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Telomeres, histone code, and DNA damage response

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Abstract

Genomic stability is maintained by telomeres, the end terminal structures that protect chromosomes from fusion or degradation. Shortening or loss of telomeric repeats or altered telomere chromatin structure is correlated with telomere dysfunction such as chromosome end-to-end associations that could lead to genomic instability and gene amplification. The structure at the end of telomeres is such that its DNA differs from DNA double strand breaks (DSBs) to avoid nonhomologous end-joining (NHEJ), which is accomplished by forming a unique higher order nucleoprotein structure. Telomeres are attached to the nuclear matrix and have a unique chromatin structure. Whether this special structure is maintained by specific chromatin changes is yet to be thoroughly investigated. Chromatin modifications implicated in transcriptional regulation are thought to be the result of a code on the histone proteins (histone code). This code, involving phosphorylation, acetylation, methylation, ubiquitylation, and sumoylation of histones, is believed to regulate chromatin accessibility either by disrupting chromatin contacts or by recruiting non-histone proteins to chromatin. The histone code in which distinct histone tail-protein interactions promote engagement may be the deciding factor for choosing specific DSB repair pathways. Recent evidence suggests that such mechanisms are involved in DNA damage detection and repair. Altered telomere chromatin structure has been linked to defective DNA damage response (DDR), and eukaryotic cells have evolved DDR mechanisms utilizing proficient DNA repair and cell cycle checkpoints in order to maintain genomic stability. Recent studies suggest that chromatin modifying factors play a critical role in the maintenance of genomic stability. This review will summarize the role of DNA damage repair proteins specifically ataxia-telangiectasia mutated (ATM) and its effectors and the telomere complex in maintaining genome stability.

Telomeres are structurally and functionally complex. They consist of an array of simple DNA repeats at the extreme end of the chromosome, with a more complex array of repeats immediately adjacent to it. Telomere behavior is abnormal in *ATM* deficient cells, both somatic as well as germ cells (Pandita et al., 1995, 1996, 1999, 2007; Pandita and Dhar, 2000; Scherthan et al., 2000; Hande et al., 2001; Pandita, 2001, 2002a, b, 2003), but the precise mechanism by which ATM regulates telomere structure and function is not known. Recent studies suggest that ATM interacts with various chromatin modifying factors that could influence telomere function (Fig. 1) (Gupta et al., 2005; Sun et al., 2005).

Chromatin, the physiological packaging structure of histones and DNA, is gaining appreciation as a factor maintaining genomic stability and as a relevant target of signaling pathways (Fischle et al., 2003; Legube and Trouche, 2003). Chromatin structure is an important determinant of protein-DNA interactions, with consequences for DNA metabolism and transcription control (Pruss et al., 1994a, b; Wallrath et al., 1994; Otten and Tapscott, 1995). After DNA damage, eukaryotic cells orchestrate a complex array of responses essential for cell survival (Hoeijmakers, 2001), coordinating checkpoint-mediated cell cycle arrest and the repair of

damaged DNA. Histone modifications by phosphorylation, acetylation, or methylation have all been linked to DNA damage repair (Fernandez-Capetillo et al., 2003). Phosphorylation of histone H2A in fission yeast (referred to as H2AX phosphorylation) or the H2A variant H2AX in mammals also has a role in checkpoint control (Fernandez-Capetillo and Nussenzweig, 2004; Nakamura et al., 2004). Recent studies have indicated that histone modifications in response to DNA damage are influenced by non-histone chromosomal proteins. We will discuss in detail the role of non-histone chromosomal proteins in telomere maintenance and DNA damage response (DDR).

Chromatin changes frequently occur at both local and global levels, and specifically telomeres acquire a chromatin structure that ensures their proper function (Sims et al., 2003). Telomere chromatin also contains nucleosomes, the basic units of chromatin, that are composed of an octamer of histones, two subunits of each H3, H4, H2A, and H2B, and DNA that wraps around the histone core (Olins and Olins, 2003). The N-terminal tails of the histones protrude from the nucleosome core and are subjected to different post-translational modifications. The types of histone post-translational modifications and the degree of DNA methylation determine the specific chromatin structure (Workman and Kingston, 1998; Strahl et al., 2002; Sims et al., 2003; Khorasanizadeh, 2004). Though mammalian telomere ends do not exist as DNA double strand breaks (DSBs) due to the terminal T-loop structures, DNA repair proteins have been shown to interact with telomeres. Whether these interactions are dependent upon any specific histone modification is under investigation. Recent studies have begun to elucidate the chromatin changes that occur upon DNA damage (Green and Almouzni, 2002; Lund and van Lohuizen, 2004; Peterson and Cote, 2004; Lydall and Whitehall, 2005; van Attikum and Gasser, 2005; Vidanes et al., 2005; Loizou et al., 2006; Wurtele and Verreault, 2006). This review will summarize the progress made on the identification and characterization of factors involved in telomere chromatin structure, histone code, and DDR.

Telomere chromatin structure

Most of the eukaryotic telomeric DNA is organized in tightly packed nucleosomes which are separated by 10–20 bp of linker DNA (Pisano et al., 2008). Several specific proteins contribute to the telomeric structure; however, the exact telomere organization is still unclear. Whereas the role played by telomeric proteins in telomere function and regulation has been widely investigated, little is known about the contribution of nucleosomes to the protection of chromosome ends. Wu and de Lange (2008) have reported that telomeres lacking TRF2, POT1a, and POT1b have no nucleosome eviction in telomeres suggesting that such proteins do not have any chromatin modifying functions. However, it is not yet clear whether depletion of TRF2, POT1a, and POT1b influences higher order chromatin structure or telomere interactions with the nuclear matrix. It will be interesting to see whether the absence of TRF2, POT1a, and POT1b influences the telomere-specific histone modifications.

Many different cellular activities coordinate the action of chromatin modifiers to modulate chromatin structure. For example, cytosine methylation, a broad variety of histone post-translational modifications, and binding of non-coding RNAs determine the status of chromatin. This has been proposed to set a code that can be read by chromatin associating complexes (Strahl and Allis, 2000; Jenuwein and Allis, 2001; Rice and Allis, 2001; Bannister et al., 2002; Kouzarides, 2002; Lachner et al., 2003; Sims et al., 2003; Martin and Zhang, 2005; Shilatifard, 2006). These chromatin modifications determine the recruitment of effectors that regulate a specific biological process, which also involves DDR to maintain the genomic stability. A challenge for the field is to identify the codes that dictate specific cellular processes as well as to identify the different pathways e.g., non-homologous end-joining (NHEJ) or homologous recombination (HR) for repair of DNA DSBs.

The most common posttranslational histone modification in mammalian cells associated with DNA DSBs is phosphorylation of H2AX, (γ -H2AX) (Fernandez-Capetillo et al., 2004). It is induced in response to external damage, forms at sites of physiological DSBs, and spreads over a region spanning megabases surrounding the lesion forming so-called nuclear foci. According to the histone code hypothesis, there are additional histone modifications at DSBs that synergize with γ -H2AX.

The histone code

Histone modifications are critical for the higher order organization of the DNA. The language of covalent histone modifications that correlate with transcription regulation was proposed and defined as the histone code in 2000 (Strahl and Allis, 2000; Turner, 2000). The histone code hypothesis is based on the fact that distinct modifications manifested at specific histone tail residues serve as domains for interaction with specific proteins and such interactions compartmentalize chromatin into heterochromatin and euchromatin. In the DNA repair pathways, some chromatin modifications seem to play a critical role in marking the lesions or recruiting the factors involved in repair thus facilitating the function of repair proteins.

Histone phosphorylation

Histone phosphorylation has been associated with transcriptional regulation, DNA repair, and chromatin condensation. The best characterized histone phosphorylation event is the phosphorylation of H2AX (γ -H2AX) that has been shown by different laboratories to be linked to the ability of the cells to sense, respond, and repair lesions in DNA (Fernandez-Capetillo et al., 2004). The most common kinases that phosphorylate H2AX in response to DNA damage are ATM, ATR, and DNA-PKcs. Phosphorylation of H2AX has been associated with recruiting repair proteins in the vicinity of DNA damage. Aurora kinases and mitogen and stress activated kinases have been shown to be responsible for phosphorylation of serine 10 and serine 28 on histone H3, phosphorylation events that mark the compaction of chromatin during mitosis (Hsu et al., 2000; Nowak and Corces, 2004). The 14-3-3 σ protein isoforms have been identified as molecules bearing a protein domain with high affinity for phosphorylated and phosphoacetylated H3 tails (Macdonald et al., 2005). Recently, phosphorylation of serine 1 on histone H4 has also been implicated in chromatin compaction (Krishnamoorthy et al., 2006).

Chromatin structure needs to return to the original state and the DNA damage response signal to be turned off once the DNA break has been repaired. Two mechanisms could remove chromatin marks acquired by the introduction of DSBs: removal of histone modifications or replacement with unmodified histones. The enzymes responsible for removing the phosphate group from H2AX are PP2A (mammalian) and Pph3 (yeast) (Chowdhury et al., 2005; Keogh et al., 2006). These phosphatases are required for the removal of ionizing radiation (IR) induced γ -H2AX foci and for efficient recovery from DNA damage. Histone phosphorylation is followed or accompanied by acetylation of the histone residues for evacuation of histone variants from the damage site (Kusch et al., 2004).

Histone acetylation

The major purpose of acetylation is to induce decondensation of chromatin. Acetylation neutralizes the positive charge of lysine residues decreasing their interaction with negatively charged DNA. In humans, five different families of histone acetyltransferases (HATs) have been identified as being responsible for acetylation of lysines at positions 9, 14, 18, and 23 on histone H3, and lysines at positions 5, 8, 12, and 16 on histone H4. Acetylated lysine residues serve as binding platforms for proteins that contain the bromodomain protein module, found in some transcription factors as well as in subunits of chromatin-remodeling complexes (Dhalluin et al., 1999; Winston and Allis, 1999; Jacobson et al., 2000; Hassan et al., 2002).

HAT activities are usually found in large multi-subunit complexes. Overall, acetylation of lysine residues on histones is associated with transcriptional activation and DNA repair (Grunstein, 1997; Kuo and Allis, 1998; Santos-Rosa et al., 2002). Acetylation has to be followed by deacetylation of histones, which allows the acquisition of a closed chromatin state.

Histone methylation

Histone methylation of lysine residues has been linked to the detection and repair of DNA DSBs (Huyen et al., 2004; Sanders et al., 2004). In mammalian cells, methylation of lysine 79 on histone H3 was shown to be necessary to target 53BP1 to DNA DSBs (Huyen et al., 2004; Sanders et al., 2004). In yeast, the HMT (histone methyl-transferase) Set9 is responsible for H4K20 methylation and is also required for the localization of Crb2, the 53BP1 homolog, to sites of DNA breaks. Loss of Set9 results in increased DNA damage sensitivity of break repair (Huyen et al., 2004; Sanders et al., 2004). A recent study of the 3D structure of the tudor domain of 53BP1 in the presence of a histone H4 peptide demonstrates a direct interaction between 53BP1 tandem tudor domains and histone H4 specifically dimethylated at lysine 20 (H4K20me₂). Similarly, crystallographic and NMR analysis revealed that Crb2 also contains tandem tudor domains with a conserved H4K20me₂ binding site. In vivo data analysis revealed that H4K20me₂, and not H3K79me, is the binding site for 53BP1 in the context of DNA DSBs (Botuyan et al., 2006). Thus, dimethylated histone H4K20 has a conserved role in the recruitment of factors participating in the repair of DNA damage. Based on the proposed model, the simultaneous recognition of γ -H2AX and H4K20me₂ might be needed to bring 53BP1 to DNA DSBs (Botuyan et al., 2006). It has been demonstrated that alterations in H4K20 trimethylation occur in thymus tissue upon whole-body exposure to irradiation (Pogribny et al., 2005). It remains to be determined whether histone methylation has any impact on telomere metabolism.

As yet, it is not clear whether methylation of histones affects the overall charge of the nucleosomes like acetylation, but methylation of histones does provide sites for binding of chromatin-associating activities. Thus, it seems that such modifications may have a role in DDR. Recent studies have shown that DNA damage influences the chromatin associated movement of a non-histone chromatin modifying factor (HP1 β), indicating a relationship between HP1 β and the DNA damage response (Ayoub et al., 2008). HP1 β colocalizes with methylated lysine 20 of histone H4 (H4K20me₃) which is also a binding motif for 53BP1 (unpublished data). In addition, HP1 β has been shown to directly interact with histone methyl-transferase Suv(3)9h1, bringing together two characteristics of chromatin regulator motifs: the CHROMO (of HP1 β) and SET (of Suv(3)9h1) domains. This interaction may also play a key role in the formation of senescence-associated heterochromatin foci (SAHF), which accumulate in nuclei during cellular senescence and are thought to be part of the oncogene induced senescence (OIS) pathway that opposes tumorigenesis (Braig et al., 2005; Narita and Lowe, 2005). Histone demethylation is believed to antagonize the function of histone lysine methylation in diverse biological processes and such modification can reverse the process of DDR. We will discuss below the role of HP1 proteins in telomere metabolism and DNA repair.

Histone ubiquitylation and sumoylation

Ubiquitin and SUMO are bulky signaling modules that are expected to induce dramatic changes in the structure of chromatin. Histones are the most abundant ubiquitylated proteins (Jason et al., 2002; Zhang, 2003; Osley, 2004; Shilatifard, 2006) that are not targeted for degradation as are other ubiquitylated proteins. In fact, ubiquitylation of histones plays a key role in transcriptional activation and repression, heterochromatic silencing, and DNA repair (Osley, 2004; Shilatifard, 2006). The significance of ubiquitination has become evident as RAP80, an ubiquitin-interaction motif containing protein, is associated with a BRCA1/BARD1 complex through its interaction with Abraxas (CCDC98, coiled coil domain containing 98). The

ubiquitin-interaction motifs of RAP80 are critical for targeting this protein complex to DNA DSB sites (reviewed in Yan and Jetten, 2008). In addition, it has been reported that after binding phosphorylated H2AX, ATM-phosphorylated MDC1 is recognized by the FHA domain of RNF8, which subsequently binds the E2 conjugating enzyme UBC13. This complex catalyzes K63-linked polyubiquitination of histones H2A and phosphorylated H2AX and is then recognized by the ubiquitin-interaction motifs of RAP80, facilitating the recruitment of the BRCA1/BARD1/CCDC98/RAP80 protein complex to sites of DNA DSBs (reviewed in Yan and Jetten, 2008).

Besides histone ubiquitination, it has been shown that histone H4 is sumoylated in mammalian cells (Shiio and Eisenman, 2003). SUMO is a small ubiquitin-related molecule of approximately 100 amino acids that modifies proteins. Histone sumoylation has been shown to mediate gene silencing in mammalian cells through recruitment of histone deacetylase and HP1 (Shiio and Eisenman, 2003). HP1 has been implicated in DDR (Sharma et al., 2003). Whether ubiquitination or sumoylation has a role in telomere stability is yet to be established.

In addition to the residue modification of histones discussed above that influence DDR, histones ADP-ribosylated at arginine and glutamate residues have been linked to DNA repair and cell proliferation. All core histones H2A, H2B, H3, and H4 as well as linker histone H1 can be mono-ADP-ribosylated. Several studies have shown that histones are mono-ADP-ribosylated in vivo in response to DNA damage, and a crosstalk between this and other postranslational modifications of histones such as acetylation or phosphorylation has been suggested (Hassa et al., 2006). However, the functional role of mono-ADP-ribosylation for chromatin structure regulation in telomere metabolism remains to be determined.

Factors common in telomere maintenance and DNA damage response

Perusal of literature reveals that there is increasing evidence that most DNA damage response proteins are directly or indirectly involved in telomere maintenance (Fig. 2). Cells defective in DNA DSB repair proteins including ATM, Ku, DNA-PKcs, RAD51D, and the MRN (MRE11/RAD50/NBS1) complex show a faulty telomere metabolism (Slijepcevic, 2006). We will discuss some of the most common proteins involved both in telomere metabolism as well as DDR.

ATM

Cells derived from individuals with ataxia-telangiectasia display genomic instability and defective DNA repair (Pandita and Hittelman, 1992a, b; Pandita, 2002a, 2003). The first report describing the association of ATM with DNA damage repair and telomere metabolism was described in 1995 (Pandita et al., 1995). Subsequently, the role of ATM in telomere function became clear by comparing the meiotic prophase 1 of spermatocytes from mice with and without ATM, revealing that ATM inactivation results in aberrant telomere clustering during meiotic prophase (Pandita et al., 1999, 2007; Scherthan et al., 2000; Pandita, 2001, 2002a, 2003). These studies prompted several groups to determine whether ATM is physically associated with telomeres. Chan et al. (2001) suggested that a primary function of the ATM-family kinases in telomere maintenance is to act on the telomere itself rather than to activate the enzymatic activity of telomerase. Recent studies have shown that cells expressing mutated telomerase (containing mutated template-bearing RNA) have activated ATM as detected by foci formation. However, transient depletion of ATM resulted in the lack of telomere fusions (Stohr and Blackburn, 2008), further suggesting the role of ATM in modulating chromatin status to provide a template for DNA ligation. ATM is associated with chromatin (Gately et al., 1998; Scherthan et al., 2000), and a fraction of ATM is detected in nuclear aggregates (Andegeko et al., 2001). The striking correlation between the appearance of retained ATM and of phosphorylated histone H2AX (γ -H2AX) and the rapid association of a fraction of ATM

with γ -H2AX foci are consistent with a major role of ATM in the early detection of DSBs and subsequent induction of cellular responses (Fig. 3) (Andegeko et al., 2001). It has been reported that the ATM protein interacts with telomere-repeat-binding factor 1 (TRF1; also known as Pin2) (Kishi et al., 2001; Kishi and Lu, 2002), and that lack of TRF2 results in apoptosis in an ATM-dependent fashion (Karlseder et al., 1999). These reports further support the role of ATM in telomere maintenance. Interestingly, it has been shown that ATM function influences the fraction of telomeres anchored to the nuclear matrix (Smilenov et al., 1999). Subsequent studies revealed that the central DNA damage response protein ATM is physically associated with telomeres (Takata et al., 2004; Verdun et al., 2005). These results are not surprising as ATM is a protein kinase that interacts with several substrates and is implicated in mitogenic signal transduction, chromosome condensation, meiotic recombination, cell-cycle control, and telomere maintenance (Fig. 1) (Pandita, 2002a, 2003). Besides ATM, several of its effectors influence both DDR as well as telomere stability as discussed below.

c-Abl

The activation of nuclear c-Abl tyrosine kinase by ionizing radiation requires a functional ATM protein (Baskaran et al., 1997; Shafman et al., 1997), and c-Abl in turn associates with the catalytic unit of telomerase (hTERT) in human cell lines through binding of its Src-homology 3 (SH3) domain to a region (amino acids 308–316) of hTERT (Kharbanda et al., 2000). Ionizing radiation induces c-Abl-dependent tyrosine phosphorylation of hTERT, which thereby inhibits hTERT activity and negatively regulates telomere length (Kharbanda et al., 2000).

14-3-3 σ

14-3-3 σ has been implicated in the G2 checkpoint (Chan et al., 1999). Its association with different kinases in the cytosol and on the nuclear membrane might contribute to kinase activation during intracellular signaling, and the protein appears to sequester the mitotic initiation complex Cdc2/cyclinB1 in the cytoplasm after DNA damage (Xing et al., 1997). Cells with both copies of the gene inactivated displayed frequent loss of telomeric repeat sequences, enhanced frequencies of chromosome end-to-end associations, and terminal nonreciprocal translocations. These phenotypes correlated with a reduction in the amount of G-strand overhangs at the telomeres and an altered nuclear matrix association of telomeres in these cells. Since the p53-mediated G1 checkpoint is operative in 14-3-3 σ ^{-/-} (gene symbol: *SFN*) cells, the observed chromosomal aberrations occurred preferentially in G2 after exposure to IR, corroborating the role of the 14-3-3 σ protein in G2–M progression. Dhar et al. (2000) also reported that, even in untreated cycling cells, occasional chromosomal breaks or telomere-telomere associations trigger a G2 checkpoint arrest followed by repair of these aberrant chromosome structures before entering M phase. Since 14-3-3 σ ^{-/-} cells are defective in maintaining G2 arrest, they enter M phase without repair of the aberrant chromosome structures and undergo cell death during mitosis. Together, these studies provide evidence for the correlation between a dysfunctional G2–M checkpoint control, genomic instability, and loss of telomeres in human cells mediated by a downstream effector of ATM (Fig. 1).

Mammalian Rad9

The mammalian homologue of *Schizosaccharomyces pombe* Rad9 was isolated in 1996 (Lieberman et al., 1996), and the gene product in human cells is phosphorylated by ATM in response to DNA damage (Chen et al., 2001). Together with hRad1 and hHus1, hRad9 forms a nuclear complex that resembles PCNA (proliferating cell nuclear antigen) and is believed to sense DNA damage (St Onge et al., 1999; Venclovas and Thelen, 2000). However, the cellular colocalization of hRad9 with the phosphorylated form of histone H2AX (γ -H2AX) after DNA damage is independent of ATM function (Greer et al., 2003), raising the possibility that hRad9 may also have a direct DNA damage-sensing function. Recent studies have revealed that

inactivation of mammalian Rad9 increases chromosome end-to-end associations and the frequency of telomere loss (Pandita et al., 2006). This telomere instability correlated with enhanced S- and G2-phase-specific cell killing, delayed kinetics of γ -H2AX focus appearance and disappearance, and reduced chromosomal repair after IR exposure, suggesting that Rad9 plays a role in cell cycle phase-specific DNA damage repair (Pandita et al., 2006). Furthermore, mammalian Rad9 interacted with Rad51, and inactivation of mammalian Rad9 also resulted in decreased homologous recombinational repair, which occurs predominantly in the S and G2 phases of the cell cycle (Pandita et al., 2006). Together, these findings provide evidence for a role of mammalian Rad9 in telomere metabolism and DDR in order to maintain genomic stability.

DNA-PKcs

Gilley et al. (2001) reported that a deficiency in DNA-PKcs (DNA-dependent protein kinase catalytic subunit) severely disrupts telomere capping function but does not affect telomere length. Furthermore, telomerase activity is not affected by a DNA-PKcs deficiency. DNA-PKcs-deficient mouse embryonic fibroblasts also exhibited elevated levels of chromosome fragments and breaks, which correlates with increased telomere fusions. Thus DNA-PKcs is a telomere maintenance protein which functions specifically in telomere capping and not in telomere length control.

Ku

Ku is a heterodimer of Ku70 and Ku80 and is crucial for NHEJ repair (Taccioli et al., 1994; Critchlow and Jackson, 1998; Kanaar et al., 1998). It binds site-specifically to particular DNA sequences (Giffin et al., 1996; Ludwig et al., 1997; Galande and Kohwi-Shigematsu, 1999), functions in site-specific recombination of V(D)J gene segments (Nussenzweig et al., 1996), and plays an important role at the telomere (Boulton and Jackson, 1996; Porter et al., 1996; Gravel et al., 1998; Hsu et al., 1999; Gasser, 2000). During the repair of DNA DSBs, Ku binds nonspecifically with high affinity to DNA ends (Mimori et al., 1986; Paillard and Strauss, 1991; Cary et al., 1997; Dynan and Yoo, 1998). However, telomeric ends are capped or bound by specific telomere proteins that serve to conceal and disguise the telomeric DNA end, thereby preventing telomere end fusion events as well as cellular DNA damage signaling. Several groups have found that Ku is in close proximity to the mammalian telomere and that loss of Ku in virally transformed Ku-deficient mouse cell lines resulted in telomere fusion events (Bailey et al., 1999; Hsu et al., 1999). Ku forms a high-affinity protein/protein interaction with TRF1 to localize to internal regions of telomeric DNA, and thus Ku provides an essential telomere capping function in primary mammalian cells to prevent telomere fusions. Therefore, Ku functions in a different way at the telomere than during DNA DSB repair by the NHEJ pathway.

MRN

The components of the MRN complex (MRE11, RAD50, and NBS1/XRS1) play a critical role in DNA DSB repair in eukaryotes, and in yeast all three MRN components bind telomeres (Lombard and Guarente, 2000; Nakamura et al., 2002; Takata et al., 2005). The role of the MRN complex in telomere maintenance parallels its function in processing DNA DSBs during repair. The MRN complex together with ATM promotes activation of the ATR kinase in the G2 phase post IR exposure. During homologous recombination, the key step in the activation mechanism is a Mre11-dependent resection of DSBs which leads to the formation of a replication protein A (RPA)-coated ssDNA (Jazayeri et al., 2006). A similar situation is observed at yeast telomeres, where Mre11 is implicated in telomere resection and loading of Mec1p during late S phase (Larrivee et al., 2004). These observations indicate that the telomeric function of Mre11 might reflect the role of Mre11 in recognizing and processing of DNA DSBs.

Heterochromatin protein 1 (HP1)

In eukaryotes, there are two major types of chromatin: heterochromatin and euchromatin (Li et al., 2002). Heterochromatin corresponds to the relatively gene-poor, late-replicating, repetitious sequences found near centromeric and telomeric locations. In contrast, euchromatin contains single copy sequences, including the majority of genes, and replicates relatively early in the cell cycle. Euchromatin and heterochromatin are further distinguished by specific histone tail modifications. In addition, euchromatin and heterochromatin show differences in non-histone chromosomal protein constituents. One of the best-studied examples of a non-histone chromatin protein is heterochromatin protein 1 (HP1) initially identified in *Drosophila* and named for its predominant localization to centromeric heterochromatin (Li et al., 2002).

In mammals, there are three HP1 isoforms termed HP1 α , HP1 β (M31, CBX1), and HP1 γ (M32) (Singh et al., 1991; Eissenberg and Elgin, 2000; Jones et al., 2000). All HP1 isoforms share a high degree of sequence similarity although they localize to distinct chromosomal territories; HP1 α and HP1 β are usually found at sites of constitutive heterochromatin, while HP1 γ has a more euchromatic distribution (Saunders et al., 1993; Wreggett et al., 1994). The best studied isoform, HP1 β , is a small 26 kDa protein with two sequence-related domains, the N-terminal chromodomain (CD) and the C-terminal chromoshadow domain (CSD), which are separated by a 'hinge' region (reviewed in Khorasanizadeh, 2004).

Telomeres are associated with the nuclear matrix and are thought to be heterochromatic. In human cells the overexpression of HP1 isoforms HP1^{Hs α} or HP1^{Hs β} results in reduction of the G-strand overhangs and overall telomere sizes (Sharma et al., 2003). Cells overexpressing HP1^{Hs α} or HP1^{Hs β} also display a higher frequency of chromosome end-to-end associations, spontaneous chromosomal damage, and reduced chromosome damage repair than the parental cells (Sharma et al., 2003).

Recent studies have revealed that DNA DSB formation is followed by ATM dependent chromatin relaxation (Ziv et al., 2006). ATM's effector in this pathway is KRAB-associated protein (KAP-1, also known as TIF1b, KRIP-1, or TRIM28) (Ziv et al., 2006). KAP-1 is phosphorylated in an ATM-dependent manner on Ser824 exclusively at DNA damage sites. Knocking down or mimicking constitutive phosphorylation of KAP-1 leads to chromatin relaxation (Ziv et al., 2006) while deletion of the KAP-1 phosphorylation site leads to loss of DSB-induced chromatin decondensation and renders the cells hypersensitive to DSB-inducing agents. These results suggest that chromatin relaxation is a critical regulatory point in the DNA-damage response pathway. KAP-1 also recruits HP1 proteins to form small HP1-containing heterochromatin domains that repress gene activity (Lechner et al., 2000; Schultz et al., 2002), and expression of mutant HP1 β results in abrogation of damage-induced H2AX phosphorylation (Ayoub et al., 2008). Further studies have shown that DNA damage influences the chromatin associated movement of HP1 β , supporting the relationship between HP1 β and DDR (Ayoub et al., 2008). HP1 β colocalizes with methylated lysine 20 of histone H4 (H4K20me3) which is also a binding motif for 53BP1 (unpublished). These studies further support the view that HP1 β influences both telomere metabolism as well as DDR.

Shelterin and DNA damage response

Shelterin is a complex formed by TRF1, TRF2, TIN2, Rap1, TPP1, and POT1, the major telomere-specific proteins that associate with telomeres to protect chromosome ends (Fig. 2) (de Lange, 2005). Three shelterin subunits, TRF1, TRF2, and POT1, directly recognize (TTAGGG)_n repeats. They are interconnected by three additional shelter-in proteins, TIN2, TPP1, and Rap1, forming a complex that allows cells to distinguish telomeres from sites of DNA damage. Shelterin is a dynamic structural component of the telomere and is emerging as a protein complex with DNA remodeling activity that acts together with several associated

DNA repair factors to change the structure of telomeric DNA, thereby protecting chromosome ends. Although telomere interacting proteins TRF2 and POT1, which are part of the shelterin complex, have been linked independently to repression of the activation of two DNA damage response pathways at chromosome ends, it has been a matter of great debate whether these proteins have any role in sensing global DNA damage or play any critical part in DNA DSB repair. Certainly, depletion of both proteins from an in vitro DNA end joining assay had no effect on ligation, thus it is an open question whether they have any specific role in DNA DSB repair in vivo. However, these structural proteins may have a specific role in staging the proteins that are required for the normal stability of telomeres.

Conclusions and future prospects

All organisms respond to interruptions in genomic stability by launching the DDR. DNA damage response can be considered a signaling transduction pathway where the DNA damage is detected by 'sensors' (Fig. 3) that trigger the activation of a transduction system composed of protein kinases and a series of adaptor proteins, some of which are known to interact with telomeres (Figs. 1, 2). This kinase cascade amplifies the initial DNA damage signal and triggers the activation of effector proteins (Figs. 1, 3) (Scott and Pandita, 2006). The end result is a delay in cell cycle progression until the damage has been repaired or the inability to properly repair DNA damage is detected, inducing either apoptosis or senescence. Two pathways for the repair of DSBs, NHEJ and HR, have evolved in eukaryotes (Scott and Pandita, 2006). These pathways, like processes such as transcription and replication, act on DNA of DSBs or telomeres that are embedded in chromatin. A growing body of evidence supports a role for chromatin-modifying and remodeling activities and their proper signaling in DSB repair and telomere maintenance by these pathways. Further evidence for a role of chromatin structure in DNA damage repair comes from studies showing that treatment of cells with agents that induce changes in chromatin structure lead to activation of DNA damage response pathways and enhanced telomerase activity in the absence of DSBs (Bakkenist and Kastan, 2003; Hunt et al., 2007; Agarwal et al., 2008). This indicates that chromatin modulation is an integral component of DDR as well as telomere stability. The connection between chromatin structure and DNA repair has been made, but only limited studies have clearly demonstrated the role of specific chromatin modifications (histone code) in DNA repair processes and telomere maintenance. Although the relationship between telomeres and DDR is highly dynamic, their functionality may be intertwined by specific histone codes, which need to be identified. Deciphering novel epigenetic mechanisms like the histone code behind DDR and telomere stability will add to our understanding of genomic stability, which will be of enormous value for therapeutic research.

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Fig. 1.

Role of ATM in cellular responses. ATM kinase activity increases immediately after exposure to ionizing radiation (IR). ATM mediates the early stages of the rapid induction of several signaling pathways, which include regulation of the cell-cycle checkpoint controls, activation of the DNA-DSB (double-strand break) repair pathway, activation of stress responses, and maintenance of telomeres. 'P' with black solid arrows indicates reported phosphorylation events; dashed arrows represent possible signaling steps and do not imply direct interaction between proteins; 'C' indicates sequestering in cytoplasm; 'R' indicates repair complexes; and 'T' indicates a role for the protein in telomere metabolism. ATM influences telomere function through direct interaction with TRF1. In addition, c-Abl phosphorylates hTERT and negatively regulates telomerase activity. The other downstream effector of ATM in telomere metabolism is 14-3-3σ. Abbreviations: AP-1: Apetala1 transcription factor; BRCA1: breast cancer susceptibility gene product 1; cAbl: Abelson protein tyrosine kinase; Cdc2: cyclinB; CDC25: cell division cycle 25; Cdk2: cyclin-dependent kinase; CHK: checkpoint kinase; CyA, CyB, CyE: cyclinA, cyclin B, cyclin E; FANCD2: Fanconi anaemia protein; hSSB1: human single strand DNA binding protein; hTERT: human catalytic unit of telomerase; JNK: Jun N-terminal protein kinase; KAP1: co-repressor of KRAB associated proein; MDC1: mediator of damage checkpoint 1; MRE11: meiotic recombination 11 gene product; MDM2: mouse double minute 2 (p53-binding protein); NBS1: Nijmegen breakage syndrome 1 protein (p95); RAD50: a radiation-damage-repair-associated protein; SMC1: structural maintenance of chromosome 1; TRF1: telomere-repeat-finding factor 1; MOF and Tip60 are members of the MYST family and have histone acetyltransferase activity, which has been implicated in ATM function.

Fig. 2. Schematic for DNA damage response at dysfunctional telomeres. Shelterin subunits (TRF1, TRF2/Rap1, TIN2/TPP1, and POT1) protect telomeres. Loss of shelterin subunits results in loss of telomere protection. The exact mechanism is not known but it can happen with or without overt change in the structure of the DNA (the latter is depicted). Upon loss of protection, the telomere is then associated with DNA damage response factors. Telomere damage is associated with ATM activation (in absence of ATM, ATR kinase is activated), which can lead to DNA double-strand break (DSB) repair along with p53-dependent G1/S arrest or induction of either apoptosis or senescence.

Fig. 3.

Major regulatory steps in the process of DNA damage response (DDR). DNA double strand break (DSB) repair involves the recognition of damage, modification of chromatin at the site of DNA damage, and recruitment of repair factors accompanied with the processes involved in cell cycle check points. Several proteins have been reported to have multiple functions that are involved in the regulation of the DNA DSB repair, whether the damage requires be repaired by either the nonhomologous end-joining (NHEJ) or homologous recombination (HR) pathway. The most commonly known histone modifications appearing immediately after DNA damage are γ -H2AX foci.