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Roles of NtGTBP1 in telomere stability

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Telomeres are protective nucleoprotein structures at the ends of linear eukaryotic chromosomes. In contrast to double-stranded-specific telomere-binding proteins, the cellular roles of single-stranded-specific telomeric proteins are not well understood in higher plants. Three highly conserved tobacco G-strand-specific telomere-binding protein paralogs (NtGTBP1, NtGTBP2 and NtGTBP3) were identified and characterized. All three NtGTBPs were able to bind specifically to the plant single-stranded telomeric repeat elements in vitro with similar affinities. Suppression of *NtGTBP1* by means of the RNAi-mediated gene knock-down method resulted in developmental defects in transgenic tobacco plants accompanied by lengthened telomeres, extra-chromosomal telomeric circles and abnormal anaphase bridges. These results suggest that the downregulation of *NtGTBP1* results in genome instability. NtGTBP1 prevented in vitro strand invasion, a prerequisite process for inter-chromosomal telomeric recombination. Therefore, tobacco NtGTBP1 is one of the essential factors for telomere stability. Because abnormal telomeric elongation and recombination due to the suppression of *NtGTBP1* are reminiscent of the recombinational telomere lengthening mechanism that purportedly operates in telomerase negative cancer cells, it is of interest to investigate whether telomeric recombination is associated with cell death in animal systems.

Key words: genome stability, inter-chromosomal recombination, single-stranded telomere-binding proteins

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Extreme ends of linear eukaryotic chromosomes maintain telomeres, which contain protective complexes of proteins and DNA repeats.[1,2] Telomeric DNA repeats consist of two parts: double-stranded and single-stranded DNA sequence elements. Telomere sequences are protected by specialized sequence-specific non-histone DNA binding proteins. In higher plants, Myb domain-containing double-stranded DNA binding proteins (TRFs) are relatively well characterized and appear to be functionally conserved with mammalian TRFs.[3-5] However, situation of single-stranded telomeric binding proteins is complicated. Pot1, a well-known shelterin complex protein, has single-stranded telomere repeat binding activity in yeasts and mammals but no DNA binding activity in Arabidopsis, despite the fact that it is necessary for the proper maintenance of telomere integrity.[6-8] These results led us to investigate other proteins that potentially bind to single-stranded telomeric ends. Because some reports have found that human heterogeneous nuclear ribonucleoproteins (HnRNP) homologs contain sequence-specific telomere repeat binding activity in higher plants,[9,10] we characterized tobacco NtGTBP1, a homolog of human HnRNPs, by performing in vitro gel retardation assays and phenotypic analyses of RNAi-mediated knock-down transgenic tobacco plants, in which *NtGTBP1* was downregulated.[11]

NtGTBPs, Tobacco HnRNP Homologs, are Single-Stranded Telomere-Binding Proteins

Previously, HnRNP A1 and its homologs were characterized as single-stranded telomere-binding proteins in mammalian and plant cells.[10,12] These results suggest potentially conserved roles of the HnRNP family on telomeres. We cloned three HnRNP homologs in tobacco and termed them

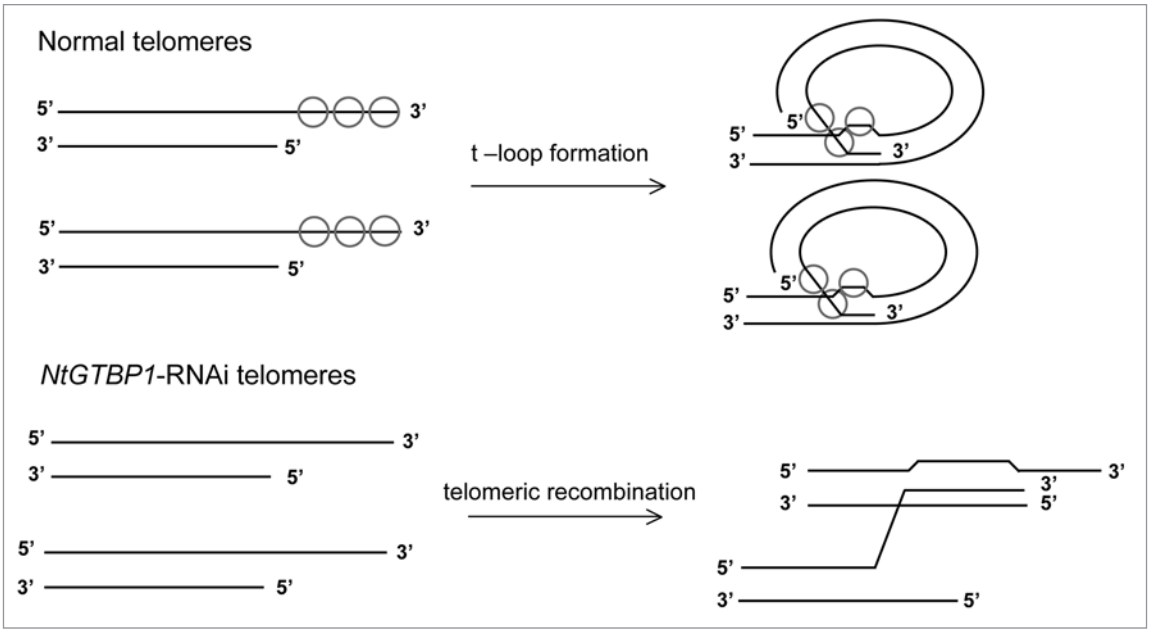

Figure 1. A schematic representation of the possible role of NtGTBP1. In normal tobacco cells, NtGTBP1 binds to single-stranded telomeric ends and inhibits inter-chromosomal telomere recombination. Properly protected telomeres by NtGTBP1 along with other telomere-binding proteins form a t-loop structure, which stabilizes the integrity of the telomere. In *NtGTBP1*-knock-down cells, aberrant telomere recombination occurs due to the absence of the recombination inhibitory function of NtGTBP1. This may cause telomere fusion and lengthening that are inevitably connected to genome instability and cellular senescence. Open circles indicate NtGTBP1.

G-strand-specific, single-stranded telomere-binding proteins (NtGTBPs). All three NtGTBPs were able to bind single-stranded telomere repeats with sequence specificity. A chromatin immuno-precipitation (ChIP) assay confirmed that NtGTBP1 was localized to the telomeres in tobacco BY2 suspension cultured cells. These results suggest that NtGTBP1 belongs to the putative inter-species conserved single-stranded telomere-binding protein family.

Suppression of *NtGTBP1* Causes Telomere Instability Accompanied by Severe Developmental Anomalies

Although single-stranded telomeric repeat binding properties of plant HnRNP homologs have been studied thoroughly, their physiological roles have not been fully elucidated.[9,10] We constructed the RNAi-mediated *NtGTBP1* knock down transgenic tobacco plants by introducing hair-pin-forming DNA of *NtGTBP1* fragments under the control of the CaMV 35S promoter. The *NtGTBP1* knock down plants showed severe developmental abnormalities, and their phenotypes were gradually intensified as plants grew to maturity. Simultaneously, the telomere lengths and recombination rates increased in accordance with their abnormal morphology. The *35S:RNAi-NtGTBP1* tobacco plants also exhibited massive extra-chromosomal telomeric circles (ECTR). These results indicate that the defective phenotypes of *35S:RNAi-NtGTBP1* plants originated from telomere instability rather than *NtGTBP1* suppression itself.

NtGTBP1 is Associated with Recombinational Stability of Telomeres

The massive ECTR resulting from the suppression of *NtGTBP1* allowed us to speculate on the cellular functions of NtGTBP1 as regards the recombinational regulation of telomeres. Because NtGTBP1 binds to the single-stranded telomeric sequence specifically, we considered that the binding of NtGTBP1 to single-stranded extreme telomeric ends blocks an invasion step in the homologous recombination processes. In vitro strand invasion assays indeed showed that NtGTBP1 binding to telomeres was correlated to the inhibition of single-strand invasion. This finding in conjunction with the telomere lengthening and ECTR formations in *NtGTBP1* knock down plants show that these characteristics coincide with the recombinational telomere lengthening mechanism proposed in telomerase-negative cancer cells.[13] In our hypothesis, the association of NtGTBP1 with single-stranded sequences protects telomeres from inter-chromosomal recombination and helps telomeres form a proper T-loop (**Fig. 1**). In contrast, in the absence of NtGTBP1, single-stranded telomeres invade other telomeres, resulting in abnormal recombination.

In telomerase-negative animal cancer cells, recombination between telomeres is used as a tool for telomere length maintenance. However, our data suggest that aberrant recombination between telomeres has a causal effect on chromosomal instability and the creation of abnormal phenotypes, which subsequently lead to unusual cell death in tobacco plants. Therefore, it is of interest to examine whether telomeric recombination is connected to cell death in animal systems.

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