

Extra-telomeric impact of telomeres: Emerging molecular connections in pluripotency or stemness

Received for publication, October 30, 2019, and in revised form, May 21, 2020. Published, Papers in Press, May 22, 2020, DOI 10.1074/jbc.REV119.009710

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Edited by Eric R. Fearon

Telomeres comprise specialized nucleic acid–protein complexes that help protect chromosome ends from DNA damage. Moreover, telomeres associate with subtelomeric regions through looping. This results in altered expression of subtelomeric genes. Recent observations further reveal telomere length–dependent gene regulation and epigenetic modifications at sites spread across the genome and distant from telomeres. This regulation is mediated through the telomere-binding protein telomeric repeat–binding factor 2 (TRF2). These observations suggest a role of telomeres in extra-telomeric functions. Most notably, telomeres have a broad impact on pluripotency and differentiation. For example, cardiomyocytes differentiate with higher efficacy from induced pluripotent stem cells having long telomeres, and differentiated cells obtained from human embryonic stem cells with relatively long telomeres have a longer lifespan. Here, we first highlight reports on these two seemingly distinct research areas: the extra-telomeric role of telomere-binding factors and the role of telomeres in pluripotency/stemness. On the basis of the observations reported in these studies, we draw attention to potential molecular connections between extra-telomeric biology and pluripotency. Finally, in the context of the nonlocal influence of telomeres on pluripotency and stemness, we discuss major opportunities for progress in molecular understanding of aging-related disorders and neurodegenerative diseases.

The ends of eukaryotic chromosomes have specialized nucleotide-protein complexes called telomeres. In mammalian cells, they are capped by a complex of six proteins, TRF1, TRF2, POT1, RAP1, TIN2, and TPP1, known as shelterin (1–3). The shelterin proteins have distinct roles. TRF1 and TRF2 bind to double-stranded telomeric DNA, whereas POT1 binds to single-stranded telomeric DNA. RAP1 associates with TRF2, whereas TPP1 and TIN2 primarily associate with POT1 (4) (Table 1). Together the shelterin proteins form subcomplexes that vary in ssDNA and dsDNA binding. These result in two broad functions: first, protection of telomeres to evade DNA damage repair at chromosome ends (which, when affected, results in chromosome end fusions and genomic instability (5, 6)) and, second, regulation of the recruitment of telomerase (the catalytic reverse transcriptase that synthesizes telomeres) to telomere ends to maintain the

length of telomeres (7–9). The involvement of telomeres in cellular homeostasis, aging, and disease risk (10–12); initiation and progression of cancer (13–15); and variation of telomere length, during evolution and in different species (16–18), have been extensively reviewed (12).

Relatively recent work shows association of shelterin proteins outside telomeres across the genome (19–21), suggesting functions that are extra-telomeric, or beyond telomeres. Extra-telomeric functions include how telomeres influence gene expression in the subtelomeric regions (~10 Mb from telomeres (22, 23)), telomere length–dependent transcriptional activity, and epigenetic modifications at sites distant from telomeres (24). In addition, a large body of work suggests a role of telomeres, particularly telomere length, in self-renewal or pluripotency (25–28) (Table 1). Herein, we discuss literature that potentially bridges these two developing aspects, keeping in mind aging-related disorders that involve premature differentiation of stem cells (29).

Telomeres: Gene regulation, epigenetics, and genome organization

The role of telomeres in gene regulation first came to light in 1990. Gottschling *et al.* (30) noted heritable silencing of transgenes inserted within 4 kb from telomeric ends in yeast cells and reported this to be due to telomere position effect (TPE). Several years later, TPE was observed at chromosome 22 telomere in human lymphoblastoid cell lines (31). Extensive research followed to understand the TPE-related silencing of genes in subtelomeric regions of fungi and other organisms, such as *Trypanosoma brucei*, *Plasmodium falciparum*, *Schizosaccharomyces pombe*, *Drosophila melanogaster*, *Pneumocystis carinii*, and *Candida glabrata* (32). It was also observed that genes (*e.g.* *ISG15*, *DSP*, and *C15*) positioned ~10 Mb further from telomeres than found in TPE were down-regulated through physical association of telomeres. This was denoted as TPE-over long distance (TPE-OLD), which involves the long telomeres looping back to the chromatin, causing gene repression and shortening of telomeres, dissociating the loop leading to gene activation (Fig. 1) (22). Recent work shows telomerase reverse transcriptase gene *hTERT* is also regulated by TPE-OLD (33). TPE or TPE-OLD has been implicated in disorders such as idiopathic mental retardation, ring chromosome 17, and facio-scapulo-humeral dystrophy (32–34).

Recent findings show that telomere length influences transcription of genes as far as ~60 Mb away from telomeres. It was

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Table 1
Diverse functions of shelterin proteins

| Shelterins | Telomeric DNA binding | Chromatin organization | Gene regulation | Maintenance of the dedifferentiated state | Cancer stem cell | References |
|------------|-----------------------|------------------------|-----------------|---|------------------|------------------------------|
| TRF1 | dsDNA | ✓ | ✗ | ✓ | ✗ | 4, 55–57, 86, 96, 97 |
| TRF2 | dsDNA | ✓ | ✓ | ✓ | ✓ | 4, 24, 58–62, 76, 77, 98–106 |
| RAP1 | ✗ | ✗ | ✓ | ✓ | ✓ | 4, 19, 46–52 |
| POT1 | ssDNA | ✓ | ✗ | ✓ | ✗ | 4, 109, 110 |
| TIN2 | ✗ | ✓ | ✓ | ✗ | ✗ | 4, 53, 54 |
| TPP1 | ✗ | ✗ | ✗ | ✓ | ✗ | 4, 107, 108 |

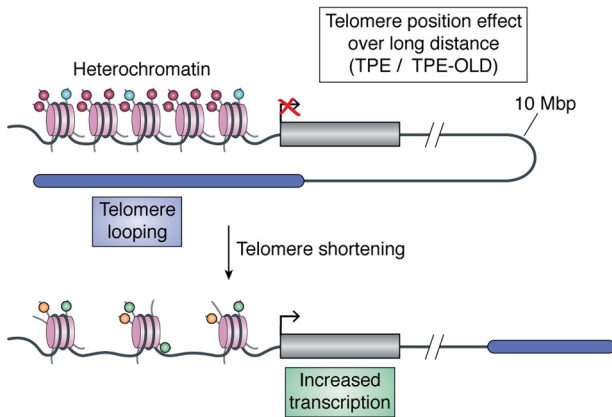


Figure 1. TPE-OLD. Physical association of relatively long telomeres by looping to the subtelomeric regions results in transcriptional repression of genes located in the subtelomeres. In relatively short telomeres, the looping is lost, and genes become transcriptionally active.

demonstrated that this was because TRF2 binding across the genome (*i.e.* extra-telomeric sites) depended on telomere length—and TRF2 occupancy at promoters affected expression of target genes (24). TRF2 is known to bind to the G-rich TTAGGG motif present as repeats at the telomeres (35). Therefore, in human cells with elongated telomeres (*i.e.* increased number of TTAGGG repeats), telomeric TRF2 binding was enhanced as expected. On the other hand, extra-telomeric TRF2 binding was reduced relative to cells with shorter telomeres (with isogenic background) (24). TRF2 levels in the nucleus, however, remained unaltered in cells with long or short telomeres, consistent with a previous report showing relatively unchanged abundance of nuclear TRF2 in different types of cells with short/long telomeres (36). Based on this, it was postulated that redistribution of TRF2 binding between telomeric and extra-telomeric sites occurs as telomeres elongate (24). This is denoted as the telomere sequestration and partitioning (TSP) model, which describes altered extra-telomeric TRF2 binding in long/short telomeres resulting in differential expression of TRF2-target promoters (Fig. 2). Moreover, altered epigenetic state of the TRF2-target promoters (*e.g.* modification of histone activation (H3K4Me1 and H3K4Me3) and suppression (H3K27Me3) marks) was evident (24).

Notable in this context are observations of extra-telomeric binding of TRF2 and/or TRF1 constituting 50 TRF2 (20) and 68 sites common to TRF1/TRF2, respectively (21). A majority of the extra-telomeric sites constituted telomere-like TTAGGG sequences and are therefore called interstitial telomeric sequence. Recent work, however, has revealed more extensive binding of TRF2 across the genome, with more than 20,000

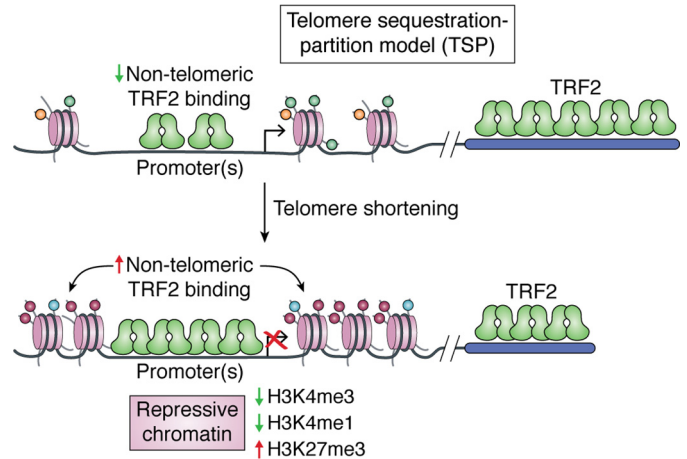


Figure 2. TSP. The model implies partitioning of TRF2 between telomeric and extra-telomeric sites. Longer telomeres sequester more TRF2, thereby depleting TRF2 binding at extra-telomeric sites. Conversely, when telomeres shorten, an increase in TRF2 binding at promoters influences TRF2-mediated chromatin modifications and transcription.

sites from TRF2 ChIP-Seq in HT1080 fibrosarcoma cells. These interstitial TRF2 binding sites comprised G-rich repeat sequences, including interstitial telomeric sequence (37). In addition, ~12,500 TRF2 peaks mapped within 20 kb of transcription start sites. Several of these promoters were tested and found to be epigenetically modified, and transcriptionally regulated, in a TRF2-dependent way (37).

Nucleosomes, the basic units of chromatin packaging in cells, comprise a complex of H2A, H2B, H3, and H4 histone proteins. Modifications (*e.g.* methylation or acetylation) of histone proteins are therefore closely related to how chromatin is packaged. These are known as epigenetic modifications that can impact gene regulation. Short telomeres, and consequent DNA damage, were noted to result in reduced histone biosynthesis (38, 39), affecting the state of chromatin.

Several studies further show shortened telomeres to be associated with genome-wide altered DNA methylation, nucleosome positioning, and histone modifications (reviewed in Ref. 40). Similar observations were also made during stem cell pluripotency, cell senescence, and cancer cell differentiation (41, 42).

Another line of investigation implicated the shelterin factor RAP1 more directly. On telomere shortening RAP1 was found to affect nucleosome occupancy, down-regulate histone genes, and increase expression of senescence-associated genes (40, 41, 43, 44). Together, these suggest a broader role of telomeres, particularly short telomeres, in the epigenetic state of the genome.

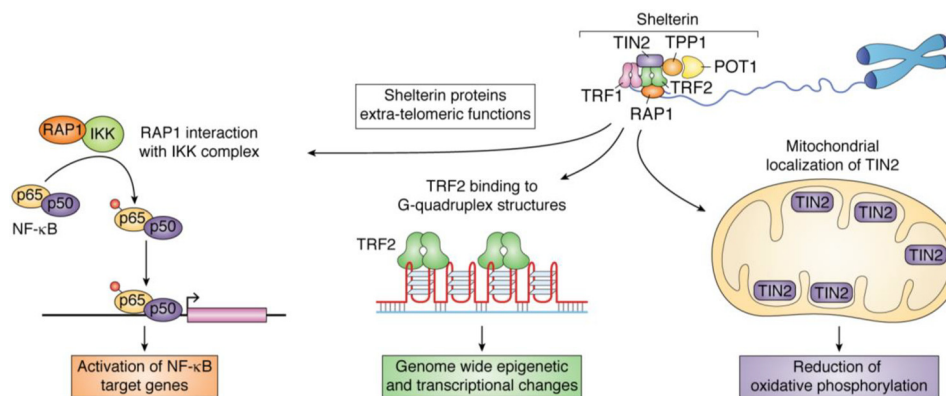


Figure 3. Extra-telomeric functions of shelterin proteins independent of telomeres. Examples of noncanonical function(s) of shelterin proteins are shown. Extra-telomeric binding of TRF2 to G-quadruplex-forming sequences across the genome induces epigenetic and transcriptional changes. NF- κ B signaling is modulated through the shelterin factor RAP1: telomere-independent interaction of RAP1 with the IKK complex results in phosphorylation of the NF- κ B p65 subunit, leading to activation of NF- κ B target gene(s). Mitochondrial localization of TIN2, another shelterin protein, was reported to negatively regulate oxidative phosphorylation.

Functions of shelterin proteins independent of chromosome-end protection

In 2010, Martinez *et al.* (19) found extra-telomeric binding of RAP1 to the TTAGGGTTAGGG consensus motif in mice; 70% of RAP1 binding was intragenic or proximal to coding regions. Based on this, they described potential RAP1 target genes in the mouse genome. In RAP1-deficient mice, about one-third of the deregulated genes were noted to be RAP1 target genes, implicating RAP1-mediated gene regulation. In a later report, genome-wide RAP1 ChIP-Seq in telomerase-deficient mice showed altered RAP1 binding on telomere shortening (45). Further, 63 genes in the human genome were reported to have RAP1 occupancy (20).

It was reported that RAP1 interacted with the I κ B kinase, a function not expected of shelterin. This resulted in activation of NF- κ B, leading to up-regulation of NF- κ B target genes (46). It was therefore postulated that expression of NF- κ B targets might be telomere length-dependent (Fig. 3) (47) (Table 1).

Extra-telomeric function of RAP1 was also noted in positive regulation of *PPAR α* and *PGC1 α* genes in mice, which affected cellular metabolism related to obesity (48, 49). RAP1 interaction with other co-factors was also reported in mesenchymal stem cell-based therapy for myocardial infarction and inflammation-dependent disorders (50, 51). Recently, Zhang *et al.* (52) observed another key function of RAP1 in epigenetic regulation of the *RELN* promoter in hematopoiesis (Table 1). Together, these show RAP1 functions that are clearly independent of its canonical role in the protection of telomeres.

Noncanonical extra-telomeric function was also observed for another shelterin protein, TIN2. A truncated isoform of TIN2, hTIN2S, was found to localize outside telomeres and affect heterochromatin organization (53). However, in cells with elongated telomeres, the dual localization was lost, such that hTIN2S redistributed from nontelomeric chromatin to telomeres exclusively. This is consistent with the TSP model described above (Fig. 2), where redistribution of a shelterin protein with change in telomere length would be expected. TIN2 was also shown to localize to mitochondria and regulate oxi-

dative phosphorylation and glucose metabolism (Fig. 3) (54) (Table 1).

Noncanonical function of TRF1 was noted in phosphorylation of nontelomeric TRF1 by Aurora-A, which resulted in mitotic abnormality (55). This was also suggested by the role of TRF1 in chromosome segregation by positively regulating Aurora-B's centromeric function (56). Further, a crystallographic study showed that TRF1 interacts with TERB1, crucial for X-Y chromosome pairing, during meiosis (57) (Table 1).

A telomere-independent role(s) was also reported for TRF2 in the transcriptional activation of *HS3ST4* (58) and *PDGFR β* (59) and repression of the cell cycle-dependent kinase inhibitor *CDKN1A* (*p21*) (60). TRF2 was further found to associate with core histone proteins (61, 62), including the RE-1-silencing factor (REST) repressor complex (60). In addition to these, a role of telomere-independent TRF2 was reported in natural killer cell activation, angiogenesis, and intrinsic aspects like nucleosome formation and chromatin compaction (63) (Table 1).

As discussed above, recent work also showed TRF2-mediated regulation of genes spread across the genome. This involved interaction of TRF2 with DNA secondary structures called G-quadruplexes within promoters (37). It is important to mention here that sequences with potential to form G-quadruplex structures are enriched in regulatory regions throughout the genome across genera (64–69), and evidence suggests that G-quadruplexes might influence local epigenetic modifications (70–72).

Telomeres in 3D—correlation of telomere architecture and cell state

Telomeres are known to be organized within the nuclear matrix through interactions with lamin. Further telomeric association with distant parts of chromatin by looping are also known. Several studies show that these three-dimensional associations (*i.e.* nonlocal or extra-telomeric interactions) can impact cellular functions.

Early work from the Blackburn group (73) found telomeres to be motile with rapid motions of the telomere ends within the

nucleus. Moreover, individual telomeres in a nucleus showed heterogeneity in motility. Relatively short uncapped telomeres in cancer cells had more motility than cells with long telomeres, possibly due to untethering of telomeres from the nuclear matrix (73–75). Furthermore, TRF2 association with lamin A/C proteins was observed to promote physical association of telomeres with interstitial chromatin through looping (76, 77) (Table 1).

Telomere-associated change in chromatin organization was noticed in other cell types also. A study in 2014 reported altered 3D telomeric architecture in buccal cells derived from Alzheimer’s disease (AD) patients of mild, moderate, and extreme pathology using three-dimensional (3D) microscopy and quantitative fluorescence *in situ* hybridization. Telomere aggregates and overall numbers increased in mild to severe AD along with a decrease in telomere length (78). A follow-up study of the same parameters between AD and control group buccal cell samples found similar results (79).

Tichy *et al.* (80) reported altered telomere length and inconsistency in telomeric foci in the muscle stem cells of Duchenne muscular dystrophy patients when compared with the control group. On the other hand, muscle-stem cell telomere length remained unchanged between young and old healthy mice (80). This was similar to what was previously noted for humans and macaques (81), although mouse somatic tissues have longer telomeres and higher telomerase activity than humans and other primates.

Similar observations were noted in other cases. For example, esophageal squamous cell carcinoma cells have altered 3D telomere organization compared with normal epithelial cells from the same patient with relatively long telomeres (82), and thyrospheres constituting stem cells from four subgroups of papillary thyroid carcinoma patients were found to have telomeric localization that was unique for each subgroup (83).

These data, describing studies of telomere-dependent gene expression and chromatin folding mediated through telomeres or telomere-associated factors, provide a clear view of how extra-telomeric functions may contribute to the role of telomeres in basic biological processes. Now we turn to the functions and implications of telomeres in integrated systems during health and disease.

Telomeres in cellular pluripotency and “stemness”—emerging observations

Induced pluripotent stem cells (iPSCs) have rapidly gained significance in basic and applied biological sciences (84) and serve as a facile model system for cellular differentiation and development. The important role of telomere elongation and homeostasis in formation/maintenance of iPSCs, including their implications in aging, is known (25, 85). In the following sections, we discuss the importance of telomeres in self-renewal and chromosome stability in iPSCs and consider emerging literature on how extra-telomeric function of telomere-associated factors might influence pluripotency.

Telomere elongation and pluripotency

The presence of relatively long telomeres has been generally observed in pluripotent cells. For example, reprogrammed iPSCs

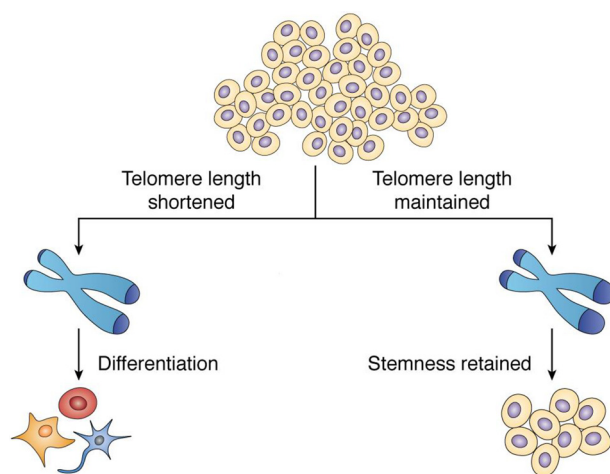


Figure 4. Role of telomeres in stem cell homeostasis. Importance of telomere length in maintaining stemness. Stem cells with relatively long telomeres are reported to retain stemness, whereas reduction of telomere length is generally observed during differentiation and/or in differentiated cells.

obtained from mice showed elongated telomeres in the pluripotent cells (86); iPSCs generated from dyskeratosis congenita patient samples had relatively long telomeres (87), and human fibroblast TIG-1 cells reprogrammed to iPSCs, telomeres increased from 6 to 8 Kb (88). Similarly, in many cancers like liposarcoma, hepatocellular carcinoma, and in gliocytic astrocytoma, elongated telomeres were noted in the dedifferentiated or pluripotent cells (89–91).

Consistent with this, shortening of telomeres was associated with differentiation. Telomere attrition was associated with loss of stemness markers in cardiac progenitor cells isolated from adult human heart failure cases (92); unstable differentiation was observed in ESCs with telomere dysfunction because of critically shorter telomeres (41); regenerative capacity of stem cells declined because of progressive telomere attrition in aging cells (93); and reduced proliferation and differentiation to osteoblasts was observed in mesenchymal stromal cells isolated from adults compared with those isolated from children, suggesting the impact of telomere attrition (94). On the other hand, longer life span was observed in cells that differentiated from human ESCs possessing relatively long telomeres (28) and cardiomyocytes differentiated with improved efficacy from iPSCs that had relatively long telomeres (26).

Furthermore, impaired differentiation due to poor telomere maintenance was observed in keratinocytes (27), and telomere elongation was found to be key for telomere length homeostasis in mouse embryonic stem cells (95). Together, these studies show the importance of telomere length maintenance in stem cells and how telomere shortening or attrition (with aging) impacts differentiation (Fig. 4).

Role of telomere-associated factors in pluripotency or “stemness” and disease

The pluripotency factor Oct3/4 was reported to positively regulate TRF1 during the induction and maintenance of pluripotency (96). Consistent with this, TRF1 was observed to be up-regulated in ESCs and iPSCs (82, 92), and in the presence of the small molecule ETP-47037, which inhibits TRF1,

reprogramming efficiency in mice was reduced (86). Moreover, increase in TRF1 expression was observed during *in vitro* derivation of ESCs from the inner cell mass (97) (Table 1).

Several reports implicated TRF2 in pluripotency. Self-renewal and maintenance potential was perturbed when TRF2 was deleted in alveolar stem cells (98), and human mesenchymal stem cells showed increased sensitivity to irradiation when TRF2 was knocked down (99, 100). Further, in TRF2-null mice, terminal differentiation was triggered during skin carcinogenesis (101), and increase in TRF2 was implicated in aggressive proliferation of liver cancer stem cells (102).

In the above studies, it was not clear whether the function of TRF2 was involved as a telomeric and/or extra-telomeric factor. However, we noted further work suggesting extra-telomeric function of TRF2 in stemness. This includes nuclear interaction of TRF2 with REST, which was reported to be important in maintenance of the neural stem cell population (103, 104). Further, TRF2 depletion resulted in reduced proliferation and enhanced differentiation of glioblastoma stem cells due to both telomeric dysfunction and loss of REST-mediated repression (105), and silencing of TRF2 resulted in the reduction of the Yamanaka factors in oral cancer stem cells (106) (Table 1). In addition to these, computational modeling indicated high binding affinity of TRF2 to the stem-cell factor KLF4 (106).

TPP1-mediated recruitment of the reverse transcriptase telomerase (TERT) for telomere elongation was observed. Here, abrogation of TPP1 affected the reprogramming of mouse embryonic fibroblasts (107). Later TPP1 was also shown to be important in maintaining the length of telomeres in human ESCs (108).

Depletion of POT1, on the other hand, triggered DNA damage response and thereby telomeric dysfunction, resulting in reduced survival of hematopoietic stem cells (HSCs) and bone marrow failure that mimicked the phenotypes of dyskeratosis congenita (109). Exogenous expression of *POT1* induced self-renewal of human HSCs by inhibiting generation of reactive oxygen species (Table 1) (110). In addition, POT1-mediated metabolic control and transcriptional regulation in HSCs was shown (111). Together, these implicate extra-telomeric functions of POT1 in pluripotency.

Furthermore, mutations within shelterin genes were found to be associated with hematological malignancies due to telomere deprotection, showing the importance of the shelterin factors in hematopoiesis and self-renewal (111).

The role of the noncoding RNA transcribed from telomeres called telomeric repeat-containing RNA (TERRA) in pluripotency is also notable. TERRA was found to be overexpressed and contributed to the self-renewal of mesenchymal stem cells (112). In addition, decline in TERRA resulted in differentiation, and overexpression resulted in rescue of the self-renewal activity (112). TERRA foci formation (*i.e.* clustered presence of TERRA molecules as seen in microscopy) due to elevated expression and aggregation of TERRA was reported to occur in both developing cerebellar neural progenitors and medulloblastoma (113). A more recent study showed how TERRA through a TRF1-dependent mode regulates the transcriptional state of ESCs such that a naive state is maintained (114).

Decrease in telomere length with age and associated telomere dysfunction contributes to initiation and progress of cancer (10, 115). It is also widely known that in more than 90% of human cancers, telomerase (*hTERT*)—the enzyme necessary for telomere synthesis—is reactivated, and as a result telomeres are maintained, unlike in normal adult somatic cells (12). However, despite reactivation, most cancer cells and cancer stem cells have shorter telomeres than surrounding normal cells (12, 116, 117).

Expression of *hTERT* was shown to involve TPE-OLD (33). In normal cells, the chromosome 5p telomere folds back and associates with the *hTERT* loci ~1.3 Mb away. Kim et al. (33) concluded that through this interaction telomeric TRF2 associates with the *hTERT* promoter. Further, the TRF2 interaction was lost in cells with relatively short telomeres where the telomeric loop was unable to form (33). Loss of TRF2 from the *hTERT* promoter correlated with increased *hTERT* expression. However, it was not clear whether this involved TRF2-mediated regulation or was a result of telomere-induced gene silencing as noted for several genes in earlier studies (22, 30).

More recent work, on the other hand, suggested that *hTERT* regulation is under direct transcriptional control of TRF2 (118). Here, TRF2 presence on the *hTERT* promoter was independent of telomeres (*i.e.* there was involvement of extra-telomeric TRF2). This was also clear from TRF2 occupancy at the exogenously inserted *hTERT* promoter ~46 Mb away from telomeres (118)—where looping due to physical proximity like the 5p telomere end was unlikely. Together, these leads suggest the involvement of the TSP model discussed above in *hTERT* regulation, where the presence of extra-telomeric TRF2 on the *hTERT* promoter is of interest and depends on how much TRF2 is free or sequestered at the telomere ends.

How might aspects of extra-telomeric biology impact stem cells? Stem cells, that replenish “worn out” cells, undergo telomere shortening, as reviewed earlier (112, 113), suggesting that many of the mechanisms described above could be in play. It must be mentioned here that although the literature suggests a potential role of extra-telomeric function(s) in pluripotency/stemness, evidence supporting direct causal links remains to be established to the best of our knowledge.

The platelet-derived growth factor receptor (PDGFR) was found to be significantly abrogated in the myocardium of people with increasing age, suggesting the role of PDGFR signaling in cardiomyocyte regeneration and proliferation (119). This was consistent with the telomere length-dependent differentiation of cardiomyocytes observed frequently (reviewed in Ref. 26). As mentioned above, *PDGFR-β* is a transcriptional target of extra-telomeric TRF2 (59). Furthermore, it was demonstrated that *PDGFR-β* is regulated epigenetically by TRF2 in a telomere length-dependent fashion (24). Therefore, it is likely that regulation of *PDGFR-β* by extra-telomeric TRF2, which depends on telomere length (24) (described above as TSP), plays a more direct role in telomere-dependent cardiomyocyte differentiation described above.

A recent study demonstrated the increased expression of genes related to neurogenesis and neuronal maturation in sporadic Alzheimer’s disease, suggesting a potential link between neuronal differentiation and this debilitating neurodegenerative

disease (29). Telomere shortening, a hallmark of aging, is also widely observed in neurodegenerative diseases like AD (119, 120, 122). Like the other cell types discussed above, short telomeres affect the proliferative capacity of neural stem cells and reduce the self-renewal potential of progenitors required for normal adult neurogenesis (123). Could a telomere function, and particularly an extra-telomeric function, serve as a molecular trigger underlying the pathophysiology of the disease (124)?

The recent study on pathophysiology in AD also showed that accelerated differentiation of neural stem cells in AD was associated with deregulated levels of REST (29). Notably, earlier work had reported that extra-telomeric TRF2-mediated stabilization of REST was critical for neuronal differentiation (125). Furthermore, recent findings showed that REST binding to nontelomeric chromatin was also dependent on extra-telomeric TRF2 (60). As described above, in TSP (Fig. 2), the presence of extra-telomeric TRF2 depends on telomere length. Therefore, these findings suggest a direct causal link between telomere length, extra-telomeric TRF2, and REST in neural stem cells, which might be key to neuronal differentiation. It will be fascinating to determine whether cellular renewal, rather than the more canonical aggregation hypothesis, might play a driving role in this and other degenerative diseases.

Conclusions and future perspectives

The notion that telomeres influence function beyond chromosome ends is relatively recent. Findings from many research groups, including ours, reveal this to be through two primary modes: (a) physical looping of telomeres (mostly within subtelomeric regions) (22) or (b) extra-telomeric function of shelterin factor(s) (37, 47, 54), which in the case of TRF2 depends on telomere length, as described above in the TSP model (Fig. 2) (24). The role of telomeres, particularly telomere length, has been observed closely during both pluripotency and stem cell differentiation. However, the underlying molecular processes that link telomeres to pluripotency are only beginning to emerge.

Notably, recent work has shown that TRF2 binding throughout the genome results in epigenetic modifications. Further, this depends on telomere length (24). Together, these findings contribute to a new understanding of telomeric factors. Moreover, these data suggest that a novel set of protein-protein interactions are possibly induced instead of the canonical shelterin complex at telomeres. It will be interesting to explore how these interactions with TRF2 and other telomeric factors are regulated (e.g. with distinct post-translational modifications that direct nontelomeric binding).

Based on RAP1 and NF- κ B interactions (46), association of telomeric factors with proteins independent of DNA binding is another molecular aspect that might be worthwhile to study. Contextually, whether other telomeric factors associate with nuclear or cytoplasmic factors—and how such interactions are affected as telomere length changes—would be of interest.

Telomerase—the only protein that synthesizes telomeres—is overexpressed in most cancers. Recent findings suggest that telomeres exert control over telomerase through telomeric or extra-telomeric mechanisms (33, 118). Teasing out molecular

details of these controls, including whether and how the TSP model might be involved in telomere-dependent control of telomerase, remains to be studied in further detail, considering its broad and significant implications.

Taken together, these new aspects of extra-telomeric biology—dependent on telomere length (and thereby aging)—may reveal a novel understanding of the molecular processes underlying pluripotency. Moreover, whether and how, particularly in what context, premature differentiation is linked to aging through telomeres would be of interest in improving our understanding of diseases associated with aging.

Acknowledgments—We acknowledge all members of the Chowdhury group for fruitful discussions on many aspects of the review and Dr. Munia Ganguli for assisting with editing of the manuscript.

Funding and additional information—This work was supported by Wellcome Trust/DBT India Alliance Fellowship IA/S/18/2/504021 (to S. C.). S. V. and A. G. acknowledge CSIR and DBT (GAP171), respectively, for research fellowships.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: TPE, telomere position effect; TPE-OLD, TPE-over long distance; TSP, telomere sequestration and partitioning; AD, Alzheimer's disease; 3D, three-dimensional; iPSC, induced pluripotent stem cell; ESC, embryonic stem cell; TERT, telomerase reverse transcriptase; HSC, hematopoietic stem cell; TERRA, telomeric repeat-containing RNA; PDGFR, platelet-derived growth factor receptor; REST, RE-1-silencing factor.

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