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Telomeric Strategies: Means to an End

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Annu. Rev. Genet. 2010. 44:243–69

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi:
10.1146/annurev-genet-102108-134841

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0066-4197/10/1201-0243$20.00

Key Words

telomerase, checkpoint, homologous recombination (HR), nonhomologous end joining (NHEJ), resection, survivors

Abstract

What really defines a telomere? Telomere literally is an amalgamation of the Greek words "telos," meaning end, and "mer," meaning part. In practice, it refers to the extremities of linear chromosomes. The defining functions of chromosome extremities can be summarized in two main categories. First, chromosome ends trick the cell into not identifying them as damage-induced double-strand DNA breaks (DSBs). An internal DSB immediately triggers cell-cycle arrest and is repaired to ensure that genome integrity remains undisturbed. Chromosome ends disguise themselves using assorted strategies, tailored to evade specific cellular responses. The second defining function of chromosome extremities involves self-preservation. Due to the inherent limitations of the canonical replication machinery, chromosomes gradually lose terminal DNA with successive rounds of replication. Telomeres have evolved tactics to circumvent this loss and to preserve themselves. This review focuses on highlights of telomeric strategies surrounding these two primary tasks, and finishes by discussing evidence that the full telomeric functional repertoire has yet to be defined.

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A SNAPSHOT OF CHROMOSOME ENDS

As the functional requirements of linear chromosomal extremities are conserved, the strategies used to fulfill them have many common themes across Eukarya. Early work on telomeres exploited the ciliated protozoans, whose genome fragmentation and consequent high mini-chromosome number makes them phenomenally rich sources of telomeres, a property that allowed the discovery of telomere sequences (17), telomerase (73), and the first telomere binding proteins (71, 159). This review focuses on chromosome end protection in mammals, budding yeast, and fission yeast, organisms in which genetic analysis has been particularly fruitful. The sections below describe the basics of mammalian and yeast telomere composition, and **Figure 1** provides a snapshot of their telomeres.

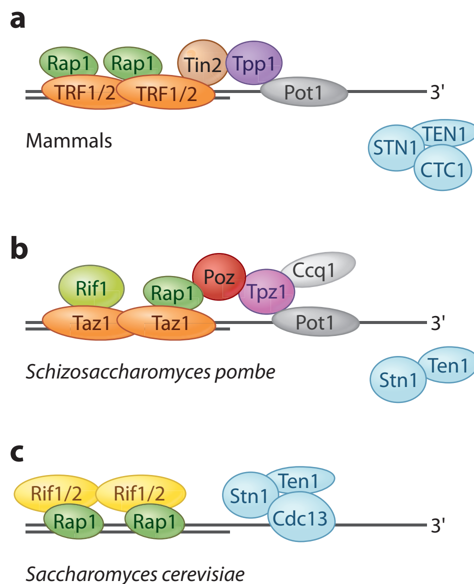

Figure 1

Schematic of telomere-specific components in (*a*) mammalian cells, (*b*) fission yeast, and (*c*) budding yeast.

Mammalian Chromosome Ends

Mammalian chromosomes end in tracts of the tandemly repeated G-rich sequence TTAGGG. Tract length ranges from approximately 10-15 kb in humans to 20-60 kb in mice (52, 77, 93). The G-rich strand forms a 3′ single-stranded (ss) overhang at the extreme terminus (116, 132). The double-stranded (ds) terminal region contains histones (117, 145, 195, 213), but its precise nucleosomal packaging is not yet defined and may differ from that of bulk chromatin. Telomeric chromatin also includes RNA transcripts of telomeric and subtelomeric sequences, named TERRA, that associate with at least a subset of chromosome ends (4, 170). When human telomeric DNA is structurally preserved (by using the DNA cross-linking agent psoralen) and purified, structures can be isolated in which the telomeric 3′ overhangs have invaded subterminal telomeric repeats of the same telomere, displacing a D-loop styled secondary structure. This structural design is termed the t-loop and has been visualized by electron microscopy (74) (**Figure 2**).

Specialized proteins coat the telomeric repeats. The ds region is bound in a sequence specific fashion by telomeric repeat binding factor 1 (TRF1) and telomeric repeat binding factor 2 (TRF2) (16, 24, 218). These are related proteins, each harboring a C-terminal Myb domain of the homeodomain subfamily but differing at their N termini, as TRF1 possesses

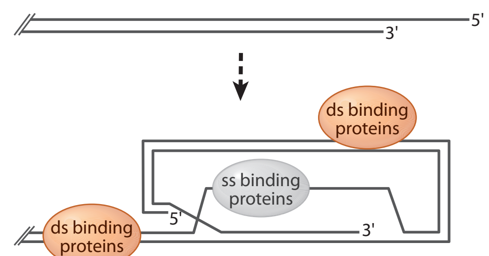

Figure 2

Schematic of t-loop formation at a mammalian telomere. Abbreviations: ds, double-strand; ss, single-strand.

an acidic N-terminal domain and TRF2 a basic N terminus (12, 13, 35, 39, 44, 61, 76). TRF1 and TRF2 recruit TIN2 and Rap1, respectively (92, 101). The terminal ssDNA is bound in a sequence specific manner by two interacting OB (oligonucleotide/oligosaccharide binding) fold-containing proteins, POT1 and TPP1; POT1/TPP1 is the mammalian counterpart of the prototypical ss telomere binding complex from ciliates, TEBPα/β, (83, 97, 109, 203, 215), known to form a tenacious complex with ss telomere sequences. TPP1 and TIN2 interact and are therefore postulated to bridge the ss and ds regions of the chromosome end (147, 215). Together these six proteins are referred to as shelterin (107, 215; reviewed in 50). This term does not signify a stoichiometric complex but rather a gathering of subcomplexes (188). Recently, a complex of three proteins, CTC1, STN1 and TEN1, collectively called the CST complex, has been found to associate with a fraction of telomeres (**Figure 1**). The members of this complex resemble replication protein A (RPA) subunits and contain putative OB folds (138), in the vein of their budding yeast counterparts (discussed below).

Yeast Chromosome Ends

Chromosomes in both fission yeast and budding yeast end in shorter G-rich tracts of approximately 300 bp, also terminating in G-rich 3′ overhangs. Unlike mammals, their telomeres consist of imperfect repeats comprising varying permutations of the sequence $(TG)_{1-3}$ in budding yeast and $TTAC(A)GG(G_{1-4})$ in fission yeast (175, 184, 206). The basic protein complement of fission yeast chromosome ends is remarkably similar to that of mammalian cells. The TRF1/2 ortholog Taz1 binds the ds telomeric repeat, and a POT1 ortholog binds the ss overhang along with its interacting partner Tpz1 (9, 42, 139). As in mammals, the ss- and ds-binding proteins interact to form a shelterin-like assemblage. Taz1 recruits Rap1 (37, 88), which interacts with Pot1 via a Poz1-Tpz1 bridge; Taz1 also recruits Rif1 (88). Ccq1 is recruited to chromosome ends by Tpz1-Pot1 (139) (**Figure 1**). Finally, fission yeast contains RPA-like Stn1 and Ten1 proteins akin to components of the CST complex described below (127).

Budding yeast chromosome ends appear to have diverged in protein composition. Budding yeast lack a TRF1/2 ortholog, and their chromosome ends are bound directly by Rap1, which contains two Myb domains with little homology to those found in TRF1/TRF2/Taz1. Rap1 recruits the additional factors Rif1 and Rif2, as well as the silent information regulator (SIR) proteins (reviewed in 75, 125). Budding yeast ss overhangs are bound by a CST complex comprising Cdc13, Stn1, and Ten1 (**Figure 1**). An extensive body of work on this complex, now partly identified in mammals and fission yeast as well, established that CST forms a telomere-specific RPA-like complex, acting as a platform for coordinating events at ss telomeres much like RPA does at ssDNA generated by unwinding activities associated with DNA replication and repair reactions throughout the genome (67, 69, 152; reviewed in 209). Although a higher-order structure that brings telomeric termini into occasional contact with subterminal sequences has been evinced in budding yeast (49, 216), there is as of yet no evidence for a t-loop–like displacement of subterminal ds repeats by yeast telomeric overhangs.

An interesting type of baggage borne by canonical telomere sequences is their propensity to self-assemble into secondary structures called G quadruplexes, which are exceedingly stable. The formation of such structures could in principle obstruct replication fork progression or confer telomere-telomere associations that would hamper chromosome segregation at mitosis. Thus, telomere-binding proteins may have important roles in preventing or resolving G-quadruplex formation. Nevertheless, the ability to form G quartets is a conserved feature of the telomeres of many organisms, suggesting a positive role for these structures. Whether G quadruplexes occur in vivo is a subject of ongoing debate (reviewed in 106).

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Telomerase: What and Why?

Although not a permanent fixture of chromosome ends, a nonetheless central telomeric module in all organisms discussed here is telomerase. Telomerase is a reverse transcriptase that synthesizes telomeric repeats using its integral RNA subunit as a template, thus playing a crucial role in telomeric self-preservation (111; reviewed in 104).

The chemistry of conventional DNA synthesis renders this process incapable of completely duplicating a linear molecule; this is referred to as the end-replication problem (**Figure 3**). DNA polymerases require a template to copy and cannot synthesize DNA de novo. Rather, they need a preexisting 3′ hydroxyl group onto which they add nucleotides; this is provided by an RNA primer, which is later removed and replaced with DNA synthesized by extending the upstream Okazaki fragment. These properties constrain semiconservative replication to the 5′ to 3′ direction and lie at the heart of the end-replication problem. Leading-strand replication of a 3′ overhanging duplex suffers as the parental strand is recessed and cannot template overhang synthesis. Conversely, lagging-strand DNA replication cannot duplicate blunt-ended molecules (which would result from loss of the 3′ overhang), as removal of the RNA primer from the terminal Okazaki fragment leaves a 5′-terminal gap (148, 207; reviewed in 105). The end-replication problem is further exacerbated by nucleolytic resection of the 5′ strand, which confers further DNA loss (see below).

Telomerase reverses these setbacks by replenishing lost telomeric repeats; hence, in the absence of telomerase, the chromosome extremities erode with time. Telomere erosion is a natural part of life in many human somatic cells as they fail to express telomerase. Once telomeres in these cells become critically short, the resulting exposed chromosome ends are recognized as double-strand DNA breaks (DSBs), triggering cell-cycle arrest and limiting cellular life span (19; reviewed in 177).

With the façade described above and a dividend of supplementary cellular machinery, telomeres are armed to undertake vital tasks that come hand-in-hand with chromosome linearity. The tasks of telomeres and their implementation are discussed below.

STRATEGY OF DISGUISE

When stripped down to its DNA component, the chromosome end is a DSB. However, DSBs trigger a number of cellular responses, collectively termed the DNA damage response (DDR), which are dangerous at chromosome ends. First, cells respond to DSBs by activating checkpoints that halt the cell cycle to allow time for repair; hence, cell division would be compromised if the cell were to react to the constitutive presence of chromosome ends as if they were DSBs. Moreover, attempts to repair natural chromosome ends can result in chromosome end-to-end fusions, rearrangements, and general genome instability. Nevertheless, some of the DDR activities that act at DSBs are important for telomeres as well. For instance, whereas the 5′ strand degradation that prepares DSBs for homologous recombination (HR)-mediated repair would be harmful if left unchecked at telomeres, a limited amount of 5′ strand degradation is necessary to insure that all telomeres have 3′ overhangs and their associated binding proteins (reviewed in 113). Hence, the principal function of telomeres is to disguise chromosome ends from DDR activities that are harmful at natural chromosome ends, while at the same time engaging and controlling those DDR activities that are necessary for telomere maintenance. In the following sections, we describe strategies used by telomeres to manipulate the repair troupe one by one, highlighting areas where interdependency is prominent.

Evading Checkpoint Activation

The damage-sensing machinery recognizes DNA aberrations and activates the checkpoint machinery, certifying that DNA integrity is maintained for the next stage of the cell cycle. In contrast to DSBs, telomeres must ensure that they do not activate checkpoints. This is a

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bona fide concern: The engineered loss of a single budding yeast telomere arrests the cell cycle (168), as does the natural telomere shortening that accompanies cellular aging in telomerase-negative primary human cells (reviewed in 183).

The avoidance of cell-cycle arrest involves dodging two chief checkpoint inducers, the ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinases. ATM polices DSBs per se, whereas ATR polices the accumulation of ssDNA that can arise as a by-product of DSB metabolism. An important complexity in considering checkpoint suppression at telomeres is the emerging realization that limited and tightly controlled activity of ATM and ATR is actually required for telomere maintenance: The recognition of telomeres by telomerase requires that specific targets be phosphorylated by these kinases at telomeres (reviewed in 178). Hence, ATM and ATR are part of the woodwork, active at telomeres even in unperturbed conditions (201, 202), making the suppression of full-blown checkpoint responses at telomeres even more challenging than initially thought.

Deterring ATM at mammalian chromosome ends. In mammalian cells, ATM and ATR appear to be independently inhibited by distinct telomere proteins. Inhibition of ATM is assigned to TRF2. In mouse embryonic fibroblasts (MEFs) in which TRF2 is deleted (26, 54) or in human cell lines where a dominant negative allele of TRF2 is overexpressed (89), ATM responds to the telomere as if it were a DSB: It phosphorylates nearby histone H2AX molecules (producing γH2AX), triggering recruitment of the signal transducer protein 53BP1 to the exposed telomere (48, 187). This enrichment of γH2AX and 53BP1 produces diagnostic foci known as telomere dysfunction induced foci (TIFs). These events culminate in activation of the cell-cycle regulator p53, leading to either cell-cycle arrest or cell death.

How TRF2 achieves inhibition of ATM is unknown, but suggested mechanisms fall into at least two categories. First, TRF2 has been proposed to promote the formation of t-loops, based on observations in which purified TRF2 appeared to induce t-loop formation of model telomere sequences in vitro (74; reviewed in 51). By sequestering the telomeric 3′ overhang within a base-paired structure, the t-loop strategy may hide the chromosome terminus from ATM's gaze. However, in vivo evidence linking TRF2 with t-loop formation is thus far lacking, and it is possible that TRF2 affects telomere state via alternative telomeric chromatin alterations or by a more direct obstruction of ATM. This latter idea has experimental support, as overexpression of TRF2 results in general downregulation of DSB-mediated ATM activation at nontelomeric sites (90, 91).

Deterring ATR at mammalian chromosome ends. The second chief checkpoint inducer that is kept at bay by the telomere is ATR. ATR responds to amassing ssDNA shrouded by RPA: A threshold length of ssDNA is required for ATR activation. For example, ATR recognizes long stretches of ssDNA that build up at stalled replication forks or resected DSBs. Therefore, the telomeric overhang could potentially activate ATR. Alternatively or in addition, if the overhang invades subterminal telomere repeats to form a t-loop, the consequently displaced D-loop will contain ssDNA that might activate the ATR checkpoint.

ATR is inhibited at mammalian telomeres by the ss-binding protein POT1. Conditional deletion of POT1 in MEFs or POT1 downregulation by shRNA in human cells leads to activation of ATR and classical downstream phenotypes—phosphorylation of the checkpoint inducers Chk1, Chk2, and histone H2AX along with formation of TIFs (54, 80, 81).

How might POT1 inhibit ATR? As RPA-bound ssDNA is required for ATR activation, current models evoke the ability of POT1 to compete with RPA for telomeric ssDNA binding. The high affinity of POT1 for ss telomere sequence along with the high local POT1 concentration conferred by its interaction with the ds telomere binding complex promote the ability of POT1 to outcompete RPA at telomeres (54). This would effectively evade ATR activation. The appearance of RPA foci at human

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telomeres upon shRNA of POT1 lends support for such a model (7). Interestingly, mice have two POT1 paralogs, POT1a and POT1b. POT1a appears to take responsibility for suppressing ATR activation, whereas POT1b restricts degradation of the telomeric 5′ strand (80; discussed below), demonstrating potentially separable functions for Pot1 in controlling generation of the telomeric 3′ overhang and disguising that same overhang from RPA.

ATR is also activated during S phase at the telomeres of MEFs in which TRF1 has been conditionally deleted. However, this ATR activation is thought to be an indirect result of the ssDNA generated by stalled replication forks that accumulate at telomeres lacking TRF1 and not a corollary of direct inhibition of ATR by TRF1 or a displacement of Pot1 from the telomeric overhang in TRF1's absence (173). The role of telomere binding proteins in promoting smooth replication fork progression is considered below.

Dodging checkpoint activation at yeast chromosome ends. The activation of checkpoints and processing/repair activities are intimately linked, making it difficult to separate mechanisms that prevent checkpoint activation from those that prevent repair. Thus, it is unclear whether yeast checkpoint suppression is enforced by direct inhibition of checkpoint activities by telomere proteins or by inhibition of telomere processing events that create checkpoint triggers. For instance, budding yeast lacking Cdc13 accumulate ss telomeric DNA and arouse a Rad9 (checkpoint signal transducer)-mediated cell-cycle arrest (68, 87, 115, 208). We now know that this checkpoint results from the role of Cdc13 in preventing excessive telomeric resection (21, 87, 103, 115, 146, 222; see below). Likewise, loss of fission yeast Pot1 results in rampant 5′ resection, converting telomeres to ssDNA and triggering a Rad3 (ATR homolog)-mediated checkpoint response (157).

Fission yeast Ccq1 plays a fascinating role in evading checkpoint activation at telomeres, as it is required specifically to suppress the checkpoint in cells containing moderately short telomeres. As mentioned previously, shortening telomeres activate some features of a checkpoint response, and these features launch telomerase action. Ccq1 appears responsible for restraining these short telomeres from activating the entire checkpoint response. Ccq1 is required for telomerase action, so a gradual decline in telomere length commences upon *ccq1*$^{+}$ deletion, just as it does upon *trt1*$^{+}$ deletion. However, *ccq1Δ* cells activate the checkpoint earlier than *trt1Δ* cells, when telomeres are still long enough to suppress checkpoint activation if Ccq1 is present. Hence, Ccq1 may orchestrate the ability of short telomeres to trigger partial checkpoint activation, which is required for telomerase action, while avoiding checkpoint-mediated arrest (139, 140, 192). This distinction again underlines the conundrum faced by telomeres, which must draw a distinction between ATM/R-mediated phosphorylation of positive telomere-specific targets and ATM/R-mediated activation of the whole cell-cycle arrest pathway.

Protection from Processing and Repair

DSBs are subject to three fundamental strokes of processing and repair—nonhomologous end joining (NHEJ), resection, and HR. Apart from evading checkpoint responses, the chromosome end must also ensure that inappropriate repair activities do not violate their integrity. Hence, telomeres must thwart NHEJ, domesticate resection, and govern HR at chromosome ends. Their engagement with each of these processes is discussed in the upcoming sections.

Protection from nonhomologous end joining. Natural chromosome ends must ensure that they are not fused by NHEJ, as this would form dicentric chromosomes that trigger severe genome instability. Such fusions lie at the heart of the breakage-fusion-bridge cycle described by McClintock (133), which, together with elucidation of the distinction between telomeres and radiation-induced breaks in fly

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chromosomes by Muller (141), originally seeded our understanding of telomeres (reviewed in 1). In yeast, NHEJ is largely restricted to the G1 phase of the cell cycle, while HR predominates in G2, when the presence of sister chromatids provides a nearby cohesed template for accurate repair (64). In mammalian cells, this dichotomy is less pronounced, with NHEJ levels being higher in G1 but not restricted to this phase.

Preventing NHEJ at mammalian chromosome ends. In mammalian cells, the protein most accountable for NHEJ inhibition is TRF2. Conditional deletion of TRF2 in MEFs or expression of a dominant negative allele in human cells results in chromosome end-to-end fusions that contain telomere sequences. These fusions require DNA ligase IV (Lig4), a ligase essential for NHEJ, demonstrating that the fusions are indeed NHEJ mediated (26, 179, 200). The fusions that result from dominant negative TRF2 are between telomeres replicated by leading-strand synthesis and occur during G2 (5), presumably because passage through S phase is required to displace wild-type TRF2, and NHEJ is most facile when the replication product is blunt ended (i.e., at the terminus created by leading-strand replication). In contrast, the fusions that result from TRF2 deletion in MEFs are all formed in G1 (26, 94). These fusions are only observable in a checkpoint-defective $p53^{-/-}$ setting because TRF2 loss arrests cells with a functioning checkpoint response before fusions can be observed. The action of NHEJ in $TRF2^{-/-}p53^{-/-}$ cells is manifest in dramatic form, as chains of fused chromosomes visible by metaphase spread (26).

The mechanism(s) by which TRF2 protects against NHEJ is a perplexing issue. NHEJ of chromosome ends requires the activity of ATM (54). It also requires downstream phosphorylation and localization of the checkpoint signal transducer 53BP1, which is thought to promote NHEJ by stimulating chromatin movements that enhance the ability of two telomeres to find each other (56). The completion of NHEJ requires the removal of 3′ overhangs, which may occur through a DNA flap-clipping step that occurs as part of the NHEJ reaction. TRF2 could impinge on several of these requirements and indeed evidence exists for the involvement of TRF2 in multiple steps. In mice, TRF2 represses ATM activation and the downstream accumulation of 53BP1 (26). It may also sequester the overhang by promoting t-loops (74; reviewed in 51). It is important to note that the telomere 3′ overhang persists in cells lacking both TRF2 and Lig4, indicating that TRF2 is not required for maintenance of the overhang itself, but rather to prevent NHEJ, whose action by definition removes endedness from chromosomes and therefore also removes their overhangs (26). In the absence of TRF2, the Ercc1/XPF endonuclease complex is required for telomeric NHEJ, possibly to remove the telomeric overhang (221). Intriguingly, artificially tethering Rap1 to telomeres blocks the NHEJ seen in human cells expressing dominant negative TRF2, suggesting that the role of TRF2 in NHEJ inhibition may be mediated in part through its ability to recruit Rap1 (169). However, the loss of Rap1 in a wild-type TRF2 setting does not precipitate telomeric NHEJ (173). This may be due to the still-intact inhibition of ATM that persists in the presence of TRF2. Alternatively, Rap1 may inhibit NHEJ redundantly with some other factor that can take over in Rap1's absence.

Apart from TRF2, POT1 also plays a minor role in NHEJ inhibition. Deletion of POT1 in MEFs results in a mild fusion phenotype (80). POT1's strategy for NHEJ repression may be distinct from TRF2 and may involve protection of the terminal overhang.

Preventing NHEJ at yeast chromosome ends. In fission yeast, the threat of NHEJ is restricted to G1. As proliferating fission yeast lack a substantial G1 phase, NHEJ levels are appreciable only in cells experiencing G1 arrest, which occurs under conditions of nitrogen starvation and upon meiotic induction (64, 198). During G1 arrest, fusions occur between *taz1Δ* telomeres in the absence of any detectable attrition,

identifying Taz1 as the primary guard against NHEJ. These fusions require the canonical NHEJ machinery: the Ku heterodimer and Lig4. Interestingly, G1-arrested *taz1Δ* cells, whose telomeres are undergoing fusions, do not appear to lose the excessive 3′-overhang signal that is characteristic of *taz1Δ* cells (63, 194); however, the NHEJ process must encompass an overhang removal step (either degradation or fill-in). Hence, from a DNA repair–centric viewpoint, Taz1 appears to carry out contradictory functions. It prevents NHEJ action, while also preventing DNA processing that in itself would be expected to inhibit NHEJ. Protection from telomeric NHEJ is also lost in the absence of Rap1 (135); therefore, the restraint exerted by Taz1 on NHEJ may be mediated by its recruitment of Rap1.

Budding yeast employ Rap1 to inhibit NHEJ between telomeres. Removal of Rap1 via a regulated degron allele results in telomere-telomere fusions that can be detected by polymerase chain reaction (PCR). The fusions depend on the canonical NHEJ machinery. It can be inferred from the sizes of the fused telomere fragments that Rap1's absence can fuse full-length telomeres (150). Rap1 achieves inhibition of NHEJ in part by recruiting the effectors Rif2 and Sir4. Interestingly, ectopic targeting of the C terminus of Rap1 to an engineered nontelomeric DSB partly inhibits its repair by NHEJ. This inhibition depends on Rif2, indicating that Rif2 inhibits NHEJ through protein interactions rather than through any telomere-specific DNA structure (124).

As in mammals, Ku is present at telomeres in both fission and budding yeast and has positive telomere related functions [e.g., in controlling 5′ resection (72, 158, 194)]. However, in yeasts, telomeric NHEJ is strongly dependent on Ku (63, 150), whereas in mammals a number of Ku-independent NHEJ pathways are also prominent at telomeres (23, 27, 167, 204). Hence, the telomere-specific factors exert a tight control on Ku, which can be viewed as a generic DNA end-binding factor that has been adopted by the NHEJ pathway and by telomeres to perform different functions.

Protection from homologous recombination. HR is a double-edged sword for telomeres, as it can be both beneficial and harmful. Therefore, telomeres employ several mechanisms to restrict HR.

Is homologous recombination a problem or a solution? Unlike NHEJ, homology-driven recombinational repair at chromosome ends is not necessarily detrimental. Exchanges between telomere sequences can potentially be reciprocal, and essentially invisible, or can result in innocuous length changes. In the presence of telomerase, any sequence loss at chromosome ends can be easily restored. On the other hand, intramolecular HR between the chromosome end and subterminal sequences in a t-loop fashion, for instance, has the potential to excise entire telomeres in a single step, making the presence of telomerase crucial for urgent telomeric replenishment. Moreover, HR between telomeres and internally located DNA sequences (e.g., interstitial telomeric repeats, which do occur in mammals) could precipitate drastic and detrimental genomic rearrangements.

In budding yeast, HR-driven telomere sequence exchanges can be seen to occur at a very low frequency in a wild-type background (189). In addition, HR-mediated copying of telomeres is exploited in all organisms discussed in this review as an alternative mechanism for telomere maintenance in cells lacking telomerase, as in these cells, telomere attrition compromises the ability of chromosome ends to suppress HR (reviewed in 131). It has even been observed that in instances where a subset of telomeres in a budding yeast cell are overelongated, a mechanism referred to as telomere rapid deletion sporadically reduces the size of these telomeres to the average telomere size in the cell. This is thought to occur via formation and excision of a t-loop–like intrachromosomal HR intermediate and requires proteins involved in HR (100; reviewed in 11). In this regard, the t-loop itself can be thought of as a semifrozen HR reaction that may be beneficial to the chromosome end, if the notion that t-loops are protective structures is borne out by experimental data.

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The potentially perilous nature of this structure is demonstrated in cells harboring an allele of TRF2 lacking its N-terminal basic domain; in these cells, the HR protein XRCC3 promotes telomere loss and a concomitant accumulation of t-loop–sized circular telomeric DNA molecules (205). Hence, t-loop formation may represent promotion of the first steps of HR in concert with TRF2-mediated inhibition of its terminal steps.

Control of HR. HR is controlled by telomere binding proteins. Mammalian Ku, TRF2, POT1, and Rap1 inhibit exchanges between sister telomeres (T-SCE) after replication (27, 149, 172). In MEFs lacking both Ku and TRF2, or in triple knockouts lacking Ku, POT1a, and POT1b, elevated levels of T-SCE can be visualised by CO-FISH (chromosome orientation fluorescence in situ hybridization), a technique in which degradable nucleotides are incorporated during S phase. Subsequent degradation leaves only the parental strands, so that at each chromosome end, one sister retains the G-rich strand while the other retains the C-rich strand, generating a characteristic pattern upon hybridization to strand-specific probes (5, 6). This technique has been valuable for assessing a number of replication issues in mammalian cells including T-SCE, which is evinced by pairs of sister telomeres in which parental strands have undergone exchange. The ability of TRF2 to inhibit sister telomere exchanges is distinct from its ability to prevent t-loop excision and is mediated by its recruitment of Rap1 (172). Curiously, Ku is redundant with either TRF2 or POT1 in its ability to prevent T-SCE, as HR only increases significantly if both Ku and either TRF2 or POT1 are missing. Why this is the case remains a mystery but may involve the ability of Ku to channel dysfunctional telomeres away from resection/HR and towards the rival NHEJ machinery.

In mice, ectopic HR between chromosome ends and interstitial telomere-like sequences is inhibited by the nucleotide excision repair protein Ercc1. This mechanism may be specific to ectopic HR, as Ercc1 does not inhibit t-loop formation in vitro. Ercc1 knockout mice display an unusual phenotype in which they accumulate small telomere-containing circular doublets called double minute chromosomes, which are products of HR between chromosome ends and internal sites (221).

HR at fission yeast telomeres is inhibited by both Ku and Taz1, as the absence of either results in unstable subtelomeric restriction digest patterns due to hyperrecombination (8, 165). In the case of *taz1Δ* cells, upregulated HR is likely to stem from fork stalling at telomeres, as both fork stalling and hyperrecombination are prevented specifically by Taz1 (136, 165; discussed below); moreover, stalled forks are known to stimulate recombination. The prominence of HR in both fission yeast and budding yeast telomere maintenance comes into sharp focus in settings in which telomerase is inactivated and is considered below (reviewed in 131). Overall, in mammals and yeast, it appears that HR type repair is not prohibited at telomeres per se, but watched over and regulated so as to allow its use in times of need.

Control of telomeric degradation. Telomeric degradation is both essential and potentially dangerous. Hence, control of 5′ telomeric resection is a major challenge for the telomeric complex.

Domestication of resection: part of everyday life. Resection of DNA 5′ ends is a key processing step at DSBs, where it plays an important role in providing both a platform for assembly of the repair machinery and the instrument for strand invasion. RPA, once assembled on the ssDNA at a resected DSB, is exchanged for Rad51, promoting homology-driven repair (reviewed in 95, 137). Although a naïve design might exclude such activities at chromosome ends, controlled resection is instead crucial at natural ends. It generates the telomeric 3′ overhang, which is both the assembly site for Pot1 and/or CST and the substrate for telomerase activity.

The length of the 3′ overhang at each telomere in every point of the cell cycle is the

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result of a multitude of processes. The product of leading-strand replication requires resection activities to generate the overhang, whereas the product of lagging-strand replication naturally acquires an overhang after removal of the ultimate RNA primer (**Figure 3**). Indeed, longer overhangs can be detected at human telomeres generated by lagging than at leading-strand synthesis (31). Nonetheless, the length of the terminal RNA primer is not sufficient to account for the observed overhang, and it is thought that resection occurs at both chromosome ends (116, 210). The action of telomerase, which extends the G strand by reverse transcription, transiently contributes to overhang generation. Finally, the C strand complementary to the G-rich telomerase product is produced by fill-in synthesis, reducing the length of the overhang (160, 217; see below).

The complete mechanism of telomeric resection and its regulation remains to be elucidated, but several key players are known. The MRN/X complex is a common player in resection at both DSBs and natural chromosome ends, but its precise biochemical function in this regard remains unclear (32, 96; reviewed in 137). In vitro, this complex exhibits 3′ to 5′ directed nuclease activity, thus acting in the opposite polarity to that required at both chromosome ends and DSBs. Hence, helicase and endonuclease activities inherent to MRN/X may contribute to resection (reviewed in 137). In addition, MRN/X may recruit additional nucleases that digest DNA in the 5′ to 3′ direction. Reduction of MRN levels in yeast or human reduces overhang signal. In mice, the simultaneous loss of MRN and TRF2 triggers NHEJ events that predominantly fuse the telomeric products of leading-strand replication (55), suggesting that MRN is involved in resecting the products of leading-strand replication; in its absence, leading telomeres remain blunt-ended and are particularly susceptible to NHEJ.

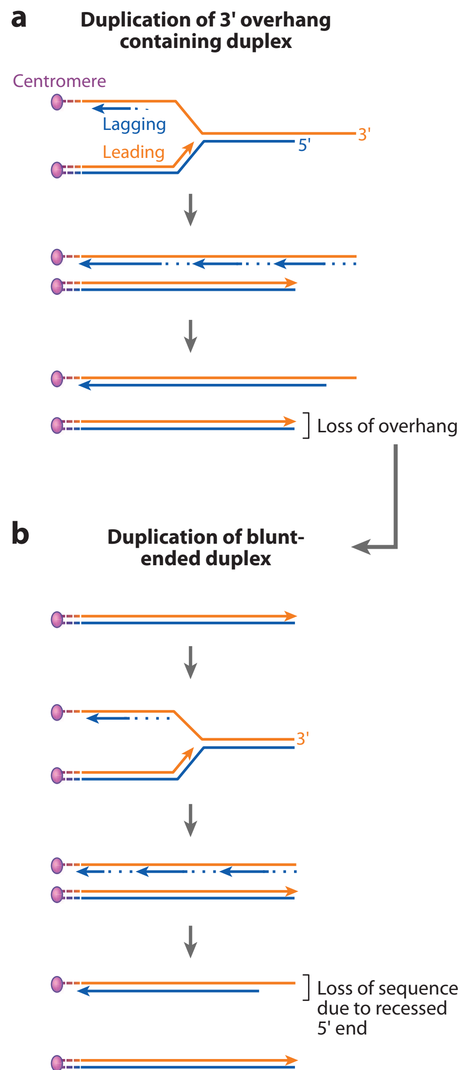

Figure 3

Schematic of the end-replication problem. The dotted lines represent the RNA primers used to initiate DNA synthesis. (*a*) Leading-strand synthesis is unable to fully duplicate a linear molecule terminating in a 3′ overhang. (*b*) Lagging-strand synthesis is unable to fully replicate blunt-ended molecules.

The additional players in budding yeast 3′ overhang generation mirror those factors involved in DSB resection (20). Initial telomere processing by MRX is assisted by the Sae2 endonuclease (ortholog of human CtIP) and can be alternatively carried out by Sgs1, the budding yeast RecQ helicase (discussed below) together with Dna2, a nuclease required for Okazaki fragment maturation. The 5′ to 3′ exonuclease ExoI can also contribute to resection.

In fission yeast, most analysis of the genetic requirements for 3′ overhang generation has been performed in a *taz1Δ* background, as the loss of Taz1 confers an elevated 3′ overhang signal easily visible by native in-gel hybridization. In a *taz1Δ* context, MRN contributes to overhangs at telomeres in a manner akin to mammals and budding yeast. The Dna2 nuclease also plays a part in resection of *taz1Δ* telomeres, whereas Exo1 appears dispensable (193, 194).

Reining in resection. While the 3′ overhang is at the heart of several key events in telomere homoeostasis, resection must be tightly guarded to avoid loss of the entire ds telomere tract and the proteins it binds. Indeed, overhang length is regulated, ranging from 12–14 nucleotides (nt) in G1-phase budding yeast to approximately 65 nt in S-phase budding yeast (211, 212) to 50–150 nt in interphase human cells (116, 132). The precise mechanisms underlying this size control remain a mystery, but hints are emerging as to how proteins control this risky business.

The proteins that bind telomeric overhangs and prevent ATR activation also thwart runaway resection. This circuitous strategy, where limited resection is required for the binding of the same factors that limit further resection, provides a negative feedback mechanism for controlling resection. Mice lacking both POT1 paralogs undergo extensive 5′ telomeric resection, acquiring long overhangs that activate ATR-dependent cell-cycle arrest (80). Likewise, fission yeast *pot1-ts* cells undergo runaway resection at restrictive temperature. The phenotype is spectacular in that the entire telomere is converted into ssDNA within the first S phase following Pot1 inactivation, triggering telomere loss and checkpoint activation (157). In human, Pot1's rule over resection is particularly intriguing. The 5′ terminal sequence of chromosome ends is fixed in human cells (i.e., it always ends in [...ATC-5′]), but becomes deregulated in the absence of POT1 so that the 5′ nucleotide is randomized within the AATCCC-5′ repeat (82, 174).

Despite the fact that budding yeast appear to lack a Pot1 ortholog, it was in this organism that rampant resection was first observed through inactivation of a conditional allele of Cdc13 (*cdc13-1*) (68). Cdc13 acts in concert with Stn1 to control resection. This resection of *cdc13-1* telomeres is suppressed by deletion of either Exo1 or the checkpoint protein Rad24, branding them as key violators of resection control (114, 222).

STRATEGY OF SELF-PRESERVATION: THE MOMENT OF TRUTH

The entire edifice of telomeric architecture faces its greatest challenge during S phase, when in order to ensure the fidelity of its own maintenance, it must disassemble enough to allow access to the replication machinery, coordinate the replicative processes needed for faithful telomeric duplication, and reassemble the protective complex, all the while ensuring that overzealous repair factors are not granted inappropriate access to the telomeric DNA.

Semiconservative Telomere Replication: Hurdles Close to the Finish Line

The vast majority of telomere repeats are synthesized by conventional DNA replication. As is true throughout the genome, this necessitates DNA duplex unwinding and chromatin disruption, issues expected to be compounded at a region encrusted with DNA binding proteins and perhaps even TERRA molecules. In line with this expectation, early two-dimensional gel electrophoretic studies of budding yeast telomeric replication intermediates suggested that the telomere protein complex obstructs replication, as replication pause sites were seen at telomeres. This replication fork stalling is greatly exacerbated in budding yeast lacking Rrm3, a helicase that travels with the replication fork and is thought to promote replication through stable DNA-protein complexes (2, 3, 18, 85, 119, 196).

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Against this backdrop of viewing the telomere binding proteins as obstacles to replication, it came as a surprise that fission yeast Taz1 is actually required for efficient passage of replication forks through telomeres. Stalled replication forks accumulate at *taz1Δ* telomeric stretches whether they are located at chromosomal termini or ectopically inserted within the genome. Hence, naked telomere repeats are difficult to replicate, and this difficulty is alleviated by Taz1. Unlike many of Taz1's functions, its ability to promote fork progression does not require Rap1 (136); hence, Taz1 may act simply by coating the ds repeats, preventing spurious secondary structures from forming when the repeats are unwound by replicative helicases. Alternatively, it may interact with the replication machinery or regulate the superhelical density of the telomeric region (70).

Whether the budding yeast ds telomere binding protein ScRap1 also promotes telomere replication has yet to be determined. However, the function of Taz1 in promoting telomere replication appears to be conserved in mammalian TRF1, as single molecule analysis (by DNA combing, in which long chromosomal fragments are stretched onto glass slides and visualized by hybridization with fluorescent probes) shows an accumulation of stalled replication forks at telomeres in MEFs lacking TRF1. This replication defect is also manifested by multiple telomeric FISH signals at individual metaphase chromosome ends, suggesting that paused forks lead to intermittent regions of ssDNA at telomeres lacking TRF1 (128, 173).

The idea that replication of naked telomeric DNA requires special attention also has precedent from studies implicating the RecQ-type Werner syndrome helicase (WRN), mutation of which causes the eponymous human premature aging syndrome, in human telomere replication. Cells lacking WRN function lose telomeres specifically from those sister chromatids copied by lagging-strand DNA replication; this telomere loss is suppressed by expression of telomerase, which replenishes the telomere tract (34, 45, 46). Along with evidence that additional helicases (Dog1 and RTEL) assist with the removal of secondary structures such as G quartets (36, 57; reviewed in 214), these studies suggest that the repetitiveness and/or G richness of the telomere repeat necessitate a variety of ironing-out processes.

Although helicases like WRN promote telomere maintenance, they can also wreak havoc at dysfunctional telomeres, in a manner analogous to the potentially deleterious actions of a number of DNA repair factors (e.g., NHEJ factors and nucleases). The replication defect at *taz1Δ* telomeres can have disastrous consequences for the cell. Although *taz1Δ* telomeres are highly elongated (due to telomerase inhibition by Taz1; see below), they are lost immediately rather than gradually upon telomerase deletion. *taz1Δ* telomeres are also hyper-recombinogenic (165). Moreover, *taz1Δ* telomeres become lethally entangled with one another at cold temperatures (134). These phenotypes are all suppressed by decreasing the activity of the fission yeast RecQ helicase, Rqh1, although fork stalling itself persists (136, 165). The mechanism by which Rqh1 transduces stalled telomeric forks into DNA breakage, hyper-recombination, and entanglement has yet to be ascertained, but a simple model would invoke conversion of stalled telomeric forks by Rqh1 (perhaps in concert with nucleases) to a structure that prevents resumption of fork progression. Hence, a number of factors collaborate to ensure that problems associated with replicating the bulk of the telomere repeats are surmounted even before the fork reaches the chromosome terminus and encounters the classical end-replication problem.

Enlisting telomerase: at telomeres and telomeres only. The engagement of telomerase is in some senses the most complex moment of telomere biology, as it requires the interplay of controlled DDR activation and two DNA synthesizing machines, the semiconservative replication apparatus and the telomerase reverse transcriptase holoenzyme. When it works, telomerase engagement can confer immortality to a cell line (19).

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The arrival of telomerase at telomeres requires not only a complex ribonucleoprotein particle assembly process (reviewed in 40) but also the actions of numerous factors that control the specificity and timing of the telomerase-telomere interaction. These factors have been best studied in budding yeast and are the subject of several reviews (166, 178). Apart from the telomerase RNA, which hybridizes with telomere overhangs and helps bring telomerase specifically to natural chromosome ends, Cdc13 and the telomerase accessory factors Est1 and Est3 are required for telomerase recruitment and action (60, 84, 98, 153), both of which are tightly coupled to replication fork passage (58, 59, 122, 186). Cdc13 is thought to serve as a platform for handing off the telomeric overhang from RPA and the conventional replication machinery to telomerase (67, 153). Interactions between Cdc13 and Est1 are crucial for telomerase binding, as is the interaction between Est1 and telomerase itself (60, 153; reviewed in 166, 178). The Ku heterodimer also contributes by interacting with a specific hairpin region within the telomerase RNA (33, 65, 155, 182).

The checkpoint factors ATM (budding and fission yeast Tel1) and ATR (budding yeast Mec1, fission yeast Rad3) are crucial for telomerase activity, as loss of both confers a telomerase-negative phenotype in both budding yeast and fission yeast (112, 140, 143, 164). Telomeric targets for ATM and ATR kinases (notably including Cdc13) are beginning to be identified, along with evidence that the corresponding phosphorylation events are relevant for telomerase action (reviewed in 178). In addition, phosphorylation of Cdc13 by the cyclin dependent kinase CDK1 promotes efficient telomerase recruitment (102, 197). Hence, a network of cell-cycle regulated modifications coordinates the readiness of telomerase to commence action upon passage of the replication fork through telomeres; elucidation of this network has begun.

The mechanisms underlying fission yeast telomerase regulation are only just starting to emerge (reviewed in 53, 178). Taz1, Rap1, Rif1, and Poz1 are key components of the telomere counting mechanism, as their absence stimulates telomeric overelongation by telomerase (37, 42, 88, 135, 139). Fission yeast harbor an Est1 ortholog whose removal causes a telomerase-minus phenotype (10). The Pot1-associated factor Ccq1 is also required for the recruitment of telomerase to telomeres (139, 192). Pot1/Tpp1 appear to play important roles in mammalian telomerase recruitment and control, and human EST1a and EST1b may also interact with telomerase (162, 163, 180). However, our understanding of the details of telomerase recruitment in mammalian cells is still in its infancy.

Given that recruitment of telomerase to telomeres requires several proteins, it seemed initially counterintuitive that specific proteins are also required to prevent telomerase from engaging with randomly placed DSBs in the genome. Addition of telomere sequence to a DSB by telomerase is a dangerous act, as it confers the loss of all centromere-distal sequences. Nevertheless, in vitro studies show that nontelomeric primers can in fact be extended by telomerase, and strikingly, in budding yeast lacking the Pif1 helicase, telomerase is 1000-fold more likely to add telomere repeats to DSBs than in its presence (120, 142, 171). Natural telomeres are also lengthened in the absence of Pif1 (171, 219). Pif1 is able to unwind DNA-RNA hybrids in vitro, and its ability to restrain telomerase is thought to be linked to this biochemical property (22). Furthermore, Pif1 is phosphorylated and inhibited by the checkpoint machinery upon damage, channeling telomerase restraint towards DSBs but not natural telomeres (118).

Fission yeast possess a single Pif1 ortholog, but this protein is essential, making its role in the suppression of random telomere addition to DSBs difficult to address (156, 220). A study monitoring the fate of HO-endonuclease-induced DSBs in fission yeast showed that a small proportion are subjected to telomere addition by telomerase; levels of telomere addition are elevated by mutations that reduce HR activity, suggesting that HR opposes telomere addition to DSBs (47). The Pif1

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helicase is also conserved as a single ortholog in human and mouse, and while it interacts with telomerase, its telomeric function is not clear (130, 181). Unexpectedly, *pif1* knockout-mice show no phenotype, leading to the proposal that Pif1's predicted telomeric functions may be carried out redundantly with unknown factors (181).

Enlisting telomerase: at the right time and only at the shortest telomeres. Telomere length differs between species, but for a given species telomeres are maintained within a set range, indicating that a homeostatic mechanism must exist. This is important for preventing telomeres that approach critically short lengths (i.e., lengths too short to recruit sufficient telomere binding proteins to effect chromosome end protection) from shortening further and triggering senescence; extremely long telomeres may also be detrimental, as they might titrate out telomeric factors present at limiting concentrations.

Short telomeres bind fewer telomere proteins, whereas longer telomeres bind many, thus conferring the beginnings of a counting mechanism if some of these proteins provide feedback inhibition of telomerase activity. Elegant budding yeast experiments in which Rap1 was artificially tethered to telomeres showed that Rap1 inhibits telomerase action in a manner proportional to the number of tethered Rap1 molecules (123). Moreover, studies in which specific telomeres were abruptly foreshortened by excising them with site-specific recombinases showed that telomerase activity increases in *cis*, at the shortened telomeres, without affecting other telomeres in the cell (121).

The concepts underlying telomere length regulation were further crystallized by Teixeira et al. (189), who devised a method for monitoring telomerase-mediated extension at single telomeres over a single cell cycle. This study showed that only approximately seven percent of telomeres, comprising the shortest telomeres in the cell, are elongated by telomerase in each S phase; longer telomeres tend to be ignored by telomerase. Moreover, telomerase tends to add a similar number of nucleotides to each telomere on which it acts; i.e., telomerase activity appears to be quantized. Hence, this work suggested a binary model for telomerase action in which telomeres are either extendible or nonextendible, refocusing efforts to discover exactly what transition occurs as telomeres erode to lengths short enough to confer preferential telomerase activity. Such studies are the subject of several recent reviews (113, 166, 178). In brief, shortened telomeres trigger some of the events that occur at DSBs, like MRN/X recruitment and ATM/Tel1 activation, which trigger a cascade of downstream events that result in preferential telomerase recruitment. Nonetheless, although a description of which molecules appear at short versus long telomeres is now forthcoming, whether the short telomeres are distinguished solely by a low local concentration of counting proteins like Rap1 or rather by a structural transition in the DNA (e.g., t-loop unfolding or stalled replication forks) that accompanies this low local telomere protein concentration is an unanswered question.

The concepts underlying yeast telomerase regulation are likely to be conserved in mammals, with human cancer cells representing crucial and informative exceptions. When mice with and without telomerase are mated, the short telomeres derived from the telomerase-deficient mice are preferentially elongated by the introduced telomerase enzyme, indicating that murine telomerase exerts a preference for short telomeres (78). A similar counting mechanism is thought to exist in human cells. TRF1 overexpression results in shortened telomeres, whereas overexpression of a dominant negative allele of TRF1 results in telomere elongation (199). As with Rap1 in budding yeast, TRF1 could effectively count the number of telomeric repeats and provide feedback regulation. TRF1 interacts with Pot1, which further interacts with telomerase via its partner protein Tpp1. In cells that harbor a Pot1 truncation that renders it unable to bind ss telomeric DNA, telomeres lengthen dramatically and continually,

most likely reflecting a disturbance in the chain of command from TRF1 to the terminus (108).

Telomerase-Independent Modes of Bypassing the End-Replication Problem

Although telomerase appears to be the most widely embraced solution to the end-replication problem, it is not ubiquitous. Its absence from human somatic cells means that somatic cancer cells, which boast a long replicative life span, need to improvise (reviewed in 161). In most cases, they solve the end-replication problem by expressing telomerase (176; reviewed in 1). However, 10% to 15% of cancer cells maintain telomeres by an alternative strategy termed alternative lengthening of telomeres (ALT) (25). This strategy utilizes HR-mediated copying of the telomeric sequence onto shortening telomeres from an assortment of templates, including telomeres of other chromosomes, telomeres of sisters, extrachromosomal telomeric sequence-containing DNA, and even the same telomere (reviewed in 30). Fusion of an ALT cell and a normal cell results in a hybrid cell that represses ALT, illustrating that this strategy of telomere maintenance is subdued by telomerase-positive telomeres (154). Why telomeric HR is sanctioned in ALT cells is still not fully understood. The destabilized state of the telomere, along with extrachromosomal telomere-containing DNA circles, may serve to siphon away telomeric proteins whose activities would normally inhibit HR (reviewed in 30).

HR-dependent telomere maintenance was originally described in budding yeast, where this is the main mode of survival in cells lacking telomerase. The survivors are called Type I or Type II survivors, classified roughly according to whether subtelomeric sequences are recombined using a Rad51-dependent pathway (Type I) or telomeric sequences are recombined using a Rad50-dependent pathway (Type II) (110, 190; reviewed in 131). Fission yeast can also use this tactic in the absence of telomerase, forming so-called linear survivors, but sparingly. This is due to stringent inhibition of HR by Taz1, as cells lacking both Taz1 and telomerase favor this mode of survival (136, 144).

Fission yeast cells are unusual in having only three chromosomes per haploid genome, allowing them to escape from chromosome linearity-related issues by circularizing their chromosomes (144; reviewed in 53). The low chromosome number confers a high probability that cells harboring critically short telomeres prone to repair/fusion reactions will sustain three intramolecular fusions without forming lethal interchromosomal fusions. Such survivors, called circular survivors, lack telomere sequences and also lack the need for them, as they lack termini. Interestingly, despite the fact that fission yeast evolved linear chromosomes, circular survivors are viable notwithstanding a number of deficiencies (e.g., slower growth and severely compromised meiosis) compared to linear survivors (86, 144).

Preserving Chromosome Linearity in the Absence of Canonical Telomere Repeats

The capacity to buffer the end-replication problem of linear chromosomes independently of canonical telomere sequence repeats has been observed in both yeast and Dipterans. Budding yeast lacking telomerase, HR, and the exonuclease ExoI can maintain linear chromosomes without telomeres using the so-called PAL (palindrome-dependent) mechanism (126). PAL chromosomes terminate in large palindromes, originating from smaller inverted repeats. It is postulated that degradation of telomere-less chromosome ends renders the inverted repeats single stranded, allowing them to fold into hairpins and subsequently prime the synthesis of upstream regions, forming extensively palindromic chromosome ends (**Figure 4**).

Fission yeast can highjack nontelomeric heterochromatin to maintain chromosome linearity in the absence of telomerase. These cells, termed HAATI (heterochromatin

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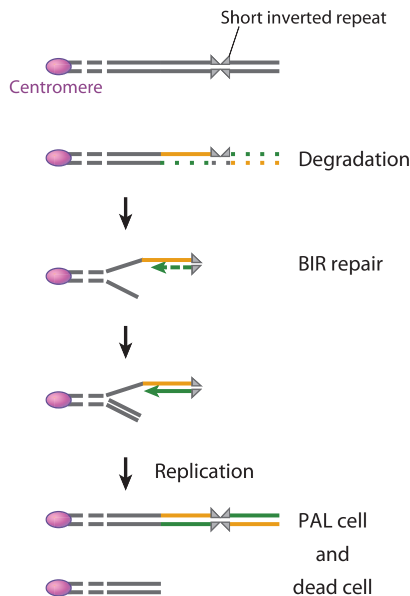

Figure 4

Model for budding yeast palindrome-dependent (PAL) survival, which can occur in the absence of telomerase, Exo1 and HR. Chromosomes contain short inverted repeats (*gray triangles*) that, upon degradation of chromosome ends, fold into hairpins and prime synthesis of a complementary strand by break-induced replication (BIR). The next replication round converts this strand into a chromosome containing large inverted repeats at its ends.

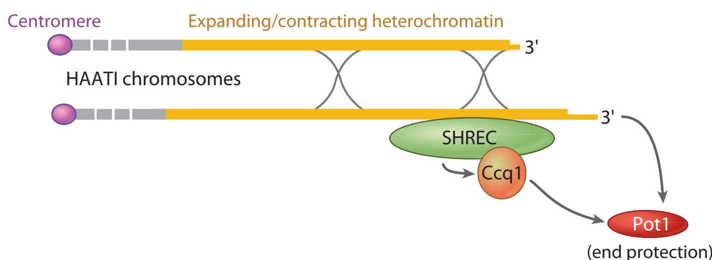

Figure 5

Model for fission yeast HAATI (heterochromatin amplification-mediated and telomerase-independent) survival. Each chromosome terminates in tracts of rDNA that undergo constant homologous recombination (HR), resulting in continual expansion and contraction of the region and buffering centromere-proximal sequences from the end-replication problem. The rDNA is assembled into heterochromatin, which associates with Ccq1 via the SHREC complex. Ccq1, in collaboration with the terminal 3′ rDNA overhangs, brings Pot1 to the chromosome ends independently of canonical telomere sequences.

amplification-mediated and telomerase-independent) (86), replace canonical telomeres with heterochromatic repeat sequences, most frequently the rDNA. Strikingly, while the rDNA is restricted to either end of chromosome III in wild-type cells, it spreads to the ends of all three HAATI chromosomes. Moreover, these chromosomes appear to terminate in 3′ overhangs of rDNA sequence. The heterochromatin assembly machinery, Pot1/Ccq1, and the recombination machinery are crucial to HAATI formation. As Ccq1 is known to bridge the Pot1 complex with the so-called SHREC heterochromatin complex (139, 185), the foregoing observations suggest a model for HAATI: Continual HR among rDNA repeats at each chromosome end insulates the remainder of the genome from the end-replication problem. At the same time, SHREC recruits Pot1 via Ccq1, and the rDNA ss 3′ overhang at the chromosome terminus also contributes to Pot1 recruitment (**Figure 5**). Pot1 in turn prevents rampant 5′ chromosomal degradation, allowing HAATI cells to survive indefinitely. Indeed, HAATI comprises the most frequent telomerase-minus survival mode when cells are grown under competitive conditions.

The HAATI strategy is reminiscent of that used by *Drosophila melanogaster* to maintain linear chromosomes. Flies lack canonical telomeres and telomerase. Instead, their chromosomes end in mixed arrays of retrotransposable elements that are maintained primarily by retrotransposition (reviewed in 151). However, these elements are dispensable at chromosome ends, and no specific sequence is required for chromosome end maintenance (14, 15, 29, 62, 99; reviewed in 28, 129). Nonetheless, fly chromosome ends are heterochromatic and are bound by terminus-specific factors that inhibit the DDR. A small set of these factors, including HOAP and HipHop, have been identified so far (29, 66). The mode of action of these factors remains to be elucidated, but much like HAATI, it is clear that they cooperate with heterochromatin factors to prevent lethal end fusions (62; reviewed in 28, 129). Hence, fly telomere maintenance and HAATI

chromosome end-protection mechanisms are highly related and may embody the universal requirements for chromosome end protection.

NOT JUST FOR AGING AND CANCER: SEX TOO

Meiosis, the specialized pair of nuclear divisions that achieves haploidization of the germ line and drives genetic diversity, presents telomeres with roles that diverge dramatically from those observed during proliferative growth. During the initial stages of meiosis, telomeres gather together at the nuclear periphery to form the so-called bouquet structure. The bouquet is highly conserved in many eukaryotes from yeast to human (reviewed in 41) and may therefore confer one of the most fundamental functions of telomeres.

The function of the bouquet is best understood in fission yeast, where it persists throughout meiotic prophase and contacts the spindle organizing center [called the spindle pole body (SPB)] (**Figure 6**). Bouquet formation requires Taz1, Rap1, and a pair of meiosis-specific proteins, Bqt1 and Bqt2, which connect the telomere with the SPB; disruption of any of these components destabilizes and/or abolishes bouquet formation (37, 38, 43, 88, 191). For several decades, it had been assumed that the bouquet serves to promote the pairing and recombination of homologs that occur during meiosis. By bringing all the chromosome ends together within a limited nuclear volume, the homology search would be restricted spatially and thus simplified (reviewed in 79). Indeed, reduced levels of meiotic recombination are observed in the absence of stable bouquet formation. However, the modest reduction in recombination levels is disproportionate to the severe defects in meiotic chromosome segregation seen in the absence of the bouquet. Instead, the principal function of the bouquet appears to lie in controlling formation of the meiotic spindle. In the absence of the bouquet, monopolar spindles, unstable spindles, and multiple spindles are observed. These spindle defects are associated with problems in SPB duplication and/or separation (191). Hence, the connection between the telomere bouquet and the SPB appears to regulate SPB separation, which in turn is required for spindle formation. An understanding of the mechanisms by which telomeres influence the meiotic SPB and spindle may shed light on a role for centromeres in

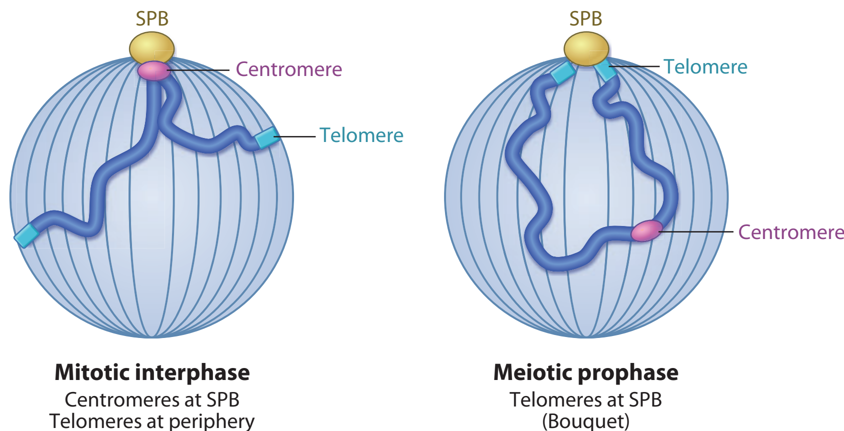

Figure 6

The fission yeast meiotic bouquet. During mitotic interphase, centromeres are attached to the spindle pole body (SPB; the fission yeast centrosome equivalent), whereas telomeres are located at the nuclear periphery. Upon induction of meiosis, the telomeres cluster at the SPB, and the centromeres move away from the SPB, forming the bouquet.

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mitotic spindle formation, as centromeres contact SPBs during mitotic interphase. Hence, telomere studies may suggest general principles of chromosome dynamics.

CONCLUDING REMARKS

Telomeres are intriguingly manipulative structures. They distinguish chromosome ends from DSBs, preventing harmful DDRs from threatening the integrity of individual chromosomes, and ensure complete chromosomal replication. To accomplish these feats, telomeres indulge in those very pathways that threaten chromosome ends, seducing components of the DDR machinery like nucleases and end-joining factors, but keeping a tight leash on their activities. Understanding the intricacies of chromosome end protection may eventually allow researchers to devise strategies for mitigating the impact of telomere dysfunction on organismal aging and disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank our current and former lab members for many fruitful discussions. Special thanks go to Ofer Rog for critical comments on the manuscript. Research in our laboratory is funded by Cancer Research UK and the European Research Council.

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Volume 44, 2010

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