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Finding a place in the SUN: telomere maintenance in a diverse nuclear landscape

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Telomeres function in the context of a complex nuclear milieu in which telomeres tend to occupy distinct subnuclear regions. Indeed, regulation of the subnuclear positioning of telomeres is conserved from yeast to human, raising the age-old question: to what extent is location important for function? In mitotically dividing cells, the positioning of telomeres affects their epigenetic state and influences telomere processing and synthesis. In meiotic cells, telomere location is important for homologue pairing, centromere assembly and spindle formation. Here we focus on recent insights into the functions of telomere positioning in maintaining genome integrity.

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Introduction

In proliferating cells, telomeres assure the complete replication of chromosome ends by engaging the reverse transcriptase telomerase, which carries a telomere repeat-specifying RNA template [1,2]; telomerase activity prevents chromosome shortening after each round of semi-conservative DNA replication, the biochemistry of which prevents duplication of linear DNA termini. These termini consist of double-stranded (ds) telomeric G-rich repeat DNA with extremities comprising a single-stranded (ss) G-rich 3′ overhang. The ds and ss telomere regions provide the platform for a suite of proteins known collectively as shelterin [3]. Mammalian shelterin comprises six proteins [4]: two dsDNA binding proteins, TRF1 and TRF2, bridged to a ssDNA overhang binding protein, POT1, by TIN2, which interacts with both TRF1/TRF2 and the POT1 binding protein TPP1. The sixth shelterin component is the TRF2 binding partner Rap1 (human ortholog of yeast Repressor/Activator Protein 1). Fission yeast Taz1, the ortholog of TRF1 and TRF2, binds telomeric dsDNA and is bridged to Pot1 in a similar fashion to the mammalian TRF1/TRF2-TIN2-TPP1-POT1 connection [5]. The resulting telomere complexes regulate telomerase activity and prevent chromosome ends from being recognized as DNA double strand breaks.

High-resolution live-cell microscopy has revealed that telomeres position within distinct regions of eukaryotic nuclei. A longstanding question has been whether positioning and movements of telomeres are essential for telomeric function or are byproducts of mitotic completion or telomere interacting factors; here, we address this question. We liken the nucleus, with its varied constituent microenvironments, to an island with interior and shoreline regions sporting distinct habitats, conducive to distinct activities and influenced by surrounding currents (Figure 1).

Going to the shore: mechanisms that move and position telomeres

Telomere positioning is regulated by interactions between shelterin components and nuclear structures such as the nuclear membrane (NM, a double membrane including an inner NM, INM, and an outer NM, ONM). Key relevant NM ingredients include the nuclear pore complexes (NPC), A-type and B-type lamins, and proteins harboring lamin binding domains, of which the LEM domain, a 45-residue double α-helical motif, is prominent. LEM proteins are conserved in yeasts (e.g. *S. pombe* Lem2 and Man1), where they fulfill lamin-like functions despite the absence of canonical nuclear lamina [6]. Moreover, the so-called 'linker of nucleoskeleton and cytoskeleton' (LINC) complexes, conserved from mammals to yeasts, are formed by interactions of SUN domain-containing INM proteins with KASH-domain ONM proteins. Variable domains within the cytoplasmic extensions of KASH proteins attach to cytoplasmic elements such as microtubules or actin. The nucleoplasmic domains of SUN proteins interact with lamins and chromosome-binding proteins. In the space between INM and ONM, SUN monomers form a triple helical coiled-coil with a hydrophobic groove that is required for KASH peptides to bind. LINCs, therefore, span the NM and transmit mechanical forces between cytoplasmic elements and chromosomes [7,8].

In budding yeast (*S. cerevisiae*; most extensively studied [9,10]), telomeres are tethered at the INM. The SUN-domain protein Mps3, the NPC, and the large acidic protein Esc1 provide independent platforms at the

Figure 1

Island analogy for the functions of subnuclear positioning. Like an island with distinct habitats in the interior and diverse shoreline regions, the nucleus consists of microenvironments that are conducive to different cellular processes. The numbers indicate analogous cellular processes taking place within different regions. For example, telomere maintenance (1 and 2) may be optimal at multiple sites with common features; DNA damage repair (3) is promoted by shuttling to distinct peripheral locations that may be only transiently occupied; gene expression (4) leads to transient localization to specific sites (nuclear pore complexes) optimal for 'launching' of transcripts, while suppression of expression (5) is promoted by localization to specific neighboring microenvironments (represented by sandy shores, adjacent to nearby cliffs but with distinct biochemical and biophysical properties).

INM for telomere tethering. Mps3 and Esc1 independently tether telomeres by interacting with Sir4 (silent information regulator 4, which interacts with Rap1) [11,12]. During S phase, Mps3 has been reported to tether telomeres via direct interaction with the telomerase accessory protein Est1 [13]. Est1 interacts with the telomerase RNA (Tlc1), which can also interact with yKu [14], a ring-shaped heterodimer of Ku70 and Ku80 that loads at DNA ends (those generated by breakage as well as telomeres). Ku promotes repair by non-homologous end-joining (NHEJ), and in budding yeast also stimulates telomerase activity. As yKu's binding to DNA ends and the Tlc1 RNA are mutually exclusive, a model has been proposed in which Tlc1 transfers yKu to short telomeres to stimulate accessibility to telomerase [15]. Mps3 may coordinate these activities, bringing together NM-yKu-associated telomeres and NM-yKu-associated telomerase. Nonetheless, short telomeres destined to be telomerase substrates are selectively released from the NM during S-phase [16] and forced tethering of a telomere to the NM causes shortening of that telomere [17]. Hence, while establishment of the telomerase–telomere interaction

may be facilitated at the NM, a compartment away from the NM may be required for telomerase-mediated synthesis.

Disruption of the yKu-telomerase-Mps3 tethering pathway causes increased recombination of subtelomeric repeats, raising the possibility that this pathway protects telomeres from inappropriate recombination [18]. Moreover, as yKu binds all telomeres and constrains 5′ nucleolytic resection [19], its function in promoting NHEJ must be inhibited at telomeres to prevent lethal telomere fusions. This inhibition requires Rap1 [20], which tethers telomeres during G1 when NHEJ is most active. NHEJ inhibition requires multisumoylated, presumably inactive, forms of Rap1 to be cleared by the SUMO-targeted ubiquitin ligase (STUbL) Uls1 [21••]. As some STUbLs have been shown to concentrate at NPC sites along the NM [22], this localization may ensure robustness of Rap1 function. The observation that Mps3- and Esc1-mediated tethering function prominently in S and G1, respectively, along with emerging evidence for subnuclear 'hubs' of sumoylation and ubiquitylation activity [23••], likely reflect differences between specific NM zones that promote telomere function at different cell cycle stages.

In the fission yeast *Schizosaccharomyces pombe*, telomere-NM tethering occurs through direct interaction of Rap1 with the INM component, Bqt4 [24]. The conserved Fun30 chromatin remodeler Fft3 also functions in telomere tethering independently of Bqt4 [25]. As Fft3's ATP-dependent DNA helicase activity is implicated in the role of 'insulators' that prevent invasion of euchromatin into heterochromatic regions, there appears to be a link between chromatin structure and tethering. However, loss of Clr4, the sole *S. pombe* histone H3 lysine-9 methyltransferase essential for gene silencing [26], confers derepression of telomeric gene expression but does not sever telomeres from the NM [27••]. As Fft3 associates with regions 50–100 kb centromere-proximal to telomeres, it may regulate positioning by maintaining a long-range telomeric chromatin structure that is distinct from silent chromatin [25]. Furthermore, telomere tethering is cell cycle regulated by Cdc2 (Cdk) phosphorylation of Rap1, which severs its interaction with Bqt4 [28]. Cdk-mediated release of telomeres at the onset of mitosis promotes faithful chromosome segregation. The presence of Bq4-independent tethering pathways might explain the partial nature of the release of telomeres by this Cdk1-mediated severing.

In the metazoan *Caenorhabditis elegans*, interaction between SUN-1 and POT-1 tethers telomeres to the NM [29••]. This tethering requires SUMO. In dividing cells of telomerase deficient *C. elegans*, telomeres cluster and localize to the NM while undergoing recombination-based alternative telomere maintenance mechanisms (ALT). This clustering is reminiscent of the ALT-associated PML body (APB) localization of telomeres in human ALT cells [30]. Telomere positioning at APBs requires sumoylation of shelterin components by the SMC5/6 complex, known for its functions in DNA damage repair and maintenance of repetitive ribosomal DNA [31,32]. SMC5/6-mediated telomere positioning at APBs promotes the recombination reactions that comprise ALT. Clustering at the INM in *C. elegans*, or APBs in human cells, may enhance recombination by spatially juxtaposing telomeres and positioning them at hubs of processing and recombination activities.

Partying inland or at the beach: mammalian telomeres position non-selectively

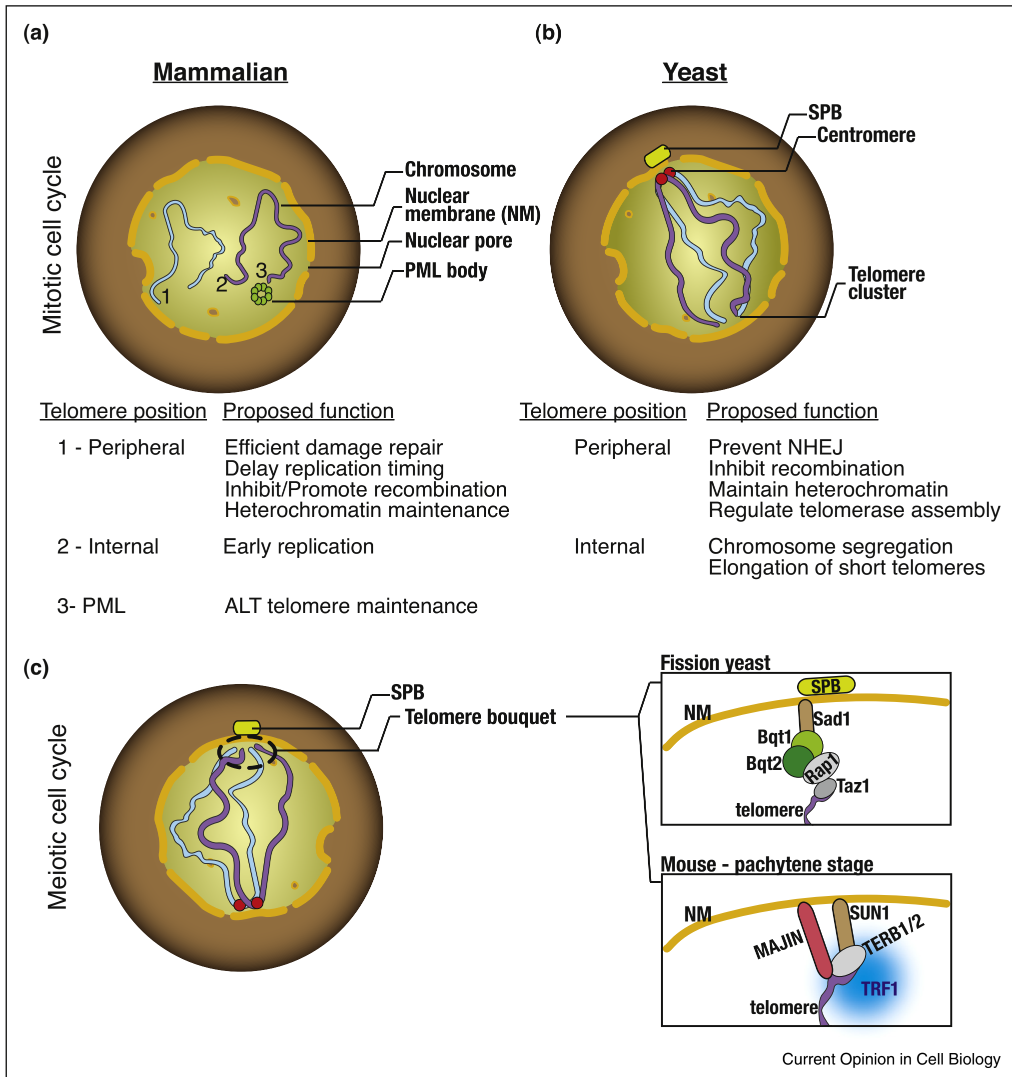
In contrast to the peripheral positioning of telomeres in *C. elegans* and yeast, telomeres of somatic human cells appear to position throughout the nuclear volume (Figure 2a,b) while interacting with the 'nuclear matrix' and promyelocytic leukemia (PML) bodies [33,34]. These interactions with large nuclear assemblies may explain the observation that the movements of human telomeres are highly confined compared to those of an actively expressing gene [35••]. Telomeric confinement may contribute to inhibition of recombination and end-fusion (discussed below). A subset of telomeres tend to localize to the nuclear periphery [36]. This localization appears to be determined by sequence context, rather than telomere length or telomerase activity.

Curiously, peripheral positioning of the telomeric region of chromosome 4q has been suggested to contribute to the etiology of the common muscular dystrophy syndrome Facioscapulohumeral dystrophy (FSHD). Telomere 4q has been shown to position at the NM, via an uncharacterized mechanism, throughout the cell cycle in normal myoblasts, myotubes, fibroblasts and lymphoblasts [37,38]. In FSHD patients, contraction of the subtelomeric macrosatellite repeat D4Z4 was shown to enhance NM tethering of telomere 4q [39]. The relationship between D4Z4 repeat size and telomere tethering is complex; while a single D4Z4 repeat can tether telomere 4q via interaction with A-type lamins, multimerized D4Z4 repeats do not generally confer tethering [36,39]. Since D4Z4 size alterations in FSHD correlate with enhanced tethering, D4Z4-mediated tethering is thought to mediate a subtle change in telomere location at the periphery that leads to substantial alterations in gene expression spanning several megabases. This suggested association between changes in telomere 4q tethering and disease implies profound roles for such tethering.

Choosing a gazebo at the beach: NM microenvironments and their effects

Transcriptionally silent loci and gene poor regions tend towards peripheral positioning in virtually all eukaryotes [40,41]. However, the NM as a whole harbors not only transcriptionally silent but also transcriptionally active regions [42]. In mice, for example, tethering of

Figure 2



Telomeres adopt cell-cycle specific positions in yeast and mammals. **(a)** In cultured mammalian cells, telomeres localize both internally and at the periphery. In the absence of telomerase, ALT occurs near PML bodies where telomeres are spatially juxtaposed for recombination. The table underneath lists proposed functions of telomere locations. **(b)** In fission yeast and budding yeast, telomeres cluster at the NM away from the yeast centrosome (SPB); centromeres localize beneath the SPB. **(c)** During meiosis, the telomere bouquet forms. In fission yeast (top right inset), centromeres move away from the SPB as telomeres gather at the SPB. This switch of SPB-LINC partners provides a transient window where telomeres are in close proximity to centromeres. This proximity has been proposed to function in centromeric assembly; the bouquet itself is required for proper meiotic recombination and spindle formation. In mice (bottom right inset), telomere remodeling takes place upon bouquet formation. In pachytene, TRF1 appears as a cloud around telomeric FISH signals, while TERB1/2 and MAJIN appear tightly colocalized with these signals.

muscle-related genes to lamin A appears to promote their expression and myogenic differentiation, while tethering to lamin B delays differentiation [43]. In yeast, actively transcribing genes associate with NPCs at the NM [44,45••,46]. Notably, highly transcribed regions need to be severed from NPC attachments while they are replicated to prevent excessive topological tension from compromising replication fork stability [47]. These observations highlight the possibility of superhelical constraints introduced by tethering; such constraints are likely to vary between tethering mechanisms and cell cycle stages. Hence, the nuclear periphery consists of neighboring but distinct microenvironments that have distinct effects on chromatin.

NM tethering of boundaries between heterochromatin and euchromatin [48] may partition these chromatin regions into distinct neighboring microenvironments that

promote distinct chromatin states. As with other correlations between chromatin state and NM localization, the cause–effect relationships between boundaries and NM tethering warrant precise experimental tests.

As peripheral telomeres specifically replicate late [36], factors at the NM may impose late replication timing. A key factor in conferring late replication in budding yeast [49,50], fission yeast [51] and mammals [52,53•] is the phosphatase binding protein Rif1, first identified as a yeast telomere length regulator and later a player in several DNA metabolism contexts [49,50,54]. Depletion of Rif1 also alters chromatin structure [53•] as detected by changes in the size of DNA loops released following nuclear high salt extraction (nuclear halo assay). Since Rif1 colocalizes with Lamin B [53•], peripheral Lamin B-associated microenvironments may sequester a subset of Rif1 molecules to regulate the dephosphorylation of local proteins that hinder accessibility to replication initiation.

Nuclear localization has also been implicated in processing dysfunctional telomeres and nontelomeric double strand breaks in a number of systems, including mouse embryonic fibroblasts. Loss of TRF2, for example, leads to activation of the DNA damage response protein 53BP1 at telomeres. Association of 53BP1 appears to promote SUN1/2-mediated, dynamic microtubule-dependent, telomeric mobility [55,56•]. This increase in mobility appears to expedite telomere fusions. 53BP1-associated telomeres may interact directly with LINC and thereby the cytoskeleton. Alternatively, telomeres may transiently visit the SUN-associated nuclear microenvironment, where the physical properties of the chromatin fiber are altered to allow higher mobility and efficient repair.

Sexual revelations: meiosis illuminates unexpected telomere functions

Subnuclear organization dramatically changes when cells enter the meiotic cell cycle [57]. In early stages of meiosis, telomeric NM tethering is required for directed movement of telomeres into a widely conserved bundle termed the telomere 'bouquet' (Figure 2c) [58]. The bouquet performs essential functions, promoting meiotic homologue pairing, proper spindle formation and successful chromosome segregation [59,60]. While LINC is required for telomere-NM tethering during bouquet formation, the specific proteins that connect telomeres to LINC vary. Rap1 links fission yeast telomeres to the SUN protein Sad1 via two meiotic prophase specific proteins, Bqt1 and Bqt2 [61]. The Rap1-Bqt1/2-Sad1 bridge is linked to the centrosome (called the spindle pole body or SPB) by interaction of Sad1 with the KASH-domain protein Kms1, which contacts the SPB on the ONM. This contrasts with mitotically proliferating cells, in which centromeres interact with Sad1 and position beneath the SPB throughout interphase [62,63]. The transition from mitotic centromere-LINC-SPB interactions to meiotic telomere–LINC–SPB interactions requires dynein motor-dependent and microtubule-dependent movement of the telomere-LINC complex along the NM toward the SPB.

Mouse Rap1 is dispensable for meiotic bouquet formation during spermatogenesis [64]; instead, TRF1 interacts with the meiosis-specific heterodimer TERB1-TERB2, which interacts with SUN1 in the NM [65••,66]. Moreover, remodeling of telomeric proteins occurs during mid-prophase. A key component at this stage is MAJIN, which contains a putative transmembrane domain, binds TERB1/2 and has non-sequence specific DNA binding ability. In the pachytene stage of meiotic prophase when homologous chromosomes have synapsed and crossovers occur, telomeric remodeling ensues. In this process, coined 'telomere cap exchange', the immunofluorescence pattern of TRF1 on telomeres changes markedly. Prior to cap exchange, TRF1 immunofluorescence signals appear as foci that overlap tightly with telomeric FISH foci. After cap exchange, the TRF1 signal appears as a halo surrounding each telomeric FISH signal, which now overlaps with TERB1/2/MAJIN immunofluorescence. This remodeling requires CDK activity, which is suggested to prompt the telomeric dislodgement of TRF1 and its replacement by MAJIN–DNA interactions. Such dissociation of TRF1 raises the possibility of yet another function of positioning, in this case for the TRF1 'halo' region. In non-meiotic cells, dislodgement of TRF1 from telomeres leads to its rapid ubiquitylation and degradation [67]. It is therefore notable that the proposed meiotic displacement of TRF1 does not cause its degradation. The post-cap exchange TRF1 halo may be a subnuclear microenvironment within which TRF1 is protected from degradation; it also may be a region in which telomeres transiently lacking shelterin binding are protected from deleterious processing/fusion reactions. The post cap-exchange MAJIN-TERB1/2 complex may also function directly in protecting chromosome ends [65••]. While its function remains enigmatic, the remodeled telomere may alter the rigidity of the chromosome axis via assembly of a specific telomere-INM interface with particular biophysical properties.

In addition to the role of telomere positioning in promoting meiotic homology search and recombination [57], recent studies have uncovered unexpected roles of such telomere dynamics. First, the telomere bouquet was shown to directly promote proper formation of meiotic spindles [60]. As the bouquet is dismantled before initiation of spindle formation, this must reflect processes upstream of initiation. Indeed, the bouquet promotes SPB insertion into a NM fenestration to nucleate spindle assembly. In bouquet deficient cells, defects in meiotic spindle formation occur in only ~50% of the population. The residual ability of bouquet-deficient cells to accomplish proper spindle formation stems from interchangeability of centromeres and telomeres; when telomeres fail to localize to the LINC complex, centromeres can associate with LINC

and substitute for telomeres [68••]. Indeed, ectopic tethering of centromeres to the LINC-SPB region restores proper spindles. Hence, centromeres and telomeres share an as yet mechanistically undefined ability to locally induce NM remodeling events that allow spindle formation.

A role in promoting robust centromeric kinetochore assembly has emerged as yet another function for meiotic telomere positioning [69••]. Centromeres were found to have a tendency to disintegrate and lose their essential kinetochore components upon meiotic induction. The juxtaposition of centromeres and the telomere-bouquet promotes centromere reassembly. In the absence of the bouquet, not only do centromeres become vulnerable to disassembly, but also pericentromeric heterochromatin is dismantled. As pericentric heterochromatin is crucial for establishment of new centromeres, its absence may mediate the inability of centromeres to reassemble in this setting. As a result, some chromosomes fail to attach to the spindle and mis-segregate in bouquet deficient settings. Insertion of a telomere sequence stretch on a circular chromosome lacking telomeres is sufficient to restore centromere reassembly in *cis*. Therefore, telomeres generate a microenvironment conducive to pericentromeric heterochromatin, supporting the notion that the NM consists of microenvironments with cell cycle stage-specific functions.

Conclusions

Studies exploring the functions of specific microenvironments within the nucleus are leading to a nuclear map replete with distinct zones serving specific functions. By analogy with the shoreline of an island, the properties of that shoreline (like the NM) are endlessly diverse, with different forms of life and different biochemical reactions thriving in each locality. The properties of nuclear microenvironments facilitate a diverse array of processes including DNA damage repair, chromosome segregation and gene expression. In some instances, cells may have evolved redundant mechanisms that ensure performance of essential processes, albeit inefficiently, when functional microenvironments fail to assemble. The evolution of the genetic manipulation toolbox, 3D cell culture techniques and higher-resolution live microscopy will lead to deeper understanding of nuclear microenvironments in physiologically relevant settings such as live tissues.

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