Research news

The main focus of the work in 2005 was to examine telomeres from an evolutionary perspective. The order of monocotyledonous plants Asparagales is attractive for studies of telomere evolution as it includes three phylogenetically distinct groups with telomeres composed of TTTAGGG (*Arabidopsis*-type), TTAGGG (human-type) and unknown alternative sequences, respectively. To analyze the molecular causes of these switches in telomere sequence (synthesis), genes coding for the catalytic telomerase subunit (TERT) of representative species in the first two groups have been cloned. Multiple alignments of the sequences, together with other TERT sequences in databases, suggested candidate amino acid substitutions grouped in the Asparagales TERT synthesizing the human-type repeat that could have contributed to the changed telomere sequence. Among these, mutations in the C motif are of special interest due to its functional importance in TERT. Furthermore, two different modes of initial elongation of the substrate primer were observed in Asparagales telomerases producing human-like repeats, which could be attributed to interactions between the telomerase RNA subunit (TR) and the substrate.

The evolutionary response was analysed of a protein complement of Asparagales telomeres to the evolutionary change of its sequence from Arabidopsis type to human type, and, to the final loss of human type-sequence. Nuclear protein extracts were tested from plants with human-type telomeres (e.g., *Scilla peruviana*) and from phylogenetically younger plants without any known minisatellite telomeres (*Allium cepa*) for a presence of proteins binding specifically the single-strand overhang of a G-rich strand of telomeric DNA. The G-rich overhang is a highly conserved telomere domain since it functions as a telomerase substrate and a target site of a number of regulatory proteins (for example, Cdc13 and Pot1). Using EMSA it was found that these extracts still contain proteins preferentially binding the ancestral *Arabidopsis*-type sequence. More detailed analyses have shown that, e.g., *Allium* extracts contain proteins of 30 and 38 kDa, which bind both Arabidopsis and human telomeric sequences, 18 kDa protein which associates only with *Arabidopsis*-type sequence and, finally, a 24 kDa protein which forms complexes only with human-type sequence.

Recent papers:

Fajkus J., Sýkorová E., Leitch A.R.: *Techniques in plant telomere biology*
BioTechniques 38, 2005, 233-243

Neplechová K., Sýkorová E., Fajkus J.: *Comparison of different kinds of probes used for*

analysis of variant telomeric sequences. Biophysical Chemistry 117, 2005, 225 – 231

Fajkus J., Sýkorová E., Leitch A.R.: *Telomeres in evolution and evolution of telomeres* Chromosome Res. 13, 2005, 469-479

Kuchař M: *Plant telomere-binding proteins.* Biol. Plant. 50, 2006, 1-7

Sýkorová E., Leitch A.R., Fajkus J.: *Asparagales telomerases which synthesize the human type of telomeres.* Plant Mol. Biol. 60, 2006, 633-646

Sýkorová E., Fajkus J., Mezníková M., Lim K.Y., Neplechová K., Blattner F.R., Chase M.W., Leitch A.R.: *Minisatellite telomeres occur in the family Alliaceae but are lost in Allium.* Am. J. Bot. 93, 2006, 814-823