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Defending the end zone: studying the players involved in protecting chromosome ends

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Abstract

The linear nature of eukaryotic chromosomes leaves natural ends susceptible to triggering DNA damage responses. Telomeres are specialized nucleoprotein structures that comprise the “end zone” of chromosomes. Besides having a specialized sequences and structures, there are six resident proteins at telomeres that play a prominent role in protecting chromosome ends. In the review, we will discuss this team of proteins called shelterin and how they are involved in regulating DNA damage signaling, repair and replication at telomeres.

Keywords

telomere; shelterin; DNA damage response; NHEJ

Introduction

The main goal of cell division is the accurate transfer of genomic information from a cell to its progeny. Breaks in chromosomal DNA threaten the genomic integrity of future cells, so sensitive and complex mechanisms have evolved for recognizing and dealing with such insults. The DNA damage response (DDR) consists of sensors of damage, which trigger a signaling cascade that activates potent cell cycle checkpoints that lead to cell cycle arrest or DNA repair to join broken DNA ends[1]. Rampant breaks and repairs lead to massive genomic instability, a phenotype first noticed by Muller and McClintock in the genomes of irradiated fruit flies and maize, respectively[2]. The linear nature of eukaryotic chromosomes presents a difficult problem for cells: how could the DDR machinery distinguish the normal ends of chromosomes from sites of DNA breaks? Or alternatively, how could it mask normal DNA ends from inappropriately activating this response so that cells can propagate? This obstacle has been deemed the “end protection problem”[3]. The fact that recombination and genomic instability, like that seen with induction of DNA breaks, did not occur with normal chromosome ends, led Muller and McClintock to postulate that these ends possess special qualities.

In addition to the end protection problem, linear chromosomes are susceptible to a second end-related phenomenon called the “end replication problem”. Because the DNA polymerase machinery is unable to completely replicate the lagging chromosome strand, each cell division contributes to progressive erosion of chromosomal ends. This shortening

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poses a potential problem, since the continuous loss of chromosomal DNA in each daughter cell results in the erosion of vital genetic information, eventually adversely affecting cellular homeostasis. It is estimated that 50–200 base pairs of genomic DNA is lost with each round of DNA replication, resulting in a total loss of 2–10 kb of DNA over the lifetime of long-lived organisms. So how could cells protect important genes from being lost through this process?

In work by Blackburn, Greider and Szostak, that was awarded the 2009 Nobel Prize in Physiology and Medicine[2], the structure of the chromosome ends was discovered to be specialized nucleoprotein structures that comprise the “end zone” of chromosomes called telomeres. Telomeres consist of long stretches of TTAGGG repeats that solve this problem by first acting as a buffer of non-coding sequences that prevent more important genes from being lost. Second, the GT-rich telomeric sequence serves as substrate for the telomerase enzyme complex, which is required to maintain the size of the end zones by adding more of the TTAGGG repeats *de novo* to chromosomal ends.

Telomeres also solve the end protection problem. The DNA tracts of the end zone are not blunt ended. Instead, they terminate in a long single stranded (ss) run of the G-rich sequence on the 3' strand, called the 3' G-rich overhang. This long single stranded tail of DNA postulated to fold back onto itself to invade the duplex portion of telomeres, forming what is called the t-loop[4]. The t-loop effectively sequesters the free ends of chromosomes, protecting them from being sensed as double strand breaks. The second solution to the end protection problem comes in the form of a six protein complex called shelterin[5], which appear to bind exclusively to telomeres. An expanding body of research is elucidating the complex roles of these proteins in the protection of chromosomal ends and the maintenance of genomic stability. In this review, we will briefly discuss how these players team up to defend the end zone, as well as the DNA damage sensing and repair responses that are triggered when these molecules are removed. We will focus on current knowledge about mammalian telomere biology derived from experiments based primarily on human cell lines or mouse genetic systems. Finally, we will discuss some of the experimental systems used to study the different shelterin components and the DNA damage response at telomeres.

Shelterin: meet the starting line-up

The team of proteins charged with protecting the end zone is called the shelterin complex[5], and consists of six main players: TRF1, TRF2, TIN2, RAP1, TPP1 and POT1 (Figure 1). Most are essential to survival of mammalian cells, as depletion of shelterin components either drives cells into cellular senescence or results in early embryonic lethality. Together, these six proteins form a complex which is charged with protecting chromosome ends from activating a DNA damage response, inhibiting inappropriate repair mechanisms and maintenance of telomeric length and structure. However, each protein plays a unique role in telomere homeostasis.

The two main proteins that anchor the complex to the double stranded telomeric DNA are telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2), which recognize TTAGGG repeats and bind directly through their SANT/Myb domain[6]. Although TRF1 and TRF2 have similar structure and protein binding domains, they recruit different proteins to telomeres [7] and play unique roles in protection of the end zone.

TRF1 is a negative regulator of telomere length[8, 9]. It is essential to cellular function as its depletion results in cellular senescence[10–12], TRF1 may act as a docking protein to recruit other factors to telomeres as it can interact with non-shelterin proteins that can be found at telomeres, including PINX1, ATM, BLM, DNA-PKcs, Tankyrase 1 and Tankyrase 2[13]. A recent conditional knockout approach to delete *Trf2* in mouse embryo fibroblasts (MEFs)

revealed novel functions for Trf2: that it may be important for preventing telomere replication mistakes and protects telomeres against a fragile-telomere phenotype [10, 11]. Furthermore, Trf1 deficiency leads a modest increase in sister telomere fusions, suggesting that it may play a role in telomere end protection[11]. However, the role for Trf1 in mediating telomere end protection requires further research, since *in vitro* experiments using purified proteins showed that in contrast to TRF2, TRF1 is unable to inhibit end-joining of telomeric substrates[14].

TRF2 plays a prominent role in end protection as its loss is very toxic to cells, initiating a potent DDR to result in cellular senescence and very dramatic end-to-end chromosome fusion phenotype indicative of improper repair[15, 16]. TRF2 also may play a role in stabilizing telomere secondary structure as it has been shown to facilitate t-loop formation *in vitro*[17]. TRF2 recruits and facilitates the interaction between the repressor/activator protein 1 (RAP1) and the end zone[18] and also interacts with a variety of non-shelterin proteins that play roles at telomeres, including ATM, the MRN complex, WRN, BLM, Ku86, ERCC1/XPF, PARP1 and PARP2[13]. As with TRF1, the importance of these interactions has not been fully elucidated.

TRF1 and TRF2 are linked together by the TRF1-interacting nuclear protein 2 (TIN2). TIN2 has also been called the linchpin of shelterin, as it is able to bind TRF1, TRF2 and TPP1, acting as a bridge between the double stranded and single stranded telomere binding proteins. Stability of the entire six protein complex appears to depend upon TIN2[19]. While it can bind to both TRF1 and TRF2, TIN2 exists in stoichiometric quantities enough to bind each molecule of TRF1 or TRF2, suggesting that it may not always bind to both proteins concurrently and that the shelterin complex may not always exist with six components[20]. It was recently discovered that TIN2 exists in two isoforms in human cells. One of these isoforms, TIN2L, was shown to bind tightly to nuclear matrix proteins suggesting a role in anchoring telomeres to the nuclear matrix[21].

TPP1 can interact with TIN2 and POT1, providing the essential link for the recruitment of POT1 to telomeres[22]. As with other members of shelterin, homozygous loss of Tpp1 is incompatible with survival[23] and the depletion of Tpp1 through genetic ablation[23], RNA interference[24], or expression of a mutant that cannot interact with POT1[24] leads to cellular consequences consistent with POT1 loss. TPP1 can also interact with telomerase, suggesting that it may play role in telomere length maintenance by modulating telomerase access to telomeres[25].

The single-stranded telomere binding protein, POT1, has two highly conserved oligonucleotide/oligosaccharide binding folds (OB folds) that show specificity for single-stranded telomeric sequences and are essential for binding the 3' overhang[26]. Its recruitment mainly depends on TPP1[22]. POT1 plays a role in regulating telomere length as knockdown of POT1 or Tpp1 in cells leads to telomere elongation[24, 27]. It is also a vital player in protecting the telomere end zone, since knockdown of POT1 also triggers chromosomal fusions and cell death[28]. Mice have two orthologs of the gene, named Pot1a and Pot1b[29, 30]. Pot1a is essential to cells as targeted deletion of this gene in the mouse is embryonic lethal very early on in development and leads to chromosomal fusions in cultured cells[29, 30]. On the other hand, Pot1b is not essential for survival as Pot1b^{-/-} mice are viable, and its deficiency in cells does not produce as severe a phenotype compared to loss of Pot1a alone or deletion of both Pot1a and Pot1b[30, 31]. Recent studies have implicated Pot1b in telomeric length maintenance; in particular, the regulation of C-strand resection for the formation of the 3' overhang, which is greatly elongated following Pot1b loss[30–32].

The final member of the shelterin team one of the most evolutionarily conserved members of shelterin, RAP1. In *S. cerevisiae*, rap1 binds directly to telomeric sequences, where it is involved in the negative regulation of telomere length, inhibition of fusions, and recruiting Sir proteins for silencing of gene near the telomeric region[33]. However, in human cells, RAP1 is recruited to telomeres by TRF2, and its association with the end zone is dependent on this interaction[18]. *In vitro* studies in human cell lines suggest that RAP1 may play a role in telomere length regulation independent of its ability to localize to telomeres[34]. Biochemical assays suggest that TRF2/RAP1 work in conjunction to inhibit end-to-end chromosome fusions[14]. Recent dissections of the roles of TRF2 vs. RAP1 involved the generation of a fusion protein that allowed RAP1 localization to telomeres without TRF2[35]. Using this approach, localization of RAP1 to telomeres independent of TRF2 did not interfere with DNA damage signaling, but was sufficient to inhibit NHEJ[35] suggesting that RAP1 plays a role in repression of DNA repair rather than damage sensing.

DNA damage sensing and signaling at telomeres

The canonical response to DNA double strand breaks in cells is a complex multi-step cascade similar to classical signal transduction pathways. In this model, DNA damage acts as a stimulus that is detected by DNA damage sensing factors. These factors further activate the signal transducing kinase factors which amplify the response, resulting in the upregulation of downstream effector proteins[36]. At double strand breaks, the initial sensing of damage occurs through factors, such as the Mre11/Rad50/Nbs1 (MRN) complex, which recruits and activates the DDR signal transduction protein from the phosphatidylinositol 3-kinase-like kinases family: ataxia-telangiectasia mutated (ATM). ATM is considered a master controller for responses to DNA double strand breaks and serves as a kinase to propagate the DNA damage signal by recruitment factors like the phosphorylated histone variant H2AX (γ -H2AX), the mediator of DNA damage checkpoint1 (MDC1), and p53 binding protein 1 (53BP1)[37]. An alternative signaling pathway is routed through the ATM- and Rad50-related protein (ATR)[37]. While ATM responds mainly to IR damage and DSB, ATR responds mainly to UV damage, single stranded breaks and replication fork stalls. These types of damage leave DNA exposed as a single stranded section of nucleotides. In this pathway, replication protein A (RPA) recognizes and binds to the ssDNA. It then recruits ATR/ATRIP, which transduces the DNA damage signal to downstream factors. Both ATM and ATR propagate damage signals through phosphorylation of downstream checkpoint molecules, such as Chk1, Chk2 and the tumor suppressor p53, which can lead to cell cycle arrest[38].

The theory that telomeres are involved in protection of chromosome ends from the DDR was first demonstrated in cells and mice with severe telomere shortening[39]. The role shelterin proteins play in suppressing this response has been teased out through the use of mouse embryonic fibroblasts (MEFs) with genetic ablation of various combinations of these factors. These studies take advantage of a technique that detects the DDR proteins, including γ -H2AX or 53BP1, at the sites of DNA damage and along the surrounding chromatin as DNA damage foci. Co-localization of these damage foci with telomere signals are called telomere-induced damage foci (TIFs)[40]. These techniques revealed that Trf2 and Pot1a play separate roles in telomere end protection[41].

First, Trf2 knockdown experiments show that cells respond to loss of Trf2 in a similar manner as IR-induced DSB, with the MRN complex required for the initial sensing of damage at dysfunctional telomeres[42, 43] and ATM necessary for propagation of the signal[24, 41]. Cells deficient in either ATM or components of the MRN complex (Mre11 or Nbs1) were unable to form TIFs in response to Trf2 loss. However, ATR deficiency had no effect[24, 41–43]. On the other hand, Tpp1 or Pot1a/b disruption leads to a DNA damage

signaling response that is dependent upon the ATR pathway. MEFs that have been depleted of Pot1a/b either through genetic ablation or through Tpp1 disruption easily formed TIFs in the absence of ATM, but failed to do so in the absence of ATR [24, 41]. These results suggest that Trf2 acts mainly to repress ATM-mediated DNA damage responses at dysfunctional telomeres, while Pot1a/b proteins are involved in repression of the ATR pathway.

How TRF2 and POT1 act to inhibit these pathways is still unclear. Paradoxically, many components of DNA repair pathways can be found normally in the end zone without activating an apparent DNA damage response[44]. Given that TRF2 can interact with these non-shelterin telomeric factors, perhaps it directly suppresses their activation. In fact, there is some evidence that the TRF2 interaction with ATM directly inhibits its activity[45]. However, POT1 has not been shown to interact with non-shelterin components. Instead, it is postulated that the binding of the POT1 to the 3' overhang provides protection through competitive blockade of RPA binding to the single stranded protrusion, preventing the activation of the ATR pathway [46].

Mechanisms of repairing dysfunctional telomeres

To protect the integrity of the genome, the DDR machinery is dispatched to fix DNA breaks by recombining the broken ends, generating end-to-end fusions. However, this process may be detrimental in the context of large amounts of DNA breaks, since these fusions form unstable dicentric chromosomes which break as they are pulled towards opposite ends of the dividing cell. The subsequent break must also be repaired and so a cycle of chromosome breakage and fusions, also called the bridge-fusion-breakage (BFB) cycle, begins[47]. With each round of improper fusions and random breaks, gene losses and amplifications accumulate and the resulting genomically unstable cells serve as a perfect nidus for cancer formation[47]. Because each chromosome end represents a potential target for this process, repression of repair processes at normal telomere ends is essential for genomic stability.

Mammalian cells have developed two primary pathways for repairing DSB: classical nonhomologous end-joining (C-NHEJ) and homologous recombination (HR)[1]. C-NHEJ is an error-prone repair mechanism that joins two blunt ends of a break[48]. Briefly, the free ends are recognized and bound by the Ku70/86 protein complex, which aligns the DNA breaks. DNA-PKcs, another kinase of the PIKK family, is recruited to the damage sites by Ku70/80 and facilitates the activation of further downstream effectors to initiate DNA end processing through nucleases such as Artemis. DNA ligase IV (Lig4), in complex with XRCC4, performs the final act of ligating the two ends together[48]. The effects of dysfunctional telomeres that are repaired by the C-NHEJ pathway can be visualized on a chromosome metaphase spread as end-to-end fusions. C-NHEJ appears to be the primary method of repair of telomeres that have lost Trf2, as depletion of Trf2 from MEFs results in rampant chromosome end-joining processes that lead to dramatic fusion of chromosome ends[49]. This repair phenotype is dependent on the canonical C-NHEJ pathway because it requires Ku70[50] and Lig4[49] proteins. It also requires upstream signaling from ATM[41, 49] and 53BP1, which is involved in chromatin mobility to position ends to facilitate end joining[51].

The MRN complex, which is important for initial detection of damage, is also involved in the repair process, as cells with Mre11 or Nbs1 deficiency cannot form end-to-end fusions efficiently in the setting of Trf2 abrogation[43]. Recent work has given insight into how Mre11 specifically plays a role in end processing for DNA repair. Mre11 has been shown to have both endonuclease and exonuclease activities *in vitro*[52]. Using an Mre11 nuclease domain mutant[53], recent data suggest that Mre11 nuclease activity is important for

creating the proper substrate for C-NHEJ when Trf2 is lost[42]. The ERCC1/XPF nuclease complex has also been implicated in the processing of the G-overhang after Trf2 loss[54]. In both cases, the G-overhang is unable to be removed after Trf2 loss, and the remaining single stranded tail, still bound by Tpp1/Pot1a/b, acts as a barrier to C-NHEJ[42, 54]. Future studies may help us to understand the interplay between Mre11 and ERCC1/XPF in this resection process.

A more accurate method of DNA repair is HR, which uses the homologous sequence from sister chromatids as templates to replicate the damaged sequence[48]. The initial step of HR involves resection of the DNA ends to form single stranded overhangs. This process also requires MRN complex and Rad50 to secure the separate DNA ends and the associated protein CTIP to help process the ends into single strands that can then invade the sister chromatid[48]. Visualization of this process at telomeres can also be seen on metaphase spreads through the use of a labeling technique called chromosome orientation FISH (CO-FISH)[13]. This technique differentially labels the G-rich and C-rich strands so that sister chromatids can be distinguished as individual green and red signals on normal telomeres. Telomere recombination events through HR, called telomere sister chromatid exchanges (T-SCEs), will co-label with both red and green probes[55]. In contrast to TRF2, POT1 does not seem to play a prominent role in repressing C-NHEJ as its loss leads to few end-to-end fusions in both human cells[28] and mouse cells depleted of Pot1a[29, 30]. Instead, POT1 appears to repress HR-mediated repair at telomeres, since Pot1a depletion in MEFs is accompanied by increased T-SCEs[29], suggesting that the resulting unprotected single stranded G-overhang may serve as a substrate for HR.

C-NHEJ was once thought to play a prominent role in the inappropriate repair of critically shortened telomeres in the telomerase knockout mice (56). Because severe erosion of telomere tracts leaves chromosome ends without the protective end zone, they are thought to be ideal substrates for C-NHEJ. However, a more extensive study demonstrated that chromosomal fusions in cells with severely shortened telomeres does not depend on two major components of the NHEJ pathway, DNA-PKcs and DNA ligase IV (Lig4)[57]. This study suggests that inappropriate repair of critically short telomeres may not be solely mediated by C-NHEJ. Alternative NHEJ (A-NHEJ) pathways are currently being explored as possible mechanisms for repair of dysfunctional telomeres [48]. Future work may help to sort out the mechanism of A-NHEJ at telomeres and the role shelterin plays in repressing this pathway.

Shelterin's role in the generation and protection of the 3' overhang

The unique structure of telomeres is an important line of defense against DDR activation. The t-loop was first visualized via electron microscopy of crosslinked human telomeric DNA [4] and later confirmed in telomeric chromatin [58]. While t-loop formation is not well understood, it is thought to be formed when the single stranded overhang loops back and invades the duplex telomeric sequences, forming the large lariat shaped t-loop. However, there is also a smaller D-loop of displaced telomeric DNA at the site of invasion and this structure is reminiscent of the D-loop formed during HR. Given that many HR factors are known to localize to telomeres [44], it is postulated that both are formed through similar processes. If this is the case, the process that prevents resolution of the Holliday junctions is not known. Perhaps the coating of POT1 on the displaced strand inhibits this process. *In vitro* studies also suggest that TRF2 plays a role in facilitating the formation of the t-loop [17].

Beyond the t-loop, evidence that shelterin plays an important role in regulating the proper replication of telomeres and generation of the overhang is accumulating. The length of G-

rich overhang is independent of telomerase activity and thought to be produced through nuclease resection of the C-strand. In mouse cells, Pot1b acts as a negative regulator of this nuclease process as Pot1b deficiency leads to elongation of the G-rich overhang without overall telomere lengthening[30, 32, 59]. However, the nuclease(s) that performs the C-strand resection are yet unknown. Exo1 was ruled out when MEFs that are genetically deficient for both Pot1b and Exo1 still showed increased overhang production[32].

While the MRN complex is involved in the DNA damage sensing and processing of dysfunctional telomere ends for NHEJ, increasing evidence suggests that the MRN complex, especially Mre11, is also involved in normal telomere end processing. First, the MRN complex can be found at normal telomeres in a cell cycle dependent manner, in the G2 phase[44]. Furthermore, Mre11 has been implicated to play a role in the generation of the 3' overhang in a telomerase-dependent manner in human cells as knockdown of Mre11 leads to reduction of the overhang[60]. Recent data has shown that depletion of Trf2 in the setting of MRN complex deficiency results in dramatic reduction of chromosome end-to-end fusions due to failure to activate ATM signaling. Interestingly, this situation results in an increase of leading-strand chromatid type fusions[42, 43, 61]. Chromatid-type fusions are generated during G2, after DNA replication. Immediately following DNA replication, telomeres created by leading-strand DNA synthesis are initially blunt-ended, and it is postulated that this blunt end could be recognized as a DSB during G2[44]. In contrast, lagging-strand synthesis results in telomeres possessing a 3' single-strand overhang that is postulated to provide protection from C-NHEJ-mediated repair. It is thought that TRF2 localization to newly replicated leading-strand telomeres would normally suppress the DDR. In the absence of TRF2, C-strand resection by the MRN complex is necessary to generate the protective 3' overhang[42, 43, 61].

Recent data suggests that a 5' to 3' exonuclease called SMN1B/Apollo may also play a role in the generation of the 3' overhang. This protein was originally identified as a shelterin associated protein and localizes to telomeres via direct binding to TRF2[62, 63]. Initial experiments involving knockdown of SMN1B/Apollo by siRNA leads to cellular senescence and TIF formation in S-phase and duplicated telomere ends, suggesting a role for SMN1B/Apollo in telomere end processing during replication[62, 63]. Recent work using SMN1B/Apollo knockout MEFs reveal a 50% reduction in 3' single-strand overhang, a phenotype rescued by wild type SMN1B/Apollo but not by mutants that do not possess nuclease activity[64]. These results suggest that SMN1B/Apollo's nuclease activity plays an important role in end resection to generate 3' overhang at telomeres. Importantly, SNMIB/Apollo null MEFs exhibit an increased incidence of chromatid-type fusions involving leading-strand telomeres, consistent with its role in protecting leading-strand telomeres after DNA replication. Mutations within its conserved nuclease domain abolish this end-protective phenotype, suggesting that SNMIB/Apollo is a pivotal 5'–3' exonuclease required for generation of the protective 3' single-stranded overhangs at leading-strand telomeres after DNA replication to prevent engagement of C-NHEJ[64].

***In vivo* consequences of shelterin disruption**

Most data on the *in vivo* consequences of telomere dysfunction has been obtained from mouse models based on genetic targeting of the telomerase complex, either the RNA component (*mTR* or *mTERC*) or the reverse transcriptase protein component (mTERT) [39] and focused on telomere dysfunction induced by severe telomere shortening. Because deficiency of most shelterin components leads to early embryonic lethality, the bulk of our knowledge about these defenders of the end zone has been elucidated in cell culture experiments. However, studies focusing on the *in vivo* consequences following shelterin

disruption, using tissue-specific conditional knockout or transgenic mouse models, are beginning to emerge.

Pot1b is a rare exception in that it is the only shelterin component that is not essential and its knockout in mice produce viable offsprings. However, Pot1b^{-/-} animals show abnormalities in highly proliferative organs, including increased apoptosis in intestines and testes[59], similar to the late generation telomerase knockout mice[65]. Interestingly, combined Pot1b deficiency with telomerase haploinsufficiency appear to have phenotypes similar to those seen in dyskeratosis congenita (DC)[32, 59]. DC is a human disease associated with mutations in the telomerase complex that manifests characteristic phenotypes including bone marrow failure, nail dystrophy and skin hyperpigmentation. All of these characteristics were observed in independent Pot1b knockout, telomerase heterozygous mouse lines[32, 59]. These results suggest an essential role for Pot1b and other shelterin components in maintaining highly proliferative tissue compartments, a notion supported by the observation that some cases of DC in humans is due to mutations in TIN2[66].

Although homozygous deletion of Tpp1 is incompatible with survival and leads to cellular consequences consistent with Pot1a loss[23], there exists a mouse model of adrenocortical dysplasia (acd) that was found to have a mutation in the Tpp1 gene[67]. The Tpp1/Acd mutation leads to splice variants of Tpp1 that depletes the protein from telomeric ends and results in chromosomal aberrations[68]. Acd^{acd/acd} mice have skin abnormalities, poor growth and survival, and defects in the development of organs derived the urogenital ridge, manifesting in adrenal insufficiency, hydronephrosis, and infertility due to gonadal defects[67]. These traits can be reversed when p53 is deficient, except for germ cell atrophy[69]. The animals also show skeletal deformities resembling caudal regression syndrome[67], a phenotype that is attributed to telomere dysfunction triggering p53-dependent apoptosis[70]. Mice harboring the Tpp1/Acd mutation combined with deficiency of p53 also showed accelerated tumorigenesis, with a shift in spectrum from lymphomas and sarcomas to carcinomas in a p53^{+/-} background[69], reminiscent of the cancer phenotypes observed in the telomerase knockout mouse model[71].

One method of circumventing embryonic lethality is to conditionally manipulate shelterin protein expression in a tissue specific manner. Interestingly, while Trf2 loss is extremely toxic to MEFs in culture, a model of hepatocyte-specific Trf2 deletion yielded mice that showed no signs of liver dysfunction[72]. Despite evidence of DDR activation, Trf2 depleted hepatocytes did not activate p53, and cell death or senescence was not observed even after partial hepatectomy. However, hepatocytes lacking Trf2 were unable to undergo cell division, instead continuing to grow and replicating the genome without dividing. These unusual results suggest a phenotype specific to liver tissue, in that liver regeneration can occur by endoreduplication without cell division, thereby circumventing problems due to chromosome fusions. Examination of a mouse model in which Trf2 was conditionally overexpressed in the basal cell and stem cells of the epidermis of mice resulted in signs of premature degeneration in the skin, including dryness, alopecia, and hyperpigmentation, as well as development of spontaneous squamous cell carcinomas in areas of skin exposed to light[73], phenotypes that were accelerated when combined with telomerase deficiency[74]. These results suggest that overexpression of Trf2 promotes cancer formation in highly proliferative tissues.

Trf1 loss has also been studied in the skin, as the Trf1 conditional knockout mice was crossed with a K5-driven Cre transgene[11]. The skin phenotype includes severe proliferative defects, potent DDR activation in the targeted cells without changes in telomere length, and a defect in the epidermal stem cell compartment. The importance of the p53

tumor suppressor pathway was highlighted once again when these animals were crossed onto a p53 deficient background – in addition to rescued mortality, these animals with dual Trf1 and p53 deficiency spontaneously developed invasive squamous cell carcinomas on the tail and ears. These results suggest that Trf1 may play an important role in maintaining genomic stability.

Taken together, these mouse models confirm what has been shown in cell culture experiments: disruption of the shelterin complex leads to potent DDR activation *in vivo*. Furthermore, in the setting of checkpoint deficiency, such as p53 loss, telomere dysfunction due to uncapping of shelterin proteins can generate genomic instability and promote tumor formation, similar to mouse models of severe telomere attrition[71]. However, these phenotypes may be tissue specific, with the proliferative epithelial compartments, such as the skin, being more susceptible than other organs, such as the liver. Future work may utilize these systems to study telomere dysfunction in other epithelial organs systems, such as the gastrointestinal tract or breast.

Conclusions

The 2009 Nobel Prize in Physiology and Medicine to Blackburn, Greider and Szostak highlighted the important roles that telomere and telomerase play to solve the end replication problem to maintain genome stability. Emerging evidence suggests that the core telomere complex, shelterin, help solve the end protection problem to defend the end zone. Shelterin components interact with members of the DDR machinery to promote normal telomere metabolism (for example, regulating telomere length and the generation of the 3' overhang), but also must repress their deleterious effects at telomeres (for example, inappropriate repair to produce end-to-end chromosome fusions). Altering shelterin component levels in both cells and mouse models result in genome instability, and in the setting of p53 deficiency could promote neoplastic transformation. The observation that TIN2 is mutated in human DC suggests that shelterin components may play important roles in preventing the onset of proliferative disorders in stem cell compartments, including cancer and aging. These phenotypes should be further explored using conditional tissue specific approaches in mouse models of shelterin deficiency.

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Figure 1. Shelterin: defender of the end zone

Shelterin is composed of six players: TRF1, TRF2, TIN2, RAP1, TPP1 and POT1. TRF1 is a negative regulator of telomere length and is required to prevent replication fork stalling at telomeres. TRF2-RAP1 protects telomeres from engaging in an ATM-dependent DNA damage response (DDR), while TPP1-POT1 binds to the single-stranded overhang to protect telomeres from activating an ATR-dependent DDR. Inappropriate activation of repair pathways at telomeres results in chromosome fusions and genome instability. TPP1-POT1 also regulates telomerase access to telomeres to influence telomere length.