Review

Fission yeast telomeres forecast the end of the crisis

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**Abstract**

Recent years have placed fission yeast at the forefront of telomere research, as this organism combines a high level of conservation with human telomeres and precise genetic manipulability. Here we highlight some of the latest knowledge of fission yeast telomere maintenance and dysfunction, and illustrate how principles arising from fission yeast research are raising novel questions about telomere plasticity and function in all eukaryotes.

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1. Introduction

From investigators of maize chromosomes to human cancer biologists, scientists have encountered the disproportionately large impact a minute genomic region, the telomere, has on wider genome stability. While the ciliates have been powerful models for their high telomere number and biochemistry, budding yeast for ease of genetic manipulation and human cell lines for their obvious applicability to humans, fission yeast has emerged as a valuable counterpart. First isolated from cultures used to brew beer in east Africa, *Schizosaccharomyces pombe* is characterized by a rod shape and division by medial fission, features that differ markedly from *Saccharomyces cerevisiae*, as fission and budding yeasts diverged around 600 million years ago. Fission yeast combines a high level of conservation with mammalian telomere components and precise genetic manipulability, allowing airtight control experiments not always accessible with mammalian systems. At the same time, fission yeast provides several unique features, such as the ability to survive complete telomere loss by circularization of each of its three chromosomes, that confer unusual experimental opportunities. This review provides a non-exhaustive summary of fission yeast telomere regulation, highlighting the newly expanded fission yeast end-capping complex, insights into telomerase recruitment mechanisms and what happens when telomerase is dispensed with entirely, ending with the role of telomeres in meiosis, an aspect of telomere biology which is so far best studied in fission yeast.

2. Structure of fission yeast telomeres

Fission yeast telomeres were cloned and sequenced by Sugawara and Szostak in the 1980s, revealing a degenerate telomere repeat \( (G_2\text{-}6TTAC[A]) \) comprising the terminal \( \sim 300 \text{ bp} \) of each chromosome. Chromosomes I and II also contain around 19 kb of loosely repetitive sequences, defined as sub-telomeric-element 1 (STE1), 2 (STE2) and 3 (STE3) according to a conserved restriction pattern, extending from the telomeric repeats towards the centromere. On chromosome III, the rDNA repeats extend from the telomeric boundary inwards, although subtelomeric repeats are present between the rDNA blocks and the telomeric repeats in at least some strains [1]. The heterochromatic nature of these sequences was first hinted at by work showing that, like fission yeast centromeres and budding yeast telomeres, fission yeast telomeres exert a so-called telomere position effect (TPE), repressing transcription of genes inserted near...
telomeres. Like mammals but unlike budding yeast, *S. pombe* is equipped with a RNAi machinery which is involved in silencing at both centromeres and the mating type locus [2,3]. Notably, TPE is not affected by RNAi pathway inactivation [2,4]. Indeed, genetic evidence suggests that TPE is exerted mainly by the telomere-specific proteins [5] along with general heterochromatin proteins whose terminal recruitment depends on telomere proteins; in contrast, heterochromatin assembly in more proximal subtelomeric regions depends on the RNAi pathway [4].

A one-hybrid screen for telomere-specific DNA binding proteins identified Taz1 as the inaugural member of the fission yeast telomere protein complex. Like mammalian TRF1 and TRF2, Taz1 contains a helix-loop-helix double stranded DNA (dsDNA) binding domain of the homodeoxodomain subset of Myb domains as well as a TRFH (telomere repeat factor homology) domain [5,6]. Rap1 and Rif1 were uncovered via sequence searches for orthologs of *S. cerevisiae* proteins. Despite the presence of two Myb-domains, fission yeast Rap1 lacks DNA binding ability and localizes to telomeres by interacting with Taz1 [7]. Nonetheless, a small amount of Rap1 binding is detected at a subset of telomeres in the absence of Taz1 [8]. Rif1’s reported mode of telomeric localization is also via Taz1 binding (despite its name, Rap1-Interacting-Factor). Genetic observations suggest that Rap1 and Rif1 compete for the same binding site on Taz1 as deletion of rap1+ leads to increased binding of Rif1 [7]. This apparent simple organization of the Taz1/Rap1/Rif1 complex still holds mysteries. For instance, do all three proteins have a uniform distribution along the telomere, through the cell cycle or between different telomeres? How does the interplay between Rap1 and Rif1 binding work? And if residual levels of Rap1 and Rif1 bind telomeres lacking Taz1, how does this occur and does it depend on telomeric chromatin or perhaps the nearby single-strand telomere binding complex? Finally, how does the balance of Taz1, Rap1 and Rif1 change during telomere shortening and what are the consequences of these changes?

### 2.1. The tip of the cap

A conserved feature of telomeres is the presence of a 3’ overhang of the G-rich telomeric strand protruding from the dsDNA repeats and a recurrent concern of eukaryotes is the protection of this overhang. For a long time, the archetypal overhang binding complex was ciliate TEBPβ/TEBPb, a dimer of proteins bearing single stranded DNA (ssDNA)-binding oligonucleotide/oligosaccharide-fold (OB-fold) domains which confer high affinity and specificity for the telomeric ss G-strand [9]. Sequence comparison unveiled fission yeast Pot1 as the ortholog of ciliate TEBPb and allowed the subsequent discovery of mammalian POT1 [10]. Mammalian shelterin was also shown to harbor an ortholog of TEBPb, TPP1, which is required for telomeric Pot1 recruitment [11–15]. However, the Ishikawa group identified three additional players in the fission yeast telomere complex, Tpz1, Poz1 and Ccq1, by a one-hybrid screen for telomere-specific DNA binding proteins identified Taz1 as the inaugural member of the fission yeast telomere protein complex. Like mammalian TRF1 and TRF2, Taz1 contains a helix-loop-helix double stranded DNA (dsDNA) binding domain of the homodeoxodomain subset of Myb domains as well as a TRFH (telomere repeat factor homology) domain [5,6]. Rap1 and Rif1 were uncovered via sequence searches for orthologs of *S. cerevisiae* proteins. Despite the presence of two Myb-domains, fission yeast Rap1 lacks DNA binding ability and localizes to telomeres by interacting with Taz1 [7]. Nonetheless, a small amount of Rap1 binding is detected at a subset of telomeres in the absence of Taz1 [8]. Rif1’s reported mode of telomeric localization is also via Taz1 binding (despite its name, Rap1-Interacting-Factor). Genetic observations suggest that Rap1 and Rif1 compete for the same binding site on Taz1 as deletion of rap1+ leads to increased binding of Rif1 [7]. This apparent simple organization of the Taz1/Rap1/Rif1 complex still holds mysteries. For instance, do all three proteins have a uniform distribution along the telomere, through the cell cycle or between different telomeres? How does the interplay between Rap1 and Rif1 binding work? And if residual levels of Rap1 and Rif1 bind telomeres lacking Taz1, how does this occur and does it depend on telomeric chromatin or perhaps the nearby single-strand telomere binding complex? Finally, how does the balance of Taz1, Rap1 and Rif1 change during telomere shortening and what are the consequences of these changes?

### 2.2. An outsider: the telomerase RNA

Telomerase adds terminal sequences by reverse-transcribing its own RNA template. While the fission yeast telomerase catalytic protein subunit (Trt1) was one of the first to be identified, the RNA resisted discovery for a surprisingly long time. Recently, a pair of groups broke this blockade. The Zakian group developed a two-step purification method involving immunoprecipitation (IP) of the catalytic subunit of telomerase followed by a second IP of the expected trimethyl-guanine (TMG) cap of the RNA. RT-PCR using primers containing the reverse complement (UAACCG) of the minimal common sequence of the degenerate fission yeast telomerase repeats allowed identification of the telomerase RNA, TER1. The Baumann group concomitantly utilized a similar biochemical approach to purify RNAs that associate with Trt1. Like its budding yeast orthologue (TLC1), TER1 is a long RNA (1213 nt compared to 450 for human hTER) and mediates the interaction of Trt1 and Est1 [26,27].

As previously mentioned, *S. pombe* telomeres are characterized by a mostly invariant 5’-GGTTAC-3’ core repeat, interrupted by short stretches of guanosine varying in length. Mutational analyses of the TER1 template unveiled variation in the register in which TER1 realigns each time telomerase translocates between cycles of copying the 3’-CCAUGT模板, promoting heterogeneity between the 5’-GGTTAC-3’ repeats [26]. Moreover, the two adjacent C residues in the template are subject to slippage, allowing formation of variable-length G-stretches [26,27]. These G stretches are important for proper telomeric protein binding [5,27].

Characterization of TER1 maturation revealed an unexpected role for the spliceosome in telomerase biogenesis. Several species of TER1 can be detected in cells, ~5% of them representing polyadenylated transcripts longer than the most abundant, mature form that associates with Trt1. The precursor TER1 transcript contains an intron which, if present in an mRNA, would be excised by the spliceosome in two steps, a first step in which the intron 5’ end is cleaved and ligated to the so-called splicing branch point (an
adenoSine usually located near the intron 3’ end), and a second in which its 3’ end is cleaved and the flanking exons are spliced. Intriguingly, the mature form of TER1 is instead generated by an incomplete splicing reaction in which only the first step occurs; indeed, splicing of the two TER1 exons generates an inactive form of TER1 that cannot support telomere maintenance. An unusually long distance from the splicing branch point within the TER1 intron to its 3’ splice site favours uncoupling of the first and second steps of the splicing reaction, a phenomenon that would appear detrimental if it occurred while splicing essential mRNAs but that could be exploited as a mechanism for regulating alternative splicing or non-coding RNA maturation [28].

3. Setting the scene for telomere protection

Taz1 and Pot1 coordinate the activities required for telomere protection, Fig. 1. Taz1 inhibits telomerase, so that taz1Δ telomeres are highly elongated [5]. At the same time, it promotes replication fork passage, so that telomere elongation in the absence of Taz1 is limited by compromised telomeric replication [29]; these two observations may be related in that compromised semi-conservative replication might stimulate telomerase activity (see below). Taz1 also limits telomeric resection, as excessive levels of telomeric HR might stimulate telomerase activity (see below).

In contrast, telomeric protection becomes lethally compromised in pot1Δ cells, which lose all telomeric repeats (along with several kb of the subtelomere; see below) and survive only by chromosome circularization. A recent report unveiled extensive telomeric 5’ resection as the proximate outcome of Pot1 loss. To circumvent the technical problem that telomeres are entirely lost by the time a pot1Δ colony is isolated, preventing elucidation of the events that precipitate this telomere loss, a temperature sensitive allele of pot1 (pot1Δ) was isolated. Inactivation of this allele results in rampant C-strand degradation followed by abrupt loss of the entire telomere in the first ensuing S-phase. Intriguingly, while taz1Δ telomeres are subject to inappropriate DNA repair reactions (NHEJ and HR), they remain refractory to checkpoint activation, while pot1Δ telomeres activate ataxia telangiectasia mutated and Rad3 related (ATR)-mediated cell elongation, suggesting that the persistence of Pot1 at taz1Δ telomeres is sufficient to prevent ATR from triggering cell cycle arrest even in the face of some aspects of the DDR [33] (considered further below).

4. Telomere replication and telomerase recruitment

It is self-evident that in order to provide the chromosome end protection required for genome stabilization, telomeres need to ensure their own complete replication. However, faithful telomere duplication is a complicated affair. Not only must chromosome end-replication contend with the classic end-replication problem inherent to the biochemical properties of DNA polymerases [34–36], but also resection ensues following replication. Moreover, despite being described as a major issue for telomere replication, the end-replication problem is not the only difficulty. When dealing with telomeres, the semi-conservative DNA replication machinery faces two additional sticky issues: the repetitiveness of telomeric sequences and barriers such as DNA binding proteins.
4.1. Replication of the bulk telomere

The semi-conservative DNA replication machinery synthesizes the vast majority of telomeric repeats [37]. Hence, problems in ‘conventional’ replication will impinge significantly on telomere maintenance. The G-richness of telomeric sequences favours the formation of higher order structures called G-quadruplexes, in which Hoogsteen base pairing among G residues confers a planar cyclic structure of very high stability, and several lines of evidence support the biological relevance of G-quadruplex structures [38,39]. These structures might represent a significant impediment to fork progression. Another well-described obstacle to fork progression is the presence of DNA-bound proteins. Notably, this property of replicating chromatin can be exploited in nature, as reflected by ‘programmed’ stalling as seen, for example, at the fission yeast RFB (Replication Fork Barrier) sequence, which mediates co-directionality between rDNA replication and transcription by promoting a polar arrest of the replication fork following recruitment of the proteins Sw1 and Sw3 [40]. To deal with obstacles to fork progression, replication factors are usually accompanied by helicases involved in higher order structure resolution or protein clearance. For instance, budding yeast Rrm3 helicase facilitates replication fork progression through telomeres [41], where the plethora of bound proteins was expected to create an obstacle to fork progression, an idea that has found experimental support in budding yeast [41,42]. However, in fission yeast, two-dimensional gel analyses of telomere replication revealed that on the contrary, the absence of Taz1, replication forks stall at telomeric repeats. Hence it is the naked telomere sequences that impede fork progression, and this is alleviated by Taz1 binding. This role is unique to Taz1, as deletion of rap1" does not impede fork progression, despite the telomere being dysfunctional. Stalled forks also accumulate at internally located telomere sequences lacking Taz1, regardless of whether the G-rich strand is replicated by the leading-strand or the lagging strand machinery. Hence, Taz1 stood as the first example of a DNA binding protein that instead of opposing fork progression facilitates it. This counter-intuitive function for a DNA binding protein has recently been reinforced by observations in mammalian cells, in which the absence of TRF1 leads to the appearance of fragile-site phenotypes at telomeres. Single-molecule (DNA combing) analyses show that TRF1 prevents replication fork stalling, a function that may be mediated by the recruitment of the helicases BLM and RTEl1 as shRNA of either induces fragile telomeres in a manner that appears epistatic with deletion of TRF1 [43]. The stalled replication forks at taz1.A telomeres trigger a series of phenotypes that may illuminate general telomere maintenance mechanisms. First, despite their increased length compared to wt, taz1.A telomeres are lost immediately upon trt1" deletion, in sharp contrast to wt cells, which exhibit the incremental telomere shortening expected for the end-replication problem [29]. This catastrophic telomere loss reflects the inability of the semi-conservative replication machinery to reach the ends of taz1.A telomeres. Second, taz1.A telomeres show elevated levels of recombination, both in a trt1" and a trt1.A context. In the former, hyper-recombination is evinced by restriction pattern instability in the subtelomeric region [44], and in the latter, it results in the emergence of so-called ‘linear survivors’ in which variable-length telomeres are constantly replenished through recombination (see below). Third, taz1.A telomeres become entangled at cold temperatures and fail to segregate properly at mitosis [45]. A distinguishing feature of these phenotypes is that, like the presumably causative replication defect itself, Rap1 is dispensable for preventing them.

Intriguingly, the activity of the fission yeast RecQ helicase, Rqh1, promotes all of these deleterious phenotypes notwithstanding its often-reported positive role in promoting telomere mainte-

nance and inhibiting recombination. Furthermore, sumoylation of Rqh1, while having no discernable effect on its non-telomeric functions, promotes its helicase activity at taz1.A telomeres; removal of either Rqh1 sumoylation or Rqh1 helicase activity suppresses the hyper-recombination, entanglement and abrupt loss upon trt1" deletion seen at taz1.A telomeres. The mechanism by which sumoylated Rqh1 affects these telomeres remains unknown but several scenarios can be imagined. For instance, sumoylation might target Rqh1 towards the unwinding and dissolution of stalled telomeric replication forks. Notably, Rqh1 activity is also the culprit underlying the catastrophic telomere loss seen in cells harboring both mutated RPA (rad11-D223Y) and taz1" deletion [46], again highlighting the need for a robust telomere complex to restrain RecQ activity at chromosome ends.

Notably, the entanglement of taz1.A telomeres seen at cold temperatures is also suppressed by a mutated allele (top2-191) of the gene encoding DNA topoisomerase II (Top2; [45,47]). Top2 is a widely conserved homodimeric enzyme that controls DNA topology by creating transient double strand breaks (DSBs) and promoting DNA strand passage through these DSBs. Intriguingly, the suppression of taz1 cold sensitivity afforded by top2-191 is genetically dominant, indicating that the mutated enzyme gains a function that allows it to promote viability with greater efficiency than wt Top2; furthermore, this activity can be conferred by mutated Top2-191 molecules that lack DNA decatenation activity. In vitro biochemical characterization of Top2-191 showed that the ‘191” mutation slows down the catalytic cycle, prolonging the lifetime of catalytic intermediates in which DNA is ‘embraced’ by Top2; indeed, other mutations that stabilize this ‘closed clamp’ Top2 intermediate also suppress taz1.A telomeric entanglements [47]. Hence, Top2 has a non-canonical activity that promotes telomere stability, perhaps by stabilizing catenanes behind stalled telomeric forks, in turn allowing the cell to detangle the unreplicated region without building up excessive superhelical stress. Disentangling Top2’s activities at telomeres will shed light on the molecular nature of telomeric entanglement.

4.2. A paradox…

A requirement of replication fork passage through a given telomere for telomerase recruitment at that telomere has been established for a long time. Nevertheless, despite the obvious fact that all telomeres sustain replication forks, seminal studies in budding yeast established that telomerase acts only on a small sub-fraction of telomeres in a given cell cycle – the shortest telomeres in the cell [48]. The recognition of short telomeres as preferred telomerase substrates requires that these short telomeres are, to some extent, recognized as DNA damage [49]. Accordingly, the PI3 kinases Tel1 (fission yeast ataxia telangiectasia mutated; ATM) and Rad3, which are crucial to DNA damage recognition, play redundant essential roles in telomere maintenance as even in presence of telomerase, a rad3.Atel1.A strain loses telomeres immediately and survives only via chromosome circularization [50]. This dramatic result suggests that telomerase activation requires either Rad3 or Tel1. Hence, telomere maintenance models must encompass the paradox that partial activation of the DDR, so potentially dangerous at chromosome ends, is required for telomerase engagement.

The Nakamura group developed a rapid plasmid loss assay that allowed investigation of the early events prompted by rad3” or tel1” deletion. Chromatin immunoprecipitation experiments revealed a decrease of telomere bound Top2 and Ccq1 upon loss of Rad3 and Tel1 [51]. As Ccq1 interacts with Trt1 and ccq1.A cells fails to recruit telomerase [19], control of telomeric Ccq1 recruitment stands as a straightforward idea for the lack of telomere maintenance in rad3.Atel1.A cells. Along with the identification of Pot1 associated factors as proteins whose telomeric abundance
is controlled by the PI3 K kinases, Ishikawa’s group invoked a regu-
latory model based on feedback interactions between distinct
telomeric sub-complexes composed of Pot1-Tpz1-Ccq1 and Taz1-
Rap1. According to this model, at long telomeres, a high local con-
centration of Taz1/Rap1 promotes interactions with Pot1/Tpz1/
Ccq1 via Pzo1, leading to a “closed” conformation disfavoring telo-
merase recruitment. The “closed” conformation could imply a
higher order structure of telomeres, but this has not been thor-
oughly addressed yet. At short telomeres, the concentration of
bound Taz1/Rap1 decreases, reducing the potential for interactions
with Pot1/Tpz1/Ccq1. This would favor an “open” telomere state
and allow recruitment of telomerase via Ccq1. Together, these
observations suggest that Rad3 and Tel1, by controlling the
amount of bound Pot1-Tpz1-Ccq1 complex, allow telomeres to
oscillate between an extendible and non-extendible state [18,51].

4.3. Is replication fork stalling a necessary evil?

The model presented in the previous section suggests that a
counting mechanism based on protein-complex concentration
controls telomerase accessibility. While such a model may explain
the preference of telomerase for short telomeres, a second mecha-
nism may augment or mediate the effects of protein concentration.
Observations from our lab suggest that replication fork stalling at
taz1Δ telomeres can generate an excellent substrate for telomerase
recruitment. According to this idea, as wt telomeres shorten, they
lose the ability to recruit sufficient levels of Taz1 to ensure smooth
replication fork passage. The resulting stalled forks, or the products
of their processing, would trigger telomerase activity (Dehé, Rog,
Ferreira & Cooper, in preparation).

4.4. A question of timing

Telomere replication necessitates a tight interplay between rep-
lication machinery, checkpoint activation, resection and telomerase
recruitment. A key question is how does the cell choreograph these
events? Most of our knowledge on cell cycle regulated dynamics of
telomeric factors comes from the tremendous work carried out in
budding yeast, but recently, this issue has also been raised in fission
yeast. The Nakamura group confirmed the coupling between replica-
tion fork arrival at telomeres and telomerase recruitment [52,53].
Surprisingly, however, the leading strand polymerase (Polɛ) appears
to arrive at telomeres significantly earlier than the lagging strand
polymerases (Polɛ/Polδ). Such an uncoupling between the timing
of leading and lagging strand replication would be expected to result
in ssDNA at S-phase telomeres, and the accumulation of RPA and the
DNA damage factor Rad26, both of which bind ssDNA, prior to lag-
ning strand telomeric replication, reinforces this possibility. RPA
and Rad26 could further stimulate local resection activities, as could
a general displacement of telomere binding proteins during telo-
mere replication [52]. The coordination of all these events should be-
come clear in the near future.

5. After the end is gone, a matter of survival

Deletion of fission yeast trt1Δ leads to a senescence phenotype
reminiscent of those found in most eukaryotes; chromosome ends
suffer critical attrition at ~100 generations, when telomeres, along
with their ability to shield chromosome ends from full-blown DDRs,
are lost [54]. Senescent cells elongate, as is characteristic of fission
yeast checkpoint activation, and deprotected ends undergo inter-
and intra-chromosomal fusions that are detrimental for cell viabil-
ity. Nonetheless, as is the case in all organisms studied including
telomerase-negative human cancer cells, survivors arise. Crucially,
the types of survivors that emerge depend on the selection protocol
used, and can comprise one of at least three distinct survivor types,
one in which chromosomes circularize, one in telomeres are main-
tained by recombination, and one in which canonical telomeres
are replaced by tracts of non-telomeric heterochromatin, Fig. 2.

5.1. Circular survivors

After successive re-streaking for single colonies on plates, trt1Δ
cells display intra-chromosomal fusions associated with loss of the
telomere and at least 4–7 kb of subtelomeric sequences (STE). This
so-called circular mode of survival is a fission yeast particularity
reflecting the high statistical chance for a cell with only three chro-
mosomes to sustain three circularizations without forming lethal
interchromosomal fusions. While NHEJ had been the assumed
pathway for these fusions, this idea was challenged by the observa-
tion that they can occur in the absence of NHEJ pathway compo-
nents [55].

A recent study shed light on the mystery of NHEJ-independent
chromosome end-fusions by probing the mechanism of chromo-
some circularization of pot1Δ cells (which lose the ability to en-
gage telomerase as well as protection from 5′ resection; see above).
While pKu70, Lig4, Rhp51, MRN and Rad3 are all dispens-
able for the formation of pot1Δ circular survivors, Rad22 (Rad52
ortholog), Rqh1, Rad16 (XPF/ERCC4 ortholog) and Swi10 (DNA re-
pair endonuclease) are all required. These observations exclude a
requirement for NHEJ for pot1Δ survival and suggest the involve-
ment that they can occur in the absence of NHEJ pathway compo-
nents [55].

One of the key questions is whether the telomere attrition in cells
harboring other alterations of the shelterin complex, such as in
taz1Δ, taz1Δ/Δ, rad22Δ and rad1Δ cells (which lose the ability to en-
gage telomerase as well as protection from 5′ resection; see above),
occurs. Strikingly, deletion of rad16Δ compromises the survival of
trt1Δ cells as well, suggesting that SSA is the major pathway of end-fusion
not only in the absence of Pot1 but in any telomere loss setting
[55]. Therefore, one can ask if NHEJ plays any role in circulariza-
tion, as it does in the telomere fusions formed in G1-arrested taz1Δ
and rap1Δ cells? Cells undergoing telomere attrition upon trt1Δ
or pot1Δ deletion retain a largely G2 cell cycle, providing a likely
explanation for the NHEJ-independence of the fusions. Indeed,
when pot1Δ/Δ taz1Δ/Δ diploids are sporulated, the intra-chromo-
somal fusions of pot1Δ taz1Δ spores contain telomeric repeats,
arguing that they are products of NHEJ [55], as might be expected
as the meiosis that precedes sporulation encompasses a G1-ar-
rested state, during which the taz1Δ/Δ background will result in
NHEJ-mediated fusions [32,56]. Presumably, SSA is blocked by the
presence of Pot1 at taz1Δpot1Δ telomeres, while NHEJ is not.
It will be informative to analyse the genetic requirements of taz1Δ
pot1Δ fusions with and without G1-arrest situations to test these
ideas and decipher the rules dictating how fusions arise from dif-
ferent types of telomere dysfunction.

Chromosomal circularizations has also been observed after telo-
mere attrition in cells harboring other alterations of the shelterin
complex, such as in taz1Δ, pozlΔ ccq1Δ, rad3Δtel1Δ and
rad3ΔMRNΔ cells [18,50]. We suppose that what all these cells
have in common is a de-regulated ability to restrain end resection
along with an inability to engage telomerase for compensatory
telomere synthesis, perhaps in some cases secondary to an inabil-
ity to recruit Pot1.

5.2. Linear survivors

Linear survivors arise at variable rates in liquid culture, where
their faster growth allows them to out-compete the more frequent
circular survivors. The ‘linears’ have been difficult to analyse genetically as they often spontaneously circularize their chromosomes. However, while crisis occurs more rapidly in \textit{taz1}^{D} \textit{trt1}^{D} cells than in \textit{trt1}^{D} single mutants, the absence of Taz1 allows the maintenance of stable linear chromosomes in those cells that do survive [57], presumably because the stalled forks which accumulate at \textit{taz1}^{D} telomeres trigger recombination [29,44]. The Nakamura group has used these stable linear survivors to dissect the mechanisms by which they maintain telomeres. Linear chromosome maintenance in \textit{taz1}^{D} \textit{trt1}^{D} survivors depends on the Rad22 recombinase and fits with the role of Taz1 as an inhibitor of telomeric recombination in the presence or absence of telomerase [44]. Correspondingly, Taz1 reintroduction results in circularization of linear \textit{taz1}^{D} \textit{trt1}^{D} chromosomes. The Rad22 recombination-based \textit{taz1}^{D} \textit{trt1}^{D} survival mechanism displays similarities with budding yeast type II survival, as both require the MRN complex and Tel1 [57].

The unexpected newcomer in our conception of control of the recombination-based mechanism is none other than telomerase itself. The Nakamura group found that introduction of catalytically inactive Trt1 (Trt1^{D743A}) prompts \textit{taz1}^{D} \textit{trt1}^{D} linear survivors to circularize their chromosomes. Est1 is required for this circularization, suggesting that it requires \textit{bona fide} recruitment of Trt1^{D743A} to telomeres [57]. How Trt1^{D743A} inhibits telomere recombination is still not understood. The idea of a protective role for telomerase (in addition to its role in telomere repeat addition) has been proposed in the past, and while these observations on Trt1^{D743A} may reflect such a role, the catalytically dead Trt1 may also be exerting a dominant negative effect, perhaps binding in a non-physiological manner to telomeres due to loss of the translocation activity that may require the catalytic cycle. These issues can be resolved with experimental tests of genetic dominance and/or biochemical tests of the properties of Trt1^{D743A}. Notably, \textit{taz1}^{D} telomeres are subjected to increased levels of recombination in the presence of wt telomerase [44], arguing against a simple model in which Trt1 inhibits recombination.

Curiously, Rap1 also appears crucial for linear survival, as \textit{taz1}^{D} \textit{trt1}^{D} \textit{rap1}^{D} chromosomes circularize. This observation again raises the question of how Rap1 promotes \textit{taz1}^{D} \textit{trt1}^{D} telomere recombination if the telomeric recruitment of Rap1 requires Taz1? As mentioned above, Rap1 foci are present at a fraction of \textit{taz1}^{D} undergoing meiosis [8]. The newly characterized Pot1/Tpz1 complex might hold the solution, as residual Poz1 could potentially retain some Rap1, whose presence might even reinforce the stabil-
ity of the Pot1 complex, in turn allowing linear chromosome maintenance and explaining the rap1Δ effect. Indeed, the mysteries underlying Rap1 deepen as a positive role in telomeric recombination seems in contradiction with mammalian RAPI, recently shown to suppress recombination-mediated telomeric sister chromatid exchange.

Ccq1, which is directly involved in telomere maintenance via its telomerase recruitment function, also plays a role in suppressing telomeric recombination. ccq1Δ deletion leads initially to telomere attrition following similar kinetics to those seen in trt1Δ cells. However, unlike the latter, ccq1Δ cells maintain moderately short telomeres in a manner reminiscent of budding yeast type I survivors, utilizing recombination of subtelomeric repeats that preserve a short terminal telomere stretch. The genetic requirements for ccq1Δ telomere maintenance suggest a mechanism involving resection by either Rad50 or Exol1 followed by Rhp51/Rhp54-mediated, but Rad22-independent, strand exchange. This recombination depends on the G2/M DNA damage checkpoint and notably, ccq1Δ cells elongate after 4 days whereas trt1Δ cells only elongate after 7 days – i.e., ccq1Δ telomeres activate the checkpoint at longer lengths than those which trigger checkpoint activation in trt1Δ telomeres. Hence, Ccq1 is involved at the delicate coordination between telomerase recruitment, which is promoted by ATR activity, with simultaneous suppression of the entire ATR-mediated cell cycle arrest pathway [19], Fig. 1.

5.3. Surviving like a fly

A third type of trt1Δ survivor, indeed the most frequent type seen under competitive growing conditions, was first recognized via its distinct level of sensitivity to DNA damaging agents. While circular trt1Δ survivors are exquisitely sensitive to these agents, linear trt1Δ survivors are as resistant as wt; the third survivor type, dubbed HAATI (heterochromatin amplification-mediated and telomerase independent), shows an intermediate level of (DSB) sensitivity. Extensive gel electrophoretic analysis demonstrated that HAATI cells retain linear chromosomes but are devoid of terminal telomeric repeats. The structure of HAATI chromosomes was initially difficult to ascertain, as they share with circular chromosomes an inability to enter gels; the terminal fragments of HAATI chromosomes also fail in gel entry, as they sustain a persistent secondary structure (most likely the continual presence of Rhp51/Rad50-dependent recombination intermediates) that prevents gel migration. However, while reintroduction of Trt1 to circular strains fails to affect Chr I or II, it confers telomere addition and gel entry to all HAATI chromosomes, allowing analysis of their genomes. This analysis revealed a striking level of genomic reorganization in HAATI cells, manifest by two alternate patterns: In the most frequent HAATI subtype, the rDNA spreads from the subtelomeric regions of Chr III to the termini of all three chromosomes; strikingly, 3' overhangs of rDNA sequence are detectable at these cells' chromosome ends. In the much less frequent HAATI subtype, the rDNA remains restricted to Chr III but STE repeats spread from the subtelomeric regions of Chr I and II to multiple sites scattered throughout all three chromosomes. Consistent with the heterochromatic nature of rDNA and STE sequences, HAATI survival requires the heterochromatin assembly machinery.

Remarkably, despite the absence of canonical telomeres in HAA-

T1 cells, Pot1 is crucial for HAATI formation and maintenance. This feature, along with further genetic and structural analysis of HAATI chromosomes, suggests the following model for HAATI survival: the central feature of this survival mode is the constant expansion and contraction of heterochromatic (usually rDNA) repeats at each chromosome end. This constant rearrangement insulates the rest of the chromosome from the end-replication problem, while at the same time contributing to Pot1 recruitment. Pot1 is recruited both through interactions with terminal heterochromatin (via the heterochromatin binding complex SHREC, which binds Ccq1 and in turn, Pot1) and through interactions with the (non-telomeric) 3' overhangs at HAATI chromosome termini. In turn, Pot1 protects HAATI chromosome ends from rampant 5' resection. Reintroduction of Trt1 confers telomere addition as HAATI chromosomes do harbor 'ends' on which Trt1 can act. Once telomeres are added to HAATI chromosomes, their genomes become stable indefinitely, regardless of the extent to which rDNA (or STE) sequences have changed locations relative to wt cells [58].

Intriguingly, this epigenetic mechanism of telomere independent telomere maintenance is reminiscent of the approach used by Drosophila melanogaster to maintain linear chromosomes. Flies lack telomeric repeats and while retrotransposons are often found at their chromosome termini, they are dispensable for chromosome end maintenance. Nonetheless, while no specific sequence is required, Drosophila chromosome ends are packaged into heterochromatin which interacts with specific and essential end-protection factors, much like the ability of 'generic' heterochromatin to interact with Pot1 in HAATI cells. The conservation of such a mechanism between fission yeast and flies would suggest that it represents a universal, 'stripped down' solution to the end-replication problem, and may even contribute to the immortality of telomerase-minus human cancer cells.

6. Let’s talk about sex

Meiosis is no less than the key mechanism driving evolution and promoting genetic diversity. Meiosis compensates for the genome doubling that occurs at fertilization by halving the diploid number of chromosomes. This occurs through a single round of replication, pairing and recombination between homologs and two successive divisions. A conserved feature of the early stages of meiosis is the gathering of all telomeres at the nuclear envelope, usually next to the centromere, in a polarized arrangement in which the gathered telomeres resemble bundled flower stems and was therefore called the “bouquet” by early 20th century cytologists. The bouquet stage coincides with the pairing of homologous chromosomes, and therefore was proposed to facilitate the homology search. Fission yeast has a particularly long and striking bouquet stage and is the first organism in which mutations disrupting bouquet formation were available, thus standing as a windfall for studies of this highly conserved structure.

Haploids of opposite mating type undergo sexual differentiation, mating, and meiosis, ultimately forming an ascus that contains four haploid spores when exposed to nitrogen starvation. A complex series of chromosome movements takes place during this process. The SPB (fission yeast centromere) co-localizes with centromeres during mitotic interphase [59] but upon sexual differentiation (pheromone stimulation), telomeres gather next to the SPB. Following mating, the two haploid SPBs fuse and the centromeres separate from the SPB, leaving the telomere bouquet as the only link between chromosomes and SPB [60]. Bouquet formation is mediated by Taz1 and Rap1 as well as the heterochromatin associated factors Rik1 and Clr4 [8]. Furthermore, two meiosis specific proteins, Bqt1 and Bqt2, bridge the SPB (via Sad1) with the telomere bound Taz1/Rap1 complex and two non-meiosis specific proteins, Bqt3 and Bqt4, are required for telomere-nuclear envelope interactions through both mitotic and meiotic cell cycles. During meiotic prophase, the SPB and the associated bouquet are pulled back and forth by cytoplasmic microtubules, dragging the chromosomes via their telomeres into an elongated shape referred to as the “horsetail” [60]. During the horsetail stage, Rec12-mediated DSBs promote meiotic recombination [61]. The horsetail movements cease before the first meiotic division (meiosis I) and the
bouquet dissociates concomitantly with SPB division [62]. Following insertion of the SPBs into the nuclear membrane, a bipolar spindle is generated between them, allowing segregation of the homologs (reductional division) and then sister chromatids (equal division; meiosis II).

Despite the traditional view assigning a role for the bouquet in meiotic homolog pairing and recombination, mutants disrupting the bouquet only show mild recombination defects. Strikingly, however, bouquet-deficient strains suffer a severe SPB defect at meiosis I and fail to form proper spindles. Indeed, in the absence of the bouquet, monopolar, unstable, misplaced or multiple meiotic spindles are formed, in turn generating chromosome missegregation. Thus, the telomere plays a prominent role in triggering proper SPB duplication and spindle formation [62]. These observations suggest unanticipated mechanisms by which telomeres in particular, and perhaps heterochromatic regions in general, have a profound impact on spindle organization.

7. Conclusions

Fission yeast research has made a number of unusual contributions to the telomere field over the last several years, both augmenting our understanding of classical telomere questions like how to protect chromosome ends and control telomerase, and raising more new-wave questions. For instance, how many ways can we survive without telomeres, and what roles might telomeres play that have barely been realized yet? We hope and expect that the fission yeast telomere field will expand and continue to contribute to our understanding of telomere biology in health and disease.

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References