence HRS60 and the TR in tobacco chromosomes (Fajkus et al. 1995b).

All sequences previously localized in *S. latifolia* subtelomeres (Bůžek et al. 1997; GenBank AF251504–8) are highly repetitive. The 19Bst sequence is of a different type and, therefore, was analysed further in silico. The first analysis was focused on the possible presence of a coding sequence. The telomere-proximal location does not exclude this possibility, as exemplified by the subtelomeric rDNA genes of *A. thaliana* (Copenhaver and Pikaard 1996). However, comparison of the 43FtC19 DNA sequence with those in GenBank (BLASTN search) gave no positive results. The 43FtC19 sequence was also evaluated with Genescan software (Burge and Karlin 1997) using a calculation matrix based on *A. thaliana*. Furthermore, sequences of all six possible translational reading frames were analysed using the software SSEARCH (Smith and Waterman 1981; Pearson 1991). Analysis using BLASTP and SSEARCH showed similarity with a putative protein of *A. thaliana* (protein CAB79198.1) in one of the six possible reading

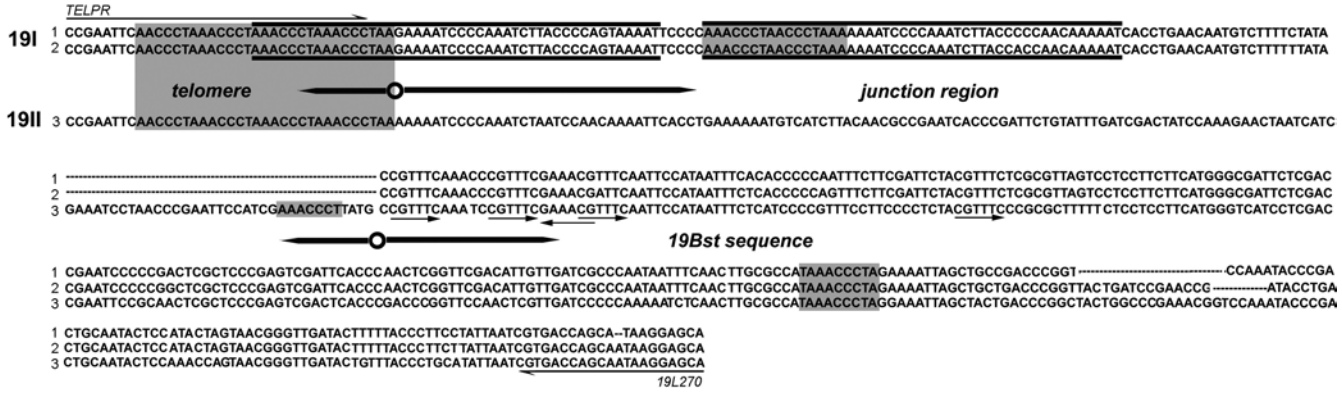

Fig. 3 Attachment of the 19Bst sequence to the telomere. Two groups of clones, **19I** (2) and **19II** (3), obtained from genomic DNA of *S. latifolia* by PCR using the primers TELPR and 19L270, differ in the telomere junction region, and are aligned with the corresponding region of the 43FtC19 clone (1). The repeating motif in the spacer of the **19I** group is marked with *two thick lines* and telomeric motifs are denoted by *grey boxes*. The *short arrows* show repeats that have the potential to form a hairpin loop next to the junction region

frames of 19Bst. The SSEARCH program further identified significant similarity to a hypothetical protein of *Oryza sativa* (protein AAD38275.1).

To test for transcription of the hypothetical coding region of the 43FtC19 clone, the cloned fragment p19/2.7K was used as a probe for hybridization with total RNA from leaves. A negative result was obtained. The 18S rDNA probe was then used for rehybridization (not shown), as a positive control. Of course, our failure to find 19Bst transcripts in this experiment does not exclude the possibility that a region of the 19Bst sequence forms part of a protein-coding gene, which is silent in leaves either due to its subtelomeric position or the developmental timing of its expression. It is, however, worth noting that both matches in protein databases were to hypothetical proteins predicted from genome sequencing data by the same program (Genescan).

Further in silico analysis was focused on the search for MAR motifs to evaluate a possible role of 19Bst in binding to the nuclear matrix. Previous biochemical and cytogenetic data have indicated that telomeres bind chromosomes to the nuclear lamina (Comings 1980; Bass et al. 2000). In a loop model of the higher-order structure of chromatin, MARs occur at a frequency of less than one per 100 kb. Individual chromatin loops may, however, differ substantially from this average, not only between species, but also with respect to chromosomal position. It has been observed at meiotic prophase that terminally located telomeric chromatin loops appear to be smaller then loops of the same telomeric DNA sequence positioned interstitially (Heng et al. 1996). As telomeres are very uniform in sequence, further MARs should occur in subtelomeres, which are commonly made up of large blocks of satellite tandem repeats. Therefore, the software MARFinder (Singh et al. 1997) was employed to find MARs based upon the simultaneous occurrence of 20 DNA patterns that have been found in the neighborhood of MARs. It follows from the analysis of the 43FtC19 sequence using this software that although several motifs that occur frequently in MARs are present, MAR function cannot be attributed to any particular region of the sequence.

In situ analysis of TAS

The above results obtained by molecular approaches were complemented by cytogenetic data obtained from fluorescence in situ hybridization (FISH) to metaphase chromosomes and extended DNA fibres (EDF-FISH). The subtelomeric location of the sequence 19Bst on *S. latifolia* chromosomes was demonstrated using FISH (Fig. 4a). 19Bst exhibited similarly located fluorescent signals to those reported previously for the 15Ssp sequence (Sykorova et al. 2001). However, the 15Ssp sequence, in agreement with its higher copy number, was found at subtelomeres of almost all chromosome arms, while the 19Bst signals were observed in the subtelomeric regions of only about 20 chromosome arms (including one arm of the X-chromosome and excluding the Y chromosome). Most of these Bst19-signals probably correspond to TR-proximal parts of 19Bst, whose copy number (22) exceeds the number of complete 19Bst copies by four- to fivefold (see above). The strong fluorescent signal may indicate the existence of sequences similar to Bst19 in subtelomeric regions, although such sequences have not been found by PCR to date.

The arrangement of 15Ssp and 19Bst sequences relative to X43.1 and telomere sequences along a single extended DNA molecule can be resolved using EDF-FISH. Figure 4b shows the direct attachment of the X43.1 sequence (biotin labelled, green track) to the telomere (digoxigenin labelled, red dot). The appearance of the telomeric signal as a single dot agrees with previous results of EDF-FISH detection of telomere sequences in rice, which are of comparable size to those in *S. latifolia* (Ohmido et al. 2000). Furthermore, the X43.1 sequence was used as reference probe to determine accurately the relative positions of 15Ssp (Fig. 4c) and 19Bst (Fig. 4d) sequences with respect to the telomere. The green signal for 15Ssp or 19Bst was located between the red signal track given by X43.1 and the single red signal dot corresponding to the telomere. Thus, using EDF-FISH, both 15Ssp and 19Bst sequences can be shown to be positioned between the telomere and the X43.1 sequence.

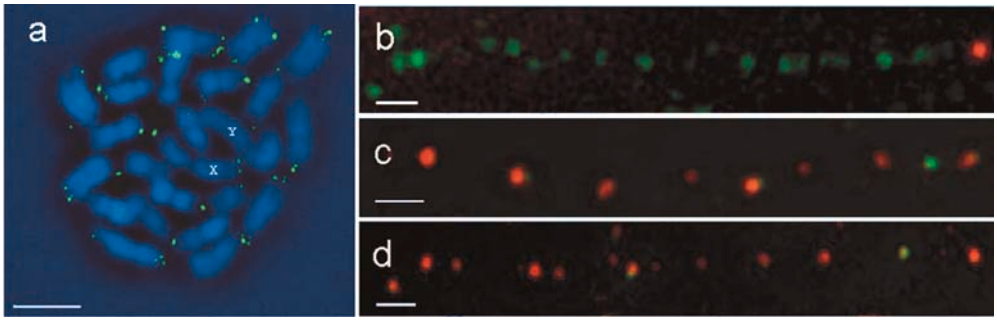

Fig. 4a–d In situ analysis of telomere-associated sequences. The subtelomeric location of the newly characterised 19Bst sequence was demonstrated using FISH on metaphase chromosomes of *S. latifolia* (**a**). Chromosomes X and Y are marked with corresponding letters. The *bar* corresponds to 5 μm. Variations in the relative arrangement of X43.1, 15Ssp, 19Bst and TR were visualized using EDF-FISH (**b** – **d**). The *bars* represent 1 μm. The direct attachment of X43.1 (*green signal track*) to TR (*red signal dot*) is shown in **b**. The 15Ssp sequence (**c**) and the Bst19 sequence (**d**) were detected as *green dots* between X43.1 (*red signal track*) and TR (*terminal red signal dot*)

It can be concluded that the spectrum of variation in the mode of attachment of X43.1 to telomeres involves both the new DNA sequences described here, and the previously characterized high-copy-number tandem repeats 15Ssp and X43.1. In the respective repeats, our previous data on their chromatin structure (Sykorova et al. 2001), together with the present detailed information on sites of their attachment to telomeres, suggest that a specific chromatin structure, usually ascribed exclusively to telomeres, extends to the telomere-associated regions. The novel 19Bst sequence described here was shown at both molecular and cytogenetic levels to act as a spacer between X43.1 and the telomere in a subset of chromosome arms, including one arm of chromosome X, while it is apparently absent in chromosome Y. All telomere-TAS junctions are non-random and highly conserved in a given type of TAS, which corresponds to our previous findings for two tobacco TASs, HRS60 (Fajkus et al. 1995b) and TAS49 (Horakova and Fajkus 2000). Taking advantage of the EDF-FISH technique, results from the DNA sequence level could be integrated with the chromosome picture. Although this study presents only a partial characterization of the structure of subterminal chromosome regions, it suggests that this chromosome domain has a highly individual character.

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