

# Shelterin Complex at Telomeres: Implications in Ageing

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**Abstract:** Different factors influence the development and control of ageing. It is well known that progressive telomere shortening is one of the molecular mechanisms underlying ageing. The shelterin complex consists of six telomere-specific proteins which are involved in the protection of chromosome ends. More particularly, this vital complex protects the telomeres from degradation, prevents from activation of unwanted repair systems, regulates the activity of telomerase, and has a crucial role in cellular senescent and ageing-related pathologies. This review explores the organization and function of telomeric DNA along with the mechanism of telomeres during ageing, followed by a discussion of the critical role of shelterin components and their changes during ageing.

**Keywords:** ageing, sheltering complex, telomeres

## Introduction

Telomeres are nucleoprotein structures at the end of eukaryotic chromosomes, which consist of DNA repeating sequences (TTAGGG) and individual proteins. These structures maintain genome integrity by capping the chromosome terminus.<sup>1,2</sup> Telomeres are associated with specific proteins composed of six-subunits called either shelterin. Telomeres associated with shelterin complex play an essential role in chromosome protection and regulation of telomere length. In each round of replication, due to the “end replication problem”, telomeres become slightly shorter.<sup>3-5</sup> Telomeres progressively shorten during ageing both in in vitro and in vivo conditions. When the telomere reaches a critical length, this leads to cell cycle arrest, cellular senescence, and apoptosis. Therefore, telomere length can regulate the lifespan of the cells. On the other hand, some mutations in telomere-binding proteins, render cells to escape from telomere shortening and become immortal.<sup>6</sup> However, It is now documented that senescent cells secrete factors that influence age-associated diseases while remaining viable but not dividing for long periods.<sup>7</sup> Accumulation of senescent cells during ageing reflects a gradual increase in different types of damage in different tissues.<sup>8,9</sup> Therefore, senescent cells often exhibit high levels of various forms of damage accumulation overtime in an old organism, including DNA damage and oxidative modifications.<sup>10</sup> One of the most significant markers of ageing in both microorganisms and senescent cells are short telomeres.<sup>11</sup> In addition, dysfunctional/damaged telomeres result in senescence in postmitotic cells.<sup>12</sup> Therefore, the present review attempts to explore the organization and function of telomeric DNA, the mechanisms through which telomeres influence ageing, and the critical role of shelterin components therein.

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## Organization and Function of Telomeric DNA

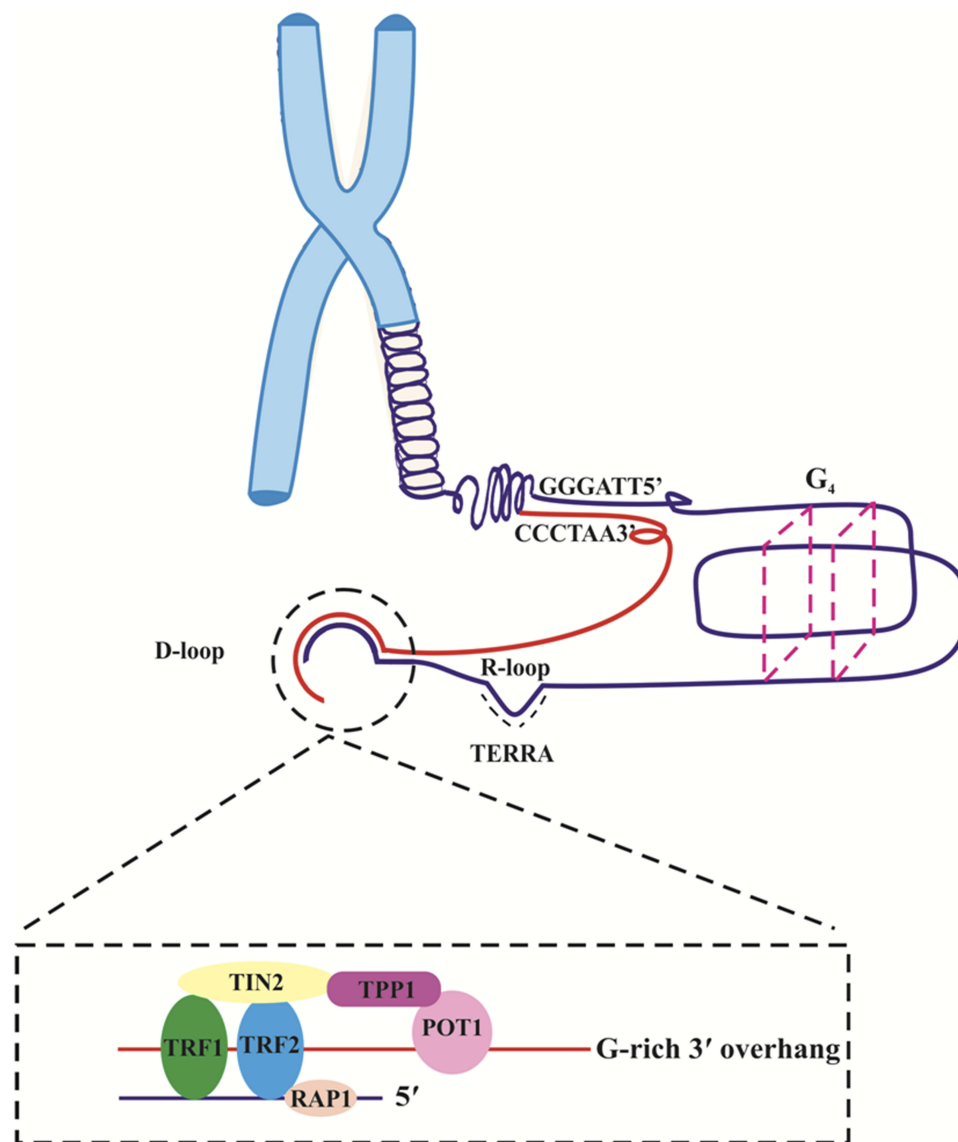
Mammalian telomeric DNA with a length of 5–15 kb in human is composed of TTAGGG repeats, the 3' end of which demonstrates a G rich mono-strand overhang stretched out beyond its counterpart.<sup>13</sup> The G-rich single-stranded overhang can fold back and invade the double-stranded region of the telomere.<sup>14</sup> Telomeres in mammals and many other organisms form t-loops. T-loop is formed by invasion 3' overhang into telomeric DNA.<sup>15,16</sup> Also, evidence suggest that 3' overhang pairs with the C-rich strand and displaces the G-rich strand into a D loop.<sup>17,18</sup> Besides, t-loop hides the 3' end from telomerase and DNA repair activities and prevents from false activation of DNA repair types of machinery such as ataxia telangiectasia mutated (ATM) and Rad3-related protein (ATR) signalling pathways.<sup>19–21</sup> Telomeres are made up of G-quadruplex (G4).<sup>22</sup> Studies providing novel insights into G4 and G4-related protein interactions have revealed a potential involvement of G4s in essential processes such as initiation of DNA replication and telomere maintenance.<sup>23</sup> Also, it has been suggested that G4s have regulatory roles in oncogene promoter regions, transcription, and translation.<sup>24</sup>

Telomerase is a ribonucleoprotein (RNP) enzyme, with two essential subunits: telomerase reverse transcriptase (TERT) and telomerase RNA (TR). Telomerase recognizes the 3'-OH at the end of chromosomes and extends telomeres by using associated RNA molecules as a template.<sup>25</sup> Recent studies have indicated that telomeric DNAs are not transcriptionally silenced and telomere position effect phenomenon occurs when a normally euchromatic gene is inserted into the telomere of a eukaryotic chromosome.<sup>26,27</sup> Despite these findings, it has been revealed that telomeric DNA is transcribed by RNA polymerase II from telomere-repetitive nucleotide region, found on the 3' end of the chromosome, to the long non-coding RNA termed telomeric repeat-containing RNA (TERRA).<sup>28,29</sup> Accumulated data indicate that TERRA transcription has a pivotal role in the regulation of heterochromatin formation at telomeres and in the protection of DNA from deterioration or fusion with neighbouring chromosomes.<sup>29,30</sup> Also, TERRA is considered a scaffold molecule is promoting the recruitment of proteins and enzymatic activities at the 3' end of the chromosome.<sup>31,32</sup> TERRA forms DNA-RNA hybrid structures at telomeres, known as R-loops.<sup>33</sup> TERRA influenced heterochromatin formation at telomeres by interacting with several heterochromatin-associated proteins such as methyl-CpG-binding protein (MeCP2)

and heterochromatin protein 1 (HP1), and heterochromatic histone modifications, especially H3 K9me3.<sup>34–36</sup> Also, as highlighted above, TERRA is a critical regulatory factor in controlling the telomeric length and telomerase activity in telomeres through interaction with various protein complexes such as shelterin and CST as a trimeric complex including Ctc1, Stn1, Ten1<sup>28,32,37</sup> and predominantly with the hnrpA1. Indeed human hnRNP A1 can disrupt the higher-order structure of human telomeric DNA<sup>38,39</sup> and TERRA act as a scaffold in the telomere-neighbouring region and inhibits hnRNPA1 localization at the telomere.<sup>40</sup>

In human cells, the shelterin complex is composed of six proteins, including TRF1, TRF2, POT1, RAP1, TIN2 and TPP1<sup>41</sup> (Figure 1). CST is a trimeric protein complex consisting of CTC1, STN1/OBFC1, and TEN1. Both Shelterin and CST complexes are involved in capping, protecting and regulating telomeres via prevention of DNA repair machinery from detecting double-strand DNA breaks and induction of folding telomeric DNA homologous end joining (NHEJ), protects the telomeres from ATM activation, controls TERRA transcription, and facilitates unwinding of t-loop to allow telomerase access to the telomeric DNA.<sup>42</sup> Furthermore, it has been shown that RAP1 cooperates with TRF2 in repressing aberrant homologous recombination (HR) at telomeres.<sup>43</sup> Hence, TRF2/RAP1 heterodimer has an important role in protecting telomeres.

TPP1/POT1 heterodimer complex performs an important protective activity at telomeres by inhibiting ATR kinase and promoting telomerase activity at telomeres.<sup>44</sup> TIN2, as the main core of the shelterin complex, has a pivotal role in the suppressing of ATM and ATR signalling pathways through interactions with POT1/TPP1 heterodimer and in stabilizing TRF2 at the telomeric DNA.<sup>45–47</sup> As mentioned earlier, the CST complex is important for telomeric DNA maintenance in a multifaceted manner. Indeed, the CST complex promotes efficient replication of telomeric DNA, and thus it has a positive role in maintaining telomere stability. CST depletion leads to delaying C strand synthesis and enhancing the rates of fragile telomeres, which results in the maintenance of extended G-overhangs.<sup>48</sup> The CST complex performs these actions by regulating DNA polymerase alpha-primase (pol-alpha-primase).<sup>49</sup> Besides, this complex contributes to genome maintenance by controlling the access of telomerase to the chromosome terminus, which is a process closely associated with the shelterin complex.



**Figure 1** Telomere organization and function. Telomeres consist of a repetitive sequence TTAGGG and related to protective proteins, shelterin. Telomeric DNA, shelterin complex and TERRA make secondary structures such as G-quadruplexes (G<sub>4</sub>), T-loop, and R-loop, respectively. Collectively, these structures protect the ends of chromosomes.

## Mechanism of Telomere Shortening During Ageing

Telomeres are subject to shortening during each cell division due to end replication problem.<sup>50</sup> Telomerase enzyme, as reverse transcriptase, solves this problem by adding the TTAGGG sequence to the 3'-end of the chromosomes and maintains telomere length and cellular immortality.<sup>51</sup> Telomerase is expressed in embryonic stem cells, and its expression is silenced in most adult somatic tissues. However, this is not sufficient to maintain the telomere length.<sup>52–54</sup> Excessive shortening of chromosomes results in the loss of essential genes in their end.<sup>55</sup> Indeed, when the length of telomeres becomes critically short, the

secondary protective structure does not occur; thus the cells enter replicative senescence.<sup>13</sup> Meanwhile, shelterin complex can act as a critical regulator for the activity of telomerase. It serves as a negative regulator of telomerase since it is attached to telomeres in a manner dependent on their length (Table 1).<sup>56</sup>

Excessive telomere shortening due to a defect in the function of telomerase or any components of the shelterin activates the DNA damage response (DDR) at chromosome ends by ATM/ATR where DDR system enables the NHEJ,<sup>57</sup> leading to end-to-end chromosomal fusion, increased HR, and genomic instability. On the other hand, activation of DDR system stimulates the tumour

**Table 1** The Role of the Components of Shelterin Complex in Telomeres and Telomerase Regulation

Components of Shelterin	Important Functions	Type of Regulator for Telomerase
TRF1	Length regulation, capping, telomere replication, DDR, mitosis, polymerization of microtubules, prevention of telomere fragility,	Negative regulator
TRF2	Length regulation, capping, DDR	Negative regulator
TIN2	Length regulation, bridging shelterin	Negative regulator
TPP1	Capping, telomere recruitment, bridging shelterin, Length regulation, prevention of telomere fragility	Connection
POT1	Protection of 3' overhang from illegitimate recombination, DDR, regulating telomere length, catastrophic chromosome instability, and abnormal chromosome segregation.	Negative regulator
RAP1	Length regulation, DDR, subtelomeric silencing, prevention of telomere recombination and telomere fragility, transcriptional gene regulation, NF-κB pathway regulators, losses of site-specific histone, maintenance of chromosomal integrity.	Positive regulator

suppressors p53 and p21, which in turn induces cell cycle checkpoint activation to take time for DNA repair, apoptosis or senescence in the cell, finally causing ageing.<sup>54</sup>

## Telomeric Repeat Binding Factors

The telomeric repeat binding factors 1 and 2 (TRF1 and TRF2) are components of the shelterin complex which directly connected to double-stranded telomeric 5'-TTAGGG-3' repeat and have a similar domain structure. These proteins are often in the form of dimer binding to repeat sequences 5'-TTAGGG-3' of telomeric DNA. On the other hand, TRF1 and TRF2 are connected to TIN2 and RAP1, respectively.<sup>51,54</sup> It has been shown that TRF1 and TRF2 participate in DNA double-strand break repair at non-telomeric regions.<sup>58</sup> Both TRF1 and TRF2 regulate telomerase activity, but neither plays a role in regulating telomerase gene expression.<sup>59</sup> TRF1 suppresses telomerase action on telomere region, while TRF2 activates a telomeric degradation without any effect on telomerase.<sup>60</sup> Thus, increasing the expression of TRF1 and TRF2 acts as a negative regulator for telomere length, and also an inhibitor for telomerase enzyme.<sup>61</sup>

### TRF1

It has been shown that accessibility of TRF1 is essential for the maintenance of telomere, and downregulation of TRF1 can activate the ATR kinase and consequently induce fragile site phenotypes at the telomere. TRF1 is a telomeric protector against repair systems, and its availability is crucial for telomere function. Therefore, telomere length is associated with ageing, and the role of telomere length regulators such as TRF1 and TRF2 is so crucial in this regard. On the other

hand, TRF1 localizes not only telomeres but also to mitotic spindles and has a decisive regulatory role in microtubule polymerization in vitro.<sup>61,62</sup>

A study assessing the integrity of telomeres and the stability of telomere protection during ageing in endothelial cells (EC) showed that telomere length decreased by 62%. This telomere dysfunction is accompanied by a 33% decrease in TRF1 mRNA expression and a 24% reduction in the TRF1 protein.<sup>63</sup> Thus, decreased telomere length in ageing cells causes negative feedback and inhibits TRF1 gene expression.<sup>64,65</sup> Furthermore, telomere length does not affect the amount of the shelterin subunits.<sup>66</sup> However, a study conducted on the neonatal human dermal fibroblasts showed that the protein level of the TRF2 decrease significantly during ageing.<sup>67</sup>

The critical role TRF1 plays in telomere biology, and disease has remained unclear. TRF1 depletion in mouse embryonic fibroblasts (MEFs) did not change telomere length. On the other hand, P53 is the protector of the genome which exerts its tumour suppression activity by regulating a large number of downstream targets involved in cell cycle arrest, DNA repair, and cellular senescence.<sup>68</sup> Instead, it has been reported to lead to rapid induction of p53/RB-dependent cellular senescence simultaneous with the accumulation of full damage foci at telomeric DNA.<sup>68,69</sup> This persistent DNA damage activates phosphorylation of the ATM/ATR kinases, and their downstream effectors, the kinases CHK1 and CHK2, resulting in cell cycle arrest.<sup>69</sup> Cells deficient in TRF1 show abundant end-to-end telomere fusions involving both chromosomes and sister chromatids.<sup>70</sup> These mice die

and show reduced skin thickness and skin stratification, as well as severe skin hyperpigmentation. Newborn mice also have focal dysplasia in the epithelia of the palate, the non-glandular stomach, oesophagus, tongue and skin. These pathologies are accompanied by activation of a persistent DDR at telomeres, which induces activation of the p53/p21 and p16 pathways, resulting in an *in vivo* cell cycle arrest. The latter produces dramatic alterations in the properties of the epithelial stem cells. Thus, morphological development of the hair follicles and the sebaceous glands is completely impaired.<sup>68,70</sup>

## TRF2

TRF2 suppresses an ATM-dependent DDR at chromosome ends and inhibits end-to-end chromosome fusions and classical NHEJ by folding telomeric DNA into a t-loop.<sup>42</sup> Moreover, TRF2 and RAP1 are essential to prevent t-loop cleavage by repressing the activation of PARP1 (poly ADP-ribose polymerase 1) to protect telomeres from HR-mediated repair in mammals.<sup>43</sup> Investigation of the effect of TRF2 deletion showed that end-to-end chromosome fusion strongly depends on the binding of 53BP1 to damaged chromosome. In the absence of 53BP1, this fusion can be rescued.<sup>71</sup> Also, in hepatocytes, the inhibition of the NHEJ pathway and DDR by the elimination of 53BP1 does not have a considerable impact on phenotypes.<sup>72</sup> In contrast, in epidermal cells, TRF2 depletion stimulates a rapid DDR that leads to inhibition of cell proliferation, and cell death.<sup>73</sup> This distinct different outcome is achieved due to the diverse nature of tissues.<sup>73</sup>

A new mouse model with excessive short telomeres has been generated by overexpressing the TRF2 telomere-binding protein, known as K5-TRF2 mice.<sup>74</sup> It was reported that in the presence of telomerase and regular enzyme activity, telomere length could be short, leading to precocious ageing and increased cancer.<sup>75</sup> In K5-TRF2 mice, by increasing the TRF2 expression, short telomeres are possible only in the presence of a nuclease called XPF. With the removal of XPF nucleases, it has been observed that telomeres begin to expand even with TRF2. It is proposed that this enzyme rapidly degrades telomeres by TRF2 overexpression.<sup>74–76</sup> Therefore, telomere-binding proteins have a direct role during ageing, which may be independent of telomerase, and they may serve as a new tool to understand the consequences of critical telomere shortening as having a crucial effect on human life and ageing pathologies.

## TIN2

As an adaptor protein, the TRF1-interacting protein 2 (TIN2) plays a linking role at the shelterin complex. Also, this vital protein can bind TRF1 and TPP1-POT1 complex, which construct the bridge among different shelterin components.<sup>54</sup> Furthermore, attachment of TIN2 to TRF1 induces changes in TRF1 conformation and stabilizes the telomeric structure, and these changes inhibit telomerase access to telomeres.<sup>77,78</sup> Therefore, TIN2 is an inhibitor of the telomerase enzyme, and the absence of TIN2 is useful for telomere accessibility and elongation.<sup>79,80</sup>

On the other hand, it has been illustrated that shelterin components play critical roles in the activation of DDR mediators. For instance, two of the most essential DDR regulators are ATM and ATR, the activation of which is caused by depletion of TIN2.<sup>45,81,82</sup> Interestingly, TIN2 can act independently of TPP1. That is, a truncated form of TIN2 lacking the TPP1 interaction can compel telomerase-dependent telomere extension.<sup>79</sup> Recently, several studies have been done on the biological correlation of TIN2 with other shelterin components during ageing.<sup>79,83</sup> In this regard, mutations in TIN2 are associated with premature ageing phenotypes.<sup>84</sup> It was shown that heterozygous TIN2-R282H mutation in telomerase-positive human cells via a knock-in approach does not interfere with the spatial structure of other shelterin components on telomeres. However, TIN2-R282H mutation either activates the telomeric DNA damage signalling or presents different aspects of telomere instability related to telomerase activity. These observations indicate that TIN2 has a direct role in mediating telomere length induced by telomerase, apart from its position in telomere protection.<sup>80</sup> Accumulating evidence has shown that TIN2 has a role in mitochondria.<sup>84,85</sup> Meanwhile, mitochondrial metabolism has been demonstrated to regulate ageing.<sup>86,87</sup> Therefore, further investigation of the correlation between TIN2, mitochondrial metabolism, and ageing is a hot topic. Regarding different studies, we think that new research is needed to verify the direct effect of TIN2 in mitochondrial metabolism, DNA damage response, and ageing.

## POT1

Increasing data have shown that one of the critical issues in the field of telomere length control is to manage how diversely proteins bound to the duplex telomeric DNA regulate telomerase.<sup>88,89</sup> Protection of telomeres 1 (POT1) has illustrated this vital challenge. POT1 as a critical component



of the shelterin complex is directly connected to 3' single-stranded G-overhang. Also, POT1 can bind to TRF1<sup>90</sup> via protein-protein interactions. G-quadruplex formation inhibits telomere elongation; however, POT1 by displacing/replacing a g-quadruplex structure promotes telomerase activity.<sup>91</sup> Besides, POT1 regulates telomere length and telomere capping, and it has a vital role in the regulation of telomerase activity on telomeres. Furthermore, several functions of this protein include protection of chromosome ends from recombination, catastrophic chromosome instability, and abnormal chromosome segregation.<sup>92</sup> Many studies have been conducted on the association between the function of POT1 with senescence.<sup>93,94</sup> For instance, depletion of POT1 in mouse embryonic fibroblasts (MEFs) and chicken cells was found to lead to a detrimental DNA damage response in telomeres resulting in telomere dysfunction-induced foci (TIFs).<sup>92</sup> According to one study, the loading of POT1 to telomeric single strand DNA (ssDNA) regulates telomere elongation. In addition, it was shown that PTOP, as a new telomere protein, interacts with both TIN2 and POT1. This novel protein binds to the POT1 and recruits it to telomeres. Therefore, suppression of PTOP inhibits the localization of POT1 to telomeres.<sup>95</sup> In humans, the shelterin complex has a single POT1 protein, while in mice the genome has two POT1 orthologs, namely Pot1a and Pot1b, each has its function at telomeres. For instance, Pot1a is involved in the inhibition of the DNA damage response at telomeres, while Pot1b is required in maintaining telomere terminus structure.<sup>96,97</sup> Hockemeyer and colleagues observed that POT1a and POT1b were predominantly not necessary for the suppression of telomere fusions, and they reported that human telomerase requires one POT1 protein, as opposed to mouse telomeres which have two.<sup>97</sup> Meanwhile, POT1a loss through tumour suppressor P53 leads to ageing induction.<sup>98,99</sup> It was also found that POT1 is associated with repressing the ATR pathway, and deletion of POT1a or POT1b did not change telomere length.<sup>97,100</sup> Recently, increasing evidence has indicated that many components of shelterin complex such as Pot1b control hematopoietic stem cell (HSC) activity and survival during ageing.<sup>101</sup> Furthermore, Pot1a knockdown raises DNA damage response and represses self-renewal in mouse HSC.<sup>102</sup> Moreover, overexpression of Pot1a inhibits DNA damage response, maintains self-renewal activity, and rejuvenates aged HSCs.<sup>103</sup> Yu et al demonstrated that at the time of DNA double-strand breaks, POT1 arrives at DNA damage sites.<sup>58</sup> It suppresses the efficiency of non-homologous end-joining (NHEJ), fixes DNA double-strand breaks, and

promotes NHEJ fidelity.<sup>58</sup> Also, POT1 overexpression represses the protein stability of Lig3, which is the principal regulator of alternative NHEJ (alt-NHEJ), hence inhibits the efficiency of alt-NHEJ.<sup>58</sup>

## TPP1

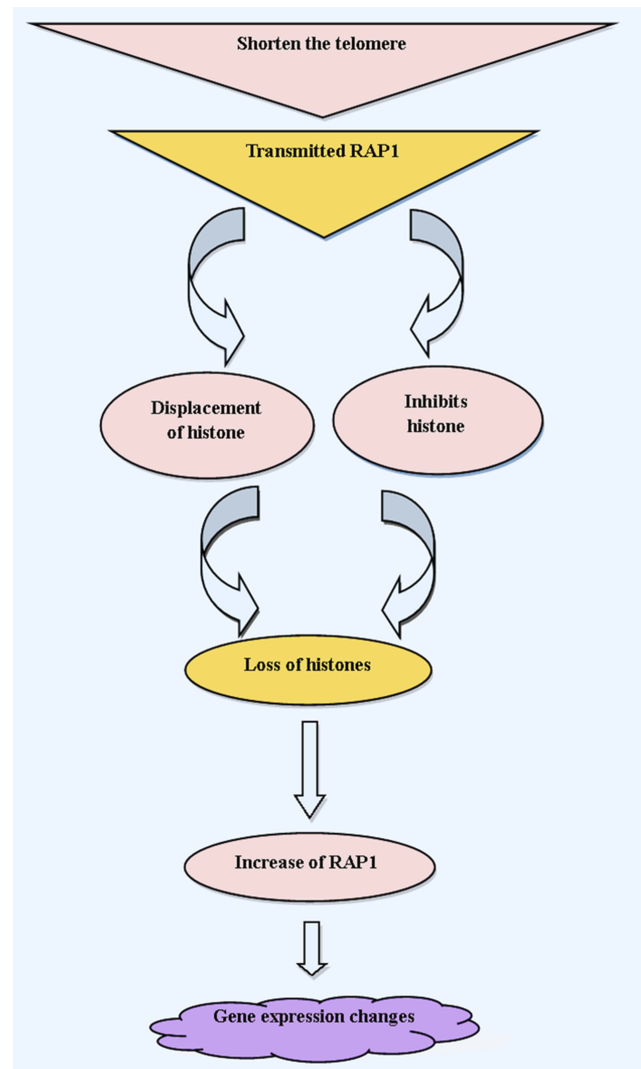
One of the six components of the shelterin complex is TPP1, which is called TIN2, PTP1 or PIP1. This adaptor protein not only binds TIN2 and POT1, but mainly interacts with POT1.<sup>54,104</sup> Concerning the role of TPP1 in linking TIN2 and other components of shelterin complex with POT1, any mutation leads to structural alteration in TPP1 for recruiting of the shelterin complex.<sup>105,106</sup> One of the most critical functions of TPP1 is regulation of telomerase recruitment to telomeres via the interaction with telomerase reverse transcriptase (TERT), as a catalytic part of the telomerase, for telomere maintenance.<sup>106–108</sup> Besides, through mediators of DNA damage response such as ATM and ATR, TPP1 plays a pivotal role in growth arrest.<sup>109</sup> Therefore, POT1 and TPP1 bind as a heterodimer to ss (single-strand) telomere DNA to inhibit DNA damage responses from capped telomeres.<sup>14,110</sup> On the other hand, it was shown that mouse cells lacking TPP1 exhibit an increase in chromosomal fusions with non-homologous chromosomes.<sup>111</sup> Since this protein is a binding subunit, it has been shown that the conditional elimination of TPP1 in MEFs causes a simultaneous removal of POT1a and POT1b from telomeres. In contrast, the other subunits of the shelterin components are not altered in the telomeres.<sup>104</sup> Regarding the vital role of TPP1 and POT1 in telomere end protection via repression of DNA damage response and also chromosome fusion, it was illustrated that depletion of TPP1 elicits a robust DNA damage response at telomeres, which is mediated by ATR and results in an increase of ss telomeric DNA.<sup>44,104</sup> Also, TPP1-depleted cells seem to recapitulate the telomere dysfunction phenotypes observed in Pot1a/b double knockout cells, in agreement with the proposed role for TPP1 in recruiting POT1a and POT1b to chromosome ends.<sup>104</sup> As a nicotinamide dinucleotide (NAD<sup>+</sup>)-dependent deacetylases, Sirtuin 1 is a protein encoded by the *SIRT1* gene in humans, and it is involved in different cellular events including DNA repair and ageing.<sup>112</sup> Notably, *SIRT1* suppresses age-related mesenchymal stem cells (MSCs) senescence by mechanisms that include enhanced TPP1 expression, increased telomerase activity, and reduced DNA damage.<sup>113</sup> Furthermore, according to a study using genome engineering of human embryonic

stem cells by knockdown and overexpression of shelterin subunit in human tumour cells, TPP1 recruits telomerase to telomeres via a region termed the TEL-patch.<sup>114</sup> The TEL-patch of TPP1 is genetically essential for telomere elongation and thus, long-term cell viability.<sup>114</sup> Meanwhile, TPP1 provides a necessary step of telomerase activation as well as feedback regulation of telomerase by telomere length, which is necessary to determine the appropriate telomere length set point in human embryonic stem cells.<sup>114</sup> Hence, it has been revealed that TPP1 has multiple vital roles in telomere elongation and stem cell telomere length homeostasis.<sup>106,115</sup> Recently, it was found that in *Tpp1*-deficient MEFs and mice with *Tpp1* deletion, induction of telomere damage foci and cell cycle arrest.<sup>116</sup> Moreover, similar to TRF1 deficiency, TPP1-null mice died, which in turn indicating severe skin hyperpigmentation, as well as defective hair follicle morphogenesis.<sup>116</sup> These phenotypes are rescued via p53 suppression, demonstrating that p53 is a central effector of proliferative defects related to TPP1 deletion. Unpredictably, TPP1 deletion leads to decreased TERT binding to telomeres and enhanced telomere shortening in MEFs and mice.<sup>116</sup> Overall, according to different reports in the case of TPP1 function, and its relationships with other components of shelterin complex including, TIN2 and POT1, this protein plays a linking role in appropriate telomere maintenance. Also, as mentioned above, via recruitment of telomerase, TPP1 has a vital role in the control of telomere length.

## RAP1

Repressor/activator protein 1 or RAP1 is another part of the shelterin complex that can only connect to telomeres by binding to TRF2.<sup>51,117</sup> The highly conserved RAP1 is encoded by *TERF2IP* gene, which is the only component of the shelterin complex that is not vital in mice. Hence, in contrast to other shelterin subunits, RAP1 does not have a protective role in telomeres. One of the most essential contradictions about this protein is that some studies have indicated that RAP1 plays a dual role at the telomere. Several studies demonstrated that at telomere, RAP1 was implicated in preventing non-homologous end joining, whereas other studies reported that RAP1 was involved in preventing homologous recombination at telomere.<sup>118–122</sup> One the other hand, it was shown that TRF2 recruited RAP1 to telomeric repeats, and that the status of RAP1 is dependent on TRF2 in mouse cells.<sup>123</sup> RAP1 has many other functions in both the nucleus and cytoplasm, including transcriptional repression of telomere-proximal genes,

and transcriptional activation of hundreds of mRNA-encoding genes, including highly transcribed ribosomal proteins and glycolytic enzyme-encoding genes.<sup>124</sup> It has been revealed the mice that were deleted RAP1 are viable but have shorter telomeres and developed skin hyperpigmentation at adulthood.<sup>125</sup> Thus, RAP1 is not necessary for mouse viability and telomere capping, whereas it is implicated in the protection of telomere recombination, fragility and length.<sup>125</sup> Also, RAP1 plays a crucial role in the regulation of gene expression due to adjacency with subtelomeric regions of chromosomes.<sup>118,126,127</sup> RAP1 mainly activates gene expression, but sometimes acts as an inhibitor (notably at telomeres and the silent mating loci).<sup>128,129</sup> In healthy cells, RAP1 only covers the

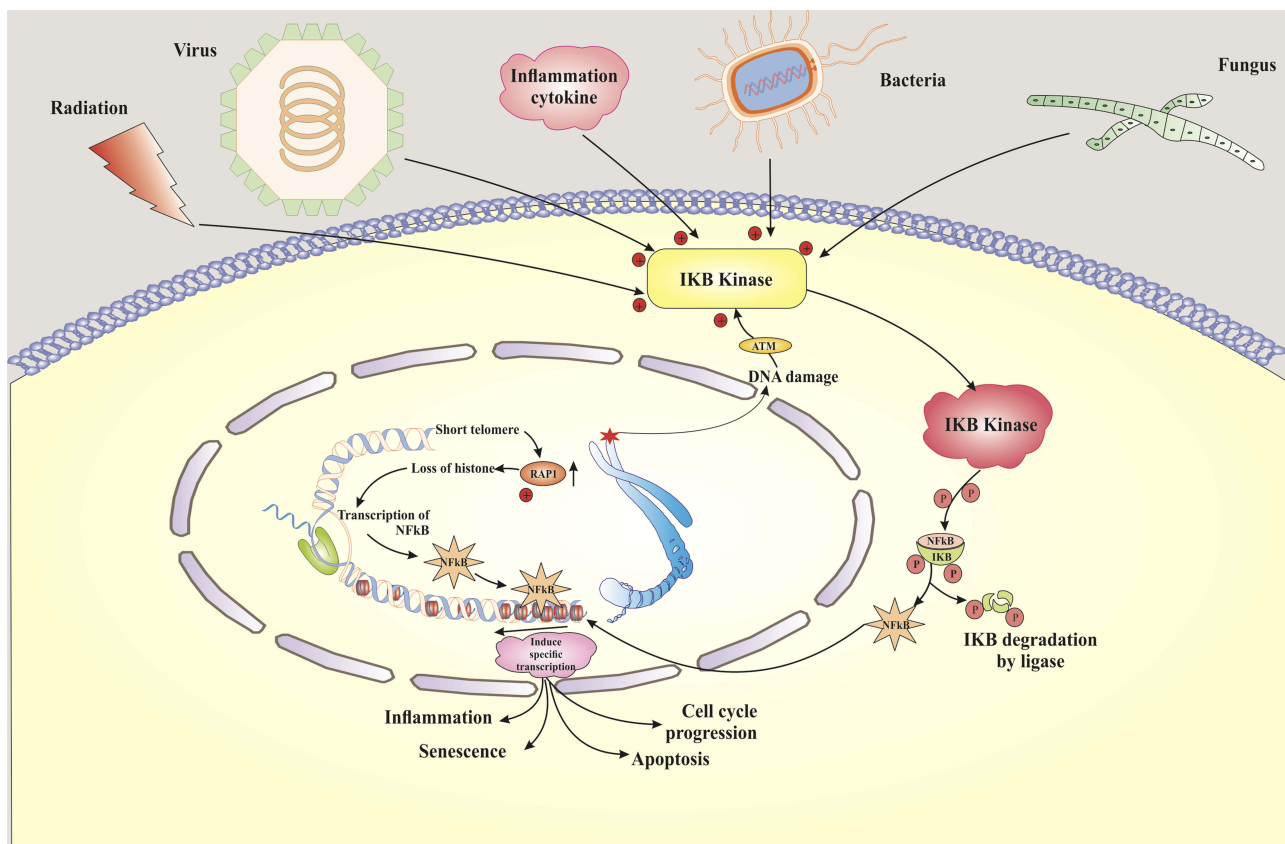


**Figure 2** Schematic representations show the mechanism of ageing by telomere shortening, and RAP1 effects on histone, as well as alterations of gene expression involved in ageing.

telomeric and subtelomeric region of the chromosome. Still, in senescent cells, due to critically short telomeres, RAP1 is transmitted to promoters of natural and new RAP1 targets at senescence (NRTS).<sup>127,130</sup> By connecting to the new promoter, RAP1 causes the displacement of histone. Eventually, this displacement leads to site-specific histone losses. Furthermore, RAP1 is transmitted to the promoters of histone genes and inhibits their transcription. As a result of cells that are senesced, RAP1 increases with decreasing histone levels, which in turn results in more RAP1 occupancy at NRTS at senescence<sup>131,132</sup> (Figure 2).

One of the non-telomeric activities of RAP1 is its regulatory role in the NF-κB signalling pathway.<sup>133</sup> It has been indicated that increased expression of RAP1 in senescence cells induces NF-κB pathway, shortens the telomere and starts ageing, and that releasing the RAP1 from telomeres to other regions contributes to the activation of NF-κB.<sup>125,134,135</sup> Mechanistically, despite its inability to bind directly to DNA, Rap1 can exclusively modulate NF-κB-dependent transcription in mammals.<sup>133,136</sup> Meanwhile, mammalian Rap1 interacts with IKKs, and depends on

their interaction with IKKs for their ability to activate NF-κB.<sup>136</sup> Rap1 is essential for IKK-mediated P65 phosphorylation, but not IκBα.<sup>137</sup> Also, NF-κB signalling pathway seems to be one of the primary mediators of ageing that is activated by genotoxic, oxidative, and inflammatory stresses.<sup>138</sup> On the other hand, NF-κB pathway modulates expression levels of cytokines, growth factors, and genes which are involved in controlling the senescence.<sup>139</sup> Also, the transcriptional activity of NF-κB is increased in most of the diseases associated with age. In mouse models, inhibition of NF-κB leads to delayed onset of age-related symptoms and pathologies<sup>138</sup> (Figure 3). Taken together, accumulating evidence has shown that RAP1 not only plays a crucial role in protecting the telomere ends from the attacks via the different DNA repair mechanisms but also has multiple functions in specific signalling pathways and metabolism. Given the controversy over the particular purpose of RAP1, further investigation is necessary to evaluate this protein comprehensively. For instance, the role of RAP1 in signalling pathways involved senescent cells could be a line of inquiry.



**Figure 3** Schematic representation shows the mechanism of regulatory RAP1 through NF-κB pathway, which leads to the induction of cellular senescence.



## Conclusion

Regulation of shelterin biology during ageing is still poorly understood, even though shelterin-related complexes are involved in ageing-related pathologies. Each of these proteins has a unique role during ageing. TRF1 and TRF2 are two components of the shelterin complex which regulate the telomerase activity. TIN2 is an inhibitor of the telomerase enzyme. POT1 is another member of the shelterin complex which regulates telomere length and telomere capping. Moreover, it has a role in the regulation of telomerase activity on telomeres. TPP1 provides an essential step in telomerase activation and the feedback regulation of telomerase by telomere length. RAP1 is another member of the shelterin complex that can only connect to the telomeres by binding to the TRF2. RAP1 is also one of the NF- $\kappa$ B pathway regulators. Excessive telomere shortening due to a defect in the components of the shelterin complex activates ageing. Understanding the role and mechanism of action of every protein in this complex concerning ageing may assist in the design of drugs to delay ageing.

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## Disclosure

The authors report that there are no conflicts of interest in this work.

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