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Telomere dynamics in genome stability

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The past several years have seen an increasing interest in telomere recombinational interactions that provide many functions in telomere capping, in telomere size homeostasis and in overcoming the catastrophic effects of telomerase deficiency. Several key recombination mechanisms have emerged from recent investigations. In the yeasts, these mechanisms include exchange between subtelomeric regions and telomere sequences, rapid telomere expansion and telomere deletion. These processes proceed by pathways that use both the cellular recombination machinery and novel mechanisms such as rolling circle replication. The insights gained from recent studies extend our understanding of similar processes in higher eukaryotes and suggest that the recombinational dynamics of telomeres have additional roles that contribute to genomic stability and instability.

Telomeres: from curiosity to limelight

Initial interest in the termini of linear chromosomes was based on the unusual mutagenic characteristics of these DNA ends. Muller's [1] classic studies on the inviability of terminal deletions in *Drosophila*, coupled with McClintock's [2] seminal demonstration that telomeres are refractory to fusion events, provided the first hint that telomere integrity is important for genome stability.

The curious nature of telomeres is not restricted to their 'cap' function. Indeed, semi-conservative DNA replication cannot explain the replication of terminal sequences of linear DNA – a puzzle that has been termed the 'end-replication problem' [3]. Without a solution to this problem, several rounds of primer loss and exonucleolytic cleavage would lead to chromosome dysfunction and cellular inviability. The finding that telomeres are composed of simple sequence DNA comprising guanine and thymine [4] and the breakthrough discovery of 'telomerase', a reverse transcriptase ribonucleoprotein that adds these (G+T)-rich repeats onto the 3′ ends of pre-existing telomeric substrates [5], initiated the modern era of investigations into telomere dynamics.

In the absence of telomerase, cells can adopt one of several alternative fates (Figure 1). First, short dysfunctional telomeres can fuse through non-homologous end joining, forming either chromosome circles or dicentric chromosomes that subsequently lead to deleterious cycles of breakage, fusion and bridging, and to massive genomic instability. Second, the ataxia-telangiectasia-mutated checkpoint protein kinase (ATM) pathway can promote an apoptotic response in cells carrying dysfunctional telomeres – a process that occurs in eukaryotes ranging from humans to *Drosophila* [6,7]. Third, in both yeast and human cells, inviability caused by the loss of telomerase can be overcome by homologous recombination between telomeric or subtelomeric sequences.

In this review, we focus on the mechanism, regulation and physiological significance of telomeric homologous recombination – in both the presence and the absence of telomerase – which functions to lengthen, shorten or rearrange telomeric and subtelomeric regions.

Homologous recombination: an alternative means of telomere maintenance

Recombinational pathways of telomere maintenance have been tested most extensively in the nuclear and linear mitochondrial genomes of yeasts, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Candida albicans* [8–12]. The involvement of telomere recombination was first indicated by the *RAD52*-dependent survival of telomerase-negative cells [8]. In the yeast *S. cerevisiae*, two principal pathways, type I subtelomeric amplification and type II telomeric repeat amplification, account for most telomeric formation among cell survivors [9,13] (Figure 2). Extensive studies in *K. lactis* have identified a similar process known as 'recombinational telomere elongation'. For simplicity, we refer to the latter two as type II recombination, although mechanistic differences remain a possibility.

Type I recombination

Type I survivors (see Glossary) of telomerase-negative lethality do not elongate telomere tracts. Rather, the number of Y′ telomeric ends or 'Y′ elements' adjacent to the telomere tract increases [8]. Y′ elements in *S. cerevisiae* are present at most telomeres in two main polymorphic forms that, when amplified, are separated by

Glossary

ALT: alternative lengthening of telomeres found in a multiplicity of carcinomas and post-senescent cells following crisis.
BLM: Bloom's-syndrome-related human ortholog of Sgs1.
Bouquet structures: a specific meiotic form of telomeric clustering.
D-loop: single-stranded DNA displaced by invading DNA.
H2AX: isoform of histone H2A used in DNA repair.
Yku70 heterodimer: multifunctional complex involved in telomere capping.
Sgs1: a yeast 3′→5′ helicase required for type II survival.
T-circles: circles derived from telomeric DNA.
T-loops: terminal loops formed by invasion of 3′ overhang into basal repeats.
Type I survivors: cells that survive owing to the amplification of subtelomeric repeats.
Type II survivors: cells that survive owing to the elongation of telomeric simple sequence tracts.
Y′ elements: several variants of a 6.7-kilobase subtelomeric repeat.

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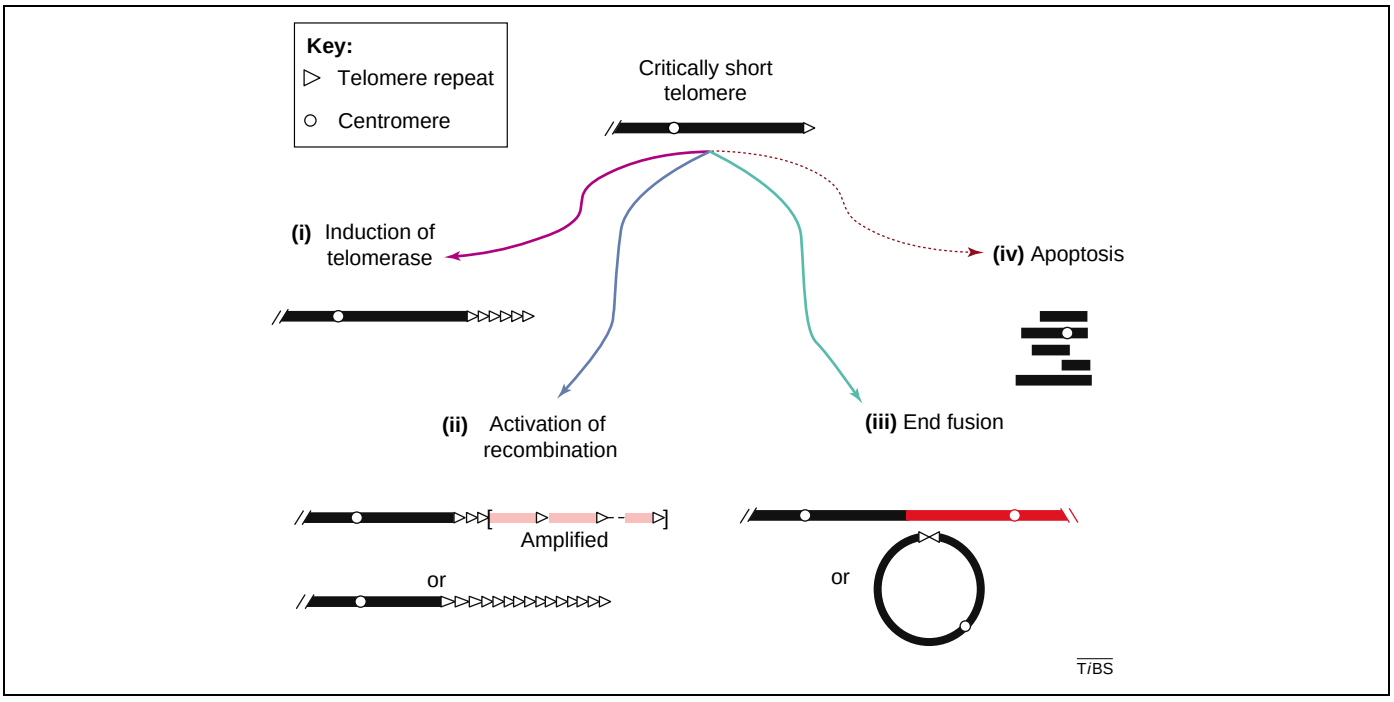

Figure 1. Multiple cellular fates arising from a critically short telomere. Depending on the pathway acting on the shortened telomere, the cell adopts different fates: (i) maintenance of telomere length in telomerase-proficient cells; (ii) elongation of telomeres by recombination mechanisms (normally in telomerase-deficient cells); (iii) failure of telomere capping with concomitant formation of unstable dicentric chromosomes formed by end-to-end fusion; (iv) apoptosis. Triangles indicate the telomeric tract; pink lines indicate amplified subtelomeric sequences; dotted lines indicate that the process does not take place in yeast.

short tracts (50–150 bp) of telomeric sequence. The basis for survival remains uncertain. Possibilities include the addition of Y′-associated telomeric sequence or the presence of a non-essential subtelomeric 'buffer zone'.

Type I survivors of telomerase-negative lethality in *S. cerevisiae* (Figure 2a) are dependent on the RecA homolog and strand invasion protein Rad51, as well as on the Rad54 helicase and the Rad55–Rad57 complex that stabilizes the Rad51 'RecA-like' recombination filament [14]. The 5′-to-3′ exonuclease Exo1 is likely to be required for resection before invasion [15,16]. These findings are most consistent with an interchromosomal break-induced replication step (Figure 3) after strand invasion between telomeric and subtelomeric sequences [17]. Break-induced replication is likely to amplify Y′ repeats by means of the invasion of the 3′ end of a truncated telomere into the

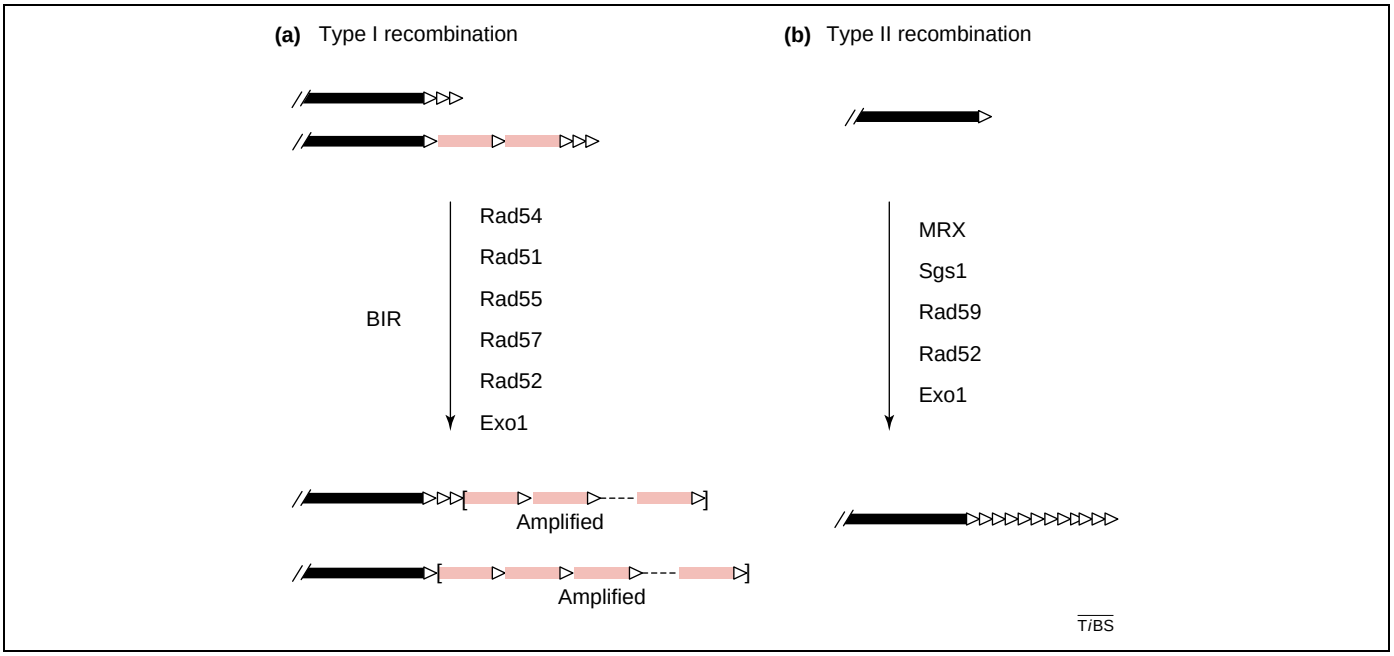

Figure 2. Two classes of yeast telomeric recombination. In telomerase-deficient cells, either type I **(a)** or type II **(b)** recombination can maintain the telomere structure. In type I recombination, the Y′ element (pink box) is amplified by invasion of a 3′ end of the truncated telomere into interstitial Y′ element-associated telomere tracts; in type II recombination, the G-rich terminal duplex DNA (open triangles) is elongated. The principal gene products involved in each process are shown. For simplicity, type II events in *S. cerevisiae* and recombinational telomere elongation events in *K. lactis* are assumed to be similar; however, there might be some mechanistic differences.

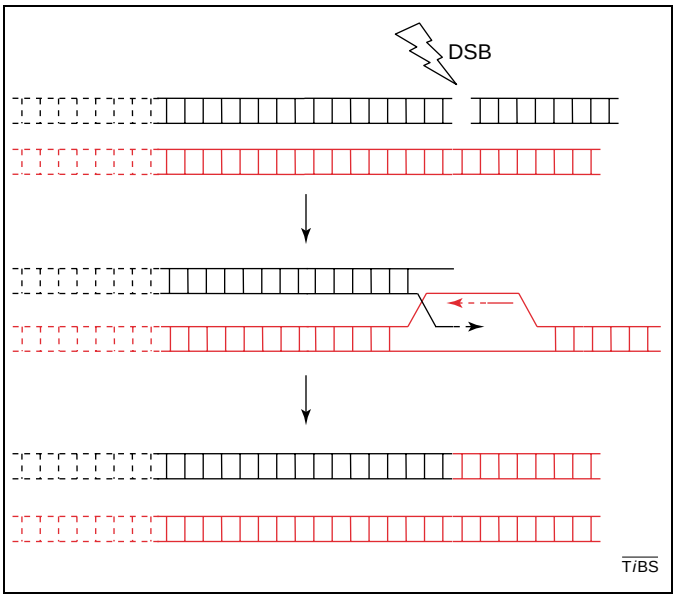

Figure 3. Break-induced replication. If one end of a double-strand break (DSB) has sequence homology elsewhere in the genome, invasion of a single strand from the broken end (black) to the homologous duplex (red) primes DNA replication, resulting in duplication of one chromosome arm (red). This mechanism is called 'break-induced replication' and could maintain the telomere in the absence of telomerase.

Y′-associated telomeric tracts of another chromosome [17,18]. Such invasion is subsequently followed by replication at the end of the heterologous chromosome. Survivors of the type I class require the continual presence of the major homologous recombination protein Rad52. The slow growth and possible inefficiency of type I survivors often result in the accumulation of type II survivors after extensive growth [12,13].

Type II recombination

In the yeasts, type II survivors of telomerase-negative lethality are uniquely characterized by a diffuse heterogeneous distribution of terminal restriction fragments that represent amplified telomeric repeats. Type II recombination has been observed in the yeasts *S. cerevisiae* and *K. lactis* and in the mitochondria of numerous species of *Candida* [10]. Type II survivors are also found in *S. pombe* under conditions in which the telomere-binding protein Taz1 is lost [11]. Importantly, Rad52 must be continuously present, indicating that type II survivors, like type I survivors, require continual recombination.

Some of the genetic requirements for type II recombination reflect the detection and processing of telomeric 3′ termini. The highly conserved and multifunctional MRX complex comprising Mre11, Rad50 and Xrs2 [19,20] associates with the telomere, where it subsequently recruits either an ATM or an ataxia-telangiectasia-related checkpoint protein kinase (ATR) homolog (Tel1 or Mec1, respectively) [21], depending on complex parameters of both the cell cycle and telomere size. The MRX complex might also act downstream of ATM as a nuclease–helicase complex that, together with the principal 5′-to-3′ exonuclease Exo1, forms the telomeric 3′ strand overhang [15,16]. Consistent with this hypothesis, both Tel1 and Mec1 are required for type II recombination [22].

The dependence of type II recombination on helicases is likely to be the consequence of the unwinding of telomeric DNA before or during processing. Among these DNA helicases are SGS1 [an ortholog of the human Werner's syndrome (WRN) and Bloom's-syndrome (BLM) proteins] and Def1, a helicase-associated protein of unknown function [23,24]. In addition, Rad59 is required for type II recombination and probably accounts for strand invasion activity [9]. Interestingly, the repair enzyme DNA polymerase δ is also required for type II recombination, indicating that semi-conservative replication is associated intimately with the recombination process [22]. Notably, loss of mismatch repair enzymes in yeast stimulates the production of recombinational survivors [25], raising the possibility that some of these proteins might block recombination between the irregular telomeric repeats present in yeast. In human cells, by contrast, loss of the mismatch repair enzyme MSH6 results in very few cells using recombination [ALT (alternative lengthening of telomeres cells); see later], suggesting that MSH6 might have a positive role [26].

Cyclin B in conjunction with Cdc28 is also required for type II recombination and, to some extent, for type I recombination, suggesting that telomeric recombination is under cell-cycle control [27]. Such regulation is similar to the recently reported dependence of HO (the 5′→3′ endogenous endonuclease)-induced recombination on Cdc28 [28].

Type II survivors – going in circles?

Several models of yeast type II recombination have emerged. So far, most studies favor a rolling circle model of recombinational elongation [29] [Figure 4a(i)]. The first step in this process is the formation of circles, and the identification of these predicted, small, single-stranded and double-stranded telomeric circles, called 'T-circles', in low abundance in the nucleus [30–32] and in mitochondria [30,33,34] is consistent with this model. Indeed, the ubiquity of such T-circles is highlighted by their presence in vertebrate cells, where they can be formed by recombination-mediated excision of elongated telomeres [35–37].

The second step involves recombination between the highly recombinogenic telomeric ends of truncated yeast telomeres with T-circles [38], giving rise to the elongated telomere [39]. The telomere is not, however, intrinsically recombinogenic. Indeed, longer telomeres in yeast are less recombinationally active after elongation [40,41]. These results are most consistent with an ability of excised T-circles to recombine with endogenous telomeres. Furthermore, molecules resembling rolling circle intermediates have been identified both in *Kluyveromyces* nuclei and *Candida* mitochondria [32,34]. Possibly the 'smoking gun' that implicates circular DNA as a substrate for elongation is the conversion of conditionally synthesized T-circles into telomeres [31]. In addition, rolling circle replication has been recapitulated *in vitro* by using telomeric nanocircles as a substrate [42].

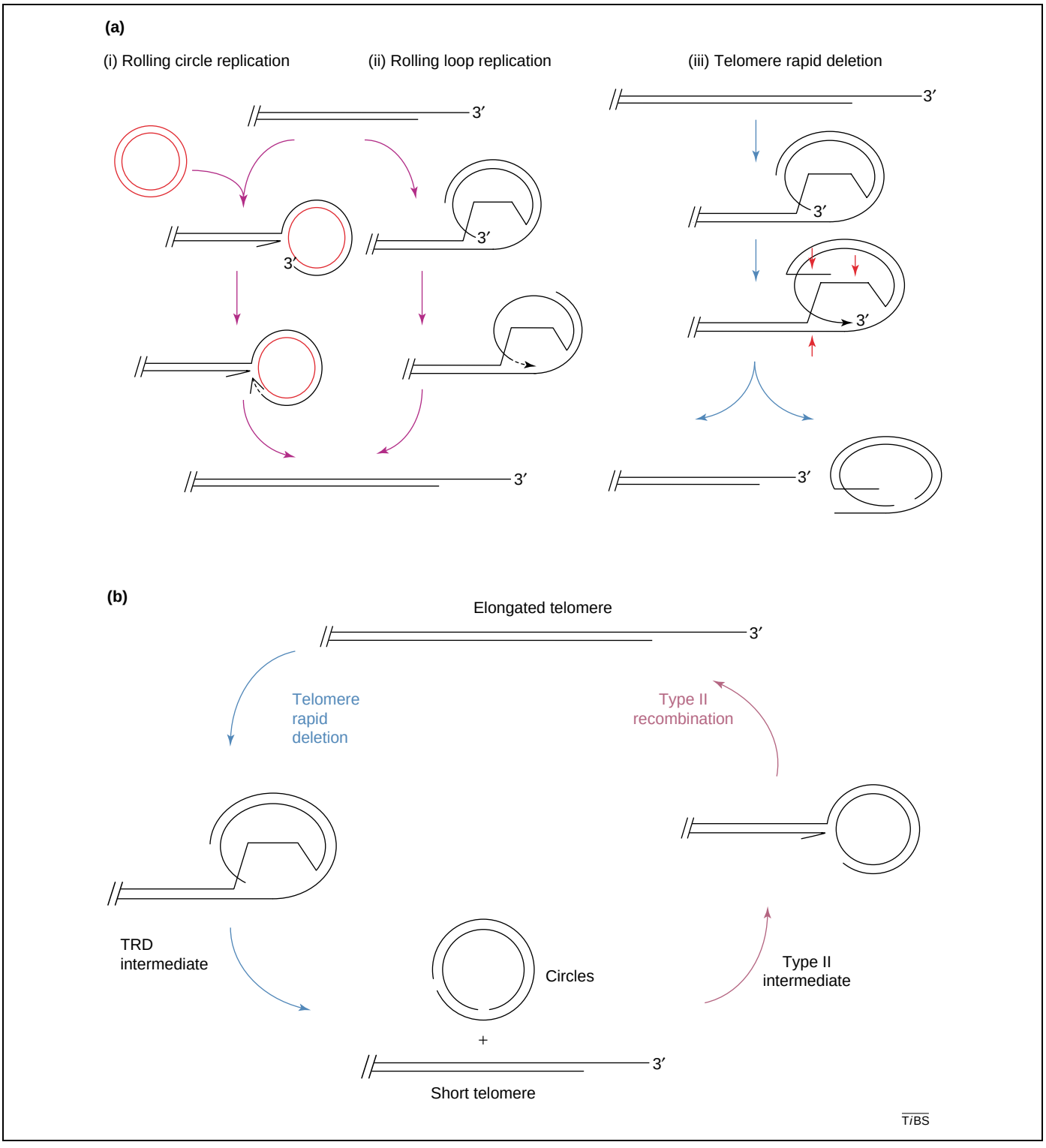

Figure 4. Telomeric strand interactions. **(a)** Mechanistic comparison between type II recombination and telomeric rapid deletion (TRD). The proposed mechanisms of rolling circle replication (i) and rolling loop replication (ii) constitute the two 'roll and spread' models of type II recombination and are shown in comparison to recombinational excision by TRD (iii). All three mechanisms proposed proceed through looped intermediates but have markedly different outcomes. In (i), continual reiterative replication of short circles occurs after invasion into the shortened repeats; in (ii), invasion of a 3′ terminus into a D-loop is followed by continual reiterative replication of a T-loop; in (iii), vertical resolution of a Holliday junction yields truncated telomeres. Replication has a different role in each pathway, ranging from continual (i, ii) to repair (iii) synthesis. The roll and spread models (i, ii) therefore follow a break-induced replication pathway. **(b)** Type II recombination and TRD can act as a crude homeostatic mechanism. TRD might give rise to short circles that are then used as substrates for type II or ALT recombination, reforming the elongated telomere. The coexistence of such elongation (type II) and contraction (TRD) mechanisms might maintain cyclic telomere homeostasis. During TRD, an elongated telomere goes via a looped intermediate (the TRD intermediate) to yield a shorter telomere and nicked circles. Such circles could then be used in type II recombination, which also proceeds via a looped intermediate (the type II intermediate) to give rise to a longer telomere.

T-circles are also generated in a unique subset of mammalian cells ([43]; and see later) from telomeric loop or 'T-loop' structures. T-loops, first identified in human cells [44], are thought to form after invasion of the 3′ terminus into more basal telomeric sequences, reminiscent of a recombination intermediate. Indeed, Holliday junction resolution of such an intermediate might provide the T-circles required for amplification [40] [Figure 4a(iii)]. T-loops have now been found in species as diverse as *S. pombe* and trypanosomes [11,45,46]. T-loop resolution and subsequent re-invasion might arise from an alternative means of telomere repeat amplification [47] [Figure 4a(ii)]. Several rounds of replication through the T-loop predict the formation of rapidly elongated telomeres with a T-loop unit size. At present, such a T-loop extension (also termed a 'rolling-loop' [47]) has not been observed.

These models of yeast type II recombination are not, however, mutually exclusive. Regardless, all homology-based models face a potential barrier to recombination in organisms such as *S. cerevisiae* and *S. pombe* that carry irregular repeats. Such imperfect partial repeats might be subject to a unique mechanism referred to as 'short tract homologous recombination' that can act on sequences containing homologous regions as small as 30 bp [48,49].

In *K. lactis*, several variants have been produced by mutation of the 30-bp RNA template that encodes the 25-bp repeat. The rolling circle model can explain elongation of one of the telomeres but not its spread to other telomeres. Notably, when cells are engineered to contain a variant on the initial elongated telomere that was not present in the progenitor cells, this variant sequence spreads to other shortened telomeres in telomerase-negative strains [10,50] (Figure 5). High rates of invasion of critically short telomeres [50] into the elongated telomere, followed by break-induced replication to the chromosomal end, are thought to give rise to multiple elongated tracts [51].

Novel routes to terminal protection

Signals other than erosion of the telomere can provoke type-II-like recombination in both yeast and humans. First, the presence of single strands in *cdc13* mutants stimulates type II recombination independent of Rad50 in yeast [52], presumably through partial uncapping, rather than through loss of telomeric sequence. Second, at a semi-permissive temperature for growth, *yku70 cdc13-1* but not *yku70* mutants undergo senescence even though telomere size is identical in both types of mutant cell. The *yku70 cdc13-1* senescent cells can undergo a Rad50-independent, Rad51-dependent pathway of telomere repeat amplification that bears similarity to type II recombination but is coupled with the formation of long single-stranded telomeric repeats [53]. Indeed, this pathway predominates in *yku70 cdc13-1* cells even in the presence of a third mutation in the type II machinery. In an analogous manner, overproduction of a dominant-negative allele of the vertebrate telomere-binding factor 2 (TRF2) results in senescence without loss of telomere size [54].

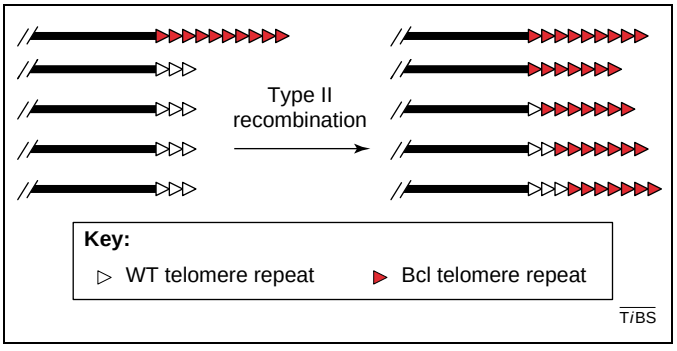

Figure 5. Telomeric and subtelomeric spreading. In telomerase-negative strains of *K. lactis*, variant telomeric repeat sequences (e.g. Bcl repeats) from an elongated telomere spread to other, shortened telomeres. After type II recombination, the recipient telomeres might or might not contain the wild-type (WT) repeats. If Bcl repeats are present in a normal length telomere, however, such spreading is a minority event. Similar break-induced replication pathways can give rise to the amplification of Y′ element repeats.

These important studies indicate that both the state of telomere capping and the telomere length result in senescent states that can be overcome by seemingly unique mechanisms of telomere elongation. These data also suggest that there is a high level of plasticity in telomere recombination. The most extreme example of this plasticity are survivors of telomere erosion in the absence of telomerase, recombination and the 5′-to-3′ nuclease ExoI. This survival occurs through a mechanism referred to as the 'palindrome-dependent mechanism', in which degradation leads to initial cell-cycle arrest, adaptation to arrest without DNA repair, the formation of short palindromes and, finally, DNA-repair-mediated senescence through the conversion of short terminal palindromes into large terminal palindromes, leading to a senescent state [55]. Several pathways for elongation also seem to be present in human cells [56]. This recombinational flexibility is likely to be a consequence of selection for genomic stability.

Telomeric rapid deletion: put it in reverse!

Studies from our laboratory have defined a process in yeast, termed 'telomeric rapid deletion' (TRD), that reduces over-elongated telomeres to wild-type size, thereby contributing to telomere size regulation [47]. Interestingly, the degree of deletion is dependent on the length of the majority of telomeres on the heterologous chromosomes, and an increased abundance of elongated telomeres rarely leads to their deletion to wild-type size. A correlation between the length of most telomeres and the rate of telomere loss has also been found in mammalian cells [57].

In yeast, the deletion process itself is recombinational on the basis of its requirement for Rad52 function. Furthermore, mutations in *HPR1* (hyper-recombination 1) that confer high rates of deletions between direct repeats [58] also increase the rate of TRD. Similar to type II recombination, TRD is dependent on Mre11 and Rad50 (but not on Xrs2) and is enhanced in the absence of the Yku70 heterodimer, reflecting a loss of cap function [40,59]. TRD is independent of the nuclease activity of Mre11, suggesting that Mre11 acts in another function such as monitoring of the telomeric 3′ structural state [60]. Analysis of physically marked telomeres has indicated that TRD is an intrachromatid recombination process [40].

In the current working model [Figure 4a(iii)], the 3′ overhang end invades more basal telomeric repeats, giving rise to a prototypical Holliday junction. The Holliday junction is then resolved to form the deleted telomere and either linear or circular extrachromosomal byproducts.

Notably, 'TRD-like' events dependent on the Mre11, Rad50 and Xrs2 (MRN) complex – the human homolog of the MRX complex – also occur in human immortalized cells, apparently as a consequence of T-loop excision [36,37]. There have also been many descriptions of catastrophic deletions in vertebrate cells, as reviewed elsewhere [47]. Given this evolutionary similarity, it is tempting to speculate that the expansion and deletion of telomeric sequences might have conserved mechanistic elements.

Recent experiments have revealed that a specific allele of the yeast *STN1* gene in *K. lactis* encodes a mutant protein that forms a complex with Cdc13, producing long telomere restriction fragments that are highly single-stranded in nature [61]. Transformation of these cells with wild-type *STN1* results in the immediate conversion of elongated telomeres to wild-type length – an effect that is unique to this mutation. It remains uncertain, however, whether this Stn1-mediated pathway that acts on extended single-stranded DNA is similar to the TRD pathway that acts on elongated duplex DNA. Rather, the Stn1-repressed deletion process might rely on elimination of the single strand through exonucleolytic activities. Such a mechanism might also help to explain the residual levels of TRD that are observed in the absence of Rad52 in *S. cerevisiae* [40].

Can type II recombination and TRD balance telomere size?

In both yeast and humans, telomere homeostasis is usually dependent on the 'counting' of the number of telomere-binding proteins bound to repeats. In turn, counting these repeats is linked to the formation and dissolution of an as yet unknown structure. In yeast telomerase-negative cells, however, the observed distribution of telomere restriction fragments is diffuse but does not lead to continual elongation or deletion in the constitutive presence of Rad52 [13]. Thus, a balance, albeit crude, between TRD and type II recombination might occur even in telomerase-negative cells that could provide a degree of genomic stability.

For example, because telomeres that are critically short are more recombinogenic, an increase in the rate of type II recombination is expected among shorter telomeres. Severely elongated telomeres, by contrast, might be capable of excision through TRD, potentially giving rise to the characteristic heterogeneous distribution of fragments (Figure 4). Indeed, work on the yeast *C. albicans* has shown that both telomerase and recombinational systems can coexist to produce physiologically relevant size homeostasis [62]. Another example of the co-use of both telomerase and recombination pathways involves the *POL12* gene encoding the B subunit of polymerase α. Loss of *RAD52* in the *pol12-216* allele actually exacerbates the telomerase-mediated elongation defect – a finding that has led to the proposal that elongation is limited by TRD or a similar deletion mechanism [63].

Unique aspects of telomeric recombination in vertebrates

The ALT pathway

Vertebrates also possess a recombinational pathway that maintains telomere length in immortalized telomerase-negative cells – a process known as the 'alternative lengthening of telomeres' (ALT) [64]. Like the pathways in type II yeast survivors, ALT confers highly diffuse telomere restriction fragments, ranging from the very short to the grossly elongated that often reach the mobility limit of >20 kb. This distribution of telomere restriction fragments suggests that there are rapid changes in both elongation and deletion. Examination of catastrophic loss of telomeres by cytological and physical methods suggests that telomere trunication is likely to take place in a single step in what might be a vertebrate form of TRD [37,65,66]. Consistent with this idea, deletion of the terminal loop forms small circles in ALT cells that might be a requirement for vertebrate rolling circle replication [36,37]. Yet, at least a fraction of ALT cells seem to use a different pathway to achieve long telomeres through high rates of interchromosomal crossing over [67] – a mechanism of recombination that differs from the break-induced recombination observed in yeasts.

Other forms of recombination that are present in ALT cells seem to be the consequence of specific forms of telomeric hyper-recombination, including sister chromatid recombination between telomeric tracts – a process termed 'telomere sister chromatid exchange' (T-SCE). These recombination events were identified by an elegant modification of co-fluorescence *in situ* hybridization in which an initial telomeric single chromatid signal was replaced by a paired chromatid signals representing the two sister chromatids [68,69]. Although T-SCE is likely to elongate one of two sister chromatids by unequal homologous recombination, it cannot explain the overall elongation of telomeres. Nonetheless, the presence of T-SCE is an excellent marker of ALT cells. Another theory suggests that T-SCE is able to resolve DNA entanglements during S phase in ALT cells [70]. Because ALT is likely to represent more than one pathway, such results are not necessarily contradictory.

ALT-associated promyelocytic bodies and telomeric recombination

A striking and unique characteristic of most ALT cells is the presence of a specialized form of nuclear promyelocytic (PML) bodies called 'ALT-associated PML bodies' (APBs). These structures have several specific features that are not typical of PML bodies. First, APBs, unlike PML bodies, undergo DNA replication in late S/G2 phase [71,72]. Second, the main human telomere-binding factors, TRF1 and TRF2, localize to APBs together with short ($\sim$1 kb) extrachromosomal telomeric DNA of unknown function [72,73]. Third, a large and curious set of proteins that participate in telomere recombination and in DNA repair in normal cells localize to APBs in ALT cells [71]. These include WRN and BLM, the human homologs of Sgs1

helicase, as well as the MRN trimeric complex [74]. Another class of familiar recombination proteins localize to APBs, including RAD51 and RAD52, as well as p53 and γ-phosphorylated histone isoform H2AX [72,75]. These data suggest that APBs associate with DNA damage proteins that unexpectedly have an intimate linkage with telomere end protection and recombination.

Although many of the components of APBs are likely to be important in telomere recombination, the mechanistic relationship of ALT to yeast type II recombination remains unknown. Because many of these APB proteins have functions in telomerase-positive cells, the context of the complexes might decide the fate of the 3′ end in recombination. The association of ALT phenotypes with APBs is, however, not universal. Indeed, some cell lines lacking APBs can switch between the ALT and telomerase pathways, whereas others form more complex subtelomeric rearrangements, indicating the presence of several classes of ALT [76,77].

Physiological functions of telomere recombination?

Several recent studies suggest that type II recombination, TRD and ALT have roles beyond rescuing cells from inviability. First, *C. albicans* maintains both telomerase and recombinational modes of telomere additions under normal growth conditions, suggesting the presence of an efficient recombinational signaling pathway that is absent or inefficient in other organisms tested. Second, type II recombination is regulated by the mating-type locus in *S. cerevisiae*, further suggesting that this pathway has an elusive cellular role [78]. Third, chromatin structure in telomeric regions might also be involved in type II recombination; this speculation is based on the preferential targeting of Ty1 transposable elements to subtelomeric regions in *S. cerevisiae* [79]. Telomeric recombination in vertebrates might also have a specific role during development because sister chromatid exchange takes place in the stem cells of telomerase-negative mice [80].

Perhaps the clearest indication that telomere recombination has a physiological role is meiotic TRD [81]. As opposed to a rate of 0.3% per telomere per cell cycle in mitotic cells, meiotic TRD returns elongated telomeres to their wild-type length at a rate of $>20\%$ per meiosis, in part owing to the ability of telomeres to form bouquet structures – spindle-pole-mediated congregations of telomeres found in early stages of meiosis. This type of increase in telomeric recombination seems to be restricted to TRD, because no other interchromosomal or intrachromosomal telomeric events have been observed. This has led to the proposal that meiotic TRD might have a role in resetting telomeres to wild-type size before subsequent mitotic growth. In summary, the emerging picture is that telomeric recombination has a multiplicity of roles depending on the needs of the system. These needs range from a means of survival during cellular crisis to a part of telomere size homeostasis and genomic stability during development.

Concluding remarks

The telomere is recombinationally dynamic, providing cells with greater flexibility to deal with various aberrant and normal processes in different organisms. Among these processes are gross telomere expansion – apparently mediated through several mechanisms – telomere deletion and subtelomeric rearrangement. The mechanisms underlying these processes include break-induced and rolling circle replication.

Telomerase is considered to be a target for oncogenic therapy because of its ability to immortalize cells containing dysfunctional short telomeres [82]. Indeed, activation of telomerase is a well-known means of overcoming crisis. However, recent evidence has indicated that numerous types of tumor and mouse embryonic fibroblasts can also use the ALT pathway. Although 85% of tumors use the telomerase pathway, a subset of the remaining 15% uses the ALT pathway [83] in a tissue-type and tumor-specific fashion. For example, carcinomas use the ALT pathway only very rarely; by contrast, a large fraction of soft tissue sarcomas uses this pathway including osteosarcomas, liposarcomas and astrocytomas [56,84]. The mechanistic basis for telomere maintenance in these cells remains fertile ground for future research. Distinct targeted therapies will probably be necessary that are based on the mechanism of immortalization.

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