The DNA damage response at dysfunctional telomeres, and at interstitial and subtelomeric DNA double-strand breaks

Keiko Muraki¹,²* and John P. Murnane²

¹Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan
²Department of Radiation Oncology, University of California, San Francisco, 2340 Sutter Street, San Francisco, CA 94143-1330, USA

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In mammals, DNA double-strand breaks (DSBs) are primarily repaired by classical non-homologous end joining (C-NHEJ), although homologous recombination repair and alternative NHEJ (A-NHEJ), which involve DSB processing, can also occur. These pathways are tightly regulated to maintain chromosome integrity. The ends of chromosomes, called telomeres, contain telomeric DNA that forms a cap structure in cooperation with telomeric proteins to prevent the activation of the DNA damage response and chromosome fusion at chromosome termini. Telomeres and subtelomeric regions are poor substrates for DNA replication; therefore, regions near telomeres are prone to replication fork stalling and chromosome breakage. Moreover, DSBs near telomeres are poorly repaired. As a result, when DSBs occur near telomeres in normal cells, the cells stop proliferating, while in cancer cells, subtelomeric DSBs induce rearrangements due to the absence of cell cycle checkpoints. The sensitivity of subtelomeric regions to DSBs is due to the improper regulation of processing, because although C-NHEJ is functional at subtelomeric DSBs, excessive processing results in an increased frequency of large deletions and chromosome rearrangements involving A-NHEJ.

Key words: C-NHEJ, A-NHEJ, processing, rearrangements, subtelomere

DSB REPAIR MECHANISMS

The integrity of the genome is essential for cell survival. However, genomes are continuously threatened by DNA lesions that occur during cellular metabolism, such as DNA replication, and by external insults, such as ultraviolet or ionizing radiation (IR). DNA double-strand breaks (DSBs) are one of the most cytotoxic lesions and, if not repaired properly, can induce gross chromosome rearrangements, including chromosome loss, deletions, amplification and translocations. These rearrangements can activate oncogenes or cause the loss of tumor suppressor genes, and, therefore, drive cells to cancer. Furthermore, DSBs are sometimes generated in a programmed manner, such as meiotic recombination or immune cell development, including class-switch recombination and variable, diversity and joining (V(D)J) recombination. Cells are equipped with multiple mechanisms to repair DSBs. In mammals, classical non-homologous end joining (C-NHEJ), which is a sequence-independent repair pathway, is the primary DSB repair pathway (Fig. 1A). However, DSBs can also be repaired by homologous recombination repair (HRR), which is an accurate repair mechanism (Fig. 1C), or alternative NHEJ (A-NHEJ), which is commonly involved with deletions and chromosome translocations (Fig. 1D). These pathways compete with each other, and are tightly regulated in a cell cycle-dependent manner.

Repair of DSBs by C-NHEJ C-NHEJ is the primary DSB repair pathway in mammalian cells, and is involved in the repair of 95% of DSBs. C-NHEJ involves sequence-independent end joining, and is mediated by the KU70/KU86 heterodimer (KU complex, Fig. 1A). The KU complex binds to DSBs to protect the ends from nuclease digestion and to tether the ends to keep them in close proximity to facilitate repair. The KU complex then recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to phosphorylate downstream proteins. DNA-PKcs also associates with the nuclease Artemis to process ends prior to rejoining. The KU com-
plex subsequently recruits the DNA ligase IV-XRCC4 complex, which ligates the DNA ends together (Lieber, 2010a).

The function of 53BP1 in C-NHEJ In addition to the KU complex, p53-binding protein 1 (53BP1) is also rapidly recruited to DSBs and is involved in C-NHEJ. 53BP1-deficient mice are hypersensitive to IR (Morales et al., 2003; Ward et al., 2003). 53BP1 works as a molecular scaffold for the DNA damage response (DDR) in combination with RAP1-interacting factor 1 (RIF1), REV7/MAD2 mitotic arrest deficient 2-like 2 (MAD2L2) and Pax2 trans-activation domain-interacting protein (PTIP). 53BP1 prevents exonuclease-mediated resection of DSBs, which inhibits C-NHEJ, but is required for HRR-mediated repair (Bunting et al., 2010). 53BP1 also functions in both heterochromatin relaxation (Ziv et al., 2006; Goodarzi et al., 2008) (see below) and chromatin mobility at DSBs. This chromatin mobility function of 53BP1 is mediated in combination with the linker of nucleoskeleton and cytoskeleton (LINC) complex and microtubules (Lottersberger et al., 2015). The mechanism involved in the mobility of the ends of DSBs functions in C-NHEJ at dysfunctional telomeres (Dimitrova et al., 2008) (see below), class switch recombination (Manis et al., 2004; Ward et al., 2004; Reina-San-Martin et al., 2007) and long-range joining of V(D)J recombination (Difilippantonio et al., 2008). 53BP1 also plays a role in the synopsis of the two ends of the DSB, and can facilitate the joining of the ends of distantly formed DSBs, although the molecular mechanism for synopsis remains uncertain (Difilippantonio et al., 2008).

53BP1 is recruited to DSBs in multiple ways (reviewed by Panier and Boulton, 2014). 53BP1 recognizes monomethylated or dimethylated histone H4 lysine 20 (H4K20) using the Tudor domain (Pei et al., 2011). The Tudor domain of 53BP1 is also involved in the recognition of the methylation of histone H3 lysine 79 (H3K79) by Dot1L (Huyen et al., 2004). 53BP1 also recognizes the E3 ubiquitin ligase RING finger motif and FHA domain.
53BP1-binding protein RIF1 is involved in the protection of DSBs

RIF1 is recruited to DSBs and forms foci through its interaction with 53BP1 that has been phosphorylated at S/T-Q motifs by ATM (Chapman et al., 2013). RIF1 is involved in protecting DSBs from processing, and thus stimulates C-NHEJ while inhibiting HRR and A-NHEJ (Silverman et al., 2004; Chapman et al., 2013; Escribano-Díaz et al., 2013; Feng et al., 2013). As a result, the absence of RIF1 downregulates C-NHEJ and diminishes cell survival (Buonomo et al., 2009; Escribano-Díaz et al., 2013). This inhibition of processing by RIF1 may be due to the recruitment of REV7/MAD2L2. The recruitment of REV7/MAD2L2 to DSBs is dependent on H2AX, MDC1, RNF8, RNF168, RIF1 and ATP-dependent phosphorylation of 53BP1, but independent of PTIP. Like 53BP1, REV7/MAD2L2 is involved in the protection of DSBs from 5'-3' end resection, and promotes C-NHEJ (Boersma et al., 2015; Xu et al., 2015).

The binding of PTIP by 53BP1 has multiple roles at DSBs

PTIP, which is a subunit of the MLL3/4 histone methyltransferase complex and regulates gene transcription by controlling histone H3 lysine 4 (H3K4) trimethylation (Cho et al., 2007), is another 53BP1 effector protein that functions in the DDR (reviewed by Muñoz and Rouse, 2009). PTIP prevents resection at DSBs, and thereby prevents HRR and stimulates C-NHEJ. Deletion of PTIP promotes resection of DSBs and rescues the defect in HRR in the absence of breast cancer 1 (BRCA1) (Callen et al., 2013). However, another group has reported that PTIP functions in HRR (Wang et al., 2010).

The mechanism of PTIP recruitment to DSBs remains unclear. In human cells, PTIP is involved in 53BP1 recruitment to DSBs using two pairs of C-terminal BRCT domains of PTIP which bind to 53BP1 that has been phosphorylated at serine 25 by ATM (Muñoz et al., 2007; reviewed by Muñoz and Rouse, 2009). On the other hand, in mouse embryonic fibroblasts (MEFs), PTIP is recruited to DSBs through the interaction with phosphorylated 53BP1, and RIF1 and PTIP are recruited independently to DSBs using different phosphorylation sites of 53BP1 (Callen et al., 2013). Another group found that PTIP and 53BP1 are recruited to DSBs independently, although both proteins are recruited downstream of RNF8 (Gong et al., 2009). 53BP1-PTIP interaction is also required for phosphorylation of Chk2 and p53, which in turn phosphorylate, activate and upregulate many proteins in response to DSBs (Jowsey et al., 2004).

C-NHEJ requires ATM and KAP1 for repair of DSBs in heterochromatin

Although most DSBs are repaired within a few hours via C-NHEJ in an ATM-independent manner, about 10–15% of DSBs are repaired much more slowly (Riballo et al., 2004). Among these are DSBs located in heterochromatin, whose repair requires ATM-mediated phosphorylation of KRAB-associated protein 1 (KAP1, also known as TIF beta, TRIM 28 or KRP1-1) (Goodarzi et al., 2008). Phosphorylated KAP1 is accumulated at DSBs and induces the dissociation of CHD3 nucleosome remodeler from heterochromatin, which results in heterochromatin relaxation (Goodarzi et al., 2011). In the absence of KAP1 phosphorylation, DSBs in heterochromatin persist. On the other hand, KAP1 knockdown alleviates the requirement for ATM for repair of heterochromatic DSBs, indicating that the KAP1-mediated relaxation of heterochromatin is required for the repair of DSBs in heterochromatin (Ziv et al., 2006; Goodarzi et al., 2008; Shibata et al., 2011). The accumulation of phosphorylated KAP1 at DSBs, and KAP1-mediated DSB repair at heterochromatin, are also dependent on MDC1, RNF8, RNF168 and 53BP1 (Noon et al., 2010). Thus, heterochromatin that contains DSBs must be decreased in density and expanded for repair to occur (Kruhlak et al., 2006).

Some DSBs require the nuclease activity of Artemis for C-NHEJ to occur

Some ‘dirty’ breaks induced by IR require processing by Artemis for C-NHEJ; Artemis is recruited to DSBs by DNA-PKcs. High linear energy transfer radiation, such as alpha-particles or carbon ions, introduces complex clustered damage, which is repaired more slowly than most damage introduced with X or gamma rays. This complex clustered damage is Artemis-dependent, while etoposide-introduced damage does not require Artemis (Riballo et al., 2004; Shibata et al., 2011). Artemis was originally found to be involved in the cleavage of the hairpin intermediate generated during V(D)J recombination, which is defective in patients with radiosensitive severe combined immune deficiency (Moshous et al., 2001). Phosphorylation of Artemis by DNA-PKcs activates the nuclease activity of Artemis (Ma et al., 2002). Although ATM is not required for V(D)J recombination, it is required for Artemis-mediated processing of DSBs, indicating that the processing required for DSB repair and V(D)J recombination is differently regulated (Riballo et al., 2004).
MRE11- and BRCA1-mediated processing determines the repair pathway

When a DSB is not repaired rapidly by C-NHEJ, the MRE11-RAD50-NBS1 (MRN) complex binds to both ends of the DSB and thwarts them together (reviewed by D’Amours and Jackson, 2002). MRN also induces autophosphorylation of ATM at serine 1981, which activates the ATM kinase activity that is required for the DDR (Bakkenist and Kastan, 2003; reviewed by Kastan and Lim, 2000).

In addition to its role in binding to DSBs and activating ATM, MRE11 also possesses nuclease activity. This nuclease activity of MRE11 is stimulated by its binding to CtBP-interacting protein (CtIP), and functions in generating the short single-stranded 3' overhangs at DSBs that are required for HRR and A-NHEJ (Fig. 1B). Thus, the inhibition of CtIP causes cellular sensitivity to DSB-inducing agents, such as the topoisomerase I inhibitor camptothecin, the topoisomerase II inhibitor etoposide, and bleo (Sartori et al., 2007). The single-stranded DNA overhangs required for HRR are generated via two steps. First, in the processing step, the MRE11-CtIP complex removes small oligonucleotide(s) to generate a short protruding end. Next, in the resection step, exonuclease I (EXOI) generates a long single-stranded 3' overhang (Garcia et al., 2011; Shibata et al., 2014). The Sgs1/Bloom syndrome helicase (BLM) complex and BRCA1 are also involved in the generation of this long single-stranded 3' overhang (Moynahan et al., 1999; Bhattacharyya et al., 2000; Truong et al., 2013; reviewed by Moynahan and Jasin, 2010). The single-stranded DNA is then bound by replication protein A (RPA), which triggers ATR-dependent checkpoint signaling (Jazayeri et al., 2006; Sartori et al., 2007). RAD51 recombinase then displaces RPA on the single-stranded DNA to form a long nucleoprotein filament, which promotes strand invasion and recombination with the homologous sequence on the sister chromatid (Mimitou and Symington, 2008; Symington and Gautier, 2011). Because HRR uses the homologous template as a repair substrate, it is largely error-free (Fig. 1C).

Mutations in BRCA1 can cause predisposition to a high incidence of breast or ovarian cancers (Miki et al., 1994; Stratton et al., 1997; reviewed by Moynahan, 2002; Wooster and Weber, 2003). BRCA1 also associates with the MRE11-CtIP complex, stimulates the nuclease activity of MRE11 (Yu et al., 2006; Chen et al., 2008a; Huertas and Jackson, 2009; Yun and Hiom, 2009) (see below), and is involved in the removal from DSBs of RIF1, which protects DSB ends from processing. BRCA1 is therefore involved in RAD51 filament formation and stimulates HRR (Moynahan et al., 1999; Bhattacharyya et al., 2000; Huber et al., 2001; Schlegel et al., 2006).

MRE11-CtIP-mediated processing is also involved in A-NHEJ (Rass et al., 2009; Xie et al., 2009; Lieber, 2010b; Zhang and Jasin, 2011; Truong et al., 2013). A-NHEJ is an evolutionarily conserved mechanism that often uses short tracts of microhomology at the repair junctions, and is commonly involved in the formation of deletions and translocations (Fig. 1D; reviewed by McVey and Lee, 2008). A-NHEJ is therefore also called microhomology-mediated end joining or backup NHEJ. During A-NHEJ, DSBs are detected and stabilized by poly(ADP-ribose) polymerase 1 (PARP1) (Audebert et al., 2004; Wang et al., 2006), and the nuclease activity of MRE11-CtIP processes the ends to generate a short 3' overhang (Rass et al., 2009; Xie et al., 2009; Zhang and Jasin, 2011). DNA ligase III then joins the ends at sites with or without microhomology within the 3' single-stranded overhangs. DNA ligase I can substitute for DNA ligase III during A-NHEJ, although less efficiently (Simsek et al., 2011). A-NHEJ can occur without the extensive resection of DSB ends that is required for HRR, and therefore A-NHEJ is not dependent on BRCA1, EXOI or BLM (Yun and Hiom, 2009; Truong et al., 2013).

The choice between DSB repair pathways is cell cycle-dependent

The choice of which pathway is used for DSB repair is largely regulated by the competition between DSB end protection and end processing. C-NHEJ is the predominant repair pathway in G1 phase (reviewed by Symington and Gautier, 2011; Zimmermann and de Lange, 2014). Once a DSB is processed, it is no longer a substrate for C-NHEJ and must be repaired via HRR or error-prone A-NHEJ. The sister chromatid, which is used as the homologous template during HRR, is available only in S/G2 phase, and therefore processing in G1 phase leads to a loss of heterozygosity or chromosome rearrangements through the A-NHEJ pathway. Therefore, DSB end protection is critical in G1 phase, while processing of DSBs is restricted. As mentioned earlier, this end protection is mediated by binding of 53BP1, RIF1, REV7/MAD2L2 and PTIP. RIF1 interacts with 53BP1 and localizes to DSBs mainly in G1 phase, and blocks BRCA1 accumulation at DSBs (Chapman et al., 2013; Escribano-Díaz et al., 2013; Feng et al., 2013).

In S/G2 phase, in which sister chromatids are available, and therefore HRR is functional, MRE11-CtIP nuclease activity is upregulated (see below), and BRCA1-CtIP promotes resection by inhibiting the accumulation of 53BP1 and RIF1 at DSBs (Chapman et al., 2012a, 2013; Escribano-Díaz et al., 2013; Feng et al., 2013). HRR also has a key role in repairing DSBs that are generated during replication fork stalling (O’Driscoll and Jeggo, 2006), which also occurs during S phase. Therefore, choosing the appropriate repair pathway during the different stages of the cell cycle is important for preserving genetic integrity, and competition of RIF1 with BRCA1 and CtIP plays a pivotal role in determining which DSB repair pathway is used (reviewed by Symington and Gautier,
Cell cycle-dependent regulation of BRCA1 and CtIP  
CtIP phosphorylation at serine 327 and threonine 847 is performed by cyclin-dependent kinase (CDK) in S/G2 phase (Wang et al., 2013). CtIP serine 327, which is well conserved and phosphorylated when cells enter S phase, is required for CtIP stability, MRE11-CtIP-BRCA1 complex formation and DSB repair by HRR (Yu and Chen, 2004; Yu et al., 2006; Chen et al., 2008a; Huertas and Jackson, 2009; Yun and Hiom, 2009). Corresponding to the presence of CDK, the nuclease activity of the MRE11-CtIP complex is upregulated in S/G2 phase, when the sister chromatids are present (Jazayeri et al., 2006). However, the MRE11-CtIP complex can process DSBs for A-NHEJ in G1 phase independent of serine 327 phosphorylation or BRCA1, and CtIP depletion decreases cell survival when DSBs are generated in G1 phase, as well as in S/G2 phase (Yun and Hiom, 2009). CtIP threonine 847 phosphorylation is necessary for the localization of CtIP to DSBs and for the removal of RIF1 to allow processing, which stimulates HRR and A-NHEJ (Escribano-Díaz et al., 2013; Truong et al., 2013; Wang et al., 2013).  
MRE11-CtIP activity is also regulated at the protein level in a cell cycle-dependent manner. CtIP is present at low levels in G1 phase, increases during progression through S/G2 phase, and then undergoes proteasome-mediated degradation in the next G1 phase (Germani et al., 2003; Buis et al., 2012). The level of CtIP decreases in cells lacking MRE11, while the level of BRCA1 is not affected (Buis et al., 2012; Truong et al., 2013).  

DSB repair pathways compete with each other  
The KU complex stimulates C-NHEJ and prevents processing by MRE11-CtIP, thus preventing HRR or A-NHEJ. As a result, the inhibition of KU increases the frequency of HRR and A-NHEJ (Bennardo et al., 2008; Fattah et al., 2010; Bunting et al., 2012; Truong et al., 2013). Conversely, once the DNA ends are processed, they are no longer a substrate for KU complex binding or C-NHEJ. Thus, CtIP suppresses C-NHEJ via processing (Shibata et al., 2011). The KU complex also competes for DNA end binding with PARP1 (Wang et al., 2006), and the inhibition of KU rescues cellular sensitivity to PARP1 inhibition in BRCA1-deficient cells (Bunting et al., 2012) (see below). Processing and resection in the absence of KU is mediated by MRE11-CtIP, and therefore the inhibition of MRE11 abrogates the increase in HRR and A-NHEJ normally seen in KU-deficient cells (Truong et al., 2013).  
In response to DSBs, 53BP1-RIF1 is recruited to the damage sites, and protects the ends from 5' processing in a cell cycle-dependent manner. Thus, 53BP1 also plays a pivotal role in determining repair pathway choice by stimulating C-NHEJ, and suppressing HRR and A-NHEJ (reviewed by Zimmermann and de Lange, 2014). In the absence of 53BP1, the efficiency of HRR (Bunting et al., 2010) and A-NHEJ (Bothmer et al., 2010) is increased. When multiple DSBs exist, C-NHEJ can promote misrepair by joining incorrect ends, forming toxic chromosome aberrations, including radial chromosomes. In the absence of BRCA1, 53BP1 inhibits HRR and is involved in the formation of radial chromosomes when cells experience multiple DSBs in S phase. When both BRCA1 and 53BP1 are absent, the loss of 53BP1 allows DSBs to be processed by CtIP, which promotes HRR and prevents radial chromosome formation (Cao et al., 2009; Bouwman et al., 2010; Bunting et al., 2010).  
Once processing is initiated, the single-stranded DNA overhang that has been generated must be repaired via HRR or A-NHEJ. This may explain why HRR-deficient cells are sensitive to the inhibition of PARP1, which is required for A-NHEJ. Therefore, PARP1 inhibitors, such as orpharib, can be used for treatment of BRCA1-negative breast and ovarian cancers (Farmer et al., 2005; Fong et al., 2009). Conversely, the loss of 53BP1 rescues the sensitivity of BRCA1-deficient cells to PARP1 inhibitors (Bouwman et al., 2010; Bunting et al., 2010; Chapman et al., 2012b). However, although the loss of 53BP1 can rescue HRR caused by the loss of processing due to BRCA1 deficiency, it cannot rescue the HRR defect caused by the loss of proteins involved in the subsequent steps of HRR such as XRCC2, BRCA2 and PALBP2 (Bouwman et al., 2010; Bunting et al., 2010).  

Like 53BP1, RIF1, REV7/MAD2L2 and PTIP are also involved in protecting DSBs from processing. A deficiency of RIF1, REV7/MAD2L2 or PTIP can partially rescue the sensitivity of BRCA1-deficient cells to PARP1 inhibitors and radial chromosome formation, but not that of cells deficient in BRCA2, which works downstream of processing in HRR (Pellegrini et al., 2002; Callen et al., 2013; Escribano-Díaz et al., 2013; Zimmermann et al., 2013; Boersma et al., 2015). In the absence of BRCA1 and 53BP1, the processing for HRR is mediated by CtIP (Bunting et al., 2010). While the effect of BRCA1 deficiency on HRR is suppressed by the loss of 53BP1 or RIF1, CtIP depletion is not rescued by the loss of 53BP1 or RIF1.  

THE RESPONSE TO DYSFUNCTIONAL TELOMERES  
Telomeres cap the chromosome ends  In mammals, telomeres cap the chromosome ends, and thereby distinguish natural chromosome ends from DSBs. Telomeres are composed of thousands of tandem repeats of TTAGGG, which span 4–12 kb in humans and 50 kb in mice, with a 3' overhang of 12–300 nucleotides. A six-protein complex called shelterin binds to telomeric DNA, and functions in telomere end protection. Two of these proteins, TRF1 and TRF2, bind directly to double-stranded telomeric DNA,
while another shelterin protein, POT1, in complex with TPP1, binds to single-stranded telomeric DNA. RAP1 is recruited to telomeric DNA through an interaction with TRF2, and TIN2 tethers the POT1-TPP1 complex to telomeres through an interaction with TRF1 and TRF2 (Fig. 2A; reviewed by Palm and de Lange, 2008).

The 3' overhang of the telomere tucks back into the double-stranded telomeric repeats and forms a lasso-like structure called a t-loop, which sequesters the real chromosome end and prevents DDR activation and chromosome end-to-end fusion by C-NHEJ (Fig. 2B). TRF2 is essential for forming the t-loop structure (Griffith et al., 1999; Stansel et al., 2001; Doksani et al., 2013). In S phase, TRF2 recruits Werner syndrome helicase (WRN) and RTEL1 to unwind t-loops for replication fork progression and telomere elongation by telomerase (Opresko et al., 2004; Barber et al., 2008; Vannier et al., 2012, 2013; Sarek et al., 2015; reviewed by Blackburn, 2001; Palm and de Lange, 2008). Disruption of RTEL1 results in SLX4-mediated t-loop excision and formation of extrachromosomal telomeric repeats (ECTRs) and telomere shortening (Vannier et al., 2012) (see below).

**Telomere heterochromatin regulates telomere function** Telomeres and the regions adjacent to telomeres, called subtelomeres, contain epigenetic marks that are characteristic of heterochromatin, which is enriched with dimethylated or trimethylated histone H3 lysine 9 (H3K9), trimethylated H4K20 and histone hypoacetylation, and is bound by heterochromatin protein HP1 (Gonzalo et al., 2006; reviewed by Blasco, 2007). Methylation of H3K9 is carried out by the histone methyltransferases SUV39H1 (suppressor of variegation) and SUV39H2 (García-Cao et al., 2004), while methylation of H4K20 is carried out by SUV4-20H2 (Benetti et al., 2007b). Furthermore, extensive DNA methylation is also observed in subtelomeric regions, and is maintained by DNMT1, DNMT3a and DNMT3b DNA methyltransferases (Gonzalo et al., 2006). Telomeric chromatin also contains shelterin, and shows a slightly shorter nucleosome spacing compared with non-telomeric heterochromatin (Tommerup et al., 1994; Lejnine et al., 1995). The heterochromatic modification at telomeres and subtelomeres is involved in the regulation of telomere length, telomere replication, and repression of recombination at telomeres (García-Cao et al., 2004; Gonzalez et al., 2006; Benetti et al., 2007b). On the other hand, telomere shortening decreases trimethylation of H3K9 and H4K20 at telomeres and subtelomeres (Benetti et al., 2007a). The H3K9 deacetylase SirT6 is also involved in the formation of telomere heterochromatin; it maintains trimethylation of H3K9 at telomeres and subtelomeres in S phase, subtelomere gene silencing and repression of replication-associated telomere defects, such as telomere loss or abnormal telomere structure (Michishita et al., 2008; Tennen et al., 2011).

**Telomere dysfunction induces the DDR** When TRF2 or POT1-TPP1 is inhibited, telomeres can no longer form t-loops and become dysfunctional, resulting in activation of the DDR and chromosome fusion. Similarly, when telomeric DNA becomes too short, so that there are not enough binding sites for shelterin, telomeres also become dysfunctional. However, in normal cells with intact cell cycle regulation, these dysfunctional telomeres activate the DDR, but do not lead to chromosome fusion, indicating that some telomere function remains (reviewed by Cesare and Karlseder, 2012).

The DDR at telomeres is mediated by 53BP1, MDC1, RNF8 and RNF168, and by phosphorylated H2AX, ATM, RAD17 and MRE11, which accumulate at dysfunctional...
induced foci (TIFs) (Takai et al., 2003). When the telomeres and form foci called telomere dysfunction-induced foci (TIFs) (Takai et al., 2003). TRF2 prevents the activation of ATM (Karlseder et al., 2004) and the downstream effectors Chk2 and p53 (Celli and de Lange, 2005) at telomeres. In contrast, POT1-TPP1 prevents ATR activation by binding to single-stranded overhangs and thus rendering them inaccessible to RPA, which would otherwise promote the activation of ATR and the downstream effector Chk1 (Zou and Elledge, 2003; Hockemeyer et al., 2007; Gong and de Lange, 2010). Telomeric DNA that becomes too short also activates the DDR (d’Adda di Fagagna et al., 2003; Herbig et al., 2004). In S phase, t-loops open for replication fork progression, which causes a temporary activation of the DDR (Verdun et al., 2005). The cellular consequences of activating the DDR by dysfunctional telomeres include activation of the p53-p21 pathway in mouse, or both the p53-p21 and the Rh-p16 pathways in human, which drives cellular growth arrest, cellular senescence and apoptosis (Smogorzewska and de Lange, 2002; Deng et al., 2008).

**Multiple mechanisms can cause telomere shortening** The cytosine-rich strand of the telomeric sequence, which is replicated by lagging-strand synthesis, is not completely replicated, because the final RNA primer binds to a random site about 100 nucleotides from the terminus, and this final RNA primer is removed after replication (Olovnikov, 1973; Chow et al., 2012). The ends of lagging daughter strands are initially random after replication, but are soon processed to generate a CCAATC-5’ terminus during late S or S/G2 phase (Chow et al., 2012). Furthermore, the ends of leading daughter strands are initially blunt, but are processed to generate the 3’ overhang (Lingner et al., 1995) (see below). Due to the incomplete replication of the terminus of the DNA sequence, and the processing of the ends of both strands, approximately 50–200 bp of telomeric repeat sequences are lost in each mammalian cell cycle.

In addition to gradual telomere shortening with each cell division, there are multiple pathways that can lead to acute telomere attrition, in which large blocks of telomeric sequence are lost in a single event. The guanine-rich sequence of telomeric DNA can form a higher-order structure called a G-quadruplex, which poses problems for DNA replication. Therefore, telomeres are difficult regions to replicate. The helicases BLM, WRN and RTEL1 function to unwind G-quadruplexes, and facilitate lagging-strand synthesis (Crabbe et al., 2004; Vannier et al., 2012; Zimmermann et al., 2014). In Schizosaccharomyces pombe, Taz1 is involved in replication fork progression at telomeric repeats, and its inhibition causes replication fork stalling when telomeric DNA is encountered (Miller et al., 2006). In mammals, TRF1 is involved in replication at telomeres, and TRF1 depletion causes replication fork stalling at telomeres and telomere fragility (Martínez et al., 2009; Sfeir et al., 2009). In human embryonic stem cells, telomeres are highly susceptible to replication stress, which can induce telomere dysfunction, indicated by abnormal telomere structures and TIF formation (Janson et al., 2015).

The structure of t-loops resembles the intermediate of homologous recombination, and aberrant recombination at t-loops can therefore lead to rapid telomere shortening and ECTR formation. This occurs when the KU complex is compromised (Wang et al., 2009), or when mutations in TRF2 induce abnormal resolution of t-loop structures, which is mediated by X-ray repair cross-complementing 3 (XRCC3), NBS1 and SLX4 (Zhu et al., 2003; Wang et al., 2004).

HRR between the telomeres of sister chromatids (telomere sister chromatid exchange; T-SCE) can be detrimental to cells, because an unequal exchange can result in a short dysfunctional telomere on one of the sister chromatids. The KU complex, which protects DSB ends and represses HRR (Pierce et al., 2001), also represses T-SCEs in combination with TRF2 (Celli et al., 2006) and POT1 (Palm et al., 2009; Sfeir and de Lange, 2012).

The guanine triplets in telomeric DNA are sensitive to oxidative stress and ultraviolet (UV) light, because oxidative damage and UV-induced pyrimidine dimers are poorly repaired in telomeres (Kruk et al., 1995; Oikawa and Kawanishi, 1999; Oikawa et al., 2001). This oxidative damage compromises DNA replication, and telomere shortening or telomere loss therefore occurs in cells undergoing oxidative stress (von Zglinicki, 2002).

**Dysfunctional telomeres lead to chromosome end-to-end fusions** The mechanism of end-to-end fusion as a consequence of telomere dysfunction depends on the type of telomere dysfunction (Fig. 2C). TRF2 inhibition induces chromosome end-to-end fusion via C-NHEJ, and fusion is dependent on DNA ligase IV (Smogorzewska et al., 2002; Celli and de Lange, 2005). As with DSBs, the KU complex is involved in C-NHEJ at dysfunctional telomeres, and therefore inhibition of the KU complex reduces chromosome fusions 10-fold when TRF2 function is compromised. The KU complex is also involved in the protection of dysfunctional telomeres from processing in concert with TRF2. In the absence of both TRF2 and the KU complex, T-SCE, which occurs through HRR, is increased and telomere end-to-end fusion via A-NHEJ is observed (Celli et al., 2006; Rai et al., 2010). In contrast to fusions in cells that are deficient in KU or TRF2, fusions in the absence of POT1-TPP1 occurs via
A-NHEJ. Chromosome end-to-end fusion in the absence of POT1-TPP1 occurs independently of 53BP1 and ATM, but requires CtIP and ATR (Rai et al., 2010).

Under some circumstances, chromosomes with telomeres that become too short can also fuse, which occurs through a mechanism that is different from that seen in TRF2-deficient cells, as shown by the fact that it is independent of DNA-PKcs and DNA ligase IV (Maser et al., 2007) and 53BP1 (Rai et al., 2010). Instead, chromosomes with short telomeres appear to fuse via A-NHEJ, as shown by extensive deletions and attenuated telomere signals, indicating the involvement of processing, and the presence of microhomology at fusion junctions (Capper et al., 2007; Letsolo et al., 2010; Rai et al., 2010), and such fusion is dependent on DNA ligase III (Jones et al., 2014).

Movement of dysfunctional telomeres is involved in chromosome-end to-end fusion In addition to its roles in protecting DSBs and telomeres from processing, 53BP1 is also involved in chromosome movement at dysfunctional telomeres, which increases the chances that two telomeres will encounter one another and, thus, facilitates end-to-end fusions (Dimitrova et al., 2008). Increased telomere mobility requires phosphorylation of the 53BP1 N-terminal 28 S/T-Q motifs by ATM (Lottersberger et al., 2013). This mobility is mediated by the transmembrane proteins SUN1 and SUN2, which are components of the LINC complex that links 53BP1 to microtubules (Lottersberger et al., 2015). Knockout of SUN1 and SUN2 decreases the mobility of dysfunctional telomeres and chromosome end-to-end fusion in the absence of TRF2 (Lottersberger et al., 2015). However, 53BP1 accumulates at dysfunctional telomeres in the absence of SUN1 and SUN2, and knockout of SUN1 and SUN2 does not affect Chk2 phosphorylation or TIF formation (Lottersberger et al., 2015), indicating that at dysfunctional telomeres, the DDR and mobility are regulated differently. Fusions that result from the absence of TRF2 primarily occur in G1 phase, and are therefore observed in mitosis as the ‘chromosome’ type, in which both chromatids on two different chromosomes are fused together (Dimitrova and de Lange, 2009).

RIF1 (Zimmermann et al., 2013), REV7/MAD2L2 (Boersma et al., 2015) and PTIP (Callen et al., 2013) are also involved in telomere end-to-end fusion when TRF2 is depleted. However, in contrast to 53BP1, RIF1 (Zimmermann et al., 2013), REV7/MAD2L2 (Boersma et al., 2015) and PTIP (Lottersberger et al., 2015) are not involved in the mobility of dysfunctional telomeres.

Multiple mechanisms by which TRF2 prevents C-NHEJ TRF2 prevents C-NHEJ by multiple mechanisms. As mentioned earlier, TRF2 is involved in the formation of t-loops (Fig. 2B) (Griffith et al., 1999; Stansel et al., 2001; Doksani et al., 2013), which sequences the ends of chromosomes and prevent access by the Ku complex (de Lange, 2005; Dimitrova and de Lange, 2009). The wrapping of DNA around the TRFH domain of TRF2 has recently been proposed to promote t-loop formation (Amiard et al., 2007; Benarroch-Popivker et al., 2016). TRF2 prevents ATM activation at chromosome termini (Karlseder et al., 2004; Verdun et al., 2005), and thus prevents C-NHEJ at telomeres, possibly because of the presence of heterochromatin (Denchi and de Lange, 2007). The iDDR (inhibitor of DNA damage response) domain of TRF2 prevents the accumulation of the E3 ubiquitin ligase RNF168 at telomeres, and thereby prevents the activation of 53BP1 (Okamoto et al., 2013). As t-loop structures open for replication fork progression in S phase, a temporary activation of ATM occurs at telomeres (Verdun et al., 2005); however, as long as TRF2 is present, telomeres do not fuse in S phase, indicating that TRF2 maintains a residual function to prevent C-NHEJ.

After DNA synthesis, the guanine-rich strand of telomeres, which is replicated by leading-strand synthesis, is initially blunt-ended, and therefore must be processed to generate a single-stranded overhang (Lingner et al., 1995). The 3’ overhang is essential for POT1-TPP1 binding, t-loop formation and prevention of C-NHEJ, and is therefore essential for telomere end protection. TRF2 recruits MRE11 and Apollo to telomeres for the processing that is required to generate the single-stranded overhang (Chai et al., 2006; Lenain et al., 2006; van Overbeek and de Lange, 2006; Chen et al., 2008b; Deng et al., 2009; Dimitrova and de Lange, 2009; Wu et al., 2010). Inhibition of Apollo causes loss of the 3’ overhang, an increased frequency of the formation of telomere damage foci, and an increase in end-to-end fusion and cellular senescence (van Overbeek and de Lange, 2006). A mutation in Apollo that prevents its binding to TRF2 also causes loss of 3’ overhangs, telomere damage foci, and premature aging (Touzot et al., 2010). The processing by Apollo is followed by further EXO1-mediated end resection, which is finally fine-tuned by a fill-in synthesis step by the CTC1-SFTN1–TEN1 complex (Miyake et al., 2009; Wu et al., 2012). The TRF2-Apollo interaction is restricted to S/G2 phase, and occurs after leading-strand synthesis. This interaction is mediated by S/G2 phase-specific NBS1 phosphorylation by CDK, which dissociates NBS1 from TRF2 and enables TRF2 to recruit Apollo (Rai et al., 2017).

TRF2 also maintains single-stranded overhangs, and inhibition of TRF2 causes the loss of the single-stranded overhang due to MRE11 and ERCC1-XPF nuclease activity. Depletion of ERCC1-XPF prevents loss of the single-stranded overhangs and chromosome end-to-end fusions in the absence of TRF2 (Zhu et al., 2009). TRF2 also prevents Ku complex heterotetramerization at telomeres by interacting with the alpha-5 region of Ku70, and thus prevents synopsis of chromosome ends (Ribes-Zamora et
Resection at dysfunctional telomeres In concert with 53BP1, RIF1 is also involved in the protection of telomeric termini from 5’ processing by CtIP, and EXOI aided by BLM, so that when 53BP1 or RIF1 are compromised, hyper-resection occurs, resulting in elongated 3’ overhangs (Sfeir and de Lange, 2012; Lottersberger et al., 2013; Kibe et al., 2016). Therefore, the dramatic increase in chromosome end-to-end fusion observed in TRF2-deficient cells is not observed in cells deficient in 53BP1 or RIF1. Instead, the telomeres in cells deficient in both TRF2 and 53BP1 or both TRF2 and RIF1 are processed by CtIP or EXOI, and hyper-elongated 3’ overhangs are formed (Lottersberger et al., 2013; Zimmermann et al., 2013). REV7/MAD2L2 also accumulates at dysfunctional telomeres and prevents 5’-3’ end resection by CtIP or EXOI, and therefore stimulates C-NHEJ and prevents T-SCE in a 53BP1- and RIF1-dependent, and PTIP-independent, manner (Boersma et al., 2015).

Although processing is essential for telomere function, too much processing is toxic to cells. Processing of telomere single-stranded overhangs by Apollo is regulated by POT1-TPP1 (Wu et al., 2012). POT1-TPP1 also prevents CtIP-, EXOI- and BLM-mediated resection of telomeres (Kibe et al., 2016). POT1-TPP1 regulates the formation of CCAATC-5’ at the end of the single-stranded overhang (Hockemeyer et al., 2005), and prevents the hyper-elongation of the single-stranded 3’ overhang (Hockemeyer et al., 2007) and chromosome end-to-end fusion by A-NHEJ (Rai et al., 2010; Sfeir and de Lange, 2012). Finally, because 3’ overhangs are substrates for homologous recombination, loss of POT1-TPP1 also results in an increased frequency of T-SCE. Recently, POT1 mutations were found in many cancer patients (reviewed by Lazzzerini-Denchi and Sfeir, 2016). Although the mechanism by which POT1 mutations lead to tumor formation is not fully understood, the elongation of the 3’ single-stranded overhangs in POT1-deficient cancer cells may facilitate A-NHEJ.

Alternative lengthening of telomeres (ALT) cells use recombination to maintain telomeres Most cancer cells use telomerase to elongate telomeres; however, about 10–15% of cancer cells do not express telomerase, and instead maintain telomeric DNA via recombination. This mechanism is called alternative lengthening of telomeres (ALT) (reviewed by Cesare and Reddel, 2010; Pickett and Reddel, 2015). Telomeres maintained by ALT are highly heterogeneous in length due to both gradual and rapid changes in telomere length (Murnane et al., 1994), show a greatly elevated level of recombination at telomeres (Dunham et al., 2000; Varley et al., 2002; reviewed by Cesare and Reddel, 2010), and contain ECTRs (Cesare and Griffith, 2004), which can serve as replication templates to elongate telomeres. The ALT mechanism requires the MRN complex to process the chromosome ends to form a single-stranded 3’ overhang for recombination, as well as HRR factors, such as RAD51, RAD52, BLM and BRCA1. Inhibition of the MRN complex therefore causes telomere shortening in ALT cells. Telomeres maintained by ALT are not fully protected, and show elevated activation of the DDR at telomeres, but are defective for DNA damage cell cycle checkpoints (Cesare et al., 2009; Lovejoy et al., 2012). The frequency of chromosome fusions within the population of cells maintaining telomeres by ALT correlates with the frequency of rapid deletion events, indicating that ALT is associated with dysfunctional telomeres and chromosome rearrangements (Murnane et al., 1994; Lovejoy et al., 2012).

THE SENSITIVITY OF SUBTELOMERIC REGIONS TO DOUBLE-STRAND BREAKS

The sensitivity of subtelomeric regions to DSBs in normal cells In contrast to most DSBs, which are efficiently repaired by C-NHEJ or HRR, IR-induced DSBs near telomeres are poorly repaired, persist for a long time, and are associated with cellular senescence (Fumagalli et al., 2012; Hewitt et al., 2012). This deficiency in DSB repair near telomeres is mediated by TRF2, as shown by the fact that forced recruitment of TRF2 near DSBs can inhibit DSB repair (Fumagalli et al., 2012).

DSBs can occur naturally at telomeres. Telomeres and subtelomeric regions are poor substrates for DNA replication, and, as a result, replication fork progression is delayed or sometimes stalls at or near telomeres (Martínez et al., 2009; Sfeir et al., 2009). Telomeric proteins are essential for replication through telomeres, as shown by the fact that the loss of the telomeric protein TRF1 or its ortholog Taz1 increases replication fork stalling at telomeres or telomere-subtelomere junctions (Miller et al., 2006; Martinez et al., 2009; Sfeir et al., 2009). Forced progression through the cell cycle by the Ras oncogene also causes replication fork stalling and chromosome breakage near telomeres, which has been associated with oncogene-induced cellular senescence (Suram et al., 2012). This ability of telomeres to act as sensors of genotoxic stress and drive cells to senescence has been proposed as a mechanism for suppressing cancer progression (reviewed by Suram and Herbig, 2014).

Chromosome rearrangements due to subtelomeric DSBs Despite the expression of telomerase, a high rate of spontaneous telomere loss can continue to occur in cancer cells (Fouladi et al., 2000; Gisselsson et al., 2001). This spontaneous telomere loss has been proposed to result from replication fork stalling and collapse due to replication stress, which leads to DSB formation and telomere loss because of the sensitivity of subtelomeric regions to DSBs in normal cells. In contrast to most DSBs, which are efficiently repaired by C-NHEJ or HRR, IR-induced DSBs near telomeres are poorly repaired, persist for a long time, and are associated with cellular senescence (Fumagalli et al., 2012; Hewitt et al., 2012). This deficiency in DSB repair near telomeres is mediated by TRF2, as shown by the fact that forced recruitment of TRF2 near DSBs can inhibit DSB repair (Fumagalli et al., 2012).
McClintock reported that telomere loss can induce chromosome instability through breakage-fusion-bridge (BFB) cycles (McClintock, 1941). BFB cycles can be initiated by fusion of chromosomes without telomeres, involving either fusions between different chromosomes or fusions of sister chromatids. Chromosome fusions as a consequence of telomere loss occur via A-NHEJ, as shown by the fact that they commonly involve large deletions and by the presence of microhomology at fusion junctions (Fouladi et al., 2000; Lo et al., 2002b). During BFB cycles, the fused chromosomes break during mitosis due to the presence of two centromeres (Fig. 3A). Because these broken chromosomes still lack a telomere in the daughter cells, the BFB cycle is then repeated in the next cycle, which can induce extensive chromosome instability involved with gene amplifications or loss of heterozygosity (Fig. 3B). A defect in p53-mediated cell cycle checkpoints or apoptosis is required for the BFB cycle to occur (Lo et al., 2002a, 2002b; Murnane, 2006, 2010). DSBs near telomeres can also induce other types of chromosome rearrangements, such as nonreciprocal translocations, duplications, inversions, ring chromosomes and aneuploidy. If the broken chromosome acquires a new telomere through the duplication of the end of another chromosome, the donor chromosome remains stable, although this results in an allelic imbalance (Fig. 3C) (Subatier et al., 2005). However, if the broken chromosome acquires a telomere through a nonreciprocal translocation, the donor chromosome loses the telomere, which transfers the chromosome instability to the donor chromosome (Fig. 3D) (Subatier et al., 2005). Thus, even the loss of a single telomere can induce the instability of multiple chromosomes.

**The mechanism responsible for the sensitivity of subtelomeric regions to DSBs** Using a transgene-based DSB induction system, Zschenker et al. found that large deletions and gross chromosome rearrangements occur at a much higher frequency at subtelomeric DSBs than at DSBs at most interstitial sites. Furthermore, the large deletions at subtelomeric DSBs are much greater in size than those at interstitial DSBs (Zschenker et al., 2009). This sensitivity of subtelomeric regions to DSBs has been shown to extend at least 100 kb from telomeres (Kulkarni et al., 2010), and has been estimated to extend 168–396 kb from telomeres (Muraki et al., 2012). This increased sensitivity is mediated by the proximity of telomeric repeat sequences, as shown by the fact that the presence of TTAGGG repeats near a DSB at interstitial sites also causes a high frequency of large deletions (Miller et al., 2011).

Although the mechanism responsible for the increased sensitivity of subtelomeric regions to DSBs is not known, evidence shows that it is a result of excessive processing of DSBs and is not due to a direct deficiency in C-NHEJ (Fig. 4). Consistent with this conclusion, the single-stranded DNA-binding protein RAD51 accumulates at subtelomeric DSBs, indicating the accumulation of processed single-stranded DNA at subtelomeric DSBs (Alcaraz Silva et al., 2017). In addition, the binding of ATM is decreased at subtelomeric DSBs, while ATR binding is increased (Alcaraz Silva et al., 2017), also suggesting that subtelomeric DSBs accumulate single-stranded DNA.
DNA, resulting in a transition from ATM to ATR. This excessive processing at subtelomeric DSBs is carried out partly by MRE11, as shown by the fact that the inhibition of MRE11 exonuclease activity by Mirin decreases the frequency of large deletions (Muraki et al., 2015), and by the increased recruitment to the DSBs of BRCA1 and CtIP, which cooperate with MRE11 in the processing of DSBs (Alcaraz Silva et al., 2017). However, the involvement of other nuclease(s) in the extensive processing of subtelomeric DSBs is also suggested, because Mirin does not reduce the frequency of large deletions at subtelomeric DSBs to the level observed at interstitial DSBs (Muraki et al., 2015).

In addition to large deletions, the increased processing of DSBs near telomeres also causes a high frequency of gross chromosome rearrangements. Gross chromosome rearrangements at subtelomeric DSBs occur via A-NHEJ, as shown by the presence of microhomology at the junctions of the chromosome fusions (Muraki et al., 2013). Although gross chromosome rearrangements often occur in combination with large deletions, they sometimes occur in their absence, demonstrating that large deletions and gross chromosome rearrangements can occur independently (Zschenker et al., 2009). The increased frequency of gross chromosome rearrangements at subtelomeric DSBs is also observed in yeast (Ricchetti et al., 2003) and mouse embryonic stem cells (Lo et al., 2002b). In contrast, the efficiency of HRR is the same at interstitial and subtelomeric DSBs, indicating that the processing that occurs during late S/G2 phase for HRR is not affected at subtelomeric DSBs (Miller et al., 2011).

Small deletions, which occur through C-NHEJ, occur at the same frequency at interstitial and subtelomeric DSBs (Muraki et al., 2015). Similarly, KU70 binding is the same at interstitial and subtelomeric DSBs (Alcaraz Silva et al., 2017). Combined, these results indicate that C-NHEJ is functional near telomeres. Therefore, the increased frequency of large deletions and gross chromosome rearrangements at subtelomeric DSBs is not due to a direct defect in C-NHEJ, supporting the conclusion that the sensitivity of subtelomeric regions to DSBs is due to excessive processing.

**Telomere crisis and cancer** When telomeres in normal cells become critically short following extended cell division, the cells cease proliferating or die due to senescence or apoptosis, which limits the number of cell divisions; this is called the Hayflick limit (Hayflick and Moorhead, 1961). However, if cells acquire mutations in genes critical to the senescence or apoptosis pathways, they can continue proliferating and can eventually reach a state called crisis (reviewed by Shay and Wright, 2005). In crisis, telomeres are so short that extensive chromosome end-to-end fusions occur, leading to chromosome instability and usually cell death (Shay and Wright, 2005). Some cells survive crisis by acquiring the ability to maintain telomeres. However, these survivors contain chromosome fusions formed during crisis through A-NHEJ, which have been shown to be involved in tumor development (Rai et al., 2010; Jones et al., 2014; Maciejowski and de Lange, 2017). Similarly, chromosome fusions as a consequence of telomere loss in tumor cells expressing telomerase also appear to involve A-NHEJ (Fouladi et al., 2000; Lo et al., 2002b). Therefore, dysfunctional telomeres in cells with intact cell cycle checkpoints inhibit tumor progression by inducing cellular senescence or apoptosis, which work as tumor barriers, while telomere dysfunction in cancer cells that are defective in checkpoint proteins promotes tumorigenesis (Deng et al., 2008).

In addition to BFB cycles, in some cases, fused chromosomes can also undergo catastrophic shattering near telomeres, called chromothripsis (Maciejowski et al., 2015; reviewed by Maciejowski and de Lange, 2017). When dicentric chromosomes are formed, they persist through mitosis and cytokinesis, and long chromatin bridges between daughter cells are formed, which disrupts the formation of a nuclear envelope. During the transient nuclear envelope rupture at interphase, the DNA in the bridge structures becomes partially single-stranded and the bridges are resolved by TREX1, which is a major cytoplasmic 3’ exonuclease. The fragmented chromosomes are then reincorporated back into the nuclear genome, but in random order and orientation. During the process of chromothripsis, clustered base substitutions arise at the boundary of rearranged sequences, exhibiting C>T and C>G changes at TpC dinucleotides, a phenomenon called kataegis. Kataegis is attributable to the activity of APOBEC3A/B, which targets single-stranded DNA and deaminates cytosine residues (Harris et al., 2002; Nik-Zainal et al., 2012; Roberts et al., 2012, 2013; Chan et al., 2015).
Consequences of the sensitivity of subtelomeric regions to DSBs The sensitivity of subtelomeric regions to DSBs has had a large impact on the human genome. Human subtelomeres contain blocks of homologous sequences that are shared on different chromosomes, although these homologous sequences are located at different distances from the telomeres (Riethman, 2008). The presence of these homologous sequences at subtelomeres can lead to mispairing of chromosomes at meiosis, a high rate of recombination between different chromosomes, and a high rate of unequal sister chromatid exchange (Rudd et al., 2007). Therefore, subtelomeric regions are highly dynamic (Linardopoulou et al., 2005).

Subtelomeric regions contain many transcript families in the shared homologous blocks. Some of these transcripts are pseudogenes, but some encode functional proteins (Ambrosini et al., 2007). Many olfactory receptor gene families are contained in the homologous blocks in human subtelomeric regions (Trask et al., 1998). This plasticity and variability of subtelomeres might therefore be the birthplace of new genes which would help organisms to adapt to new environmental conditions (Mefford and Trask, 2002). Finally, many human genetic disorders are caused by rearrangements in subtelomeric regions (reviewed by De Vries et al., 2003).

CONCLUSIONS AND PERSPECTIVES

DSBs are very hazardous to chromosome integrity, and cells are equipped with multiple pathways to repair DSBs. C-NHEJ is the primary pathway for repair of DSBs, although HRR and A-NHEJ are also involved. These DSB repair pathways are tightly regulated by the competition between DSB end protection and end processing in a cell cycle-dependent manner, because homologous templates for HRR are available only in S/G2 phase. Telomeres prevent the activation of the DDR at the chromosome termini, and therefore dysfunctional telomeres induce chromosome end-to-end fusion, which occurs through either C-NHEJ or A-NHEJ. Telomere dysfunction, telomere loss and subtelomeric DSBs induce senescence or apoptosis in cells with intact checkpoints, while they induce chromosome rearrangements in cells without functional cell cycle checkpoints, which promotes tumor formation. Subtelomeric regions are prone to DNA rearrangements in cancer because of their sensitivity to DSBs. This sensitivity to DSBs is due to excessive processing, which leads to large deletions and gross chromosome rearrangements involving A-NHEJ. Understanding the mechanism responsible for the sensitivity of subtelomeric regions to DSBs may provide new insights leading to new approaches for cancer therapy.

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