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Nuclear organization in DNA end processing: Telomeres vs double-strand breaks

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ABSTRACT

Many proteins ligands are shared between double-strand breaks and natural chromosomal ends or telomeres. The structural similarity of the 3' overhang, and the efficiency of cellular DNA end degradation machineries, highlight the need for mechanisms that resect selectively to promote or restrict recombination events. Here we examine the means used by eukaryotic cells to suppress resection at telomeres, target telomerase to short telomeres, and process broken ends for appropriate repair. Not only molecular ligands, but the spatial sequestration of telomeres and damage likely ensure that these two very similar structures have very distinct outcomes with respect to the DNA damage response and repair.

1. The double-strand break response

DNA double-strand breaks (DSB) are among the most deleterious types of DNA damage in the nucleus, particularly if an intact template is not immediately available for repair [1]. To ensure cell survival, DSBs trigger a conserved cascade of events called the DNA damage checkpoint, that arrests the cell cycle and stimulates repair [2]. The Ser/Thr PI3-like kinases ATM (Tel1 in the budding yeast, *Saccharomyces cerevisiae*) and ATR (Mec1 in *S. cerevisiae*) mediate the checkpoint signaling by phosphorylating other effector kinases, thereby promoting the recruitment of the repair machinery.

DSBs are repaired through two main pathways in eukaryotic cells. Non-homologous end joining (NHEJ) is active throughout the cell cycle and involves the direct religation of the broken ends [3], while homologous recombination (HR) is primarily active in S and G2 phases, and makes use of a DNA template for repair following the resection of DNA ends at the break. The ensuing single-strand DNA (ssDNA) overhang both mediates strand invasion and triggers checkpoint kinase activation to arrest the cell cycle.

Initially, DSBs are sensed by the Mre11–Rad50–Nbs1 (MRN) complex (Mre11–Rad50–Xrs2 or MRX in yeast), which rapidly localizes to DNA ends and promotes a short range 5′ end resection when it is stimulated by CtIP (Sae2 in yeast), a SUMO-E2 ligase activator [4]. MRN/MRX competes for end binding with the Ku70/80 heterodimer: the Ku complex limits ssDNA formation and the activity of resection proteins, and channels repair into NHEJ [5]. Conversely, the initial cleavage catalyzed by Mre11 together with Sae2 dismantles Ku binding, thereby promoting long-range resection. Ku has a much higher DNA binding affinity for blunt dsDNA ends than for short overhangs [6].

The ssDNA stretches created by MRX/Sae2 are coated by replication protein A (RPA) that promotes further resection by recruiting Exo1 endonuclease and/or a complex of Dna2–Sgs1 (DNA2-BLM in mammalian cells) [7–9]. The resected ssDNA filament bound by RPA recruits the ATR/ATRIP (Mec1/Ddc2 in yeast) kinase, and its activity is further stimulated by the 9–1–1 complex. The 9-1-1 complex binds the ss-dsDNA junction at resected breaks, and stimulates ATR/Mec1 through the BRCT-domain protein, TOPBP1 (or Dbp11 in yeast) [10,11]. RPA is later replaced by Rad51, forming a protein-ssDNA filament that is essential for homology search and invasion into the double-stranded template DNA.

In contrast to iATR/Mec1, the ATM/Tel1 kinase is recruited by the C terminus of Nbs1 (Xrs2 in yeast) to DSBs [12]. ATM/Tel1 is activated, phosphorylating itself and the C-terminal tail of histone H2AX (γH2AX or P-Ser129 in yeast histone H2A). ATM/Tel1 modifies other proteins, but γH2AX has the unique propensity to spread from the site of damage, providing a platform for the binding of many other DNA repair enzymes [13]. Whereas in mammals, ATM is specifically activated by DSBs, ATR also responds to replication stress and other lesions that generate ssDNA [14,15]. In budding yeast, the ATR homolog Mec1 plays a much more central role in DSB processing than Tel1 (the ATM homolog), possibly, because resection and ssDNA formation are more efficient in this organism [16]. Indeed, as its name indicates, yeast Tel1 yeast is largely

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specialized for functions at telomeres; its loss leads to constitutively short TG repeats at chromosome ends [17]. At telomeres, even when they are short, resection is suppressed and Tel1 activity leads to the recruitment of telomerase, as discussed below.

2. The problem passed by telomeres

The natural ends of eukaryotic chromosomes, or telomeres, expose a short protruding 3′ end, just like resected DSBs, although, telomeres contain repetitions of a TG-rich sequence, specifically $(TG_{1-3})_n$ in budding yeast, and TTAGGG in most vertebrates. The length of this repetitive region varies from 300 to 400 bp in yeast to ~15 kb in humans, and up to 100 kb in certain strains of mice. One of the main functions of telomeres is to ensure complete replication of chromosomes. Replication is discontinuous on the lagging strand and makes use of RNA primers, which are removed at the end of the polymerization process [18]. At chromosomes ends, this mechanism leads to a small loss of the terminal sequence each cell cycle, which would eventually lead to unique chromosomal DNA erosion. To compensate for this, a specialized replicative polymerase called telomerase acts at telomeres [19]. Telomerase is a protein–RNA complex whose RNA moiety serves as an internal primer that templates DNA synthesis at chromosomal ends. It is generally in very low abundance and its recruitment is highly regulated. On average, fewer than one telomere in 10 is elongated by telomerase each cell cycle [20], with shorter telomeres being favored, suggesting that there may be a switch from an inaccessible to an accessible structure prior to elongation. Nonetheless, telomerase is not only bound at short telomeres (Fig. 1).

A second crucial function of the telomere is to suppress the DNA damage response (DDR) and block the action of the repair machinery that would normally act on the terminal 3′ ssDNA overhang as a repair substrate. If DSB repair takes place at telomeres, cells undergo potentially lethal telomere fusions, translocations and genomic instability [21]. This is normally prevented by a set of proteins that have evolved to specifically recognize telomeric DNA, forming the telosome. The telosome is composed of a ssDNA binding component and a sequence-specific double-strand repeat binding component. In budding yeast, the former is the Cdc13–Stn1–Ten1 (CST) complex, while ds repeats are bound by Rap1, which in turn binds the Rap1-interacting factors, Rif1 and Rif2, as well as Sir3 and Sir4, two yeast-specific nucleosome-binding factors involved in gene silencing. Higher eukaryotes possess a single telosome complex called Shelterin that integrates both ss- and ds-DNA binding activities. In addition, mammalian telomeres form secondary structures called t-loops, by folding back of the 3′ overhang to anneals with upstream telomeric repeats and displace the TG-rich strand of the repeat DNA [22]. This may physically sequester the chromosome end from the DSB signaling machinery, at least until replicative polymerases pass through. The human TTAGGG-binding factor, TRF2, which similar to Rap1 has a Myb-like DNA binding domain, is required for the formation of such structures even in vitro [23].

In most species, telomeric repeat binding factors also nucleate a domain of repressed chromatin that spreads inwards from the chromosomal end. In yeast, the Rap1 binds the ds TG repeat roughly every 18 bp in a sequence-specific manner, and recruits Sir4 to nucleate SIR-mediated gene repression [24]. This requires at least 20 Rap1 binding sites, perhaps due to competition between Rif1/2 and Sir3/4 for binding the Rap1C-terminus [25]. This subtelomeric heterochromatin may help to distinguish telomeres from breaks, as heterochromatin tends to cluster in nuclear subcompartments, either adjacent to the nuclear envelope or around the nucleolus [26]. Cdc13, which binds the ssDNA at telomeres in a sequence-specific manner, further distinguishes breaks from chromosomal ends, as its binds resected TG ssDNA 40-fold more efficiently than random ssDNA at a resected break [27]. Cdc13 is a target of the Tel1 kinase (ATM), and its phosphorylation promotes telomere elongation through interaction with Est1 of the telomerase complex [28,29]. On the other hand, RPA, a similar trimeric complex that binds ssDNA with little sequence specificity, stabilizes the resected end and activates the Mec1/ATR checkpoint kinase.

3. Double-strand break repair proteins with telomeric functions

Interestingly, despite the presence of the telosome and its suppression of the DDR, a number of DNA repair proteins have a functional role at telomeres, as illustrated in Fig. 1. For example, the MRN/MRX complex is involved in telomere maintenance in both higher eukaryotes and yeast [30]. During the DSB response, Mre11 is one of the earliest sensors of damage, recruiting ATM/Tel1 as well as helping to activate ATR/Mec1, by initiating resection to form a ssDNA overhang. At telomeres, MRX preferentially binds short telomeres [27], yet there it may inhibit resection: in a *cdc13-1* mutant, in which telomeres are uncapped, MRX binding was proposed to hide the ends from other exonuclease activities [31] and possibly promote telomerase binding [32,33]. Consistently, mutations in any MRX subunit lead to constitutively short telomeres, just like deletion of *tel1* or yKu [34].

The roles of MRN at mammalian telomeres may be simpler. It was shown that functional telomeres are indeed perceived as DSBs in late S/G2 in mammalian cells, when they are replicated: at that point, ATM is transiently phosphorylated, although it does not activate Chk2 nor p53, and cell-cycle progression is not delayed. ATM can be recovered at telomeres with MRN, suggesting that a localized damage response is started at telomeres [35], resembling the association of Tel1 at short yeast telomeres bound by MRX [27]. Finally, an enzymatic assay based on nucleotide addition by terminal transferase showed that chromosome ends in S/G2 are accessible, confirming the disruption of the t-loop [35]. Thus, unpairing of the t-loop coincides with MRN binding, ATM activation and telomerase recruitment.

Ku is another conserved, end-binding protein that has a dual role in DSB repair and in telomere protection. At DSBs, it competes with Mre11 for the binding of DSBs immediately after break induction, and promotes repair by the NHEJ pathway, by recruiting ligase IV and other enzymes involved in end religation [36]. In budding yeast, yKu80 mutants display elevated rates of ectopic recombination [37] presumably because NHEJ is compromised. Yeast Ku also directly interacts with telomerase RNA and contributes to the recruitment of the enzyme to telomeres. Finally, the loss of yKu also leads to a physical release of telomeres from their sequestration by Mps3, a SUN domain protein of the inner nuclear membrane [38]. Indeed, Ku has multiple roles at telomeres, and yeast Ku (yKu) was among the first proteins shown to contribute to telomeric silencing, length maintenance and the spatial organization of telomeres [34,39,40].

In *S. cerevisiae*, telomeres cluster in 6–8 foci at the nuclear periphery [41] in a manner dependent on yKu80 and Sir4. The targeting of yKu80 to a randomly localized chromosomal locus was sufficient to shift it to the nuclear periphery [42], in a manner dependent on Mps3 and Est1 [38]. However, due to its interaction with Sir4, yKu also contributes to the nucleation of subtelomeric gene repression [34,39,40], which can be separated genetically from its anchorage function [42]. Finally, genetic screens have identified mutants [43,44] that separate the function of yKu in DSB repair from its roles at telomeres. This genetic approach showed that through binding Tlc1 (the RNA moiety of telomerase) yKu helps to recruit telomerase and suppress recombination. This latter may be aided by an *S*-phase sequestration of telomeres [38].