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Review article

Stretching, scrambling, piercing and entangling: Challenges for telomeres in mitotic and meiotic chromosome segregation

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A B S T R A C T

The consequences of telomere loss or dysfunction become most prominent when cells enter the nuclear division stage of the cell cycle. At this climactic stage when chromosome segregation occurs, telomere fusions or entanglements can lead to chromosome breakage, wreaking havoc on genome stability. Here we review recent progress in understanding the mechanisms of detangling and breaking telomere associations at mitosis, as well as the unique ways in which telomeres are processed to allow regulated sister telomere separation. Moreover, we discuss unexpected roles for telomeres in orchestrating nuclear envelope breakdown and spindle formation, crucial processes for nuclear division. Finally, we discuss the discovery that telomeres create microdomains in the nucleus that are conducive to centromere assembly, cementing the unexpectedly influential role of telomeres in mitosis.

The essentiality of telomeres for the duplication and segregation of linear eukaryotic chromosomes has been known for decades (Szostak and Blackburn, 1982) as the problem of distinguishing chromosome ends from DNA double strand breaks (DSBs) was recognized in the 1930s (Muller, 1938; McClintock, 1941), and the end replication became apparent along with definition of the fundamental properties of the semi-conservative DNA replication machinery (Lingner et al., 1995; Olovnikov, 1973; Watson, 1972). The primary danger invoked by the spectre of telomere loss was that of chromosome end-fusions that form dicentric chromosomes that break at mitosis or cytokinesis, creating further unprotected chromosome ends and creating catastrophic genome instability. This cycle of instability is known as the breakage-fusion-bridge or BFB cycle. Here we focus on recent studies that shine a spotlight on mitosis, illuminating surprising roles for telomeres in this crucial cell cycle denouement in which replicated chromosomes finally distribute to daughter cells.

Telomeres generally consist of tandemly repeated G-rich sequences terminating in a 3' overhang (Blackburn and Challoner, 1984; Henderson and Blackburn, 1989; Klobutcher et al., 1981) bound by a group of proteins collectively called Shelterin (de Lange, 2005). Human telomeres comprise the repeated hexanucleotide TTAGGG bound by six shelterin proteins, TRF1, TRF2, POT1, RAP1, TIN2, and TPP1; both the telomere sequence and shelterin components are widely conserved, with some variation, across eukaryotes (Rog and Cooper, 2008). Shelterin protects chromatin ends from being treated as deleterious double strand breaks (DSBs); hence, extensive 5' resection and end-fusion are both prohibited by Shelterin, as is the checkpoint activation triggered by DNA damage responses (DDRs). Shelterin also prevents erosion due to the end replication problem by recruiting and controlling telomerase, a reverse transcriptase equipped with a dedicated RNA subunit that templates the addition of telomere repeats to chromosome ends, replenishing sequences lost upon semi-conservative DNA replication. The essential role of telomerase-mediated telomere replenishment for continued cell growth has been confirmed in multiple systems, such as yeasts (Lundblad and Szostak, 1989; Nakamura et al., 1997), mice (Blasco et al., 1997; Lee et al., 1998), and human (Bodnar et al., 1998). Moreover, defective telomere maintenance is causative of human diseases, such as Dyskeratosis Congenita (DKC) (Mitchell et al., 1999; Vulliamy et al., 2004) and aplastic anemia (Vulliamy et al., 2002; Yamaguchi et al., 2005), where mutations in telomerase subunits or shelterin limit growth of highly proliferative tissues like bone marrow.

Normal human cells can only proliferate for a limited number of generations before entering senescence, a state of irreversible growth arrest when proliferative potential is exhausted (Hayflick and Moorhead, 1961). This limitation stems from the repression of telomerase (Nakamura et al., 1997; Meyerson et al., 1997; Kilian et al., 1997; Nakayama et al., 1998; Kim et al., 1994; Harley et al., 1992). Once telomeres erode to a threshold length, their ability to prevent DDR is lost and the cell cycle is arrested (Zou et al., 2004; d'Adda di Fagagna et al., 2003; Shay et al., 1991; Smogorzewska and de Lange, 2002). This telomere erosion-led senescence can be considered

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a tumor suppressive mechanism (Chin et al., 1999; Pereira and Ferreira, 2013); indeed, telomerase is re-activated in most cancers consisting of immortal cells (Nakamura et al., 1997; Meyerson et al., 1997; Kilian et al., 1997; Nakayama et al., 1998; Kim et al., 1994). At the same time, dysfunctional telomeres can also promote tumorigenesis (Blasco et al., 1997; Rudolph et al., 1999), as telomere loss or dysfunction increases mutation rate and chromosome re-arrangements (Hackett et al., 2001). In mammalian cells lacking telomere-mediated checkpoints to arrest proliferation, dysfunctional telomeres become a source of cancer-promoting genome instability (Chin et al., 1999; Maciejowski and de Lange, 2017; Artandi et al., 2000).

The BFB cycle originally described by Barbara McClintock places mitotic nuclear division as the moment of truth for parsing the outcome of telomere dysfunction. Here we discuss recent work that probes the timing and mechanism by which telomere fusions lead to chromosome breakage during BFB cycles. Moreover, additional roles of telomeres in governing mitosis have been revealed. Damage to telomeres during S-phase can lead to telomere entanglements that present a distinct set of challenges to the mitotic apparatus. Sister chromatid cohesion can persist at telomeres and lead to anaphase bridges. Prolonged mitosis can lead to telomere deprotection. Finally, during meiotic nuclear divisions, telomeres not only organize the homolog alignment needed for meiotic recombination and reductional chromosome segregation, but also actively promote both spindle assembly and centromere assembly. This review will focus on these emerging behaviors of telomeres, focusing on new insights into how telomeres coordinate or confound the climactic nuclear division stage of the cell cycle.

1. Ending up with two centromeres

1.1. Dealing with the aftermath of telomere fusions

Covalently ligated chromosome end-fusions are a prominent consequence of telomere loss or dysfunction (Lee et al., 1998; Hackett et al., 2001; Nakamura et al., 1998; van Steensel et al., 1998). Fusions can result from non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ) or single-strand annealing (SSA). NHEJ is the predominant pathway for repairing DSBs genomewide in the G1 phase of cell cycle (Ferreira and Cooper, 2004) and acts on unprotected telomeres during G1 (Mieczkowski et al., 2003; Heacock et al., 2004; Ferreira and Cooper, 2001; Smogorzewska et al., 2002). In S- or post-S-phase cells, Ku independent pathways requiring exposure of complementary single-strand DNA (ssDNA) dominate the end-fusion spectrum in fission yeast (Almeida and Ferreira, 2013). Similarly, MMEJ can mediate fusion of mammalian chromosome ends after telomere de-protection or attrition (Sfeir and de Lange, 2012; Rai et al., 2010; Jones et al., 2014). SSA involves annealing of longer tracts of ssDNA and occurs between telomeres of fission yeast lacking telomerase, as this organism spends most of its time in the G2 cell cycle stage in which NHEJ is rare (Wang and Baumann, 2008).

Despite end-fusions arising via different mechanisms, they generate dicentric chromosomes whose fate can follow two paths. If both centromeres attach to spindle microtubules from the same pole, the dicentric chromosome may segregate without undergoing breakage. In contrast, attachment of the two centromeres to opposite poles results in BFB events. A key question has been exactly what process results in chromosome breakage. For instance, the possibility that spindle elongation stretches the dicentrics to the point of scission is unlikely, as the forces generated by spindle elongation are too weak to sever the phosphodiester backbone (Houchmandzadeh et al., 1997; Nicklas, 1983). One challenge in deciphering the precise fate of fused dicentric chromosomes is that of visualizing them in the first mitotic division after fusion occurs.

The problems associated with inexact observation of the first mitosis following dicentric formation were solved using ingenious systems developed in budding yeast. Its centromeres are defined by a single CENP-A-containing nucleosome binding to a specific DNA sequence; this CENP-A binding can be rendered conditional by placing an inducible promoter immediately upstream of the centromere. Robust transcription inactivates the centromere, and this can be reversed by repressing the promoter; hence, the centromere can be activated and inactivated at will (Hill and Bloom, 1989, 1987). As predicted, visualization of the first mitosis following acquisition of a second active centromere on a single chromosome shows that the two centromeres on one chromosome attach to opposite poles in ~half the population (Thrower and Bloom, 2001). In the other half of the population, dicentric chromosome breakage frequently leads to recombinational excision of the second centromere (Hill and Bloom, 1989). This system was extended to study cells in which the Shelterin protein Rap1 is inactivated, leading to telomere fusions; the resulting dicentric chromosomes were stabilized by conditionally inactivating one centromere (Pobiega and Marcand, 2010). Upon reactivation of the conditional centromere, the fate of the dicentric chromosome was monitored. Surprisingly, dicentric chromosome breakage is a more controlled process than initially assumed. The dicentric chromosomes derived from end fusions often break at the telomere fusion sites, restoring the parental chromosomes. This 'fragility' of the fused telomere sequences, in inverted orientation, may offer a safeguard against accidental fusions (Pobiega and Marcand, 2010). Fusions between nontelomeric sites were also induced by transforming cells with fragments in which DNA from two different chromosomes flanked a selectable marker. In this case, breakage of the dicentric preferentially occurs within 25- to 30-kb of either of the two centromeres (Lopez et al., 2015). While such breakage is often lethal, it can result in deletion of one centromere, converting a dicentric chromosome to a stable monocentric chromosome (Lopez et al., 2015). In all cases, the breakages are not a result of spindle forces, as mitotic exit is required for the breakage; dicentric chromosomes persist in cells arrested in late anaphase. Indeed, breakage occurs only when the cells undergo cytokinesis, and requires the attendant actomyosin ring contraction (Lopez et al., 2015). Intriguingly, the centromeres are closely apposed with the centrosomes not only as they separate during spindle elongation, but also after spindle dissolution, when the chromosome bridges appear to confer a 'snapping-back' of the centrosomes towards the actomyosin ring; the localization of centromeres to this region may allow the cytokinetic furrow to exert pressure on the pericentromeres, leading to their breakage (Lopez et al., 2015). Consistently, a role for cytokinesis in breaking chromatin bridges was suggested in mammalian cells, in which chromatin bridges accumulate when cytokinesis is inhibited (Fenech, 2006; Janssen et al., 2011); positioning of chromosomes at the site of cytokinetic furrow ingression renders them likely to show DDR foci and undergo missegregation. Moreover, induction of ectopic kinetochore formation on a mammalian chromosome leads to a chromatin bridge whose breakage can be reduced by preventing cytokinesis (Gascoigne and Cheeseman, 2013).

Meanwhile, multiple mechanisms arise to contain or resolve dicentric chromosomes in various eukaryotes, allowing escape from their detrimental effects (Stimpson et al., 2012). Dicentric chromosomes can be stabilized through epigenetic inactivation of the kinetochore (Daniel, 1979; Earnshaw and Migeon, 1985) or through deletion of intercentromeric or centromeric sequences (Hill and Bloom, 1989; Jager and Philippsen, 1989; Page and Shaffer, 1998; Sullivan and Willard, 1998). These are likely the consequences of the genome instability imposed by BFB cycles (Pennaneach and Kolodner, 2009).

1.2. Chromothripsis: the shattering outcome of human telomere fusions

Insights into how and when dicentric chromosomes break in human cells experiencing telomere dysfunction have illuminated key