THE EMBO JOURNAL

Focus Review

Taming the tiger by the tail: modulation of DNA damage responses by telomeres

David Lydall*

Centre for Integrated Systems Biology of Ageing and Nutrition, Institute for Ageing and Health and Institute of Cell and Molecular Biosciences, Newcastle University, Tyne and Wear, UK

Telomeres are by definition stable and inert chromosome ends, whereas internal chromosome breaks are potent stimulators of the DNA damage response (DDR). Telomeres do not, as might be expected, exclude DDR proteins from chromosome ends but instead engage with many DDR proteins. However, the most powerful DDRs, those that might induce chromosome fusion or cell-cycle arrest, are inhibited at telomeres. In budding yeast, many DDR proteins that accumulate most rapidly at double strand breaks (DSBs), have important functions in physiological telomere maintenance, whereas DDR proteins that arrive later tend to have less important functions. Considerable diversity in telomere structure has evolved in different organisms and, perhaps reflecting this diversity, different DDR proteins seem to have distinct roles in telomere physiology in different organisms. Drawing principally on studies in simple model organisms such as budding yeast, in which many fundamental aspects of the DDR and telomere biology have been established; current views on how telomeres harness aspects of DDR pathways to maintain telomere stability and permit cell-cycle division are discussed.

Telomeres, the chromosome ends

Telomeres are the natural ends of linear chromosomes and are found on most eukaryotic nuclear chromosomes. When the concept of telomeres was proposed it was suggested that 'the terminal gene must have a special function, that of sealing the end of the chromosome' (Muller, 1938). Of course, at this time, the nature of a gene was not clear and therefore there could have been little idea about the special function that would allow the terminal gene to cap (seal) the chromosome end. Since then much has been discovered about the

*Corresponding author. Institute for Ageing and Health, Newcastle University, Henry Wellcome Laboratory, Newcastle upon Tyne, Tyne and Wear, NE4 5PL, UK. Tel.: +44 191 248 1207; Fax: +44 191 248 1101; E-mail: D.A.Lydall@ncl.ac.uk

nucleic acid and protein constituents of chromosome ends and about the mechanisms by which these structures cap chromosome ends to ensure that telomeres are much more stable and inert than internal chromosome breaks (Blackburn *et al*, 2006). It has also become clear that telomere capping is not only important for maintaining genetic stability and protecting against cancer, but also for permitting cell division and protecting against ageing (Stewart and Weinberg, 2006; Aubert and Lansdorp, 2008; Deng *et al*, 2008; Jeyapalan and Sedivy, 2008). Before describing how telomeres cap chromosome ends it is worth considering how cells respond to chromosome breaks elsewhere.

DNA damage responses to double strand breaks

A double strand break (DSB) is the DNA lesion that most resembles a telomere. All cell types from bacteria with circular chromosomes to human cells with linear chromosomes have evolved a powerful DNA damage response (DDR) to DSBs and other types of DNA damage. DSBs are perhaps the most potent inducers of DDRs because a single unrepaired DSB can lead to loss of DNA distal to the break when chromosomes are segregated to daughter cells. The importance of the DDR is illustrated by the fact that many DDR protein functions are conserved through evolution, in bacteria, archaea and eukaryotes.

In essence, the DDR comprises three coordinated responses: DNA repair pathways reverse lesions in DNA; checkpoint pathways inhibit cell-cycle progression while repair occurs; and apoptosis ensures that cells with high levels of DNA damage are killed rather than permitted to divide and pass on damaged genomes. Two major DNA repair pathways engage with DSBs: non-homologous end joining (NHEJ) fuses broken chromosome ends together, whereas homologous recombination (HR) uses sequence homology to repair DSBs (Frank-Vaillant and Marcand, 2002; Kim *et al*, 2005).

Scores of proteins contribute to cellular responses to DSBs and some of these are listed in Table I. Some proteins engage early with DSBs, others engage later, and with the final repair outcome depending on competition between different repair pathways (Lisby *et al*, 2004; Symington and Heyer, 2006; Kanaar *et al*, 2008). The left part of Figure 1 shows some of the budding yeast proteins binding to a DSB as it undergoes HR repair and the right part shows some of the same proteins and telomere-specific proteins playing roles in telomere maintenance. Interestingly, in budding yeast many of the 'early' DDR proteins at DSBs are involved in physiological telomere maintenance, whereas 'late' DDR proteins seem, generally, to have less of a role in telomere maintenance. Late DDR proteins do have important functions in the case of

Table I DNA Damage response proteins and telomeres

Budding yeast	Human cells	DSB arrival	Telomere length	Biochemical function
Rad50	Rad50	Early	Short	Mre11, Rad50 and Xrs2 complex, functions in
Mre11	Mre11	Early	Short	meiotic recombination, checkpoint signalling
Xrs2	Nbs1	Early	Short	
Tel1	ATM	Early	Short	PIKK recruited by Mre11 complex to DSBs
Sae2	CTIP		Normal	Nuclease recruited to DSBs
Yku70	Ku70	Early	Short	Ku70/Ku80 heterodimer, which functions in NHEJ
Yku80	Ku86	Early	Short	
Lig4	Lig4	Late	Normal	DNA ligase for NHEJ
Mec1	ATR	Late	Normal	Mec1, a PIKK and Ddc2, recruited to RPA-coated ssDNA
Ddc2	ATRIP	Late		
Chk1	Chk1		Normal	Downstream checkpoint kinase
Rad53	Chk2		Normal	Downstream checkpoint kinase
Exo1	Exo1	Late	Normal	5′–3′ exonuclease, DSB and mismatch repair
Sgs1	BLM/WRN	Late	Normal	Helicase
Dna2	Dna2			Helicase/nuclease required for DNA replication
Rad51	RAD51	Late	Normal	RecA orthologue, HR strand exchange
Rad52	RAD52	Late	Normal	Required with Rad51 during HR
Rad9	53BP1	Late	Normal	Checkpoint mediator, binds near DSBs
Rad17	Rad1	Late	Normal	Checkpoint, components of 911 complex loaded at DSBs
Ddc1	Rad9	Late	Normal	
Mec3	Hus1	Late	Normal	
Rad24	Rad17	Late	Normal	Checkpoint, Replication Factor C type subunit, works with small RFC subunits to load 911 complex

telomere failure, either in back up mechanisms of telomere maintenance, such as alternative lengthening of telomeres (ALTs), or inhibiting cell-cycle progression if telomeres are uncapped (Lydall and Weinert, 1995; Enomoto et al, 2002; Lundblad, 2002; IJpma and Greider, 2003).

The simplest way to repair a DSB is to fuse the ends back together, and this is essentially what the NHEJ repair pathway does. NHEJ depends on little or no homology between the ends being joined, and therefore carries the risk the DNA ends that were not adjacent previously may be joined, causing a chromosomal translocation. Different eukaryotic organisms use variations on a core set of proteins to perform NHEJ. DSBs are recognised by the Ku heterodimer, which forms a ring and binds DSB ends with high affinity and is an early recruit to DSBs (Figure 1C). In many eukaryotes, Ku interacts with accessory factors, such as DNA PK (DNA-dependent protein kinase), a PI3-kinase-like protein kinase (PIKK), XRCC4 and polymerases and nucleases that fill in or trim DNA ends before ligation. In all eukaryotes DNA ligase IV is required to fuse (ligate) the ends. In budding yeast, the Mre11 complex contributes to NHEJ, and recent evidence suggests that the Mre11 complex also contributes to NHEJ in mammals (D'Amours and Jackson, 2002; Deriano et al, 2009).

The other major route to repair DSBs is HR. HR has the advantage that homology between the DSBs site and homologous DNA sequences, mean that repair by HR can occur without error, with complete fidelity. Recently, there has been much progress in dissecting some of the early steps of HR (Gravel et al, 2008; Mimitou and Symington, 2008; Raynard et al, 2008; Zhu et al, 2008). Some early aspects of HR are indicated in Figure 1A, C, E and G. The Mre11 complex, comprising Mre11, Rad50 and Xrs2 in budding yeast, is recruited rapidly to DSB ends, at about the same time as the Ku complex. The Mre11 complex does two things at the lesion, it recruits the PIKK, Tel1 (orthologue of ATM), which signals cell-cycle arrest through its kinase activity. Tel1 as the name suggests contributes to telomere homeostasis. Second, and in combination with Sae2/CTIP, the Mre11 complex is important for initiating resection to generate ssDNA, an important intermediate in HR. Resection further from the break appears to be due to several complementary nuclease and helicase activities, including Dna2, Exo1 and Sgs1. Dna2 and Exo1 possess nuclease activity and Dna2 and Sgs1 helicase activity. ssDNA generated at DSBs is bound by the hetero-trimeric ssDNA-binding complex, replication protein A (RPA). RPA is important for regulating recruitment of HR proteins such as Rad52 and Rad51, and checkpoint proteins.

During HR repair, checkpoint pathways recognise that repair is incomplete and signal cell-cycle arrest. Several checkpoint proteins bind close to DSBs. The Mre11/Tel1 complex contributes to a weak checkpoint pathway, termed the TM pathway (Usui et al, 2001). A complementary and more potent checkpoint pathway depends on a different PIKK, Mec1 (ATR), which binds with its partner Ddc2 to RPA-coated ssDNA. Full checkpoint kinase activation and cell-cycle arrest after DSBs also depend on the loading of the 911 complex, comprising Rad17, Mec3 and Ddc1 in budding yeast, which is loaded onto to DNA by the Rad24/RFC complex, comprising Rad24 and the four small Replication Factor C subunits (Figure 1G) (Majka et al, 2006). Rad9, another checkpoint protein, binds in the vicinity of DSBs, in part, through its interaction with chromatin and
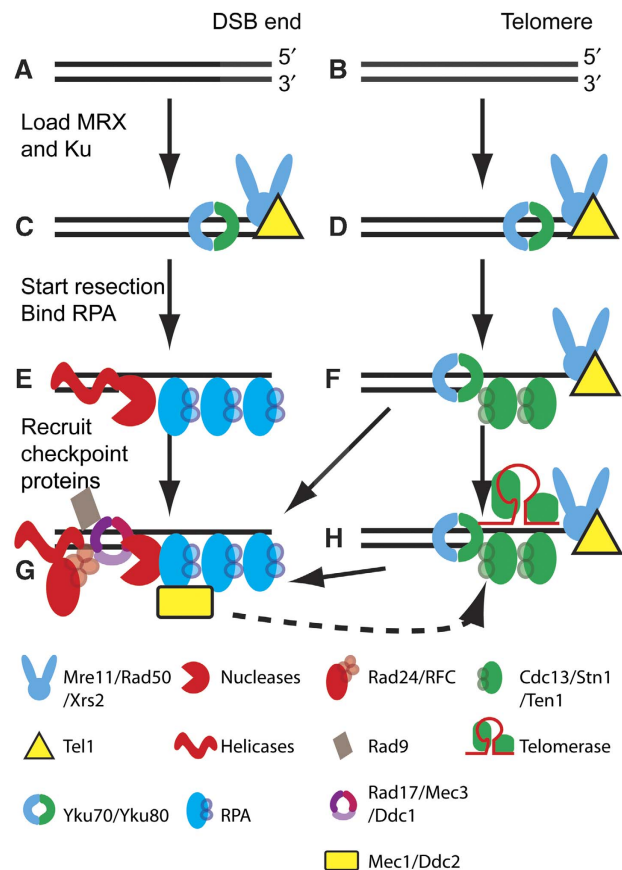
Figure 1 DDR proteins at budding yeast telomeres and DSBs. (A, C, E, G) show the recruitment of DNA damage response proteins to a DSB undergoing HR. (B, D, F, H) show the role of DDR and telomere-capping proteins in forming a capped telomere. (A) A blunt ended DSB. (B) A leading strand telomere after DNA replication. (C) Rapid recruitment of Mre11, Rad50 and Xrs2, Tel1 and Yku70/Yku80 to DSBs. (D) Rapid recruitment of Mre11, Rad50, Xrs2, Tel1 and Yku70/Yku80 to a telomere. (E) Nuclease- and helicase-dependent production of ssDNA generates a substrate for RPA binding. (F) Telomeric (G rich) ssDNA, which is partially Mre11 dependent, provides a substrate for Cdc13, Stn1 and Ten1 binding. (G) RPA-coated ssDNA helps recruit not only HR proteins such as Rad51/Rad52 (not shown) but also checkpoint proteins Rad24, the Rad17, Mec3, Ddc1 heterotrimeric ring. Mec1 and, its partner, Ddc2 bind RPA and help contribute to kinase-dependent signal transduction cascades that can lead to not only cell-cycle arrest, but also a capped telomere (dashed line between G and H). Rad9, essential for signalling cell-cycle arrest at DSBs and *cdc13-1* uncapped telomeres, is recruited in part through the interaction with the methylated histone H3 lysine 79. (H) Telomerase is recruited to telomeres, in part, through interactions with Yku80, and with Cdc13.

Figure 2 Telomeres and DSBs. A cartoon showing a DSB, in the centre, and two telomeres. The rays emanating from each type of end illustrate the potency of each type of end for inducing DNA damage responses, such as DNA repair and cell-cycle arrest. Telomeres can be estimated to be at least a 1000-fold less potent inducers of cell-cycle arrest compared with DSBs.

It is possible to make a crude calculation of the comparative checkpoint stimulating activities of telomeres versus DSBs. A single un-repaired DSB in the budding yeast genome causes cell-cycle arrest before entry into anaphase for many generation times (Sandell and Zakian, 1993; Michelson et al, 2005). Budding yeast has 16 chromosomes and if a DSB were made in G1 and replicated without being repaired, this could lead to a maximum of four internal ends in G2 but the same yeast cell would contain 64 inert telomeric DNA ends, which do not induce arrest. Therefore, telomeres in yeast must be at least 16 times less potent than DSB-induced ends in activating cell-cycle arrest. Furthermore, if native telomeres induced even a 1% cell-cycle delay such growth-inhibited cells would be out competed by cells that better hid chromosome ends from the DDR. An induced DSB induces arrest for several generation times (Michelson et al, 2005), which can be conservatively calculated as a four-fold or 400% cell-cycle delay. Therefore, it seems reasonable to estimate that a budding yeast DSB is $64/4 \times 400$ or 6400-fold more potent stimulator of checkpoint pathways than a telomere. A conservative estimate is therefore that a budding yeast telomere is at least a 1000-fold less potent than an internal chromosome end at inducing cell-cycle arrest (Figure 2).

Harnessing DNA damage responses for telomere maintenance

One way to ensure that telomeres do not activate DDRs would be to exclude DDR proteins from telomeres. Powerful biological damage defence responses are sometimes excluded from specific locations as a mechanism of attenuating responses that might do more harm than good. For example, the potentially harmful effects of the immune response are limited in organs such as the eye and brain (Ferguson et al, 2002; Streilein, 2003; Caspi, 2006). Although DDRs are clearly attenuated at telomeres, it is evident that rather than being excluded from telomeres many DDR proteins bind telomeres and indeed have critical functions in telomere maintenance and physiology, as well as in cellular responses to telomere uncapping.

Why do DDR proteins bind and have such important functions at telomeres rather than being excluded from the ends of chromosomes? Perhaps the major reason is simply that it would be too difficult to exclude DDR proteins from telomeres given the clear requirement that DDR proteins have

methylated histone, H3K79, and phosphorylated, H2A(X), and is also critical for cell-cycle arrest in response to DSBs.

The DDR, similar to many biological defence mechanisms, is potentially harmful. For example, DNA repair enzymes often transiently amplify DNA damage during the process of DNA repair, for example, during resection of DSBs undergoing HR repair. Similarly, if the DDR responded to telomeres as it does to DSBs elsewhere in the genome, chromosome ends might be 'repaired' by NHEJ leading to chromosome fusions and/or activate checkpoint pathways, leading to perpetual cell-cycle arrest. For these reasons it is critical that functional telomeres do not stimulate the most powerful DDRs.
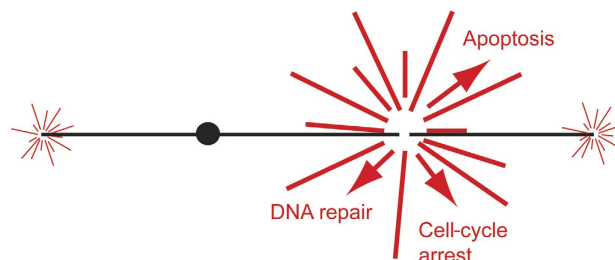
high-affinity for DNA damage elsewhere in the genome. In addition, passage of the replication fork, which is associated with many DDR proteins, through telomeres would make it difficult to exclude DDR proteins from telomeric ends.

In principal, telomere-capping proteins and DDR proteins have opposing goals when interacting with chromosome ends. The goal of telomere-capping proteins is to stably maintain the DNA end at telomeres, whereas the goal of DDR proteins is to 'repair' these ends. However, the evidence suggests that telomere-capping proteins and DDR proteins have evolved to work together, interdependently, to maintain telomere and genetic stability. Thus, one view is that telomeres have evolved to harness the power of the DDR pathways at chromosome ends. If so, then perhaps telomeres, similar to a skilled judo practitioner, use their opponent's weight, strength and momentum for their own benefit or, as suggested by the title of this review, the most powerful, tiger-like aspects of the DDR are tamed at telomeres.

NHEJ, HR and checkpoint proteins all play roles in physiological telomere maintenance. DDRs occur in the context of chromatin, and vary through the cell cycle, as does the function of telomere-capping proteins. However, for simplicity the effects of the cell cycle and chromatin on DDRs at the telomeres will only be superficially discussed in this review. A number of complementary reviews on the interplay between DDRs and telomere function have been published recently and are excellent sources for alternative and more detailed views on these and other relevant areas of telomere biology (Verdun and Karlseder, 2007; Longhese, 2008; Rog and Cooper, 2008; Sabourin and Zakian, 2008).

Diversity in telomere structures

To understand the roles of DDR proteins at telomeres it is necessary to understand telomere structure. Many eukaryotic telomeres are similar in structure, including human and yeast telomeres (Blackburn et al, 2006). However, some radically different approaches to telomere capping are used by organisms like *Drosphila,* which does not use telomerase (Louis and Vershinin, 2005). In human and yeast cells, telomeric DNA is composed of G-rich repetitive sequences with the G-rich strand ending in a 3′ single stranded DNA overhang (Figure 3A). The chromosomal 'end replication problem', which conventional DNA replication enzymes cannot replicate the end of linear DNA molecules, is solved in yeast and human cells using telomerase, a reverse transcriptase-like enzyme, which synthesises G-rich DNA without the need for an DNA template (Greider and Blackburn, 1985). The 3′ overhang structure at telomeres provides not only a suitable substrate for telomerase (Lingner and Cech, 1996) but also resembles one half of a resected DSB, an intermediate in HR repair (Figure 3B). In many eukaryotes, including human cells, the 3′ overhang can invade the double stranded DNA, to form a t loop, which is similar to the d loop, an intermediate in HR (Griffith et al, 1999)(Figure 3C). The t loop structure probably contributes to protecting the end of the chromosome from the DDR. Other structures, such as G quartets, which form in G-rich DNA, are likely to form at telomeres and may also contribute to telomere function.

Recent experiments show that 3′ overhangs are not found in all organisms that use telomerase to maintain telomere length. *Caenorhabditis elegans*, which expresses telomerase,
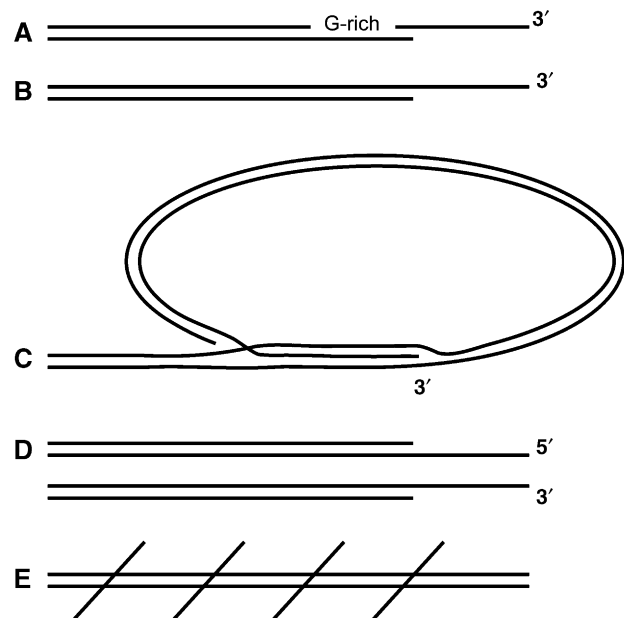

Figure 3 Diverse chromosome end structures. (**A**) A telomere chromosome end as found in yeast and mammalian cells. The G-rich 3′ strand is maintained by telomerase activity, which overcomes the end replication problem. The complementary C-rich strand is maintained by conventional DNA replication machinery. (**B**) A DSB in the process of HR, after processing to generate a 3′ overhang, an intermediate in the HR repair process. (**C**) A t loop, found at the end of mammalian telomeres when the 3′ G-rich overhang at telomeres invades double stranded DNA. (**D**) *C. elegans* telomeres, maintained by telomerase, contain both 3′ and 5′ ssDNA overhangs. (**E**) Drosophila telomeres containing arrays of transposons at chromosome ends. The diagonal lines represent the junctions between individual repeats.

contains 5′ and 3′ overhangs at telomeres (Figure 3D) (Raices et al, 2008). Furthermore, the fruit fly *Drosophila* does not express telomerase and instead large transposon element arrays are found at its chromosome ends (Figure 3E) (George et al, 2006). It is not yet established whether single strand overhangs or t loops are found at telomeres in *Drosophila* but it seems that there needs be no special DNA sequence at *Drosophila* telomeres and that therefore the telomeres are epigenetic in nature (Rong, 2008). Overall, when the spectrum of functional telomeric DNA structures is considered, it is perhaps to be expected that the role of DDR proteins at telomeres will be numerous and diverse.

Gain and loss of telomeres

Chromosome and telomere structure are very stable over evolutionary time periods. For example, the chromosomal structure of apes and humans is very similar, with evidence for just a single chromosome fusion event occurring during evolution (Hartl and Jones, 2009). However, chromosome stability is most likely a product of the stabilising influence of meiosis and sexual cycles, which act to limit chromosomal changes, rather than because of the innate stability of chromosome and/or telomere structures. In some organisms, such as *Tetrahymena* and in the largely non-sexual yeast *Candida*, in tumours and in the laboratory, chromosomal structure and hence telomere position can be remarkably

flexible (Maser and DePinho, 2002; Maringele and Lydall, 2004b; Blackburn *et al*, 2006; Murnane, 2006; Titen and Golic, 2008; Polakova *et al*, 2009). Indeed, the enzyme telomerase was first purified from *Tetrahymena*, a protozoan organism that as part of its developmental programme divides its large chromosomes into thousands of smaller segments each of which needs telomeres to be added *de novo* (Blackburn *et al*, 2006). Therefore, it is clear that eukaryotic cells can readily gain or lose telomeres.

Most eukaryotic genomes are linear and most bacterial genomes circular but this is not universally the case, for example the bacteria that causes Lyme disease, *Borrelia* contains linear chromosomes (Tourand *et al*, 2007). Furthermore, in the laboratory, linear chromosomes can become circular and *vice versa*. For example, when fission yeast *Schizosaccharomyces pombe*, which contains three linear chromosomes, is cultured in the absence of telomere maintenance mechanisms, such as the telomere-capping protein, Pot1, chromosomes can become circularised (Baumann and Cech, 2001). In the opposite direction, the bacterium *Escherichia coli*, which contains a single circular chromosome, can be manipulated to grow with a linear chromosome by the insertion of N15 phage telomeres (Cui *et al*, 2007). Thus, it seems that in principal both bacteria and eukaryotes could live with either linear or circular chromosomes, and that all cell types are very flexible with respect to chromosomal structure.

Telomere length homeostasis

In all organisms telomeres need to be resistant to DNA repair activities, such as nucleases and ligases that are active at DSBs. In addition, in dividing cells, the end replication problem needs to be solved and telomeres should not stimulate checkpoint pathways. Many organisms, including human, yeast and *Tetrahymena* use telomerase to solve the end replication problem (Blackburn *et al*, 2006). Telomerase adds telomeric DNA to chromosome ends without the need of a chromosomal DNA template, and therefore telomerase activity needs to be properly regulated to ensure that telomeres do not extend indefinitely because, if for no other reason, generating lots of unnecessary telomeric DNA this would be extremely wasteful. Telomerase does not act on every telomere in every cell cycle, rather telomerase is activated stochastically with short, rather than long, telomeres being the most likely substrates for telomere extension (Teixeira *et al*, 2004; Chang *et al*, 2007). A balance between telomerase inhibitory activities, regulated in part by telomeric length, and telomerase recruiting/activating activities regulate telomere length (Bianchi and Shore, 2008). DNA repair activities also contribute to telomere length homeostasis, for example telomere rapid deletion can reduce the size of very long telomeres (Li and Lustig, 1996; Bucholc *et al*, 2001; Pickett *et al*, 2009). Therefore, telomere length measurement is a simple readout of all the competing activities that contribute to telomere maintenance. Mutant cells with a phenotype of short telomeres could, for example, be less efficient at recruiting telomerase to short telomeres or less efficient at inhibiting the DNA repair activities that act to reduce telomere length. Mutant cells with a phenotype of unusually long telomeres might promiscuously engage telo-

merase activity or fail to engage telomere rapid deletion pathways.

How telomeres inhibit DNA damage responses

When telomeres fail and become uncapped telomeric DNA ends can induce all three major consequences of the DDR at DSBs—HR, NHEJ and checkpoint activation. The mechanisms by which functional telomeres inhibit downstream DDRs are unclear but most likely depend on telomeric DNA sequences, the proteins localised at or near telomeres, location (e.g. nuclear periphery) and structures of telomeric DNA. Elegant experiments in yeast clearly show that a DSB placed adjacent to a telomere is a much less potent inducer of cell-cycle arrest than a DSB placed internally in the chromosome (Michelson *et al*, 2005).

In mammalian cells a stable complex of six proteins, TRF2, TRF1, TIN2, Rap1, TPP1 and POT1, collectively termed Shelterin, binds telomeric DNA (de Lange, 2005). As the name suggests, Shelterin protects the ends of chromosomes from being recognised as DSBs, but in addition components of Shelterin both inhibit and activate telomerase activity (de Lange, 2005; Wang *et al*, 2007). Analogous proteins bind telomeric DNA in all organisms, these include Taz1 and Pot1 in fission yeast (Cooper *et al*, 1997; Baumann and Cech, 2001) and Rap1 and Cdc13 in budding yeast. In *Drosophila*, HOAP and HP1 do not bind specific telomeric sequences, but bind the ends of chromosomes to mark them epigenetically as 'telomeres' (Cenci, 2009). In the absence of many of these proteins, NHEJ, HR and checkpoint-dependent cell-cycle arrest can be induced.

Cdc13, Stn1, Ten1 are three essential budding yeast proteins, which bind telomeric ssDNA and behave like a telomere-specific RPA complex (Gao *et al*, 2007) (Figure 1). Cdc13 has at least two roles at telomeres—recruiting telomerase to chromosome ends and protecting chromosome ends from numerous DDR activities (Nugent *et al*, 1996; Jia *et al*, 2004; Zubko *et al*, 2004). One way that Cdc13, Stn1 and Ten1 protect against DDR pathways is that they outcompete the RPA complex for binding to telomeric ssDNA (Figure 1). Presumably, the lower levels of RPA near telomeres reduce the recuitment of DNA repair proteins such as Rad52 and Rad51 and also reduce the likelihood that RPA-bound ssDNA recruits and activates the Mec1/Ddc2-dependent checkpoint kinase cascade. Interestingly, in certain circumstances when aspects of the DDR are disabled or telomeres are being maintained by ALT-like mechanisms, cells can grow in the absence of these normally essential telomere-specific ssDNA-binding proteins (Larrivee and Wellinger, 2006; Petreaca *et al*, 2006; Zubko and Lydall, 2006).

In budding yeast, the essential protein, Rap1, binds telomeric dsDNA, as well as other genomic locations, for example at mating type loci (Shore and Nasmyth, 1987). Rap1 in turn interacts with two other non-essential proteins, Rif1 and Rif2, and these proteins seem to inhibit telomerase activity because *rif1Δ* and *rif2Δ* mutants contain very long telomeres (Wotton and Shore, 1997). Part of the mechanism by which Rif1 and Rif2 inhibit telomere lengthening is by inhibiting the Tel1 protein from binding telomeres (Hirano *et al*, 2009). Rap1 binding at telomeres inhibits NHEJ (Pardo and

Marcand, 2005). In budding yeast, systematic genome-wide screens have identified approximately 300 gene products that affect telomere length homeostasis, showing that telomere length control is complex and regulated by numerous processes and pathways (Askree *et al*, 2004; Gatbonton *et al*, 2006; Shachar *et al*, 2008). Interestingly, among the many proteins that affect telomere lengths are two critically important DDR protein complexes, the Ku complex and the Mre11 complex, which are discussed below in more detail.

Mre11 complexes and telomere homeostasis

In budding yeast, the Mre11/Rad50/Xrs2 complex (Mre11, Rad50 and Nbs1 in mammals) is recruited early to DSBs and is involved in HR, NHEJ and checkpoint activation (D'Amours and Jackson, 2002; Lisby *et al*, 2004; Sabourin and Zakian, 2008) (Table I; Figure 1). The Mre11 complex interacts with many other DDR proteins to contribute to these three different responses to DSBs. In all organisms examined, the Mre11 complex has roles in telomere maintenance. For example, loss of Mre11 results in a short telomere phenotype in budding yeast and human cells (Ritchie and Petes, 2000; Ranganathan *et al*, 2001) and high levels of telomere fusions in *Drosophila* (Bi *et al*, 2004; Ciapponi *et al*, 2004).

To activate checkpoint pathways, the Mre11 complex component, Xrs2 (Nbs1) protein, interacts through its C terminus with the PIKK, Tel1 (ATM) (Nakada *et al*, 2003). Tel1 and the Mre11 complex seem to act in a single pathway for telomere length maintenance in budding yeast because single and double mutants have similarly short telomere lengths (Ritchie and Petes, 2000). However, in other contexts, for example DSB repair and meiosis, the Mre11 complex functions independently of Tel1 (Ritchie and Petes, 2000; Carballo *et al*, 2008). Sae2, which works with the Mre11 complex to process meiotic DSBs and to regulate resection at meiotic and mitotic DSBs, seems to have little role at telomeres because *sae2Δ* mutants show, in contrast to *mre11Δ* and *tel1Δ* mutants, a minor telomere length phenotype in yeast (Kim *et al*, 2008).

There are at least two ways in which the Mre11 complex could contribute to maintain telomere length homeostasis. The Mre11 complex could increase telomerase activity at chromosome ends, contribute to telomere capping or both. There is evidence for both. In budding yeast, genetic experiments show that Mre11 and telomerase work in the same pathway for telomerase activation at telomeres (Nugent *et al*, 1998). Interestingly, in *Drosophila*, which caps telomeres in the absence of any telomerase activity, loss of Mre11 or ATM leads to a failure of telomere capping and telomere fusion (Bi *et al*, 2004; Ciapponi *et al*, 2004). These observations in *Drosophila* clearly show that Mre11 and ATM have roles in telomere capping that are completely independent of telomerase activity. In budding yeast, there is also evidence for the Mre11 complex having a role capping telomeres in *cdc13-1* mutants, defective in an essential telomere-capping protein (Foster *et al*, 2006). In *Drosophila*, the Mre11 complex and ATM contribute to capping by providing a substrate for binding of the heterochromatin factors HP1 and HOAP binding (Cenci, 2009).

In yeast and mammalian cells, the Mre11 complex is involved in making the 3′ overhang at the end of telomeres (Larrivee *et al*, 2004; Chai *et al*, 2006). Mre11-independent mechanisms to generate 3′ overhangs also exist and different mechanisms for 3′ overhang generation most likely occur on leading and lagging strand telomeres (Lydall, 2003). The 3′ overhang may help chromosome capping in at least three ways: first a 3′ overhang, rather than a blunt end, is a necessary substrate for telomerase (Lingner and Cech, 1996); second, as mentioned earlier, essential telomere-capping proteins, such as Cdc13/Stn1/Ten1, in budding yeast bind to the ssDNA telomeric overhang (Gao *et al*, 2007); third, a 3′ overhang is necessary to form a t loop. In yeast, Mre11 is necessary for recruiting Tel1 specifically to short telomeres and for telomere extension at these short telomeres (Hector *et al*, 2007; Sabourin *et al*, 2007). The Tel1 pathway inhibits NHEJ at telomeres (Chan and Blackburn, 2003). In mammals, the Mre11 complex binds mammalian telomeres every cell cycle and contributes to telomere length control (Verdun *et al*, 2005; Verdun and Karlseder, 2006; Wu *et al*, 2007). In summary, the Mre11 complex is likely to have numerous roles at telomeres and seems to have evolved to have different roles in different organisms.

Tel1/ATM and Mec1/ATR redundancy at telomeres

In budding yeast, fission yeast and *Drosophila* there is evidence that the Tel1 (ATM) and Mec1/(Rad3, ATR) PIKKs have redundant roles in telomere maintenance. *In vitro* the two PIKKs are capable of phosphorylating a very similar range of substrates but the kinases have different roles *in vivo* (Baldo *et al*, 2008). In budding yeast, *mec1 tel1* double mutants are incapable of maintaining telomeres and, similar to telomerase deficient cells, use recombination-dependent mechanisms of telomere elongation (Ritchie *et al*, 1999). Similarly, in fission yeast, *rad3 tel1* double mutants are incapable of maintaining telomeres and generate survivors by creating circular chromosomes (Naito *et al*, 1998). In *Drosophila*, ATM and ATR function together to help recruit the heterochromatic factors HOAP and HP1 to telomeres (Oikemus *et al*, 2006; Rong, 2008).

Interestingly, in budding yeast, *mec1* mutants have a very minor effect on telomere length, whereas *tel1* mutants have profound effects on telomere length (Longhese *et al*, 2000). This is in contrast to the relative importance of the two proteins in signalling checkpoint-dependent cell-cycle arrest, in which Mec1 has a much more important function (Usui *et al*, 2001; Mantiero *et al*, 2007). The telomere-length phenotype of the double mutants could perhaps best be explained if Mre11/Tel1 complex bind and become activated at blunt telomeres or telomeres with short telomeric ssDNA regions, when Cdc13, Stn1 and Ten1 bind the G-rich ssDNA (Figure 1D, F, H). The preferential binding of Cdc13, Stn1 and Ten1 to G-rich ssDNA at telomeres would help inhibit Mec1/Ddc2 binding, because Mec1/Ddc2 has high affinity for RPA-coated ssDNA. However, if telomeres shorten further, because for example the Mre11 complex is non-functional, there is a higher probability that RPA binds ssDNA near telomeres (intriguingly, Rpa2 and Rpa3 have higher affinity for telomeric ssDNA than random DNA (Gao *et al*, 2007)). At such telomeres, Mec1/Ddc2 will bind near telomeres and

can, assuming resection is not excessive, contribute as a back up mechanism for telomere protection that only engages if Tel1 fails to recruit telomerase activity to short telomeres (dashed arrow between Figure 1G and H). If the two PIKKs, Mec1/Rad3 and Tel1/ATM are missing, chromosomes circularise (fission yeast), or engage recombination-dependent, telomerase-independent, pathways of telomere maintenance (budding yeast) (McEachern and Haber, 2006).

The KU dimer and telomere homeostasis

Ku is an abundant heterodimeric ring protein, comprising Ku70 and Ku80, with high affinity for DSBs. Ku, similar to the Mre11 complex discussed above, is an early recruit to DSBs (Frank-Vaillant and Marcand, 2002; Kim *et al*, 2005; Wu *et al*, 2008) (Figure 1). Ku contributes to NHEJ but seems to inhibit HR, in part, by inhibiting resection of DSBs to create the 3′ ssDNA that is an intermediate in HR (Lee *et al*, 1998; Barlow *et al*, 2008). Ku has a role in telomere maintenance in budding yeast, mice, human cells, *Drosophila* and indeed all organisms examined (Myung *et al*, 2004; Fisher and Zakian, 2005; Celli *et al*, 2006). Ligase IV, which ligates DNA molecules during NHEJ, does not seem to have a role at telomeres because unlike *yku70Δ* and *mre11Δ* mutants, *lig4Δ* mutants show no telomere length phenotype (Teo and Jackson, 1997). This shows that it is not NHEJ *per se* that contributes to telomere length homeostasis.

Interestingly, the effect of Ku on telomere length in different organisms can vary dramatically. In budding and fission yeast, human cells and trypanosomes, the effect of deleting Ku is reduction in telomere length, often dramatically. Although in *Arabidopsis* and *Drosophila*, the effect of deleting Ku is to lengthen telomeres (reviewed in (Fisher and Zakian, 2005)). Therefore, it can be inferred that Ku performs different functions at telomeres in different organisms.

In budding yeast, Ku performs at least four functions at telomeres. (1) Ku binds telomerase *TLC1* RNA and is important for recruiting telomerase to telomeres (Stellwagen *et al*, 2003). (2) Ku protects telomeres from nuclease activities and this helps stop telomeres undergoing recombination or stimulating checkpoint-dependent cell-cycle arrest (Fellerhoff *et al*, 2000; Maringele and Lydall, 2002). (3) Ku is required for the silencing of telomeres, helping recruit the Sir2/3/4 complex (Boulton and Jackson, 1998). (4) Ku is required to localise some telomeres to the nuclear periphery (Laroche *et al*, 1998).

Given the numerous roles for Ku at budding yeast telomeres it is not surprising that not all of the roles of Ku are conserved and that the effects of deleting Ku in different organisms vary. For example, if in plants and *Drosophila*, Ku ensures that telomerase is inhibited or transposons move less readily to chromosome ends, this would explain telomere the length increases observed in these organisms when Ku is inactivated (Riha and Shippen, 2003; Melnikova *et al*, 2005).

Interplay between Ku and Mre11 complexes

The Ku and MRX complexes, which are early and independent recruits to DSBs (Figure 1), have redundant roles in telomere capping in budding yeast. When the Ku complex and the MRX complex are simultaneously deleted from budding yeast, cells no longer maintain telomere length using telomerase and instead use HR-dependent mechanisms to maintain telomere length (analogous to *mec1Δ tel1Δ* mutants above) (DuBois *et al*, 2002; Maringele and Lydall, 2004a). Furthermore, both Ku and MRX seem to have independent roles to the essential Cdc13, Stn1 and Ten1 complex in telomere capping because they contribute to the robust growth of temperature-sensitive *cdc13-1* mutants at permissive temperatures (Polotnianka *et al*, 1998; Addinall *et al*, 2008).

HR proteins and telomere homeostasis

In budding yeast, proteins recruited late to DSBs, such as Rad51 and Rad52, tend to have a lesser or non-detectable role in telomere length homeostasis. Two genome-wide screens in budding yeast showed that about 300 to 4500 non-essential budding yeast genes have important functions in telomere length maintenance. The Ku and MRX complexes were identified as important for telomere length homeostasis but interestingly very few other DNA repair genes involved at DSBs were identified by these systematic screens (Askree *et al*, 2004; Gatbonton *et al*, 2006; Shachar *et al*, 2008). However, in mammals there is a role for HR proteins in telomere homeostasis (Tarsounas *et al*, 2004).

Checkpoint proteins and telomere homeostasis

In budding yeast, many of the DNA damage checkpoint proteins including the 911 complex (Rad17, Mec3 and Ddc1), the clamp loader (Rad24, RFC) and Rad9 have comparatively small effects on telomere length in comparison with Ku or Mre11 proteins and are late recruits to DSBs (Longhese *et al*, 2000) (see Figure 1). However, all of these checkpoint proteins have critical roles in responding to uncapped telomeres as discussed below (see *cdc13-1* below). However, in *C. elegans*, genes encoding components of the 911 complex and its clamp loader have critical roles maintaining telomere length (Boerckel *et al*, 2007). In *C elegans*, telomere loss rates in *telomerase* mutants, *911* mutants and *telomerase 911* double mutants, suggest that telomerase and the 911 complex belong in the same pathway of maintaining telomere length (Boerckel *et al*, 2007). Indeed, *MRT2*, encoding the Rad1 component of the 911 complex, was first identified on its telomere loss phenotype (Ahmed and Hodgkin, 2000). In mouse and human cells, the 911 complex also has critical roles in telomere maintenance (Francia *et al*, 2006).

Telomerase limitation

Understanding DNA repair and checkpoint activation at uncapped telomeres in human cells is critically important for understanding carcinogenesis and ageing. In most human somatic cells insufficient telomerase is expressed to counteract the end replication problem. Consequently, in these cells telomeres shorten with each cell division until telomeres reach a critically short length, are no longer functional and are perceived like DSBs (Takai *et al*, 2003; d'Adda di Fagagna *et al*, 2003). This leads to p53- and p21-dependent cell-cycle arrest in G1 (Artandi and Attardi, 2005). This checkpoint-dependent cell-cycle arrest induced by short telomeres is thought to act as a tumour suppressor mechanism because it acts to limit indefinite cell division. In the absence of

proficient checkpoint controls (e.g. in p53 defective cells) cells continue to divide with ever shorter telomeres and high levels of genetic instability are induced, presumably as chromosome ends become less and less 'telomeric' and more DNA repair events, such as NHEJ, lead to chromosome fusions and subsequent breakage fusion bridge cycles (Artandi and Attardi, 2005). Short telomeres seem to be the substrates for DNA repair events that lead to telomere fusion events (Capper *et al*, 2007). After a period of 'crisis' characterised by high levels of genetic instability, comparative genetic stability is often restored in cancer cells when telomerase is reactivated or ALT mechanisms are engaged (Stewart and Weinberg, 2006; Cesare and Reddel, 2008).

Experiments in yeast may shed light on the variety of plausible ALT mechanisms because yeast can be genetically manipulated to grow in the absence of telomerase. In such yeast cells telomeres shorten and cells enter a period of crisis, when growth is slow and checkpoint pathways are activated by the dysfunctional telomeres (Enomoto *et al*, 2002; IJpma and Greider, 2003). However, after this period of slow growth, yeast cells often regain the ability to extend telomeres by using HR (*RAD52*)-dependent mechanisms (Lundblad, 2002; McEachern and Haber, 2006). Very recent experiments show that the requirement for HR in contributing to ALT can vary between different strain backgrounds (Grandin and Charbonneau, 2009; LeBel *et al*, 2009). Further experiments in budding yeast have also shown that when telomerase, HR and other aspects of the DDR are inactived (*rad52∆, exo1∆, mre11∆, sgs1∆*), budding yeast cells are often able to divide without any discernable mechanisms for capping chromosome ends (Maringele and Lydall, 2004b, 2005; Lee *et al*, 2008). Such cells cannot divide indefinitely, losing DNA at chromosome ends because of the end replication problem and nuclease activities, and therefore to counteract loss of DNA from chromosome ends, large palindromes often form at chromosome ends.

In budding yeast, Exo1, which is a major nuclease involved in generating ssDNA at DSBs and uncapped telomeres, also accelerates the rate at which telomerase deficient budding yeast cells enter telomere-initiated senescence and also contributes to the recombination-dependent recovery from *tlc1∆*-initiated senescence (Bertuch and Lundblad, 2004; Maringele and Lydall, 2004a). Interestingly, this role of Exo1 is conserved in mammals because in telomerase knockout mice, Exo1 contributes to ssDNA generation and early death of telomerase deficient mice (Schaetzlein *et al*, 2007).

Experimental uncapping of telomeres

Even in yeast, when telomerase genes can be entirely deleted, telomere attrition caused by insufficient telomerase expression takes a long time to induce somewhat asynchronous and therefore difficult to study DDRs (Enomoto *et al*, 2002; Nautiyal *et al*, 2002; IJpma and Greider, 2003). Therefore, it is largely through artificial but experimentally tractable manipulations that much has been learnt about the role of DDR proteins at uncapped telomeres. Telomere uncapping has been induced by gene deletion, siRNA and use of dominant negative or temperature-sensitive alleles. The results of a number of different experiments, in a number of different model systems, show that there is no universal response to telomere-capping defects and uncapped telo-meres can be subject to NHEJ, HR and induce different types of checkpoint pathways.

cdc13-1

The budding yeast temperature-sensitive *cdc13-1* mutation affects the essential Cdc13 protein that binds telomeric ssDNA in budding yeast (Hartwell *et al*, 1973) (Figure 1F). Under restrictive conditions *cdc13-1* mutants rapidly and efficiently activate checkpoint-dependent cell-cycle arrest before anaphase, with greater than 90% of cells arrested at this point within 2 h. While arrested, *cdc13-1* mutants accumulate large regions of ssDNA at telomeres but not at other locations in the genome (Garvik *et al*, 1995). Cdc13-1 seems to be completely non-functional at 36°C (Garvik *et al*, 1995) but passage of the replication fork through the telomere is necessary for ssDNA to be generated at telomeres (Vodenicharov and Wellinger, 2006). The ssDNA at telomeres in *cdc13-1* mutants resembles a DSB-end, initiating 5′–3′ resection to generate a 3′ overhang that is an intermediate of HR, and ssDNA can be detected up to 30 kb from chromosome ends (Booth *et al*, 2001; Zubko *et al*, 2004). All the DNA damage checkpoint proteins that play roles in inducing cell-cycle arrest after DSBs also play a role in inducing cell-cycle arrest after telomere uncapping. In fact, *cdc13-1* was the tool first used to identify or show that Mec1, Mec3, Rad17, Rad24 and Rad53 checkpoint proteins played a role in checkpoint pathways (Weinert and Hartwell, 1993; Weinert *et al*, 1994). In addition to its role in telomere capping, Cdc13 has a separate function of recruiting telomerase to telomeres. Cells containing the *cdc13-2* allele, which is defective in interacting with the Est1 subunit of telomerase, enter telo-mere-initiated senescence at the same rate (i.e. over many generation times) as cells deleted of telomerase activity (Nugent *et al*, 1996).

Although telomeres of *cdc13-1* mutants resemble DSBs undergoing HR there are some differences. A number of recent papers have shown that the 5′–3′ dsDNA exonuclease, Exo1, the Mre11 complex and Sae2, the Sgs1 helicase and the Dna2 helicase/nuclease all contribute to DSB resection (Gravel *et al*, 2008; Mimitou and Symington, 2008; Raynard *et al*, 2008; Zhu *et al*, 2008). At DSBs, it seems that the Mre11/Sae2 complex is important for initiating resection, and that Exo1 and Sgs1/Dna2 are important for resection further from the site of the initial break. Interestingly, at uncapped *cdc13-1* telomeres the Mre11 complex is not required to initiate resection and instead the Mre11 complex contributes to capping (Foster *et al*, 2006). The difference between *cdc13-1* telomeres and DSBs is probably for a combination of reasons, first some ssDNA must already exist at telomeres for Cdc13-1 to bind telomeric DNA at permissive temperatures (Figure 1F), and second the Mre11 complex contributes to telomere capping through its interaction with Tel1. The Mre11 complex is not involved in signalling cell-cycle arrest of *cdc13-1* mutants (Foster *et al*, 2006).

Just as found at DSBs, Exo1 is not the only nuclease generating ssDNA at *cdc13-1* telomeres (Zubko *et al*, 2004). The roles of Sgs1 and Dna2, recently reported to play roles in resection at DSBs, at *cdc13-1* telomeres have not so far been reported. However, interestingly there is a Rad24-, Rad17-, Mec3- and Ddc1 (checkpoint sliding clamp and checkpoint

clamp loader)-dependent nuclease activity (ExoX) that is active at *cdc13-1* telomeres (Lydall and Weinert, 1995; Booth *et al*, 2001; Zubko *et al*, 2004). There are at least two reports that a similar activity is functional at DSBs (Aylon and Kupiec, 2003; Dubrana *et al*, 2007). It will be interesting to see how or if the Rad24-dependent nuclease activity relates to the Sgs1-, Dna2- or Mre11-dependent activities.

Resection at DSBs and uncapped telomeres needs to be properly regulated. Studies in *cdc13-1* mutants have shown that Rad9-dependent and checkpoint kinase-dependent mechanisms inhibit resection at uncapped telomeres (Lydall and Weinert, 1995; Jia *et al*, 2004; Lazzaro *et al*, 2008). It seems there are at least two routes to inhibit resection at *cdc13-1* telomeres and that these mechanisms are also probably active at DSBs. First, Rad9 binds histone H3 methylated on the K79 residue close to uncapped telomeres. H3K79 methylation is Dot1-dependent and is found in about 90% of H3 molecules (van Leeuwen *et al*, 2002). Dot1 contributes to the Rad9-dependent inhibition of resection, as well as Rad9-dependent cell-cycle arrest (Lazzaro *et al*, 2008). Second, a Rad9-, Rad24-, Rad53- and Mec1-dependent kinase cascade phosphorylates Exo1 and inhibits its nuclease activity (Morin *et al*, 2008). However, other mechanisms affecting the mobility of telomeric DNA are suggested by the finding that a Rad9 orthologue, 53BP1, contributes to telomere mobility and NHEJ in mammalian cells with capping defects (Dimitrova *et al*, 2008). In addition, Rad9, which contains a BRCA1 domain, and/or other proteins may interact with phosphorylated H2AX near DSBs and uncapped telomeres to inhibit resection.

Extensive studies of *cdc13-1* mutant cells have generated much insight into cellular responses to telomere uncapping and DSBs. The *cdc13-1* defect is useful in part because it is tunable, in the sense that by simply increasing temperature by small increments (e.g. 0.5°C), telomeres can become slightly less telomeric and slightly more DSB-like, and subtle effects of DDR pathways on growth of cells with telomere-capping defects can be distinguished (Addinall *et al*, 2008; Morin *et al*, 2008). Furthermore, *cdc13-1*-induced DNA damage is better tolerated than DNA damage induced elsewhere because growth of *cdc13-1* mutants is improved by inactivation of checkpoint pathways, whereas growth of cells with less localised chromosomal damage, for example caused by defects in DNA-ligase (*cdc9*), is exacerbated by inactivation of the same checkpoint pathways (Weinert and Hartwell, 1993). Systematic genetic, transcriptomic and mathematical modelling of *cdc13-1*-induced responses suggest that this allele, first identified nearly 40 years ago, still has much to teach about the interplay between telomeres and the DDR (Proctor *et al*, 2007; Addinall *et al*, 2008; Greenall *et al*, 2008).

Lack of Ku

In budding yeast, loss of Ku causes a short telomere phenotype, as discussed above, but also a temperature-sensitive telomere-capping defect. At DSBs, Ku seems to inhibit resection and HR, whereas favouring the NHEJ pathway of repair (Frank-Vaillant and Marcand, 2002; Barlow *et al*, 2008). At high temperature, such as 37°C, *yku70Δ* mutants accumulate excessive levels of Exo1-dependent ssDNA in subtelomeric regions and thus telomeres in Ku mutants look like intermediates in HR (Maringele and Lydall, 2002).

Interestingly, cell-cycle arrest of *yku70Δ* mutants at high temperature depends on only some of the checkpoint genes that respond to the *cdc13-1* defect, such as Rad9, Chk1 and Mec1 but arrest is independent of Rad17, Rad24, Mec3, Ddc1 (the checkpoint sliding clamp and clamp loader) and Rad53. Therefore, although in most contexts, for example at DSBs and in *cdc13-1* mutants, the 911 complex has a critical role in signalling cell-cycle arrest, its role is unnecessary for signalling cell-cycle arrest of Ku mutants with uncapped telomeres. Interestingly, there is also a role for spindle checkpoint genes in responding to the Ku defect, as there is in *Drosophila* mutants with telomere-capping defects, and *S. pombe taz1Δ* defective cells (see next section) (Maringele and Lydall, 2002; Miller and Cooper, 2003; Cenci, 2009).

Lack of Taz1

In *S. pombe* Taz1, an orthologue of human Trf1 and Trf2, binds the telomeric double stranded DNA. Interestingly, lack of Taz1 leads to uncontrolled telomere elongation, a cold-sensitive phenotype, and a failure of DNA replication through telomeric DNA (Ferreira *et al*, 2004). It seems that Dna2, rather than Exo1, is important for generating the high level of ssDNA observed in *taz1Δ* cells (Tomita *et al*, 2003, 2004). Interestingly, the RecQ helicase, Rqh1, analogous to Sgs1 in budding yeast, also contributes to the defects in *taz1Δ* cells, inducing telomere breakage (Rog *et al*, 2009).

Lack of Pot1

Pot1 genes encode ssDNA-binding proteins that bind the ssDNA at telomeres of *S. pombe* and mammalian cells (Lei *et al*, 2003). In many senses Pot1 is analogous to Cdc13 in *Saccharomyces cerevisiae*. Pot1 is essential for telomere function because in *S. pombe* loss of Pot1 leads to chromosome circularisation (Baumann and Cech, 2001). In mice there are two Pot1 genes, Pot1a and Pot1b. Loss of Pot1a leads to p53-dependent cell-cycle arrest, increased levels of 3′ ssDNA overhang length, elevated levels of HR and chromosome instability (Wu *et al*, 2006). Loss of Pot1b also increases the amount of ssDNA found at mouse telomeres (Hockemeyer *et al*, 2006, 2008). Loss of Pot1 from chicken cells, also increases levels of ssDNA at telomeres and induces ATR and Chk1-dependent cell-cycle arrest (Churikov and Price, 2008). However, in human cells siRNA depletion of Pot1 resulted in a loss of the G-rich 3′ overhang at telomeres (Hockemeyer *et al*, 2005). It is clear that in all organisms Pot1 contributes to telomere capping (Denchi and de Lange, 2007). Interestingly, the Pot1 complex does more than simply cap telomeres because it increases telomerase processivity and activity (Wang *et al*, 2007).

Lack of TRF2

TRF2 is one component of the mammalian shelterin complex proteins and it directly binds telomeric DNA. Human fibroblasts expressing a dominant negative version of TRF2 (Trf2ΔBΔM) induce rapid cell-cycle arrest in G1, and telomeres look like DSBs because, for example, there is the accumulation of phosphorylated H2AX, Mre11 and ATM, and telomere fusions are detected (van Steensel *et al*, 1998; d'Adda di Fagagna *et al*, 2003). Mouse cells, in which TRF2 is deleted by Cre-dependent
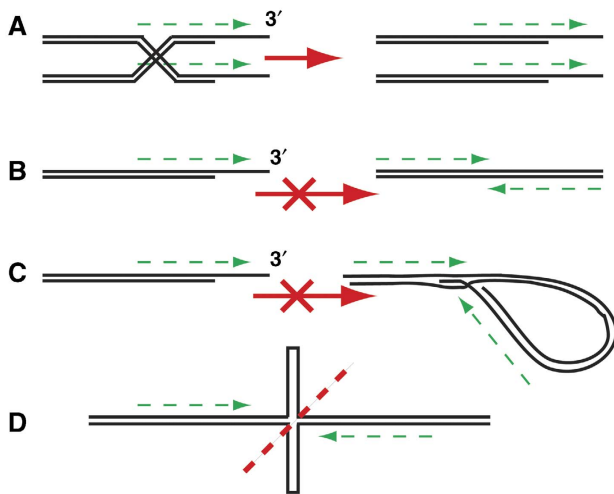
Figure 4 Telomere structure negates the harmful effects of the DDR. Black lines indicate telomeric DNA and dashed green arrows the direction of telomeric repeats. (**A**) Telomeric DNA sequences are repetitive and orientated in the same direction, centromere to telomere, and therefore should HR take place the effects are neutral. (**B**) The 3′ overhang at telomeres will inhibit NHEJ pathway of DNA repair. (**C**) If the 3′ overhang loops back to invade double stranded DNA it will form a t loop, when the 3′ end points towards the chromosome end (See Figure 2) rather than towards the centromere as shown. An invading 3′ end facing the centromere would be dangerous because of its potential to initiate break-induced replication events. (**D**) Should telomeres fuse, they will form palindromes, which have the potential to from Holliday junctions and be resolved by mechanisms that cleave Holliday junctions.

recombination, show massive levels of Ligase IV-dependent telomere fusion (Celli and de Lange, 2005).

Minimising the consequences of telomere repair

Telomere structure limits the harmful consequence of DNA repair at telomeres. Irrespective of the mechanisms by which telomeric structures inhibit downstream aspects of the DDR, such mechanisms are not perfect. Figure 4 illustrates at least four ways in which telomeric structure minimises the harmful effects of DDRs. In all eukaryotes, telomeric DNA is repetitive and orientated in the same direction (centromere to telomere). Thus, should HR occur between telomeres the effects can be largely neutral because the same type of repetitive DNA is found on all chromosome ends, and exchanges can be balanced (or equal) (Figure 4A). HR

between telomere repeats can also to unequal exchanges resulting in increases or decreases in the number of telomere repeats on individual chromosome ends. The 3′ overhang found at telomeres inhibits the NHEJ pathway of repair and makes it difficult to fuse telomeres (Figure 4B). The repetitive nature of telomeric DNA ensures that the 3′ ssDNA overhang cannot fold back on itself and base pair to generate a 3′ end that points internally towards the centromere (Figure 4C) instead the base paring ensures that a t loop pointing towards the telomere is formed (Figure 3C). A 3′ end pointing towards the centromere would be dangerous because it could initiate break-induced replication and perhaps lead to the formation of large palindromes at chromosome ends that have lost telomeric DNA repeats, as proposed in cells that have lost telomeric repeats (Maringele and Lydall, 2004b). Finally, the telomerase-dependent repeats found in most eukaryotic cells mean that should NHEJ occur between telomeres then a palindromic sequence will be formed. Palindromes, although more stable in eukaryotes than prokaryotes, can form cruciform structures and be susceptible to cleavage through resolvase activities, and therefore the fusion could be reversed. When human telomere fusions have been sequenced they invariably have lost all the telomeric DNA from at least one side of the fusion (Capper et al, 2007).

Conclusions

Telomeres and DDRs have evolved side-by-side. In all organisms, including those that maintain telomeres in the absence of telomerase, it seems that DDR proteins have critically important functions in telomere physiology as well as when telomeres fail. Just as different solutions to telomere capping have evolved in different organisms so have the roles of individual DDR proteins at telomeres in different organisms. Understanding the diverse roles of DDR proteins at telomeres of model organisms, as well as in human cells, will have important implications for the development and treatment of cancer and for understanding ageing.

Acknowledgements

References

Addinall SG, Downey M, Yu M, Zubko MK, Dewar J, Leake A, Hallinan J, Shaw O, James K, Wilkinson DJ, Wipat A, Durocher D, Lydall D (2008) A genomewide suppressor and enhancer analysis of cdc13-1 reveals varied cellular processes influencing telomere capping in Saccharomyces cerevisiae. Genetics **180:** 2251–2266

Ahmed S, Hodgkin J (2000) MRT-2 checkpoint protein is required for germline immortality and telomere replication in C. elegans [see comments]. Nature **403:** 159–164

Artandi SE, Attardi LD (2005) Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. Biochem Biophys Res Commun **331:** 881–890

Askree SH, Yehuda T, Smolikov S, Gurevich R, Hawk J, Coker C, Krauskopf A, Kupiec M, McEachern MJ (2004) A genome-wide screen for Saccharomyces cerevisiae deletion mutants that affect telomere length. Proc Natl Acad Sci USA **101:** 8658–8663

Aubert G, Lansdorp PM (2008) Telomeres and aging. Physiol Rev **88:** 557–579

Aylon Y, Kupiec M (2003) The checkpoint protein Rad24 of Saccharomyces cerevisiae is involved in processing double-strand break ends and in recombination partner choice. Mol Cell Biol **23:** 6585–6596

Baldo V, Testoni V, Lucchini G, Longhese MP (2008) Dominant TEL1-hy mutations compensate for Mec1 lack of functions in the DNA damage response. Mol Cell Biol **28:** 358–375

Barlow JH, Lisby M, Rothstein R (2008) Differential regulation of the cellular response to DNA double-strand breaks in G1. *Mol Cell* **30:** 73–85

Baumann P, Cech TR (2001) Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* **292:** 1171–1175

Bertuch AA, Lundblad V (2004) *EXO1* contributes to telomere maintenance in both telomerase proficient and telomerase deficient *Saccharomyces cerevisiae*. *Genetics* **166:** 1651–1659

Bi X, Wei SC, Rong YS (2004) Telomere protection without a telomerase; the role of ATM and Mre11 in Drosophila telomere maintenance. *Curr Biol* **14:** 1348–1353

Bianchi A, Shore D (2008) How telomerase reaches its end: mechanism of telomerase regulation by the telomeric complex. *Mol Cell* **31:** 153–165

Blackburn EH, Greider CW, Szostak JW (2006) Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med* **12:** 1133–1138

Boerckel J, Walker D, Ahmed S (2007) The Caenorhabditis elegans Rad17 homolog HPR-17 is required for telomere replication. *Genetics* **176:** 703–709

Booth C, Griffith E, Brady G, Lydall D (2001) Quantitative amplification of single-stranded DNA (QAOS) demonstrates that *cdc13-1* mutants generate ssDNA in a telomere to centromere direction. *Nucleic Acids Res* **29:** 4414–4422

Boulton SJ, Jackson SP (1998) Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J* **17:** 1819–1828

Bucholc M, Park Y, Lustig AJ (2001) Intrachromatid excision of telomeric DNA as a mechanism for telomere size control in Saccharomyces cerevisiae. *Mol Cell Biol* **21:** 6559–6573

Capper R, Britt-Compton B, Tankimanova M, Rowson J, Letsolo B, Man S, Haughton M, Baird DM (2007) The nature of telomere fusion and a definition of the critical telomere length in human cells. *Genes Dev* **21:** 2495–2508

Carballo JA, Johnson AL, Sedgwick SG, Cha RS (2008) Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. *Cell* **132:** 758–770

Caspi RR (2006) Ocular autoimmunity: the price of privilege? *Immunol Rev* **213:** 23–35

Celli GB, de Lange T (2005) DNA processing is not required for ATM-mediated telomere damage response after TRF2 deletion. *Nat Cell Biol* **7:** 712–718

Celli GB, Denchi EL, de Lange T (2006) Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. *Nat Cell Biol* **8:** 885–890

Cenci G (2009) Drosophila cell cycle under arrest: uncapped telomeres plead guilty. *Cell Cycle* **8:** 990–995

Cesare AJ, Reddel RR (2008) Telomere uncapping and alternative lengthening of telomeres. *Mech Ageing Dev* **129:** 99–108

Chai W, Sfeir AJ, Hoshiyama H, Shay JW, Wright WE (2006) The involvement of the Mre11/Rad50/Nbs1 complex in the generation of G-overhangs at human telomeres. *EMBO Rep* **7:** 225–230

Chan SW, Blackburn EH (2003) Telomerase and ATM/Tel1p protect telomeres from nonhomologous end joining. *Mol Cell* **11:** 1379–1387

Chang M, Arneric M, Lingner J (2007) Telomerase repeat addition processivity is increased at critically short telomeres in a Tel1-dependent manner in Saccharomyces cerevisiae. *Genes Dev* **21:** 2485–2494

Churikov D, Price CM (2008) Pot1 and cell cycle progression cooperate in telomere length regulation. *Nat Struct Mol Biol* **15:** 79–84

Ciapponi L, Cenci G, Ducau J, Flores C, Johnson-Schlitz D, Gorski MM, Engels WR, Gatti M (2004) The Drosophila Mre11/Rad50 complex is required to prevent both telomeric fusion and chromosome breakage. *Curr Biol* **14:** 1360–1366

Cooper JP, Nimmo ER, Allshire RC, Cech TR (1997) Regulation of telomere length and function by a Myb-domain protein in fission yeast. *Nature* **385:** 744–747

Cui T, Moro-oka N, Ohsumi K, Kodama K, Ohshima T, Ogasawara N, Mori H, Wanner B, Niki H, Horiuchi T (2007) Escherichia coli with a linear genome. *EMBO Rep* **8:** 181–187

d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP (2003) A DNA damage checkpoint response in telomere-initiated senescence. *Nature* **426:** 194–198

D'Amours D, Jackson SP (2002) The MRE11 complex: at the crossroads of DNA repair and checkpoint signalling. *Nat Rev Mol Cell Biol* **3:** 317–327

de Lange T (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* **19:** 2100–2110

Denchi EL, de Lange T (2007) Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature* **448:** 1068–1071

Deng Y, Chan SS, Chang S (2008) Telomere dysfunction and tumour suppression: the senescence connection. *Nat Rev Cancer* **8:** 450–458

Deriano L, Stracker TH, Baker A, Petrini JH, Roth DB (2009) Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. *Mol Cell* **34:** 13–25

Dimitrova N, Chen YC, Spector DL, de Lange T (2008) 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* **456:** 524–528

DuBois ML, Haimberger ZW, McIntosh MW, Gottschling DE (2002) A quantitative assay for telomere protection in Saccharomyces cerevisiae. *Genetics* **161:** 995–1013

Dubrana K, van Attikum H, Hediger F, Gasser SM (2007) The processing of double-strand breaks and binding of single-strand-binding proteins RPA and Rad51 modulate the formation of ATR-kinase foci in yeast. *J Cell Sci* **120:** 4209–4220

Enomoto S, Glowczewski L, Berman J (2002) MEC3, MEC1, and DDC2 are essential components of a telomere checkpoint pathway required for cell cycle arrest during senescence in Saccharomyces cerevisiae. *Mol Biol Cell* **13:** 2626–2638

Fellerhoff B, Eckardt-Schupp F, Friedl AA (2000) Subtelomeric repeat amplification is associated with growth at elevated temperature in yku70 mutants of Saccharomyces cerevisiae. *Genetics* **154:** 1039–1051

Ferguson TA, Green DR, Griffith TS (2002) Cell death and immune privilege. *Int Rev Immunol* **21:** 153–172

Ferreira MG, Miller KM, Cooper JP (2004) Indecent exposure: when telomeres become uncapped. *Mol Cell* **13:** 7–18

Fisher TS, Zakian VA (2005) Ku: A multifunctional protein involved in telomere maintenance. *DNA Repair (Amst)* **4:** 1215–1226

Foster SS, Zubko MK, Guillard S, Lydall D (2006) MRX protects telomeric DNA at uncapped telomeres of budding yeast cdc13-1 mutants. *DNA Repair (Amst)* **5:** 840–851

Francia S, Weiss RS, Hande MP, Freire R, d'Adda di Fagagna F (2006) Telomere and telomerase modulation by the mammalian Rad9/Rad1/Hus1 DNA-damage-checkpoint complex. *Curr Biol* **16:** 1551–1558

Frank-Vaillant M, Marcand S (2002) Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol Cell* **10:** 1189–1199

Gao H, Cervantes RB, Mandell EK, Otero JH, Lundblad V (2007) RPA-like proteins mediate yeast telomere function. *Nat Struct Mol Biol* **14:** 208–214

Garvik B, Carson M, Hartwell L (1995) Single-stranded DNA arising at telomeres in cdc13 mutants may constitute a specific signal for the RAD9 checkpoint. *Mol Cell Biol* **15:** 6128–6138

Gatbonton T, Imbesi M, Nelson M, Akey JM, Ruderfer DM, Kruglyak L, Simon JA, Bedalov A (2006) Telomere length as a quantitative trait: genome-wide survey and genetic mapping of telomere length-control genes in yeast. *PLoS Genet* **2:** e35

George JA, DeBaryshe PG, Traverse KL, Celniker SE, Pardue ML (2006) Genomic organization of the Drosophila telomere retrotransposable elements. *Genome Res* **16:** 1231–1240

Grandin N, Charbonneau M (2009) Telomerase- and Rad52-independent immortalization of budding yeast by an inherited-long-telomere pathway of telomeric repeat amplification. *Mol Cell Biol* **29:** 965–985

Gravel S, Chapman JR, Magill C, Jackson SP (2008) DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev* **22:** 2767–2772

Greenall A, Lei G, Swan DC, James K, Wang L, Peters H, Wipat A, Wilkinson DJ, Lydall D (2008) A genome wide analysis of the response to uncapped telomeres in budding yeast reveals a novel role for the NAD+ biosynthetic gene BNA2 in chromosome end protection. *Genome Biol* **9:** R146

Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* **43:** 405–413

Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T (1999) Mammalian telomeres end in a large duplex loop. *Cell* **97:** 503–514

Hartl DL, Jones EW (2009) *Genetics: Analysis of Genes and Genomes*. Boston, MA: Jones and Bartlett

Hartwell LH, Mortimer RK, Culotti J, Culotti M (1973) Genetic control of the cell division cycle in yeast: V. Genetic analysis of cdc mutants. *Genetics* **74:** 267–286

Hector RE, Shtofman RL, Ray A, Chen BR, Nyun T, Berkner KL, Runge KW (2007) Tel1p preferentially associates with short telomeres to stimulate their elongation. *Mol Cell* **27:** 851–858

Hirano Y, Fukunaga K, Sugimoto K (2009) Rif1 and rif2 inhibit localization of tel1 to DNA ends. *Mol Cell* **33:** 312–322

Hockemeyer D, Daniels JP, Takai H, de Lange T (2006) Recent expansion of the telomeric complex in rodents: two distinct POT1 proteins protect mouse telomeres. *Cell* **126:** 63–77

Hockemeyer D, Palm W, Wang RC, Couto SS, de Lange T (2008) Engineered telomere degradation models dyskeratosis congenita. *Genes Dev* **22:** 1773–1785

Hockemeyer D, Sfeir AJ, Shay JW, Wright WE, de Lange T (2005) POT1 protects telomeres from a transient DNA damage response and determines how human chromosomes end. *EMBO J* **24:** 2667–2678

IJpma A, Greider CW (2003) Short telomeres induce a DNA damage response in Saccharomyces cerevisiae. *Mol Biol Cell* **14:** 987–1001

Jeyapalan JC, Sedivy JM (2008) Cellular senescence and organismal aging. *Mech Ageing Dev* **129:** 467–474

Jia X, Weinert T, Lydall D (2004) Mec1 and Rad53 inhibit formation of single-stranded DNA at telomeres of Saccharomyces cerevisiae cdc13-1 mutants. *Genetics* **166:** 753–764

Kanaar R, Wyman C, Rothstein R (2008) Quality control of DNA break metabolism: in the 'end', it's a good thing. *EMBO J* **27:** 581–588

Kim HS, Vijayakumar S, Reger M, Harrison JC, Haber JE, Weil C, Petrini JH (2008) Functional interactions between Sae2 and the Mre11 complex. *Genetics* **178:** 711–723

Kim JS, Krasieva TB, Kurumizaka H, Chen DJ, Taylor AM, Yokomori K (2005) Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells. *J Cell Biol* **170:** 341–347

Laroche T, Martin SG, Gotta M, Gorham HC, Pryde FE, Louis EJ, Gasser SM (1998) Mutation of yeast Ku genes disrupts the sub-nuclear organization of telomeres. *Curr Biol* **8:** 653–656

Larrivee M, LeBel C, Wellinger RJ (2004) The generation of proper constitutive G-tails on yeast telomeres is dependent on the MRX complex. *Genes Dev* **18:** 1391–1396

Larrivee M, Wellinger RJ (2006) Telomerase- and capping-independent yeast survivors with alternate telomere states. *Nat Cell Biol* **8:** 741–747

Lazzaro F, Sapountzi V, Granata M, Pellicioli A, Vaze M, Haber JE, Plevani P, Lydall D, Muzi-Falconi M (2008) Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *EMBO J* **27:** 1502–1512

LeBel C, Rosonina E, Sealey DCF, Pryde F, Lydall D, Maringele L, Harrington LA (2009) Telomere maintenance and survival in Saccharomyces cerevisiae in the absence of telomerase and RAD52. *Genetics* (e-pub ahead of print 20 April 2009)

Lee JY, Mogen JL, Chavez A, Johnson FB (2008) Sgs1 RecQ helicase inhibits survival of Saccharomyces cerevisiae cells lacking telomerase and homologous recombination. *J Biol Chem* **283:** 29847–29858

Lee SE, Moore JK, Holmes A, Umezu K, Kolodner RD, Haber JE (1998) Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94:** 399–409

Lei M, Podell ER, Baumann P, Cech TR (2003) DNA self-recognition in the structure of Pot1 bound to telomeric single-stranded DNA. *Nature* **426:** 198–203

Li B, Lustig AJ (1996) A novel mechanism for telomere size control in Saccharomyces cerevisiae. *Genes Dev* **10:** 1310–1326

Lingner J, Cech TR (1996) Purification of telomerase from Euplotes aediculatus: requirement of a primer 3′ overhang. *Proc Natl Acad Sci USA* **93:** 10712–10717

Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* **118:** 699–713

Longhese MP (2008) DNA damage response at functional and dysfunctional telomeres. *Genes Dev* **22:** 125–140

Longhese MP, Paciotti V, Neecke H, Lucchini G (2000) Checkpoint proteins influence telomeric silencing and length maintenance in budding yeast. *Genetics* **155:** 1577–1591

Louis EJ, Vershinin AV (2005) Chromosome ends: different sequences may provide conserved functions. *Bioessays* **27:** 685–697

Lundblad V (2002) Telomere maintenance without telomerase. *Oncogene* **21:** 522–531

Lydall D (2003) Hiding at the ends of yeast chromosomes: telomeres, nucleases and checkpoint pathways. *J Cell Sci* **116:** 4057–4065

Lydall D, Weinert T (1995) Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. *Science* **270:** 1488–1491

Majka J, Niedziela-Majka A, Burgers PM (2006) The checkpoint clamp activates Mec1 kinase during initiation of the DNA damage checkpoint. *Mol Cell* **24:** 891–901

Mantiero D, Clerici M, Lucchini G, Longhese MP (2007) Dual role for Saccharomyces cerevisiae Tel1 in the checkpoint response to double-strand breaks. *EMBO Rep* **8:** 380–387

Maringele L, Lydall D (2002) EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Δ mutants. *Genes Dev* **16:** 1919–1933

Maringele L, Lydall D (2004a) EXO1 plays a role in generating type I and type II survivors in budding yeast. *Genetics* **166:** 1641–1649

Maringele L, Lydall D (2004b) Telomerase- and recombination-independent immortalization of budding yeast. *Genes Dev* **18:** 2663–2675

Maringele L, Lydall D (2005) The PAL-mechanism of chromosome maintenance: causes and consequences. *Cell Cycle* **4:** 747–751

Maser RS, DePinho RA (2002) Connecting chromosomes, crisis, and cancer. *Science* **297:** 565–569

McEachern MJ, Haber JE (2006) Break-induced replication and recombinational telomere elongation in yeast. *Annu Rev Biochem* **75:** 111–135

Melnikova L, Biessmann H, Georgiev P (2005) The Ku protein complex is involved in length regulation of Drosophila telomeres. *Genetics* **170:** 221–235

Michelson RJ, Rosenstein S, Weinert T (2005) A telomeric repeat sequence adjacent to a DNA double-stranded break produces an anticheckpoint. *Genes Dev* **19:** 2546–2559

Miller KM, Cooper JP (2003) The telomere protein Taz1 is required to prevent and repair genomic DNA breaks. *Mol Cell* **11:** 303–313

Mimitou EP, Symington LS (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* **455:** 770–774

Morin I, Ngo HP, Greenall A, Zubko MK, Morrice N, Lydall D (2008) Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. *EMBO J* **27:** 2400–2410

Muller HJ (1938) The remaking of chromosomes. *Collecting Net* **13:** 181–195

Murnane JP (2006) Telomeres and chromosome instability. *DNA Repair (Amst)* **5:** 1082–1092

Myung K, Ghosh G, Fattah FJ, Li G, Kim H, Dutia A, Pak E, Smith S, Hendrickson EA (2004) Regulation of telomere length and suppression of genomic instability in human somatic cells by Ku86. *Mol Cell Biol* **24:** 5050–5059

Naito T, Matsuura A, Ishikawa F (1998) Circular chromosome formation in a fission yeast mutant defective in two ATM homologues. *Nat Genet* **20:** 203–206

Nakada D, Matsumoto K, Sugimoto K (2003) ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* **17:** 1957–1962

Nautiyal S, DeRisi JL, Blackburn EH (2002) The genome-wide expression response to telomerase deletion in Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* **99:** 9316–9321

Nugent CI, Bosco G, Ross LO, Evans SK, Salinger AP, Moore JK, Haber JE, Lundblad V (1998) Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr Biol* **8:** 657–660

Nugent CI, Hughes TR, Lue NF, Lundblad V (1996) Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. *Science* **274:** 249–252

Oikemus SR, Queiroz-Machado J, Lai K, McGinnis N, Sunkel C, Brodsky MH (2006) Epigenetic telomere protection by Drosophila DNA damage response pathways. *PLoS Genet* **2:** e71

Pardo B, Marcand S (2005) Rap1 prevents telomere fusions by nonhomologous end joining. *EMBO J* **24:** 3117–3127

Petreaca RC, Chiu HC, Eckelhoefer HA, Chuang C, Xu L, Nugent CI (2006) Chromosome end protection plasticity revealed by Stn1p and Ten1p bypass of Cdc13p. *Nat Cell Biol* **8:** 748–755

Pickett HA, Cesare AJ, Johnston RL, Neumann AA, Reddel RR (2009) Control of telomere length by a trimming mechanism that involves generation of t-circles. *EMBO J* **28:** 799–809

Polakova S, Blume C, Zarate JA, Mentel M, Jorck-Ramberg D, Stenderup J, Piskur J (2009) Formation of new chromosomes as a virulence mechanism in yeast Candida glabrata. *Proc Natl Acad Sci USA* **106:** 2688–2693

Polotnianka RM, Li J, Lustig AJ (1998) The yeast Ku heterodimer is essential for protection of the telomere against nucleolytic and recombinational activities. *Curr Biol* **8:** 831–834

Proctor CJ, Lydall DA, Boys RJ, Gillespie CS, Shanley DP, Wilkinson DJ, Kirkwood TB (2007) Modelling the checkpoint response to telomere uncapping in budding yeast. *J R Soc Interface* **4:** 73–90

Raices M, Verdun RE, Compton SA, Haggblom CI, Griffith JD, Dillin A, Karlseder J (2008) C. elegans telomeres contain G-strand and C-strand overhangs that are bound by distinct proteins. *Cell* **132:** 745–757

Ranganathan V, Heine WF, Ciccone DN, Rudolph KL, Wu X, Chang S, Hai H, Ahearn IM, Livingston DM, Resnick I, Rosen F, Seemanova E, Jarolim P, DePinho RA, Weaver DT (2001) Rescue of a telomere length defect of Nijmegen breakage syndrome cells requires NBS and telomerase catalytic subunit. *Curr Biol* **11:** 962–966

Raynard S, Niu H, Sung P (2008) DNA double-strand break processing: the beginning of the end. *Genes Dev* **22:** 2903–2907

Riha K, Shippen DE (2003) Ku is required for telomeric C-rich strand maintenance but not for end-to-end chromosome fusions in Arabidopsis. *Proc Natl Acad Sci USA* **100:** 611–615

Ritchie KB, Mallory JC, Petes TD (1999) Interactions of TLC1 (which encodes the RNA subunit of telomerase), TEL1, and MEC1 in regulating telomere length in the yeast Saccharomyces cerevisiae. *Mol Cell Biol* **19:** 6065–6075

Ritchie KB, Petes TD (2000) The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. *Genetics* **155:** 475–479

Rog O, Cooper JP (2008) Telomeres in drag: dressing as DNA damage to engage telomerase. *Curr Opin Genet Dev* **18:** 212–220

Rog O, Miller KM, Ferreira MG, Cooper JP (2009) Sumoylation of RecQ helicase controls the fate of dysfunctional telomeres. *Mol Cell* **33:** 559–569

Rong YS (2008) Telomere capping in Drosophila: dealing with chromosome ends that most resemble DNA breaks. *Chromosoma* **117:** 235–242

Sabourin M, Tuzon CT, Zakian VA (2007) Telomerase and Tel1p preferentially associate with short telomeres in S. cerevisiae. *Mol Cell* **27:** 550–561

Sabourin M, Zakian VA (2008) ATM-like kinases and regulation of telomerase: lessons from yeast and mammals. *Trends Cell Biol* **18:** 337–346

Sandell LL, Zakian VA (1993) Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* **75:** 729–739

Schaetzlein S, Kodandaramireddy NR, Ju Z, Lechel A, Stepczynska A, Lilli DR, Clark AB, Rudolph C, Wei K, Schlegelberger B, Schirmacher P, Kunkel TA, Greenberg RA, Edelmann W, Rudolph KL (2007) Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. *Cell* **130:** 863–877

Shachar R, Ungar L, Kupiec M, Ruppin E, Sharan R (2008) A systems-level approach to mapping the telomere length maintenance gene circuitry. *Mol Syst Biol* **4:** 172

Shore D, Nasmyth K (1987) Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* **51:** 721–732

Stellwagen AE, Haimberger ZW, Veatch JR, Gottschling DE (2003) Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. *Genes Dev* **17:** 2384–2395

Stewart SA, Weinberg RA (2006) Telomeres: cancer to human aging. *Annu Rev Cell Dev Biol* **22:** 531–557

Streilein JW (2003) Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *J Leukoc Biol* **74:** 179–185

Symington LS, Heyer WD (2006) Some disassembly required: role of DNA translocases in the disruption of recombination intermediates and dead-end complexes. *Genes Dev* **20:** 2479–2486

Takai H, Smogorzewska A, de Lange T (2003) DNA damage foci at dysfunctional telomeres. *Curr Biol* **13:** 1549–1556

Tarsounas M, Munoz P, Claas A, Smiraldo PG, Pittman DL, Blasco MA, West SC (2004) Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* **117:** 337–347

Teixeira MT, Arneric M, Sperisen P, Lingner J (2004) Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states. *Cell* **117:** 323–335

Teo SH, Jackson SP (1997) Identification of Saccharomyces cerevisiae DNA ligase IV: involvement in DNA double-strand break repair. *EMBO J* **16:** 4788–4795

Titen SW, Golic KG (2008) Telomere loss provokes multiple pathways to apoptosis and produces genomic instability in Drosophila melanogaster. *Genetics* **180:** 1821–1832

Tomita K, Kibe T, Kang HY, Seo YS, Uritani M, Ushimaru T, Ueno M (2004) Fission yeast Dna2 is required for generation of the telomeric single-strand overhang. *Mol Cell Biol* **24:** 9557–9567

Tomita K, Matsuura A, Caspari T, Carr AM, Akamatsu Y, Iwasaki H, Mizuno K, Ohta K, Uritani M, Ushimaru T, Yoshinaga K, Ueno M (2003) Competition between the Rad50 complex and the Ku heterodimer reveals a role for Exo1 in processing double-strand breaks but not telomeres. *Mol Cell Biol* **23:** 5186–5197

Tourand Y, Lee L, Chaconas G (2007) Telomere resolution by Borrelia burgdorferi ResT through the collaborative efforts of tethered DNA binding domains. *Mol Microbiol* **64:** 580–590

Usui T, Ogawa H, Petrini JH (2001) A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol Cell* **7:** 1255–1266

van Leeuwen F, Gafken PR, Gottschling DE (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109:** 745–756

van Steensel B, Smogorzewska A, de Lange T (1998) TRF2 protects human telomeres from end-to-end fusions. *Cell* **92:** 401–413

Verdun RE, Crabbe L, Haggblom C, Karlseder J (2005) Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. *Mol Cell* **20:** 551–561

Verdun RE, Karlseder J (2006) The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. *Cell* **127:** 709–720

Verdun RE, Karlseder J (2007) Replication and protection of telomeres. *Nature* **447:** 924–931

Vodenicharov MD, Wellinger RJ (2006) DNA degradation at unprotected telomeres in yeast is regulated by the CDK1 (Cdc28/Clb) cell-cycle kinase. *Mol Cell* **24:** 127–137

Wang F, Podell ER, Zaug AJ, Yang Y, Baciu P, Cech TR, Lei M (2007) The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* **445:** 506–510

Weinert TA, Hartwell LH (1993) Cell cycle arrest of cdc mutants and specificity of the RAD9 checkpoint. *Genetics* **134:** 63–80

Weinert TA, Kiser GL, Hartwell LH (1994) Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes Dev* **8:** 652–665

Wotton D, Shore D (1997) A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in Saccharomyces cerevisiae. *Genes Dev* **11:** 748–760

Wu D, Topper LM, Wilson TE (2008) Recruitment and dissociation of nonhomologous end joining proteins at a DNA double-strand break in Saccharomyces cerevisiae. *Genetics* **178:** 1237–1249

Wu L, Multani AS, He H, Cosme-Blanco W, Deng Y, Deng JM, Bachilo O, Pathak S, Tahara H, Bailey SM, Behringer RR, Chang S (2006) Pot1 deficiency initiates DNA damage checkpoint activa-

tion and aberrant homologous recombination at telomeres. *Cell* **126:** 49–62

Wu Y, Xiao S, Zhu XD (2007) MRE11-RAD50-NBS1 and ATM function as co-mediators of TRF1 in telomere length control. *Nat Struct Mol Biol* **14:** 832–840

Zhu Z, Chung WH, Shim EY, Lee SE, Ira G (2008) Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* **134:** 981–994

Zubko MK, Guillard S, Lydall D (2004) Exo1 and Rad24 differentially regulate generation of ssDNA at telomeres of *Saccharomyces cerevisiae cdc13-1* mutants. *Genetics* **168:** 103–115

Zubko MK, Lydall D (2006) Linear chromosome maintenance in the absence of essential telomere-capping proteins. *Nat Cell Biol* **8:** 734–740