BIOLOGIA PLANTARUM 53 (1): 1-4, 2009

MINIREVIEW

Protect and regulate: recent findings on plant POT1-like proteins

G. ROTKOVÁ[1], E. SÝKOROVÁ[1,2] and J. FAJKUS[1,2]*

Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic[1]
Department of Functional Genomics and Proteomics, Faculty of Science, Masaryk University, Kamenice 5, CZ-62500 Brno, Czech Republic[2]

Abstract

Single-stranded DNA-binding proteins form protective caps at the chromosome ends and their binding is important in the regulation of telomerase access to telomeres. This group of proteins is represented by POT1 proteins described in yeast, humans and other model organisms. Here we review recent findings obtained in *Arabidopsis* POT1-like paralogs, namely the observed diversity in their interaction features and corresponding functions.

Additional key words: *Arabidopsis*, double-stranded DNA, single-stranded DNA, telomeres.

Telomeres are specialized nucleoprotein structures at the ends of eukaryotic chromosomes. In most higher eukaryotes they are formed by double-stranded DNA terminated by 3'-overhangs of G-rich single-stranded DNA (ssDNA). A number of proteins participate in telomere homeostasis and these proteins can be classified into three groups (Kuchar and Fajkus 2004, Kuchar *et al*. 2006). The first binds to the double-stranded part of telomeric DNA, the second comprises single-stranded-DNA-binding proteins, which bind the 3'-overhang, and the third associates with telomeres indirectly, by protein-protein interactions.

Single-stranded DNA-binding proteins form protective caps at the chromosomes ends and their binding is important in the regulation of telomerase access to telomeres. The first protein identified to bind telomeric ssDNA was described in *Oxytricha nova* (Gottschling and Zakian 1986) and is now a key protein in the study of telomere biology. This telomere end-binding protein (TEBP) consists of two subunits, α and β, which do not interact in the absence of telomeric DNA. The presence of DNA triggers the formation of a ternary complex α:β:ssDNA. Both α and β subunits contain conserved domains, the oligosaccharide/oligonucleotide binding motifs (OB folds). TEBPα contains three OB folds: the first two are located at the N terminus of TEBPα and are involved in DNA binding. The third OB fold is involved in mediating protein-protein interactions with TEBPβ. The β subunit contains a single OB fold that is involved in binding the ssDNA and in the interaction with TEBPα in the α:β:ssDNA complex. The β subunit alone cannot interact with the α subunit or the ssDNA (Fang and Cech 1993, Gottschiling and Zakian 1986, Gray *et al*. 1991). Telomeres in budding yeast are protected by ScCdc13p, a distant relative of *Oxytricha* TEBP (Nugent *et al*. 1996). The crystal structure of ScCdc13p bound to its substrate DNA revealed that it also forms an OB fold at the DNA-binding site (Mitton-Fry *et al.* 2004). Database searches have identified in *Schizosaccharomyces pombe* an open reading frame named POT1 (protection of telomeres) that contains a region with some similarity to α subunit of TEBP. This protein is thought to have a role in the stabilization of chromosome termini (Baumann and Cech 2001). Pot1 orthologs have since been identified in other organisms, including humans (Baumann *et al*. 2002).

Received 11 October 2007, *accepted* 15 February 2008.
Abbreviations: OB - oligosaccharide/oligonucleotide binding motifs; TEBP - telomere end-binding protein; TR - telomerase RNA subunit.
Acknowledgements: We thank Professor Andrew R. Leitch (Queen Mary, University of London, UK) for critical reading of the manuscript. This work was supported by the GA ASCR (IAA600040505), GACR (521/05/0055) Ministry of Education (LC06004) and institutional support (MSM0021622415, AVOZ50040507, AVOZ50040702).
* Corresponding author; fax: (+420) 549492654, e-mail: fajkus@sci.muni.cz

POT1-related proteins appear to act as both positive and negative regulators of telomere length. It is thought that the N-terminal DNA binding domain and amino acids close to the C terminus are essential for POT1 function (Bunch *et al*. 2005). Biochemical and cytological data indicate that POT1 binds the single-stranded G-rich extension at the ends of telomeres and prevents access to nucleases, telomerase and other DNA-modifying enzymes. C-terminal fragments of the protein probably have dominant-negative effect by displacing endogenous Pot1 from telomeres, which results in dramatic lengthening of telomeres. Upon further reduction of POT1 at the ends of chromosomes the opposite effect is observed. including loss of telomeric DNA and chromosome end fusions (Bunch *et al*. 2005). In humans, POT1 can regulate telomere synthesis either positively or negatively. Positive regulation of telomere elongation by telomerase (Colgin *et al*. 2003), could occur by trapping the G-overhangs in unfolded (telomerase-extendable) conformations (Zaug *et al*. 2005), or by promoting telomerase-recruitment and processivity when acting in a functional complex with another telomere-associated protein, TPP1 (Wang *et al*. 2007, Xin *et al*. 2007). Negative regulation of telomere extension could be mediated by restricted access of telomerase to the 3'-terminus of the G-overhang (Kelleher *et al*. 2005).

Baumann *et al*. (2002) were the first who consider POT1-like proteins in plants. Three genes coding for POT1-like proteins have been identified in *Arabidopsis thaliana* and partially characterized. Unfortunately, the use of different nomenclature for these proteins has

Table 1. ID numbers, present nomenclature and previous names for *Arabidopsis thaliana* POT1-like proteins.

Accession number	Database ID	Present name	Alternative names	Publications where they were used
At2g05210	BT012568	AtPOT1a	AtPOT1-1	Tani and Murata 2005
			AtPOT1	Shakirov *et al*. 2005, Surovtseva *et al*. 2007
			AtPOT1;1	Rossignol 2005, Rossignol *et al*. 2007
At5g06310	BAD99149	AtPOT1b	AtPOT1	Kuchar and Fajkus 2004
			AtPOT1-2	Tani and Murata 2005
			AtPOT2	Shakirov *et al*. 2005, Surovtseva *et al*. 2007
			AtPOT1;2	Rossignol 2005, Rossignol *et al*. 2007
At2g04395	NP_671775	AtPOT1c	AtPOT1;3	Rossignol 2005, Rossignol *et al*. 2007

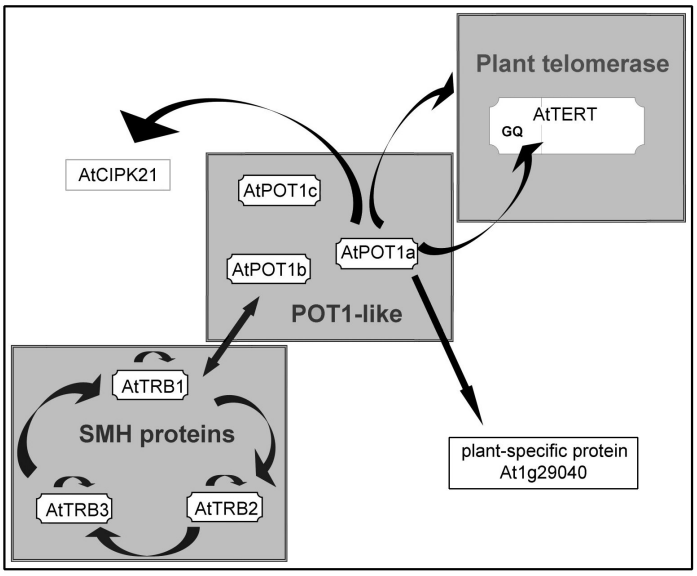

Fig. 1. Interactions of *Arabidopsis* POT1-like proteins. According to Rossignol (2005) and Rossignol *et al*. (2007), AtPOT1a interacts with protein kinase AtCIPK21, with a plant-specific protein of unknown function (At1g29040) and with the N-terminal fragment of the telomerase catalytic subunit AtTERT (containing a conserved GQ-motif), but not with the full-length AtTERT. In recent findings of Surovtseva *et al.* (2007), AtPOT1a is also able to interact with the whole nucleoprotein complex of plant telomerase. AtPOT1b protein interacts with AtTRB1 protein (Kuchař and Fajkus 2004) and other members of the SMH family of proteins (Prochazkova Schrumpfova *et al*. 2008). The ability of SMH proteins to form homo- and heteromeric protein-protein complexes (Schrumpfova *et al.* 2004, Prochazkova Schrumpfova *et al*. 2008, Mozgova *et al.* 2008) is also shown.

complicated interpretation (see Table 1). To circumvent any potential confusion a unified nomenclature has been agreed by authors of the primary papers on POT1-like proteins in plants (D. Shippen, J. Fajkus and M. Murata), and the three proteins are now termed AtPOT1a, AtPOT1b and AtPOT1c (*cf*. Table 1 for nomenclature, pseudonyms and references). Most of the research effort up to now has been focused on AtPOT1a and AtPOT1b proteins. The gene (or pseudogene) coding for the third *A. thaliana* POT1-like protein, AtPOT1c, has probably arisen as a partial duplication of the AtPOT1a gene, and its function remains unclear (Rossignol *et al*. 2007).

RT-PCR of full length AtPOT1a and AtPOT1b cDNAs revealed that three different splicing variants for AtPOT1a and two for AtPOT1b gene exist. Northern blot hybridization and Western analysis failed to reveal tissue specificity for any variants of the AtPOT1a and AtPOT1b genes (Tani and Murata 2005), which contrasts with human Pot1 where one of five splicing variants was tissue-specific (Baumann *et al*. 2002).

The yeast two-hybrid system and complementary immunoprecipitation techniques have been used to understand the functions of plant POT1 proteins and to search for interaction partners (Fig. 1). Kuchař and Fajkus (2004) showed that AtPOT1b interacted with AtTRB1 protein, the latter belonging to a family of SMH (Single-myb-histone) proteins, which bear a unique triple motif structure containing an N-terminal myb-like domain, a central GH1/GH5 histone globular domain and a C-terminal coiled-coil domain (Marian *et al.* 2003). Proteins of this family in *Arabidopsis* show not only specific binding to telomeric DNA, but also formation of homo- and heteromeric protein-protein complexes (Schrumpfova *et al.* 2004). Interestingly, the N-terminal domain of AtPOT1b forming the OB-folds (which is presumably involved in the interaction with single-stranded telomeric DNA) participates also in protein-protein interaction with AtTRB1, being suggestive of regulatory role of these interactions (Prochazkova Schrumpfova *et al.* 2008). *Arabidopsis* plants over-expressing the N-terminal domain of AtPOT1b show a mild telomere shortening, but severe growth defects, sterility, and massive genome instability (Shakirov *et al.* 2005). The genome instability indicates that AtPot1b contributes to chromosome end protection.

Similar studies on AtPOT1a have identified three interacting partners (Rossignol 2005, Rossignol *et al.* 2007): a protein kinase AtCIPK21 which belongs to the family of kinases involved in calcium signalling and potentially in DNA damage signalling, a plant-specific protein of unknown function (At1g29040) and the N-terminus of the telomerase catalytic subunit AtTERT (Fig. 1). That N-terminal fragment corresponds to one of the catalytically inactive splicing variants termed AtTERT V (I8) and contains a conserved GQ motif and a flexible linker with nuclear localisation signals (Sykorova *et al.* 2006, for review see Autexier and Lue 2006). In addition, this domain can participate in binding the telomerase RNA subunit, TR (Friedman and Cech 1999, Moriarty *et al.* 2002), and to telomeric DNA (Lue 2005, Jacobs *et al.* 2006). Since no interaction was observed with the full-length AtTERT variant, the authors concluded that the alternatively spliced TERT form, AtTERT V(I8), acts to displace AtPOT1a bound to telomeres allowing AtTERT to be recruited to telomeres and to synthesize DNA repeats. Alternatively, phosphorylation signals or other modifications of full-length AtTERT structure are necessary to make its N-terminus accessible for interaction with AtPOT1a *in vivo*, and the interaction mediates recruitment of AtTERT to telomeres. Functional studies of AtPOT1a (Shakirov *et al.* 2005) have shown that overexpression of a C-terminal fragment of AtPOT1a (lacking the OB-fold motifs) results in modest telomere shortening, but plants show no signs of genome instability and growth defects. Immunoprecipitation of telomerase with an antibody against AtPOT1a demonstrates a dynamic association of AtPOT1a to telomeres (peaking in S-phase) (Surovtseva *et al.* 2007).

In conclusion these data suggest that AtPOT1a and AtPOT1b proteins are highly diverged in both sequence (they have 49 % overall sequence similarity) and function: while AtPOT1a functions mainly in telomerase regulation, AtPOT1b contributes to chromosome end-protection and genome stability. Thus, the functional diversity of *Arabidopsis* POT1-paralogs may provide a good example of acquired gene function following gene duplication, perhaps associated with segmental duplication or polyploidy in the ancestry of the lineage leading to *Arabidopsis thaliana*.

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