Journal of Plant Biology, September 2001, 44(3) : 168-171

SHORT COMMUNICATION

Expression of Telomerase Activity is Closely Correlated with the Capacity for Cell Division in Tobacco Plants

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Telomerase is a specialized RNA-directed DNA polymerase that adds telomeric repeats to the ends of linear eukaryotic chromosomes. This activity is developmentally regulated in mammals. Here, we investigated the expression of telomerase activities in various cell types of tobacco plants using the telomere repeat amplification protocol (TRAP) assay. The greatest telomerase activity was detected in BY-2 suspension culture cells, while a relatively high level of activity was also found in roots. Because these two cell types contain a high proportion of actively dividing cells, our results indicate a close correlation between telomerase activity and the capacity for division in tobacco cells. Consistent with this observation was the very low level of telomerase activity in stems, leaves, and flowers, all tissues that had negligible activity of cell division. The specific expression of telomerase in actively dividing plant cells suggests that the pattern of telomerase regulation is largely conserved between higher plants and mammals.

Keywords: BY-2 cells, cell division, telomerase, telomere, tissue-specific expression, tobacco

Telomeres are specialized nucleoprotein complexes found at the ends of linear eukaryotic chromosomes (Blackburn, 1991). They are essential for the maintenance of chromosome integrity and for protection from end-to-end fusion with other chromosomes or from exonucleolytic degradation (Greider, 1996). These complexes consist of tandem repeats of short, G-rich sequence elements, such as TTAGGG in human (Moyzis et al., 1988) or TTTAGGG in higher plants (Richards and Ausubel, 1988). Telomeres are synthesized and maintained through telomerase, a ribonucleoprotein complex that conducts specialized reverse transcriptase activity using its own RNA subunit as the template (Zakian, 1995; Linger and Cech, 1998; Nugent and Lundblad, 1998). In mammalian cells, telomerase activity is closely associated with the program of cell proliferation and dedifferentiation. In humans, for example, telomerase is highly active in tumor cells and in germ-line and embryonic cells, but its activity is not detected in most normal somatic tissues (Kim et al., 1994). It has been suggested that telomerase activity and telomerase-mediated stabilization of telomere length are intimately linked with the cell's capacity for proliferation (Allosopp et al., 1992; Autexier and Greider, 1996).

Although this specialized complex has been studied extensively in animal systems, the understanding of plant telomerases is limited. In maize and barley, telomerase is highly expressed in young immature embryos, while significantly lower activity is observed in young endosperms (Heller et al., 1996; Killan et al., 1998). In addition, very low activities have been detected in the differentiated tissues of these species, including the roots, internodes, and leaves (Heller et al., 1996; Killan et al., 1998). Fitzgerald et al. (1996) have also measured high levels of telomerase activity in soybean suspension cultures, with activity being only moderate in soybean root tips, and low or nonexistent in various vegetative tissues.

Riha et al. (1998) investigated the developmental pattern of telomerase expression in *Melandrium album*, a model dioecious plant. The highest activity was detected in germinating seedlings and root tips, with slightly lower activities in callus culture, immature floral buds and axillary shoots, and almost no activity in leaves and quiescent seeds. All of these results support the hypothesis that telomerase activity is closely associated with the capacity for cell division in higher plants. In addition, when *Arabidopsis* telomerase-null mutants were generated, Fitzgerald et al. (1999) found that telomere length decreased by about 500 bp per generation. Although those plants could survive up to 10 generations without telomerase, the last five generations of the telomerase-deficient mutants showed severe developmental defects in both vegetative and reproductive organs. This confirms that telomerase functions to maintain the integ-

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rity of telomeres in higher plants (Riha et al., 2001).

We have been interested in elucidating the molecular mechanism for regulation of telomerase activity during the plant cell cycle. Therefore, our objective here was to monitor the levels of telomerase activity using the telomere repeat amplification protocol (TRAP) assay in various tissues of tobacco plants, as well as in tobacco BY-2 suspension cells. We wished to determine whether expression of telomerase activity was intimately correlated with the capacity for cell division.

MATERIALS AND METHODS

Plant Material

The different types of mature tissues, including leaves, stems, roots, and flowers, were obtained from fully-grown *Nicotiana glutinosa* (2n) plants. The tobacco BY-2 (*Nicotiana tabacum* L. cv. bright yellow-2) suspension cell line was maintained in a Murashige-Skoog salt medium (Murashige and Skoog, 1962) on a rotary shaker (120 rpm) at 27°C in the dark. Every week, 10 mL of stationary-phase cells were transferred to 90 mL of fresh media for culturing.

Telomerase Activity Assay

We monitored levels of telomerase enzyme activity using a modified version of the TRAP assay as described by Fitzgerald et al. (1996) and Riha et al. (1998). Our most critical modification to that procedure was the use of an internal standard (IS) in the PCR amplification, as described by Killan et al. (1998). A 184-bp DNA fragment derived from the multicloning site region of a Bluescript SK plasmid was used for our IS. The TRAP assay was carried out in 40 μL of a reaction mixture comprising 50 mM Tris-acetate, pH. 8.3, 50 mM potassium glutamate, 0.1% Triton X-100, 1 mM spermidine, 1 mM DTT, 50 μM each of dNTP, 5 mM $MgCl_2$, 10 mM EGTA, 100 ng/μL BSA and 0.5 unit Taq polymerase (Promega). After the addition of tobacco cell extracts containing 1 μg of total proteins and 50 ng of GC (21) forward primer (CACTATCGACTACGCGATCGG), the telomerase reaction was allowed to proceed at 24°C for 45 min. Fifty ng of $(C_3TA_3)_3$ reverse primer (CCCTAAACCCTAAACCCTAAA) and 1.5 ng of IS DNA containing GG(21) and $(C_3TA_3)_3$ primer sequences at its 5'- and 3'-end, respectively, were then added. The reaction mixture was covered with 50 μL paraffin oil and heated to 94°C. Products of the telomerase reaction were amplified by 30 cycles of PCR, each cycle consisting of 30 s at 94°C, 30 s at 65°C, and 90 s at 72°C, with an additional 5 min at 72°C in an automatic thermal cycler (Perkin-Elmer/Cetus). PCR products were separated on a 10% nondenaturing polyacrylamide gel. The gel was then stained in 0.01% SYBR Green I (Molecular Probes) for 45 min, and scanned on a PhosphorImager.

RESULTS AND DISCUSSION

One property particular to plants, but not animals, is that cell division is localized to meristems, i.e., specialized regions of shoots and roots. Because telomere length was shown to be declined during differentiation in plants (Killan et al., 1995), we presumed that expression of telomerase activity would be localized to the meristematic tissues in mature plants. To evaluate this hypothesis, we used TRAP assays of tobacco cell extracts from various tissue types to compare their in-vitro telomerase activities (Fig. 1). A relatively high level of activity was detected in the root tissue, which is consistent with the presence of the root apical meristem. In contrast, telomerase activities were much lower in mature stems and flowers, and none was detected in mature leaf tissues. We next investigated the telomerase activity in BY-2 suspension culture

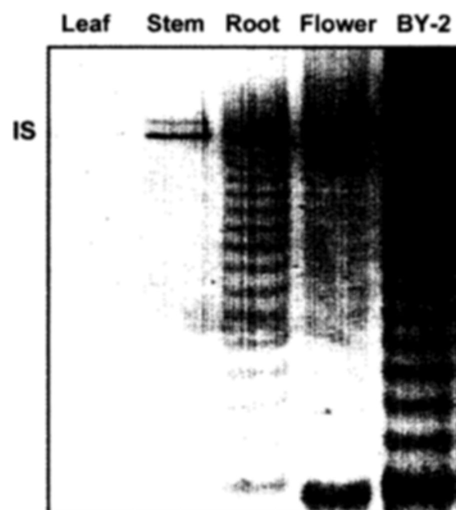

Figure 1. Regulation of telomerase expression in tobacco plants. Levels of telomerase activity were estimated in various cell types, including leaf, stem, root, flower, and BY-2 suspension cells. Reaction mixture of the TRAP assay contained 1 μg of total extract protein as described in 'MATERIALS AND METHODS'. The 184-bp internal standard DNA band (IS) is indicated.

cells that were in the phase of active cell division. BY-2 cells possessed the highest telomerase activity among various cell types examined (Fig. 1). Thus, these results are in line with the notion that the expression of telomerase activity is associated with the capacity for cell division in diverse tissues of tobacco plants.

To further investigate a possible relationship between telomerase activity and the capacity for cell division, we monitored telomerase levels during the 9-d culture period for BY-2 suspension cells. Telomerase activity was low during Days 1 and 2 of culturing (Fig. 2B). This low, basal expression of telomerase activity began to increase markedly on the third day, and reached a maximum at Day 5. This time point corresponded with maximal cell division (Fig. 2A). Subsequently, expressions of telomerase activity decreased. In 9-day-old culture cells with no capacity for cell division, the amount of telomerase activity was negligible (Fig. 2B). These results indicate that cell division

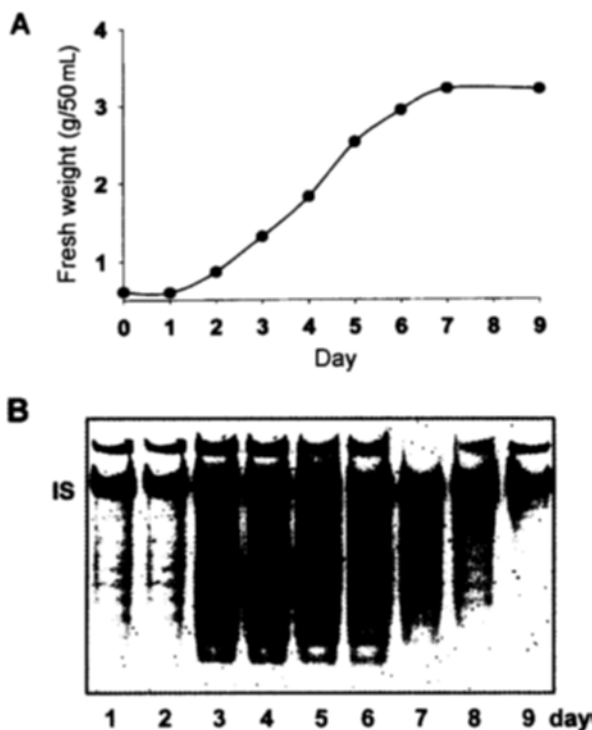

Figure 2. A. Growth curve of BY-2 cells during the 9-d culture period. The tobacco BY-2 suspension cell line was maintained in a Murashige-Skoog salt medium on a rotary shaker (120 rpm) at 27°C in the dark. **B.** Expression of telomerase activity during 9 d of culture for BY-2 suspension cells. Reaction mixture of the TRAP assay contained 1 μg of total extract protein. The 184-bp internal standard DNA band (IS) is indicated.

and telomerase activities are also intimately connected in tobacco BY-2 cells.

In conclusion, high levels of telomerase activity were detected via TRAP assays in tobacco BY-2 suspension culture cells and roots, both of which contained high proportions of actively dividing cells. Likewise, very low telomerase expressions in mature stems, leaves, and flowers corresponded with their negligible activity of cell division. Therefore, our results suggest that telomerase activity is closely associated with cell division in tobacco. This selective expression in actively dividing plant cells may be evidence that the pattern of telomerase regulation is highly conserved between plants and mammals. Future characterization of the molecular mechanism for telomerase expression during the cell cycle would enhance our understanding of the complex regulatory pathway for cell proliferation in higher plants.

ACKNOWLEDGMENTS

This work was supported by a grant from the Korea Research Foundation (Project No. KRF-2000-2-0821) to W.T.K.

Received September 19, 2001; accepted September 28, 2001.

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