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Recent progress in plant telomere biology

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ABSTRACT

Telomeres are the specialized structures consisting of highly repetitive DNA and associated proteins. The primary function of telomeres is to protect the chromosomal ends from fusion and from the attack of nucleases. As in the case of yeast and mammals, telomere is essential for the normal development and differentiation in plants which has been shown by study using telomerase-deficient Arabidopsis. However in spite of recent remarkable advances in plant telomere biology, the mechanism of telomere homeostasis, a fundamental concern, remains yet to be elucidated. In this review, we introduce the recent findings in plant telomere biology and discuss how the length of plant telomere might be regulated.

Key words : telomerase, telomere, telomere binding protein

Eukaryotic chromosomes meet a serious problem during cell division, especially replication. Due to their linearity, RNA primers are used to synthesize the lagging strands of the chromosomes and the last RNA primer is not replaced by DNA, which is called 'end replication problem' (1). If special machinery is not present to maintain the end of chromosomes, then the chromosomal ends will be progressively shortened and in the end the shortening will bring about genomic instability and cell death. Eukaryotic cells, thus, have developed a mechanism to prevent the progressive erosion of their chromosomal end, telomere. Though most are still unknown in plant field, we here summarize the recent progress in plant telomere biology.

Telomeric DNA and telomerase

Eukaryotic chromosomes end with a tandem array of repeated sequences, telomeric DNA. Although the primary sequences are not identical, the telomeric DNA is enriched by G residues. The repeat unit is TTAGGG in most vertebrates, TTGGGG in *Tetrahymena* and TTAGGC in *C. elegans* (2). Plants also possess the G-rich repeats at the end of chromosomes and the repeated sequence in Arabidopsis is TTTAGGG (3-6). Telomeric DNA is composed of long double stranded and short single stranded DNAs. The single stranded G-rich overhang was known to invade the duplex telomeric DNA and form a lariat-like structure, called t-loop (7) and this structure is thought to conceal the G-rich 3' overhang and protect it from the action of nucleases or DNA break repair systems. Recently, this structure was also found in pea (8) indicating that t-loop is a conserved way to maintain and protect the chromosomal ends in eukaryotes.

Although telomeric DNA itself is resistant to the action of nuculeases by forming t-loop structure, in the continuously dividing cells such as cells in the meristems, non-replacement of the last RNA primers and the exposure of the linear DNA during S phase cause the gradual shortening of chromosomal ends **(Fig 1)**. High repetition of simple DNA sequence and controlled length regulation at the chromosomal

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ends gave rise to the finding of telomerase, a specialized reverse transcriptase (9-11). The reverse transcriptional activity of telomerase can be detected using TRAP (telomeric repeat amplification protocol) assay and the RNA-dependent DNA polymerization of telomerase can be supported by RNase treatment **(Fig 2)**. *In vitro* telomerase activity can be reconstituted with telomerase catalytic subunit, TERT and RNA template, TR (12, 13). However, several associated proteins are also required *in vivo* for assembly, recruitment to the chromosomal ends and fine tuning of telomerase (14). Because telomerase is essential for the completion of DNA replication, it has been shown that the loss of telomerase in mice caused the progressive shortening of telomere and eventually cell death and disastrous developmental defects (15, 16). This is also the case in Arabidopsis. Although the template RNA for telomerase is not identified yet in Arabidopsis, disruption of telomerase reverse transcriptase, AtTERT by T-DNA insertion led to the progressive reduction of telomere length, 250 to 500 bp loss per generation (17). In this mutant Arabidopsis, severe developmental defects appeared from sixth generation similarly to telomerase-deficient mice and none survived after tenth generation due tothe genome instability (18), suggesting that telomerase also plays a key role in plant development.

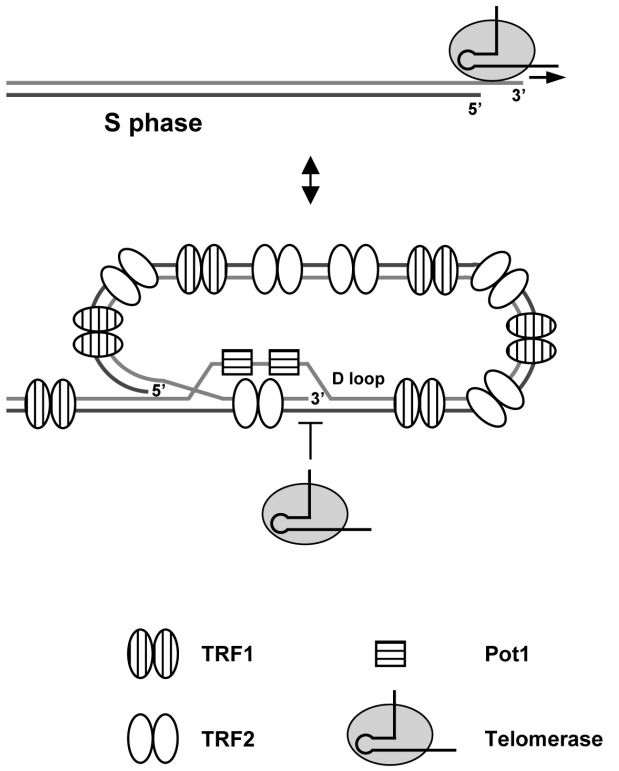

Fig 1. Dynamic structure of human telomere. Chromosomal DNA is thought to be linearized and permit the access of telomerase during DNA replication (S phase), while at the other stages chromosomal end is thought to form t-loop by the intrusion of 3′ G-rich overhang into the duplex telomeric DNA. TRF2 stabilizes t-loop and inhibits the access of telomerase by interacting with Pot1.

Telomere-binding protein

Several proteins directly interacting with telomeric DNA are critically required for the maintenance of telomere although telomerase is a key factor to regulate the telomere length. Best known are TRF1 (TTAGGG repeat-binding factor1) and TRF2 which bind the double stranded telomere DNA (**Fig 1**). TRF1 regulates telomere length depending on telomerase activity (19, 20). TRF2 is involved in the regulation of telomere length (20), the protection of chromosomal ends (21) and the facilitation of t-loop formation (22). In yeast, Rap1 was found to interact with yeast telomere DNA (23) and play a crucial role in the regulation of telomere length (24-27). The shared motif of proteins mentioned above is Myb-related DNA binding domain and using sequence similarity proteins such as RTBP1 from rice (28), AtTBP1 from Arabidopsis (29) and NgTRF1 from tobacco (30) were identified to specifically interact with the double stranded plant telomeric DNA. It was recently reported that the level of NgTRF1 negatively correlated with telomere length in tobacco BY-2 cells leading to the alteration of cell viability (31) suggesting that NgTRF1 may function as TRF1 in human and Rap1 in yeast to regulate telomere length as counting molecules.

Proteins which bind the single stranded telomeric DNA are also very important for the maintenance of telomere because eukaryotic chromosomes end with single stranded G-rich overhangs. Cdc13 binds the 3′ overhang to protect the chromosomal end and interacts with Est1 to recruit telomerase in budding yeast (32, 33). In fission yeast and human, Pot1 (protection of telomeres1) plays a similar role to

Fig 2. TRAP assay (43). Plant extracts were prepared by grinding of 7-day-grown Arabidopsis. The GG substrate primer (5′-CACTATCGACTACGCGATCGG-3′) and the antisense telomeric repeat primer (5′-CCCTAAACCCTAAACCCTAAA-3′) were used as forward and reverse primers, respectively. Telomerase extension reactions were carried out with 200 nM GG primer, and 100 μM concentrations of each dNTP. Extracted plant protein (1.5 μg) was added to the reaction mixture, and the mixture was incubated at room temperature for 45 min. The DNA samples were amplified by PCR using 200 nM forward primer and 200 nM reverse primer for 30 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s, with an additional 10-min extension step at the end. The amplified products were resolved by 10% nondenaturing polyacrylamide gel and visualized by staining with SYBR Green (Molecular Probes). As controls, telomerase extension reaction was performed without plant extract or with RNase-treated plant extract.

Cdc13 to protect the chromosomal end and to regulate telomere length by interacting with TRF1 (**Fig 2**) (34, 35). In human, hnRNP (heterogeneous nuclear ribonucleoprotein) family also binds the single stranded telomeric DNA (36). hnRNP A1/UP1 (proteolytic fragment of hnRNP A1 lacking splicing activity) binds both the single stranded telomeric DNA and telomerase RNA (37), and is involved in the regulation of telomere length possibly by recruiting telomerase to the very end of chromosomes (38). In plants, many reports have shown the presence of protein factors which specifically interact with the single stranded telomeric DNA (39-42). Several proteins which bound the plant single stranded telomeric DNA were identified from Arabidopsis using affinity chromatography and MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) analysis (43). STEP1 (single stranded telomere-binding protein1) was the first plant protein that directly interacted with the single stranded telomere and modulated telomerase activity *in vitro* (43). Although it is localized to the nucleus, its *in vivo* role however is yet to be answered. Arabidopsis seems to have two Pots, AtPot1 and AtPot2, and these Pots were recently reported to differentially function in the regulation of telomere length and the protection of chromosomal ends (44).

Conclusion

In the past decade, many researchers exerted to expand the knowledge on plant telomere biology. Despite recent advances, compared to yeast and mammalian field, many questions are yet to be addressed. The primary question is how telomere length is controlled in plants. In *Saccharomyces cereviciae*, Rap1 which contains Myb DNA binding motif and binds the duplex telomere, acts as a counting molecule. Rap1 perceives the number of yeast telomere DNA repeat by direct binding and the number of bound Rap1 determines whether telomerase adds the telomere repeat to chromosomal end (45, 46). In human, TRF1 negatively regulates telomere length by recruiting Pot1 to chromosomal end to inhibit the action of telomerase (35) and hRap1 is also involved in the control of telomere length by interacting with TRF2 (47, 48). It was reported that even in plants telomerase activity and telomere length are developmentally regulated (49, 50). NgTRF1, in this context, could be regarded as a plant homolog of TRF1 since it inhibits telomerase activity *in vitro* and its *in vivo* level negatively correlates with telomere length (31), and RTBP1 and AtTBP1 might have similar function in rice and Arabidopsis, respectively (28, 29). Although the direct interaction of AtPot1 and AtPot2 with the plant single strand telomere is not evident, the alteration of telomere length in transgenic Arabidopsis implicates that both AtPots behave similar to human Pot1 (44). STEP1 which is a splicing variant of cp31 lacking transit peptide

and contains two RNA-binding motifs, directly interacts with plant single stranded telomere and inhibits telomerase activity *in vitro* (43) implying that STEP1 might protect chromosomal ends and regulate the accessibility of telomerase. Of particular and immediate interest is how they operate *in vivo*. Possibly, it would be greatly helpful to reveal the relationship between telomerase and known telomere binding proteins.

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