RESEARCH ARTICLE

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A mutation in yeast Tel1p that causes differential effects on the DNA damage checkpoint and telomere maintenance

Abstract ATM/ATR homologs are the central elements of genome surveillance mechanisms in many organisms, including yeasts, flies, and mammals. In *Saccharomyces cerevisiae*, most checkpoint responses depend on the ATR ortholog Mec1p. The yeast ATM ortholog, Tel1p, so far has been implicated in a specific DNA damage checkpoint during S-phase as well as in telomere homeostasis. In particular, yeast cells lacking only Tel1p harbor short but stable telomeres, while cells lacking both Tel1p and Mec1p are unable to maintain telomeric repeats and senesce. Here, we present the characterization of a new mutation in the *TEL1*-gene, called *tel1-11*, which was isolated by virtue of a synthetic lethal interaction at 37°C with a previously described *mec1-ts* mutation. Interestingly, telomere and checkpoint functions are differentially affected by the mutant protein Tel1-11p. The Tel1p-dependent checkpoint response is undetectable in cells containing Tel1-11p and incubated at 37°C, but basic telomere function is maintained. Further, when the same cells are incubated at 26°C, Tel1-11p confers full proficiency for all telomere functions analyzed, whereas the function for DNA-damage checkpoint activation is clearly affected. The results thus strongly suggest that the different cellular pathways affected by Tel1p do not require the same level of Tel1p activity to be fully functional.

Keywords ATM/ATR · *TEL1* · DNA-damage checkpoint · Telomeres · Yeast

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Introduction

In eukaryotes, maintaining genome integrity relies on a set of surveillance mechanisms, called checkpoints. These checkpoints are responsible for proper detection and repair of DNA damage caused by environmental stresses or irregularities during DNA replication. Pivotal elements of all known eukaryotic checkpoints are homologs of mammalian ATM/ATR kinases (ataxia-telangiectasia-mutated/ATM and Rad3-related (Nyberg et al. 2002). In budding yeast, Mec1p (an ATR ortholog), plays a prevalent role in G1, S, and G2/M checkpoints. In addition to sensitivity to all DNA-damaging agents, Mec1p-deficient cells display increased gross chromosomal rearrangements (GCR) and aberrant patterns of DNA replication (Santocanale and Diffley 1998; Myung et al. 2001; Cha and Kleckner 2002). A downstream target of Mec1p-dependent phosphorylation is another essential kinase, Rad53p (Sanchez et al. 1996). The essential function of Mec1p and Rad53p is thought to be up-regulation of nucleotide pools in response to replication and DNA damage, as alleviation of inhibition of nucleotide synthesis in *sml1* mutants rescues the lethality of cells lacking Mec1p or Rad53p (Zhao et al. 1998).

The checkpoint function of Tel1p, the budding yeast ortholog of ATM, appears more furtive, as Tel1p-deficient cells do not suffer from obvious sensitivity to DNA-damaging agents and there is no major defect in genome stability, as assessed by GCR rates (Morrow et al. 1995; Myung et al. 2001). In addition, Tel1p appears not to be involved in the induction of nucleotide synthesis, at least after DNA damage (Zhu and Xiao 2001). Tel1p's contribution to cellular checkpoint activity might be masked by the prevailing activity of Mec1p. For example, a Tel1p deficiency exacerbates checkpoint defects and causes large increases of GCR rates displayed by *mec1* mutants (Morrow et al. 1995; Myung et al. 2001). Furthermore, in response to DNA damage, Rad53p can be phos-

phorylated in both Mec1p and Tel1p-dependent manner, although Tel1p's dependent phosphorylation is minor and is detectable mainly in cells lacking Mec1p (Sanchez et al. 1996; Vialard et al. 1998; Clerici et al. 2001; Nakada et al. 2003b).

According to a current view, most DNA damage is converted to DNA intermediates with single-stranded DNA (ssDNA). These intermediates are readily sensed by ATR-dependent pathways, whereas the ATM-dependent pathway is thought to respond to DNA double-strand breaks (DSB). By extension, this might explain why the checkpoint function provided by Mec1p prevails over that of Tel1p (Nyberg et al. 2002). Supporting this view, a striking demonstration of a Tel1p-dependent checkpoint function could be made in yeast cells in which conversion of DSB into ssDNA intermediates is blocked by *sae2* or *rad50S* mutations. In mitotic cells lacking both Mec1p and Sae2p, the sensitivity to methyl methanesulfonate (MMS) is partially suppressed and such cells are capable of Rad53p phosphorylation and cell cycle arrest. It was shown that these checkpoint functions are dependent on Tel1p and Mre11p (also referred to as Tel1p- and Mre11p-dependent checkpoint or TM-checkpoint; (Usui et al. 2001). However, Mre11p has multiple implications in G1 and G2/M checkpoints (D'Amours and Jackson 2002) and it remains unclear how specific its implication in a S-phase DNA-damage checkpoint is. In yeast, Mre11p forms a complex with Rad50p and Xrs2p, called the MRX-complex. Interestingly, under particular conditions and in response to DNA damage, both Mre11p and Xrs2p have been reported to be phosphorylated in a Tel1p-dependent fashion (D'Amours and Jackson 2001; Usui et al. 2001; Nakada et al. 2003b).

An additional challenge for DNA replication and therefore genomic stability is the faithful maintenance of chromosomal termini, the telomeres. As could be predicted, subsets of proteins involved in checkpoint pathways have a role to play in telomere maintenance. Indeed, certain mutations in *MEC1* or *RAD53* cause a shortening of telomeric repeat tracts and a decrease in telomere position effect (TPE, telomeric chromatin-dependent transcriptional inhibition) (McAinsh et al. 1999; Ritchie et al. 1999). Furthermore, mutations in genes of the Rad17-Mec3-Ddc1 complex, which is involved in the Mec1p-dependent pathway, can also affect telomere length (Corda et al. 1999; Longhese et al. 2000); Dionne and Wellinger, unpublished data). Furthermore, Mec1p has been shown to associate with telomeres during late S phase (Takata et al. 2004). More recent data on the assembly of repair complexes at DSB as well as studies on the association of proteins at telomeres suggest that the MRX-complex and Telp1p are among the first factors to associate to such DNA-ends (Lisby et al. 2004; Takata et al. 2005). Taken together, while a deficiency in the Mec1p-dependent pathway clearly has some influence at telomeres, no telomere-specific function could be assigned to its components yet.

In contrast, both Tel1p and its mammalian ortholog ATM have been directly implicated in telomere homeostasis (Pandita 2002). Both in mice and human cells, an ATM deficiency provokes accelerated telomere loss and other phenotypic changes such as genomic instability (Metcalfe et al. 1996; Wong et al. 2003). In yeast, the *TEL1* gene had initially been identified in a screen for mutations affecting telomere length and Tel1p-deficient cells have very short telomeric repeat tracts (Lustig and Petes 1986). Later genetic analyses placed *TEL1* and the genes encoding the MRX-complex) in the same pathway as telomerase (Ritchie et al. 1999; Ritchie and Petes 2000). The same analyses showed that in the absence of these proteins, cells rely on Mec1p to maintain short but stable telomeres, which suggests that the function of Mec1p at telomeres is redundant with or masked by the Tel1p and the MRX-complex in normal cells. In the complete absence of ATM-like kinases, yeast cells are not able to maintain telomeric DNA and senesce after about 60–80 generations, a phenotype also displayed by cells lacking telomerase components (Ritchie et al. 1999; Chan et al. 2001). One characteristic of this senescence phenotype is the emergence of survivors: these are cells that can maintain telomeric DNA in a telomerase-independent fashion but relying on homeologous recombination (Lundblad and Blackburn 1993). Finally, telomerase and Tel1p have recently been associated with a chromosome protective function that may be independent of active telomere lengthening (Chan and Blackburn 2003). Intriguingly, the two ATM-like kinases, Mec1p and Tel1p, show mutually exclusive association with telomeres at distinct stages of the cell cycle (Takata et al. 2004). However, the molecular mechanisms for Mec1p- or Tel1p-dependent telomere dynamics remain unclear and hypotheses are dependent on model systems and conditions used (Takata et al. 2004); reviewed in (Chakhparonian and Wellinger 2003). It would therefore be of interest to be able to analyze mutant Tel1p proteins that cause deficiencies in only one pathway, but not for others. There is a precedence suggesting that the functions of proteins involved in DNA-damage checkpoint and in telomere homeostasis may be separable. Cells expressing a particular mutant Mre11p protein, called Mre11-tsp, display short telomeres, similar to those observed in cells lacking Mre11p altogether (Chamankhah et al. 2000). However, these cells are not sensitive to an exposure to MMS when grown at 24°C, while they are sensitive to MMS at 34°C. These data show that the various functions of Mre11p can be affected in a differential fashion and perhaps separable.

Here we describe the characterization of a mutation in the *TEL1* gene, generating a protein we call Tel1-11p. Cells harboring this protein display properties that are consistent with the hypothesis that the functions of Tel1p at telomeres and in the DNA-damage checkpoint have different requirements for Tel1p function. We analyzed three parameters to characterize the effects of the Tel1-11p on cells. Checkpoint functionality was

assessed as resistance to MMS-exposure and induction of Rad53p-phosphorylation in cells that contained the Tel1-11p in combination with *mec1Δsml1Δsae2Δ* mutations. Telomere functions were assessed as overall telomere length and, independently, the ability to stably maintain a certain telomeric repeat tract when Tel1-11p expression is combined with *mec1Δsml1Δ* mutations. The results show that when the cells are grown at 26°C, Tel1-11p confers completely wild-type telomere homeostasis and its checkpoint function is slightly affected. Upon growth of the cells at 37°C, telomeres are shortened, but still stably maintained, even in the absence of Mec1p. However, the checkpoint function is severely impaired or absent at this temperature, since after exposure of cells to MMS, Rad53p phosphorylation is undetectable. These data thus strongly suggest a differential requirement for Tel1p activity in telomere maintenance as compared to the checkpoint function.

Materials and methods

Strains and plasmids

Saccharomyces cerevisiae strains used in this study are listed in Table 1. All yeast cells were grown at temperatures indicated in either complete YPD supplemented at need with nourseothricin at 100 μg/ml (WERNER Bioagents, Germany) or G-418 at 150 μg/ml (Sigma). Alternatively, synthetic medium YC supplemented with required amino acids and bases was used. Genetic manipulations were as described (Zakian and Scott 1982; Rose et al. 1990). Transformations were performed according to a modified lithium acetate method (Gietz et al. 1995).

Haploid strains were produced by micro-dissection and genotypes confirmed by marker segregation and Southern analyses, if needed. For construction of the diploid RWY51 (Table 1), the *mec1-ts* allele (Weinert et al. 1994) was recreated in diploid BY4705 (Brachmann et al. 1998) by integrating a copy of *mec1-t*s linked to the *HIS3* gene into the *MEC1* locus, creating *mec1::mec1-ts::HIS3* (T. Weinert, unpublished data). Subsequently, the *tel1Δ::LEU2* and *sml1Δ::TRP1* deletions were introduced into that strain by successively replacing the open reading frames (ORF) of the corresponding genes with the respective selection markers using a PCR-mediated gene disruption method (Brachmann et al. 1998). The same technique was used to introduce the *sae2Δ::kanMX4* and *rad52Δ::kanMX4* deletions into strains as indicated in Table 1. *E. coli* strain DH5α was used for plasmid manipulation according to standard procedures (Sambrook et al. 1989).

Screen for mutations conferring conditional synthetic lethality with *mec1-ts* (see Fig. 1 for a general outline)

RWY51-24A cells (*mec1-ts ade2 ura3*) carrying a p[*MEC1, ADE2, URA3*] plasmid were subjected to EMS-induced mutagenesis. Briefly, exponentially growing cells were collected, washed twice and resuspended in 1.5 ml of 0.01 M potassium phosphate buffer at pH 7.0, sonicated lightly and incubated for 30 min in presence of 2% EMS, leading to about 40–50% lethality. They were then washed thrice in water, plated on YC medium containing 10 mg/l adenine and incubated at 37°C. An aliquot of cells was also incubated at 26°C as a scoring control. Cell growth at 37°C was observed up to 7 days after plating and white non-sectored colonies were selected as clones dependent on the plasmid. About 50,000 colonies were screened in this manner. In order to purify the background and select clones that were affected at a single locus, candidate strains were backcrossed to wild type three times. Since these backcrosses involved at least two loci (the *mec1-ts* allele and the unknown mutation, *xxx*), haploid segregants after microdissection were expected to follow the segregation ratio of 4:1:1 (tetratype:parental ditype:non-parental ditype). For example, for the strain leading to the eventual identification of the *tel1-11* allele, 34 tetrads score yielded 24:6:4 (T:PD:NPD) tetrads. Furthermore, when a *mec1-ts tel1-11* strain was crossed to *mec1-ts* strain, haploid segregants after microdissection followed the segregation ratio of 2:2 (*mec1-ts* phenotype:*mec1-ts tel1-11* phenotype) for 29 of 31 tetrads analyzed, confirming a single mutation unlinked to *MEC1*. Next, we assessed whether the selected clones contained a mutation in any of the genes known to display synthetic lethality with *mec1-ts* at 37°C, namely *TEL1*, *MRE11*, *XRS2*, *RAD50*, or *RAD52*. This was achieved by (1) crossing candidate double mutants (*mec1-ts*, *xxx*) with respective double mutants and checking the viability of diploids at 37°C; and (2) by complementing haploid double mutants (*mec1-ts*, *xxx*) with plasmids carrying one of the genes above (Fig. 1a). These experiments identified at least one mutation in *RAD50* and two in *TEL1*. Haploid cells harboring the mutation in *RAD50*, or one of the mutations in *TEL1*, together with the *mec1-ts* allele displayed short telomeres when grown at 26°C, as expected, whereas one of the *TEL1* alleles, named *tel1-11*, conferred wt telomere lengths (Fig. 1b). This latter allele was isolated and re-introduced into the genome as described below.

Allele rescue and sequencing

See Fig. 2a for a general outline of the *TEL1* locus. Plasmid p316TGR contains the *TEL1* gene region fragments −4,582 to −4,159 and −388 to +9,133 with respect to the translation initiation codon. These regions were connected by a polylinker containing a *Sac*II recognition site and the fragment was inserted into the *Sac*I–*Sal*I sites of pRS316 (Sikorski and Hieter 1989). For allele rescue, p316TGR was digested with *Sac*II and *Nhe*I to create a gap in *TEL1* from −4,159 to +8,830 and the gapped plasmid was transformed into yeast strains carrying either *TEL1* or the *tel1-11* alleles. The

Table 1 Strains used in this study

| Strain	Genotype	Source/remarks
BY4705 (diploid)	*MATα/MAT**a**ade2Δ::hisG/ade2Δ::hisG his3Δ200/his3Δ200 leu2Δ0/leu2Δ0 lys2Δ0/ lys2Δ0 met15Δ0/met15Δ0 trp1Δ63/trp1Δ63 ura3Δ0/ura3Δ0*	Brachmann et al. (1998)
U953-61A	*MAT**a**ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100 mec1Δ::TRP1 sml1Δ::HIS3*	Zhao et al. (1998)
RWY51	Same as BY4705, addition of *mec1::mec1ts::HIS3/MEC1 tel1Δ::LEU2/TEL1 sml1Δ::TRP1/SML1*	This study (derived from BY4705, see Materials and methods)
RWY51-7B	*MATαade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0*	Spore of RWY51
RWY51-28C	Same as RWY51-7B	Spore of RWY51
RWY51-4A	Same as RWY51-7B but *MAT**a***	Spore of RWY51
RWY51-7D	Same as RWY51-7B but *tel1Δ::LEU2*	Spore of RWY51
RWY51-27A	Same as RWY51-7D but *MAT**a***	Spore of RWY51
RWY51-23A	Same as RWY51-7B but *mec1::mec1ts::HIS3 tel1Δ::LEU2*	Spore of RWY51
MCY5122	Same as RWY51, addition of *sae2Δ::kanMX4/SAE2*	This study
RWY51-24A	Same as RWY51-7B but *mec1::mec1ts::HIS3*	This study
Clone 11	Derived from RWY51-24A after EMS mutagenesis; original isolate harboring *tel1-11*, used for backcrosses	This study
Clone 5	Derived from RWY51-24A after EMS mutagenesis; original isolate harboring a mutation in *RAD50*	This study
MCY5125	Same as MCY5122	This study
MCY5122-7C	*ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 mec1::mec1ts::HIS3 sml1Δ::TRP1 tel1Δ::LEU2 sae2Δ::kanMX4*	Spore of MCY5122
MCY5125-11A	Same as MCY5122-7C	Spore of MCY5125
MCY5122-3D	*ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 mec1::mec1ts::HIS3 TEL1 sml1Δ::TRP1 sae2Δ::kanMX4*	Spore of MCY5122
RWY517	*MAT**a**/MATαade2-1/ade2Δ::hisG ura3-1/ura3Δ0 his3-11/his3Δ200 trp1-1/trp1Δ63 leu2-3,112/leu2Δ0 mec1::TRP1/MEC1 sml1Δ::HIS3/SML1 TEL1/tel1Δ::LEU2*	Diploid formed by mating of U953-61A with RWY51-7D
RWY517-5B	*MAT**a**ade2 ura3 his3 trp1 leu2 mec1Δ::TRP1 sml1Δ::HIS3 tel1Δ::LEU2*	Spore of RWY517
RWY51-30C	*ade2 ura3 his3 trp1 leu2 mec1Δ::TRP1 sml1Δ::HIS3 TEL1*	Spore of RWY517
MCY511-1	Same as RWY51-23A in which the *tel1Δ::LEU2* allele was changed by integration of the *tel1-11::natR* allele	This study
MCY511-3	Same as MCY511-1	This study
MCY56-4	Same as RWY51-23A in which the *tel1Δ::LEU2* allele was changed by integration of the *TEL1::natR* allele	This study
MCY56-10	Same as MCY56-4	This study
MCY511-9	Same as RWY51-23A in which the *tel1Δ::LEU2* allele was changed by integration of the *natR* gene	This study
MCY56-3	Same as MCY511-9	This study
MCY721	*MAT**a**/MATαade2/ade2Δ::hisG ura3/ura3Δ0 his3/his3Δ200 trp1/trp1Δ63 leu2/ leu2Δ0 mec1Δ::TRP1/mec1::mec1-ts::HIS3 sml1Δ::HIS3/SML1 tel1Δ::LEU2/tel1-11::natR*	Diploid formed by mating of MCY511-1 with RWY517-5B
MCY721-1B	*ade2 ura3 his3 trp1 leu2 mec1Δ::TRP1 sml1Δ::HIS3 tel1-11::natR*	Spore of MCY721
MCY741	*MAT**a**/MATαade2Δ::hisG/ade2 his3Δ200/his3 leu2Δ0/leu2 lys2Δ0/lys2 met15Δ0/met15 trp1Δ63/trp1 ura3Δ0/ura3 mec1::mec1ts::HIS3/mec1Δ::TRP1 sml1Δ::TRP1/sml1::HIS3 tel1Δ::LEU2/tel1-11::natR sae2Δ::kanMX4/SAE2*	Diploid formed by mating MCY721-1B and MCY5122-7C
MCY742	*MAT**a**/MATαade2Δ::hisG/ade2 his3Δ200/his3 leu2Δ0/leu2 lys2Δ0/lys2 met15Δ0/met15 trp1Δ63/trp1 ura3Δ0/ura3 mec1::mec1ts::HIS3/mec1Δ::TRP1 sml1Δ::TRP1/sml1::HIS3 TEL1/tel1-11::natR sae2Δ::kanMX4/SAE2*	Diploid formed by mating of MCY721-1B with MCY5125-11A
MCY743	Same as MCY742	Diploid formed by mating of MCY721-1B with MCY5122-3D
MCY7412-18B	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::TRP1 sae2Δ::kanMX4 tel1-11::natR*	Spore of MCY741
MCY7422-7C	Same as MCY7412-18B	Spore of MCY742
MCY7431-11A	Same as MCY7412-18B	Spore of MCY743
MCY7412-18A	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::TRP1 sae2Δ::kanMX4 TEL1*	Spore of MCY741
MCY7411-4B	Same as MCY7412-18A	Spore of MCY741
MCY7422-7B	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::TRP1 sae2Δ::kanMX4 tel1Δ::LEU2*	Spore of MCY742
MCY7431-16D	Same as MCY7422-7B	Spore of MCY743
MCY751	Same as BY4705 but *mec1::mec1ts::HIS3/MEC1 tel1-11::natR/tel1Δ::LEU2*	Diploid formed by mating of MCY511-1 with RWY51-27A
MCY751-1D	*ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1-11::natR*	Spore of MCY751
MCY751-2B	Same as MCY751-1D	Spore of MCY751
MCY751-1A	*ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tel1Δ::LEU2*	Spore of MCY751
MCY751-2C	Same as MCY751-1A	Spore of MCY751

Table 1 (Contd.)

Strain	Genotype	Source/remarks
MCY1700	Same as RWY517 with the addition of *RAD52/rad52Δ::kanMX4*	This study
MCY1700-7A	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::HIS3 tel1Δ::LEU2 rad52Δ::kanMX4*	Spore of MCY1700
MCY1700-2C	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::HIS3 TEL1 rad52Δ::kanMX4*	Spore of MCY1700
MCY11	*MAT**a**/MATαade2/ade2 his3/his3 leu2/leu2 lys2/lys2 met15/met15 trp1/trp1 ura3/ura3 mec1Δ::TRP1/MEC1 sml1Δ::HIS3/SML1 TEL1/tel1-11::natR*	Diploid formed by mating of MCY751-1D with RWY51-30C
MCY111	Same as MCY11 with the addition of *RAD52/rad52Δ::kanMX4*	This study
MCY111-6A	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::HIS3 tel1-11::natR rad52Δ::kanMX4*	Spore of MCY111
MCY111-10A	Same as MCY111-6A	Spore of MCY111
DFY030	*ade2 his3 leu2 lys2 met15 trp1 ura3 mec1Δ::TRP1 sml1Δ::HIS3 tel1Δ::LEU2 RAD52*	Spore of MCY1700

plasmids resulting from this allele rescue (Fig. 2b) were named p316GRtel1-11 and p316GRTEL1, respectively. The complete *tel1-11* coding region on the p316GRtel1-11 plasmid was sequenced at least twice using internal *TEL1* primers and the assembled sequence revealed a single point mutation (G3955A), leading to a E to K

Fig. 1 Schematic presentation of the conditional synthetic lethality screen that allowed identification of the *tel1-11* allele. **a** Experimental chart flow. *xxx* represents a mutant allele of candidate genes conferring synthetic lethality at 37°C if combined with the *mec1-ts* allele. **b** Cells with the indicated genotypes were grown for at least 90 generations at 26°C on plates and then allowed to grow for an additional five generations in liquid culture prior to DNA extraction and TRF analyses. *Lane 1* RWY51-23A (*mec1-ts tel1Δ*); *lane 2* RWY51-24A (*mec1-ts TEL1*); *lane 3* clone 11 (*mec1-ts tel1-11*); *lane 4* clone 5 (*mec1-ts rad50*)
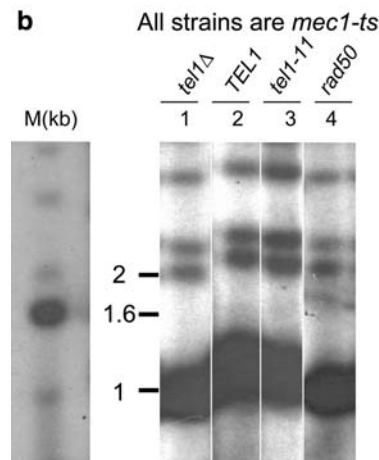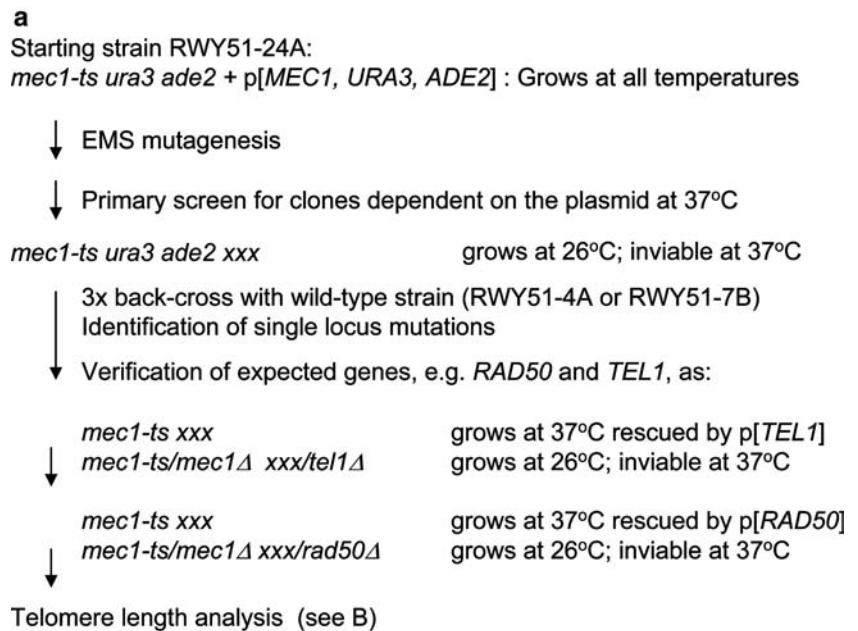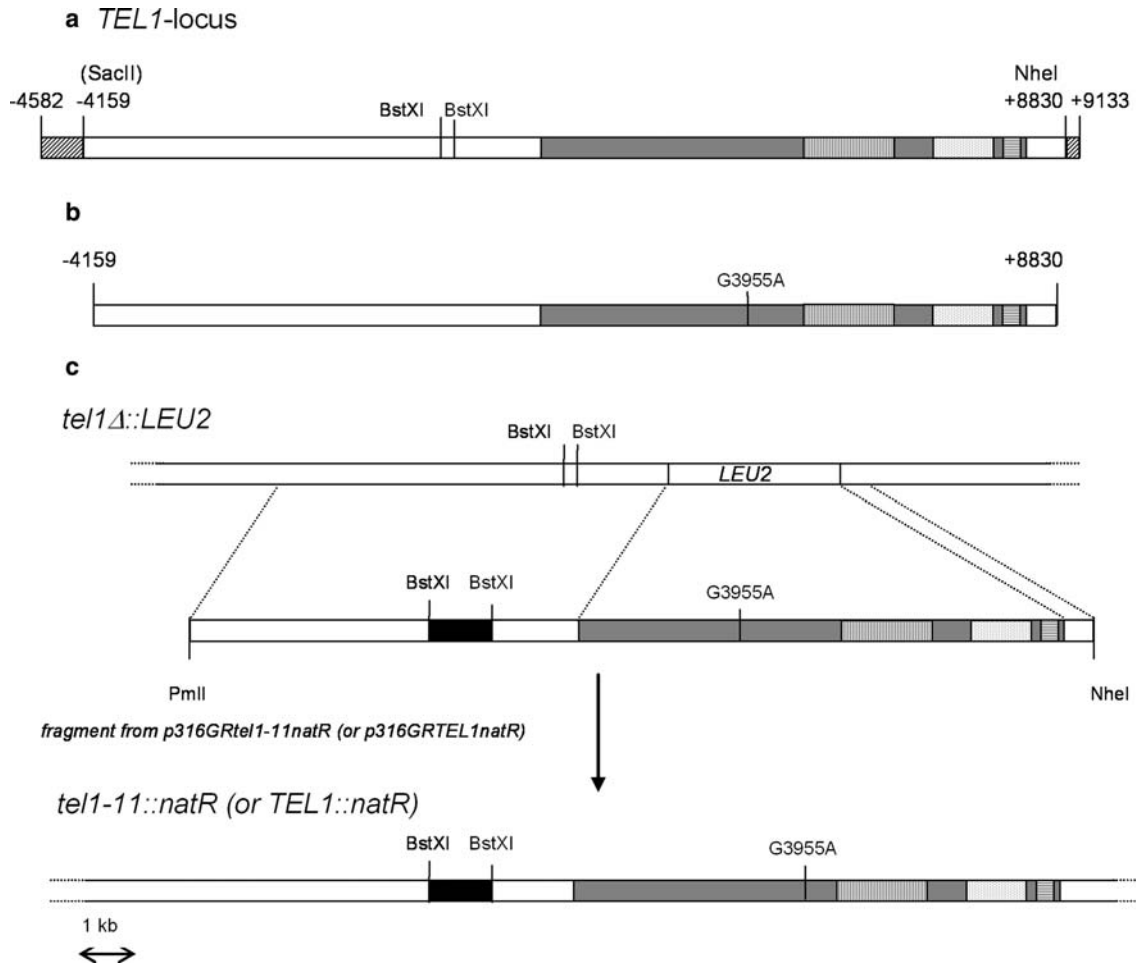
Fig. 2 Organization of the *TEL1* locus. **a** *TEL1* genomic region; **b** Fragments recovered by gap repair from *TEL1* or *tel-11* carrying strains (mutation in *tel1-11* is indicated); **c** Introduction of *tel-11::natR* or *TEL1::natR* into the genome. Featured are *TEL1* ORF (*gray box*), domains: FAT (*vertically striped box*), kinase (*dotted box*) and FATC (*horizontally striped box*) (Bosotti et al. 2000). Marker genes [*LEU2*, *natR* (*black box*)] and flanking fragments used for gap repair (*hatched boxes*) are also indicated. *Vertical bars* represent: G3955A—the position of the mutation revealed by sequencing, *Bst*XI—the sites delimiting the region that was replaced by *natR*; *Pml*I, *Sac*II and *Nhe*I—sites used for gap repair or genomic integration of *TEL1* alleles. The *Sac*II site is indicated in brackets as it does not belong to the genomic sequence but only to the p316TGR plasmid; it consequently disappeared during the gap repair

change at amino acid position 1319. Further, a *Spe*I-fragment spanning the region from −808 to +6,300 was subcloned and sequenced using flanking T3/T7 and internal *TEL1* primers, revealing no additional mutations in this entire region. In order to be able to follow *TEL1*-alleles through crosses, plasmids p316GRtel1-11natR and p316GRTEL1natR were constructed by inserting a PCR-amplified *natR* selection marker (conferring resistance to nourseothrecin (Goldstein and McCusker 1999) into the *Bst*XI sites upstream of the *TEL1* gene of p316GRtel1-11 and p316GRTEL1 plasmids, respectively (Fig. 2c). Integration of the *natR*-linked *tel1-11* or *TEL1* alleles into the genome was performed by transformation of the RWY51-23A strain with *Pml*I- *Sal*I fragments (Fig. 2c). Replacement of *tel1Δ::LEU2* by *tel1-11::natR, TEL1::natR or tel1-Δ::LEU2::natR* was verified by Southern blotting and phenotype analysis of the corresponding strains (data

not shown). Strains with or without the *natR* gene integrated upstream from different alleles of *TEL1* gene behaved similarly in all assays used (data not shown). Plasmid p316TEL1 contained a *TEL1* genomic fragment −808 to ∼+9,133 in the *Spe*I–*Sal*I sites of pRS316 and was used interchangeably with p316GRTEL1 in complementation assays.

Yeast senescence assays and growth tests

Senescence was assessed by visual analysis of colony-formation and growth on solid-rich medium (YPD) under given conditions for a number of generations, and further confirmed by telomere analysis (see below; Lundblad and Szostak 1989). Formation of an average colony from a single cell was estimated to require ∼20 divisions, and growth in a 5 ml liquid culture to
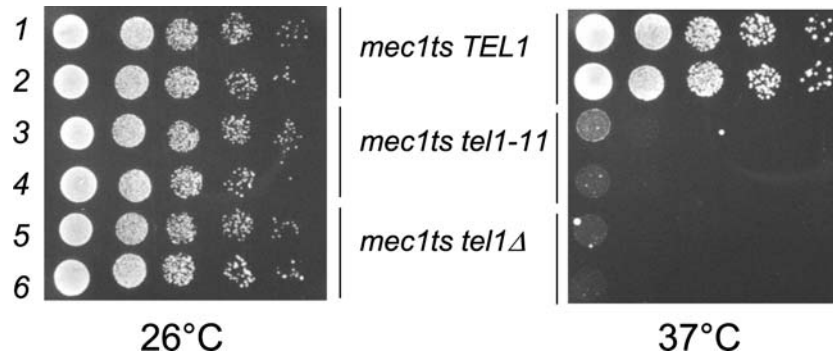
Fig. 3 At 37°C the *tel1-11* mutation is lethal when combined with the *mec1-ts* mutation. Growth tests of integrative mutants were performed as described in Materials and methods. About ten cells were plated on the most diluted spots. Strains used are: *1* MCY56-4 (*mec1-ts TEL1*); *2* MCY56-10 (*mec1-ts TEL1*); *3* MCY511-1 (*mec1-ts tel1-11*); *4* MCY511-3 (*mec1-ts tel1-11*); *5* MCY56-3 (*mec1-ts tel1Δ*); *6* MCY511-9 (*mec1-ts tel1Δ*)

saturation ~5 generations. Overall ability to grow was evaluated by "spot dilution tests". The cell concentration of an initial liquid culture was determined by measuring the OD600 and corresponding numbers of cells were spotted in serial tenfold dilutions on YPD medium.

MMS sensitivity assays

Stationary cultures were diluted to ~0.5–1.5×10^6 cells/ml and allowed to re-grow to exponential phase (~0.5–1.5×10^7 cells/ml). For permanent exposure to MMS, cell growth was assessed by spot dilution tests on YPD medium containing indicated concentrations of MMS. Scoring was generally performed after a 3 or 4-day growth before MMS concentration declined due to its degradation. For acute exposure to MMS, MMS was added directly (final concentration 0.01%) to a culture which was usually 5–20 ml. These MMS-treated cultures were incubated for 90 min at the indicated temperatures, then washed thrice with water and survival was assessed by spot dilution tests on YPD medium lacking MMS. Colony scoring was generally performed after a 3-day growth, as no distinct additional colonies appeared later.

Analysis of telomere length

Telomere length was analyzed as described (Wellinger et al. 1993). Briefly, isolated genomic DNA was digested with the *Xho*I restriction enzyme, which cuts once in the conserved telomere proximal Y′ repeat element. Many yeast telomeres contain such a Y′-element and this digestion releases a diagnostic ~1.2 kb terminal restriction fragment (TRF), which includes the terminal ~0.35 kb TG$_{1-3}$ repeats. This DNA is then analyzed by Southern blotting using specific TG$_{1-3}$-repeat probes, as described previously (Wellinger et al. 1993).

Western analysis

After acute exposure to MMS (see above), protein extracts from 5 to 20 ml aliquots of the cultures were prepared using a modified TCA method. The same cultures were also used to assess survival by spot dilution tests. Proteins were separated on an 8% acrylamid-bis-acrylamid gel (ratio 37.5:1) according to standard techniques (Sambrook et al. 1989) and transferred onto Hybond-P membrane according to supplier's instructions (Amersham Pharmacia Biotech). Anti-Rad53p polyclonal rabbit antibody was a kind gift from Frederic Sweeney and Daniel Durocher (Samuel Lunenfeld Research Institute, University of Toronto). Chemiluminescent detection of antigen–antibody complexes was carried out with horseradish peroxidase-conjugated anti-rabbit secondary antibodies in combination with ECL + detection reagent (Amersham Pharmacia Biotech).

Results

Isolation of a mutation in the *TEL1* gene that is lethal when combined with a *mec1-ts* mutation

Cells harboring the *mec1-ts* allele grow normally at 26°C and are only mildly impaired at 37°C (Fig. 3). They are moderately resistant to the DNA-damaging agent MMS at both 26 and 37°C, and arrest properly upon a single HO-induced DSB at 26°C, but not at 36°C (Weinert et al. 1994). Interestingly, the *mec1-ts* mutation confers lethality when combined with a deletion of *TEL1* (Fig. 3), *MRE11* or *RAD50* (data not shown). This suggests that an essential function of Mec1p is provided by these genes in *mec1-ts* cells at 37°C. The lethality of *mec1-ts tel1Δ* cells at 37°C is suppressed by a deletion of *SML1* or over-expression of *RNR1* (data not shown), indicating that this essential function is related to regulation of nucleotide synthesis (Zhao et al. 1998). However, it is not clear to which extent the essential function of the *mec1-ts*-encoded protein is compromised, as the

mec1-ts allele has not been characterized in full detail. We initially wished to determine which, if any, other genes would belong to the same pathway as the *TEL1* and MRX-genes. Therefore, we performed a plasmid-loss based genetic screen (Kranz and Holm 1990) for mutations that would cause lethality when combined with the *mec1-ts* mutation at 37°C, but not at 26°C. Primary candidate mutants were identified as *mec1-ts* cells that could not lose a plasmid harboring a wild-type copy of *MEC1* gene at 37°C, but could lose the same at 26°C (see Materials and methods). As expected, we recovered single-locus mutants that affected the *RAD50* and *TEL1* genes. Curiously however, one of the single recessive mutations isolated in our screen, clone 11, while genetically linked to *TEL1*, conferred normal TRF sizes when the cells were grown at 26°C (see below). These initial results indicated that this mutation in *TEL1* did not yield a short telomere phenotype at 26°C, yet it conferred synthetic lethality when combined with the *mec1-ts* allele at 37°C.

In order to ensure that the mutation indeed occurred in the *TEL1* gene, the mutated gene was cloned by gap repair using a plasmid bearing a gapped copy of *TEL1* (see Materials and methods). The complete region corresponding to a functional *TEL1* gene was sequenced and shown to contain one single nucleotide divergence with the *TEL1* wild-type sequence. The detected guanine to adenine substitution is predicted to provoke the amino acid change E1319K in the Tel1p sequence. The corresponding allele was thus named *tel1-11* and the protein encoded by it Tel1-11p.

In order to further characterize this mutation, we integrated the *tel1-11* into the genome of the strain RWY51-23A in replacement of *tel1Δ::LEU2* as described in Materials and methods. The analysis of the resultant strain proved that *tel1-11* conferred synthetic lethality with *mec1-ts* at 37°C, but did not impair the growth of *mec1-ts* cells at 26°C (Fig. 3). We then characterized the *tel1-11* allele with respect to three functions that the Tel1p protein is known to accomplish: normal telomere length maintenance, maintenance of telomeres in *mec1Δsml1Δ* cells and activation of a DNA-damage checkpoint.

In cells expressing Tel1-11p, telomere lengths are normal at 26°C and shortened at 37°C

In order to assess the telomere functions of Tel1-11p, congenic spores bearing corresponding alleles of *TEL1* were derived from diploid heterozygous strains MCY751 (*MEC1/mec1-ts tel1Δ/tel1-11*) or RWY51 (*MEC1/mec1-ts TEL1/tel1Δ*). These heterozygous diploids, when grown at 26°C, possessed normal telomeres (data not shown). Haploid segregants were sub-cultured by re-streaking on plates at 26 or 37°C followed by a liquid culture at the same conditions, genomic DNA was isolated and subjected to TRF analysis. In cells harboring certain *TEL1* alleles or upon a complete loss of

the *TEL1* gene, telomeres shorten to attain their minimal stable length after about 150 generations (Ritchie et al. 1999; Lustig and Petes 1986). After growth for 145 generations at 26°C, the presence of Tel1-11p still conferred stable telomere lengths, which were indistinguishable from cells containing the wild-type Tel1p (Fig. 4a). As reported before, strains lacking Tel1p (*tel1Δ*) bore short stable telomeres. When the cells were subcultured at 37°C, telomeres of *tel1-11* strains gradually shortened, but after about 145 generations, they were still slightly longer than those of cells lacking Tel1p altogether (Fig. 4b, lanes 7–12). When these *tel1-11* cells were shifted back to 26°C, their telomeres regained wild-type length within 45 generations (Fig. 4b, lanes 1–6). These data indicate that Tel1-11p confers some temperature sensitivity in terms of normal telomere length
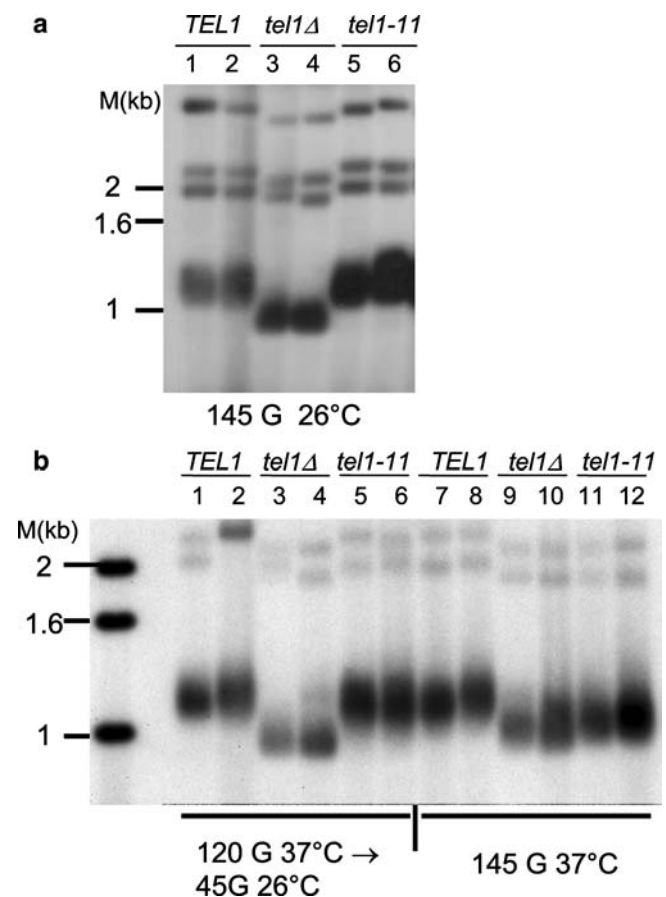

Fig. 4 The Tel1-11p confers a normal telomere length phenotype at 26°C and shortened telomeres at 37°C. **a** Cells harboring the indicated alleles of the *TEL1* gene were cultivated at 26°C for 145 generations and TRF lengths were assessed as described in Materials and methods. **b** Cells as in (**a**) were cultivated for 120 generations at 37°C, then split into two subcultures; one was kept for an additional 25 generations at 37°C (*lanes 7–12, right*), the other was cultivated for the next 45 generations at 26°C (*lanes 1–6, left*). *M* molecular weight marker, *G* generations. Signals between 1.0 and 1.6 kb are TRFs from Y' telomeres; some non-Y' TRFs of >1.6 kb can be seen. Strains used are *1, 7* RWY51-7B (*TEL1*); *2, 8* RWY51-28C (*TEL1*); *3, 9* MCY751-1A (*tel1Δ*); *4, 10* MCY751-2C (*tel1Δ*); *5, 11* MCY751-1D (*tel1-11*); *6, 12* MCY751-2B (*tel1-11*)

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maintenance: this function appears to be fully supplied when cells are grown at 26°C, but compromised at 37°C.

## *Mec1Δ sml1Δ tel1-11* cells can maintain short, but stable telomeres at 37°C

As a second criterion for the functioning of Tel1-11p at telomeres, we asked whether *tel1-11* cells depended on Mec1p to maintain a critical telomere length without entering a senescence crisis. To that end, we first compared the growth and telomere TRF length patterns of cells that were grown at 37°C with the following genotypes: *mec1Δsml1ΔTEL1 rad52Δ*, *mec1Δsml1Δtel1-11 rad52Δ* and *mec1Δsml1Δtel1Δrad52Δ*. No striking difference could be observed in the growth of Mec1-deficient cells possessing a *TEL1* or *tel1-11* allele for 120 generations analyzed, whereas cells with a *tel1Δ* allele displayed a clear senescent phenotype after ~60 generations (Fig. 5a). Furthermore, at the end of the analyses, *mec1Δsml1Δtel1-11 rad52Δ* cells harbored short and stable telomeres (Fig. 5b). As a possible indication of a stable maintenance of the telomeres in this strain, they were clearly longer than those of *mec1Δsml1Δtel1Δ* cells and there was no indication of telomeric rearrangements typical for survivors, which maintain their telomeres by homeologous recombination (Fig. 5b, Lundblad and

Blackburn 1993; Teng and Zakian 1999). These data indicate that even when cells were grown continuously at 37°C, cells lacking Mec1p and expressing Tel1-11p can stably maintain telomeric repeats and do not display phenotypes that would indicate a complete loss of telomerase activity at the telomeres, such as observed in the cells completely lacking both Mec1p and Tel1p. Thus, although Tel1-11p cannot fully supply the function to maintain normal length telomeres at 37°C, this protein is proficient in providing the functions required for stable maintenance of telomeric repeats in the absence of Mec1p at all temperatures.

The DNA-damage checkpoint function is undetectable in cells expressing Tel1-11p grown at 37°C

In the presence of Mec1p, strains expressing Tel1-11p did not display any obvious growth defects or MMS sensitivity at either 26 or 37°C (data not shown). This was expected though, since otherwise wild-type cells but lacking Tel1p altogether were shown to behave similarly (Sanchez et al. 1996). However, the contribution of Tel1p to the a DNA-damage checkpoint was demonstrated in cells that contain *mec1Δsml1Δsae2Δ* mutations (Usui et al. 2001). We therefore introduced the *tel1-11* allele into that background by crossings (see Table 1). In

Fig. 5 In the absence of Mec1p, Tel1-11p does not confer a senescence phenotype even when the cells are grown at 37°C. a Subculturing of strains on plates were performed at 37°C for the indicated number of generations as described in Materials and methods. To the right, the relevant genotypes of the strains cultured in the respective quadrants of the plates are indicated. Note that *mec1Δsml1Δtel1Δrad52Δ* cells (*top left quadrant*) did not grow beyond 80 generations. Strains used are: MCY1700-7A (*mec1Δsml1Δrad52Δtel1Δ*); MCY1700-2C (*mec1Δsml1Δrad52ΔTEL1*); MCY111-6A, MCY111-10A (*mec1Δsml1Δtel-11 rad52Δ*). b Cells with the indicated genotypes were grown for at least 120 generations at 37°C on plates and were then allowed to grow for an additional five generations in liquid culture prior to DNA extraction and TRF analyses as described in Materials and methods. *Lane 1* MCY1700-2C (*mec1Δsml1Δrad52ΔTEL1*); *lane 2* MCY111-6A (*mec1Δsml1Δrad52Δtel1-11*); *lane 3* DFY 030 (*mec1Δsml1ΔRAD52 tel1Δ*)
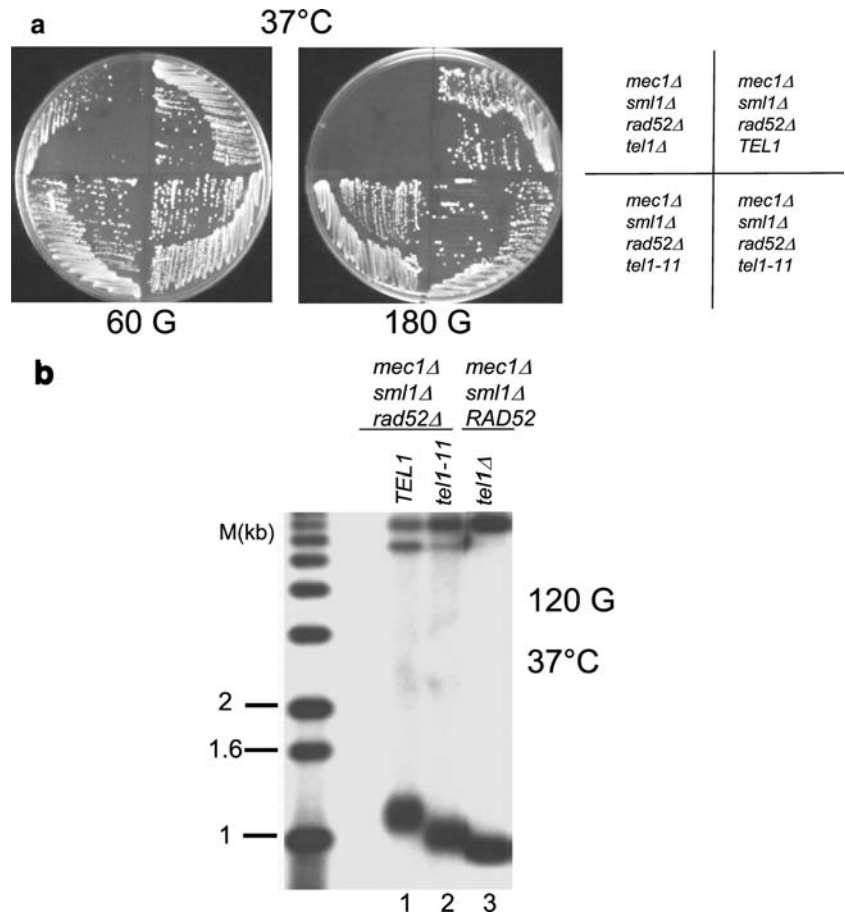
this setting, cells possessing a functional checkpoint provided by Tel1p are relatively resistant to MMS exposure and able to phosphorylate Rad53p. Unfortunately, we could not use *mec1Δsml1Δsae2Δtel1Δ* cells in tests for checkpoint function, as cells with this combination of mutations displayed a very early onset of senescence (see Fig. 6 for example). Thus, growth of *mec1Δsml1Δsae2Δ* cells expressing Tel1-11p was compared to *mec1Δsml1Δsae2Δ* cells expressing wild-type Tel1p in the presence of MMS (Fig. 6). At 26°C, *mec1Δsml1Δsae2Δtel1-11* cells were no more sensitive to 0.01% MMS than *mec1Δsml1Δsae2ΔTEL1* cells (Fig. 6, bottom left). At 37°C, *mec1Δsml1Δsae2Δtel1-11* cells clearly were more sensitive to 0.01% MMS exposure than *mec1Δsml1Δsae2ΔTEL1* cells (Fig. 6, bottom right).

In a parallel assay, the cells were exposed to 0.01% MMS for a limited time (90 min, acute exposure) at 26 or 37°C and the extent of Rad53p phosphorylation was analyzed in total protein extracts from the cells exposed to these conditions. Phosphorylation was deduced from the appearance of slowly migrating forms of Rad53p using an anti-Rad53 antibody (Vialard et al. 1998). After acute exposure to MMS at 26°C, Rad53p was nearly completely phosphorylated in *mec1Δsml1Δsae2ΔTEL1* cells and at least partially phosphorylated in *mec1Δsml1Δsae2Δtel1-11* cells (Fig. 7). When these same

Fig. 6 At 37°C, the *tel1-11* allele confers sensitivity to MMS exposure when combined with *mec1Δsml1Δsae2Δ* mutations. *Top* Growth tests in the absence of MMS by spot dilution were performed as described in Materials and methods. Cells used for these tests were grown for ~30 generations before plating. About ten cells were plated on the most diluted spots. *Note the senescence of cells lacking all ATM-like kinases (*mec1Δsml1Δsae2Δtel1Δ*). Strains used are: *1* MCY7411-4B (*mec1Δsml1Δsae2ΔTEL1*); *2* MCY7412-18A (*mec1Δsml1Δsae2ΔTEL1*); *3* MCY7412-18B (*mec1Δsml1Δsae2Δtel1-11*); *4* MCY7422-7B (*mec1Δsml1Δsae2Δtel1Δ*); *5* MCY7422-7C (*mec1Δsml1Δsae2Δtel1-11*). *Bottom* Growth tests of strains in presence of MMS were performed as described in Materials and methods. About one cell was plated on the most diluted spots. Strains used are: *1* MCY7412-18A (*mec1Δsml1Δsae2ΔTEL1*); *2* MCY7412-18B (*mec1Δsml1Δsae2Δtel1-11*)

cells were replated after the acute exposure on media without MMS at 26°C, a slight reduction in viability was observed for the *mec1Δsml1Δsae2Δtel1-11* cells when compared to the in *mec1Δsml1Δsae2ΔTEL1* cells (data not shown), which correlates well with the incomplete phosphorylation of Rad53p (Fig. 7). In contrast, at 37°C, Rad53p phosphorylation could only be detected in *mec1Δsml1Δsae2ΔTEL1* cells, but not in *mec1Δsml1Δsae2Δtel1-11* cells (Fig. 7) and the latter cells did not regrow on plates without MMS incubated at 37°C (data not shown). This correlation between analyses of re-growth and Rad53p-phosphorylation upon exposure to MMS demonstrates that in the genetic backgrounds used here, cells expressing Tel1-11p are severely compromised for the DNA-damage checkpoint at 37°C, while this function is quite proficient, when the assays were performed at 26°C.

Discussion

Tel1p has been implicated in both telomere maintenance and a DNA-damage checkpoint, sometimes referred to as TM-checkpoint (Greenwell et al. 1995; Usui et al. 2001). In addition, the MRX-complex has been associated with Tel1p for both of these functions (Tsukamoto et al. 2001; Usui et al. 2001). Although the first indication that these functions can be separated was provided by the discovery of an *mre11-ts* allele (Chamankhah et al. 2000), it remained unclear whether Tel1p contributes differentially to telomere maintenance and the DNA-damage checkpoint or whether its functions at telomeres are dependent on the same type and level of activity also required for the checkpoint. Here, we show that Tel1p contributes differentially to normal and basic telomere functions and DNA-damage checkpoints.

This conclusion is derived from the analyses of phenotypes conferred to cells by a new mutation in the *TEL1* gene, called *tel1-11*. The mutation was isolated by virtue of its synthetic lethal interaction with a *mec1-ts* allele at 37°C. First, to assess the functions of *tel1-11*-encoded protein (Tel1-11p) in terms of maintenance of telomeric repeats, we analyzed two different phenotypes. The first relates to the ability of Tel1p to maintain a telomeric repeat tract of wild-type length, which we will refer to as the normal telomere function. The second is operationally defined as the activity which, in the absence of Mec1p, is required to maintain a stable telomeric repeat tract for more than 120 generations with the cells not undergoing a senescence crisis; we will call this activity the basic telomere function of Tel1p. Both of these functions are absent in cells completely lacking Tel1p (Figs. 4, 5). Intriguingly, Tel1-11p provides proficiency in both normal and basic telomere function when cells are grown at 26°C. However, in cells grown at 37°C, the mutation clearly affects normal telomere function, although not as severely as a *tel1Δ* mutation (Fig. 4). On the other hand and most significantly, in cells expressing Tel1-11p, the basic telomere function
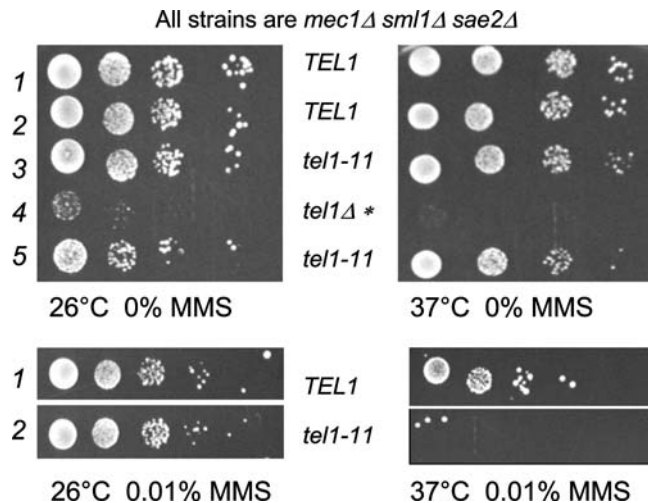
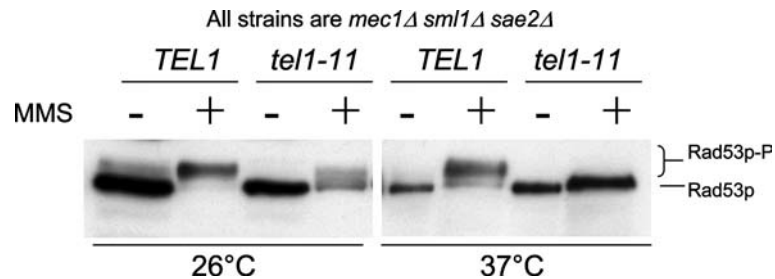All strains are *mec1Δ sml1Δ sae2Δ*

Fig. 7 Absence of detectable Rad53p phosphorylation in cells expressing Tel1-11p at 37°C. Phosphorylation of Rad53p in *mec1Δsml1Δsae2ΔTEL1* or *mec1Δsml1Δsae2Δtel1-11* cells exposed to 0.01% MMS for 90 min at 26 or 37°C. Assay conditions and detection of non-phosphorylated Rad53p (Rad53p) and phosphorylated Rad53p (Rad53p-P) was performed as described in Materials and methods. Strains used are: MCY7412-18A (*mec1Δsml1Δsae2ΔTEL1*); MCY7412-18B (*mec1Δsml1Δsae2Δtel1-11*)

remains intact at all temperatures (Fig. 5). Second, we assayed the DNA-damage checkpoint functions conferred by Tel1-11p, as defined by phosphorylation of Rad53p and cell survival in a *mec1Δsml1Δsae2Δ* background upon exposure to MMS. Compared to cells expressing wild-type Tel1p, cells harboring Tel1-11p display a reduction of the level of Rad53p phosphorylation already at 26°C; and there is no phosphorylated Rad53p detectable, when the checkpoint functions are assayed in this setting at 37°C (Fig. 7). The sensitivities of these cells to DNA-damage induced by MMS parallel these findings quite well (Fig. 6 and data not shown). We conclude that DNA-damage checkpoint functions conferred by Tel1-11p are reduced at 26°C and virtually abolished at 37°C. These data are summarized in Table 2.

Hence and most strikingly, at 37°C, the basic telomere function of Tel1p is maintained by Tel1-11p even in the absence of detectable induction of a DNA-damage checkpoint. This differential requirement for Tel1p activity in terms of the basic telomere function and the checkpoint function can be rationalized in several ways. For example, these two functions could be carried out by different activities of Tel1p, or there are different targets of a common Tel1p activity, such as its kinase activity. In this latter scenario, Tel1-11p could be deficient in interactions required to activate the checkpoint, but still able to perform its activity on the targets required for maintaining telomeric repeats in the absence of Mec1p. Alternatively, the checkpoint and the basic telomere functions could have differential requirements for a common Tel1p activity on the same target. For example, even when the cells are grown at 37°C, Tel1-11p could supply a minimal kinase activity, just sufficient to phosphorylate a critical substrate to allow the basic telomere function. However, this minimal level of activity would not be sufficient to activate the DNA-damage pathway, at least in terms of detectable Rad53p phosphorylation or resistance to genotoxic stress. Although we do not know for certain whether the mutation in *tel1-11* directly impairs Tel1p activity or causes decreased protein stability with normal levels of activity, at least HA3-tagged Tel1-11p protein levels expressed from the endogenous locus are very similar at 26 and 37°C, which is inconsistent with the mutation inducing a temperature-dependent protein instability (Supplemental Fig. 1).

The two hypotheses mentioned above are not mutually exclusive, since the mutation may actually affect different activities to different extents. The known genetic and physical interactions of Tel1p with other components of cellular checkpoint and repair mechanisms are consistent with either possibility, even though the MRX-complex has been placed in the same epistasis group as Tel1p with respect to its functions in the checkpoint as well as with respect to its functions at

Table 2 Summary of phenotypes conferred by Tel1p versus Tel1-11p

Protein	Temp. (°C)	TM -checkpoint[a]	Normal telomere length[b]	Absence of senescence in *mec1Δsml1Δrad52Δ* cells[c]
Tel1p wt	26	+	+	+
Tel1p wt	37	+	+	+
Tel1-11p	26	− +	+	+
Tel1-11p	37	− −	− +	+
(*tel1Δ*)	26	− −	− −	− −
(*tel1Δ*)	37	− −	− −	− −

Note: − − severely impaired, − + moderately impaired, + normal function
[a]Assessed by MMS sensitivity and Rad53p phosphorylation when the indicated Tel1p protein is expressed in a *mec1Δsml1Δsae2Δ* background
[b]Assessed by Southern hybridization
[c]Assessed by growth observation and telomere analysis by Southern hybridization when the indicated Tel1p protein is expressed in a *mec1Δsml1Δrad52Δ* background

telomeres (Ritchie and Petes 2000; Usui et al. 2001), suggesting a common function. However, these genetic interactions may not necessarily reflect the same functional interactions. For example, recent data suggest that in vivo, the MRX-complex associates efficiently with DSBs in wild-type as in cells lacking Tel1p (Nakada et al. 2003a). The bound MRX-complex then interacts with Tel1p via a C-terminal domain of the Xrs2p, since the association of Tel1p with the breaks is dependent on this domain (Nakada et al. 2003a). On the other hand, telomeres isolated from asynchronously growing cells do not seem to be bound by the MRX-complex at an appreciable level, as long as Tel1p or Mec1p are present (Mieczkowski et al. 2003). Therefore, on telomeres, an activity that can be supplied by Mec1p or Tel1p appears to restrict and/or regulate the binding of the MRX-complex (Mieczkowski et al. 2003). As one possible interpretation of all the data, we speculate that the mutated Tel1-11p analyzed here may be severely impaired in its interactions at DSBs, but much less affected in its telomere-related activity. This would explain the drastic loss of the checkpoint activity while the basic telomere function remained intact in cells harboring this protein (Table 2).

Qualitatively, the degree of impairment of the normal telomere function conferred by Tel1-11p at 37°C lies between that required for the basic telomere functions and that required for checkpoint function, and this function is unaffected at 26°C, as discussed above (Table 2). These data reinforce the idea that the normal telomere function as well does not require a fully functional checkpoint (Morrow et al. 1995). However, given that in cells grown at 37°C, telomeric repeat tracts are not quite as long as in wild-type cells, it is difficult to predict whether this phenotype is caused by the impairment of the checkpoint activity or is the consequence of the mutation affecting another function of Tel1p. There is circumstantial evidence that Tel1p is involved in a Mec1-dependent, but MRX-independent G2/M-checkpoint signaling cascade (Giannattasio et al. 2002), and Tel1p has also been implicated in a chromosome capping function that prevents DSB to telomere fusions (Chan and Blackburn 2003). However, the latter issue is complicated by the fact that frequencies of direct telomere to telomere fusions are not increased in the absence of only Tel1p (Mieczkowski et al. 2003). It will be important to assess whether all of these functions depend on an active kinase function of Tel1p or whether some of them are dependent on other functions of Tel1p (see above).

Taken together, our data establish that Tel1p contributes differently to normal and basic telomere functions versus the DNA-damage checkpoint. This reinforces the idea that in wild-type cells, this yeast ATM-like kinase is involved in an important regulatory step during telomere replication and this function may be separable from the checkpoint function. Atm-lacking mammalian cells also display telomere-specific phenotypes, such as increased frequencies of chromosome end-fusions and accelerated loss of telomeric repeat DNA (Metcalfe et al. 1996; Pandita 2002). Given our results, it is possible that Atm-functions required at mammalian telomeres are different and separable from other cellular Atm functions. In fact, a recent phenotypic analysis of $Terc^{-/-}$ $Atm^{-/-}$ mice raised a similar possibility (Wong et al. 2003). Therefore, an in-depth analysis of separation-of-function alleles of ATM-like kinases in yeast could yield hints about the molecular defects in A-T patients.

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