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

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Abstract

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.

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

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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), and A- 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. LINC, 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 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 Bqt4-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 and 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 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 [44-46]. 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- 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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References

1. Blackburn EH, Collins K: Telomerase: an RNP enzyme synthesizes DNA. *Cold Spring Harb Perspect Biol* 2011, 3.
2. Schmidt JC, Cech TR: Human telomerase: biogenesis, trafficking, recruitment, and activation. *Genes Dev* 2015, 29:1095–1105. [PubMed: 26063571]
3. de Lange T: How telomeres solve the end-protection problem. *Science* 2009, 326:948–952. [PubMed: 19965504]
4. de Lange T: Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005, 19:2100–2110. [PubMed: 16166375]
5. Miyoshi T, Kanoh J, Saito M, Ishikawa F: Fission yeast Pot1-Tpp1 protects telomeres and regulates telomere length. *Science* 2008, 320:1341–1344. [PubMed: 18535244]
6. Gonzalez Y, Saito A, Sazer S: Fission yeast Lem2 and Man1 perform fundamental functions of the animal cell nuclear lamina. *Nucleus* 2012, 3:60–76. [PubMed: 22540024]
7. Chang W, Worman HJ, Gundersen GG: Accessorizing and anchoring the LINC complex for multifunctionality. *J Cell Biol* 2015, 208:11–22. [PubMed: 25559183]
8. Starr DA, Fridolfsson HN: Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. *Annu Rev Cell Dev Biol* 2010, 26:421–444. [PubMed: 20507227]
9. Marcomini I, Gasser SM: Nuclear organization in DNA end processing: Telomeres vs double-strand breaks. *DNA Repair (Amst)* 2015, 32:134–140. [PubMed: 26004856]
10. Zimmer C, Fabre E: Principles of chromosomal organization: lessons from yeast. *J Cell Biol* 2011, 192:723–733. [PubMed: 21383075]
11. Bupp JM, Martin AE, Stensrud ES, Jaspersen SL: Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. *J Cell Biol* 2007, 179:845–854. [PubMed: 18039933]
12. Taddei A, Hediger F, Neumann FR, Bauer C, Gasser SM: Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. *EMBO J* 2004, 23:1301–1312. [PubMed: 15014445]
13. Osterhage JL, Talley JM, Friedman KL: Proteasome-dependent degradation of Est1p regulates the cell cycle-restricted assembly of telomerase in *Saccharomyces cerevisiae*. *Nat Struct Mol Biol* 2006, 13:720–728. [PubMed: 16862158]
14. Stellwagen AE, Haimberger ZW, Veatch JR, Gottschling DE: Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. *Genes Dev* 2003, 17:2384–2395. [PubMed: 12975323]
15. Pfingsten JS, Goodrich KJ, Taabazuing C, Ouenzar F, Chartrand P, Cech TR: Mutually exclusive binding of telomerase RNA and DNA by Ku alters telomerase recruitment model. *Cell* 2012, 148:922–932. [PubMed: 22365814]

16. Ferreira HC, Luke B, Schober H, Kalck V, Lingner J, Gasser SM: The PIAS homologue Siz2 regulates perinuclear telomere position and telomerase activity in budding yeast. *Nat Cell Biol* 2011, 13:867–874. [PubMed: 21666682]
17. Mondoux MA, Scaife JG, Zakian VA: Differential nuclear localization does not determine the silencing status of *Saccharomyces cerevisiae* telomeres. *Genetics* 2007, 177:2019–2029. [PubMed: 18073421]
18. Schober H, Ferreira H, Kalck V, Gehlen LR, Gasser SM: Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. *Genes Dev* 2009, 23:928–938. [PubMed: 19390087]
19. Maringele L, Lydall D: EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Delta mutants. *Genes Dev* 2002, 16:1919–1933. [PubMed: 12154123]
20. Pardo B, Marcand S: Rap1 prevents telomere fusions by nonhomologous end joining. *EMBO J* 2005, 24:3117–3127. [PubMed: 16096640]
- 21*. Lescasse R, Pobiega S, Callebaut I, Marcand S: End-joining inhibition at telomeres requires the translocase and polySUMO-dependent ubiquitin ligase Uls1. *EMBO J* 2013, 32:805–815. [PubMed: 23417015] The budding yeast STUbL (Uls1) is shown to be required for Rap1-mediated inhibition of NHEJ at telomeres. Uls1, by removing non-functional poly-SUMOylated Rap1, ensures that telomeres are coated with only functional Rap1, ensuring efficient NHEJ inhibition.
22. Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, Varela E, Hediger F, Gasser SM, Krogan NJ: Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 2008, 322:597–602. [PubMed: 18948542]
- 23*. Ryu T, Spatola B, Delabaere L, Bowlin K, Hopp H, Kunitake R, Karpen GH, Chiolo I: Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat Cell Biol* 2015, 17:1401–1411. [PubMed: 26502056] An essential function for sumoylation in ensuring proper HR repair within heterochromatic repeats in *Drosophila* is described. SUMO and SUMO E3 ligases prevent the untimely initiation of repair and target partially processed DNA damage to the NM, where the SUMO machinery resides. Hence, proper repair can take place away from other repetitive heterochromatic regions, preventing ectopic recombination. Tethering to the INM is mediated by the Smc5/6-interacting STUbL/RENi proteins.
24. Chikashige Y, Yamane M, Okamasa K, Tsutsumi C, Kojidani T, Sato M, Haraguchi T, Hiraoka Y: Membrane proteins Bqt3 and -4 anchor telomeres to the nuclear envelope to ensure chromosomal bouquet formation. *J Cell Biol* 2009, 187:413–427. [PubMed: 19948484]
25. Steglich B, Stralfors A, Khorosjutina O, Persson J, Smialowska A, Javerzat JP, Ekwall K: The Fun30 chromatin remodeler Fft3 controls nuclear organization and chromatin structure of insulators and subtelomeres in fission yeast. *PLoS Genet* 2015, 11:e1005101. [PubMed: 25798942]
26. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, et al.: Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 2000, 406:593–599. [PubMed: 10949293]
- 27*. Mizuguchi T, Fudenberg G, Mehta S, Belton JM, Taneja N, Folco HD, FitzGerald P, Dekker J, Mirny L, Barrowman J, et al.: Cohesin-dependent globules and heterochromatin shape 3D genome architecture in *S. pombe*. *Nature* 2014, 516:432–435. [PubMed: 25307058] The authors use genome-wide chromatin conformation capture (Hi-C) analysis to reveal two distinct forms of chromosome organization in fission yeast nuclei. First, cohesin, in a manner independent of its role in sister chromatid cohesion, is required for local (within ~100 kb) interactions along chromosome arms, creating chromatin “globules”. Second, heterochromatin, which is not required for globule formation, causes chromatin compaction. In the absence of heterochromatin and proper chromatin compaction, disparate chromosomal regions display increased levels of interaction.
28. Fujita I, Nishihara Y, Tanaka M, Tsujii H, Chikashige Y, Watanabe Y, Saito M, Ishikawa F, Hiraoka Y, Kanoh J: Telomere-nuclear envelope dissociation promoted by Rap1 phosphorylation ensures faithful chromosome segregation. *Curr Biol* 2012, 22:1932–1937. [PubMed: 22959349]

- 29*. Ferreira HC, Towbin BD, Jegou T, Gasser SM: The shelterin protein POT-1 anchors *Caenorhabditis elegans* telomeres through SUN-1 at the nuclear periphery. *Journal of Cell Biology* 2013, 203:727–735. [PubMed: 24297748] The authors describe the dynamics of telomere positioning and tethering mechanisms in the metazoan *Caenorhabditis elegans*. Telomeres are tethered to the NM in embryos and differentiated cells. In embryos, POT-1, the SUMO ligase GEI-17, and SUN-1 are all required for tethering; the dependencies differ in late development. The clustering dynamics of telomeres undergoing ALT are also described.
30. Potts PR, Yu H: The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat Struct Mol Biol* 2007, 14:581–590. [PubMed: 17589526]
31. Lehmann AR: The role of SMC proteins in the responses to DNA damage. *DNA Repair (Amst)* 2005, 4:309–314. [PubMed: 15661654]
32. Torres-Rosell J, Machin F, Farmer S, Jarmuz A, Eydmann T, Dalgaard JZ, Aragon L: SMC5 and SMC6 genes are required for the segregation of repetitive chromosome regions. *Nat Cell Biol* 2005, 7:412–419. [PubMed: 15793567]
33. Luderus ME, van Steensel B, Chong L, Sibon OC, Cremers FF, de Lange T: Structure, subnuclear distribution, and nuclear matrix association of the mammalian telomeric complex. *J Cell Biol* 1996, 135:867–881. [PubMed: 8922373]
34. Ramirez MJ, Surrallés J: Laser confocal microscopy analysis of human interphase nuclei by three-dimensional FISH reveals dynamic perinucleolar clustering of telomeres. *Cytogenet Genome Res* 2008, 122:237–242. [PubMed: 19188692]
- 35*. Chen B, Gilbert LA, Cimini BA, Schnitzbauer J, Zhang W, Li GW, Park J, Blackburn EH, Weissman JS, Qi LS, et al.: Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* 2013, 155:1479–1491. [PubMed: 24360272] The authors use a modified CRISPR/Cas method to image telomeres as well as transcribing genes in live human cells. Telomeres appear to be confined but moving with varying speeds. A transcribing gene (MUC4) appears to be less confined than telomeres, and positions near the nuclear periphery.
36. Arnoult N, Schluth-Bolard C, Letessier A, Drascovic I, Bouarich-Bourimi R, Campisi J, Kim SH, Boussouar A, Ottaviani A, Magdinier F, et al.: Replication timing of human telomeres is chromosome arm-specific, influenced by subtelomeric structures and connected to nuclear localization. *PLoS Genet* 2010, 6:e1000920. [PubMed: 20421929]
37. Masny PS, Bengtsson U, Chung SA, Martin JH, van Engelen B, van der Maarel SM, Winokur ST: Localization of 4q35.2 to the nuclear periphery: is FSHD a nuclear envelope disease? *Hum Mol Genet* 2004, 13:1857–1871. [PubMed: 15238509]
38. Tam R, Smith KP, Lawrence JB: The 4q subtelomere harboring the FSHD locus is specifically anchored with peripheral heterochromatin unlike most human telomeres. *J Cell Biol* 2004, 167:269–279. [PubMed: 15504910]
39. Ottaviani A, Schluth-Bolard C, Rival-Gervier S, Boussouar A, Rondier D, Foerster AM, Morere J, Bauwens S, Gazzo S, Callet-Bauchu E, et al.: Identification of a perinuclear positioning element in human subtelomeres that requires A-type lamins and CTCF. *EMBO J* 2009, 28:2428–2436. [PubMed: 19644448]
40. Lanctot C, Cheutin T, Cremer M, Cavalli G, Cremer T: Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. *Nat Rev Genet* 2007, 8:104–115. [PubMed: 17230197]
41. Nagai S, Heun P, Gasser SM: Roles for nuclear organization in the maintenance of genome stability. *Epigenomics* 2010, 2:289–305. [PubMed: 22121875]
42. Lemaître C, Bickmore WA: Chromatin at the nuclear periphery and the regulation of genome functions. *Histochem Cell Biol* 2015, 144:111–122. [PubMed: 26170147]
43. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M, Cohen TV, Devys D, Foisner R, Peichl L, et al.: LBR and lamin A/C sequentially tether peripheral heterochromatin and inversely regulate differentiation. *Cell* 2013, 152:584–598. [PubMed: 23374351]
44. Kumaran RI, Spector DL: A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. *J Cell Biol* 2008, 180:51–65. [PubMed: 18195101]

- 45**. Steglich B, Filion GJ, van Steensel B, Ekwall K: The inner nuclear membrane proteins Man1 and Ima1 link to two different types of chromatin at the nuclear periphery in *S. pombe*. *Nucleus* 2012, 3:77–87. [PubMed: 22156748] Genome-wide mapping (via DamID methodology) of chromosomal loci that interact with two fission yeast INM proteins, Ima1 and Man1, is described. These peripheral regions correlate with low levels of RNA-Polymerase II and nucleosome occupancy, a hallmark of repressed chromatin regions. In agreement with previous studies of other organisms, highly expressed genes are shown to be depleted from the INM. Ima1 interacts prominently with RNAi-mediated heterochromatic regions, while Man1 interacts prominently with subtelomeric regions
46. Taddei A, Van Houwe G, Hediger F, Kalck V, Cubizolles F, Schober H, Gasser SM: Nuclear pore association confers optimal expression levels for an inducible yeast gene. *Nature* 2006, 441:774–778. [PubMed: 16760983]
47. Bermejo R, Capra T, Jossen R, Colosio A, Frattini C, Carotenuto W, Cocito A, Doksani Y, Klein H, Gomez-Gonzalez B, et al.: The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. *Cell* 2011, 146:233–246. [PubMed: 21784245]
48. Noma K, Cam HP, Maraia RJ, Grewal SI: A role for TFIIC transcription factor complex in genome organization. *Cell* 2006, 125:859–872. [PubMed: 16751097]
49. Hiraga S, Alvino GM, Chang F, Lian HY, Sridhar A, Kubota T, Brewer BJ, Weinreich M, Raghuraman MK, Donaldson AD: Rif1 controls DNA replication by directing Protein Phosphatase 1 to reverse Cdc7-mediated phosphorylation of the MCM complex. *Genes Dev* 2014, 28:372–383. [PubMed: 24532715]
50. Mattarocci S, Shyian M, Lemmens L, Damay P, Altintas DM, Shi T, Bartholomew CR, Thoma NH, Hardy CF, Shore D: Rif1 controls DNA replication timing in yeast through the PP1 phosphatase Glc7. *Cell Rep* 2014, 7:62–69. [PubMed: 24685139]
51. Hayano M, Kanoh Y, Matsumoto S, Renard-Guillet C, Shirahige K, Masai H: Rif1 is a global regulator of timing of replication origin firing in fission yeast. *Genes Dev* 2012, 26:137–150. [PubMed: 22279046]
52. Cornacchia D, Dileep V, Quivy JP, Foti R, Tili F, Santarella-Mellwig R, Antony C, Almouzni G, Gilbert DM, Buonomo SB: Mouse Rif1 is a key regulator of the replication-timing programme in mammalian cells. *EMBO J* 2012, 31:3678–3690. [PubMed: 22850673]
- 53*. Yamazaki S, Ishii A, Kanoh Y, Oda M, Nishito Y, Masai H: Rif1 regulates the replication timing domains on the human genome. *EMBO J* 2012, 31:3667–3677. [PubMed: 22850674] The authors use BrdU pulse labeling to show that Rif1 knockdown in HeLa cells causes dramatic changes in replication timing. Furthermore, using careful microscopy techniques, Rif1 appears to colocalize with DNase I-insoluble nuclear structures and mid-S phase replication foci. The authors propose that Rif1 establishes mid-S phase replication domains to prevent accessibility of replication initiation factors during early S phase.
54. Dave A, Cooley C, Garg M, Bianchi A: Protein Phosphatase 1 Recruitment by Rif1 Regulates DNA Replication Origin Firing by Counteracting DDK Activity. *Cell Reports* 2014, 7:53–61. [PubMed: 24656819]
55. Dimitrova N, Chen YC, Spector DL, de Lange T: 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 2008, 456:524–528. [PubMed: 18931659]
- 56*. Lottersberger F, Karssemeijer RA, Dimitrova N, de Lange T: 53BP1 and the LINC Complex Promote Microtubule-Dependent DSB Mobility and DNA Repair. *Cell* 2015, 163:880–893. [PubMed: 26544937] DNA damage has been shown in a number of studies to trigger increased chromosomal mobility in yeast and mammals (see citations in this publication). This study shows that the increased mobility of dysfunctional telomeres lacking TRF2 depends on 53BP1 and SUN1/SUN2. When mobility is ablated either by disrupting microtubule dynamics or in the absence of functional 53BP1 or SUN1/2, telomere fusions are delayed. This study raises a potential caveat for taxane treatment in cancers that have developed resistance to PARP-inhibitors; ablating microtubule dynamics may reduce chromosome fusion products that ultimately promote cell death.
57. Klutstein M, Cooper JP: The Chromosomal Courtship Dance-homolog pairing in early meiosis. *Curr Opin Cell Biol* 2014, 26:123–131. [PubMed: 24529254]

58. Scherthan H: A bouquet makes ends meet. *Nat Rev Mol Cell Biol* 2001, 2:621–627. [PubMed: 11483995]
59. Ding DQ, Yamamoto A, Haraguchi T, Hiraoka Y: Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. *Dev Cell* 2004, 6:329–341. [PubMed: 15030757]
60. Tomita K, Cooper JP: The telomere bouquet controls the meiotic spindle. *Cell* 2007, 130:113–126. [PubMed: 17632059]
61. Chikashige Y, Tsutsumi C, Yamane M, Okamasa K, Haraguchi T, Hiraoka Y: Meiotic proteins bqt1 and bqt2 tether telomeres to form the bouquet arrangement of chromosomes. *Cell* 2006, 125:59–69. [PubMed: 16615890]
62. Funabiki H, Hagan I, Uzawa S, Yanagida M: Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. *J Cell Biol* 1993, 121:961–976. [PubMed: 8388878]
63. Hagan I, Yanagida M: The product of the spindle formation gene *sad1+* associates with the fission yeast spindle pole body and is essential for viability. *J Cell Biol* 1995, 129:1033–1047. [PubMed: 7744953]
64. Scherthan H, Sfeir A, de Lange T: Rap1-independent telomere attachment and bouquet formation in mammalian meiosis. *Chromosoma* 2011, 120:151–157. [PubMed: 20927532]
- 65*. Shibuya H, Hernandez-Hernandez A, Morimoto A, Negishi L, Hoog C, Watanabe Y: MAJIN Links Telomeric DNA to the Nuclear Membrane by Exchanging Telomere Cap. *Cell* 2015, 163:1252–1266. [PubMed: 26548954] Two new telomere binding factors are identified in mouse testis extracts: TERB2 and MAJIN. In knockout mice lacking either TERB2 and MAJIN, homologous synapsis is compromised via defects in telomere tethering to the nuclear membrane. The authors propose a “telomere cap-exchange” model whereby TERB1/2 and MAJIN replace the canonical shelterin complex in pachytene to ensure timely formation of a meiotic telomere attachment plate at the INM.
66. Shibuya H, Ishiguro K, Watanabe Y: The TRF1-binding protein TERB1 promotes chromosome movement and telomere rigidity in meiosis. *Nat Cell Biol* 2014, 16:145–156. [PubMed: 24413433]
67. Chang W, Dynek JN, Smith S: TRF1 is degraded by ubiquitin-mediated proteolysis after release from telomeres. *Genes Dev* 2003, 17:1328–1333. [PubMed: 12782650]
- 68**. Fennell A, Fernandez-Alvarez A, Tomita K, Cooper JP: Telomeres and centromeres have interchangeable roles in promoting meiotic spindle formation. *J Cell Biol* 2015, 208:415–428. [PubMed: 25688135] A mechanism is described through which meiotic spindles can form successfully in the absence of a functional telomere bouquet. In bouquet-deficient cells in which telomeres do not position near the SPB, seemingly accidental centromere-SPB association correlates with proper spindle assembly. Inducing centromere-LINC-SPB association, either by tethering a kinetochore component to the SPB or ablating dynein-driven nuclear movements, fully rescues robust spindle assembly. Hence, centromeres and telomeres can act interchangeably to control spindle formation.
- 69**. Klutstein M, Fennell A, Fernandez-Alvarez A, Cooper JP: The telomere bouquet regulates meiotic centromere assembly. *Nat Cell Biol* 2015, 17:458–469. [PubMed: 25774833] Time-lapse microscopy of fission yeast undergoing meiotic division reveals that chromosomes that fail to attach to the spindle. ~2–4% of centromeres in bouquet deficient cells lack stable association with tagged kinetochore proteins as well as the heterochromatin HP1 protein. Centromere maintenance in meiosis is shown to be inherently precarious, with reassembly promoted by the bouquet. The mechanism by which telomeres ensure robust centromere assembly appears to require the transient co-localization of telomeres with centromeres that occurs during bouquet formation.

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 (distinct regions at the shore) 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 microenvironment (represented by sandy shores, adjacent to nearby cliffs but with distinct biochemical and biophysical properties).

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 yeast (top right inset), centromeres move away from the SPB as telomeres are gathered 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.