Hiding at the ends of yeast chromosomes: telomeres, nucleases and checkpoint pathways

David Lydall

School of Biological Sciences, University of Manchester, G38 Stopford Building, Oxford Road, Manchester M13 9PT, UK
(e-mail: lydall@man.ac.uk)

Journal of Cell Science 116, 4057-4065 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00765

Summary

Telomeres stabilise DNA at the ends of chromosomes, preventing chromosome fusion and genetic instability. Telomeres differ from double strand breaks in that they activate neither DNA repair nor DNA damage checkpoint pathways. Paradoxically DNA repair and checkpoint genes play critical roles in telomere stability. Recent work has provided insights into the roles of DNA repair and DNA damage checkpoint pathways in the physiological maintenance of telomeres and in cellular responses when telomeres become uncapped. In budding yeast the Mre11p nuclease, along with other unidentified nucleases, plays critical roles in physiological telomere maintenance. However, when telomeres are uncapped, the 5′-to-3′ exonuclease, Exo1p, plays a critical role in generating single-stranded DNA and activating checkpoint pathways. Intriguingly Exo1p does not play an important role in normal telomere maintenance. Although checkpoint pathways are not normally activated by telomeres, at least four different types of telomere defect activate checkpoint pathways. Interestingly, each of these telomere defects depends on a different subset of checkpoint proteins to induce cell cycle arrest. A model for how a spectrum of telomeric states might interact with telomerase and checkpoint pathways is proposed.

Key words: Telomere, Checkpoint, DNA repair, DNA damage

Introduction

Telomeric DNA ends are inert because they induce neither DNA repair nor DNA damage checkpoint responses. The efficiency with which telomeres are hidden from checkpoint and repair processes is illustrated by the fact that a haploid budding yeast cell that has 64 telomeres enters mitosis without delay or 'repair' of the chromosome ends. In contrast, a yeast cell with a single unrepaired double strand break (DSB) does not (Lee et al., 1998; Sandell and Zakian, 1993). Therefore, special properties of telomeric DNA ends must explain why the ends of chromosomes are perceived differently from DNA ends elsewhere in the genome.

It is critical for genetic stability that telomeres and DSBs do not interconvert. If DSBs and telomeres were to switch properties, acentric fragments would be induced by DSBs that switched to telomeres and chromosome fusions would be induced by many of the telomeres that switched to DSBs. Since DSBs and telomeres rarely interconvert, it seemed reasonable to imagine that cells distinguish DSBs from telomeres by ensuring that different classes of protein bind to telomeric and DSB DNA ends. However, it is now clear that telomeres interact with numerous DNA repair and DNA damage checkpoint proteins. How DNA repair and checkpoint proteins interact at telomeres and yet induce neither DNA repair nor cell cycle arrest is a paradox that is not yet explained. Understanding this paradox will lead to a better understanding of the roles of DNA repair and checkpoint pathways not only in telomere stability but also in processing other types of DNA damage.

Here, I review recent insights into the roles of budding yeast DNA repair and checkpoint proteins in telomere physiology and pathology. Yeast telomeres are similar to those of many other organisms, including humans, and therefore lessons from budding yeast may be generally relevant. Other aspects of telomere biology are much better described in reviews on telomere-binding proteins (Cooper, 2000; McEachern et al., 2000; Rhodes et al., 2002), capping and replication (Blackburn, 2000; Blackburn, 2001; Cervantes and Lundblad, 2002; Chan and Blackburn, 2002; Dubrana et al., 2001; Evans and Lundblad, 2000; Shore, 2001), localisation (Hediger et al., 2002) and chromatin structure (Chan and Blackburn, 2002; Gasser, 2000). In addition, several recent reviews describing the roles of DNA damage checkpoint sensors, mediators and kinases in signaling cell cycle arrest have been published (Melo and Toczyski, 2002; Nyberg et al., 2002; Rouse and Jackson, 2002a).

To understand how telomeres protect chromosome ends it is important to know the DNA structures at telomeric ends and their interactions with repair and checkpoint pathways.

Telomeric repetitive DNA

The inert nature of telomeric DNA must depend, at least in part, on specific DNA sequences found at telomeres. Two properties are common to all telomeres: repetitive DNA and short 3′ ssDNA tails.

Since telomeric repeats are found at all telomeres, they are presumably essential for telomeres to escape DNA repair or checkpoint responses. Across species, there is significant variation in the type of repetitive DNA sequence that forms the basis of functional telomeres (Louis, 2002; Mefford and Trask, 2002). Thus the presence of some type of repeat, rather than

specific repeats, appears to allow telomeres to be inert. The considerable degree of variation in the type of repetitive DNA sequence that can form functional telomeres in yeast supports this view (Fig. 1). One view is that telomeric repeats allow the telomere to form a heterochromatic, silenced state, and thereby avoid repair and checkpoint pathways (Chan and Blackburn, 2002).

In most organisms the terminal telomeric repeats are generated by telomerase. Telomerase is a ribonucleoprotein with reverse transcriptase activity that circumvents the 'end replication problem'[†] by maintaining the presence of G-rich repeats at telomeres (Olovnikov, 1973; Watson, 1972). Telomerase extends the G-rich strand at the 3′ terminus of natural telomeres without a requirement for a complementary, template strand of DNA (Greider and Blackburn, 1985). Among species there is considerable variation in the precise sequence of the G-rich repeat added by telomerase (Wellinger and Sen, 1997). The C-rich strand is produced by standard, semi-conservative DNA replication.

Telomerase can generate telomeres de novo, from DSBs, if G-rich telomeric seed sequences lie close to the site of the DSB (Diede and Gottschling, 1999; Kramer and Haber, 1993; Myung et al., 2001). DSB-derived telomeres contain G-rich repeats but lack sub-telomeric X or Y′ repeats that precede G-rich repeats at natural telomeres (see below and Fig. 1). DSB-induced telomeres act as fully functional chromosome caps but have silencing properties different from those of natural telomeres (Pryde and Louis, 1999).

A single sub-telomeric X repeat precedes G-rich repeats at all natural budding yeast telomeres (Pryde and Louis, 1997). Each X repeat is based on a 473 bp core sequence that contains an ARS (autonomously replicating sequence) consensus sequence, the binding site for the origin-recognition complex and a separate Abf1 (ARS binding factor 1) binding site (Pryde and Louis, 1997; Pryde and Louis, 1999). By these criteria the core X repeat is an origin of replication (Raychaudhuri et al., 1997). In addition, approximately half the budding yeast telomeres contain one to four copies of a Y′ repeat (Louis et al., 1994; Pryde and Louis, 1997) (Fig. 1A,B). Y′ repeats are considerably larger than X repeats and have two predominant sizes, 5.2 and 6.7 kb (Lundblad and Blackburn, 1993). Y′ repeats also contain ARS consensus sequences and Abf1-binding sites and are therefore potential origins of replication (Pryde and Louis, 1997). In addition Y′ repeats encode functional helicases (Yamada et al., 1998).

X and Y′ repeats are always orientated in the same direction at telomeres, presumably to ensure that recombination between sub-telomeric repeats does not generate dicentric chromosomes. The high degree of homology between Y′ repeats on different telomeres, and the variation in Y′ repeat number between different strains, indicates that there is a high frequency of recombination at sub-telomeric Y′ repeats (Louis et al., 1994). In contrast, the X repeats share less sequence similarity and are never present as more than one copy per sub-telomere, which indicates that recombination is suppressed at X sequences.

[†]Olovnikov and Watson pointed out that the ends of linear DNA molecules (telomeres) could not be completely replicated by the normal DNA replication machinery and that this 'end replication problem' would lead to loss of telomeric DNA each cell cycle. Telomerase solves the end replication problem because it extends telomeres without the need for a template strand.

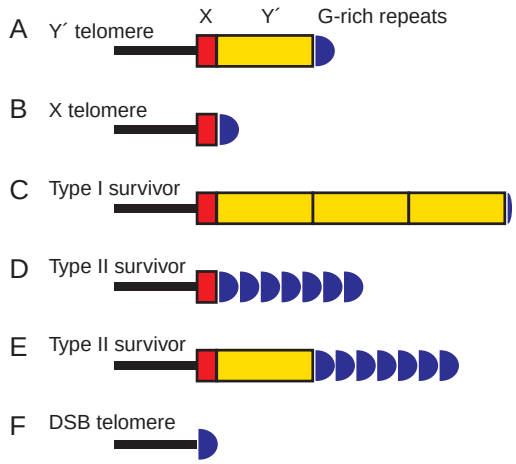

Fig. 1. Six classes of functional telomere in budding yeast. Natural telomeres of budding yeast are illustrated in A and B (Pryde and Louis, 1997) and other types of functional telomere are illustrated in C to F. The data and colouring scheme are from the website of Ed Louis (http://www.le.ac.uk/ge/ejl12/research/telostruc/EndsSmall.html). (A) Y′ telomeres contain the three major repetitive sequences found at budding yeast telomeres: G-rich, Y′ and X repeats. G-rich and X repeats are found at all telomeres. The G-rich repeats are the product of telomerase activity and are approximately 300 bp in wild-type budding yeast strains. X repeats are based on a 473 bp core sequence that contains an ARS (autonomously replicating sequence) consensus sequence, the binding site of the origin recognition complex and a separate Abf1 (ARS binding factor 1) binding site (Pryde and Louis, 1997; Pryde and Louis, 1999). Y′ repeats are considerably larger than X repeats, with two predominant sizes of 5.2 and 6.7 kb (Lundblad and Blackburn, 1993). Y′ repeats also contain ARS consensus sequences and Abf1 binding sites and are therefore potential origins of replication (Pryde and Louis, 1997). In addition, Y′ repeats encode a functional helicase (Yamada et al., 1998). (B) X telomeres contain only G-rich and X repeats (C) In the absence of telomerase, or if telomere capping is defective, cells enter crisis and generate survivors. Type I survivors lose most of the G-rich repeats but amplify Y′ repeats by recombination-dependent mechanisms. (D,E) In the absence of telomerase, Type II survivors contain highly lengthened G-rich repeats that have been maintained by recombination-dependent mechanisms. (F) If a DSB is induced close to a G-rich telomere seed sequence a telomere can be formed de novo.

Yeast and mammalian cells that do not express telomerase divide for a small number of cell divisions before entering crisis[‡]. At low frequency yeast cells in crisis generate 'survivors' that can divide and maintain telomeres by recombination-dependent mechanisms. These alternative pathways of telomere maintenance may be analogous to the telomerase-independent ALT (alternative lengthening of telomeres) pathways that exist in mammalian cells (Henson et al., 2002). In budding yeast, Type I survivors amplify Y′ repeats (Fig. 1C) whereas Type II survivors amplify the G-rich repeats (Fig. 1D,E) (Le et al., 1999; Lundblad and Blackburn, 1993; Teng and Zakian, 1999). Type I survivors, containing amplified Y′ repeats, are particularly interesting because the telomeres of these cells effectively cap chromosome ends in the absence of extensive G-rich repeats at chromosome ends.

[‡]After a number of divisions in the absence of telomerase, telomeres become critically short, and cells stop division and enter crisis.

This suggests that proteins that bind double-stranded G-rich repeats may not be essential for capping and that telomerase and other proteins binding the 3′ ssDNA tail at telomeres may be sufficient for capping. Alternatively, Type I survivors might cap telomeres by forming a heterochromatin type of structure (Chan and Blackburn, 2002).

Telomeric ssDNA overhangs

Telomeres of all organisms examined, including budding yeast, *Tetrahymena*, human and *Arabidopsis*, terminate with a short 3′ overhang of the G-rich strand (Jacob et al., 2003; Makarov et al., 1997; McElligott and Wellinger, 1997; Riha et al., 2000; Wei and Price, 2003; Wellinger et al., 1996; Wellinger et al., 1993). This ssDNA tail probably exists to provide a substrate for telomerase, which requires a 3′ overhang on its substrate to function (Lingner and Cech, 1996; Wang and Blackburn, 1997). However, 3′ tails are also important for initiating recombination at DSBs. Since recombination-dependent mechanisms of telomere maintenance can be important, the 3′ tails at telomeres may also play a critical role in recombination-dependent telomere maintenance.

In mammalian and many other cell types the 3′ overhang appears to be folded back into a sub-telomeric location to create a 't-loop' (Wei and Price, 2003). To form t-loops the 3′ ssDNA G-rich repeat loops back and invades the dsDNA G-rich repeats (Griffith et al., 1999; Wei and Price, 2003). So far there is no evidence for t-loops in yeasts, which suggests that in yeasts the 3′ overhang may be exposed. In budding yeast the 3′ overhang is more pronounced in S phase (Dionne and Wellinger, 1996; Wellinger et al., 1996; Wellinger et al., 1993) and requires the passage of the replication fork (Dionne and Wellinger, 1998). In human and tetrahymena cells the ssDNA tail is detectable at all stages of the cell cycle (Jacob et al., 2003; McElligott and Wellinger, 1997). Recent experiments suggest that in rapidly dividing mammalian cells some telomeres instead have a 5′ C strand ssDNA overhang (Cimino-Reale et al., 2003).

The 3′ ssDNA overhang at telomeres is intriguing because mitotic and meiotic DSBs are resected to generate 3′ ssDNA overhangs as a prerequisite for genetic recombination (Sugawara and Haber, 1992; Sun et al., 1991). This raises the question: why are telomeres not undergoing continual recombination events? If telomeres were in a perpetual state of recombination then cell cycle progression might be inhibited either by checkpoint-dependent signaling or physically by inter-chromatid exchanges. Although there is clear evidence for elevated rates of recombination between Y′ repeats, recombination does not seem to be occurring continually because the cell cycle proceeds on schedule. Presumably some aspect of telomere capping is important for limiting resection and recombination at telomeres. Consistent with this idea yeast strains that are defective in telomere capping and/or replication show elevated levels of ssDNA and recombination at telomeres (see below; negative regulation of nucleases).

ssDNA at telomeres is also intriguing because ssDNA is thought to be an important component of the stimulus for checkpoint-dependent cell cycle arrest (Carr, 2003; Garvik et al., 1995; Maringele and Lydall, 2002; Vaze et al., 2002; Zou and Elledge, 2003). Analysis of cell cycle arrest in response to a single DSB suggests that 10 kb of ssDNA is necessary for cell cycle arrest (Vaze et al., 2002). In budding yeast it can be calculated that >150 bp of ssDNA per telomere would be required to generate 10 kb of ssDNA. Since each telomere contains approximately 300 bp of G-rich repeats, this would represent extremely high levels of ssDNA. Therefore it may simply be that there is normally insufficient ssDNA at telomeres to activate checkpoint-dependent cell cycle arrest.

Telomere capping and replication

Telomere capping ensures that telomeric DNA ends behave differently from DSB ends. A large number of telomere-binding proteins have been identified and these contribute to telomere capping and replication. Proteins that bind dsDNA at telomeres include Trf1, Trf2, Tin2, Tankyrase and hRap1 in mammalian cells (Rhodes et al., 2002), and Rap1, Sir2, Sir3, Sir4, Rif1 and Yku70/Yku80 in budding yeast, as well as components of telomerase (Est1 and Est2) and the DNA replication machinery (Cooper, 2000). In addition there are proteins that appear to be particularly involved in binding ssDNA at telomeres. Pot1 is a ssDNA-binding protein in mammalian and fission yeast cells, and Cdc13p binds ssDNA in budding yeast (Baumann and Cech, 2001; Garvik et al., 1995; Mitton-Fry et al., 2002; Nugent et al., 1996; Rhodes et al., 2002; Wei and Price, 2003).

Cells lacking telomere-binding proteins display several different phenotypes associated with improper capping and/or replication. These phenotypes include shortened telomeres, lengthened telomeres, telomere fusions, elevated levels of ssDNA, elevated levels of recombination, telomere loss and checkpoint activation. This range of phenotypes indicates that numerous different activities are normally coordinated to maintain and cap telomeres. For example, not only does telomerase need to be recruited to telomeres successfully but its activity needs to be inhibited to ensure that telomere length does not increase indefinitely. Similarly, lagging-strand DNA synthesis needs to be regulated coordinately with telomerase activity to ensure that the length of the 3′ single-stranded overhang does not become excessive (Fig. 2C). The large number of telomere-binding proteins, and the complexity of phenotypes associated with defects in these proteins, means that is difficult to understand precisely the roles of the various proteins in telomere capping and replication.

Nucleases at telomeres

Nucleases are usually associated with DNA repair and replication processes but they are also critical for generating 3′ ssDNA overhangs at telomeres, particularly on leading strand telomeres (Fig. 2). Recent experiments in *Tetrahymena* indicate that unidentified nucleases degrade not only the 5′ strand but also the 3′ strand at telomeres (Jacob et al., 2003). Recruitment of nucleases to telomeres requires proper regulation because excessive nuclease activity might lead to telomere loss or high levels of ssDNA.

Telomere attrition occurs in mammalian and yeast cells that do not express telomerase. The end replication problem may explain the loss of telomeric DNA that occurs each cycle. However, it is equally possible that nucleases play a major role in degrading the ends of the chromosomes. Telomerase-deficient human cells lose approximately 150 bp (30-500 bp) of telomeric

DNA per generation (Huffman et al., 2000). In contrast, telomerase-deficient yeast cells lose just 3-6 bp per generation (Lundblad and Blackburn, 1993). The difference in telomere loss rate in yeast and humans may reflect differing susceptibilities of human and yeast telomeres to nucleases. It is also known that different human cell types contain ssDNA overhangs of different lengths and lose telomeric DNA at different rates (Huffman et al., 2000). These cell-type-specific differences may be due to differing nuclease activities in different cell types rather than different abilities to replicate telomeres.

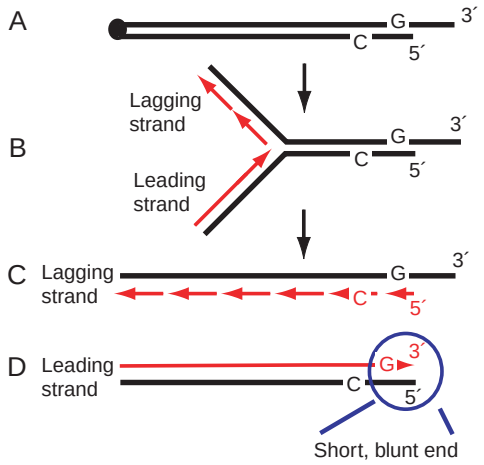

Fig. 2. Telomere replication. **(**A) Telomeres in all organisms contain a short 3′ overhang on the G rich strand. (B) A replication fork moving towards the end of the chromosome. (C) The newly replicated, lagging C strand, will generate a natural 3′ overhang when the RNA primer is removed from the final Okazaki fragment, or if the lagging strand replication machinery cannot reach the end of the chromosome. In the absence of nuclease activity the unreplicated 3′ strand will be the same length as it was prior to replication. (D) The newly replicated leading G strand will be the same length as the parental 5′ C strand, and blunt ended if the replication fork reaches the end of the chromosome. Therefore the newly replicated 3′ G strand will be shorter than the parental 3′ strand and unable to act as a substrate for telomerase because it does not contain a 3′ overhang. If the leading strand replication fork does not reach the end of the chromosome a 5′ rather than 3′ overhang would be generated, but this would not be a suitable substrate for telomerase.

Negative regulation of nucleases

Several budding yeast genes that limit the extent of ssDNA at telomeres have been identified. These include genes encoding the DNA repair protein Yku70/Yku80 (Gravel et al., 1998; Maringele and Lydall, 2002; Polotnianka et al., 1998), the telomeric ssDNA-binding protein Cdc13 (Garvik et al., 1995; Polotnianka et al., 1998), DNA polymerase α (*CDC17*) (Adams Martin et al., 2000; Carson and Hartwell, 1985) and a FLAP endonuclease (*RAD27*) (Parenteau and Wellinger, 2002). In addition, other proteins that interact with Cdc13p, including Stn1p (Grandin et al., 1997) and Ten1p (Grandin et al., 2001) along with telomerase itself (Chan et al., 2001), contribute to limiting ssDNA at telomeres. Presumably many of these gene products play roles in capping and limiting nuclease activity at telomeres or in coordinating lagging-strand replication (Fig. 3C) (Diede and Gottschling, 1999; Parenteau and Wellinger, 2002).

Interestingly, checkpoint pathways also limit ssDNA production at telomeres. *RAD9* was the first checkpoint gene to be so defined (Weinert and Hartwell, 1988) and is critical for cell cycle arrest in many strains that have telomere defects (Table 1). *RAD9* inhibits ssDNA production in strains lacking the telomere-binding protein Cdc13p (Lydall and Weinert, 1995). The mechanism by which Rad9p inhibits nuclease activity at uncapped telomeres in *cdc13-1* mutants is unclear. Rad9p is considered to be a ‘mediator’ checkpoint protein, facilitating crosstalk between upstream checkpoint kinases, such as Mec1p, and downstream checkpoint kinases, such as Chk1p and Rad53p (Melo and Toczyski, 2002; Osborn et al., 2002). Our recent experiments suggest that Rad9p also inhibits

Table 1. The role of checkpoint genes in responding to telomeric defects

S. cereviesiae gene	Human/pombe orthologue	Function	Telomere damage			
			cdc13-1	*yku70Δ*	*tlc1Δ*	Tel1p op
MEC1	ATR/Rad3	PIKKinase	Yes	Yes	Yes	No
DDC2	ATRIP/Rad26	Kinase binding	Yes		Yes	No
RAD53	CHK2/Cds1	Kinase	50%		Minor?	Yes
DUN1		Kinase	50%	No		
CHK1	CHK1	Kinase	50%	Yes		Yes
TEL1	ATM/Tel1	PIKKinase			No	
RAD9	BRCA1 Rhp9 TOPBP1	Mediator	Yes	Yes	Minor?	Yes
RAD24	RAD17	RFC like	Yes	No	Yes	
RAD17	RAD1	PCNA like	Yes	No		
MEC3	HUS1	PCNA like	Yes	No	Yes	
DDC1	RAD9	PCNA like	Yes	No		No

The roles of different checkpoint genes in causing cell cycle arrest in *cdc13-1*, *yku70Δ*, *tlc1Δ* and *TEL1* overexpressing strains are indicated. A blank indicates that the particular checkpoint gene has not been tested, 50% indicates that the checkpoint gene is only partially required for arrest (Clerici et al., 2001; Enomoto et al., 2002; Gardner et al., 1999; IJpma and Greider, 2003; Lydall and Weinert, 1995; Maringele and Lydall, 2002; Rouse and Jackson, 2002b; Sanchez et al., 1999; Viscardi et al., 2003). Checkpoint genes in bold are implicated in telomere maintenance or stability. *mec1Δ tel1Δ* double mutants and analogous *rad3Δ tel1Δ* double mutants of fission yeast are completely defective in telomere maintenance, erode telomeres and undergo telomere fusions (Craven et al., 2002; Matsuura et al., 1999; Naito et al., 1998). *cdc13-1*, *yku70Δ*, *tlc1Δ* and *TEL1* over-expressing strains accumulate ‘DNA damage’ at telomeres, but it is also possible that damage simultaneously induced elsewhere in the genome is an important stimulus for arrest. For *tlc1Δ* damage there is evidence that *RAD9* and *RAD53* play either no role (Enomoto et al., 2002) or a minor role (IJpma and Greider, 2003) in cell cycle arrest.

ssDNA production at uncapped telomeres by mediating interactions between upstream and downstream checkpoint kinases (X. Jia, T. Weinert and D. Lydall, unpublished).

Positive regulation of nucleases

The nucleases responsible for generating the 3′ ssDNA tails at leading strand telomeres have yet to be unambiguously identified in budding yeast. However, there is evidence showing that *MRX*, *EXO1* and the *RAD24* group of checkpoint genes regulate or encode nucleases with differing activities at telomeres.

MRX

The MRX nuclease complex in yeast, comprising Mre11p, Rad50p and Xrs2p, is implicated in DNA repair, cell cycle arrest and telomere maintenance (D'Amours and Jackson, 2002). Null mutations in *MRX* genes result in short telomeres in most genetic backgrounds (Ritchie and Petes, 2000; Tsubouchi and Ogawa, 2000; Wilson et al., 1999), and in one background complete loss of telomeric DNA and senescence (Kironmai and Muniyappa, 1997). Although the MRX complex has numerous biochemical activities in vitro, including 3′-to-5′ nuclease activity, it is involved in the formation of a 3′ overhang at DSBs in vivo (D'Amours and Jackson, 2002; Haber, 1998).

mrx mutants are defective at generating telomeres de novo. In an elegant series of experiments, Diede and Gottschling showed that appropriately located DSBs are resected to generate 3′ ssDNA tails before telomerase converts them to functional capped telomeres (Diede and Gottschling, 2001). In this assay *mrx* mutants were defective in formation of 3′ ssDNA tails and generation of telomeres in vivo. However, four lines of evidence suggest that *MRX*-independent mechanisms to generate ssDNA also exist. (1) *mrx* mutants can generate telomeres at DSBs but with a delay (Diede and Gottschling, 2001). (2) The nuclease activity of the MRX complex does not seem to be required for telomere maintenance (Tsukamoto et al., 2001). (3) The ssDNA-binding protein Cdc13p binds telomeres in the absence of Mre11p (Tsukamoto et al., 2001). (4) In most genetic backgrounds *mrx* mutants do not become senescent and enter crisis. If MRX were the only nuclease required to generate 3′ ssDNA overhangs at leading strand telomeres (Fig. 2D) then *mrx* mutants should be unable to recruit telomerase and should enter crisis as do telomerase-deficient cells. Therefore, *MRX*-independent nucleases or mechanisms contribute to generating 3′ ssDNA overhangs at telomeres. A strong candidate for an alternative exonuclease at telomeres is Exo1p.

EXO1

Exo1p is a conserved 5′-to-3′ exonuclease with FLAP endouclease activity that appears to function redundantly with the MRX complex in resection of DSBs and DNA repair (Lee et al., 2002; Lewis et al., 2002; Moreau et al., 2001; Tran et al., 2002; Tsubouchi and Ogawa, 2000). Exo1p is also implicated in mismatch repair (Tishkoff et al., 1997) and meiotic recombination (Khazanehdari and Borts, 2000; Kirkpatrick et al., 2000). However, unlike *mrx* mutants, *exo1Δ* mutants show no telomere length defects (Moreau et al., 2001; Tsubouchi and Ogawa, 2000). Furthermore, *mre11Δ* single and *mre11Δ exo1Δ* double mutants have telomeres of similar length, which suggests that Exo1p does not function redundantly with MRX at telomeres.

Although Exo1p appears to play no essential role in telomere physiology, it plays a critical role in regulating ssDNA levels when telomere capping is defective. Specifically, an *exo1Δ* mutation suppresses the temperature-dependent growth defects and reduces ssDNA accumulation in capping-defective *yku70Δ* and *cdc13-1* mutants cultured at non-permissive temperatures (Maringele and Lydall, 2002) (M. Zubko, S. Guillard and D. Lydall, unpublished). *EXO1* is essential for generating all the ssDNA at telomeres of *yku70Δ* mutants at 37°C but other *RAD24*-dependent nuclease(s) appear to act in concert with Exo1p at telomeres of *cdc13-1* mutants (Maringele and Lydall, 2002) (M. Zubko, S. Guillard and D. Lydall, unpublished).

The *RAD24* group

RAD17, *RAD24*, *MEC3* and *DDC1* are termed the *RAD24* group because deleting any or all of these genes results in similar checkpoint and DNA damage sensitivity phenotypes (Lydall and Weinert, 1995). In a variety of organisms, telomere defects are associated with defects in the *RAD24* group of gene products. In *C. elegans*, *mrt2* mutants lacking a *RAD17* orthologue possess short telomeres and undergo end-to-end chromosome fusions (Ahmed and Hodgkin, 2000). In *S. pombe*, mutations in genes encoding the orthologues of the *RAD24* group also result in generation of short telomeres (Dahlen et al., 1998; Matsuura et al., 1999; Nakamura et al., 2002). In budding yeast, *mec3Δ* mutants have long telomeres in one genetic background (Corda et al., 1999) but in another background *rad17Δ*, *rad24Δ* and *ddc1Δ* mutants have slightly shortened telomeres (Longhese et al., 2000).

Rad24p and the four small replication factor C subunits (Rfc2p-Rfc5p) appear to load Rad17p, Mec3p and Ddc1p, a heterotrimeric PCNA-like ring, at uncapped telomeres of *cdc13-1* mutants (Griffith et al., 2002; Kondo et al., 2001; Majka and Burgers, 2003; Melo et al., 2001; Shiomi et al., 2002). *RAD24* is important, similarly to *EXO1*, for generating ssDNA at telomeres of *cdc13-1* mutants (Booth et al., 2001; Lydall and Weinert, 1995; Maringele and Lydall, 2002) (M. Zubko, S. Guillard and D. Lydall, unpublished). However, unlike *EXO1*, *RAD24* is not important for generating ssDNA at telomeres of *yku70Δ* mutants (Maringele and Lydall, 2002). Therefore, an appealing model to explain the role the *RAD24* group in generating ssDNA at telomeres of *cdc13-1* mutants is that the Rad17p-Mec3p-Ddc1p PCNA-type complex tethers some type of nuclease(s) onto DNA. However, Rad17p, Mec3p and Ddc1p do not appear to tether Exo1p to DNA because *RAD24* and *EXO1* encode or control nucleases with different properties (Maringele and Lydall, 2002) (M. Zubko, S. Guillard and D. Lydall, unpublished).

Telomere switching and checkpoint activation

As described above, checkpoint proteins play critical roles in responding to uncapped telomeres. Blackburn and others have proposed that capped telomeres prevent telomerase, DNA repair and checkpoint pathways from being activated, whereas uncapped telomeres activate telomerase, repair and checkpoint

pathways (Blackburn, 2000; Blackburn, 2001; Chan and Blackburn, 2002). According to these models uncapped telomeres are short lived because telomerase rapidly restores the telomere length required for capping. However, the question remains as to how a cell distinguishes between telomeric and DSB ends such that telomeres induce telomerase-dependent rather than repair-dependent pathways to heal the end.

Building on the concept of capped and uncapped telomeres, I suggest that telomeres may vary between the extremes of capped, telomere-like states, and uncapped, DSB-like states. Fig. 3 illustrates this model and is based on the finding that at least four seemingly different DNA damage checkpoint pathways can be activated by uncapped telomeres. According to this model, a fully capped telomere is essentially inert within the cell (Fig. 3A), whereas a fully uncapped telomere behaves like a DSB and is a potent inducer of cell cycle arrest and DNA repair events (Fig. 3C). Between these two extremes, a spectrum of alternative states might exist and just one is shown in Fig. 3B. Telomere capping proteins favour the formation of capped telomeres (Fig. 3A), whereas nucleases favour the formation of DSB-like telomeres (Fig. 3C).

In budding yeast four different types of telomere defect activate checkpoint-dependent cell cycle arrest. Although the roles of all checkpoint genes at each type of damage have not been tested, it is clear that each type of damage relies on different subsets of DNA-damage checkpoint genes to signal arrest (Table 1). Intriguingly, spindle checkpoint genes have also been implicated in arrest of cells with telomere damage (Maringele and Lydall, 2002; Miller and Cooper, 2003). The role of spindle checkpoints in responding to telomere damage is poorly understood.

cdc13-1 mutants cultured at 36°C arrest cell division very rapidly and rely on all classes of checkpoint gene for efficient cell cycle arrest. This is completely dependent on *MEC1*, *DDC2*, *RAD9* and the *RAD24* group but only partially on *RAD53*, *DUN1* and *CHK1*. The Rad53p and Dun1p protein kinases appear to function in a pathway parallel to that involving Chk1p (and Pds1p) because double mutants are completely arrest-defective (Gardner et al., 1999; Sanchez et al., 1999).

*yku70*Δ mutants cultured at 37°C arrest more slowly than *cdc13-1* mutants and their arrest depends on only a subset of checkpoint genes. It is particularly notable that there is no role for *DUN1* [*RAD53*; see discussion in Maringele and Lydall (Maringele and Lydall, 2002)] or the members of the *RAD24* group in arrest of *yku70*Δ mutants at 37°C (Maringele and Lydall, 2002).

Telomerase-deficient yeast, in crisis, accumulate in G2/M phase of the cell cycle. Two recent papers showed that disruption of checkpoint genes reduces the G2/M delay observed in such cells (Enomoto et al., 2002; IJpma and Greider, 2003). The two papers show that the delay depends on *MEC1*, *DDC2* and the *RAD24* group of genes but less so on *TEL1*, *RAD9* and *RAD53*. Interestingly, Rad53p is phosphorylated in a *RAD9*-dependent manner in senescing cells, although neither *RAD9* nor *RAD53* plays a major role in cell cycle arrest (IJpma and Greider, 2003; Enomoto et al., 2002).

Finally, Tel1p overexpression in cells that have short telomeres induces transient checkpoint-dependent cell cycle arrest (Clerici et al., 2001; Viscardi et al., 2003). This type of damage is unique in that it is the only type of telomere-specific damage that can induce arrest independently of *MEC1* and *DDC2*. Arrest of cells overexpressing *TEL1* is transient and correlates with the length of time it takes for telomeres to stabilise at a new length. Tel1p overexpression may exaggerate a weak checkpoint that occurs each time telomeres are elongated.

In summary, each of four different types of telomere defect relies on a different subset of checkpoint genes. One explanation for these data is that each type of damage recruits and activates a different constellation of checkpoint proteins at the telomere. I suggest that in yeast different telomeric stimuli represent states that exist between the extremes of a fully capped telomere and a DSB-like telomere. Damage induced by the *cdc13-1* mutation is perhaps most ‘DSB-like’ because it relies on most of the genes required to induce arrest at DSBs, whereas *TEL1* overexpression is most ‘telomere-like’ since arrest is transient and independent of *MEC1*. It may also be relevant that different checkpoint genes respond to blunt or resected DSBs. *MEC1*, *RAD17*, *RAD24* and *MEC3* signal cell cycle arrest in meiotic cells that contain resected DSBs, but *RAD9* and *TEL1* do not (Lydall et al., 1996; Usui et al., 2001). However, all six genes, and the *MRX* genes, are essential to signal arrest in response to unresected

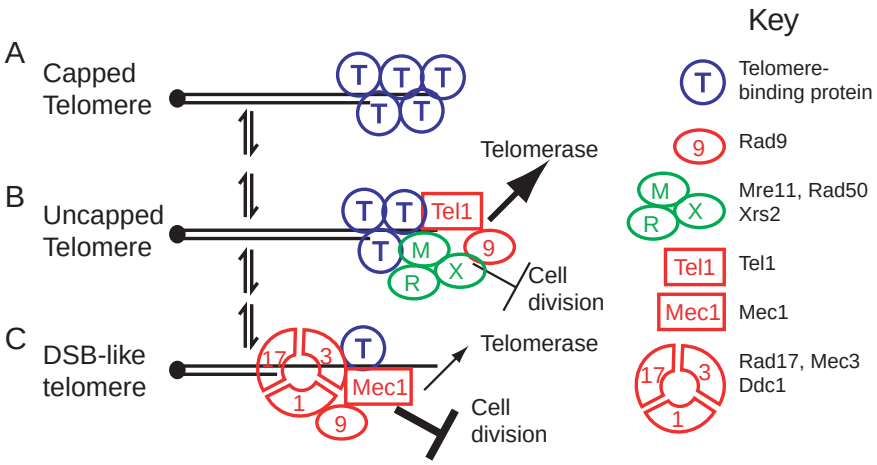

Fig. 3. A spectrum of telomeric states. A model showing three states at budding yeast telomeres. (A) A fully capped telomere that prevents checkpoint activation and repair pathways. It is capped by numerous telomere-binding proteins, indicated by T. (B) An uncapped telomere that has recruited the PIKK kinase Tel1p, the checkpoint protein Rad9p, and the MRX complex (encoded by *MRE11*, *RAD50* and *XRS2*). This type of telomere is a weak inhibitor of cell division based on the fact that the *TEL1*-dependent response to unresected DSBs is weak (Usui et al., 2001) and that Tel1p overexpression causes transient arrest (Viscardi et al., 2003). Tel1p appears to be a potent activator of telomerases and contributes to telomere capping. (C) A resected, DSB-like telomere that has recruited the core members of the DNA damage checkpoint response, including *MEC1*, *MEC3*, *RAD9*, *RAD17* and *DDC1*. This DSB-like telomere is a potent activator of cell cycle arrest but less efficient at recruiting telomerase.

(blunt) DSBs (Usui et al., 2001). Aspects of this model may also be relevant in mouse and human cells, where different checkpoint pathways respond to similar telomere defects (Smogorzewska and de Lange, 2002).

Conclusions and perspectives

Telomeres do not normally activate DNA repair and DNA damage checkpoint responses. It came as a surprise to discover that DNA repair and DNA damage checkpoint genes play important roles at telomeres. Although we are still far from understanding the precise roles of repair and checkpoint proteins at telomeres, some important clues are emerging. It is now clear that many different types of telomere defect exist and each type requires a different subset of checkpoint genes to induce arrest. It may be that a spectrum of states, each activating different checkpoint pathways, explains the roles of checkpoint proteins in telomere physiology and pathology. If so, then understanding these states will have implications not only for how cells respond to defective telomeres but also for how cells respond to damaged DNA elsewhere in the genome.

I thank Richard Blankley, Julie Cooper, Xindan Jia, Pia Longhese, Ed Louis, Laura Maringele, Misha Zubko and anonymous referees for input and comments on the manuscript. I apologise to those whose work was not cited owing to space constraints. D.L. is supported by the Wellcome Trust.

References

Adams Martin, A., Dionne, I., Wellinger, R. J. and Holm, C. (2000). The function of DNA polymerase alpha at telomeric G tails is important for telomere homeostasis. *Mol. Cell. Biol.* **20**, 786-796.

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

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

Blackburn, E. H. (2000). Telomere states and cell fates. *Nature* **408**, 53-56.

Blackburn, E. H. (2001). Switching and signaling at the telomere. *Cell* **106**, 661-673.

Booth, C., Griffith, E., Brady, G. and 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.

Carr, A. M. (2003). Beginning at the end. *Science* **300**, 1512-1513.

Carson, M. J. and Hartwell, L. (1985). CDC17: an essential gene that prevents telomere elongation in yeast. *Cell* **42**, 249-257.

Cervantes, R. B. and Lundblad, V. (2002). Mechanisms of chromosome-end protection. *Curr. Opin. Cell Biol.* **14**, 351-356.

Chan, S. W. and Blackburn, E. H. (2002). New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. *Oncogene* **21**, 553-563.

Chan, S. W., Chang, J., Prescott, J. and Blackburn, E. H. (2001). Altering telomere structure allows telomerase to act in yeast lacking ATM kinases. *Curr. Biol.* **11**, 1240-1250.

Cimino-Reale, G., Pascale, E., Alvino, E., Starace, G. and D'Ambrosio, E. (2003). Long telomeric C-rich 5′-tails in human replicating cells. *J. Biol. Chem.* **278**, 2136-2140.

Clerici, M., Paciotti, V., Baldo, V., Romano, M., Lucchini, G. and Longhese, M. P. (2001). Hyperactivation of the yeast DNA damage checkpoint by TEL1 and DDC2 overexpression. *EMBO J.* **20**, 6485-6498.

Cooper, J. P. (2000). Telomere transitions in yeast: the end of the chromosome as we know it. *Curr. Opin. Genet. Dev.* **10**, 169-177.

Corda, Y., Schramke, V., Longhese, M. P., Smokvina, T., Paciotti, V., Brevet, V., Gilson, E. and Geli, V. (1999). Interaction between Set1p and checkpoint protein Mec3p in DNA repair and telomere functions. *Nat. Genet.* **21**, 204-208.

Craven, R. J., Greenwell, P. W., Dominska, M. and Petes, T. D. (2002). Regulation of Genome Stability by TEL1 and MEC1, Yeast Homologs of the Mammalian ATM and ATR Genes. *Genetics* **161**, 493-507.

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

Dahlen, M., Olsson, T., Kanter-Smoler, G., Ramne, A. and Sunnerhagen, P. (1998). Regulation of telomere length by checkpoint genes in Schizosaccharomyces pombe. *Mol. Biol. Cell* **9**, 611-621.

Diede, S. J. and Gottschling, D. E. (1999). Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases alpha and delta. *Cell* **99**, 723-733.

Diede, S. J. and Gottschling, D. E. (2001). Exonuclease activity is required for sequence addition and Cdc13p loading at a de novo telomere. *Curr. Biol.* **11**, 1336-1340.

Dionne, I. and Wellinger, R. J. (1996). Cell cycle-regulated generation of single-stranded G-rich DNA in the absence of telomerase. *Proc. Natl. Acad. Sci. USA* **93**, 13902-13907.

Dionne, I. and Wellinger, R. J. (1998). Processing of telomeric DNA ends requires the passage of a replication fork. *Nucleic Acids Res.* **26**, 5365-5371.

Dubrana, K., Perrod, S. and Gasser, S. M. (2001). Turning telomeres off and on. *Curr. Opin. Cell Biol.* **13**, 281-289.

Enomoto, S., Glowczewski, L. and 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.

Evans, S. K. and Lundblad, V. (2000). Positive and negative regulation of telomerase access to the telomere. *J. Cell Sci.* **113**, 3357-3364.

Gardner, R., Putnam, C. W. and Weinert, T. (1999). RAD53, DUN1 and PDS1 define two parallel G(2)/M checkpoint pathways in budding yeast. *EMBO J.* **18**, 3173-3185.

Garvik, B., Carson, M. and 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.

Gasser, S. M. (2000). A sense of the end. *Science* **288**, 1377-1379.

Grandin, N., Reed, S. I. and Charbonneau, M. (1997). Stn1, a new Saccharomyces cerevisiae protein, is implicated in telomere size regulation in association with Cdc13. *Genes Dev.* **11**, 512-527.

Grandin, N., Damon, C. and Charbonneau, M. (2001). Ten1 functions in telomere end protection and length regulation in association with Stn1 and Cdc13. *EMBO J.* **20**, 1173-1183.

Gravel, S., Larrivee, M., Labrecque, P. and Wellinger, R. J. (1998). Yeast Ku as a regulator of chromosomal DNA end structure. *Science* **280**, 741-744.

Greider, C. W. and Blackburn, E. H. (1985). Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* **43**, 405-413.

Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H. and de Lange, T. (1999). Mammalian telomeres end in a large duplex loop. *Cell* **97**, 503-514.

Griffith, J. D., Lindsey-Boltz, L. A. and Sancar, A. (2002). Structures of the human Rad17-replication factor C and checkpoint Rad 9-1-1 complexes visualized by glycerol spray/low voltage microscopy. *J. Biol. Chem.* **277**, 15233-15236.

Haber, J. E. (1998). The many interfaces of Mre11. *Cell* **95**, 583-586.

Hediger, F., Neumann, F. R., van Houwe, G., Dubrana, K. and Gasser, S. M. (2002). Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. *Curr. Biol.* **12**, 2076-2089.

Henson, J. D., Neumann, A. A., Yeager, T. R. and Reddel, R. R. (2002). Alternative lengthening of telomeres in mammalian cells. *Oncogene* **21**, 598-610.

Huffman, K. E., Levene, S. D., Tesmer, V. M., Shay, J. W. and Wright, W. E. (2000). Telomere shortening is proportional to the size of the G-rich telomeric 3′-overhang. *J. Biol. Chem.* **275**, 19719-19722.

IJpma, A. and Greider, C. W. (2003). Short telomeres induce a DNA damage response in Saccharomyces cerevisiae. *Mol. Biol. Cell* **14**, 987-1001.

Jacob, N. K., Kirk, K. E. and Price, C. M. (2003). Generation of Telomeric G strand overhangs involves both G and C strand cleavage. *Mol. Cell* **11**, 1021-1032.

Khazanehdari, K. A. and Borts, R. H. (2000). EXO1 and MSH4 differentially affect crossing-over and segregation. *Chromosoma* **109**, 94-102.

Kirkpatrick, D. T., Ferguson, J. R., Petes, T. D. and Symington, L. S. (2000). Decreased meiotic intergenic recombination and increased meiosis

I nondisjunction in exo1 mutants of Saccharomyces cerevisiae. *Genetics* **156**, 1549-1557.

Kironmai, K. M. and Muniyappa, K. (1997). Alteration of telomeric sequences and senescence caused by mutations in RAD50 of Saccharomyces cerevisiae. *Genes Cells* **2**, 443-455.

Kondo, T., Wakayama, T., Naiki, T., Matsumoto, K. and Sugimoto, K. (2001). Recruitment of Mec1 and Ddc1 checkpoint proteins to double-strand breaks through distinct mechanisms. *Science* **294**, 867-870.

Kramer, K. M. and Haber, J. E. (1993). New telomeres in yeast are initiated with a highly selected subset of TG1-3 repeats. *Genes Dev.* **7**, 2345-2356.

Le, S., Moore, J. K., Haber, J. E. and Greider, C. W. (1999). RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. *Genetics* **152**, 143-152.

Lee, S. E., Moore, J. K., Holmes, A., Umezu, K., Kolodner, R. D. and Haber, J. E. (1998). Saccharomyces Ku70, Mre11/Rad50, and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94**, 399-409.

Lee, S. E., Bressan, D. A., Petrini, J. H. J. and Haber, J. E. (2002). Complementation between N-terminal Saccharomyces cerevisiae mre11 alleles in DNA repair and telomere length maintenance. *DNA Repair* **1**, 27-40.

Lewis, L. K., Karthikeyan, G., Westmoreland, J. W. and Resnick, M. A. (2002). Differential suppression of DNA repair deficiencies of yeast rad50, mre11 and xrs2 mutants by EXO1 and TLC1 (the RNA component of telomerase). *Genetics* **160**, 49-62.

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

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

Louis, E. J. (2002). Are Drosophila telomeres an exception or the rule? *Genome Biol.* **3**, reviews0007.

Louis, E. J., Naumova, E. S., Lee, A., Naumov, G. and Haber, J. E. (1994). The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. *Genetics* **136**, 789-802.

Lundblad, V. and Blackburn, E. H. (1993). An alternative pathway for yeast telomere maintenance rescues est1-senescence. *Cell* **73**, 347-360.

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

Lydall, D., Nikolsky, Y., Bishop, D. K. and Weinert, T. (1996). A meiotic recombination checkpoint controlled by mitotic checkpoint genes. *Nature* **383**, 840-843.

Majka, J. and Burgers, P. M. (2003). Yeast Rad17/Mec3/Ddc1: A sliding clamp for the DNA damage checkpoint. *Proc. Natl. Acad. Sci. USA* **100**, 2249-2254.

Makarov, V. L., Hirose, Y. and Langmore, J. P. (1997). Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell* **88**, 657-666.

Maringele, L. and 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.

Matsuura, A., Naito, T. and Ishikawa, F. (1999). Genetic control of telomere integrity in Schizosaccharomyces pombe: rad3(+) and tel1(+) are parts of two regulatory networks independent of the downstream protein kinases chk1(+) and cds1(+). *Genetics* **152**, 1501-1512.

McEachern, M. J., Krauskopf, A. and Blackburn, E. H. (2000). Telomeres and their control. *Annu. Rev. Genet.* **34**, 331-358.

McElligott, R. and Wellinger, R. J. (1997). The terminal DNA structure of mammalian chromosomes. *EMBO J.* **16**, 3705-3714.

Mefford, H. C. and Trask, B. J. (2002). The complex structure and dynamic evolution of human subtelomeres. *Nat. Rev. Genet.* **3**, 91-102.

Melo, J. and Toczyski, D. (2002). A unified view of the DNA-damage checkpoint. *Curr. Opin. Cell Biol.* **14**, 237-245.

Melo, J. A., Cohen, J. and Toczyski, D. P. (2001). Two checkpoint complexes are independently recruited to sites of DNA damage in vivo. *Genes Dev.* **15**, 2809-2821.

Miller, K. M. and Cooper, J. P. (2003). The telomere protein Taz1 is required to prevent and repair genomic DNA breaks. *Mol. Cell* **11**, 303-313.

Mitton-Fry, R. M., Anderson, E. M., Hughes, T. R., Lundblad, V. and Wuttke, D. S. (2002). Conserved structure for single-stranded telomeric DNA recognition. *Science* **296**, 145-147.

Moreau, S., Morgan, E. A. and Symington, L. S. (2001). Overlapping functions of the Saccharomyces cerevisiae Mre11, Exo1 and Rad27 nucleases in DNA metabolism. *Genetics* **159**, 1423-1433.

Myung, K., Datta, A. and Kolodner, R. D. (2001). Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in Saccharomyces cerevisiae. *Cell* **104**, 397-408.

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

Nakamura, T. M., Moser, B. A. and Russell, P. (2002). Telomere binding of checkpoint sensor and DNA repair proteins contributes to maintenance of functional fission yeast telomeres. *Genetics* **161**, 1437-1452.

Nugent, C. I., Hughes, T. R., Lue, N. F. and Lundblad, V. (1996). Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. *Science* **274**, 249-252.

Nyberg, K. A., Michelson, R. J., Putnam, C. W. and Weinert, T. A. (2002). Toward maintaining the genome: DNA damage and replication checkpoints. *Annu. Rev. Genet.* **36**, 617-656.

Olovnikov, A. M. (1973). A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J. Theor. Biol.* **41**, 181-190.

Osborn, A. J., Elledge, S. J. and Zou, L. (2002). Checking on the fork: the DNA-replication stress-response pathway. *Trends Cell Biol.* **12**, 509-516.

Parenteau, J. and Wellinger, R. J. (2002). Differential processing of leading- and lagging-strand ends at Saccharomyces cerevisiae telomeres revealed by the absence of Rad27p nuclease. *Genetics* **162**, 1583-1594.

Polotnianka, R. M., Li, J. and Lustig, A. J. (1998). The yeast Ku heterodimer is essential for protection of the telomere against nucleolytic and recombinational activities. *Curr. Biol.* **8**, 831-834.

Pryde, F. E. and Louis, E. J. (1997). Saccharomyces cerevisiae telomeres. A review. *Biochemistry (Mosc)* **62**, 1232-1241.

Pryde, F. E. and Louis, E. J. (1999). Limitations of silencing at native yeast telomeres. *EMBO J.* **18**, 2538-2550.

Raychaudhuri, S., Byers, R., Upton, T. and Eisenberg, S. (1997). Functional analysis of a replication origin from Saccharomyces cerevisiae: identification of a new replication enhancer. *Nucleic Acids Res.* **25**, 5057-5064.

Rhodes, D., Fairall, L., Simonsson, T., Court, R. and Chapman, L. (2002). Telomere architecture. *EMBO Rep.* **3**, 1139-1145.

Riha, K., McKnight, T. D., Fajkus, J., Vyskot, B. and Shippen, D. E. (2000). Analysis of the G-overhang structures on plant telomeres: evidence for two distinct telomere architectures. *Plant J.* **23**, 633-641.

Ritchie, K. B. and Petes, T. D. (2000). The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. *Genetics* **155**, 475-479.

Rouse, J. and Jackson, S. P. (2002a). Interfaces between the detection, signaling, and repair of DNA damage. *Science* **297**, 547-551.

Rouse, J. and Jackson, S. P. (2002b). Lcd1p recruits Mec1p to DNA lesions in vitro and in vivo. *Mol. Cell* **9**, 857-869.

Sanchez, Y., Bachant, J., Wang, H., Hu, F., Liu, D., Tetzlaff, M. and Elledge, S. J. (1999). Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* **286**, 1166-1171.

Sandell, L. L. and Zakian, V. A. (1993). Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* **75**, 729-739.

Shiomi, Y., Shinozaki, A., Nakada, D., Sugimoto, K., Usukura, J., Obuse, C. and Tsurimoto, T. (2002). Clamp and clamp loader structures of the human checkpoint protein complexes, Rad9-1-1 and Rad17-RFC. *Genes Cells* **7**, 861-868.

Shore, D. (2001). Telomeric chromatin: replicating and wrapping up chromosome ends. *Curr. Opin. Genet. Dev.* **11**, 189-198.

Smogorzewska, A. and de Lange, T. (2002). Different telomere damage signaling pathways in human and mouse cells. *EMBO J.* **21**, 4338-4348.

Sugawara, N. and Haber, J. E. (1992). Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Mol. Cell. Biol.* **12**, 563-575.

Sun, H., Treco, D. and Szostak, J. W. (1991). Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. *Cell* **64**, 1155-1161.

Teng, S. C. and Zakian, V. A. (1999). Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **19**, 8083-8093.

Tishkoff, D. X., Boerger, A. L., Bertrand, P., Filosi, N., Gaida, G. M., Kane, M. F. and Kolodner, R. D. (1997). Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2. *Proc. Natl. Acad. Sci. USA* **94**, 7487-7492.

Tran, P. T., Erdenez, N., Dudley, S. and Liskay, R. M. (2002).

Characterization of nuclease-dependent functions of Exo1p in Saccharomyces cerevisiae. *DNA Repair* **1**, 895-812.

Tsubouchi, H. and Ogawa, H. (2000). Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in Saccharomyces cerevisiae. *Mol. Biol. Cell* **11**, 2221-2233.

Tsukamoto, Y., Taggart, A. K. and Zakian, V. A. (2001). The role of the Mre11-Rad50-Xrs2 complex in telomerase- mediated lengthening of Saccharomyces cerevisiae telomeres. *Curr. Biol.* **11**, 1328-1335.

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

Vaze, M. B., Pellicioli, A., Lee, S. E., Ira, G., Liberi, G., Arbel-Eden, A., Foiani, M. and Haber, J. E. (2002). Recovery from checkpoint-mediated arrest after repair of a double-strand break requires Srs2 helicase. *Mol. Cell* **10**, 373-385.

Viscardi, V., Baroni, E., Romano, M., Lucchini, G. and Longhese, M. P. (2003). Sudden telomere lengthening triggers a Rad53-dependent checkpont in *S. cerevisiae*. *Mol. Biol. Cell* **14**, 3126-3143.

Wang, H. and Blackburn, E. H. (1997). De novo telomere addition by Tetrahymena telomerase in vitro. *EMBO J.* **16**, 866-879.

Watson, J. D. (1972). Origin of concatemeric T7 DNA. *Nat. New Biol.* **239**, 197-201.

Wei, C. and Price, C. M. (2003). Protecing the terminus: t-loops and telomere end-binding proteins. *Cell. Mol. Life Sci.* (in press).

Weinert, T. A. and Hartwell, L. H. (1988). The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. *Science* **241**, 317-322.

Wellinger, R. J. and Sen, D. (1997). The DNA structures at the ends of eukaryotic chromosomes. *Eur. J. Cancer* **33**, 735-749.

Wellinger, R. J., Wolf, A. J. and Zakian, V. A. (1993). Saccharomyces telomeres acquire single-strand TG1-3 tails late in S phase. *Cell* **72**, 51-60.

Wellinger, R. J., Ethier, K., Labrecque, P. and Zakian, V. A. (1996). Evidence for a new step in telomere maintenance. *Cell* **85**, 423-433.

Wilson, S., Warr, N., Taylor, D. and Watts, F. (1999). The role of Schizosaccharomyces pombe Rad32, the Mre11 homologue, and other DNA damage response proteins in non-homologous end joining and telomere length maintenance. *Nucleic Acids. Res.* **27**, 2655-2661.

Yamada, M., Hayatsu, N., Matsuura, A. and Ishikawa, F. (1998). Y′-Help1, a DNA helicase encoded by the yeast subtelomeric Y′ element, is induced in survivors defective for telomerase. *J. Biol. Chem.* **273**, 33360-33366.

Zou, L. and Elledge, S. J. (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* **300**, 1542-1548.