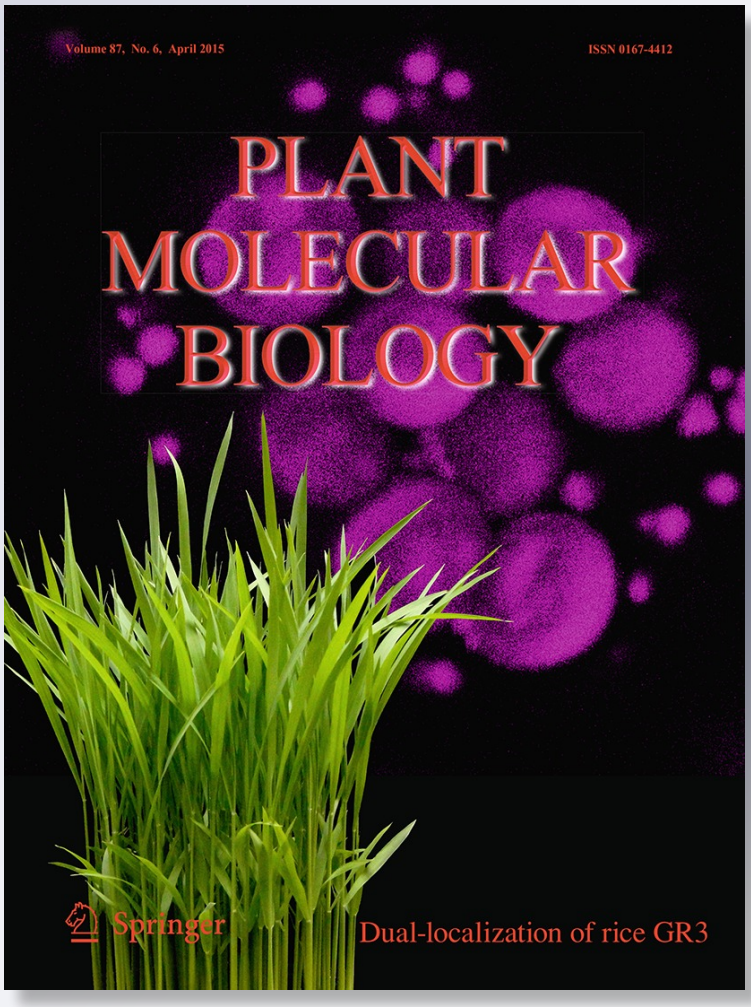
Telomere dynamics in the lower plant Physcomitrella patens

Miloslava Fojtová, Eva Sýkorová, Lucie Najdekrová, Pavla Polanská, Dagmar Zachová, Radka Vagnerová, Karel J. Angelis, et al.

Plant Molecular Biology
An International Journal on Molecular Biology, Molecular Genetics and Biochemistry

ISSN 0167-4412
Volume 87
Number 6

Plant Mol Biol (2015) 87:591-601
DOI 10.1007/s11103-015-0299-9



Springer

Plant Mol Biol (2015) 87:591–601
DOI 10.1007/s11103-015-0299-9

Telomere dynamics in the lower plant *Physcomitrella patens*

Miloslava Fojtová · Eva Sýkorová · Lucie Najdekrová · Pavla Polanská · Dagmar Zachová · Radka Vagnerová · Karel J. Angelis · Jiří Fajkus

Received: 6 January 2015 / Accepted: 16 February 2015 / Published online: 21 February 2015

Abstract A comparative approach in biology is needed to assess the universality of rules governing this discipline. In plant telomere research, most of the key principles were established based on studies in only single model plant, *Arabidopsis thaliana*. These principles include the absence of telomere shortening during plant development and the corresponding activity of telomerase in dividing (meristem) plant cells. Here we examine these principles in *Physcomitrella patens* as a representative of lower plants. To follow telomerase expression, we first characterize the gene coding for the telomerase reverse transcriptase subunit *PpTERT* in *P. patens*, for which only incomplete prediction has been available so far. In protonema cultures of *P. patens*, growing by filament apical cell division, the proportion of apical (dividing) cells was quantified and telomere length, telomerase expression and activity were determined. Our results show telomere stability and demonstrate proportionality of telomerase activity and expression with the number of apical cells. In addition, we analyze telomere maintenance in *mre11*, *rad50*, *nbs1*, *ku70* and *lig4* mutants of *P. patens* and compare the impact of these mutations in double-strand-break (DSB) repair pathways with earlier observations in corresponding *A. thaliana* mutants. Telomere phenotypes are absent and DSB repair kinetics is not affected in *P. patens* mutants for DSB factors involved in non-homologous end joining (NHEJ). This is compliant with the overall dominance of homologous recombination over NHEJ pathways in the moss, contrary to the inverse situation in flowering plants.

Keywords *Physcomitrella patens* · Telomere maintenance · Telomerase · *PpTERT* structure · DSB repair mutants

Miloslava Fojtová, Eva Sýkorová and Lucie Najdekrová have contributed equally to this study.

During manuscript preparation the pre-release of the new moss genome annotation V3.1 was made available at cosmoss.org. Our experimental results define the prediction Pp3c1_42700V1.1 as a correct gene model for the *PpTERT*.

Electronic supplementary material The online version of this article (doi:10.1007/s11103-015-0299-9) contains supplementary material, which is available to authorized users.

M. Fojtová · E. Sýkorová · L. Najdekrová · P. Polanská · D. Zachová · J. Fajkus (✉)
Faculty of Science and CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic
e-mail: fajkus@sci.muni.cz

M. Fojtová · E. Sýkorová · J. Fajkus
Institute of Biophysics, Czech Academy of Sciences, v.v.i., Královopolská 135, 61265 Brno, Czech Republic

R. Vagnerová · K. J. Angelis
Institute of Experimental Botany, Czech Academy of Sciences, v.v.i., Na Karlovce 1, 16000 Prague 6, Czech Republic

Introduction

Telomeres are the conserved terminal domains of linear chromosomes, which are essential for protection of chromosome integrity. Telomeres are chromatin structures and as such they are formed by telomeric DNA and numerous protein components (reviewed in Fajkus et al. 2005). Telomeres serve multiple roles, but their fundamental functions include demarcation of natural chromosome ends to distinguish them from unrepaired chromosome breaks and prevent telomeres from unwanted repair (the so called end-protection problem) (de Lange 2009). Failure in this

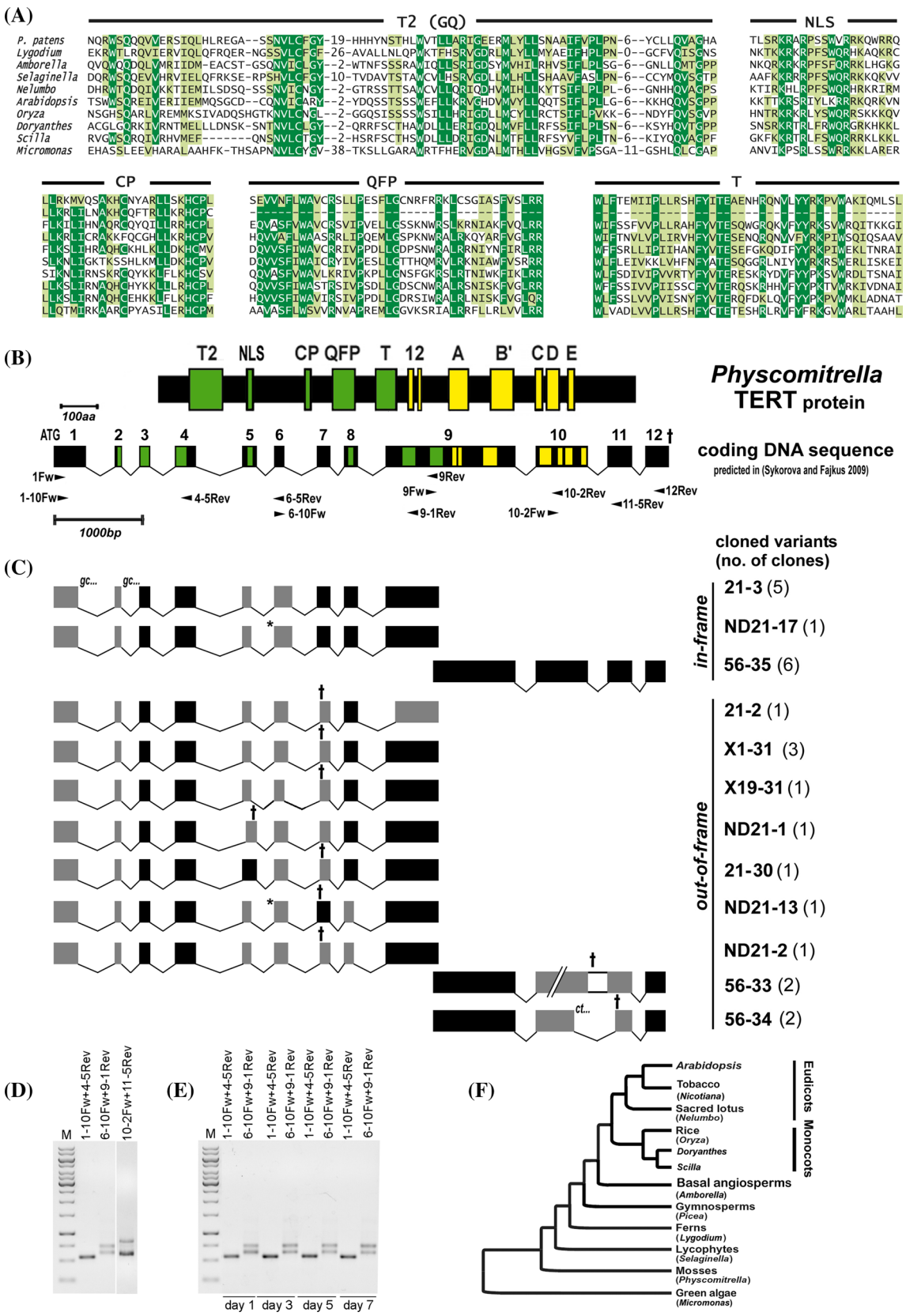
function results in chromosome fusions and subsequent genome instability [breakage–fusion–bridge cycle, BFB (McClintock 1941)]. This function is dependent on specific telomeric proteins, as well as the protective secondary structures (such as G-quadruplexes or t-loops) of telomeric DNA itself (de Lange 2009). The second major role of telomeres is to solve the end-replication problem, i.e. to ensure stable maintenance of themselves, as the conventional semiconservative replication is not able to replicate completely the 3′-end of the parental DNA strand synthesized by lagging strand synthesis (Olovnikov 1971). The most common mechanism to counteract this replicative telomere shortening is telomere elongation by a specific ribonucleoprotein complex called telomerase (Greider and Blackburn 1985, 1987). Telomerase replenishes telomeres by a reverse transcription mechanism using its catalytic protein subunit (Telomerase Reverse Transcriptase, TERT) and template RNA subunit (Telomerase RNA, TR) (Greider and Blackburn 1989) for synthesis of tandem repeats of $T_xA_yG_z$ units as TTAGGG in vertebrates or TTTAGGG in most land plants and algae (Sykorova et al. 2003b; Fulneckova et al. 2013).

Telomere and telomerase functions are highly conserved among yeasts, protozoans, plants and animals, although exceptions were described which show a telomere sequence different from its phylogenetic position (Sykorova et al. 2003a, b) or even a different (telomerase-independent) mechanism of telomere synthesis (Pich et al. 1996; Sykorova et al. 2006a). In plants, telomere biology has been studied mostly in classical model species such as *Arabidopsis thaliana* or *Nicotiana tabacum* (Fajkus et al. 1995, 1996; Fitzgerald et al. 1996) and compared to flowering plants, much less information is available in lower plants like moss and algae species (Suzuki 2004; Shakirov et al. 2010; Fulneckova et al. 2012, 2013).

Physcomitrella patens is a species of moss (bryophytes) which is a basal lineage of land plants, having diverged before the acquisition of well-developed vasculature (Fig. 1f). As the oldest living branch in land plant evolution, it stands in an important phylogenetic position for comparative studies to illuminate the evolution of the mechanisms behind the complexity of modern plants, including model organisms, such as *Arabidopsis*, and crop plants (Rensing et al. 2008). The body plans of all land plants are shaped through the actions of apical meristems, tissues composed of self-renewing stem cells that provide daughter cells for subsequent differentiation (Graham et al. 2000; Friml et al. 2006; Benkova et al. 2009) and some common pathways of apical meristem regulation may be conserved between ancestral plants and present day mosses (Prigge and Bezanilla 2010; Viaene et al. 2014).

Unlike its 450 million years younger land plant relatives, *Physcomitrella* is one of a few known multicellular organisms with a highly efficient system of homologous recombination (Schaefer 2002; Kamisugi et al. 2006). Consequently, gene targeting in *Physcomitrella* is five orders of magnitude more efficient than in angiosperms and two orders of magnitude more efficient than in mouse embryonic stem cells, thus being comparable with that observed in *Saccharomyces cerevisiae* (Kamisugi et al. 2006). In contrast to seed plants, the dominating generation in the moss life cycle is the haploid gametophyte and the regenerating moss filaments (protonemata) can be directly assayed using PCR methods without complex back-crosses (Kamisugi et al. 2006; Smidkova et al. 2010).

Fig. 1 PpTERT protein, gene, expression and mRNA splicing variants. **a**, **b** The *Physcomitrella* (Bryophytes) telomerase reverse transcriptase (TERT) subunit comprises conserved N-terminal telomerase specific regions [T2(GQ), NLS, CP, QFP, T] and reverse transcriptase motifs (1, 2, A–E) similar to other TERTs. The predicted PpTERT coding DNA sequence (**b**) from the start (ATG) to the stop (†) codon was revised and detailed analysis of cloned cDNA sequences (**c**) confirmed the existence of 12 exons in the *PpTERT* gene and a high number of splicing events leading mostly to out-of-frame variants with premature stop codons. Detailed RT-PCR analysis (primer positions shown in **b**) and sequencing of purified RT-PCR products (representative combinations obtained in RNA from 7 day protonemata shown in **d**) demonstrated the major splicing pattern in the exon 1–4 region (1–10Fw and 4–5Rev primers) which confirms data from all cloned cDNA sequences. In addition, the presence of two abundant mRNA variants corresponding to the in-frame representative clone 21–3 and the out-of-frame representative clone X1–31 (compare primer combination 6–10Fw and 9–1Rev in **d**) was revealed during protonema development (**e**). The major splicing variant of the 3′region is represented by the in-frame clone 56–35. Besides that, a low abundance of the out-of-frame variant 56–33 was observed (10–2Fw and 11–5Rev primers, shown in **d**). Phylogeny position of *Physcomitrella* (**f**) as a basal plant lineage indicates that its 12 exon *TERT* structure as ancestral to plant telomerase *TERT* genes. An overview of land plant phylogeny including the relationship among major lineages of angiosperms was adapted and modified from (Albert et al. 2013) (scientific names in parentheses). **a** Aligned sequences (phylogeny relationship shown in **f**) were (i) experimentally verified TERT sequences from Arabidopsis (Genbank AAD54276.1), rice (*Oryza sativa*, AAM21641.1), *Doryanthes excelsa* (AAX19887.1), *Scilla peruviana* (both Asparagales, AAX21217.1); (ii) transcriptome isotig from fern (*Lygodium japonicum*, isotig24217, http://bioinf.mind.meiji.ac.jp/kanikusa/) (iii) predicted TERT sequences from green alga (*Micromonas* sp. RCC299, Mamiellales, Genbank XP_002505190.1), sacred lotus (*Nelumbo nucifera*, Proteales, Genbank XP_010257254.1); (iv) revised TERT prediction from *Amborella trichopoda* (basal Magnoliophyta, AmTr_v1.0_scaffold00007, NW_006498404) and *Selaginella moellendorffii* (Lycopodiidae, SELMOscaffold_16, NW_003314277). **b** Positions of telomerase specific motifs are highlighted in the PpTERT protein and predicted coding DNA sequence. **c** Cloned cDNA sequences (clone names on right with the number of corresponding sequenced clones given in *brackets*) show usage of the alternative splice donor sites (*gc..*, *ct..*), and a high number of splicing events either corresponding (*black*) or not corresponding (*grey*) to predicted exon–intron borders illustrated in part (**b**). The alternative splice donor site in exon 6 (*) was found in two clones and it differs in three nucleotides from the major splice site



In this paper, we take advantage of the use of *P. patens* to perform a comparative investigation of telomere dynamics between this species representing lower plants, and flowering plants. In contrast to animals, telomerase is not developmentally silenced during embryogenesis in flowering plants, but remains active throughout entire plant life in dividing meristem cells (Fajkus et al. 1996; Fitzgerald et al. 1996). This results in the absence of replicative telomere shortening during individual plant development in contrast to animals (Fajkus et al. 1998; Riha et al. 1998). However, experimental support for these conclusions has been only indirect so far since plant samples used for telomere and telomerase analyses always contain unknown proportions of meristem cells in great excess of other cells. In contrast, the filaments of *P. patens* protonema grows by apical cell division and represents a perfect cell-lineage and, therefore, plant development can be pinpointed to the differentiation of a single cell. Most importantly, the percentage of apical cells can be quantified exactly, depending on the days of protonema cultivation (total cell number) and the level of protonema branching, and thus it is possible to tackle the telomere and telomerase dynamics experimentally. To do this, we characterise here the gene coding for telomerase reverse transcriptase, *PpTERT*, in *P. patens* that we have predicted previously from genome sequencing data (Sykorova and Fajkus 2009). We further perform analyses of *PpTERT* expression, telomerase activity and telomere lengths, in samples with a varying percentage of apical cells during protonema growth. Finally, we examine telomerase activity and telomere lengths in selected mutants for DNA repair factors which are potentially involved in *P. patens* telomere structure, function and maintenance, as inferred from the corresponding results obtained in land plants.

Materials and methods

Plant material

Physcomitrella patens (Hedw.) B.S.G. "Gransden 2004" wild type and the mutants *pprad50*, *ppmre11*, *ppnbs1* and *pplig4* were described previously (Kamisugi et al. 2012; Hola et al. 2013). The *pplig4* and *ppku70* mutants in the C-NHEJ repair pathway were generated by D. G. Schaefer, Neuchatel University, Switzerland and F. Nogue, INRA, Paris, France as gene replacement mutants, and were kindly provided to us by the authors.

Cultivation of *P. patens*

P. patens wild type and mutants were propagated vegetatively as described by (Knight et al. 2002). Individual plants were cultured as 'spot inocula' on BCD agar medium supplemented with 1 mM $CaCl_2$ and 5 mM ammonium tartrate (BCDAT medium), or as lawns of protonemal filaments by subculture of homogenized tissue on BCDAT agar medium overlaid with cellophane discs (Focus Packaging & Design, UK) in growth chambers with 18/6 h day/night cycle at 22/18 °C.

For subculture and preparation of 1-day-old protonemal tissue, 1-week-old tissue scraped from plates was suspended in 8 mL of BCD medium and sheared by a T25 homogenizer (IKA, Germany) at 24,000 rpm for two 1-minute cycles and let 24 h to recover in a cultivation chamber with gentle shaking at 100 rpm. This treatment yielded a suspension of 3–5 cell protonema filaments, which readily settle for recovery. Settled protonemata could be handled without excessive losses by tweezers on Petri plates.

Counting of apical cells in *P. patens* branching culture

Cells cultivated on agar plates were counted manually by eye using a stereoscopic binocular light microscope (Olympus SZX16 research stereo microscope), and the field in sharp focus was evaluated on a computer monitor. Only objects in the focused area were included for counting. Specific fields were selected randomly across the plate, and usually areas with a lower density of cells were considered. Cells were counted on ten independent plate sections containing 25–204 cells for each time period using four independently grown cultures.

Cloning and analysis of *PpTERT* gene structure

Total RNA was isolated from *P. patens* cultures using the RNeasy Plant Mini Kit (Qiagen) followed by DNaseI treatment (TURBO DNA-free, Applied Biosystems) according to the manufacturers' instructions. cDNA was prepared by reverse transcription of 1 μg of RNA using M-MuLV reverse transcriptase (NEB) and Random Nonamers (Sigma). The predicted *PpTERT* gene sequence (Sykorova and Fajkus 2009) was used for designing specific primers and cloning of the cDNA sequence (Table S1, Fig. 1 b). RT-PCR conditions for primer combinations (1Fw or 1–10Fw and 9Rev primers, 9Fw and 12Rev primers, Table S1) were optimized using gradient PCR and the *PpTERT* sequences were amplified with *DyNAzyme* II DNA Polymerase (Finnzymes) as follows: 2 min of initial denaturation and 35 cycles of 30 s at 94 °C, 30 s at 57 °C and 90 s at 72 °C. Sequences covering the overlapping cDNA regions (exons 1–9 and exons 9–12, GenBank KM886460-KM886468, KP001262, KP091459) were cloned, sequenced and manually aligned on the predicted *PpTERT* gene sequence (Sykorova and Fajkus 2009). The

prevailing splicing pattern of the *PpTERT* transcripts was verified by sequencing of the RT-PCR products spanning regions with frequent alternative splicing sites (see below, Table S1, Fig. 1c). The PpTERT protein sequence was examined for the presence of conserved motifs and manually aligned to the experimentally verified plant TERT sequences of *Arabidopsis thaliana*, *Oryza sativa*, *Doryanthes excelsa*, and *Scilla peruviana* (see (Sykorova and Fajkus 2009) for review), and to predicted sequences from the transcriptome of *Lygodium japonicum* (Aya et al. 2015) and the genome scaffolds of *Selaginella moellendorff*ii (Banks et al. 2011), *Amborella trichopoda* (Albert et al. 2013), *Nelumbo nucifera* (Ming et al. 2013) and the prasinophyte alga *Micromonas* sp. RCC299 (Worden et al. 2009).

Analysis of the *PpTERT* gene transcripts

Quantification of the *PpTERT* transcript levels was done using FastStart SYBR Green Master (Roche) and primers derived from the exon 10 coding for the reverse transcription domain of the protein (Table S1); ubiquitin was chosen as a reference gene (Harries et al. 2005). One microliter of 2× diluted cDNA (see above) was added to the 20 μl reaction mix, and the final concentration of each forward and reverse primer was 0.25 μM. Reactions were done in triplicates; the PCR cycling consisted of 15 min of initial denaturation followed by 40 cycles of 30 s at 94 °C, 30 s at 56 °C and 30 s at 72 °C. At least two biological replicates in two technical replicates were analysed. Relative *TERT* transcription was calculated by the ΔΔCt method (Pfaffl 2004). Additional RT-PCR experiments were performed to verify exon/intron structure of the *PpTERT* gene and the presence of mRNA variants during development.

Analysis of telomerase activity (TRAP—telomere repeat amplification protocol)

In vitro analysis of telomerase activity is based on the elongation of a substrate primer by the telomerase, and the extension product is then amplified by PCR. A telomerase extract from *P. patens* cultures was prepared as previously described (Fitzgerald et al. 1996; Sykorova et al. 2003a), and the maximum of telomerase activity was detected in fractions precipitated by 7.5–10.0 % PEG 8000. The quantitative version of the TRAP assay was performed according to (Herbert et al. 2006) using FastStart SYBR Green Master (Roche) and TS21 substrate primer and TelPr reverse primer (Table S1). Samples were analysed in triplicates in a 20-μl reaction mix, and at least two biological replicates (independently grown cultures) were evaluated. Relative telomerase activity was calculated by the ΔCt method (Pfaffl 2004).

Analysis of telomere lengths

Analysis of telomere lengths by the Terminal Restriction Fragments (TRF) method is based on the digestion of genomic DNA by a frequently cutting restriction endonuclease without a recognition site in the G-rich telomeric repeats. After hybridization with a radioactively labeled telomeric probe, the signal corresponds to non-digested telomeric tracts (plus subtelomeric regions up to the first restriction site upstream of the telomeres). Analysis was performed as previously described (Ruckova et al. 2008; Jaske et al. 2013). Briefly, a *P. patens* culture was homogenized in liquid nitrogen, DNA was isolated according to (Dellaporta et al. 1983) and digested by either *Mse*I or *Taq*I restriction enzymes. Southern hybridization was performed with a telomeric probe synthetized as described in (Ijdo et al. 1991). Signals were visualized on a FLA7000 phosphorimager (FujiFilm). Evaluation of fragment lengths was done by using the Gene Ruler 1-kb DNA Ladder (Fermentas) as a standard; hybridization patterns were analysed by Multi Gauge software (FujiFilm). The unweighted mean telomere length was calculated as $\sum(OD_i \times L_i)/\sum(OD_i)$, where OD_i is the signal intensity above background within interval i and L_i is the molecular weight (kb) at the midpoint of interval i.

Analysis of induced DNA double strand breaks (DSBs) and of their repair in *P. patens*

Bleomedac inj. (Medac, Hamburg, Germany) was used for Bleomycin treatment as previously described (Hola et al. 2013). One day regenerated protonemal tissues (>50 % of apical cells) from wild type and mutant lines were treated with Bleomycin for 1 h prior to nuclear extraction and analysis. DSBs were detected by a comet assay using a fully neutral N/N protocol (Olive and Banath 2006; Rensing et al. 2008; Kozak et al. 2009). Comets were stained with SYBR Gold (Molecular Probes/Invitrogen), viewed in epifluorescence with a Nikon Eclipse 800 microscope and captured and evaluated by the LUCIA Comet cytogenetic software (LIM Inc., Czech Republic). The fraction of DNA in comet tails was used as a measure of DNA damage and for calculation of the percentage of DSBs remaining (Kozak et al. 2009). Data in this study were obtained in at least three independent experiments. Measurements of blindly labeled comet slides included four independent gel replicas of 25 evaluated comets with a total of at least 300 comets analyzed per experimental point.

Results and discussion

Structure of *PpTERT* gene

In order to track a possible involvement of telomerase expression in *P. patens* telomere dynamics, we analysed *P. patens TERT* gene structure and transcription. According to the *P. patens* genome annotation v1.6 (Rensing et al. 2008), the *PpTERT* gene sequence was predicted in silico with only three exons covering the C-part of the PpTERT protein thus possibly missing specific N-terminal telomerase motifs. Taking advantage of our knowledge about *TERT* gene structure and possible mRNA splicing (Rensing et al. 2005) we predicted 12 exons in the *PpTERT* gene (6971 bp long, inside contig ABEU01012720 (Sykorova and Fajkus 2009), Fig. 1b) similarly to other known plant *TERT* genes. To verify the predicted *PpTERT* gene and mRNA structure, we cloned cDNA sequences to cover the entire *PpTERT* (GenBank KM886460-KM886468, KP001262). Our gene structure prediction differed from the experimental results only in details, e.g. alignments of cloned *PpTERT* sequences revealed that exons 1 and 2 used the alternative splice donor site GC (Fig. 1c) instead of the consensus splice site GT reported in *Physcomitrella* transcriptome analysis (Rensing et al. 2005). This splicing pattern was found in all cloned cDNA sequences (Fig. 1c) and confirmed by sequencing of RT-PCR products spanning exon 1–4 (Fig. 1b, d, e). The exon lengths and cDNA sequence also correspond to the EST sequence (Genbank CN203080) covering exons 1–4 of the putative *TERT* of the bryophyte *Syntricha ruralis*. Moreover, we identified a surprisingly high number of alternative splicing events in *PpTERT*. These comprise different combinations of alternative donor and acceptor sites, especially in the 5′region of the *PpTERT* transcript, and retention of the intron 10 (Fig. 1c). Variation between the predicted and the cloned *PpTERT* cDNA sequences originated mostly from alternative splicing of exons 5–7 that code for a non-conserved protein linker between the nuclear localisation signal (NLS) and CP telomerase motifs. Majority of identified splicing events lead to out-of-frame mRNA variants (Fig. 1c). The high number of identified alternative transcripts necessitated a detailed analysis of major splicing patterns via sequencing of RT-PCR products that span exons 1–4, 1–6, 4–7, 7–9 and 9–12 (Table S1, Fig. 1b, d). For the 5′region of *PpTERT*, the results verified the presence of two major variants (Fig. 1b–d, primer combination 6–10Fw and 9–1Rev) found in the representative clone 21–3 (in-frame variant, Genbank KM886462) and in the representative clone 31–X1 (out-of-frame, premature stop codon in exon 7). Sequencing of RT-PCR products for the 3′region of *PpTERT* showed the presence of a major variant 56–35 (in-frame, Genbank KM886460) in addition to a low abundance variant 56–33 (out-of frame, premature stop codon in intron 10) (Fig. 1b–d, primer combination 10–2Fw and 11–5Rev). The major mRNA splicing represented by in-frame variant clone 56–35 was also verified with the EST sequences from the GENBANK database (Unigene ID: 2850076).

Analysis of the PpTERT protein sequence (1301 AA, 148 kDa, pI 9.371) clearly showed the presence of all telomerase specific motifs (Fig. 1a) including the conserved NLS motif and highly conserved regions in the C-terminal extension similar to other plant TERTs (Sykorova et al. 2006a, b; Sykorova and Fajkus 2009). To compare telomerase structure from evolutionary distinct plant groups we analyzed a representative set comprising eudicots (*Arabidopsis*, sacred lotus), monocots (rice, *Doryanthes*, *Scilla*), basal angiosperm (*Amborella*), fern (*Lygodium*), lycophyte (*Selaginella*) and green alga (*Micromonas*). The experimentally verified sequences and the *Lygodium* isotig derived from transcriptome data were used for prediction or improvement of predicted TERT gene and protein structures (Fig. 1a). For example, analysis of the predicted *Amborella TERT* sequence revealed a misanotated gene structure and analysis of the corresponding genome scaffold clearly showed the presence of 12 exons similarly to the prediction from *Selaginella* scaffold 16, and the predicted *Nelumbo TERT*. Together with the presented *Physcomitrella TERT* data, these results suggest a 12 exon gene structure for an ancestral plant *TERT* (Fig. 1f) and also emphasize the necessity of experimental verification of predicted *TERT* gene structures.

RT-PCR analysis of *PpTERT* mRNA variants during development

The spectrum of *PpTERT* alternative splicing events is in agreement with the moss genome re-annotation that was reported after implementation of *Physcomitrella* transcriptome data (Zimmer et al. 2013) confirming intron retention as the most frequent form (~40 %) among gene loci with alternate transcripts (~21 % of all genes). However, its abundance in a single gene is quite unusual—for example, alternative *TERT* transcripts amount to only a few per cent in *Arabidopsis* (Zachova et al. 2013). The almost equal representation of in-frame and out-of-frame *PpTERT* variants during protonema development (Fig. 1e) may be of functional relevance, possibly related to the haploid protonema status. To our knowledge, equimolar representation of more *TERT* gene variants was only detected in the polyploid species *Nicotiana tabacum*, where three sequence variants of the *TERT* gene derived from the progenitor *N. tomentosiformis* and *N. sylvestris* genomes were identified (Sykorova et al. 2012). However, even in this case the level of the pseudogene *TERT* variant transcript was negligibly low (close to the detection limit). Potential translation of the out-of-frame *PpTERT* variant would lead to a protein comprising the N-terminal telomerase domain similar to the

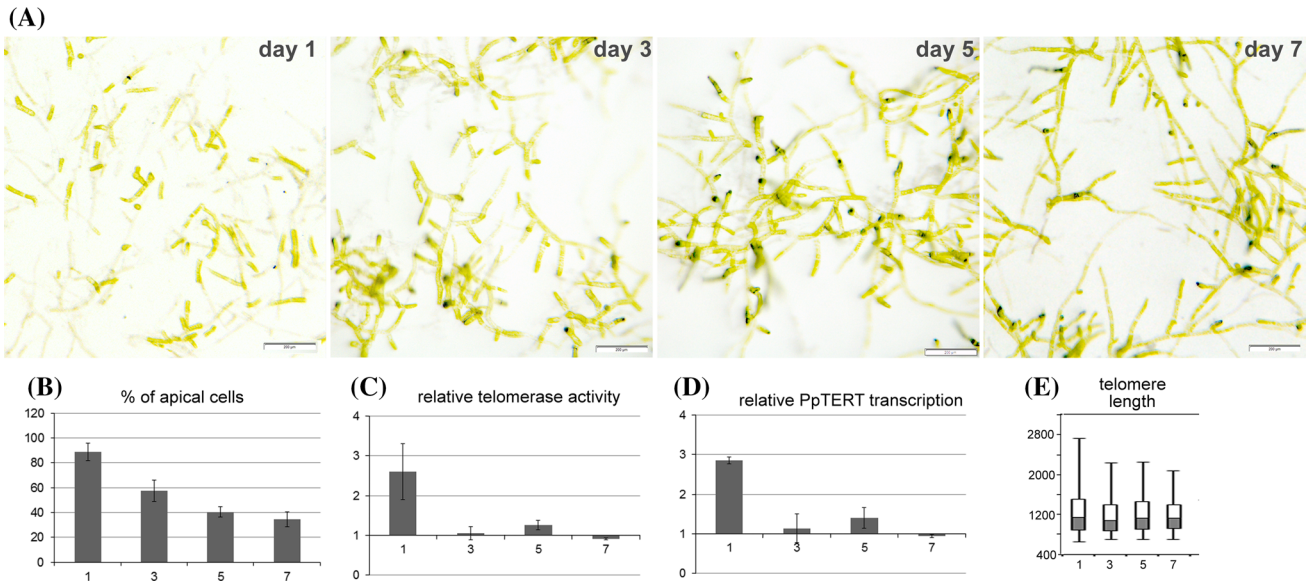


Fig. 2 Telomere dynamics in *P. patens* protonema. **a** Morphology of *P. patens* cells, **b** percentage of apical cells, **c** telomerase activity, **d** *PpTERT* gene transcription, and **e** telomere lengths in the course of 7-day culturing. A 7-day old culture was mechanically disrupted to destroy branched cells chains and cultivated under standard conditions. Samples were taken in 1, 3, 5 and 7 days after sub-culturing for respective analyses. Telomerase activity and *TERT* gene transcription were related to the values obtained for a 7-day culture. The length of telomeres is presented using a *box*-and-*whisker* plot where the bottom part (*gray*) and the top (*white*) of the *box* are the lower and upper quartiles, respectively, separated by the median. The ends of the *whiskers* represent the minimum and maximum length corresponding to the hybridization signal range. Analyses were performed using three independently grown cultures

Arabidopsis TERT V(I8) variant. This variant interacts with POT1a, one of the paralogs of POT1 (Protection of Telomeres 1), a single-stranded-DNA-binding protein (Rossignol et al. 2007) and was suggested to provide additional telomerase function and regulation (reviewed in Majerska et al. 2011). Since *P. patens* also harbours a gene coding for POT1 protein (only a single-copy) and, moreover, its role for telomere integrity has already been demonstrated (Shakirov et al. 2010), this hypothesis is plausible.

Telomere dynamics during protonema growth

When sub-culturing the *P. patens* protonemata, highly branched chains of cells were mechanically disrupted and transferred to fresh medium; during the growth phase, the protonemata lengthened and divided forming branched chains. Since in higher plants telomerase is active in organs and tissues containing dividing meristem cells (seedlings, root tips, blossoms, floral buds), we monitored the percentage of apical cells in a growing *P. patens* culture and correlated this parameter to the telomerase activity, *PpTERT* transcript levels, and telomere length. One day after sub-culturing, the level of apical cells was highest and decreased relatively sharply during the subsequent 2 days, while a further drop was significantly slower (Fig. 2a, b). Telomerase activity and *PpTERT* gene transcripts increased moderately but reproducibly in the first day after the sub-culturing (Fig. 2c, d), when about 85 % of the cells were located at the chain termini (Fig. 2b). In the following time intervals, telomerase activity and *PpTERT* transcripts were at the level comparable to standard 7-day-old culture which was used as the source culture. Importantly, the accrual of telomerase activity and transcription after sub-culturing was not correlated with a possible stress connected to the mechanical disruption of protonemata; telomerase activity and transcription in the 7-day-old culture collected from the plate and the same culture after mechanical treatment were quite comparable (data not shown). Telomere lengths were stable during the *P. patens* protonema culturing (Fig. 2e, Fig. S1) which accords with the results obtained in higher plants where telomere lengths are preserved in all tissues throughout development (Fajkus et al. 1998; Riha et al. 1998). Based on these results, telomerase activity and expression correlates with the proportion of apical cells in a protonema culture and telomere lengths remain stable during 7-day culturing in *P. patens*. We conclude that our results provide a direct experimental support of the general validity of the key principles of telomere dynamics during plant development which so far have only been predicted.

Telomere maintenance in *P. patens* mutants in selected DSB repair factors

In the *Arabidopsis thaliana* model plant, telomere homeostasis was shown to be disrupted in mutants with loss of

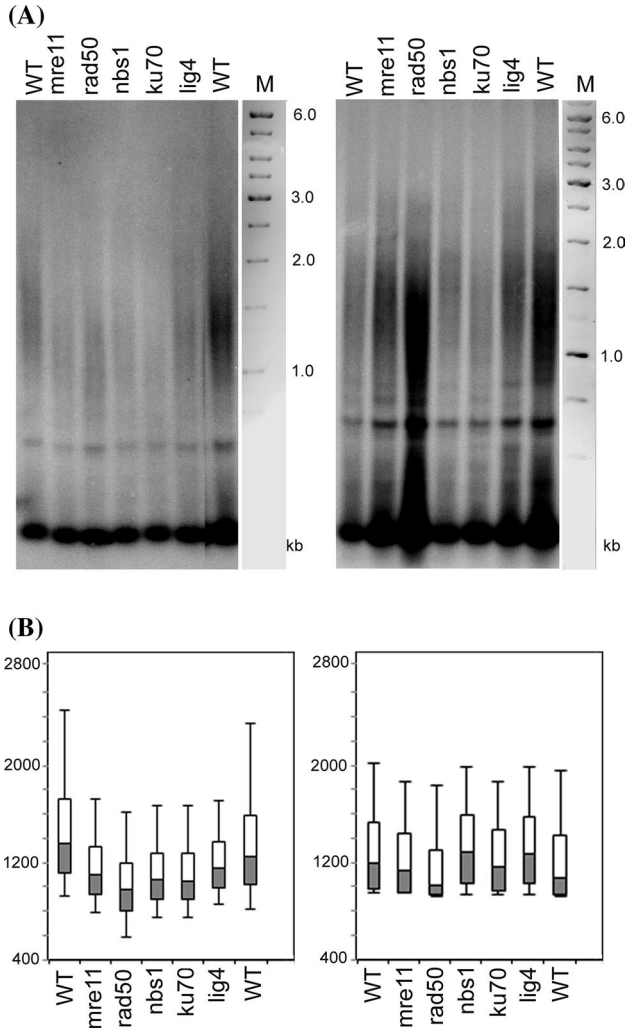


Fig. 3 Telomere length in *P. patens* mutants. The length of telomeric repeats was assayed by the TRF protocol in 7-day old cultures (two biological replicates are shown). **a** After hybridization of DNA digested by a restriction endonuclease against the telomeric probe, the radioactive signals in mutants lines reflecting the length of telomeres were either slightly shifted towards shorter lengths compared to wild type (WT) samples (*left*) or were maintained at the wild type level (*right*). Five independent analyses of telomere length were performed, and representative results are presented. **b** Hybridization signals were evaluated by MultiGauge software (Fujifilm) and are presented using a *box*-and-*whisker* plot as described in Fig. 2

function of genes coding for proteins involved in repair and recombination processes (see below). Although one of the crucial function of telomeres is prevention of aberrant recombination events at the chromosome termini, proteins participating in recombination are essential for proper telomere structure and functions and are even involved in alternative mechanisms of telomere lengthening (Draskovic et al. 2009; Amiard et al. 2011). We took advantage of the extremely efficient system of homologous recombination in *P. patens* (Schaefer 2002) which enables relatively simple and highly effective targeting of any non-essential gene, and analyse telomere length, telomerase activity and transcription of the *PpTERT* gene in mutant lines as follow: (i) In mutants in genes of the MRN complex (*mre11*, *rad50*, *nbs1*) which is involved in recognition of DSBs in DNA and subsequent phosphorylation signalling. Vertebrate cells with *MRE11* gene loss of function are even not viable, but in *A. thaliana* telomere lengthening was observed and mutants were hypersensitive to DNA damaging agents and suffered from developmental defects (Bundock and Hooykaas 2002). Similarly, RAD50 function is essential for telomere maintenance in *A. thaliana*; in plants with a T-DNA insertion in the *AtRAD50* gene a significant telomere shortening and meiotic and DNA repair defects were observed (Gallego and White 2001). Finally, *A. thaliana nbs1* mutants are without morphological defects and telomere length is at the wild type level, but in *nsb1/tert* double-mutants telomere erosion was more progressive compared to *tert* mutants suggesting interplay of NBS1 and telomerase in telomere homeostasis processes (Najdekrova and Siroky 2012). (ii) In a *ku70* mutant; KU70 protein is a part of the KU70/KU80 complex which binds and stabilizes DSBs. In *Arabidopsis*, KU70 loss led to the formation of extra-long telomeres, elongation of single-strand telomere overhangs and significantly increased level of telomeric circles (Riha et al. 2002; Gallego et al. 2003; Akimcheva et al. 2008). (iii) In a *lig4* mutant with loss of function of the major DNA ligase participating in non-homologous end joining. In *Arabidopsis*, telomeres in *lig4* mutant are more heterogeneous but have the same average length as wild type telomeres (Heacock et al. 2007).

In the *P. patens* mutants tested, telomerase activity and *TERT* gene transcripts assayed by quantitative approaches do not significantly differ from the values obtained for wild type cultures (not shown). Interestingly, lengths of telomeres in mutants, including *ku70* mutant, are similar to the wild type telomeres, and no significant changes were observed (Fig. 3, Fig. S2). Basic telomere protective functions appear to be preserved as well, as suggested by unchanged morphology and growth parameters of mutant cultures (results not shown). Altogether, malfunction of the proteins examined involved in repair and recombination pathways does not influence telomere homeostasis substantially in *P. patens*, in contrast to the common higher plant model *Arabidopsis thaliana* (Table 1). The striking absence of a telomeric phenotype in *ku70* and other mutants in NHEJ factor genes in *P. patens* presumably corresponds to the general dominance and higher efficiency of homologous recombination over NHEJ in this system, contrasting with the opposite situation in flowering plants. These results further suggest that KU70 is not directly involved in protection of telomeres in *P. patens*, contrary to the situation described in *A. thaliana* (Kazda et al. 2012).

Table 1 Comparison of the telomere phenotype in mutants with loss of function of genes involved in the DNA DSB repair pathways in plant models *A. thaliana* and *P. patens*

Mutant	*Arabidopsis thaliana*	*Physcomitrella patens*
mre11	Elongated telomeres[a]	No significant changes in telomere lengths No morphological or growth defects in protonema cultures
rad50	Shortened telomeres[b]	
nbs1	WT morphology and telomere length, in *nbs/tert* more progressive telomere erosion than in *tert* mutants[c]	
ku70	Extra long telomeres[d]	
lig4	Length of telomeres heterogeneous[e]	

[a] Bundock and Hooykaas (2002); [b] Gallego and White (2001); [c] Najdekrova and Siroky (2012); [d] Riha et al. (2002), Gallego et al. (2003) and Akimcheva et al. (2008); [e] Heacock et al. (2007)

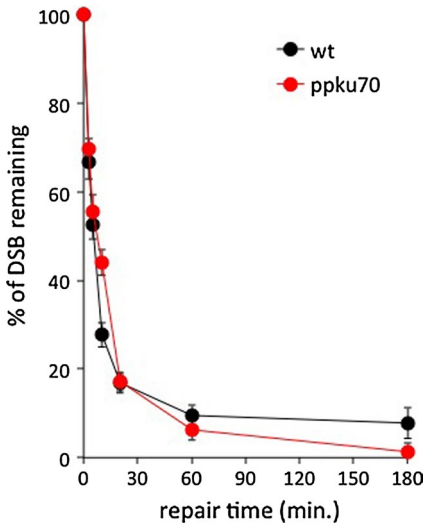


Fig. 4 DSBs repair kinetics determined by comet assay. One day regenerated protonemal tissue from wild type and *ku70* mutant lines was treated with 30 μg/ml Bleomycin for 1 h prior to nuclear extraction and analysis. DSBs were determined by the N/N protocol: *black*—wild type, *red*—*ku70*. Repair kinetics is plotted as % of DSBs remaining after 0, 3, 5, 10, 20, 60 and 180 min period of repair. Maximum damage is normalised as 100 % at t = 0 for both lines. *Error bars* show standard error

Repair kinetics of DSBs in *ku70 mutants*

In order to assess the interpretation of the surprising absence of a telomeric phenotype in *ku70* mutants, DNA repair kinetics were analysed in these mutants. The results of comet assays demonstrate (Fig. 4) that inactivation of the C-NHEJ pathway in *P. patens* by knocking out the *KU70* gene, whose product is responsible for DSB recognition, does not affect the overall ability to repair DSBs. Similarly mutations of *LIG4* (Hola et al. 2013), a factor involved in the final step of C-NHEJ, and of *MRE11*, *RAD50* and *NBS1*, components of the key repair complex MRN involved in both NHEJ and homologous recombination pathways (Kamisugi et al. 2012), do not affect overall DSB repair in *P. patens* apical cells. In this respect, the moss *P. patens* parallels the situation in *Arabidopsis* where mutants of *KU80* and *LIG4* also efficiently repair DSBs (Kozak et al. 2009; Amiard et al. 2010; Charbonnel et al. 2010, 2011) but in contrast to *Physcomitrella*, the *Arabidopsis* mutants repair DSBs during the 1st phase even faster than wt Col0 (Kozak et al. 2009). Thus, *P. patens* wild type and mutants used in these studies exercise efficient and rapid DSB repair that in the case of deprotected telomeres can detect them as substrates for joining. However, the absence of a telomere phenotype in the corresponding *P. patens* mutants suggests that the loss of neither of these factors results in telomere deprotection.

Conclusion

We report here on the characterisation of *PpTERT* gene and its major splicing variants in growing protonemata. The overall *PpTERT* exon structure and the arrangement of telomerase-specific functional motifs in the amino acid sequence is conserved with respect to the previously characterised plant *TERT*s. A surprisingly rich spectrum and high abundance of alternate splicing products was observed and subsequent studies should address their functional importance. Further, we investigated telomere maintenance in growing *P. patens* protonemata and our results demonstrate telomere length stability and association of telomerase activity and expression with dividing apical cells. These findings provide experimental evidence for previously anticipated principles governing plant telomere biology and suggest their general validity among both lower and land plants. Next, we examined *P. patens* mutants in DSB repair factors for a possible telomere phenotype. Particularly interesting is the absence of a telomere phenotype in mutants depleted of KU70, the key factor in NHEJ, whose absence in *Arabidopsis* results in extremely elongated telomeres. Our results suggest that contrary to the situation in *A. thaliana*, KU70 is not essential for protection of

telomeres in *P. patens*. Thus, our study identifies both conserved and distinct features between the telomere biology of lower and land plants which may initiate further studies directed to deeper understanding of their strategies to protect genome integrity.

Acknowledgments This work was supported by the Czech Science Foundation (13-06595S), project CEITEC (CZ.1.05/1.1.00/02.0068) of the European Regional Development Fund and project EVOGEN (CZ.1.07/2.3.00./20.0189) co-financed from European Social Fund and the State Budget of the Czech Republic.

References

Akimcheva S, Zellinger B, Riha K (2008) Genome stability in Arabidopsis cells exhibiting alternative lengthening of telomeres. Cytogenet Genome Res 122:388–395. doi:10.1159/000167827

Albert VA et al (2013) The Amborella genome and the evolution of flowering plants. Science 342:1467. doi:10.1126/science.1241089

Amiard S, Charbonnel C, Allain E, Depeiges A, White CI, Gallego ME (2010) Distinct roles of the ATR kinase and the Mre11-Rad50-Nbs1 complex in the maintenance of chromosomal stability in Arabidopsis. Plant Cell 22:3020–3033. doi:10.1105/tpc.110.078527

Amiard S, White C, Gallego ME (2011) Recombination proteins and telomere stability in plants. Curr Protein Pept Sci 12:84–92

Aya K, Kobayashi M, Tanaka J, Ohyanagi H, Suzuki T, Yano K, Takano T, Yano K, Matsuoka M (2015) De novo transcriptome assembly of a fern, *Lygodium japonicum*, and a web resource database, Ljtrans DB. Plant Cell Physiol 56:e5. doi:10.1093/pcp/pcu184

Banks JA, Nishiyama T, Hasebe M et al (2011) The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science 332:960–963. doi:10.1126/science.1203810

Benkova E, Ivanchenko MG, Friml J, Shishkova S, Dubrovsky JG (2009) A morphogenetic trigger: is there an emerging concept in plant developmental biology? Trends Plant Sci 14:189–193. doi:10.1016/j.tplants.2009.01.006

Bundock P, Hooykaas P (2002) Severe developmental defects, hypersensitivity to DNA-damaging agents, and lengthened telomeres in Arabidopsis MRE11 mutants. Plant Cell 14:2451–2462. doi:10.1105/Tpc.005959

Charbonnel C, Gallego ME, White CI (2010) Xrcc1-dependent and Ku-dependent DNA double-strand break repair kinetics in Arabidopsis plants. Plant J 64:280–290. doi:10.1111/j.1365-313X.2010.04331.x

Charbonnel C, Allain E, Gallego ME, White CI (2011) Kinetic analysis of DNA double-strand break repair pathways in Arabidopsis. DNA Repair (Amst) 10:611–619. doi:10.1016/j.dnarep.2011.04.002

de Lange T (2009) How telomeres solve the end-protection problem. Science 326:948–952. doi:10.1126/science.1170633

Dellaporta SL, Wood J, Hicks JB (1983) A Plant DNA minipreparation. Version II. Plant Mol Biol Rep 1:19–21

Draskovic I, Arnoult N, Steiner V, Bacchetti S, Lomonte P, Londono-Vallejo A (2009) Probing PML body function in ALT cells reveals spatiotemporal requirements for telomere recombination. Proc Natl Acad Sci U S A 106:15726–15731. doi:10.1073/pnas.0907689106

Fajkus J, Kovarik A, Kralovics R, Bezdek M (1995) Organization of telomeric and subtelomeric chromatin in the higher plant *Nicotiana tabacum*. Mol Gen Genet 247:633–638. doi:10.1007/Bf00290355

Fajkus J, Kovarik A, Kralovics R (1996) Telomerase activity in plant cells. FEBS Lett 391:307–309

Fajkus J, Fulneckova J, Hulanova M, Berkova K, Riha K, Matyasek R (1998) Plant cells express telomerase activity upon transfer to callus culture, without extensively changing telomere lengths. Mol Gen Genet 260:470–474

Fajkus J, Sykorova E, Leitch AR (2005) Telomeres in evolution and evolution of telomeres. Chromosome Res 13:469–479. doi:10.1007/s10577-005-0997-2

Fitzgerald MS, McKnight TD, Shippen DE (1996) Characterization and developmental patterns of telomerase expression in plants. Proc Natl Acad Sci U S A 93:14422–14427

Friml J, Benfey P, Benkova E et al (2006) Apical-basal polarity: why plant cells don't stand on their heads. Trends Plant Sci 11:12–14. doi:10.1016/j.tpnats.2005.11.010

Fulneckova J, Hasikova T, Fajkus J, Lukesova A, Elias M, Sykorova E (2012) Dynamic evolution of telomeric sequences in the green algal order Chlamydomonadales. Genome Biol Evol 4:248–264. doi:10.1093/gbe/evs007

Fulneckova J, Sevcikova T, Fajkus J, Lukesova A, Lukes M, Vlcek C, Lang BF, King E, Elias M, Sykorova E (2013) A broad phylogenetic survey unveils the diversity and evolution of telomeres in eukaryotes. Genome Biol Evol 5:468–483. doi:10.1093/Gbe/Evt019

Gallego ME, White CI (2001) RAD50 function is essential for telomere maintenance in Arabidopsis. Proc Natl Acad Sci U S A 98:1711–1716. doi:10.1073/pnas.98.4.1711

Gallego ME, Bleuyard JY, Daoudal-Cotterell S, Jallut N, White CI (2003) Ku80 plays a role in non-homologous recombination but is not required for T-DNA integration in Arabidopsis. Plant J 35:557–565

Graham LE, Cook ME, Busse JS (2000) The origin of plants: body plan changes contributing to a major evolutionary radiation. Proc Natl Acad Sci U S A 97:4535–4540. doi:10.1073/pnas.97.9.4535

Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 43:405–413. doi:10.1016/0092-8674(85)90170-9

Greider CW, Blackburn EH (1987) The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with 2 kinds of primer specificity. Cell 51:887–898. doi:10.1016/0092-8674(87)90576-9

Greider CW, Blackburn EH (1989) A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 337:331–337. doi:10.1038/337331a0

Harries PA, Pan AH, Quatrano RS (2005) Actin-related protein2/3 complex component ARPC1 is required for proper cell morphogenesis and polarized cell growth in *Physcomitrella patens*. Plant Cell 17:2327–2339. doi:10.1105/tpc.105.033266

Heacock ML, Idol RA, Friesner JD, Britt AB, Shippen DE (2007) Telomere dynamics and fusion of critically shortened telomeres in plants lacking DNA ligase IV. Nucleic Acids Res 35:6490–6500. doi:10.1093/nar/gkm472

Herbert BS, Hochreiter AE, Wright WE, Shay JW (2006) Nonradioactive detection of telomerase activity using the telomeric repeat amplification protocol. Nat Protoc 1:1583–1590. doi:10.1038/nprot.2006.239

Hola M, Kozak J, Vagnerova R, Angelis KJ (2013) Genotoxin induced mutagenesis in the model plant *Physcomitrella patens*. Biomed Res Int 2013:535049. doi:10.1155/2013/535049

Ijdo JW, Wells RA, Baldini A, Reeders ST (1991) Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. Nucleic Acids Res 19:4780. doi:10.1093/nar/19.17.4780

Jaske K, Mokros P, Mozgova I, Fojtova M, Fajkus J (2013) A telomerase-independent component of telomere loss in chromatin assembly factor 1 mutants of *Arabidopsis thaliana*. Chromosoma 122:285–293. doi:10.1007/s00412-013-0400-6

Kamisugi Y, Schlink K, Rensing SA, Schween G, von Stackelberg M, Cuming AC, Reski R, Cove DJ (2006) The mechanism of gene targeting in *Physcomitrella patens*: homologous recombination, concatenation and multiple integration. Nucleic Acids Res 34:6205–6214

Kamisugi Y, Schaefer DG, Kozak J, Charlot F, Vrielynck N, Holá M, Angelis KJ, Cuming AC, Nogue F (2012) MRE11 and RAD50, but not NBS1, are essential for gene targeting in the moss *Physcomitrella patens*. Nucleic Acids Res 40:3496–3510. doi:10.1093/nar/gkr1272

Kazda A, Zellinger B, Rossler M, Derboven E, Kusenda B, Riha K (2012) Chromosome end protection by blunt-ended telomeres. Genes Dev 26:1703–1713. doi:10.1101/gad.194944.112

Knight CD, Cove DJ, Cuming AC, Quatrano RS (2002) Moss gene technology. In: Gilmartin PM, Bowler C (eds) Molecular plant biology, vol 2. Oxford University Press, Oxford, pp 285–299

Kozak J, West CE, White C, da Costa-Nunes JA, Angelis KJ (2009) Rapid repair of DNA double strand breaks in *Arabidopsis thaliana* is dependent on proteins involved in chromosome structure maintenance. DNA Repair (Amst) 8:413–419. doi:10.1016/j.dnarep.2008.11.012

Majerska J, Sykorova E, Fajkus J (2011) Non-telomeric activities of telomerase. Mol BioSyst 7:1013–1023. doi:10.1039/C0mb00268b

McClintock B (1941) The stability of broken rnds of chromosomes in Zea mays. Genetics 26:234–282

Ming R, VanBuren R, Liu Y et al (2013) Genome of the long-living sacred lotus (*Nelumbo nucifera* Gaertn.). Genome Biol 14:R41. doi:10.1186/Gb-2013-14-5-R41

Najdekrova L, Siroky J (2012) NBS1 plays a synergistic role with telomerase in the maintenance of telomeres in *Arabidopsis thaliana*. BMC Plant Biol 12:167. doi:10.1186/1471-2229-12-167

Olive PL, Banath JP (2006) The comet assay: a method to measure DNA damage in individual cells. Nat Protoc 1:23–29. doi:10.1038/nprot.2006.5

Olovnikov AM (1971) Principle of marginotomy in template synthesis of polynucleotides. Dokl Akad Nauk SSSR 201:1496–1499

Pfaffl MW (2004) Quantification strategies in real-time PCR. In: Bustin SA (ed) A-Z of quantitative PCR. International University Line, La Jolla, pp 87–112

Pich U, Fuchs J, Schubert I (1996) How do Alliaceae stabilize their chromosome ends in the absence of TTTAGGG sequences? Chromosome Res 4:207–213. doi:10.1007/Bf02254961

Prigge MJ, Bezanilla M (2010) Evolutionary crossroads in developmental biology: *Physcomitrella patens*. Development 137:3535–3543. doi:10.1242/Dev.049023

Rensing SA, Fritzowsky D, Lang D, Reski R (2005) Protein encoding genes in an ancient plant: analysis of codon usage, retained genes and splice sites in a moss, *Physcomitrella patens*. BMC Genom 6:43. doi:10.1186/1471-2164-6-43

Rensing SA, Lang D, Zimmer AD et al (2008) The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science 319:64–69. doi:10.1126/science.1150646

Riha K, Fajkus J, Siroky J, Vyskot B (1998) Developmental control of telomere lengths and telomerase activity in plants. Plant Cell 10:1691–1698

Riha K, Watson JM, Parkey J, Shippen DE (2002) Telomere length deregulation and enhanced sensitivity to genotoxic stress in Arabidopsis mutants deficient in Ku70. EMBO J 21:2819–2826. doi:10.1093/emboj/21.11.2819

Rossignol P, Collier S, Bush M, Shaw P, Doonan JH (2007) Arabidopsis POT1A interacts with TERT-V(I8), an N-terminal splicing variant of telomerase. J Cell Sci 120:3678–3687. doi:10.1242/jcs.004119

Ruckova E, Friml J, Schrumpfova PP, Fajkus J (2008) Role of alternative telomere lengthening unmasked in telomerase knock-out mutant plants. Plant Mol Biol 66:637–646. doi:10.1007/s11103-008-9295-7

Schaefer DG (2002) A new moss genetics: targeted mutagenesis in *Physcomitrella patens*. Annu Rev Plant Biol 53:477–501. doi:10.1146/annurev.arplant.53.100301.135202

Shakirov EV, Perroud PF, Nelson AD, Cannell ME, Quatrano RS, Shippen DE (2010) Protection of Telomeres 1 is required for telomere integrity in the moss *Physcomitrella patens*. Plant Cell 22:1838–1848. doi:10.1105/tpc.110.075846

Smidkova M, Hola M, Angelis KJ (2010) Efficient biolistic transformation of the moss *Physcomitrella patens*. Biol Plant 54:777–780

Suzuki K (2004) Characterization of telomere DNA among five species of pteridophytes and bryophytes. J Bryol 26:175–180. doi:10.1179/037366804x5279

Sykorova E, Fajkus J (2009) Structure-function relationships in telomerase genes. Biol Cell 101:375–392. doi:10.1042/Bc20080205

Sykorova E, Lim KY, Chase MW, Knapp S, Leitch IJ, Leitch AR, Fajkus J (2003a) The absence of Arabidopsis-type telomeres in Cestrum and closely related genera Vestia and Sessea (Solanaceae): first evidence from eudicots. Plant J 34:283–291

Sykorova E, Lim KY, Kunicka Z, Chase MW, Bennett MD, Fajkus J, Leitch AR (2003b) Telomere variability in the monocotyledonous plant order Asparagales. Proc Biol Sci 270:1893–1904. doi:10.1098/rspb.2003.2446

Sykorova E, Fajkus J, Meznikova M, Lim KY, Neplechova K, Blattner FR, Chase MW, Leitch A (2006a) Minisatellite telomeres occur in the family Alliaceae but are lost in Allium. Am J Bot 93:814–823

Sykorova E, Rowland A, Leitch AR, Fajkus J (2006b) *Asparagales telomerases* which synthesize the human type of telomeres. Plant Mol Biol 60:633–646. doi:10.1007/s11103-005-5091-9

Sykorova E, Fulneckova J, Mokros P, Fajkus J, Fojtova M, Peska V (2012) Three TERT genes in *Nicotiana tabacum*. Chromosome Res 20:381–394. doi:10.1007/s10577-012-9282-3

Viaene T, Landberg K, Thelander M, Medvecka E, Pederson E, Feraru E, Cooper ED, Karimi M, Delwiche CF, Ljung K, Geisler M, Sundberg E, Friml J (2014) Directional auxin transport mechanisms in early diverging land plants. Curr Biol 24:2786–2791. doi:10.1016/j.cub.2014.09.056

Worden AZ, Lee JH, Mock T et al (2009) Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes Micromonas. Science 324:268–272. doi:10.1126/science.1167222

Zachova D, Fojtova M, Dvorackova M, Mozgova I, Lermontova I, Peska V, Schubert I, Fajkus J, Sykorova E (2013) Structure-function relationships during transgenic telomerase expression in Arabidopsis. Physiol Plant 149:114–126. doi:10.1111/Ppl.12021

Zimmer AD, Lang D, Buchta K, Rombauts S, Nishiyama T, Hasebe M, van de Peer Y, Rensing SA, Reski R (2013) Reannotation and extended community resources for the genome of the non-seed plant *Physcomitrella patens* provide insights into the evolution of plant gene structures and functions. BMC Genom 14:498. doi:10.1186/1471-2164-14-498