

Recombination Proteins and Telomere Stability in Plants

Simon Amiard, Charles White and Maria Eugenia Gallego*

Génétique, Reproduction et Développement, UMR CNRS 6247 - Clermont Université - INSERM U931, UFR Sciences et Technologies, Université Blaise Pascal, 24 avenue des Landais, 63171 Aubière cedex, France

Abstract: Repair of DNA damage is essential for the maintenance of the integrity and transmission of the genome in development and reproduction. Telomeres are nucleoprotein structures which protect the ends of (linear) eukaryotic chromosomes. Telomere dysfunction results in loss of this protection and the telomeres being recognised as DNA damage by the cellular DNA Damage Repair and Response (DDR) machinery, leading to senescence or cell death. Telomeric homeostasis is thus tightly controlled and many specific and non-specific proteins are involved in its regulation. Among these, DNA damage and Repair proteins contribute both to the recognition of telomere dysfunction and more surprisingly, are directly implicated in telomere homeostasis itself. Plants offer a great opportunity to study these mechanisms due to the fact that many key DNA repair and recombination proteins are non-essential in plants, in contrast to vertebrates. In the following text, after a brief summary of the current state of knowledge on telomere-specific proteins in plants, we review the DDR processes and the related proteins implicated in plant telomere stability. We focus specifically on telomere signalling and on recombination events induced by unprotected telomeres, at the origin of genome rearrangements and instability when telomere function is affected.

Keywords: *Arabidopsis thaliana*, DDR, HR, NHEJ, recombination, signalling, telomere.

INTRODUCTION

Genome stability is of primary importance for proper development and survival of all living organisms. Surveillance systems are present to block the cell cycle, to repair and to induce cell death. Double-stranded breaks (DSBs) in DNA are one of the most important threats to genome integrity because they can result in chromosomal aberrations that can simultaneously affect many genes and eventually lead to cell death [1].

Most characterised eukaryotic chromosomes are linear and thus have two ends which, if unprotected, can be recognised by the cell as DSB. Inappropriate repair of these chromosome ends can then lead to deleterious chromosome fusions and constitute an important threat for genomic integrity [2]. To avoid this, linear chromosomes throughout the living world are capped by specialised nucleoprotein structures called telomeres. Telomeres in most eukaryotes are believed to be folded into a closed "T-loop" structure which "hides" them from cellular recombination machinery, permitting cells to distinguish their natural chromosome ends from DNA breaks. Another role of the specialised telomere structure is to counteract DNA erosion arising from incomplete replication of linear chromosomes. This elongation is carried out by the telomerase, a specialized reverse transcriptase that extends chromosome 3' ends by adding several repeats of telomeric DNA using its own RNA subunit as a template.

DNA breaks can arise through replication fork stalling at obstacles such as DNA adducts, secondary structures or tightly bound proteins [3]. They also arise through the action of exogenous (IR, UV, chemical agents) and endogenous (nucleases and metabolic products such as ROS) genotoxic agents, and occur naturally in cellular processes such as meiotic recombination, V(D)J recombination and mating type switching in the yeast *Saccharomyces cerevisiae* [4]. Whatever their origin, DSB are repaired by recombination, carrying the risk of mutation and/or gross chromosomal rearrangements (GCRs) [3] and in turn to apoptosis or carcinogenesis. Considerable interest is currently focussed on the choice and mechanisms of the different homologous (HR) and non-homologous recombination (NHR) pathways which carry out this repair [5-7].

The remarkable resistance of plants to DNA/telomere damage makes them, and specifically *Arabidopsis thaliana*, particularly relevant models for studies of telomere function. This also applies to DNA repair and recombination, studies of which are greatly facilitated by the viability of key mutants. It is also of interest to note that the particular properties of telomeres were first noticed in plants (maize) and flies in the work of B. McClintock and H. Muller and gave rise to the breakage-fusion-bridge cycle (BFB) model - still in force to explain telomere-induced genomic instability [8].

In this review, we focus on the current state of knowledge concerning the intriguing relationship between telomeres and DNA damage repair proteins in plants. As expected, these proteins are able to recognize and act upon deprotected telomeres, but more surprisingly, many repair proteins directly participate to telomere homeostasis.

*Address correspondence to this author at the Génétique, Reproduction et Développement, UMR CNRS 6247 - Clermont Université - INSERM U931, UFR Sciences et Technologies, Université Blaise Pascal, 24 avenue des Landais, 63171 Aubière cedex, France;
Tel: (33) 473 407 752; Fax: (33) 473 407 777;
E-mail: megalleg@univ-bpclermont.fr

TELOMERIC PROTEINS IN PLANTS AND TELOMERE HOMEOSTASIS

Telomeric DNA is composed of long tracts of double-stranded, G-rich repeats (TTAGGG in humans and TTTAGGG in plants) and it ends with a single-stranded protrusion called the "G-overhang". In vertebrates, the "shelterin" complex is thought to remodel the end of chromosomes in a "closed" conformation, sequestering the extremities of the chromosomes and assuring their protection. This complex, composed of six proteins (TRF1, TRF2, RAP1, TIN2, TPP1 and POT1), is responsible for topological changes causing invasion of telomeric duplex DNA by the G-overhang and thus formation of the "T-loop" structure [9]. This structure is thought to be ubiquitous in higher eukaryotes and has been visualized in the common garden pea [10], as well as in human and mouse cells [11].

TRF1 and TRF2 bind telomeric double-strand DNA directly, while POT1 binding is specific to telomeric ssDNA. TRF2 and POT1 recruit their respective partners (RAP1 and TPP1) and TIN2 is considered as the "bridge" in the complex, having been shown to interact with TRF1, TRF2 and TPP1 - thus linking the double-stranded to the single-stranded part of the structure [9]. TRF1 and TRF2 are both negative telomere-length regulators, however TRF2 seems to be the major telomere caretaker given the dramatic end-to-end fusions events and the strong checkpoint response observed after conditional deletion of mouse TRF2 [12].

Many telomeric proteins are conserved amongst eukaryotes, and specifically in plants. Concerning the telomeric double-stranded DNA binding proteins, research of homologs is based on the identification of proteins possessing a "myb-like" DNA binding domain that contains the "telobox" motif required for specific telomeric sequence recognition. These "telobox myb" domains are almost identical in TRF1 and TRF2, and share strong homologies with DNA binding domains of the oncoprotein "c-myb" that gave the name to this domain [13]. The *S. pombe* Taz1 protein possesses this domain and is required for telomere homeostasis [14]. In plants however, the identification of TRF-like proteins is complicated by the existence of many proteins with the ability to bind double-stranded telomeric sequence. The first double-stranded telomeric binding proteins were found by gel mobility shift assays in maize and *Arabidopsis* crude extracts [15][16] and in tobacco, the protein NgTRF1 can bind telomeric sequences and is required for correct telomerase regulation during the cell cycle [17]. *Arabidopsis* has about 200 proteins with a "myb-like domain", of which at least 15 possess a single "telobox myb domain" [18]. These proteins are classified in three classes depending on the N-terminal or C-terminal position of the domain and their ability to bind telomeric DNA *in vitro*. So far, none of these proteins has been shown to be essential for telomere protection and this presumably indicates redundancy in plant double-stranded binding telomeric proteins. Notwithstanding, one of the *Arabidopsis* proteins (AtTBP1) has been shown to be involved in negative telomere-length regulation [19], another (AtTRP1) can interact with AtKU70 [20] and finally AtPOT1b interacts directly with the three members of the single myb-histone (smh) family (AtTRB1, AtTRB2 and AtTRB3) [21].

Telomere ssDNA binding proteins, such as POT1 or its ciliate homolog TEBP α , bind ssDNA *via* two or three OB-fold domains respectively. Recent studies have shown the implication of several plant proteins possessing this binding domain in telomere protection. The *Arabidopsis thaliana* genome encodes two Pot1-like proteins, named AtPOT1a (or AtPOT1) and AtPOT1b (or AtPOT2) [22]. Over-expression studies and analyses of mutants have shown that these proteins have different functions at telomeres: AtPOT1a contributes to the regulation of telomere length by promoting telomerase activity and AtPOT1b contributes to chromosome end protection [23]. Such evolution into two proteins with distinct functions has also been found in mouse, in which POT1a is crucial for telomere integrity and overall genomic stability and POT1b has the ability to regulate the amount of ssDNA at the telomere [24, 25].

In budding yeast, Cdc13 is the major G-overhang binding protein and binds the 3' overhang through a single OB-fold. Cdc13 does not however appear to be a POT1 ortholog and protects telomeres by forming a heterotrimeric protein complex with Stn1 and Ten1 (CST complex) [26-28]. Mutants of one of the proteins of the complex exhibit single-strand G-overhang elongation by C-strand degradation. CST, which has recently been proposed to be a RPA-like protein complex involved in telomere metabolism [29], has been isolated in *Arabidopsis* and in mammals [30-32]. In the plant, absence of Stn1 or Ctc1 (putative Cdc13 homologue) leads to severe phenotypic defects and is accompanied by massive genomic instability: telomere length decrease, telomeric fusions and appearance of extra-chromosomal telomere circles [30].

In plants, as in other eukaryotes, defects in telomeric proteins affect telomere maintenance and global genomic stability. This is also the case with the telomerase enzyme At-TERT (the catalytic subunit of telomerase), absence of which results in a slow but progressive loss of telomeric DNA through successive mutant generations [33]. Mutant plant lineages can survive up to 11 generations with worsening phenotypes, until they ultimately arrest growth in a miniature, sterile dedifferentiated state [34].

From this brief summary, we can see that telomeric proteins and architecture appear to be conserved in plants [18], and that telomeric stability is essential for plant genome integrity and development. However, due to a remarkable tolerance to telomere dysfunction, the flowering plant *Arabidopsis thaliana* constitutes a particularly useful model for studies of telomere biology.

DNA DAMAGE SIGNALLING PROTEINS IN GENOME STABILITY

Specific telomeric DNA binding proteins permit the assembly of other protein complexes, which involve telomere-specific factors as well as proteins with more general cellular functions like DNA repair, DNA damage checkpoints or chromatin modification [9]. A recent study has revealed that at least 210 proteins may interact at human telomeres [35]. Very interestingly, and *a priori* surprising given the role of telomeres in protecting against repair, DDR proteins participate very actively in telomere homeostasis. Some genetic disorders, like *Ataxia-Telangiectasia*, *Ataxia-Telangiectasia like disease (ATLD)*, *Nijmegen Breakage Syndrome* or *ATR-*

Seckel Syndrome show telomeres and DNA damage response defects revealing clear relationships between both systems [36, 37]. To clarify current understanding of the intriguing relationships between these proteins and telomeres we summarise below the general function of these proteins along with the specific role of these proteins in telomere homeostasis - with a focus on the state of knowledge concerning plants.

THE ATM/ATR SIGNALLING PATHWAY IN PLANTS

Cellular DNA damage survey mechanisms continually monitor the integrity of the DNA and function to prevent the occurrence of deleterious mutations and rearrangements. DSB signalling invokes cell-cycle arrest, chromatin remodeling, DDR repair pathways and eventually cell death or senescence. In mammals, the signalling role is assumed by three specific kinases belonging to the PI3K-like protein kinases (PIKK) family: ATM, ATR and DNA-PKcs. Once activated, PIKK can phosphorylate (and activate) hundreds of targets necessary to maintain genomic integrity [38]. The specific ways of activation by the different PIKK are still under investigation, but it is now accepted that DSB cause ATM activation in an Mre11-Rad50-Nbs1 (MRN) dependent manner. ATR is considered as the specific sensor of DNA replication damage that lead to replication fork stalling [39] and is more generally activated by a variety of lesions that have in common the generation of ssDNA. There are however clear links between these two responses, as ATM/MRN-dependent resection of DSB extremities generates single stranded DNA (ssDNA), and thus activation of ATR [40]. Whatever the origin of the single strand, ATR is recruited by its cofactor ATRIP, which indirectly recognizes ssDNA through interaction with the ssDNA-binding protein, RPA. DNA-PKcs is specifically recruited to DSB sites by interaction with the end binding heterodimer Ku70/80, that engages repair by NHEJ pathway [41].

In *Arabidopsis*, AtATM and AtATR have been characterized but no DNA-PKcs gene has been found [42]. As in mammals, both AtATM and AtATR participate in global genome stability. The *atam* mutant develops normally but is partially sterile due to defects in processing of Spo11-dependent chromosome breakage during meiosis [43]. These plants are hypersensitive to ionizing irradiation (IR) and methyl methane sulphonate (MMS), but not to UV irradiation. ATR is an essential gene in animals, but *Arabidopsis atr* mutants are viable, fertile, and like *atam* mutants, phenotypically wild-type in the absence of exogenous DNA damaging agents. *atatr* mutants are hypersensitive to hydroxyurea and aphidicolin, due to a defective G2 checkpoint response to blocked replication forks [44]. AtATR can however partially compensate for the ATM response, as the double *atam atr* mutant is completely sterile due to extreme genome fragmentation [45]. Neither AtATR, nor AtATM signalling is thus essential during normal plant development. However, signalling is primordial for correct repair of genome damage, independent of its endogenous or exogenous origin.

Phosphorylation by PIKK of the histone variant H2AX, forming γ -H2AX, is an early cellular response to the induc-

tion of DNA double-strand breaks. This epigenetic modification of H2AX plays a key role in the recruitment and accumulation of DNA repair proteins at sites of DSB damage [46-48] and detection of this phosphorylation event using antibodies to γ -H2AX has emerged as a highly specific and sensitive molecular marker for monitoring DNA damage and its repair [49]. Immunocytogenetic studies show nuclear foci, which may be counted to enumerate the number of DSBs in a cell and/or to examine the co-localization of other DNA repair proteins to the sites of double-strand damage [50].

Due to the viability of the mutants, AtATM and AtATR activation studies are facilitated and *Arabidopsis* is the only known organism in which *atm atr* double mutant is viable. Most of the studies have essentially been performed using the observation of γ -H2AX foci appearance after ionizing radiation (IR). In 2005, studies performed in the Britt lab revealed that IR induced foci are mediated essentially by AtATM and much less by AtATR [51]. They also showed that double *atm atr* mutant does not trigger any γ -H2AX foci formation after IR, reinforcing the idea that DNA-PKcs is absent and that AtATM and AtATR are the only signalling PIKK kinases in plants. The recent discovery in *Arabidopsis* of the protein AtATRIP, necessary for AtATR activation as in mammals reinforced the idea that DNA damage signalling in plant is conserved [52]. Plant homologs of the genes encoding the 9-1-1 (Rad9/Rad1/Hus1) sensor complex have also been identified and are required for resistance to DNA damaging agents like bleomycin or Mitomycin C (MMC) [53].

In addition to DNA repair, activation of DDR signalling pathway leads also to chromatin remodeling, cell cycle arrest and eventually to cell death or senescence. In *Arabidopsis*, AtATR has a minor role in transcriptional changes after irradiation and the transcriptional response to ionizing radiation is principally dependent on AtATM [43, 54, 55]. Concerning cell cycle responses, in mammals activation of ATM or ATR leads to the activation of the checkpoint kinases CHK2 or CHK1 respectively. These in turn phosphorylate the CDC25 phosphatase, leading to the "non-activation" of CDKs and to the arrest of the cell cycle progression [56, 57]. In plants, checkpoint activation is still poorly understood. Neither CHK1 nor CHK2 orthologs have been found, and the described CDC25 protein lacks the regulating domain [58]. Plants do however possess a WEE1 kinase, and this has been shown to provoke cell cycle arrest in response to activation of AtATM or AtATR [59]. Finally, a recent study reports that cell proliferation control and DNA damage response activation is independent of CDKA;1 (human Cdk1 homologue) phospho-regulation - arguing that the Cdc25/Wee1 cell cycle control may not be fully in place in *Arabidopsis* [60].

When the number of DSB reaches a certain threshold, the DNA repair machinery can become overwhelmed and the damaged cells suicide through the induction of apoptotic, programmed cell death (PCD). In mammals, this mechanism is controlled by ATM, through activation of the p53 pathway [61]. A type of programmed cell death does occur in plants in response to DNA damages [62], although no plant p53 homologs have yet been described. The recent discovery in *Arabidopsis* of the protein AtSOG1 supplies the key to un-

derstanding of DDR PCD in plants. AtSOG1 controls the expression of hundreds of genes after IR and AtSOG1 is suspected to regulate cell death in response to AtATM or AtATR activation [63]. DNA damage-induced cell death in plants occurs principally in dividing meristematic stem cells and this has recently been shown to be AtATR and AtATM dependent [64, 65]. *Arabidopsis* roots constitute an interesting stem cell niche model for these studies and further studies will clarify the details of DDR PCD in plants.

MRN COMPLEX

The highly conserved MRN complex is essential for global genome and telomere integrity, with multiple roles in DNA replication, DNA repair and signalling [37]. In mammals, the MRN complex is composed of three distinct proteins: Mre11, Rad50 and Nbs1. The binding of to DSB sites is extremely fast and promotes the tethering of the "free" extremities [66, 67] in addition to being the key element in the DNA damage signalling. Once bound to DNA, MRN recruits and activates ATM *via* interaction with Nbs1 [68] and Mre11 nuclease activity leads to the formation of single-strand oligonucleotides that further promote ATM activation [69]. Further maturation of the extremities can also lead to ssDNA formation and ATR activation [40].

In contrast to vertebrates for which null mutations in many DNA repair and recombination genes are lethal, such mutants of *Arabidopsis* are frequently viable, a fact first remarked in the *atrads50* mutant [70]. *Arabidopsis* MRN mutants are viable and *atmre11* and *atrads50* mutants are both hypersensitive to DNA damaging agents and sterile due to meiotic genome fragmentation [70-73]. The *atmbs1* mutant is also hypersensitive to MMS and MMC, but meiosis occurs normally and the plants are fertile [74]. AtNBS1 does however play a role in meiosis, as shown by the full sterility of *atmbs1 atmbs1* double mutant plants in contrast to the partial fertility of the *atmbs1* mutant. It remains to be determined whether this partial meiotic role of AtNBS1 is due to retention of some function in the described *atmbs1-1* allele. Be that as it may, the plant MRN complex is of primordial importance for correct DNA signalling and repair. Data from our lab reveal clear MRN-dependent ATM activation after DNA damage and furthermore that both AtATR and AtATM activation after IR are dependent upon MRN [122].

RPA

Replication protein A (RPA) is an ssDNA-binding protein composed of three subunits of 70, 32 and 14 kDa [75, 76]. The RPA heterocomplex is implicated in a wide range of cellular activities associated with DNA metabolism and notably DNA replication, DNA repair and HR. In plants and particularly in *Arabidopsis*, multiple RPA genes have been described: 5 for AtRPA70, 2 for AtRPA32 and 2 for AtRPA14 [77]. This presence of multiple homologs has made determination of the functional role of the RPA difficult and much work remains to be done. Some *atrpa* mutants are sensitive to DNA damage inducing agents, at least one presents meiotic defects [78, 79] and a recent report shows that inactivation of AtRPA70a by T-DNA insertion leads to increased sensitivity to genotoxic agents such as MMS,

bleomycin and hydroxyurea revealing a role of this protein in DSB repair [80].

DNA DAMAGE SIGNALLING PROTEINS IN TELOMERE STABILITY

Although ATR and ATM are present at functional mammalian telomeres, the shelterin organization avoids their activation. TPP1/POT1 are required for the repression of ATR signalling and TRF2 is the predominant repressor of ATM signalling [81], either through blockage of ATM binding by TRF2-promoted t-loop formation or direct interactions [82]. Concerning ATR inhibition or activation, POT1 and RPA may compete for binding to the ssDNA of either the 3' overhang or that excluded in the "D-loop" (displacement loop) after invasion of the ssDNA into the duplex telomeric tract [83]. In *Arabidopsis*, signalling of dysfunctional telomeres is still poorly understood, but recent work from our lab revealed a conserved mechanism in telomere signalling. Indeed, we found that in short telomere condition, both AtATM and AtATR are activated and that in single-strand telomere de-protection condition, only AtATR is activated (Amiard *et al.* in preparation).

ATM and ATR also play direct roles in telomere homeostasis. ATM inhibition in mammals provokes telomere shortening [84] and in yeast, mutation of the ATR ortholog *mec1* provokes telomere shortening [85]. In contrast to other organisms, *atm* and *atatr* mutants show no telomere length misregulation and no end-to-end chromosome fusions, indicating that AtATM or AtATR are not required for telomere homeostasis in normal growth conditions [86]. ATM and ATR do however directly participate in telomere homeostasis in *Arabidopsis*, as shown by the telomere fusions and severe developmental phenotypes of double *atm* *atatr* or *atatr* *atatr* mutants [86, 87].

Proteins of the MRN complex also directly influence telomere homeostasis. In humans, MRN is recruited to telomeres through interaction with TRF2 [88] and knockout of one protein of the MRN complex in HeLa cells leads to G-overhang shortening [89]. The MRN complex acts as a positive regulator of telomerase at yeast telomeres [90] and the MRN complex also participates in telomere homeostasis in plants [72, 91]. Absence of AtRad50 leads to elevated number of end-to-end chromosome fusions with about 50% of these fusions that contain subtelomeric sequences but no telomeric repeats, revealing a drastic degradation of a certain population of chromosome ends. Furthermore, telomeric defects in *atatr* mutants are exacerbated by the absence of AtRAD50, revealing a protective role of AtRAD50 against deleterious recombination events at shortening telomeres [92]. Chromosomal aberrations including end-to-end chromosome fusions have also been reported in *atmre11* mutants [72]. No data of telomeric defects has been reported for the *atmbs1* mutant, although it is possible that this may be due to some residual activity in the characterised *atmbs1* allele (see above). Rad50-Mre11, and probably the whole MRN complex, thus clearly plays key roles in plant telomere homeostasis.

RPA is not a constitutive telomere binding protein but is associated to telomeres during S/G2 phase, both in yeast and in humans. RPA may be required for correct replication of

telomeric sequence through unfolding G-quadruplex structures that may form within G rich telomeric DNA sequences [93]. Moreover, RPA associates to G-overhangs in *S. pombe* and is critical for telomere length maintenance [94, 95]. In plants, the *atp70* mutant presents significant, telomerase-dependent telomere lengthening [80].

DNA DAMAGE REPAIR PROTEINS IN TELOMERE STABILITY

Telomere structure acts to protect telomeres from recombination. This regulation of recombination is however lost at unprotected telomeres, which are recognized as DSB and the resulting recombinational repair gives rise to end-to-end chromosome fusions and genome rearrangements. Multiple pathways of recombination exist and the dependence on DNA sequence homology between the two recombining DNA molecules permits the separation of recombination mechanisms into two general classes : homologous and non-homologous recombination, respectively. The roles of these two pathways at telomeres are dealt with separately below.

NON-HOMOLOGOUS END JOINING PATHWAYS IN TELOMERE BIOLOGY

Non-homologous recombination (NHR) permits joining of two broken double-strand DNA ends in the absence or with minor sequence homology. The best characterised NHR pathway is non-homologous end-joining (C-NHEJ). The first step of C-NHEJ involves the Ku70/80 heterodimer, which binds dsDNA ends with strong affinity, independently of the sequence and the structure [96]. Binding of KU to the DSB ends may assist in tethering the broken ends together and in making the extreme termini accessible to downstream proteins. KU binding is followed by the action of DNA-PKcs (specific to vertebrates), Artemis and X-family polymerases to prepare the ends for ligation by XLF-Xrcc4-DNA ligaseIV.

If the protective cap of the chromosome end is altered (through, for example, the deletion of an essential telomeric protein or inactivation of the telomerase), telomeres become substrates for recombinational repair and this can generate dicentric chromosomes and establishment of the bridge-breakage-fusion cycle [97]. Chromosome ends fuse massively in TRF2-depleted mammalian cells, mostly in a DNA ligaseIV dependent way [98]. In plants, NHEJ mechanisms appear to be conserved and most of the partners are present except for DNA-PKcs (only described in vertebrates). Neither Artemis nor XLF orthologs have been described in plants; however, *Arabidopsis* does encode a putative Artemis ortholog [99] and DNA ligaseVI has a predicted Artemis domain [100]. Concerning telomere biology, absence of KU has different effects on telomere length in different organisms (discussed by Gallego and White [101]). *Arabidopsis* and rice *ku* mutants have longer telomeres, and in contrast to animal cells [102, 103], absence of KU does not lead to telomeric fusions in plants [104-106]. In the *attert atku70* mutant, the telomere C-strand is more prone to nucleolytic degradation, revealing a protective role for KU at the 5' end of the chromosome [107].

In *Arabidopsis*, the recombination pathways responsible for chromosome fusions in response to dysfunctional te-

lomeres are only partially understood. In *attert* mutants, telomeric fusions can arise through KU-dependent C-NHEJ as in mammals, however KU-independent fusion also occurs [107]. PCR based methods reveal at least two alternative end joining pathways. The first, based on short sequence homology and named Microhomology-Mediated End Joining (MMEJ), is dependent on the MRN complex. In an *attert atku70 atmre11* triple mutant, fusion still occurs revealing at least another unidentified end-joining pathway and the absence of Mre11 reduces the use of microhomologies [108]. Absence of DNA LigaseIV does not reduce the number of fusions observed in the *attert atku70* mutant, but the fusion junctions involve shorter microhomologies and include less telomeric DNA [109]. KU-independent end-joining pathways are currently the focus of much attention [96, 110], and it is likely that further studies will implicate them in plant telomere homeostasis.

HOMOLOGOUS RECOMBINATION PATHWAYS IN TELOMERE BIOLOGY

Due to the simple, highly repetitive sequence of their DNA, telomeres are prone to homologous recombination events. As described above for NHR, mammalian TRF2 is required for inhibition of HR at telomeres [9]. When TRF2 is removed in the absence of KU70, telomeres undergo extensive HR observed as telomeric sister-chromatid exchanges (T-SCEs) [111]. Dramatic loss of telomeric sequences (TRD, Telomeric Rapid Deletion) has been described in human cells expressing an N-terminal deleted form of TRF2. These are associated with extrachromosomal telomere circles (ECTCs), implicating T-loop deletion by HR [112]. Telomeres can also recombine with homologous interstitial (not at telomeres) repeats of telomeric DNA sequence. Such recombination can lead to terminal deletions and the appearance of extrachromosomal elements containing the deleted segment with the original telomeric sequence (Telomeric Double-Minute chromosomes) [113]. HR at telomeres can thus also lead to chromosomal rearrangements and constitute a real threat for genomic stability.

In *Arabidopsis*, the AtERCC1/AtRAD1 (Ercc1/Xpf) complex has recently been shown to play a protecting role against recombination between short telomeres and interstitial telomeric repeats [114]. *atercc1 attert* and *atradd1 attert* mutants exhibit dramatically enhanced telomere instability and chromosome fusions. Cytogenetic analysis confirmed that in absence of this complex, short telomeres tend to recombine with intrachromosomal telomeric sequences leading to these karyotypic rearrangements.

In human cells, HR is thought to play a role in telomere elongation in absence of telomerase and this ALT (Alternative Lengthening of Telomere) pathway is implicated in around 15% of human tumors. ALT cells exhibit long and heterogenous telomeres and the presence of extrachromosomal telomeric DNA and ALT-associated promyelocytic leukemia nuclear bodies (APBs) [115]. ALT is a recombination based mechanism in which one telomere can be extended by using the telomere from another chromatid or from extrachromosomal telomeric DNA [116]. In *Arabidopsis*, ALT is suspected to be a general mechanism of telomere elongation. This idea comes from the observation that telomeric

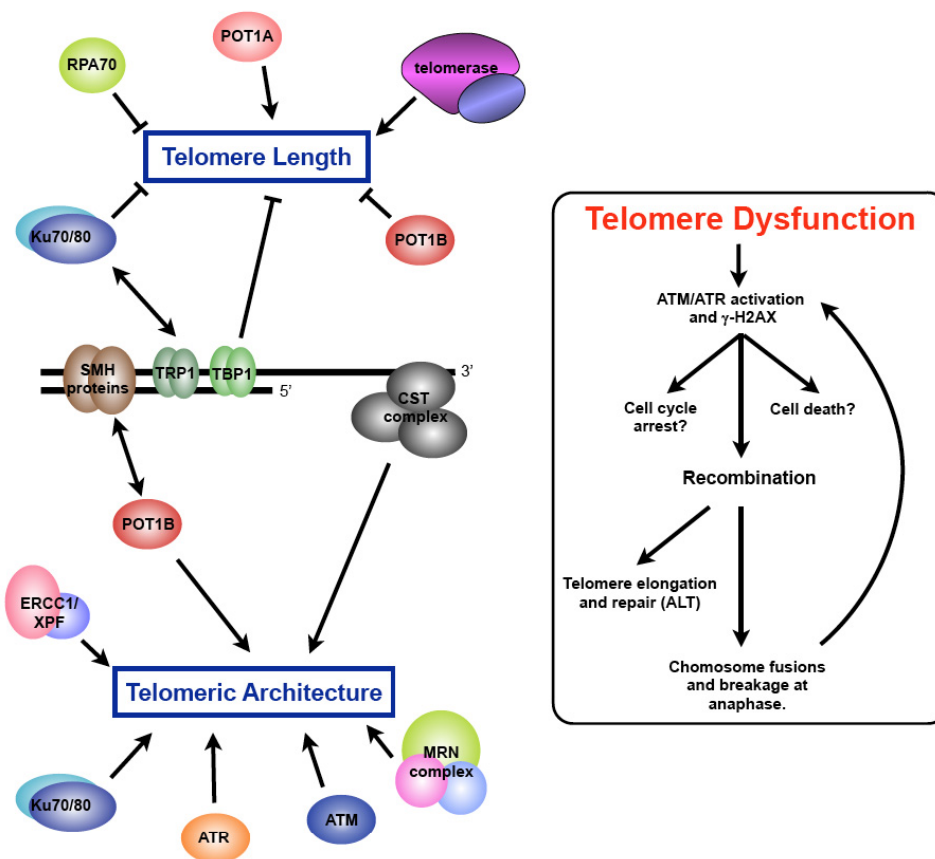


Fig. (1). Overview of telomere dynamics in the plant *Arabidopsis thaliana*. Telomeric Binding Proteins and DNA Repair proteins participate in the regulation of telomere length regulation and the maintenance of telomere architecture (see text for details). Telomere dysfunction activates ATM and/or ATR DNA damage signalling kinases, phosphorylation of histone H2AX, cell cycle arrest and potentially cell death. Recombination can repair unprotected telomeres through an ALT-like elongation or lead to chromosome or chromatid fusions.

DNA loss per generation (250-500 bp) in *atert* mutant seems to be very low considering the estimated number (around 1000) of cell divisions per seed-to-seed generation [117]. Several attempts to obtain ALT cell lines derived from *atert* mutant cells have however been unsuccessful [118] and it is now known that the AtKU complex protects telomeric extremities from homologous recombination events [119, 120]. *Arabidopsis* cell lines deficient for AtKU70 exhibit extrachromosomal telomeric circles (t-circles) and *atert atku70* cell lines show massive TRD, long and heterogeneous telomere length and telomeric circles - all features of ALT [120, 121].

CONCLUSIONS

Notwithstanding minor differences, this review underlines the strong conservation of genome maintenance proteins and pathways throughout the living world. This generalisation clearly encompasses plants and nowhere is this seen more clearly than in the study of telomeres and DNA repair and recombination Fig. (1). Plant models have been at the origin of many fundamental discoveries in genetics, and the strong conservation of functions and proteins and the viability of key plant mutants, are driving the increasing importance of plant models in studies on telomere biology and genome maintenance functions in general.

ACKNOWLEDGEMENTS

We are grateful to members of the White lab for helpful comments and discussions. We apologize to those colleagues whose work has not been cited due to space limitation. This work was supported by a grant from the Agence Nationale de la Recherche (ANR-07-BLAN-0068, "TELOPLANTE"), an European Union research grant (LSHG-CT-2005-018785, "RECBREED"), the Centre National de la Recherche Scientifique, the Université Blaise Pascal, the Université d'Auvergne and the Institut National de la Santé et la Recherche Médicale.

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