Cytogenet Genome Res 122:365–373 (2008)
DOI: 10.1159/000167824

Cytogenetic and Genome Research

# Telomere capping and cellular checkpoints: clues from fruit flies

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Accepted in revised form for publication by P. Slijepcevic, 27 August 2008.

**Abstract.** In most organisms, telomeres consist of repetitive G-rich sequences that are elongated by a specific reverse transcriptase, telomerase. A large number of proteins are recruited by these terminal repeats, forming specialized structures that regulate telomerase activity and protect telomeres from degradation and recombination. *Drosophila* lacks telomerase and telomere length is maintained by transposition of three specialized retrotransposons. In addition, unlike yeast and mammals, *Drosophila* telomeres are epigenetically determined, sequence-independent structures. However, several proteins required for *Drosophila* telomere behavior are evolutionarily conserved. These include the Mre11-Rad50-Nbs (MRN) complex and the Ataxia Telangiectasia Mutated (ATM) kinase, which are required to prevent telomeric fusions. In addition, recent studies have provided evidence that *Drosophila* uncapped telomeres elicit a DNA damage response (DDR) just as dysfunctional yeast and human telomeres. Uncapped *Drosophila* telomeres also activate the spindle assembly checkpoint (SAC) by recruiting the SAC kinase BubR1. Telomere-induced DDR and SAC both require the wild type function of the MRN complex. In addition, while DDR is mediated by ATR kinase, SAC activation requires both the ATM and ATR activities. These results indicate that the DNA repair systems play multiple roles at *Drosophila* telomeres, highlighting the importance of this model organism for investigations on the relationships between DNA repair and telomere maintenance.


Telomeres perform at least two essential functions; they counterbalance the incomplete replication of terminal DNA; they cap chromosome ends and protect them from degradation and fusion events, allowing the cell to distinguish natural chromosome termini from broken DNA ends (Ferreira et al., 2004; de Lange, 2005).

The telomere capping function is compromised in many human tumors that display frequent telomeric fusions (TFs, Hastie and Allshire, 1989; de Lange, 1995). It has been suggested that TFs are the consequence of telomere shortening that occurs in early stages of carcinogenesis, and that short telomeres are fusigenic because they cannot recruit sufficient amounts of telomere capping proteins (van Steensel et al., 1998). It is now widely accepted that telomere dysfunction is an important factor in carcinogenesis. The dicentric chromosomes generated by TFs can cause nondisjunction and chromosome breakage during anaphase, resulting in genetic instability that promotes tumor development (Maser and DePinho, 2002; Sharpless and DePinho, 2004).

In most eukaryotes, terminal DNA consists of short, repetitive G-rich sequences, which end in a 3′ overhang of the G-rich strand. This overhang is specifically elongated by a specialized reverse transcriptase, telomerase, which contains a species-specific RNA template (Nugent and Lundblad, 1998; Cristofari and Lingner, 2006). In human cells, the G-strand overhang can fold back and invade the double-stranded region of telomeres, forming a protective structure known as the t-loop (de Lange, 2005). The telomeric repeats are bound by several single strand (ss) and double strand

This work was supported by a grant for Progetti di Ricerca di Interesse Nazionale (PRIN) from Ministero dell'Università e Ricerca (MIUR) to G.C.

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(ds) DNA-binding proteins, which regulate telomerase activity and protect chromosome ends from degradation and end-to-end joining events (de Lange, 2005). In budding yeast, telomeric repeats are bound by an array of Repressor/activator protein 1 (Rap1). Rap1 interacts with the Silent information regulator proteins (Sir2, Sir3 and Sir4) and Rap1-interacting factors (Rif1 and Rif2), which are involved in the formation of subtelomeric heterochromatin and in the regulation of telomere elongation, respectively (Lundblad, 2006). *S. cerevisiae* G-tail binds Cdc13 (Cell division control protein 13), an oligonucleotide/oligosaccharide-binding (OB)-fold containing protein, that interacts with Stn1 and Ten1 controlling telomerase-mediated telomere elongation (Lundblad, 2006; Gao et al., 2007). In the fission yeast *Schizosaccharomyces pombe*, Rap1 lacks the ability to bind DNA and its recruitment at telomeres is largely dependent on Taz1 (Cooper and Hiraoka, 2006). Homologues of budding yeast Rif proteins have been also found in fission yeast telomeres, where they are recruited through the interaction with Taz1 and regulate telomere length and structure (Cooper et al., 1997). In addition *S. pombe* G-tail is bound and protected by Pot1 (Protection of telomeres 1), an OB-fold containing protein orthologous to TEBPα that binds the 3′ overhang of ciliates (Price, 2006).

Human telomeric repeats are specifically bound by three proteins: human POT1 that associates with G-tails, and the Telomeric-Repeat-binding Factors TRF1 and TRF2, which are homologous to Taz1 and bind the telomeric DNA duplex. POT1, TRF1 and TRF2 are interconnected by three additional polypeptides (human RAP1, TIN2 and TPP1) forming a six protein complex, called shelterin, that specifically binds chromosome ends (de Lange, 2005). Shelterin is thought to mediate t-loop formation and stabilization, thereby regulating telomerase action and protecting chromosome ends from degradation and fusion (de Lange, 2005). In addition shelterin mediates recruitment at telomeres of 'nonshelterin' factors, most of which are conserved proteins involved in DNA repair (de Lange, 2005).

*Drosophila* lacks telomerase activity and telomere length is maintained by transposition to chromosome ends of three specialized retrotransposons, called HeT-A, TART and TAHRE (Mason et al., 2008). Multiple copies of these elements constitute the terminal DNA array, referred to as HeT-A/TART/TAHRE (HTT) domain (see Frydrychova et al., this issue). *Drosophila* also lacks the telomeric DNA binding factors that have been described in yeast and mammals (Rap1, Cdc13, Pot1/hPot1, Taz1/TRF2 and TRF1). However, *Drosophila* telomeres are protected from fusion events just as their mammalian counterparts, and many *Drosophila* genes that control telomere behavior are conserved in mammals (Cenci et al., 2005). In the past few years *Drosophila melanogaster* has emerged as a useful model organism for dissecting the mechanisms of telomere capping. There are at least three reasons that render *Drosophila* particularly favorable for this type of studies. First, unlike other eukaryotic systems, telomere capping and telomere elongation in *Drosophila* are naturally uncoupled, thus facilitating the characterization of specific capping functions (Mason and Biessmann, 1995; Cenci et al., 2005; Pimpinelli, 2006; Mason et al., 2008). Second, *Drosophila* telomere capping is sequence-independent implying a close resemblance of *Drosophila* telomeres to DNA double-strand breaks. Third, identification and characterization of *Drosophila* mutants defective in telomere protection is straightforward. In fruit flies, thanks to the maternal contribution, mutant embryos can survive till late larval stages, allowing phenotypic analysis of the cytological consequences of mutations affecting telomere capping (Gatti and Baker, 1989; Cenci et al., 2005). In contrast, mutations in many telomere capping/DNA repair genes in mice results in early lethality preventing an assessing of defects in telomere behavior.

Here, we review the work on factors required to prevent telomeric fusions in *Drosophila,* with a focus on proteins playing also a role in DNA repair. We then describe parallels and differences between yeast, humans and *Drosophila* in the activation of cell cycle checkpoints following telomere dysfunction.

## *Drosophila* telomere capping

The isolation of mutants displaying frequent telomeric fusions in larval brain cells has led so far to the identification of eight genes required for telomere capping: *UbcD1/effete* (*eff*), *Su(var)205*, *caravaggio (cav)*, *telomeric fusion (tefu), mre11, rad50, nbs* and *without children (woc)* (Cenci et al., 2005; Rong, 2008).

*UbcD1/eff* represents the first example of a gene required to prevent telomeric fusions in *Drosophila* (Cenci et al., 1997). As the gene encodes a highly conserved class I ubiquitin (E2)-conjugating enzyme, we suggested that a failure of either poly- or mono-ubiquitination of some telomeric protein(s) leads to fusigenic chromosome ends. Although there is evidence that the putative substrate(s) of UbcD1 associates with telomeres independently of the presence of HTT elements (Cenci et al., 2003a, 2005), its molecular nature remains to be identified.

*Su(var)205* encodes the highly conserved Heterochromatin Protein 1 (HP1), one of the three HP1 family members found in both *Drosophila* and humans. The original HP1 (also known as HP1a) was first described in *Drosophila* as a key factor in heterochromatin formation. A detailed phenotypic analysis of mutations in the HP1a-encoding *Su(var)205* gene has subsequently revealed that HP1 is also required for telomere capping (Fanti et al., 1998) and silencing of HTT elements (Perrini et al., 2004). Consistent with its function at telomeres, HP1 localizes to the ends of polytene chromosomes in addition to the chromocenter and many euchromatic bands (James et al., 1989; Fanti et al., 1998, 2003). It is worth noting that different HP1 isoforms have been localized at mammalian telomeres (Aagaard et al., 2000; Koering et al., 2002; Garcia-Cao et al., 2004) suggesting that the role of HP1 at chromosome ends may be conserved between *Drosophila* and humans (Pimpinelli, 2006).

HP1 interacts with HP1/ORC2-Associated Protein (HOAP), a DNA-binding protein encoded by the *cav* gene

(Shareef et al., 2001; Badugu et al., 2003; Cenci et al., 2003b). HOAP, which contains a high mobility group (HMG) motif but is not conserved outside of fly species, is a major component of the *Drosophila* chromosome cap. HOAP has three unique characteristics. First, mutations in *cav* result in the most severe end-to-end fusion phenotype observed in *Drosophila* (Fig. 1d; Cenci et al., 2003b, 2005; Musarò et al., 2008). Second, HOAP is the only *Drosophila* protein exclusively enriched at both mitotic and polytene telomeres (Cenci et al., 2003b, 2005; Oikemus et al., 2004). Finally, loss of HOAP results in dysfunctional telomeres that activate both the DDR and the SAC (Musarò et al., 2008).

The characterization of *woc* revealed that additional capping factors co-exist with and act independently of the HP1/HOAP complex at chromosome ends. Woc is a putative transcription factor that co-localizes with the initiating forms of RNA polymerase II in most euchromatic bands of polytene chromosomes. Woc localizes also at all polytene chromosomes telomeres. Loss of Woc leads to telomeric fusions, although uncapped telomeres that lack Woc maintain normal amounts of HP1 and HOAP (Raffa et al., 2005). In addition Woc is regularly found at both *Su(var)205* and *cav* mutant telomeres. Likewise, mutations in the *rad50* and *tefu/atm* genes do not affect Woc localization at telomeres suggesting that Woc and HP1/HOAP complex play independent roles in telomere capping (Raffa et al., 2005). It has been suggested that Woc is a transcription factor with a telomere capping function just as Rap1 is in yeast (Raffa et al., 2005).

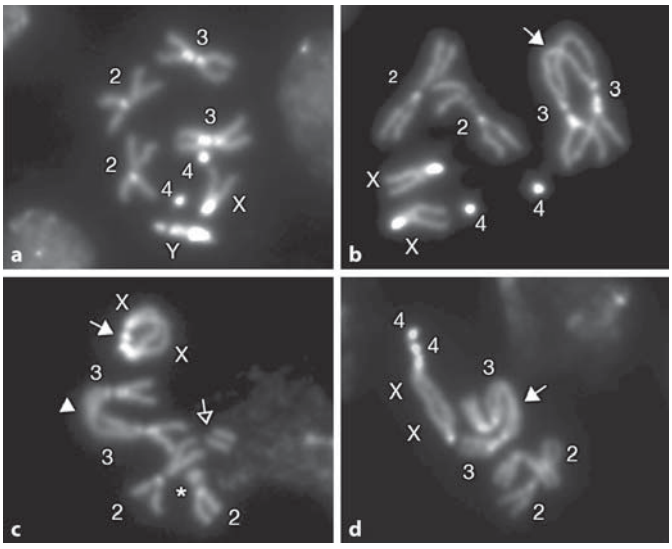


**Fig. 1.** Examples of cytological defects observed in different telomere-fusion mutants. (**a**) Control male metaphase. (**b**) Typical metaphase observed in *mre11*, *rad50* or *nbs* mutants: female metaphase showing a 3–3 dicentric chromosome generated by a TF (arrow). (**c**) Example of metaphase observed in *mei-41/atr; tefu/atm* or *tefu/atm nbs* double mutant: female metaphase containing a dicentric ring generated by XR-XR and XL-XL TFs (arrow), a 3–3 dicentric chromosome (arrowhead) and an isochromatid break involving chromosome 2 (the centric and acentric fragments are indicated by an asterisk and an empty arrow, repectively). (**d**) Example of a *cav* mutant metaphase: female metaphase containing a linear dicentric chromosome generated by one TF between two chromosomes 3 (arrow) and a tetracentric linear chromosome generated by three TFs between two chromosomes 4 and two X chromosomes.

### The role of MRN complex and ATM kinase in telomere capping

One of the essential roles of telomeres is the prevention of inappropriate repair between chromosome termini (telomeric fusions) and between DNA breaks and natural chromosome ends. It is thus paradoxical that many of the DNA repair and checkpoint proteins that respond to DNA damage elicited either by DSB or telomere dysfunction (i.e. the MRN complex and ATM/ATR kinases) are also required for the maintenance of telomere integrity.

In the mammalian system the MRN complex has been shown to play several essential functions (D'Amours and Jackson, 2002). It is involved in both modes of DNA DSB repair: the non-homologous end joining (NHEJ), and the homologous recombination (HR). The MRN complex interacts with the ATM kinase, which is at the top of a complex signaling web that mediates the response to DNA damage. Recent studies indicate that the MRN complex facilitates ATM activation through autophosphorylation at serine 1981 (Shiloh, 2006). In addition, it stimulates phosphorylation of several ATM substrates, acting as an adaptor that facilitates their binding to ATM (Shiloh, 2006). The MRN complex also enhances several Ataxia-Telangiectasia and Rad3-Related kinase (ATR) dependent phosphorylation events (Stiff et al., 2005; Zhong et al., 2005), and there is evidence that ATM and ATR can phosphorylate distinct as well as overlapping downstream proteins (Shiloh, 2006).

The first evidence of a molecular connection between the MRN complex and telomeres, comes from the finding that in mammals a portion of the cellular pool of MRE11, RAD50 and NBS1 forms a protein complex with the telomere repeat binding protein TRF2. The MRE11 and RAD50 proteins co-localize at telomeres throughout the cell cycle, while NBS1 has been found at telomeres only during S phase (Zhu et al., 2000). In addition, both the interaction with TRF2 and the telomeric localization of the MRN complex remain unaltered after gamma irradiation, suggesting a specific implication of the MRN complex in the formation and function of telomeric structure (Zhu et al., 2000).

Hypomorphic mutations in human *NBS1* or *MRE11* genes cause the genetic disorders Nijmengen Breakage Syndrome (NBS) and Ataxia Telangiectasia-Like Disorders (ATLD), respectively (Frappart and McKinnon, 2006). Null mutations affecting any member of the MRN complex lead to embryonic lethality in mice, precluding the analysis of telomere functions in the complete absence of MRN (Luo et al., 1999; Zhu et al., 2001; Theunissen et al., 2003). However, a few telomeric associations have been observed in cultured cells derived both from mice bearing a hypomorphic mutation in *Rad50* and from mice bearing the *Mre11* hypomorphic point mutation that causes ATLD (Bender et al., 2002; Theunissen et al., 2003).

**Table 1.** Human proteins homologous to *Drosophila* and yeast proteins and the corresponding genes involved in telomere maintenance and telomere-dependent cellular checkpoints. The *Drosophila* HOAP protein encoded by the *cav* gene that is not conserved in either human or yeasts, has not been included. Gene names are indicated by their corresponding abbreviations.

| Human protein | Genes | | | |
|---|---|---|---|---|
| | *D. melanogaster* | *H. sapiens* | *S. cerevisiae* | *S. pombe* |
| ATM | *tefu* | *ATM* | *tel1* | *tel1* |
| ATR | *mei-41* | *ATR* | *mec1* | *rad3* |
| ATRIP | *mus304* | *ATRIP* | *ddc2* | *rad26* |
| CHK1 | *grp* | *CHK1* | *chk1* | *chk1* |
| CHK2 | *mnk/loki* | *CHK2* | *rad53* | *mek1* |
| NBS1 | *nbs* | *NBS1* | *xrs2* | *nbs1* |
| RAD50 | *rad50* | *RAD50* | *rad50* | *rad50* |
| MRE11 | *mre11* | *MRE11* | *mre11* | *mre11* |
| BUBR1 | *bubR1* | *BUBR1* | *mad3* | *mad3* |
| CDC20 | *fzy* | *CDC20* | *cdc20* | *slp1* |
| TRF1 | – | *TRF1* | – | *taz1* |
| TRF2 | – | *TRF2* | – | *taz1* |
| HP1a | *Su(var)205* | *HP1a* | – | *swi6* |

The components of the MRN complex (MRX in *S. cerevisiae*) and its molecular architecture are highly conserved from yeast to mammals (D'Amours and Jackson, 2002); the fly orthologues (Table 1) of these genes exhibit approximately 50% similarity with their human and yeast counterparts (Ciapponi et al., 2004, 2006). In the last five years different groups have generated null mutations in the *Drosophila* homologues of *RAD50*, *MRE11* and *NBS1* (Bi et al., 2004; Ciapponi et al., 2004, 2006; Cenci et al., 2005; Oikemus et al., 2006; Rong, 2008). These null mutants die late in development, close to the time of pupal eclosion; the pharate adults exhibit phenotypic traits typically associated with high levels of chromosome instability and cell death, such as rough eyes, scalloped wings and shorter or missing bristles (Ahmad and Golic, 1999; Brodsky et al., 2000a; Bi et al., 2004; Ciapponi et al., 2004, 2006; Oikemus et al., 2006). Cell death is clearly visible in imaginal discs, where a high number of cells undergoing a p53-dependent spontaneous apoptosis has been observed (Bi et al., 2004, 2005a; Ciapponi et al., 2004, 2006; Gorski et al., 2004; Oikemus et al., 2006). Most importantly, mitotic cells from *rad50*, *mre11* and *nbs* mutants displayed both TFs (Fig. 1) and chromosome breakage, indicating that chromosome instability, and presumably cell death, are caused by extensive chromosome damage. Interestingly, although *rad50, mre11* and *nbs* mutants analyzed were all genetically null, they showed different TF frequencies; in *nbs* mutants, the frequency of TFs was approximately one half of that observed in either *rad50* or *mre11*, suggesting an Nbs-independent role of Rad50 and Mre11 in telomere protection (Bi et al., 2005b; Ciapponi et al., 2006; Oikemus et al., 2006). Similarly to the mutations in their mouse counterparts (Barlow et al., 1996; Luo et al., 1999; Kang et al., 2002; Theunissen et al., 2003) and to the mutations in the corresponding human genes, mutations in *Drosophila mre11, rad50* and *nbs* genes also cause defects in the DNA damage checkpoint (Bi et al., 2005a; Oikemus et al., 2006) and an extremely high sensitivity to the induction of chromosome breakage by X-rays (Bi et al., 2004, 2005b; Ciapponi et al., 2004, 2006; Oikemus et al., 2006).

ATM and ATR belong to the highly conserved phosphoinositide 3-kinase-related kinase (PIKKs) family. In humans, deficiency of these kinases leads to hereditary diseases associated with genetic instability, namely Ataxia Telangiectasia (AT) caused by ATM deficiency and ATR-Seckel due to hypomorphic mutations in ATR (Shiloh, 2006). Budding and fission yeast have orthologues of both ATM and ATR (Tel1 for ATM in both yeasts, Mec1 and Rad3 for ATR in *S. cerevisiae* and *S. pombe,* respectively, Table 1; for review see Shiloh, 2006; Longhese et al., 2008). In *Drosophila*, these two kinases are encoded by the *mei-41* (ATR*)* and *tefu* (ATM) genes (Table 1; Hari et al., 1995; Bi et al., 2004; Oikemus et al., 2004; Silva et al., 2004; Song et al., 2004).

In both yeast and mammals Mec1/ATR and Tel1/ATM are implicated in telomere structure and function. MRN/MRX-dependent ATM localization at telomeres is observed during S phase (Verdun and Karlseder, 2006; Hector et al., 2007; Sabourin et al., 2007) and contributes to the maintenance of telomere identity and length (Verdun et al., 2005; Longhese, 2008). Recent data indicate that in yeast Tel1 and MRX might promote telomerase recruitment and processivity at critically short telomeres (Chang et al., 2007; Gao et al., 2007). Moreover, yeast cells rely on Mec1/Rad3 to maintain short but stable telomeres in the absence of Tel1, suggesting that in normal cells the function of Mec1 at telomeres is redundant with, or masked by, Tel1 and MRX (Longhese, 2008).

Cells derived from AT patients and cells from *Atm*-deficient mice exhibit low levels of telomere associations, suggesting a role for this kinase in telomere protection (Hande et al., 2001; Pandita, 2002). Additionally, *Atm* and *telomerase* deficient mice appear to have a significant increase of telomere dysfunction with respect to single mutants, indicating that ATM deficiency and telomere dysfunction cooperate to increase chromosomal instability (Wong et al., 2003).

In line with these findings, in a number of simultaneous publications it has recently been reported that *Drosophila* mitotic cells from *tefu/atm* mutants display high levels of both TFs and chromosome breakage (Bi et al., 2004; Oikemus et al., 2004; Silva et al., 2004; Song et al., 2004). *Drosophila* ATM has been also shown to be required for viability and p53-dependent apoptosis (Oikemus et al., 2006), and to be essential for cell cycle arrest after low doses of X-irradiation but, unlike ATR and Nbs, not after high doses (Bi et al., 2005a; Oikemus et al., 2006). Flies with mutations in *mei-41/atr* have been studied in detail for many years. Mei-41/ATR is required for ionizing radiation-induced cell cycle arrest, but not for p53-dependent apoptosis (Hari et al., 1995; Ahmad and Golic, 1999; Brodsky et al., 2000a, b). *mei-41/atr* null mutants are viable and female sterile, and TFs have never been observed in mitotic cells lacking Mei-41/

ATR or its binding partner, Mus304/ATRIP (Gatti et al., 1974; Hari et al., 1995; Brodsky et al., 2000b). However, as discussed below, the ATR/ATRIP pathway plays a redundant role in *Drosophila* telomere maintenance. The molecular processes by which the MRN complex and ATM kinase prevent telomeric fusions are not well understood. Mutations in each component of the MRN complex strongly reduce but do not completely eliminate HOAP binding at mitotic telomeres, consistent with the mild TF phenotype observed in these mutants with respect to *cav* where HOAP is completely lost (Fig. 1; Bi et al., 2004; Ciapponi et al., 2004, 2006; Oikemus et al., 2006). In contrast, although *tefu/atm* mitotic chromosomes display a normal HOAP accumulation at telomeres, they exhibit a frequency of TFs similar to that observed in *rad50* and *mre11* mutants. This result provides further support to the notion that prevention of TFs does not solely depend on HOAP loading at chromosome ends. In line with this, TFs have also been found in mutants that retain HOAP localization at telomeres such as *Su(var)205* and *woc* (Cenci et al., 2003b; Raffa et al., 2005). However, it has been reported that HOAP is completely missing from telomeres of *mei-41/atr tefu/atm* and *mus304/atrip tefu/atm* double mutants (Bi et al., 2005b; Oikemus et al., 2006). Thus, it can be envisaged that when ATM is missing, the ATR/ATRIP pathway plays a compensatory function leading to the localization of HOAP at chromosome ends.

Genetic analysis of double mutants has corroborated the hypothesis that *rad50, mre11, nbs1, tefu/atm, mus304/atrip* and *mei-41/atr* function in different pathways of telomere protection (Fig. 2). One of these pathways includes *mre11, rad50* and *tefu/atm* genes as double mutant combinations involving these mutations show TF frequencies similar to those observed in the corresponding single mutants (Fig. 2; Bi et al., 2004; Ciapponi et al., 2004). In contrast, double mutant combinations between *tefu/atm*, *rad50*, *mre11* or *nbs* and either *mei-41/atr* or *mus304/atrip* cause significant increases in the TF frequency compared to single mutants (Bi et al., 2005b; Ciapponi et al., 2006; Oikemus et al., 2006). These results are consistent with the idea the *Drosophila* Mre11/Rad50/ATM telomere protection pathway is backed by an additional pathway that involves ATR and ATRIP (Fig. 2). The factors that act downstream of ATM and ATR at *Drosophila* telomeres need to be identified. Mutations in the *mnk/loki* and *grp* genes that encode the *Drosophila* homologues of the major downstream targets of these kinases in mammals (Chk2 and Chk1, respectively; Table 1), do not affect telomere stability. Furthermore, mutations in these genes do not enhance the frequency of TFs when combined with mutations in either *tefu/atm*, *mei-41/atr* or *mre11* (Bi et al., 2005b; Oikemus et al., 2006). Interestingly, mutations in *nbs* exacerbate the telomere fusion phenotype elicited by mutations in *rad50* or *tefu/atm*, suggesting that Nbs may function in both the ATM and the ATR telomere protection pathway. This finding implies that Nbs can act independently of its binding partners Mre11 and Rad50 (Ciapponi et al., 2006). However, contradictory results have been provided on this issue, since other groups have reported that both *mre11 nbs* and *tefu/atm nbs* double mutants exhibit TF

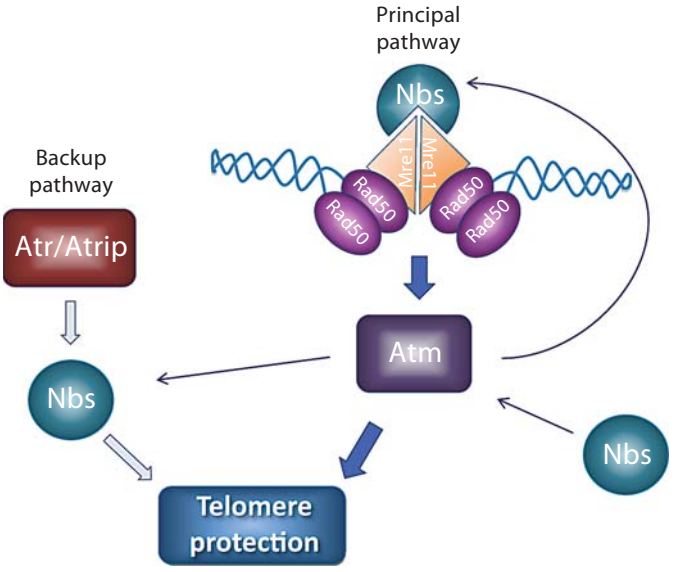


**Fig. 2.** A model for telomere protection in *Drosophila*. We propose that end-to-end fusion of *Drosophila* chromosomes is primarily prevented by an ATM-based signaling pathway, while ATR-mediated signaling acts as a backup pathway. The Nbs protein participates in both pathways, functioning both together and independently of its binding partners Mre11 and Rad50 (see text for detailed explanation).

frequencies similar to those found in single mutants (Bi et al., 2005b; Oikemus et al., 2006). Therefore definition of the Nbs contribution to the ATM- and ATR-dependent telomere protection pathways needs further investigation.

### The DNA damage response at telomeres

Recent studies in mammals, yeast and *Drosophila* converge towards the view that dysfunctional telomeres elicit a DDR (Ahmad and Golic, 1999; de Lange, 2005; Longhese, 2008; Musarò et al., 2008). In humans, evidence that dysfunctional telomeres trigger DDR comes from the observation that loss of the shelterin component TRF2 activates the ATM kinase pathway, leads to p53 up-regulation and causes a p21-mediated G1/S arrest (Karlseder et al., 1999). DNA damage factors such as phosphorylated ATM and ATR, the MRN complex, 53BP1 and γ-H2AX were subsequently found at unprotected telomeres (d'Adda di Fagagna et al., 2003; Takai et al., 2003). After disruption of the telomere protein complex or in response to telomere erosion, these factors and Rif1 are recruited to chromosome ends, where they form cytological structures that are referred to as Telomere dysfunction Induced Foci (TIFs). TIFs mimic the cellular response to DSBs induced by gamma irradiation, supporting the view that dysfunctional telomeres generate a canonical DDR (de Lange, 2005). Loss of TRF2 in DNA ligase IV-deficient mouse cells induces the formation of TIFs and activates the ATM kinase even though the 3′ overhang is intact, indicating that the overhang protection is not suf-

ficient to prevent the telomere damage response (Celli and de Lange, 2005). However, inhibition of POT1 triggers an ATR-dependent DNA damage response in $Atm^{-/-}$ mouse cells, indicating that ATM is not required for the activation of ATR (Denchi and de Lange, 2007). This finding differs from the situation at irradiation-induced DSBs, where ATM signaling is required to activate ATR, very likely by promoting the formation of ssDNA at the broken sites (Jazayeri et al., 2006).

Telomere dysfunction activates DDR also in budding yeast. Loss of Cdc13 results in an extensive single-stranded telomeric region and in a G2/M arrest mediated by a RAD9-dependent checkpoint (Garvik et al., 1995; Zubko et al., 2004). Surprisingly, however, disruption of Taz1 does not affect cell cycle progression despite the presence of aberrant telomeric phenotypes (Cooper et al., 1997; Ferreira and Cooper, 2004). It has recently been demonstrated that a DDR is not limited to cells with either dysfunctional or eroded telomeres but it is elicited also by functional telomeres. Normal telomeres of budding yeast that become suitable for elongation during S-phase bind MRX and generate a transient DDR, suggesting that they can be perceived and treated as DSBs during replication (Viscardi et al., 2007; Longhese, 2008). Analogously, DNA damage signals are triggered during replication and processing of functional human telomeres. The accumulation of ssDNA at telomeres leads first to an ATR-dependent DNA damage response. Subsequently, when telomeres are replicated, the ATM and the HR machineries act on chromosome ends to provide a protective structure to the telomeres (Verdun et al., 2005; Verdun and Karlseder, 2006). This indicates that human telomeres are not always hidden from the DNA-damage repair machinery and suggests that they elicit at least two distinct DDRs during and after replication (Verdun et al., 2005; Verdun and Karlseder, 2006). Remarkably, however, both responses do not lead to a cell cycle arrest.

### Uncapped *Drosophila* telomeres activate cell cycle checkpoints

Mutations in the *cav* gene, which encodes HOAP, lead to extensive end-to-end fusions and affect cell cycle progression (Cenci et al., 2005; Musarò et al., 2008). In *cav* mutants the mitotic index, which measures the frequency of cells engaged in mitosis relative to interphase cells, is drastically reduced compared to wild type, suggesting that loss of HOAP results in a premitotic interphase delay. This delay is not a direct consequence of TFs, since other mutants with TF frequencies comparable to that observed in *cav,* exhibit only a mild reduction of the mitotic index. The cell cycle block induced by *cav* is alleviated by mutations in *mei-41/atr*, *mus304/atrip*, *grp/chk1* and *rad50,* indicating that HOAP-depleted telomeres activate a DDR (Musarò et al., 2008). However, it is still unclear whether this response occurs during the G1/S transition, the S phase or the G2/M transition. Surprisingly, ATM was not involved in the *cav*-induced DDR. This situation contrasts with that of mammals and yeast where ATM plays a pivotal function in activating an intra-S checkpoint response following telomere dysfunction (de Lange, 2005; Longhese, 2008).

HOAP-depleted telomeres also cause cell cycle arrest during the metaphase-to-anaphase transition. Unlike other telomere-fusion mutants, *cav* mutants exhibit a severe reduction of the frequency of anaphases with respect to wild type controls. Consistent with these results, an in vivo analysis of mitotic divisions in *cav* neuroblasts revealed that *cav* mutant cells remain arrested in metaphase for a very long time. This delay in anaphase onset is not due to spindle morphology defects, as the spindles of *cav* mutant cells are not substantially different from their wild type counterparts (Musarò et al., 2008). A delay in the metaphase-to-anaphase transition is normally due to the activation of the spindle assembly checkpoint (SAC), which monitors the interaction between kinetochores and microtubules and inhibits the anaphase onset until all the chromosomes are properly aligned in a metaphase plate (Musacchio and Salmon, 2007). Mutations in genes required for the SAC in *Drosophila*, such as *zw10* and *bubR1* (Karess, 2005), partially suppressed the metaphase-to-anaphase delay in *cav* mutants but did not alleviate the interphase block, indicating that *cav*-induced SAC activation is not a consequence of the DDR.

Immunostaining experiments of *cav* mutant cells revealed that some proteins involved in the SAC such as BubR1, Cenp-meta, Zw10 and Zwilch, accumulate at the kinetochores as in wild type colchicine-treated cells. This is an expected result that confirms that HOAP-depleted telomeres activate a canonical SAC, which is normally mediated by accumulation of SAC proteins at kinetochores. Remarkably, however, BubR1 accumulated also at telomeres in almost all *cav* mutant cells, whereas this kinase is never targeted to wild type telomeres or to X-ray induced chromosome breakpoints. The molecular mechanism that drives the localization of BubR1 at unprotected telomeres is not known. However, BubR1 localization at *cav* mutant telomeres requires the wild type function of the same genes that alleviate the interphase block *(mei-41/atr, mus304/atrip, rad50, mre11,* and *grp/chk1)* as well as *tefu/atm*. An examination of other mutants with TFs, including *UbcD1/eff*, *Su(var)205* and *woc,* showed that they have a very limited ability to recruit BubR1 at telomeres, indicating that telomeric accumulation of BubR1 is specifically induced by HOAP depletion. Collectively, these analyses revealed a significant negative correlation between the frequencies of anaphases and the frequencies of BubR1-labeled telomeres, suggesting that telomeric BubR1 can actively inhibit anaphase (Musarò et al., 2008). Thus, it has been proposed that BubR1 accumulation at telomeres can directly inactivate either Fizzy (Cdc20; Table 1) or another APC/C subunit leading to a metaphase arrest.

Recent work has focused on the possible relationships between DNA damage, SAC and telomeres. DNA breaks have been found to activate the SAC in both *Drosophila* and mammalian cells, presumably by disrupting kinetochore function (Mikhailov et al., 2002; Royou et al., 2005). Loss of Taz1 in *S. pombe* causes the activation of the SAC mediated

by Mph1p and Bub1p (Miller and Cooper, 2003). Similarly, mutations in *yKu70* that result in alterations of *S. cerevisiae* telomeres, activate both the DNA damage and spindle checkpoints (Maringele and Lydall, 2002). However, it is not clear how telomere perturbations in these organisms can be perceived by the cell surveillance systems that normally control the interactions between the spindle microtubules and the kinetochores, and block the anaphase onset until all chromosomes have reached a proper bipolar alignment. Recent reports have also suggested that the SAC proteins mediate a mitotic delay in response to multiple lesions affecting DNA replication (Garber and Rine, 2002; Clerici et al., 2004; Sugimoto et al., 2004; Collura et al., 2005; Gibson et al., 2006; Zachos et al., 2007; Kim and Burke, 2008). Moreover, yeast cells lacking the DNA damage response arrest prior to anaphase after methyl methane sulfonate (MMS) treatment (Kim and Burke, 2008). Unlike a canonical SAC, this arrest requires the SAC proteins that inhibit Cdc20 and securin (Pds1), but not a functional kinetochore. Inhibition of anaphase onset also requires Mec1/ATR and Tel1/ATM, indicating that these kinases can regulate cell division by exploiting the SAC machinery (Kim and Burke, 2008).

In summary, when HOAP is missing from chromosome ends, cell cycle arrests prior to mitosis (interphase) and at the metaphase-to-anaphase transition (Fig. 3). The interphase arrest depends on the activation of DDR, suggesting that in *Drosophila*, similar to mammals and yeast, dysfunctional telomeres are perceived as DNA breaks and induce a DNA damage checkpoint. When a cell enters mitosis, unprotected telomeres that have recruited BubR1 also activate the SAC. The SAC is likely to act as a backup mechanism to prevent genomic instability caused by damaged DNA ends. Its activation would prevent the spreading of damaged chromosomes by blocking mitotic division of cells with unprotected telomeres that were able to escape the DNA damage checkpoint.

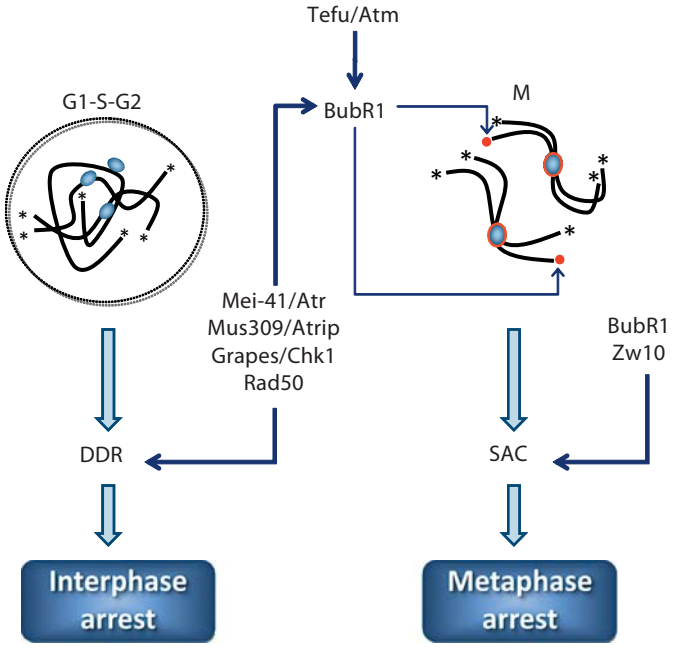


**Fig. 3.** A model for checkpoint activation induced by dysfunctional *Drosophila* telomeres. We hypothesize that unprotected telomeres (indicated with an asterisk) can induce cell cycle arrest during interphase. This block is mediated by ATR, ATRIP, Chk1 and Rad50 but not by ATM. Loss of HOAP also induces a SAC-dependent metaphase arrest. Unprotected telomeres recruit BubR1 (red circles) in an ATM, ATR, ATRIP, Chk1 and Rad50 dependent manner. Telomeric accumulation of BubR1 negatively regulates the Cdc20/APC complex (not shown) resulting in a metaphase-to-anaphase transition block.

## Conclusions

Despite the identification of a large number of proteins required for telomere maintenance, the mechanisms employed by the cell to distinguish natural ends from DSBs are not fully understood. *Drosophila* has evolved non-canonical telomeres, yet the analysis of telomeres in this organism can provide important information on telomere biology. Molecular and genetic analyses have shown that fruit fly telomeres share several features with their yeast and mammalian counterparts. For example, in all organisms, telomere maintenance and functioning requires the MRN complex as well as the ATM and ATR kinases. The absence of a telomerase-mediated telomere maintenance, and thus of an intimate connection between capping and elongation, makes *Drosophila* particularly suitable for the analysis of the mechanism of telomere protection. In this respect, it should be recalled that telomere-fusion mutants are easily detected by a cytological screening of lethal mutants, and that most of the genes required for *Drosophila* telomere capping remain to be identified (Cenci et al., 2005). *Drosophila* can also provide information on how dysfunctional telomeres affect cell cycle progression. The finding that cells with uncapped telomeres activate the SAC through BubR1 recruitment at damaged telomeres, highlights an intriguing and unsuspected connection between telomeres and cell cycle regulation. We believe that the sophisticated *Drosophila* genetics and the favorable cytology of both mitotic and polytene chromosomes will allow further dissection of the mechanism of telomere capping and checkpoint activation. We also believe that the information gathered on *Drosophila* telomeres will provide useful clues to understanding human telomere biology.

## Acknowledgments

We thank Giorgia Siriaco for critical reading of the manuscript. We are in debt to Maurizio Gatti for insightful comments on the review and helpful discussion.

## References


Aagaard L, Schmid M, Warburton P, Jenuwein T: Mitotic phosphorylation of SUV39H1, a novel component of active centromeres, coincides with transient accumulation at mammalian centromeres. J Cell Sci 113:817–829 (2000).

Ahmad K, Golic KG: Telomere loss in somatic cells of *Drosophila* causes cell cycle arrest and apoptosis. Genetics 151:1041–1051 (1999).

Badugu R, Shareef MM, Kellum R: Novel *Drosophila* heterochromatin protein 1 (HP1)/origin recognition complex-associated protein (HOAP) repeat motif in HP1/HOAP interactions and chromocenter associations. J Biol Chem 278: 34491–34498 (2003).

Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, et al: Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 86:159–171 (1996).

Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, et al: Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. Genes Dev 16:2237–2251 (2002).

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

Bi X, Gong M, Srikanta D, Rong YS: *Drosophila* ATM and Mre11 are essential for the G2/M checkpoint induced by low-dose irradiation. Genetics 171:845–847 (2005a).

Bi X, Srikanta D, Fanti L, Pimpinelli S, Badugu R, et al: *Drosophila* ATM and ATR checkpoint kinases control partially redundant pathways for telomere maintenance. Proc Natl Acad Sci USA 102:15167–15172 (2005b).

Brodsky MH, Nordstrom W, Tsang G, Kwan E, Rubin GM, Abrams JM: *Drosophila* p53 binds a damage response element at the reaper locus. Cell 101:103–113 (2000a).

Brodsky MH, Sekelsky JJ, Tsang G, Hawley RS, Rubin GM: mus304 encodes a novel DNA damage checkpoint protein required during *Drosophila* development. Genes Dev 14:666–678 (2000b).

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

Cenci G, Rawson RB, Belloni G, Castrillon DH, Tudor M, et al: UbcD1, a *Drosophila* ubiquitin-conjugating enzyme required for proper telomere behavior. Genes Dev 11:863–875 (1997).

Cenci G, Siriaco G, Gatti M: The role of HeT-A and TART retrotransposons in *Drosophila* telomere capping. Genetica 117:311–318 (2003a).

Cenci G, Siriaco G, Raffa GD, Kellum R, Gatti M: The *Drosophila* HOAP protein is required for telomere capping. Nat Cell Biol 5:82–84 (2003b).

Cenci G, Ciapponi L, Gatti M: The mechanism of telomere protection: a comparison between *Drosophila* and humans. Chromosoma 114: 135–145 (2005).

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

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

Ciapponi L, Cenci G, Gatti M: The *Drosophila* Nbs protein functions in multiple pathways for the maintenance of genome stability. Genetics 173: 1447–1454 (2006).

Clerici M, Baldo V, Mantiero D, Lottersberger F, Lucchini G, Longhese MP: A Tel1/MRX-dependent checkpoint inhibits the metaphase-to-anaphase transition after UV irradiation in the absence of Mec1. Mol Cell Biol 24:10126–10144 (2004).

Collura A, Blaisonneau J, Baldacci G, Francesconi S: The fission yeast Crb2/Chk1 pathway coordinates the DNA damage and spindle checkpoint in response to replication stress induced by topoisomerase I inhibitor. Mol Cell Biol 25: 7889–7899 (2005).

Cooper JP, Hiraoka Y: Fission yeast telomeres; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 495–523 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2006).

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

Cristofari G, Lingner J: The telomerase ribonucleoprotein particle; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 21–47 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2006).

d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, et al: A DNA damage checkpoint response in telomere-initiated senescence. Nature 426:194–198 (2003).

D'Amours D, Jackson SP: The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling. Nat Rev Mol Cell Biol 3:317–327 (2002).

de Lange T: Telomere dynamics and genome instability in human cancer; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 265–293 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1995).

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

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

Fanti L, Giovinazzo G, Berloco M, Pimpinelli S: The heterochromatin protein 1 prevents telomere fusions in *Drosophila*. Mol Cell 2:527–538 (1998).

Fanti L, Berloco M, Piacentini L, Pimpinelli S: Chromosomal distribution of heterochromatin protein 1 (HP1) in *Drosophila*: a cytological map of euchromatic HP1 binding sites. Genetica 117:135–147 (2003).

Ferreira MG, Cooper JP: Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. Genes Dev 18:2249–2254 (2004).

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

Frappart PO, McKinnon PJ: Ataxia-telangiectasia and related diseases. Neuromol Med 8:495–511 (2006).

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

Garber PM, Rine J: Overlapping roles of the spindle assembly and DNA damage checkpoints in the cell-cycle response to altered chromosomes in *Saccharomyces cerevisiae*. Genetics 161:521–534 (2002).

Garcia-Cao M, O'Sullivan R, Peters AH, Jenuwein T, Blasco MA: Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. Nat Genet 36:94–99 (2004).

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

Gatti M, Baker BS: Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. Genes Dev 3:438–453 (1989).

Gatti M, Tanzarella C, Olivieri G: Analysis of the chromosome aberrations induced by X-rays in somatic cells of *Drosophila melanogaster*. Genetics 77:701–719 (1974).

Gibson DG, Bell SP, Aparicio OM: Cell cycle execution point analysis of ORC function and characterization of the checkpoint response to ORC inactivation in *Saccharomyces cerevisiae*. Genes Cells 11:557–573 (2006).

Gorski MM, Romeijn RJ, Eeken JC, De Jong AW, Van Veen BL, et al: Disruption of *Drosophila* Rad50 causes pupal lethality, the accumulation of DNA double-strand breaks and the induction of apoptosis in third instar larvae. DNA Repair (Amst) 3:603–615 (2004).

Hande MP, Balajee AS, Tchirkov A, Wynshaw-Boris A, Lansdorp PM: Extra-chromosomal telomeric DNA in cells from Atm(–/–) mice and patients with ataxia-telangiectasia. Hum Mol Genet 10:519–528 (2001).

Hari KL, Santerre A, Sekelsky JJ, McKim KS, Boyd JB, Hawley RS: The mei-41 gene of *D. melanogaster* is a structural and functional homolog of the human ataxia telangiectasia gene. Cell 82: 815–821 (1995).

Hastie ND, Allshire RC: Human telomeres: fusion and interstitial sites. Trends Genet 5:326–331 (1989).

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

James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, Elgin SC: Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*. Eur J Cell Biol 50:170–180 (1989).

Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, et al: ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. Nat Cell Biol 8:37–45 (2006).

Kang J, Bronson RT, Xu Y: Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair. EMBO J 21:1447–1455 (2002).

Karess R: Rod-Zw10-Zwilch: a key player in the spindle checkpoint. Trends Cell Biol 15:386–392 (2005).

Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T: p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283:1321–1325 (1999).

Kim EM, Burke DJ: DNA damage activates the SAC in an ATM/ATR-dependent manner, independently of the kinetochore. PLoS Genet 4: e1000015 (2008).

Koering CE, Pollice A, Zibella MP, Bauwens S, Puisieux A, et al: Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity. EMBO Rep 3: 1055–1061 (2002).

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

Lundblad V: Budding yeast telomeres; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 345–386 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2006).
Luo G, Yao MS, Bender CF, Mills M, Bladl AR, et al: Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. Proc Natl Acad Sci USA 96:7376–7381 (1999).
Maringele L, Lydall D: EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Delta mutants. Genes Dev 16:1919–1933 (2002).
Maser RS, DePinho RA: Connecting chromosomes, crisis, and cancer. Science 297:565–569 (2002).
Mason JM, Biessmann H: The unusual telomeres of *Drosophila*. Trends Genet 11:58–62 (1995).
Mason JM, Frydrychova RC, Biessmann H: *Drosophila* telomeres: an exception providing new insights. Bioessays 30:25–37 (2008).
Mikhailov A, Cole RW, Rieder CL: DNA damage during mitosis in human cells delays the metaphase/anaphase transition via the spindle-assembly checkpoint. Curr Biol 12:1797–1806 (2002).
Miller KM, Cooper JP: The telomere protein Taz1 is required to prevent and repair genomic DNA breaks. Mol Cell 11:303–313 (2003).
Musacchio A, Salmon ED: The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 8:379–393 (2007).
Musarò M, Ciapponi L, Fasulo B, Gatti M, Cenci G: Unprotected *Drosophila melanogaster* telomeres activate the spindle assembly checkpoint. Nat Genet 40:362–366 (2008).
Nugent CI, Lundblad V: The telomerase reverse transcriptase: components and regulation. Genes Dev 12:1073–1085 (1998).
Oikemus SR, McGinnis N, Queiroz-Machado J, Tukachinsky H, Takada S, et al: *Drosophila* atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. Genes Dev 18:1850–1861 (2004).
Oikemus SR, Queiroz-Machado J, Lai K, McGinnis N, Sunkel C, Brodsky MH: Epigenetic telomere protection by *Drosophila* DNA damage response pathways. PLoS Genet 2:e71 (2006).
Pandita TK: ATM function and telomere stability. Oncogene 21:611–618 (2002).
Perrini B, Piacentini L, Fanti L, Altieri F, Chichiarelli S, et al: HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in *Drosophila*. Mol Cell 15:467–476 (2004).
Pimpinelli S: *Drosophila* telomeres; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 433–463 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2006).
Price C: Ciliate telomeres; in de Lange T, Lundblad V, Blackburn EH (eds): Telomeres, pp 465–493 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2006).
Raffa GD, Cenci G, Siriaco G, Goldberg ML, Gatti M: The putative *Drosophila* transcription factor woc is required to prevent telomeric fusions. Mol Cell 20:821–831 (2005).
Rong YS: Telomere capping in *Drosophila*: dealing with chromosome ends that most resemble DNA breaks. Chromosoma 117:235–242 (2008).
Royou A, Macias H, Sullivan W: The *Drosophila* Grp/Chk1 DNA damage checkpoint controls entry into anaphase. Curr Biol 15:334–339 (2005).
Sabourin M, Tuzon CT, Zakian VA: Telomerase and Tel1p preferentially associate with short telomeres in *S. cerevisiae*. Mol Cell 27:550–561 (2007).
Shareef MM, King C, Damaj M, Badagu R, Huang DW, Kellum R: *Drosophila* heterochromatin protein 1 (HP1)/origin recognition complex (ORC) protein is associated with HP1 and ORC and functions in heterochromatin-induced silencing. Mol Biol Cell 12:1671–1685 (2001).
Sharpless NE, DePinho RA: Telomeres, stem cells, senescence, and cancer. J Clin Invest 113:160–168 (2004).
Shiloh Y: The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 31:402–410 (2006).
Silva E, Tiong S, Pedersen M, Homola EM, Royou A, et al: ATM is required for telomere maintenance and chromosome stability during *Drosophila* development. Curr Biol 14:1341–1347 (2004).
Song YH, Mirey G, Betson M, Haber DA, Settleman J: The *Drosophila* ATM ortholog, dATM, mediates the response to ionizing radiation and to spontaneous DNA damage during development. Curr Biol 14:1354–1359 (2004).
Stiff T, Reis C, Alderton GK, Woodbine L, O'Driscoll M, Jeggo PA: Nbs1 is required for ATR-dependent phosphorylation events. EMBO J 24:199–208 (2005).
Sugimoto I, Murakami H, Tonami Y, Moriyama A, Nakanishi M: DNA replication checkpoint control mediated by the spindle checkpoint protein Mad2p in fission yeast. J Biol Chem 279:47372–47378 (2004).
Takai H, Smogorzewska A, de Lange T: DNA damage foci at dysfunctional telomeres. Curr Biol 13:1549–1556 (2003).
Theunissen JW, Kaplan MI, Hunt PA, Williams BR, Ferguson DO, et al: Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11 (ATLD1/ATLD1) mice. Mol Cell 12:1511–1523 (2003).
van Steensel B, Smogorzewska A, de Lange T: TRF2 protects human telomeres from end-to-end fusions. Cell 92:401–413 (1998).
Verdun RE, Karlseder J: The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. Cell 127:709–720 (2006).
Verdun RE, Crabbe L, Haggblom C, Karlseder J: Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. Mol Cell 20:551–561 (2005).
Viscardi V, Bonetti D, Cartagena-Lirola H, Lucchini G, Longhese MP: MRX-dependent DNA damage response to short telomeres. Mol Biol Cell 18:3047–3058 (2007).
Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, et al: Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. Nature 421:643–648 (2003).
Zachos G, Black EJ, Walker M, Scott MT, Vagnarelli P, et al: Chk1 is required for spindle checkpoint function. Dev Cell 12:247–260 (2007).
Zhong H, Bryson A, Eckersdorff M, Ferguson DO: Rad50 depletion impacts upon ATR-dependent DNA damage responses. Hum Mol Genet 14:2685–2693 (2005).
Zhu J, Petersen S, Tessarollo L, Nussenzweig A: Targeted disruption of the Nijmegen breakage syndrome gene *NBS1* leads to early embryonic lethality in mice. Curr Biol 11:105–109 (2001).
Zhu XD, Kuster B, Mann M, Petrini JH, Lange T: Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat Genet 25:347–352 (2000).
Zubko MK, Guillard S, Lydall D: Exo1 and Rad24 differentially regulate generation of ssDNA at telomeres of *Saccharomyces cerevisiae cdc13-1* mutants. Genetics 168:103–115 (2004).