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Telomeres: protecting chromosomes against genome instability

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Preface

The natural ends of linear chromosomes require unique genetic and structural adaptations to facilitate the protection of genetic material. This is achieved by the sequestration of the telomeric sequence into a protective nucleoprotein cap that masks the ends from constitutive exposure to the DNA damage response (DDR). When telomeres are unmasked, genome instability arises. Balancing capping requirements with telomere replication and the enzymatic processing steps obligatory for telomere function is a complex problem. Telomeric proteins and their interacting factors create an environment at chromosome ends that inhibits DNA repair there, however, the repair machinery is essential for proper telomere function.

Introduction

Linear DNA fragments are toxic to mammalian cells and effective mechanisms evolved to deal with them, involving signalling cascades resulting in detection, enzymatic degradation or repair of the fragments, cell cycle arrest and/or cell death. Failure to appropriately respond to broken DNA can result in unequal distribution of genetic material during cell division, in genome instability and eventually in the development of malignancies.

The natural ends of linear chromosomes resemble DNA breaks, but are an exception, where repair would lead to deleterious chromosome fusions and therefore has to be avoided. This is accomplished by specialized ribonucleoprotein structures, termed telomeres. They are composed of long tracts of double stranded G rich repeats, which in humans extend for 9–15kb, but can be as long as 100kb in rodents. The actual end of the telomere is conspicuous by the presence of a 50–300nt protrusion of single stranded repeats from the 3' end, termed the G-tail or G-overhang (Figure 1) [1]. This G-overhang is presumably the result of highly regulated post-replicative 5'-3' exonucleolytic resection of the C-rich strand.

In somatic cells that lack telomere length maintenance mechanisms the failure of lagging strand synthesis to fully replicate the parental strain [2], coupled with the processing required to generate the G-overhang result in the progressive removal of telomeric sequence in each round of replication. In stem cells, germ cells and lineage progenitor cells this telomere shortening is offset by the addition of newly synthesized repeats by the telomerase complex, which uses the 3'-OH of the G-overhang as its substrate (Box 1). It is not intuitive how the G-tail provides protection from the pathways that detect and process broken DNA, but it has been proposed that the 3' G-overhang can be sequestered into a lasso like structure known as the T-Loop [3] (Figure 1). The closed configuration of the T-loop provides a protective cap that defines the natural end of the chromosome and masks the telomere from the DNA damage response (DDR) machinery (Fig 2). Therefore, the generation of the G overhang and the manipulation of the

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tail by telomerase in telomerase positive cells is an important point of convergence of end protection and telomere length maintenance mechanisms.

Here we will discuss how functional telomeres prevent chromosomes from fusion, which factors contribute to chromosome end protection and how dysfunctional telomeres can be the source of genome instability and cancer.

Shelterin organizes and defines telomeres

The repetitive and GC rich nature of telomeric DNA endows it with the capability to form higher order DNA secondary structures, such as G-quadruplexes, which have been proposed to represent obstacles for the replication machinery [4, 5]. TTAGGG repeats have also been identified as poor substrates for nucleosome assembly *in vitro* [6] and therefore telomeric chromatin is quite distinctive 7. Given these properties, telomeres are thought to resemble fragile sites and telomeric proteins aid the DNA replication machinery in accurate duplication of the chromosome end 8, 9. This is further substantiated by the observation that the complete replication of telomeric DNA tends to occur later than other chromosomal regions [10] (ROS and JK, unpublished).

Such are the unusual properties of telomeres, a *bona fide* telomeric protein complex has evolved. In mammals this complex is termed Shelterin and consists of six individual proteins, TRF1 (Telomeric Repeat binding Factor 1), TRF2, RAP1 (Repressor/Activator Protein 1), TIN2 (TRF1 INteracting protein 1), TPP1 (TINT1/PIP1/PTOP 1) and POT 1 (Protection Of Telomeres 1) [11] (Figure 1). The double stranded telomeric repeats are bound by TRF1 and TRF2 whereas POT1 attaches to the single stranded overhang. These DNA binding modules are bridged by TPP1 and TIN2 and are crucial for chromosome end protection and telomere length regulation. TRF1 and TRF2 are constitutively present at telomeres and the proportion of TRF1 and TRF2 loaded on telomeres is important for telomere length regulation. TRF1 has DNA remodelling activity [12, 13] and has recently been shown to promote efficient replication of telomeres [8, 9]. TRF2 primarily functions in chromosome end protection by promoting topological changes in telomeric DNA [14], T-loop assembly [15, 16] and by suppression of ATM (Ataxia Telangiectasia Mutated)-dependent DDR and non-homologous end joining (NHEJ) (Fig 2) [17, 18]. TRF2 also plays a role in chromatin assembly, indicated by the finding that TRF2 overexpression causes aberrant nucleosome spacing and decreases the abundance of the core histones H3 and H4 at chromosome ends [19]. The function of RAP1 is more enigmatic. Unlike its homologue in *Saccharomyces cerevisiae*, mammalian RAP1 does not bind TTAGGG repeats and its telomeric localization is dependent on interaction with TRF2 [20], but the factor has recently been implicated in the inhibition of NHEJ *in vitro* and *in vivo* [21, 22]. POT1 contributes to telomere protection by binding to the overhang and by suppression of ATR (ATM Rad3 related protein)-dependant DDR pathways [17, 23]. Also, the high specificity of POT1 for single stranded telomeric DNA leaves the possibility open that it might bind to the displaced G-strand in the T-Loop and "lock-in" the closed configuration of this structure (Figure 1). The loading of POT1 and TPP1 onto the overhang is also an important determinant of telomere length. Current models suggest that POT1 and TPP1 compete with telomerase for access to the overhang [23]. A direct interaction between TPP1 and telomerase has been shown to bolster telomerase processivity [24, 25]. However, increased loading of POT1 along the overhang seems to block telomerase accessibility to the 3'-OH substrate.

Taken together, the shelterin complex, while consisting of six proteins only, has an immensely complex role in telomere length regulation, protection from enzymatic attack, recruitment of required enzymatic activities and control of signalling cascades from the natural chromosome ends. While our understanding of the individual roles of shelterin components is growing fast, much remains to be discovered about the transcriptional, translational and post-translational

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regulation of these components and about the importance of shelterin stoichiometry upon cell cycle changes, DNA damage and differentiation.

In a recent study, the biochemical purification of telomeric proteome produced a listing of 210 proteins that interacted with and might influence telomeric structure [26]. Many of these factors have previously been independently shown to localize to telomeres where they modulate higher order telomeric DNA and chromatin, unravel secondary DNA structures, promote resection and strand invasion of the overhang into the double stranded DNA and consequently aid in generation of the displacement loop (D-Loop) [27, 28]. In addition, there is a growing number of proteins that localize to telomeres that are involved in the assembly and regulation of telomerase in cells where this complex is expressed [29–31].

A recent addition to the telomeric RNP (RiboNucleoProtein complex) is TERRA (Telomeric Repeat containing RNA). For many years telomeres were viewed as transcriptionally inert. However, transcription of the C-strand of telomeres by RNAPII produces long UUAGGG containing transcripts that are regulated by the NMD (Nonsense Mediated Decay) pathway [32–34]. TERRA display a strong inverse correlation with telomerase activity [34, 35] and it is speculated that TERRA are non-coding structural RNAs that maintain higher order telomeric chromatin structures, either by forming highly stable DNA-RNA hybrids [34] or through interaction with TRF2 [36, 37].

Taken together, it is becoming increasingly obvious that the six-protein telomeric core complex provides the basis for a highly regulated and sophisticated network of proteins and RNA, which responds to the ever-changing environment in cells during the life span of the organism.

Molecular basis of telomere dysfunction

The interactions of telomeric DNA with shelterin and telomerase are highly regulated and essential for chromosome end protection. Defects in shelterin components and telomerase directly and adversely affect telomere structure and length, and, as a result, inappropriate changes in telomere length and/or structure are commonly invoked as the primary triggers of telomere dysfunction. Here we are first examining the proposition that changes in telomere length lead to telomere dysfunction.

The role of telomere length in end protection

In somatic cells naturally lacking telomere length maintenance pathways, replication itself and the post replicative restoration of the protective cap at chromosome ends is accompanied by a net loss of 100 to 200bp of telomeric sequence in every cell division. The molecular basis for this DNA loss is due to the inabilities of conventional polymerases to fully replicate the parenting DNA by lagging strand synthesis (termed as the 'end replication problem') [2], combined with the requirement to enzymatically generate G tails at both leading and lagging strand replication products [38–40]. As a function of the rate of replication associated telomere shortening and initial telomere length a somatic cell can only undergo a defined number of doublings before telomeres become critically short, lose their protective properties, and send cells into a terminal arrest termed replicative senescence, or cell death. This mechanism limits the replicative lifespan of individual cells and likely of some cellular compartments in organisms and therefore represents a tumor suppressive pathway that prevents cells from becoming immortal [41, 42]. Introduction of the catalytic subunit of telomerase into somatic cells is sufficient to counteract replicative telomere shortening [42, 43], however, as a consequence such cells are only rendered immortal, not transformed [44]. Ectopic expression of the catalytic subunit of telomerase extended the life span of cancer resistant mice, demonstrating a role of the telomerase complex in organismal longevity [45]. The fact that most cancers need to activate a telomere length maintenance pathway for survival emphasizes the attraction of this unifying

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principle as a potential target for cancer therapy, and a number of efforts are underway to develop *in vivo* inhibitors of telomerase.

The true length of telomeres that lose their protective function is rather unclear and subject to debate. Yeast cells carrying a single telomere devoid of any telomeric repeats are still capable of several divisions before cell cycle arrest, suggesting that telomere length is not the only determinant of proliferative potential [46]. Telomeres in primary human fibroblasts that enter senescence are readily detectable by imaging techniques that lack the sensitivity of detecting less than 0.5kb of repeat sequence [47], and the bulk of telomeres as determined by southern analysis appears around 4kb [48]. In primary mouse cells, where telomere lengths are far greater than that in human cells, replicative senescence is frequently observed after few population doublings in culture, suggesting the complete loss of telomeric sequence as trigger for the loss of proliferative potential as unlikely. At the same time ultra-short telomeres of ~13 repeats, termed T-stumps have been shown to efficiently protect chromosome ends when cellular proliferation bypasses p53 and p16 imposed checkpoints which sense critically short chromosome ends, and in cancer cells [49]. Therefore, the hypothesis of critically short telomere length, a phrase borrowed from observations of yeast telomeres and defined by sequence loss, apparently does not apply in mammals, where the telomere dependent limitation of the proliferative life span is much more complex.

We suggest that senescence could be triggered by the accumulation of damage and stress in cells, resulting from additive signals of shorter telomeres, increased replicative stress in aging cells, reactive oxygen species, chromatin changes, degradation of the nuclear envelope and pore complexes as well as protein damage [50–53]. The more a single component contributes to the total, the less additional damage is required to exceed the critical threshold, illustrated by the fact that primary cells grown under low oxygen conditions undergo many more population doublings and enter senescence with shorter bulk telomeres.

The role of telomere uncapping in genome instability

Telomeres can lose their protective function when they shorten to a critical length, or when they fail to mask themselves from recognition by the DNA damage repair machinery, even in the presence of long stretches of TTAGGG repeats. The most striking and obvious example of acute telomere uncapping is illustrated by removal of TRF2 from telomeres, either by a dominant negative allele [18, 54, 55], or by targeted deletion of the gene in mouse [56, 57]. Within a few cell divisions all chromosome ends are detected as DNA breaks and then fuse in an ATM pathway dependent manner, leading to the appearance of metaphase chromosomes as a 'plate of spaghetti' [56, 57] (Box 2). POT1, while strongly implicated in telomere protection in yeast [58], has a less pronounced capping phenotype in mammals. Mouse cells possess two POT1 proteins, POT1a and POT1b, suggesting recent expansion of the telomeric complex in rodents [59, 60], and POT1a is sufficient to repress damage signaling at telomeres. The subsequent processing of dysfunctional telomeres is virtually identical to the canonical DDR at intra-chromosomal break sites. The association of 53BP1 (p53 Binding Protein 1), a protein involved in the detection and processing of double strand breaks, with several telomeres creates large chromatin domains conducive for covalent fusion of chromosomes [61, 62]. The build-up of DDR proteins as well as changes in local telomeric chromatin structure exemplified by γH2AX, a variant of histone H2A that localizes to sites of DNA damage, can be visualized by florescence-based techniques such as Telomere-dysfunction Induced Foci (TIFs) (Box 2) that are virtually identical to the foci detected at sites of breaks and repair following irradiation-induced DNA damage [63]. TIFs are observed in almost all settings of engineered telomere dysfunction but also in cells that undergo replicative senescence, blurring the boundary between telomere uncapping and telomere length phenotypes.

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Recently, the accumulation of damage signals within cells has been shown to be required for senescence entry and that DDR components function to suppress the detrimental outcomes that arise from replicative stress [64, 65]. Therefore, the changes in telomeric chromatin and loading of DDR proteins on several telomeres could provide the impetus to exit the cell cycle and induce apoptosis or senescence, depending on cell type. How are these telomeres selected? Cell cycle dependent chromatin dynamics or cell cycle dependent phosphorylation of DDR proteins might influence the choice and timing of these events and addressing this question will be complex but essential in the future.

Many of the phenotypes observed in engineered and acute human and mouse models of telomere dysfunction only manifest in circumstances where p53 and/or pRb pathways are absent or suppressed, allowing cell growth in the presence of DNA damage signals, emphasizing the role of these essential tumor suppressor pathways (Fig 2) in detecting damaged chromosome ends. At the same the involvement of p53 and pRB suggests that dysfunctional telomeres signal in the same way as intra-chromosomal breaks. In addition, contrary to primary human cells, primary mouse fibroblasts display a significant level of telomerase activity, allowing the speculation that extension of the 3'overhang by telomerase has a role in signaling from mouse telomeres, whereas the same effects would not arise in human somatic cells.

Dysfunctional telomeres are also potential substrates for homologous recombination (HR) (Fig 2). Expression of TRF2 lacking the amino-terminal basic domain results in massive loss of telomeric sequence and the formation of extrachromosomal circular arrays of dsTTAGGG repeats, termed t-circles 66, suggesting that this domain is involved in protecting telomeres against inappropriate HR events, potentially by binding to telomeric Holliday junctions and directly inhibiting resolvase activity [67]. The basic TRF2 domain also directly interacts with WRN, a RecQ helicase missing in patients suffering from Werner Syndrome (WS). Therefore, it has been hypothesized that strand invasion that would otherwise be promoted by WRN cannot occur. The exposed G-overhang then engages in rampant HR resulting in sister-telomere loss (STL), sister chromatid exchange (SCE) and other gross chromosomal aberrations. Finally, the recently identified TERRA have been shown to interact with the basic domain of TRF2 [36] and suppression of TERRA leads to changes in heterochromatin, however the molecular pathways are not known. This lends support to the speculation that TERRA might function in the suppression of HR by forming stable higher order structures at telomeres. Solving the enigma of TERRA will undoubtedly prove challenging, judging from the difficulties encountered in analyzing other non-coding RNAs such as *Xist* (X inactive specific transcript), a transcript that plays a major role in the X chromosome inactivation process.

Recognition of telomeres as DNA damage

It is assumed that dysfunctional telomeres are recognized as damage in the same ways the cell detects intrachromosomeal double stranded breaks (Fig 2). However, many factors involved in damage recognition can be found at functional telomeres, suggesting a dual role for the damage machinery in chromosome end protection and detection of uncapped telomeres.

Interactions of telomeres with DNA damage machineries

One of the major functions of telomeres is to shield the natural chromosome ends from inappropriate repair and to distinguish them from intrachromosomal double stranded breaks. It was therefore surprising and seemed paradoxical that many proteins that have a major role in the detection of DNA damage as well as in signaling to DNA damage cascades localize to functional telomeres. The yeast MRX complex (consisting of Mre11, Rad50 and XRN2), as well as the ATM and ATR related kinases TEL1 and MEC1 are all involved in the early phases of detection of DNA damage, they are all found at yeast telomeres, and their deletions lead to telomere length and telomere deprotection phenotypes 68–71. Similarly human cells derived

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from patients suffering from Nijmegen Breakage Syndrome or Ataxia Telangiectasia display accelerated telomere shortening 72, 73, but the pathways that lead to elevated levels of TTAGGG repeat loss are not clear. Also, similarly to yeast, the mammalian MRN complex, consisting of MRE11, RAD50 and NBS1 (the homolog of yeast XRN2), ATM and ATR can be detected at telomeres, suggesting that the DNA damage machinery has a pivotal role at functional and dysfunctional telomeres [74, 75].

TRF2 and POT1 have major roles in the interaction of telomeres with the DNA damage signaling machinery. Human telomeres that lose telomeric TRF2 succumb to ATM and p53 dependent death in cells that are competent for apoptosis [55]. At the same time, TRF2 seems to be capable of directly inhibiting ATM signaling at chromosome ends, by generating an environment where the capability of ATM to autophosphorylate and self-activate is compromised [76, 77]. These preliminary findings suggested a complex role for shelterin components in suppressing ATM and ATR dependent damaging pathways (Fig 2), which was recently molecularly defined by the targeted deletion of TRF2 and POT1 in mice. Analysis of mice lacking combinations of TRF2, POT1, ATM and ATR revealed independently controlled pathways [17]. TRF2 and POT1 were shown to act separately, where TRF2 repressed ATM and POT1 prevented activation of the ATR signaling cascade. When ATM or ATR signaling was inhibited, dysfunctional chromosome ends were not detected as damage, and also evaded NHEJ dependent fusion [17]. Activation of ATM by TRF2 was dependent on the MRN complex, and it is possible that TRF2 inhibits MRN activation at functional telomeres by inhibiting it to bind to the very end of telomeres [62]. At them same time the MRN complex has been suggested to be required for proper processing of telomeres after replication and in processing of dysfunctional telomeres prior to fusion, emphasizing the multiple roles this complex plays at telomeres [62, 75, 78] (Figure 3).

Getting ever closer to the primary signal event that initiates detection of dysfunctional telomeres by the damage machinery it was discovered that binding of 53BP1 to uncapped telomeres is an essential event for NHEJ dependent end to end fusion [61]. Dysfunctional telomeres bound to 53BP1 were found to be significantly more mobile than such telomeres without 53BP1, suggesting that altering telomere mobility enhanced the chances of finding a partner telomere for fusion, facilitating NHEJ repair reactions that involve distant sites [61].

Taken together, it is becoming increasingly clear that the interactions of functional and dysfunctional telomeres with the damage machineries are highly complex and that many levels of interaction exist. Functional telomeres require the damage machinery for efficient replication of TTAGGG repeats, processing of the ends after replication and for formation of a protective cap at chromosome ends. Dysfunctional telomeres require the same machinery for detection of uncapped telomeres, for processing of those uncapped telomeres for repair and for mobility to enhance the efficiency of repair. Adding to the complexity are individual and specific interactions of shelterin components with the signaling molecules in the damage repair cascades, and at this point the field has not even scratched the surface of post transcription modifications that are expected to also play a role in protection and signaling from telomeres.

Amplification of the signal

While it is becoming clearer how telomeres interact with the damage machinery and how uncapped telomeres initiate signaling cascades, it is not known how the localized damage signal at chromosome ends gets translated to a response affecting the whole cell. ATM comes to mind as a molecule capable of amplifying damage signals, as it has been suggested that localized damage leads to phosphorylation of most of the ATM molecules in the cell, potentially via changes in chromatin structure [79] (Box 3). However, the specialized nature of the telomeric environment, where ATM is directly suppressed locally argues against this possibility [76, 77],

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supported by the finding that local ATM activation can occur at telomeres in the absence of a response that spreads throughout the nucleus [75].

Nonetheless, it is likely that chromatin plays a role in signal amplification, as changes in chromatin structure can efficiently spread through the nucleus and could be established and re-established rapidly in every cell cycle. Telomeric and subtelomeric chromatin in mice displays marks of heterochromatin, particularly trimethylation of histone H3 Lys9 (H3K9) and H4K20 (Box 3). Increasing evidence suggests that histone and DNA methylation and the enzymes involved in these events play a key role in regulating telomere length, and these events are also likely to play a role in signaling from dysfunctional telomeres [7, 80–83] (Box 3).

To date little is known about global chromatin changes upon replication associated telomere shortening or telomere dysfunction, and it is not clear yet whether the long telomeres in mice directly compare to short human telomeres. It will be exciting to see whether signaling from telomeres is involved in global changes in chromatin and histone modifications, and whether this is the mechanism that translates localized damage signals to a nucleus-wide level.

Telomeres as sources of genome instability

In 1938 Barbara McClintock noticed that chromosomes in plants that had previously been irradiated with x-rays engaged in spontaneous chromosome breakage-fusion-bridge cycles, laying the foundation for the hypothesis that aberrantly fused chromosomes would break in the subsequent cell division, leading the unequal and random distribution of genetic material into the daughter cells [84] (Figure 3). Dysfunctional telomeres that fail to be distinguished from broken DNA lend themselves perfectly to the hypothesis that loss of chromosome end protection could lead to genome instability via McClintock's breakage-fusion-bridge cycles. This hypothesis was formally proven in mice, when targeted deletion of the RNA subunit of telomerase rendered the complex inactive for telomere length maintenance [85]. As a consequence telomeres shortened progressively by approximately 5kb per generation and after four generations telomeres lacking TTAGGG signals were detected. Loss of telomeric sequences led to chromosome end-to end fusions, chromosomal abnormalities and aneuploidy, formally supporting the suggestion that loss of chromosome end protection can be the basis for genome instability in mammals.

However, these data originally seemed inconsistent with the finding that telomerase is found activated in the majority of human tumors [86] and with the finding that cells that fail to maintain their telomeres succumb to replicative senescence or cell death. Again, the p53 dependent signaling pathway emerged as a key player, when it was discovered that the deletion of p53 attenuated the adverse effects of telomere dysfunction in late generations of mice lacking an active telomerase complex. Consequently it was suggested that the loss of telomerase and p53 cooperate to promote cellular transformation [87]. Telomere attrition in a mouse model lacking p53 similarly led to breakage-fusion-bridge cycle based genome instability and to a massive increase in non-reciprocal translocations, resulting in the development of epithelial cancers, formally linking telomere dysfunction with genome instability and malignancy [88] (Figure 3). Eventually a model emerged that recognizes and emphasizes the dual role that telomerase plays [89]: When telomerase is activated in cells that entered breakage-fusion cycles and have lost tumor suppressive pathways such as p53 and pRb, telomeres are stabilized and immortality in the presence of genome instability is promoted, pushing cells towards malignancy. On the other hand, when telomerase is activated before telomeres become critically short, telomeres never reach a state where they are processed by the NHEJ machinery and fused. In this case telomerase acts to suppress the accumulation of chromosomal aberrations and genome instability, and, while controversial, it has been argued that bursts of telomerase might be useful to counteract transformation and cancer development.

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Telomere driven genome instability can also ensue in the presence of long stretches of double stranded telomeric repeats. When TRF2 was removed from telomeres by expression of a dominant negative allele, chromosome ends fused displaying long stretches of TTAGGG repeats at the fusion sites [54] (Box 2). When cells continued to cycle, the chromosomes entered fusion-breakage cycles resulting in non-reciprocal translocations and genome instability, demonstrating that a telomere capping dysfunction can lead to the same outcome as telomere dysfunction due to catastrophic loss of telomeric repeats [18]. One would expect that partial or complete loss of TRF2 therefore could be implicated in cancer formation, however few evidence for this hypothesis has been found so far. TRF1 and TRF2 have been found up regulated in gastric carcinoma and during hepatocarcinogenesis, however, it is not intuitively obvious how overexpression of these proteins might contribute to cancer formation [90, 91]. When TRF2 was overexpressed in basal and stem cells of the epidermis skin cancer levels increased in mice, again suggesting that destabilization of the shelterin complex by overexpression or removal of individual complex members can lead to malignancy [92, 93].

Telomeres, due to their G rich and repetitive nature, represent obstacles to the replication fork and require telomeric proteins for efficient duplication, as has been originally observed in *Schizosaccharomyces pombe* [94]. Targeted deletion of TRF1 in mice revealed that telomeres represented fragile sites and replication forks stalled without the aid of this telomeric factor [8]. It remains to be established whether TRF1 driven replication defects directly contribute to genome instability, but it has been noted that mice that harbor a deletion of TRF1 in the stratified epithelia display epithelia dysplasia and develop squamous cell carcinomas in a p53 negative background [9].

The RecQ helicase WRN also contributes to efficient telomere replication. Over-expression of a dominant negative allele of WRN led to the occasional loss of telomeres generated by the lagging-strand machinery, a phenotype that was counteracted by telomerase [47]. Accordingly, targeted deletion of WRN in the mouse only lead to phenotypes that resemble the human Werner Syndrome when telomerase was co-deleted [95]. However, in the case of WRN deletion there is little doubt that telomere driven genome instability ensues, as cells deficient for telomerase and WRN accumulate chromosomal aberrations and elevated recombination rates between telomeres of sister chromatids [96], and the genomic instability observed in human Werner Syndrome cells was directly dependent on telomere function [97].

Taken together there is no doubt that telomere dysfunction, be it due to loss of telomeric sequences, loss of telomere capping or end protection and due to telomere replication problems can lead to genome instability and cancer. It remains to be investigated whether mutations or aberrant expression patterns of shelterin components and shelterin associating factors play direct roles in tumorigenesis in humans, and whether such proteins lend themselves as targets for cancer therapy.

Telomere dysfunction in disease

While the link between telomeres, telomere dysfunction, telomere replication and cancer has been well established, there are no telomere- or telomerase based therapies so far. Small molecule telomerase inhibitors, while working well *in vitro* and in tissue culture, still await to pass clinical trials and to be established in standard care. Similarly, efforts to generate telomerase based cancer vaccines so far have failed to lead to viable therapies. While the approaches are promising and telomere length maintenance and telomere function make for unifying cancer targets, only time and effort will tell whether such approaches are crowned by success.

Recently telomere function has been directly implicated in two additional diseases, Dyskeratosis Congenita (DC) and Idiopathic Pulmonary Fibrosis (IPF). DC is an inherited

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disease marked by bone marrow failure, abnormal skin pigmentation, nail dystrophy and leucoplakia [98, 99]. X-linked recessive DC is caused by mutations in dyskerin, a protein that associates with a subgroup of small nucleolar RNAs, but also with the RNA component of telomerase, TERC, an association that stabilizes the telomerase complex 100. Autosomal dominant DC has been linked to mutations in TERC itself 101, in the catalytic telomerase subunit TERT [102], and also in the shelterin component TIN2 [103, 104]. One unifying feature in DC is short telomeres, and the importance of limiting telomere length has been emphasized by the observation that mice that suffer from extensive telomere shortening due to the lack of Pot1b suffer from clear signs of DC [105].

IPF is a lung disorder marked by progressive scarring, leading to destruction of lung architecture with a frequently fatal outcome. The discovery that short telomeres correlated with the disease eventually led to the finding that heterozygous mutations in TERT or TERC can be the cause of the disease [106, 107]. It is not obvious how short telomeres, usually associated with limited proliferation potential of stem cells or other rapidly dividing tissues can lead to a scarring phenotype in lungs only, but it will be exciting to learn about the implicated pathways in the future.

Conclusions and perspectives

Telomere biology has come far in the recent 70 years, from the observation by McClintock that chromosomes need protection, to the award of the 2009 Nobel price in Physiology and Medicine to Elizabeth Blackburn, Carol Greider and Jack Szostak for the discovery of telomerase and the effects of telomere shortening on cells. Telomere function has been implicated in the replicative aging process and shown to play a major role in the establishment of genome instability in cancer development. The understanding of mammalian telomeric proteins has progressed from the idea that they were simply covering the telomeric repeats, to the telomeric core complex shelterin, whose members regulate telomere length, individual signaling cascades at telomeres, aid in telomere processing, interact with the DNA damage machineries in many ways and attract many other factors to telomeres transiently. Telomeric proteins and telomere length regulation have been implicated in stem cell management and various diseases.

However, some major questions remain: It is not clear what the primary signal is that detects critically short or uncapped telomeres. It is not known how the signal gets translated and amplified throughout the nucleus, and whether this cascade plays a role in organismal aging and disease. It remains to be discovered how functional telomeres change throughout the lifespan of an organism, and whether telomeric proteins are modified during the cell cycle or during aging. It has not been understood how mutations in individual telomeric proteins impact on diseases such as DC and IPF. And finally, while the connection between telomere shortening, telomere dysfunction and cancer is now abundantly clear, this knowledge has not yet translated to disease management.

Box 1. The telomerase complex

The discovery by Greider and Blackburn that telomeric repeats are added *de novo* to the end of eukaryotic chromosomes by telomerase is one of the most important findings in the recent history of molecular biology [108]. The telomerase complex was biochemically purified and consists of the catalytic reverse transcriptase, TERT (TElomerase Reverse Transcriptase), the RNA subunit TR (Telomerase template RNA) that provides the template for repeat synthesis at chromosome ends and Dyskerin, a key auxillary protein [109]. This complex is assembled in Cajal Bodies within the nucleus and shuttled to telomeres by an accessory factor, TCAB1 (Telomerase CAjal Body protein 1) [31]. The ATPases Pontin and

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Reptin sequester this immature complex into an active conformation, whereupon it associates with the terminal exposed 3' hydroxyl group and initiates nucleotide addition at the chromosome ends [30]. For many years, it was thought that telomerase preferentially elongated the shortest telomeres in the cell [110, 111], however, recent studies have suggested that telomerase is more promiscuous and randomly targets telomeres during S-phase in cancer cells [112]. By doing so, telomerase counteracts the generational shortening of telomeres, maintaining telomere length and stability. This latter property is essential for highly proliferative cells such as stem cells and lineage progenitors, perhaps as a requisite for "stemness". Mice lacking the RNA subunit display diminished stem cell compartments and reduced stem cell proliferation, differentiation and self-renewal [113]. This manifests as a phenotype of accelerated ageing and atrophy of key tissues in those animals lacking functional telomerase [114].

Recently non-telomeric functions for mammalian TERT have been suggested, which include the regulation of global chromatin dynamics, stem cell proliferation and transcription of developmentally regulated genes [29, 115, 116].

Box 2. Cytological representation of telomere dysfunction

Covalent fusions are efficiently detected by spreading of metaphase chromosomes and marking of telomeres by fluorescent *in situ* hybridization (A). This allows for the distinction of fusions in the presence (left panel) and the absence (center panel) of telomeric repeats. The right panel represents a metaphase where all chromosomes have been fused due to deletion of TRF2 (image kindly provided by E. Lazzerini Denchi). DNA has been stained in red, and telomeric repeats in green.

Telomeres that are detected as damage can be visualized as telomere dysfunction induced foci (TIFs) (B). Cells are simultaneously stained with antibodies against factors that localize to sites of damage and with probes recognizing telomeric DNA. While the chance for telomeric colocalization with intra-chromosomal damage sites is slim, the merged images clearly designate telomeres that bind damage factors, and therefore can be considered as recognized by the damage machinery. Here the DNA of an interphase nucleus has been stained in the blue channel, telomeres in green, and γH2AX (upper panel) or 53BP1 (lower panel) in red. Yellow dots (indicated by white arrows) in the merge represent the colocalization of γH2AX or 53BP1 with telomeres.

ALT associated PML bodies (APBs) (C) are structures in cells that employ the recombination based ALT (Alternative Lengthening of Telomeres) mechanism for telomere length regulation [117,] 118. They represent loci that contain telomeric DNA, telomeric proteins and factors involved in DNA metabolism. Here the DNA of an interphase nucleus has been stained in blue, PML in green and TRF2 as marker for telomeres in red. The merge of all 3 colors is on the right. It has been proposed that APBs represent the sites of telomeric recombination, however, they could also simply serve as storage pools for proteins required for ALT and ALT by-products.

Box 3. Mammalian Telomeric Chromatin

In human chromatin, 147bp of DNA are wrapped around nucleosomes, comprised of histones H3, H4, H2A and H2B [119]. The amino terminal tails of histones are subject to an elaborate system of post-translational modification (PTM), namely through the addition and removal of acetyl, methyl, phospho and ubiquitin groups. These are viewed as epigenetic marks and function by extending the informational capacity of cells [120]. The distribution

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of these marks at diverse regions across the genome results in the simplified characterization of regions as being either heterochromatic or euchromatic.

G1 phase telomeric chromatin bears hallmarks of constitutive heterochromatin, which are H3K9me3, H4K20me3 and HP1 binding 36, 121 (top panel, right). Subtelomeric chromatin is distinctive by a regular nucleosomal distribution, harbors extensive DNA methylation 122 and histone PTMs distinct from those at telomeres (top panel, left). The replication of DNA during S-phase coincides with the disruption and restoration of the parental chromatin identity. Newly synthesized histones are acetylated at H4 lysines 5, 12 and 16 and H3 lysines 9 and 56 123. The removal of these acetyl groups at telomeres by SIRT proteins appears to be important for regulating the association of accessory proteins to telomeres, as exhibited by the relationship between H3K9ac, SIRT6 and WRN function 124 (middle panel).

Changes in chromatin structure have been shown to occur at dysfunctional telomeres [7]. These are often similar to those exhibited at sites of DNA damage, such as phosphorylation of H2AX, changes H4K20me2 and recruitment of 53BP1, implying a general "epigenetic" stress response (lower panel). However, it is still unclear whether changes seen at dysfunctional telomeres are proactive or merely responsive to changes in telomeric architecture. Nuclear reprogramming also leads to dramatic changes in telomeric chromatin and telomere length, emphasizing the dynamic and developmentally regulated nature of chromosome ends [125].

Online summary

- Telomeric proteins control telomere length and telomere integrity. The six bona fide telomeric binding proteins form shelterin, a complex that maintains chromosome end integrity.
- Telomere dysfunction can be caused by loss of telomeric repeats or by loss of protective features, both of which are essential for telomere function.
- Functional telomeres interact with the DNA damage machinery, but the machinery is prevented form processing these ends. Dysfunctional telomeres are recognized as damage and repaired.
- Repair of dysfunctional telomeres by fusion propels cells into breakage-fusion-bridge cycles, resulting in unequal distribution of genetic material into daughter cells, and hence, genome instability.
- Telomere dysfunction and the failure to maintain telomere length is emerging as the cause for a number of diseases syndromes.

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Glossary terms

G-quadruplex	G-quadruplexes consist of G quartets, where guanosine residues serve as donors and acceptors in a G-G base pair, a structure that represents an obstacle to the moving replication fork.
Fragile site	A fragile site is a location in the chromosome where breaks occur frequently.

Displacement loop	A displacement loop is a single stranded DNA loop, resulting from the invasion and pairing of a DNA end into homologous double stranded sequences.
Replicative senescence	Replicative senescence is a permanently differentiated state that cells enter when their telomeres become critically short, or a threshold of DNA damage is exceeded.
Homologous recombination (HR)	HR is a repair pathway where homologous sequences align and genetic information is copied from one DNA strand to the other.
Werner Syndrome (WS)	WS is an inherited genetic disease characterized by premature aging symptoms and the early onset of cancer.
Sister-telomere loss (STL)	STL is defined by the loss of telomeric sequences from a single sister chromatid, while the other telomere stays intact.
Sister chromatid exchange (SCE)	SCE is the HR based exchange of DNA strands between sister chromatids.
Non-reciprocal translocation	A non reciprocal translocation is defined by the transfer of genetic information from one non homologous chromosome to another.
Nijmegen Breakage Syndrome	Nijmegen breakage syndrome, is a rare syndrome characterized by chromosomal instability, as a result of mutations in the NBS1 gene.
Ataxia Telangiectasia (AT)	AT is a rare inherited disease, characterized by neurodegeneration, cancer susceptibility and radiation sensitivity, caused by mutations in the AT kinase gene.

References

1. Verdun RE, Karlseder J. Replication and protection of telomeres. Nature 2007;447:924–931. [PubMed: 17581575]
2. Watson JD. Origin of concatemeric T7 DNA. Nat. New Biol 1972;239:197–201. [PubMed: 4507727]
3. Griffith JD, et al. Mammalian telomeres end in a large duplex loop. Cell 1999;97:503–514. [PubMed: 10338214] This mansucript details the finding that telomeres can form a looped structure that could distinguish natural chromosome ends form DNA breaks.
4. Oganesian L, Bryan TM. Physiological relevance of telomeric G-quadruplex formation: a potential drug target. Bioessays 2007;29:155–165. [PubMed: 17226803]
5. Rhodes D, Giraldo R. Telomere structure and function. Curr Opin Struct Biol 1995;5:311–322. [PubMed: 7583629]
6. Cacchione S, Cerone MA, Savino M. In vitro low propensity to form nucleosomes of four telomeric sequences. FEBS Lett 1997;400:37–41. [PubMed: 9000509]
7. Blasco MA. The epigenetic regulation of mammalian telomeres. Nat Rev Genet 2007;8:299–309. [PubMed: 17363977] This publication summarizes the current knowledge of histone and DNA modifications at telomeres.
8. Sfeir A, et al. Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. Cell 2009;138:90–103. [PubMed: 19596237]
9. Martinez P, et al. Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. Genes Dev 2009;23:2060–2075. [PubMed: 19679647]
10. Raghuraman MK, et al. Replication dynamics of the yeast genome. Science 2001;294:115–121. [PubMed: 11588253]
11. Palm W, de Lange T. How shelterin protects mammalian telomeres. Annu Rev Genet 2008;42:301–334. [PubMed: 18680434]

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12. Bianchi A, Smith S, Chong L, Elias P, de Lange T. TRF1 is a dimer and bends telomeric DNA. EMBO J 1997;16:1785–1794. [PubMed: 9130722]
13. Griffith J, Bianchi A, de Lange T. TRF1 promotes parallel pairing of telomeric tracts in vitro. J Mol Biol 1998;278:79–88. [PubMed: 9571035]
14. Amiard S, et al. A topological mechanism for TRF2-enhanced strand invasion. Nat Struct Mol Biol 2007;14:147–154. [PubMed: 17220898]
15. de Lange T. Protection of mammalian telomeres. Oncogene 2002;21:532–540. [PubMed: 11850778]
16. Stansel RM, de Lange T, Griffith JD. T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. Embo J 2001;20:5532–5540. [PubMed: 11574485]
17. Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature 2007;448:1068–1071. [PubMed: 17687332] This manuscript highlights the control of the ATM and ATR dependent DNA damage response pathways by distinct shelterin components.
18. Smogorzewska A, Karlseder J, Holtgreve-Grez H, Jauch A, de Lange T. DNA ligase IV-dependent NHEJ of deprotected mammalian telomeres in G1, and G2. Curr. Biol 2002;12:1635–1644. [PubMed: 12361565]
19. Benetti R, Schoeftner S, Munoz P, Blasco MA. Role of TRF2 in the assembly of telomeric chromatin. Cell Cycle 2008;7:3461–3468. [PubMed: 18971622]
20. Li B, Oestreich S, de Lange T. Identification of human Rap1: implications for telomere evolution. Cell 2000;101:471–483. [PubMed: 10850490]
21. Bae NS, Baumann P. A RAP1/TRF2 complex inhibits nonhomologous end-joining at human telomeric DNA ends. Mol Cell 2007;26:323–334. [PubMed: 17499040]
22. Sarthy J, Bae NS, Scrafford J, Baumann P. Human RAP1 inhibits non-homologous end joining at telomeres. EMBO J. 2009
23. Loayza D, De Lange T. POT1 as a terminal transducer of TRF1 telomere length control. Nature 2003;424:1013–1018. [PubMed: 12944955]
24. Xin H, et al. TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. Nature 2007;445:559–562. [PubMed: 17237767]
25. Wang F, et al. The POT1-TPP1 telomere complex is a telomerase processivity factor. Nature 2007;445:506–510. [PubMed: 17237768]
26. Dejardin J, Kingston RE. Purification of proteins associated with specific genomic Loci. Cell 2009;136:175–186. [PubMed: 19135898]
27. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev 2005;19:2100–2110. [PubMed: 16166375]
28. de Lange T. The Protein Components of Mammalian Telomeres. Ann Reviews in Biochemistry, in preparation. 2002
29. Park JI, et al. Telomerase modulates Wnt signalling by association with target gene chromatin. Nature 2009;460:66–72. [PubMed: 19571879]
30. Venteicher AS, Meng Z, Mason PJ, Veenstra TD, Artandi SE. Identification of ATPases pontin and reptin as telomerase components essential for holoenzyme assembly. Cell 2008;132:945–957. [PubMed: 18358808] This manuscript describes the essential role of ATPases in assembly of the functional telomerase complex.
31. Venteicher AS, et al. A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. Science 2009;323:644–648. [PubMed: 19179534] This publication illustrates the requirement of the telomerase complex to pass through the Cajal bodies for assembly.
32. Luke B, et al. The Rat1p 5' to 3' exonuclease degrades telomeric repeat-containing RNA and promotes telomere elongation in Saccharomyces cerevisiae. Mol Cell 2008;32:465–477. [PubMed: 19026778]
33. Azzalin CM, Reichenbach P, Khoriauli L, Giulotto E, Lingner J. Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science 2007;318:798–801. [PubMed: 17916692]
34. Schoeftner S, Blasco MA. Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat Cell Biol 2008;10:228–236. [PubMed: 18157120] The two

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manuscripts by Azzalin and Schoeftner recognize that telomeres can be transcribed as long repeat containing RNAs.

35. Luke B, Lingner J. TERRA: telomeric repeat-containing RNA. EMBO J 2009;28:2503–2510. [PubMed: 19629047]
36. Deng Z, Norseen J, Wiedmer A, Riethman H, Lieberman PM. TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres. Mol Cell 2009;35:403–413. [PubMed: 19716786]
37. Schoeftner S, Blasco MA. Chromatin regulation and non-coding RNAs at mammalian telomeres. Semin Cell Dev Biol. 2009
38. Makarov VL, Hirose Y, Langmore JP. Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. Cell 1997;88:657–666. [PubMed: 9054505]
39. McElligott R, Wellinger RJ. The terminal DNA structure of mammalian chromosomes. EMBO J 1997;16:3705–3714. [PubMed: 9218811]
40. Wright WE, Tesmer VM, Huffman KE, Levene SD, Shay JW. Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes & Dev 1997;11:2801–2809. [PubMed: 9353250]
41. Lundblad V, Szostak JW. A mutant with a defect in telomere elongation leads to senescence in yeast. Cell 1989;57:633–643. [PubMed: 2655926]
42. Bodnar AG, et al. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279:349–352. [PubMed: 9454332]
43. Meyerson M, et al. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 1997;90:785–795. [PubMed: 9288757] The two manuscripts by Bodnar and Meyerson describe the isolation and characterization of the catalytic subunit of human mtelomerase.
44. Morales CP, et al. Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 1999;21:115–118. [PubMed: 9916803]
45. Tomas-Loba A, et al. Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell 2008;135:609–622. [PubMed: 19013273] This manuscript analyzes the effects of telomerase expression on the lifespan of mice rendered cancer resistant by the overexpression tumor suppressors.
46. Abdallah P, et al. A two-step model for senescence triggered by a single critically short telomere. Nat Cell Biol 2009;11:988–993. [PubMed: 19597486]
47. Crabbe L, Verdun RE, Haggblom CI, Karlseder J. Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. Science 2004;306:1951–1953. [PubMed: 15591207]
48. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. Science 2002;295:2446–2449. [PubMed: 11923537]
49. Xu L, Blackburn EH. Human cancer cells harbor T-stumps, a distinct class of extremely short telomeres. Mol Cell 2007;28:315–327. [PubMed: 17964269]
50. Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science 2003;299:1751–1753. [PubMed: 12610228]
51. D'Angelo MA, Raices M, Panowski SH, Hetzer MW. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. Cell 2009;136:284–295. [PubMed: 19167330]
52. Ouellette MM, McDaniel LD, Wright WE, Shay JW, Schultz RA. The establishment of telomerase-immortalized cell lines representing human chromosome instability syndromes. Hum Mol Genet 2000;9:403–411. [PubMed: 10655550]
53. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. Curr Opin Genet Dev 2001;11:98–103. [PubMed: 11163158]
54. van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. Cell 1998;92:401–413. [PubMed: 9476899] This manuscript recognizes the protective role of TRF2 at mammalian telomeres.
55. Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 1999;283:1321–1325. [PubMed: 10037601]

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56. Lazzerini Denchi E, Celli G, de Lange T. Hepatocytes with extensive telomere deprotection and fusion remain viable and regenerate liver mass through endoreduplication. Genes Dev 2006;20:2648–2653. [PubMed: 17015429]
57. Celli GB, de Lange T. DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. Nat Cell Biol 2005;7:712–718. [PubMed: 15968270]
58. Baumann P, Cech TR. Pot1, the putative telomere end-binding protein in fission yeast and humans. Science 2001;292:1171–1175. [PubMed: 11349150] This manuscript describes the isolation and characterization of the single stranded telomeric DNA binding factor POT1.
59. Hockemeyer D, Daniels JP, Takai H, de Lange T. Recent expansion of the telomeric complex in rodents: Two distinct POT1 proteins protect mouse telomeres. Cell 2006;126:63–77. [PubMed: 16839877]
60. Wu L, et al. Pot1 deficiency initiates DNA damage checkpoint activation and aberrant homologous recombination at telomeres. Cell 2006;126:49–62. [PubMed: 16839876]
61. Dimitrova N, Chen YC, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. Nature 2008;456:524–528. [PubMed: 18931659]
62. Dimitrova N, de Lange T. Cell cycle dependent role of MRN at dysfunctional telomeres: ATM signaling-dependent induction of NHEJ in G1 and resection-mediated inhibition of NHEJ in G2. Mol Cell Biol. 2009
63. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. Curr Biol 2003;13:1549–1556. [PubMed: 12956959]
64. Bartek J, Bartkova J, Lukas J. DNA damage signalling guards against activated oncogenes and tumour progression. Oncogene 2007;26:7773–7779. [PubMed: 18066090]
65. Bartek J, Lukas J, Bartkova J. DNA damage response as an anti-cancer barrier: damage threshold and the concept of 'conditional haploinsufficiency'. Cell Cycle 2007;6:2344–2347. [PubMed: 17700066]
66. Wang RC, Smogorzewska A, de Lange T. Homologous recombination generates T-loop-sized deletions at human telomeres. Cell 2004;119:355–368. [PubMed: 15507207]
67. Poulet A, et al. TRF2 promotes, remodels and protects telomeric Holliday junctions. Embo J 2009;28:641–651. [PubMed: 19197240]
68. Nakamura TM, Moser BA, Russell P. Telomere binding of checkpoint sensor and DNA repair proteins contributes to maintenance of functional fission yeast telomeres. Genetics 2002;161:1437–1452. [PubMed: 12196391]
69. Nugent CI, et al. Telomere maintenance is dependent on activities required for end repair of double-strand breaks. Curr. Biol 1998;8:657–660. [PubMed: 9635193]
70. Takata H, Kanoh Y, Gunge N, Shirahige K, Matsuura A. Reciprocal association of the budding yeast ATM-related proteins Tel1 and Mec1 with telomeres in vivo. Mol Cell 2004;14:515–522. [PubMed: 15149600]
71. Tsukamoto Y, Taggart AK, Zakian VA. The role of the Mre11-Rad50-Xrs2 complex in telomerase-mediated lengthening of Saccharomyces cerevisiae telomeres. Curr Biol 2001;11:1328–1335. [PubMed: 11553325]
72. Vaziri H, et al. ATM-dependent telomere loss in aging human diploid fibroblasts and DNA damage lead to the post-translational activation of p53 protein involving poly(ADP-ribose) polymerase. Embo J 1997;16:6018–6033. [PubMed: 9312059]
73. Ranganathan V, et al. Rescue of a telomere length defect of Nijmegen breakage syndrome cells requires NBS and telomerase catalytic subunit. Curr Biol 2001;11:962–966. [PubMed: 11448772]
74. Zhu XD, Kuster B, Mann M, Petrini JH, de Lange T. Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat Genet 2000;25:347–352. [PubMed: 10888888]
75. Verdun RE, Crabbe L, Haggblom C, Karlseder J. Functional Human Telomeres Are Recognized as DNA Damage in G2 of the Cell Cycle. Mol Cell 2005;20:551–561. [PubMed: 16307919]
76. Bradshaw PS, Stavropoulos DJ, Meyn MS. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. Nat Genet 2005;37:193–197. [PubMed: 15665826]
77. Karlseder J, et al. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. PLoS Biol 2, E240. 2004

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78. Deng Y, Guo X, Ferguson DO, Chang S. Multiple roles for MRE11 at uncapped telomeres. Nature 2009;460:914–918. [PubMed: 19633651]

79. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 2003;421:499–506. [PubMed: 12556884]

80. Benetti R, Garcia-Cao M, Blasco MA. Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. Nat Genet 2007;39:243–250. [PubMed: 17237781]

81. Benetti R, et al. Suv4–20h deficiency results in telomere elongation and derepression of telomere recombination. J Cell Biol 2007;178:925–936. [PubMed: 17846168]

82. Garcia-Cao M, O'Sullivan R, Peters AH, Jenuwein T, Blasco MA. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat Genet 2004;36:94–99. [PubMed: 14702045]

83. Gonzalo S, et al. DNA methyltransferases control telomere length and telomere recombination in mammalian cells. Nat Cell Biol 2006;8:416–424. [PubMed: 16565708]

84. McClintockBThe fusion of broken ends of sister half chromatids following chromatid breakage at meiotic anaphase. Mo. Agric. Exp Station Res. Bull1938290,1 – 48

85. Blasco MA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 1997;91:25–34. [PubMed: 9335332] This manuscript is the first one to describe the effects of telomerase inhibition on a chromosomal and organismal level in mammals.

86. Shay JW, Wright WE. Telomerase activity in human cancer. Curr Opin Oncol 1996;8:66–71. [PubMed: 8868103]

87. Chin L, et al. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 1999;97:527–538. [PubMed: 10338216]

88. Artandi SE, et al. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 2000;406:641–645. [PubMed: 10949306] This paper shows that telomere dysfunction leads to the development of epithelial cancers in aging mice lacking p53.

89. Artandi SE, DePinho RA. A critical role for telomeres in suppressing and facilitating carcinogenesis. Curr Opin Genet Dev 2000;10:39–46. [PubMed: 10679392]

90. Matsutani N, et al. Expression of telomeric repeat binding factor 1 and 2 and TRF1-interacting nuclear protein 2 in human gastric carcinomas. Int J Oncol 2001;19:507–512. [PubMed: 11494028]

91. Oh BK, Kim YJ, Park C, Park YN. Up-regulation of telomere-binding proteins, TRF1, TRF2, and TIN2 is related to telomere shortening during human multistep hepatocarcinogenesis. Am J Pathol 2005;166:73–80. [PubMed: 15632001]

92. Blanco R, Munoz P, Flores JM, Klatt P, Blasco MA. Telomerase abrogation dramatically accelerates TRF2-induced epithelial carcinogenesis. Genes Dev 2007;21:206–220. [PubMed: 17234886]

93. Munoz P, Blanco R, Flores JM, Blasco MA. XPF nuclease-dependent telomere loss and increased DNA damage in mice overexpressing TRF2 result in premature aging and cancer. Nat Genet 2005;37:1063–1071. [PubMed: 16142233]

94. Miller KM, Rog O, Cooper JP. Semi-conservative DNA replication through telomeres requires Taz1. Nature 2006;440:824–828. [PubMed: 16598261]

95. Chang S, et al. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nat Genet 2004;36:877–882. [PubMed: 15235603]

96. Laud PR, et al. Elevated telomere-telomere recombination in WRN-deficient, telomere dysfunctional cells promotes escape from senescence and engagement of the ALT pathway. Genes Dev 2005;19:2560–2570. [PubMed: 16264192]

97. Crabbe L, Jauch A, Naeger CM, Holtgreve-Grez H, Karlseder J. Telomere dysfunction as a cause of genomic instability in Werner syndrome. Proc Natl Acad Sci U S A 2007;104:2205–2210. [PubMed: 17284601]

98. Mason PJ. Stem cells, telomerase and dyskeratosis congenita. Bioessays 2003;25:126–133. [PubMed: 12539238]

99. Mason PJ, Wilson DB, Bessler M. Dyskeratosis congenita -- a disease of dysfunctional telomere maintenance. Curr Mol Med 2005;5:159–170. [PubMed: 15974869]

100. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature 1999;402:551–555. [PubMed: 10591218]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

101. Vulliamy T, et al. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 2001;413:432–435. [PubMed: 11574891]
102. Vulliamy TJ, et al. Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. Blood Cells Mol Dis 2005;34:257–263. [PubMed: 15885610]
103. Savage SA, et al. TINF2, a component of the shelterin telomere protection complex, is mutated in dyskeratosis congenita. Am J Hum Genet 2008;82:501–509. [PubMed: 18252230]
104. Walne AJ, Vulliamy T, Beswick R, Kirwan M, Dokal I. TINF2 mutations result in very short telomeres: analysis of a large cohort of patients with dyskeratosis congenita and related bone marrow failure syndromes. Blood 2008;112:3594–3600. [PubMed: 18669893]
105. Hockemeyer D, Palm W, Wang RC, Couto SS, de Lange T. Engineered telomere degradation models dyskeratosis congenita. Genes Dev 2008;22:1773–1785. [PubMed: 18550783]
106. Alder JK, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A 2008;105:13051–13056. [PubMed: 18753630]
107. Armanios MY, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med 2007;356:1317–1326. [PubMed: 17392301]
108. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 1987;51:887–898. [PubMed: 3319189] This manuscript is the first one to suggest that telomerase is required for elongation of chromosome ends in eukaryotes.
109. Cohen SB, et al. Protein composition of catalytically active human telomerase from immortal cells. Science 2007;315:1850–1853. [PubMed: 17395830]
110. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 2001;107:67–77. [PubMed: 11595186]
111. Marcand S, Brevet V, Gilson E. Progressive *cis*-inhibition of telomerase upon telomere elongation. EMBO J 1999;18:3509–3519. [PubMed: 10369690]
112. Zhao Y, et al. Telomere extension occurs at most chromosome ends and is uncoupled from fill-in in human cancer cells. Cell 2009;138:463–475. [PubMed: 19665970]
113. Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol 2007;3:640–649. [PubMed: 17876321]
114. Rudolph KL, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 1999;96:701–712. [PubMed: 10089885]
115. Masutomi K, et al. The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. Proc Natl Acad Sci U S A 2005;102:8222–8227. [PubMed: 15928077]
116. Sarin KY, et al. Conditional telomerase induction causes proliferation of hair follicle stem cells. Nature 2005;436:1048–1052. [PubMed: 16107853]
117. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 1995;14:4240–4248. [PubMed: 7556065]
118. Yeager TR, et al. Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer Res 1999;59:4175–4179. [PubMed: 10485449]
119. Luger K. Structure and dynamic behavior of nucleosomes. Curr Opin Genet Dev 2003;13:127–135. [PubMed: 12672489]
120. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693–705. [PubMed: 17320507]
121. Caslini C, Connelly JA, Serna A, Broccoli D, Hess JL. MLL associates with telomeres and regulates telomeric repeat-containing RNA transcription. Mol Cell Biol 2009;29:4519–4526. [PubMed: 19528237]
122. Lister R, et al. Human DNA methylomes at base resolution show widespread epigenomic differences. Nature. 2009
123. Probst AV, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. Nat Rev Mol Cell Biol 2009;10:192–206. [PubMed: 19234478]
124. Michishita E, et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 2008;452:492–496. [PubMed: 18337721]

125. Marion RM, et al. Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. Cell Stem Cell 2009;4:141–154. [PubMed: 19200803]

Biographies

Jan Karlseder was educated in Austria and got his Ph. D. from the University of Vienna. He then worked as a postdoc in Titia de Lange's laboratory at the Rockefeller University, before joining the Salk Institute as faculty in 2002. The Karlseder laboratory is interested in telomere metabolism and aging, using mammalian cells and nematodes as model systems.

Rodderick J. O'Sullivan achieved his BA at the Trinity College in Dublin, Ireland, and got his Ph. D. at the Institute of Molecular Pathology in Vienna, Austria. During his doctoral thesis he explored chromatin modifications in mammalian cells. He joined the Karlseder laboratory as a postdoc in 2007 and is exploring epigenetic changes during replicative aging.

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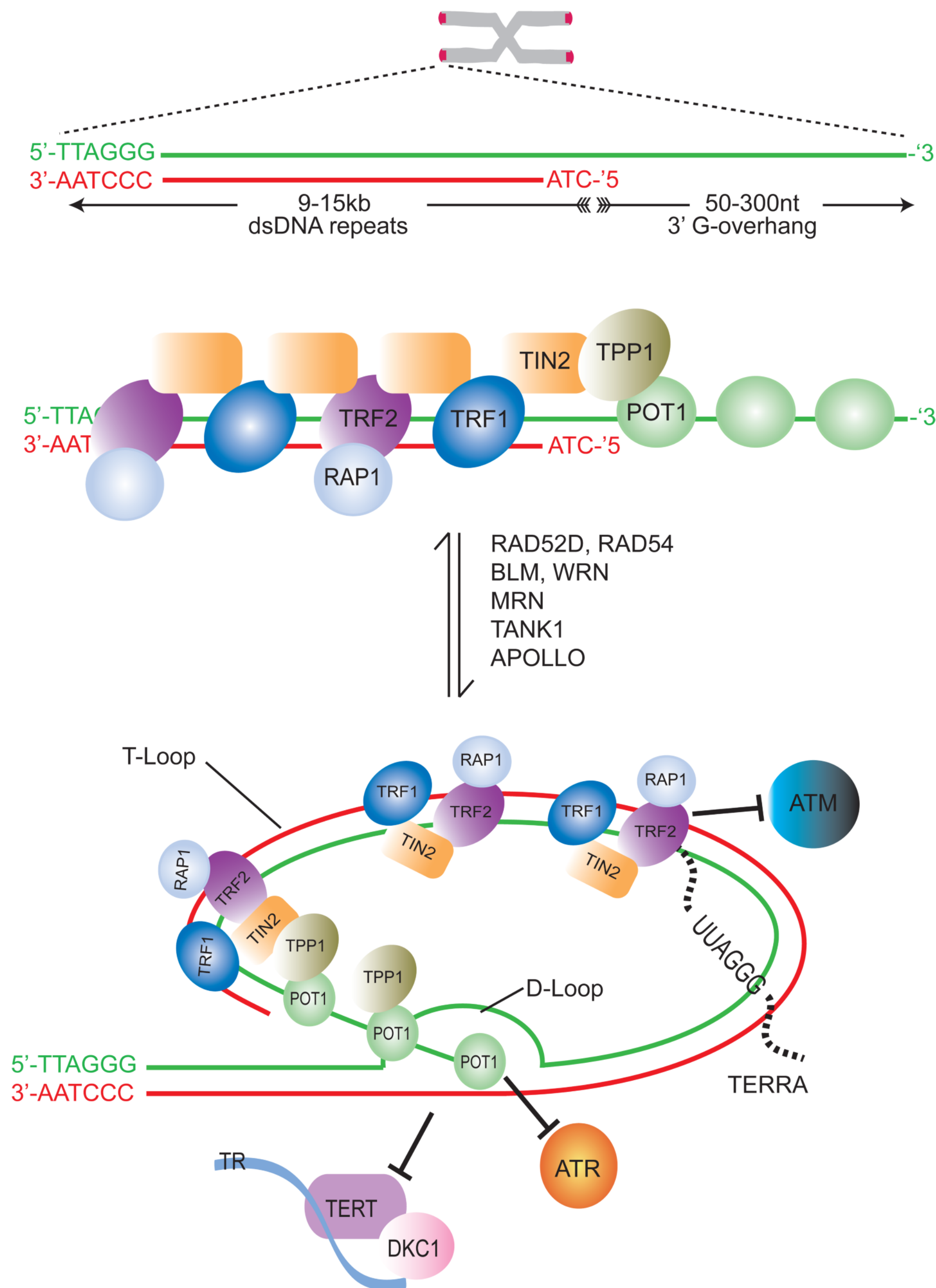

Figure 1. The structure of human telomeres

Human telomeres consist of many kilobases of TTAGGG repeats, with a G rich leading strand and a C rich lagging strand. The G strand extends in the 3' direction, forming the G-tail. The shelterin complex [27], consisting of the double stranded telomeric repeat binding factors TRF1 and TRF2, the TRF2 interacting factor RAP1, the bridging molecules TIN2 and TPP1 and the telomeric protection factor POT1, together covering the double and single stranded repeats. Shelterin members interact with a large number of other factors that transiently localize to telomeres, frequently in a cell-cycle dependent manner. These factors aid in the generation of a protective structure at chromosome ends, here referred to as telomeric loop, or T-loop. The T loop is generated by invasion of the single stranded G-overhang into the double stranded

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TTAGGG repeats. The looped structure protects telomeres on several levels. Invasion effectively sequesters the G-tail, and allows distinction of natural chromosome ends from double stranded breaks. The ATM dependent signaling cascade is inhibited by TRF2 and the ATR signaling pathway by POT1. Telomerase is likely inhibited by the complex, and it is suspected that TERRA play a role in this inhibition.

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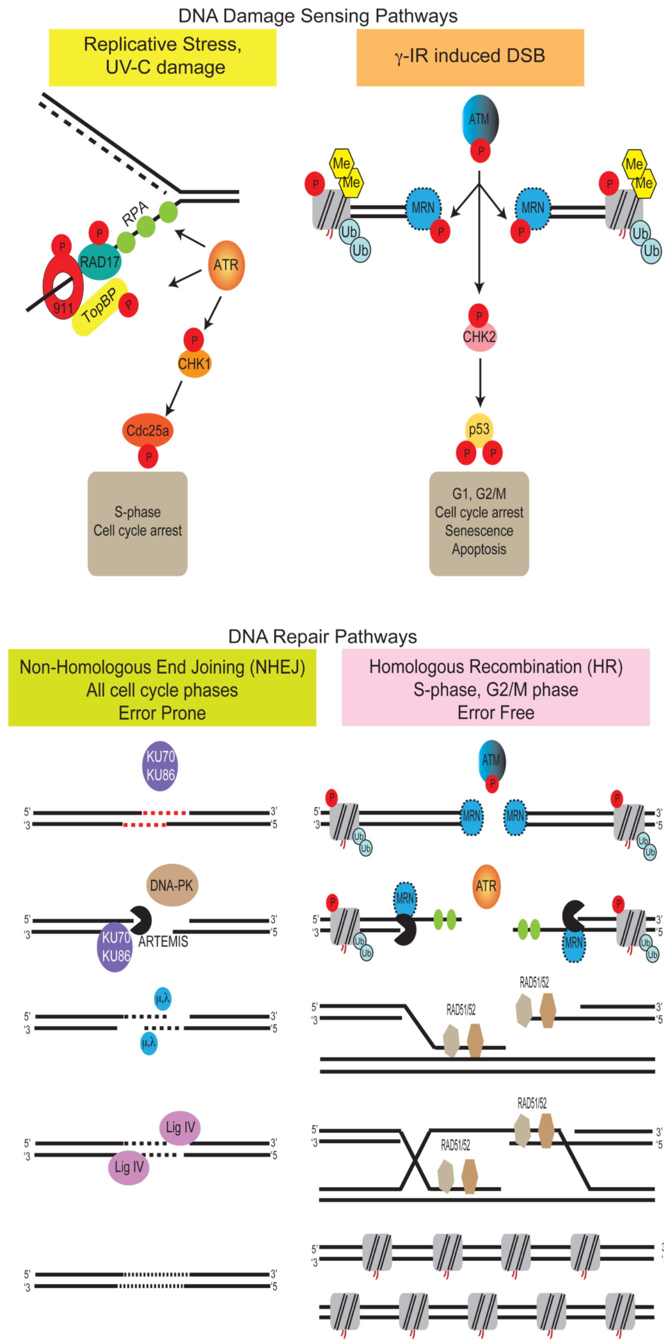

Figure 2. (A) Damage Sensing Pathways

In the event of single stranded breaks or fork stalling ATR is activated and RPA (Replication Protein A) binds to the exposed strands. ATR then phosphorylates RAD17, the 9-1-1 complex and TopBP1 (Topoisomerase IIβ Binding Protein 1), as well as CHK1 which amplifies the signal and mediates cell cycle arrest via Cdc25a. At double strand breaks (DSBs) the chromatin structure surrounding the break is dynamically re-structured, exemplified by ATM dependent phosphorylation of H2AX and modification of adjacent chromatin. The sensing of the DSB by the MRN complex triggers targeting of downstream mediators and activation of DNA repair pathways. The key event is the ATM dependent activation of CHK2 and p53, inducing arrest

in G1 and G2/M phases of the cell cycle. Failure to repair results in permanent cell cycle arrest, senescence or apoptosis.

(B) DNA Repair Pathways. The two primary pathways for the DNA repair are NHEJ and HR. NHEJ is the major pathway as it functions throughout the cell cycle. NHEJ requires sensing of the lesion by Ku70/80, activation of the DNA-PK (DNA dependent Protein Kinase) complex and 3'-5' endonucleolytic resection of the break site. The break is then filled in by DNA Polymerases υ and λ and the repaired ends are fused by DNA Ligase IV. However, NHEJ is error-prone and defects in NHEJ are frequently linked with cancer. If DNA is damaged during S-phase the cell employs the error free HR pathway. ATM and MRN mediate recognition and resection of the break, the ssDNA overhang is detected by ATR and RPA, which promote association of RAD51/RAD52. The HR machinery mediates the synthesis of a new DNA strand using the overhang sequence as template. This mechanism ensures that the original DNA sequence can be faithfully restored and genetic integrity is maintained.

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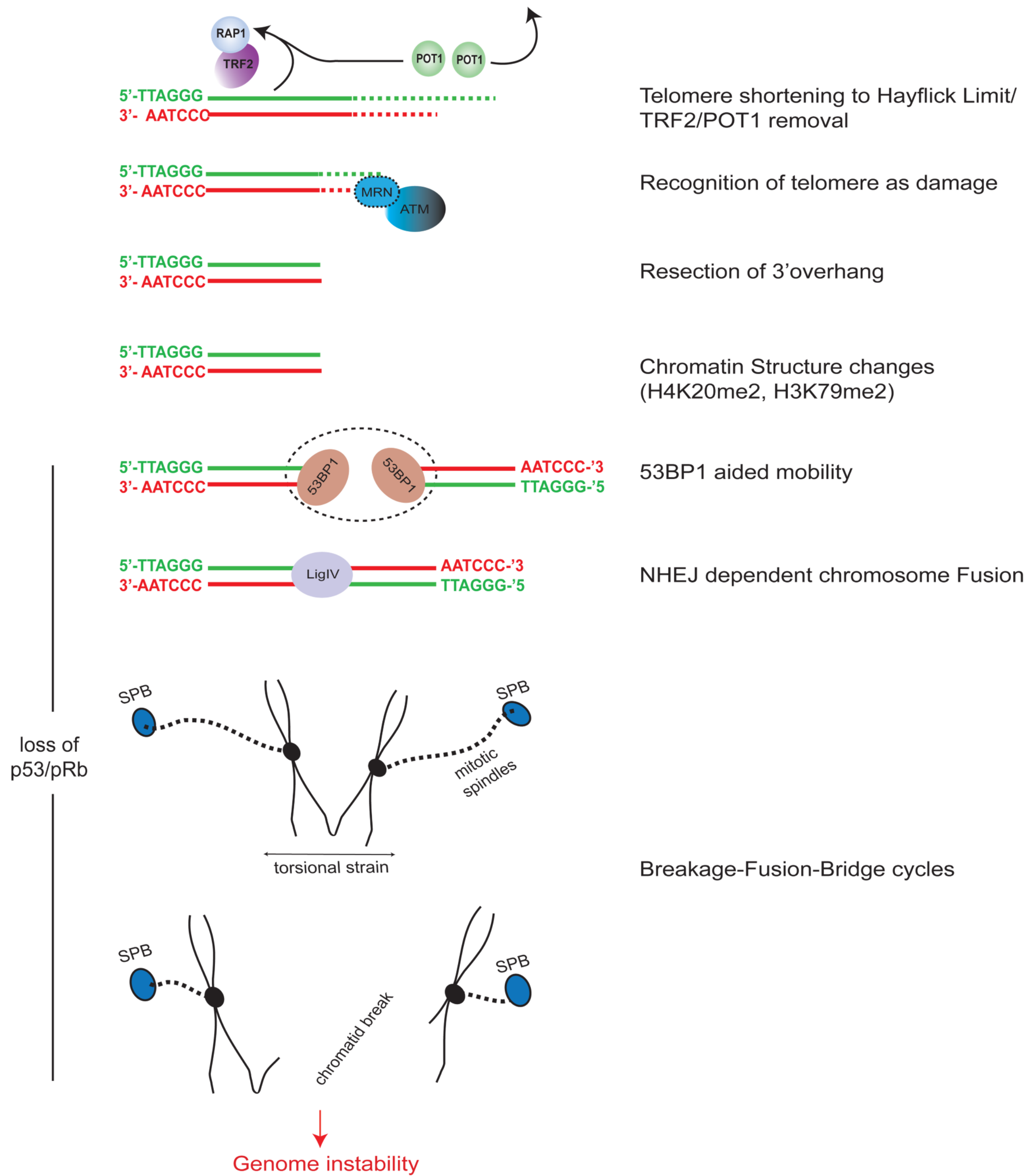

Figure 3. Telomeres as the cause of genome instability

When telomeres lose protection, either due to extensive loss of TTAGGG repeats or due to loss of protective factors such as TRF2 and POT1, they are recognized as damage in a pathway that depends on the MRN complex and the AT kinase. Next the G overhang is lost, then the chromatin structure changes and 53BP1 is recruited to allow for greater mobility, which facilitates NHEJ dependent covalent fusion of chromosome ends. In the absence of the p53 and pRb dependent tumor suppressor pathways cells containing nuclei with fused chromosome ends continue to cycle. When the fused chromosomes pass through mitosis they break randomly, leading to unequal distribution of genetic material in the daughter cells. These

fusion-breakage-bridge cycles continue through the following cell divisions, leading to multiple non-reciprocal translocations and genome instability.

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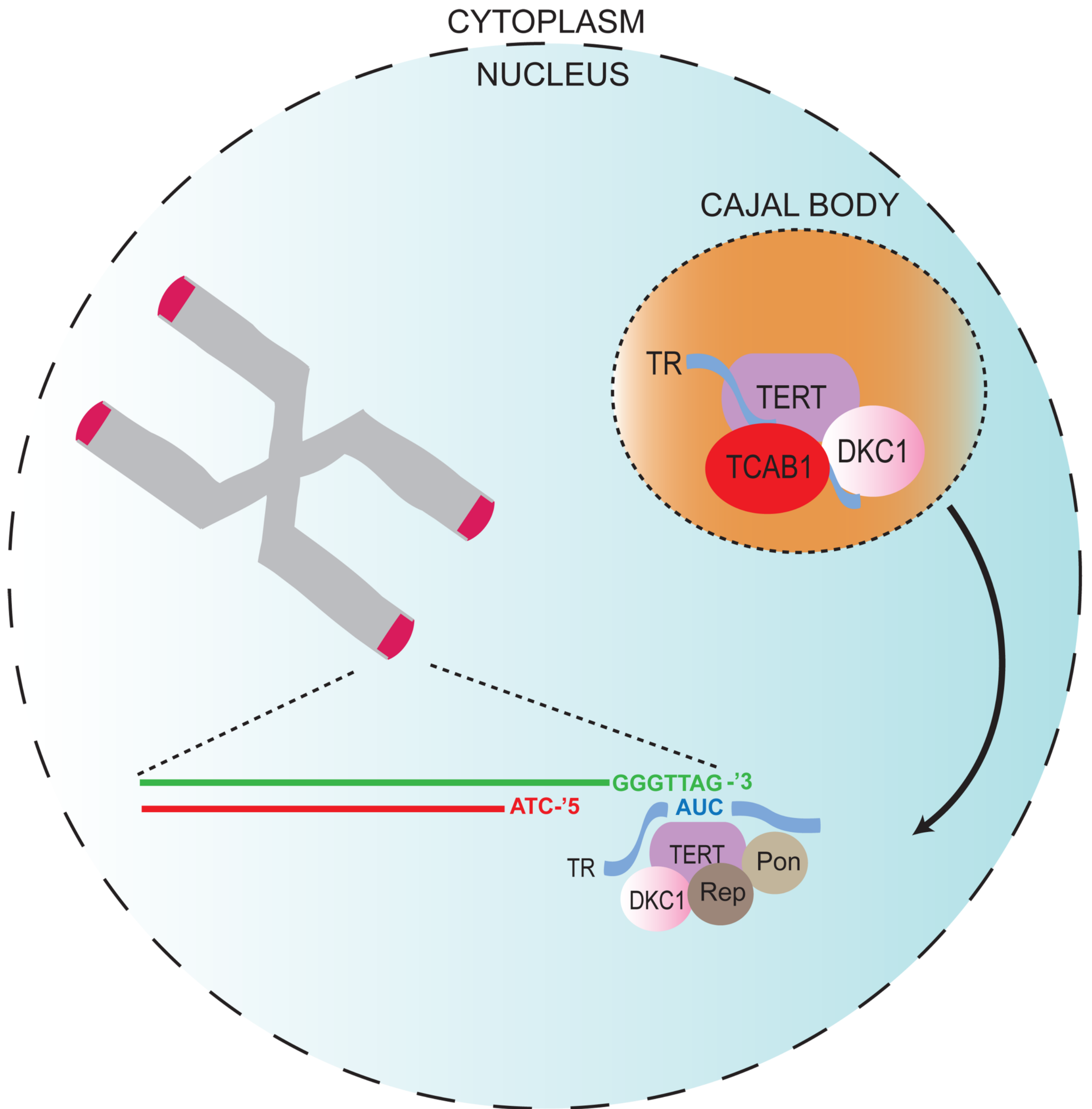

Box 1 Figure.

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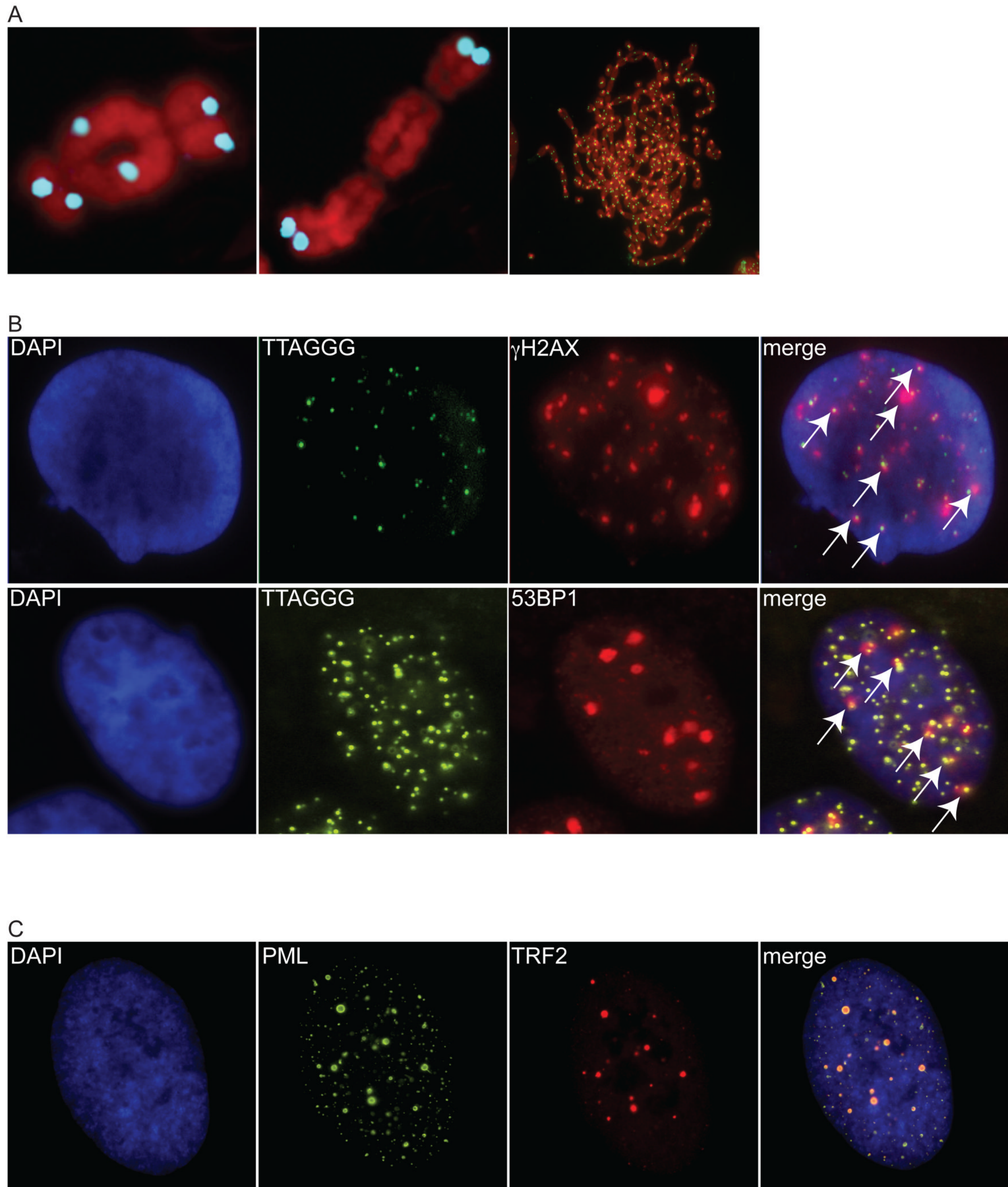

Box 2 Figure.

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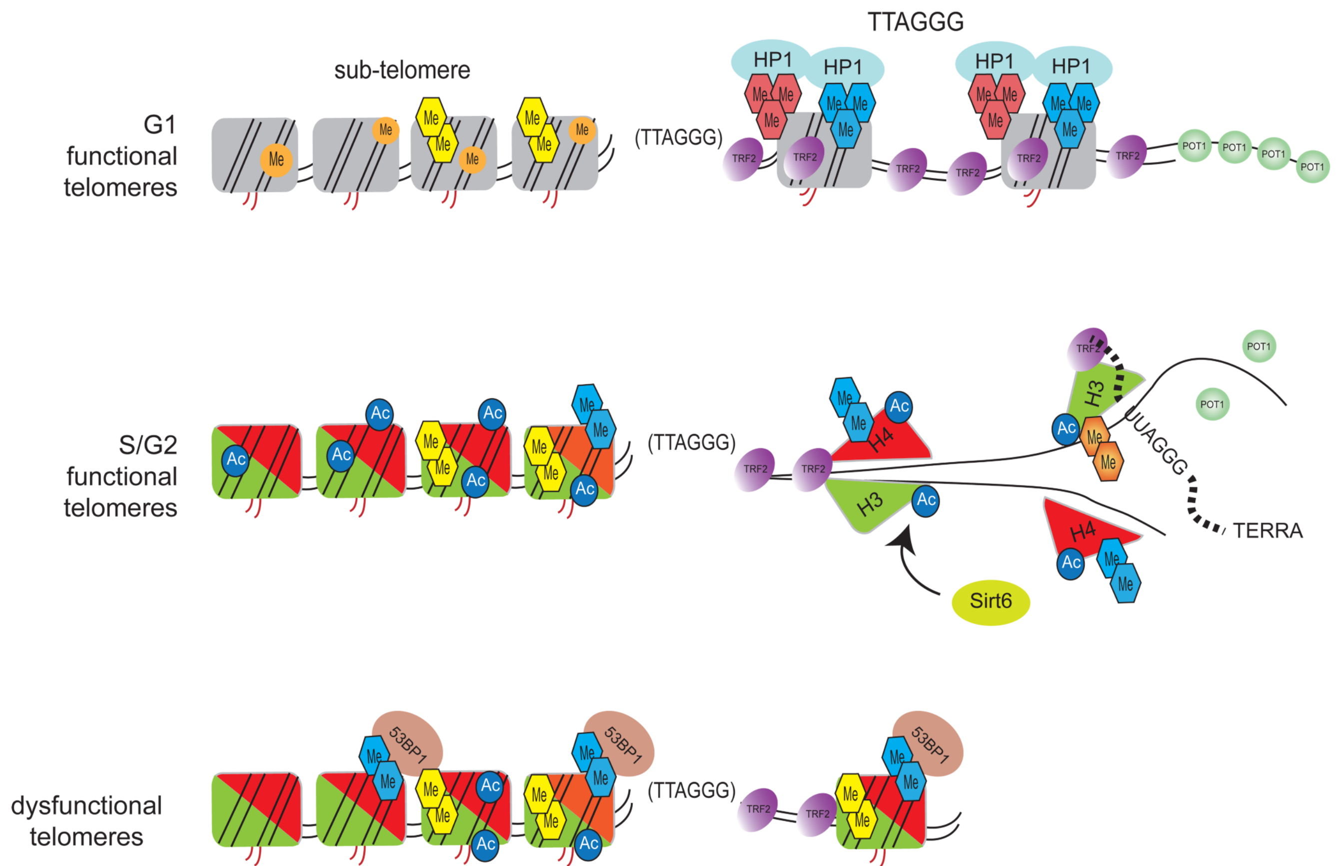

Box 3 Figure.