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REVIEW

Plant telomere-binding proteins

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Abstract

Telomere-binding proteins have recently been recognised not only as necessary building blocks of telomere structure, but namely as components which are of central importance to telomere metabolism being involved in regulation of telomere length as well as in protective (capping) function of telomeres. Although the knowledge on plant telomeric DNA-binding proteins lags behind that in human and yeast, recent data show both analogies and plant-specific features in the composition and interactions of telomeric proteins. This review focuses primarily on proteins with known amino acid sequence. These can be classified into following groups: *1*) the family of proteins with Myb domain at C-terminus, *2*) proteins with Myb domain at N-terminus, both binding double-stranded DNA of telomeric repeats TTTAGGG, *3*) the single-stranded DNA-binding proteins, and *4*) other proteins that act also in non-telomeric chromatin regions. Proteins with C-terminal Myb domain reported as IBP family were previously found in human, whereas Smh family representing proteins with Myb domain at N-terminus was identified only in plants. Also RRM family of the single-stranded DNA-binding proteins is likely to be plant specific.

Additional key words: telomere-associated proteins, DNA-binding, *Arabidopsis thaliana.*

Introduction

Telomere is a specialised chromatin domain composed of telomere DNA and various telomere-associated proteins. In many plants, telomeric DNA contains specific G-rich repeat sequence TTTAGGG (Richards and Ausubel 1988) and in contrast to animals the number of tandem repeat is relatively stable in different tissues during development of plant (Riha *et al*. 1998). Most of the telomeric DNA is duplex, but the most distal end of the telomere contains a single-stranded 3´-overhang called the G tail. Because protein-free DNA ends are vulnerable to degradation and are subject of fusion, telomere-associated proteins provide a sort of protective cap to the ends of linear chromosomes (Bryan and Cech 1999). During DNA replication the chromosome ends are not completely copied. That is why the 5´-terminus of telomeres is extended by a mechanism different from conventional replication, most frequently by telomerase, a reverse transcriptase with an RNA component that serves as a template for the synthesis of *de novo* telomeric repeats. In plant cells the telomerase activity was first observed by Fajkus *et al*. (1996) using a direct assay, and independently by Fitzerald *et al*. (1996) and Heller *et al.* (1996) with the use of the telomeric repeat amplification protocol (TRAP). Fitzgerald *et al.* (1999) and Oguchi *et al.* (1999) cloned and characterised independently the catalytic subunit of telomerase AtTERT from *Arabidopsis thaliana*.

It has been known that besides telomerase, a number of telomere-associated proteins participates in telomere structure and function. In plants DNA-binding activities specific to telomeric sequences were first observed by gel mobility shift assays with synthetic oligonucleotides and crude nuclear protein extracts of *Arabidopsis* or maize (Regad *et al.* 1994, Zentgraf 1995). Some telomer-binding proteins bind specifically to telomeric double- or single-strand DNA (telomeric DNA-binding proteins). The telomere-repeat double-stranded DNA-binding proteins share a characteristic Myb domain referred to as a telobox, which is responsible for binding to DNA sequences (Bilaud *et al.* 1996). Well characterized members of this protein group include human proteins TRF1 and TRF2 (Broccoli *et al.* 1997, Chong *et al.*

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1995, Konig *et al.* 1998), protein Rap1 from *Saccharomyces cerevisiae* (Shore and Nasmyth 1987, Hanaoka *et al.* 2001) and protein Taz1 from *Schizosaccharomyces pombe* (Cooper *et al.* 1998, Nimmo *et al.* 1998). TRF1 contributes to telomere stability by negative regulation of telomerase (van Steensel and De Lange 1997), whereas TRF2 appears to mediate formation of the T-loop involved in protecting telomeres from end-to-end fusion (Van Steensel *et al.* 1998, Griffith *et al.* 1999). Protein Taz1 function in telomere-length regulation and protein Rap1 has dual function and is required for telomeric transcriptional repression and telomere length regulation (Konig *et al.* 1996, Shore 1997, Lieb *et al.* 2001). This group includes also plant myb-like proteins OsRTBP1 from *Oryza sativa,* AtTBP1 and AtTRP1 from *Arabidopsis thaliana* (Yu *et al.* 2000, Chen *et al.* 2001, Hwang *et al.* 2001). All these proteins contain Myb domain at their C-termini. However, another family of telomeric DNA-binding proteins with Myb-domain at the N-terminus has been found. This family includes at present only plant proteins, especially protein PcMyb1 from *Petroselinum crispum*, AtTRB1, AtTRB2 (syn. AtTBP3) and AtTRB3 (syn. AtTBP2) from *A. thaliana* and Smh1 from *Zea mays* (Feldbrugge *et al.* 1997, Marian *et al.* 2003, Schrumpfova *et al.* 2004). Another group represents proteins, which are able to bind specifically to single-stranded G-strand telomeric DNA. For example protein STEP1 from *A. thaliana* (Kwon and Chung 2004) and NtGTBP1 from *Nicotiana tabacum* (Hirata *et al.* 2004) contain two RNA recognition motifs (RRMs) which enable the specific ssDNA binding.

In addition to specific telomer-associated proteins the telomeres contain proteins, which occur also in non-telomeric regions possessing special functions on telomeres (AtKu70, AtKu80, AtRad50, AtMre11) and finally common proteins (*e.g.* core and linker histones, non-included in this paper). In this contribution a review of specific plant telomeric DNA-binding proteins is given.

Proteins with Myb domain in C-terminal region

The Myb domain was previously found in transcription factors to consist of tandem repeats R1, R2 and R3, each in size of about 52 amino acids, but the plant proteins of this group contain Myb domain with one repeat (Rabinowicz *et al.* 1999, Rosinski and Atchley 1998, Stracke *et al.* 2001). Probably, the number of Myb repeats has been reduced during evolution of myb-like proteis (Ganter *et al.* 1999). Each Myb domain repeat contains helix-helix-turn-helix structure, which intercalates into major groove of double-stranded DNA (Jin and Martin 1999). This group indicated as IBP family includes plant proteins with single Myb domain at C-terminus (RTBP1 from rice, AtTRP1 and AtTBP1 from *Arabidopsis*; Konig *et al.* 1998, Chen *et al.* 2001, Hwang *et al.* 2001) as well as the already characterized telomeric DNA-binding proteins TRF1, TRF2, Rap1 and Taz1. These plant proteins contain further a highly conservative region behind the Myb domain towards C-end called Myb-extension (Myb-ext) and a central domain (CD) in the central portion of the protein. Both these regions are characteristic for this protein family and have not been observed in other protein groups (Karamysheva *et al.* 2004). The genes coding for these proteins are ubiquitously expressed in various organs. Also maize protein IBP, which is similar to proteins RTBP1, AtTRP1 and AtTBP1, belongs to this protein family but so far it has not been tested whether it binds specifically to telomeres. On the other hand, IBP1 protein is known to bind specifically to promoter including direct telomere repetition AGGGTTT (Lugert and Werr 1994).

RTBP1 (rice telomere-binding protein 1) from *Oryza sativa* was reported as the first cloned protein whose product is able to bind double-stranded telomeric DNA in plants. Protein binds DNA with two or more duplex TTTAGGG repeats. Isolated Myb domain is able to bind specifically telomere sequence as a homodimer and for this binding the internal sequence GGGTTT in the two-telomere repeats is critical (Yu *et al.* 2000). That observation is consistent with the model of the TRF1-DNA complex showing that base-specific contacts are made within the sequence GGGTTA (Konig *et al.* 1998).

Protein AtTBP1 (*Arabidopsis thaliana* telomere-binding protein 1) is encoded by a single-copy gene localised at chromosome V in *Arabidopsis thaliana*. Amino acid sequence of Myb domain of this protein is in a great accordance with domain sequence of rice protein RTBP1 (80 % of identity and 87 % of similarity). The fragment of protein (amino acid positions 461 - 640) containing Myb domain binds specifically double-stranded DNA of four-telomere repeats $(TTTAGGG)_4$ as a homomultimer. However, for the protein fragment binding the minimum length of telomeric DNA of two telomere repeats is required (Hwang *et al.* 2001).

The single-copy gene AtTRP1 (*A. thaliana* telomere relating protein 1) occurs also at the chromosome 5 in *A. thaliana* and codes for protein bearing Myb domain with glutamin-rich regions on both sides of the Myb domain. These regions are critical for protein binding to DNA. Additionally, AtTRP1 contains short clusters of acidic residues and two nuclear localisation signals NLS at its N-terminus. Protein AtTRP1 is also similar to several plant proteins binding to certain promoter sequences, for example to protein HPPBF1 (AFO72536, *A. thaliana*). Using gel retardation assay a specific binding of the protein to dsDNA containing at least five GGTTTAG repeats has been observed. However, the protein forms a complex with the sequence $(GGTTTAG)_8$ more efficiently than with the sequence $(GGTTTAG)_5$ (Chen *et al.* 2001). Gel retardation assays showed that the protein fragment (amino acid positions 326 - 578) without N-terminal region binds telomere repeats more efficiently than the full-length protein and is able to bind telomeric sequence with four repeats $(GGTTTAG)_4$. It is interesting, that in cell extract of *Arabidopsis* the shorter form in the size of 67 kDa instead of expected 83 kDa protein has been identified. Moreover, this 67 kDa protein reported as ATBP1 can bind specifically to $(GGTTTAG)_4$ as well as the above-mentioned fragment (326 - 578 aa). The differences in these two proteins suggest that there may be two different telomeric DNA-binding proteins or the ATBP1 could be one of the proteolytic products of AtTRP1. Using a method of co-immunoprecipitation, the homomerization of protein AtTRP1 has been detected (Karamysheva *et al.* 2004).

Recently, further proteins TRFL1, TRFL2, TRFL4 and TRFL9 (TRF-like protein) have been found in *A. thaliana* in which binding to telomeric dsDNA *in vitro* was observed and homomerization was detected by immunoprecipitation. Simultaneously, interaction of AtTRP1 with TRFL1 and TRFL9 has been detected *in vitro*. For specific binding to telomeric DNA the proteins TRFL4, TRFL9 require minimum of four repeats $(TTTAGGG)_4$ and proteins TRFL1 and TRFL2 require even six repeats. Localisation experiments showed accumulations of all these proteins in a cell nucleus (Karamysheva *et al.* 2004).

Proteins with Myb domain in N-terminal region

For this protein group reported as a SMH (single myb histone) family a unique triple motif structure is characteristic and was not observed at any animal or fungal proteins (Marian *et al.* 2003). The first motif is the Myb domain that occurs at N-terminus in this group. The Myb domain of these proteins contains also only one of the three repeats such as the proteins with Myb domain at C-terminus. The second domain towards C-terminus is the globular GH1/GH5 domain, which was originally found in the globular region of histone H1. Its secondary structure consist of three α-helices forming a three-helical bundle and two following β-strings forming β-turn (Calikowski *et al.* 2000). The third common region is coiled-coil domain near to C-terminus, which is likely to stabilise dimer formation and which was identified in some transcriptional factors (Lupas *et al.* 1991). These proteins are represented in small gene families in number depending on the particular specie. They are likely to occur in most plants but have not been found in *Chlamydomonas reinhardtii* that have different telomeric sequence TTTTAGGG (Petracek *et al.* 1990).

Protein PcMyb1 from *Petroselinum crispum* represents one of the studied plant proteins that were expected to bind specifically telomeric DNA. Feldbrugge *et al.* (1997) has referred to a dual function of this protein which may interact with telomeric DNA as well as regulatory promoter elements of chalconsynthase gene (*MRE*$^{\mathrm{CHS}}$). A similar dual function was previously reported at yeast protein Rap1 which participates in telomere regulation and functions as a transcription factor (Konig *et al.* 1996, Shore 1994). PcMyb1 contains one repeat R1 of the Myb domain.

In *Arabidopsis thaliana* two telomeric DNA-binding proteins AtTRB2 and AtTRB3 (telomere repeat binding factor, reported also as ATTBP3 and ATTBP2, respectively) have been found on the basis of amino acid sequence homology with protein PcMyb1 (Schrumpfova *et al.* 2004). The Myb domain represents one repeat that have slightly greater sequence identity with the R2 Myb domains than with the R1 or R3 Myb domains (Marian *et al.* 2003). Both proteins share a great similarity and probably belong to the same protein family with protein AtTRB1. A series of retardation assays revealed specific binding of proteins AtTRB2 and AtTRB3 to telomeric DNA containing minimum of two repeats $(TTTAGGG)_2$. However, binding of AtTRB1 to telomeric DNA has not been tested. Using two-hybrid system interaction between proteins AtTRB1, AtTRB2 and AtTRB3 and multimerization of each protein has been identified. So the both proteins are likely to bind DNA as dimers (Kuchar and Fajkus 2004, Schrumpfova *et al.* 2004).

In the same way, a protein Smh1 (single myb histone 1) from *Zea mays* has been found on the basis of comparison with the Myb domain of human protein TRF1 and Myb domain consensus sequence. Also in this case the Myb domain represents a single sequence homologous to R2. Smh1 is small basic protein that binds specifically two or more telomeric repeats TTTAGGG *in vitro*. Sequence-non-specific binding of a single-stranded DNA has also been observed (Marian *et al.* 2003).

ssDNA telomere-binding proteins

In this group of telomere-associated proteins only one family of plant proteins, which comprise RNA recognition motif (RRM) (Keene and Query 1991), has been well characterised as yet. This domain occurs in proteins in two tandemly repeated copies and commonly enables proteins to bind RNA or single-stranded DNA. So far, two proteins, STEP1 (*A. thaliana*) and NtGTBP1 (*Nicotiana tabacum*), have been found to bind specifically telomeric single-stranded DNA, in particular the G-rich strand, but they do not bind either the C-rich strand, or the double-stranded DNA of telomere. Analogous protein GBP1 has been found in

Chlamydomonas reinhardtii (Petracek *et al.* 1994) and these proteins are likely to be present in most plants.

The protein STEP1 (single-stranded telomere-binding protein 1) has been found in protein extract of *Arabidopsis* on the basis of specific binding to G-rich strand of telomeric single-stranded DNA. The protein STEP1 is one of two products encoded by the gene for RNA-binding protein 3 (acc. 475720, AAA18380). The full-length product is expressed in chloroplasts and it does not bind single-stranded DNA. The shorter protein STEP1 lacks the N-terminal chloroplast transit peptide and the acidic domain, and is localised into nucleus. Experiments carried out *in vitro* suggest that the protein STEP1 binds to telomeric ssDNA overhangs and inhibits telomerase-dependent lengthening of telomeres by its binding (Kwon and Chung 2004). The single-stranded DNA-binding is mediated by the tandem pair of the RNA-binding domains RBD1 and RBD2 (RRM1 a RRM2), which occur at the C-terminus of the protein. Both domains co-operate together, so any of the RBDs alone is not sufficient for binding to telomeric DNA sequences. For ssDNA binding the protein requires three or more tandem TTTAGGG repeats.

Analogous to previous protein, the tobacco protein NtGTBP1 (G-strand telomere-binding protein) contains two RRMs and binds specifically two or more telomeric repeats. The protein derived from cDNA sequence is longer than protein NtGTBP1 and on the basis of sequence comparison it is apparent that the C-terminal region of 36-kDa protein is removed by proteolytic cleavage to form a 28-kDa NtGTBP1 (Hirata *et al.* 2004).

Additionally, another possible candidate for specific telomeric ssDNA-binding protein could be *Arabidopsis* protein AtPot1 (accession number BAB08953) that contains a sequence homologous to the Pot1-like domain previously discovered in human telomeric ssDNA-binding protein Pot1 (Baumann *et al.* 2002). However, binding ability of AtPot1 to telomeric ssDNA has not been known yet. The human protein hPot1 is known to interact with the TRF1 protein complex (Loayza and De Lange 2003, Liu *et al.* 2004). Analogously, AtPot1 interacts with AtTRB1 and the interaction is limited to this protein only, while there is no interaction with the other *Arabidopsis* members of the same family (AtTRB2 and AtTRB3). The interaction has been detected by two-hybrid system and confirmed by co-immunoprecipitation (Kuchar and Fajkus 2004).

Moreover, binding activities specific to G-rich strand of plant telomeric DNA sequence have been detected in rice (Kim *et al.* 1998) and mung bean (Lee *et al.* 2000). Fulnecková and Fajkus (2000) have detected the 40 kDa protein in telomerase negative tissues of tobacco and *Silene latifolia* plants. This protein formed sequence-specific complexes with single-stranded G-rich telomeric overhang, thus causing species-nonspecific inhibition of telomerase due to blocking the telomerase from access to its substrate.

Other telomere-associated proteins

The plant telomeres include also proteins identified at non-telomeric regions of chromosomes. Some of these proteins may posses a special function at telomeres. For example two human proteins hKu70 and hKu80, which are known to mediate the non-homologous end joining (NHEJ) pathway to repair DNA double-strand breaks, have been found to be localised into ends of human chromosomes (Hsu *et al.* 1999).

Two proteins AtKu70 and AtKu80 from *A. thaliana* have been isolated on the basis of the sequence similarity to the human protein hKu70 and hKu80. Yeast two-hybrid analysis demonstrated that AtKu70 and AtKu80 form a heterodimer, and electrophoretic mobility-shift assays revealed that this heterodimer binds to double-stranded telomeric and non-telomeric DNA sequences, but not to single-stranded DNA. The AtKu70/AtKu80 heterodimer possesses single-stranded DNA-dependent ATPase and ATP-dependent DNA helicase activities (Tamura *et al.* 2002). Deficiency of AtKu70 and AtKu80 results in the lengthening of telomeres (Bundock *et al.* 2002, Riha *et al.* 2002, Gallego *et al.* 2003). In case of plants lacking both AtKu70 and catalytic subunit of telomerase AtTERT, the longer G-overhangs also occurred despite that total telomere lengths shortened approximately two or three times faster than in case of plants with only inactive AtTERT. G-overhangs in Ku mutants are longer probably due to an aberrant maintenance of the complementary C-rich strand (Riha and Shippen 2003). These observations indicate that AtKu70 and AtKu80 are involved in telomere metabolism. One possible role of AtKu70 at telomeres is a maintenance of the telomeric C-rich strand by action on proteins that control telomere elongation (Bundock *et al.* 2002). Moreover, Ku regulates also extension of the telomeric G-rich strand by acting as a negative regulator of telomerase (Riha and Shippen 2003). Two-hybrid system revealed also interaction of AtKu70 with the telomeric DNA-binding protein AtTRP1 and this finding corresponds to previously identified interaction of human protein Ku70 with the telomeric DNA-binding protein TRF2 (Kuchar and Fajkus 2004, Song *et al.* 2000).

Two other proteins of *Arabidopsis*, which are also involved in DNA double-strand break repair, homologues of human proteins Rad50 and Mre11, AtRad50 and AtMre11 have been characterised. These proteins form a complex which has been identified in all eukaryotic organisms examined to date, so this complex is likely to have been highly conserved during evolution (Bundock and Hooykaas 2002, Gallego and White 2001). Analysis of mutant plant containing a T-DNA insertion in the AtRAD50 gene presented progressive shortening of telomeric DNA and cell senescence in homozygous (rad50/rad50) plants. However, cell population passing through a crisis, from which only a fraction of cells survives, was observed. These survivors posses longer telomeres as compared with wild-type cells. This observation suggests the existence of AtRad50-independent mechanism for telomere maintenance. The telomere repeat amplification protocol (TRAP) assay showed that AtRad50 is not needed for expression of the telomerase activity itself but rather for its *in vivo* action on chromosome ends (Gallego and White 2001). On the other hand, mutation of AtMre11 in phosphoesterase domain causes surprisingly lengthening of telomeres in contrast to other examined organisms (yeast, human), and this mutation is dominant for increased telomere length (Bundock and Hooykaas 2002). Bundock and Hooykaas (2002) have proposed that AtMre11 may be bond to AtTRP1 and regulate the telomere length by its action on proteins that inhibit telomere elongation. These results suggest the role of these proteins in telomere maintenance in higher eukaryotes. Although the human proteins co-localise at the telomeres (Zhu *et al.* 2000), localisation of AtRad50 and AtMre11 has not been observed until now (Bundock and Hooykaas 2002).

Table 1. List of proteins mentioned in this contribution.

Protein group	Characteristic domain	Protein
dsDNA-binding proteins	C-terminal Myb domain	AtTRP1, AtTBP1, IBP, TRBP1, TRFL1, TRFL2, TRFL4, TRFL9
	N-terminal Myb domain	PcMyb1, AtTRB1, AtTRB2, AtTRB3, Smh1
ssDNA-binding proteins	RRM	STEP1, NtGTBP1
	Pot1 domain	AtPot1
Other telomere-associated proteins	Ku70/80 consensus	AtKu70, AtKu80
	RAD50 consensus	AtRad50
	phosphoesterase domain	AtMre11

Conclusion

The telomeres are composed of telomeric DNA repeats and telomere-associated proteins and the telomere structures seem to be highly conserved among many eukaryotes. Most of those plant proteins were found on the basis of amino-acid sequence similarity with already known telomere-binding proteins from other organisms. The mentioned double-stranded DNA-binding proteins bear the Myb domain that is highly conserved and is present as a single sequence in contrast to previously found transcription factors, which has two or three Myb repeats. However, some proteins of both families act also as transcription factors. This finding suggests a possible dual function for some of these proteins, telomeric and transcriptional. The myb-like proteins include two different families: the IBP family (proteins with Myb domain at C-terminus) and the SMH family (proteins with Myb domain at N-terminus), where the IBP-type proteins probably correspond to previously characterised human and yeast myb-like telomeric DNA-binding proteins, whereas the SMH proteins represent a novel group. The SMH-type proteins have a unique triple motif structure and have been identified only in plants. The family of single-strand binding proteins bearing RNA recognition motif has been characterised exclusively in plants as well. Although the proteins bind telomeric DNA *in vitro*, it is not known yet whether these proteins occur at telomeres *in vivo*. Future analysis with gene knock-out plants will be important for defining the biological role of these proteins. The identification and the functional characterisation of the proteins at chromosomal ends facilitate understanding of telomere function.

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