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Involvement of the Checkpoint Protein Mec1p in Silencing of Gene Expression at Telomeres in *Saccharomyces cerevisiae*

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Yeast strains with a mutation in the *MEC1* gene are deficient in the cellular checkpoint response to DNA-damaging agents and have short telomeres (K. B. Ritchie, J. C. Mallory, and T. D. Petes, Mol. Cell. Biol. 19: 6065–6075, 1999; T. A. Weinert, G. L. Kiser, and L. H. Hartwell, Genes Dev. 8:652–665, 1994). In wild-type yeast cells, genes inserted near the telomeres are transcriptionally silenced (D. E. Gottschling, O. M. Aparichio, B. L. Billington, and V. A. Zakian, Cell 63:751–762, 1990). We show that *mec1* strains have reduced ability to silence gene expression near the telomere. This deficiency was alleviated by the *sml1* mutation. Overexpression of Mec1p also resulted in a silencing defect, although this overexpression did not affect the checkpoint function of Mec1p. Telomeric silencing was not affected by mutations in several other genes in the Mec1p checkpoint pathway (null mutations in *RAD9* and *CHK1* or in several hypomorphic *rad53* alleles) but was reduced by a null mutation of *DUN1*. In addition, the loss of telomeric silencing in *mec1* strains was not a consequence of the slightly shortened telomeres observed in these strains.

Our study concerns the relationship between two pathways in *Saccharomyces cerevisiae*: the pathway controlling reversible silencing of genes inserted near the telomeres, or telomere position effect (TPE), and the pathway regulating the response of the cell to DNA damage. TPE in *S. cerevisiae* was first reported by Gottschling et al. (14). Mutations in genes encoding telomere-binding proteins (Rap1p), Rap1p-interacting proteins (the Sir proteins, Rif1p and Rif2p), the histones H3 and H4, and proteins controlling posttranslational modifications of histones affect telomeric silencing (22); many of these mutations also affect the silencing of mating-type information at *HML* and *HMR* (2).

Silencing at the telomere is thought to involve interactions between the Rap1, Sir3p, and Sir4p proteins and the amino termini of histones H3 and H4 (17); subsequent "spreading" of silencing from the telomeric repeats to adjacent regions may involve posttranslational modifications of the histones (18). The net effect of these modifications is to reduce the availability of DNA in the silenced regions for the binding of transcription factors (3). Telomeric silencing also affects the timing of DNA replication. Telomeric sequences are replicated late in the S period in wild-type cells (27), and this replication delay is lost in strains in which TPE is eliminated (35).

In response to DNA damage, yeast cells induce various DNA repair enzymes and arrest the cell cycle in order to repair the damage (11, 40). Different gene products are involved in the early steps of recognizing DNA damage, in transmitting the DNA damage signal, and in responding to the DNA damage signal. The checkpoint proteins relevant to our study are Mec1p, Rad53p, Rad9p, Dun1p, and Chk1p (1, 39, 43). In current models of checkpoint pathways, Mec1p transduces signals from proteins that sense damaged DNA or delayed DNA replication to proteins that block the cell cycle or induce expression of DNA repair genes.

Mec1p is a very large protein with a protein/lipid kinase motif shared with the yeast Tel1p and the human ATM protein (19). Phosphorylation of Rad53p, Rad9p, and Dun1p requires Mec1p (12, 21, 38). Rad9p may be involved both in sensing DNA damage (23) and in activating functions downstream of Mec1p (12, 38). Both Rad53p and Dun1p are protein kinases that function in a damage response pathway downstream of Mec1p, with Rad53p functioning upstream of Dun1p (1, 13, 43). Both *rad53* and *mec1* strains are deficient in the transcriptional induction of various DNA repair genes, including *RNR1-3* (43). Chk1p functions downstream of Mec1p in a G_2-M checkpoint pathway different from that regulated by Rad53p and Dun1p (31).

Although the pathways controlling telomeric silencing and checkpoints appear to have separate functions, two recent results suggest overlaps. First, a mutation in the *S. cerevisiae* checkpoint gene *MEC3* increases TPE (7). Second, a mutation in *rad3*$^+$, a *Schizosaccharomyces pombe* gene homologous to *MEC1*, results in increased telomere length and reduced TPE (9, 25). Below, we show that mutations in *MEC1* and in *DUN1* lead to substantially reduced TPE.

MATERIALS AND METHODS

Plasmids. Several plasmids containing *URA3* adjacent to telomeric sequences derived from different chromosomes were used in our studies. The plasmid pAD-UCA (14) was used to insert *URA3* near telomere VII_L (Fig. 1a). Plasmid pV-UCA (identical to pV-R URA3-TEL [14]) contained DNA derived from telomere V_R on a *Hin*dIII fragment. In the construction of pPG70, this fragment was replaced by a fragment generated by PCR amplification of telomeric XV_L sequences (primers 5′ GGATCCCAAGCTTGAATATTACGTACTTATG and 5′ GGATCCCAAGCTTCTCGAGGAGAACTTCTAG) followed by *Hin*dIII treatment.

Several plasmids with *MEC1* were derived from YEp-*MEC1* (*MEC1* in the *URA3*-containing high-copy-number plasmid pRS426, provided by S. Elledge); since *Escherichia coli* transformed with *MEC1*-containing plasmids grows slowly, we generated these derivatives by recombination in yeast. We replaced *URA3* with *HIS3* (resulting in the plasmid pRC5) by transforming a strain containing YEp-*MEC1* with a fragment generated by amplifying the *HIS3* gene of pRS303 (33) with primers (5′ ATGTCGAAAGCTACATATAAGGAACGTGCTGCTA CTCATCCTAGTCCTGTGATTGTACTGAGAGTGCACC and 5′ TTTTGC TGGCCGCATCTTCTCAAATATGCTTCCCAGCCTGCTTTTCTGTAACT GTGCGGTATTTCACACCG) that had 5′ homology to DNA sequences flanking *URA3* in YEp-*MEC1* and 3′ homology to sequences flanking *HIS3*.

To construct a plasmid with *MEC1* on a *CEN*-containing plasmid (pRS4), we cotransformed (into the *mec1-21* strain RCY109-1c) a 9-kb *Bsa*I-*Nae*I fragment

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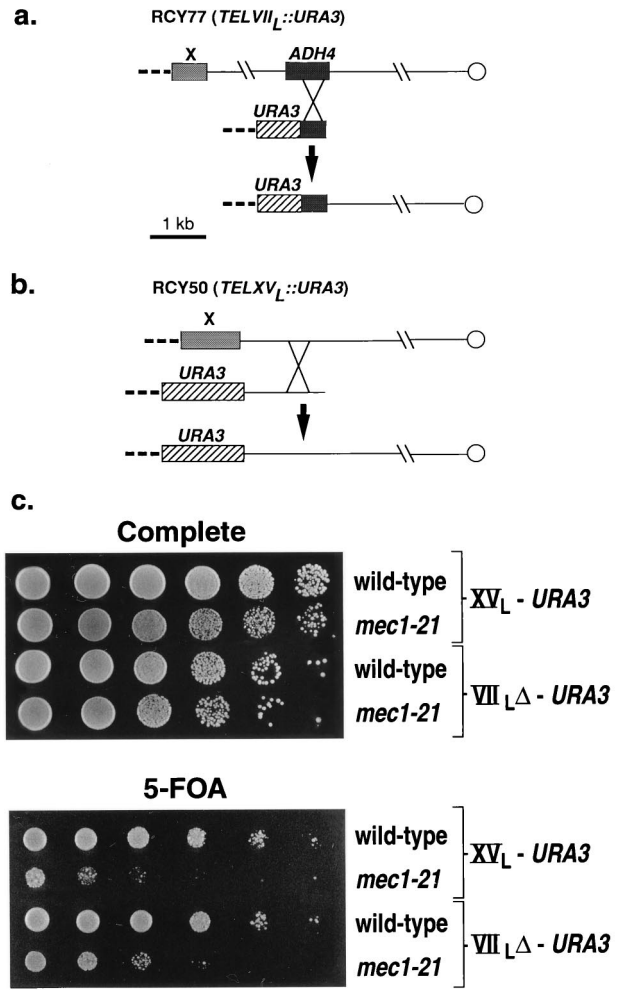

FIG. 1. Construction of strains to monitor telomeric silencing and assay for telomeric silencing. As in previous studies (14), the *URA3* gene was introduced at the telomere by transforming a *ura3* mutant strain with a linear DNA fragment in which the *URA3* gene was adjacent to telomeric sequences. Poly(TG_{1-3}) repeats are indicated by dashed lines. (a) RCY77 (*TELVII$_L$::URA3*) was constructed by transforming W303a with an *Eco*RI/*Sal*I restriction fragment derived from the plasmid pAD-UCA (14). (b) RCY50 (*TELXV$_L$::URA3*) was derived from W303a by transformation with an *Eco*RI fragment of pPG70 (see Materials and Methods). (c) TPE was monitored by plating serial dilutions of various strains onto media lacking or containing 5-FOA. Since the *URA3* insertion in *TELVII$_L$::URA3* strains is more strongly silenced than the *URA3* insertion in *TELXV$_L$::URA3* strains, 1:5 serial dilutions were used for strains shown in the top two rows, and 1:10 dilutions were used for strains shown in the bottom two rows. The effects of *mec1-21* on TPE at two different telomeres are shown. Top rows, RCY109-2b; second rows, RCY109-1c; third rows, RCY110-5d; fourth rows, RCY110-1b.

of YEp-*MEC1* (containing the *MEC1* gene and flanking vector sequences) and *Bam*HI- and *Sal*I-treated pR313 (*CEN*-containing vector with *HIS3*). The *HIS3*-containing high-copy-number vector pRS423 (6) was used as a control in some experiments. We also constructed a high-copy-number *LEU2*-containing plasmid (pRC11) with an insertion of *RNR1*. A *Pst*I-*Kpn*I fragment derived from the plasmid YEp24-(*RNR1*) (37) was ligated to the *Pst*I- and *Kpn*I-treated vector YEplac181 (15).

Yeast strains. All strains used in this study were isogenic with W303a (**a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100* [36]) except for alterations introduced by transformation. To monitor TPE, we constructed derivatives of W303a with the *URA3* gene inserted near the telomeres of different chromosomes (Fig. 1). The plasmids and restriction fragments used in the constructions were as follows: for RCY50, (*TELXV$_L$::URA3*), an *Eco*RI fragment of pPG70, and for RCY77 (*TELVII$_L$::URA3*), an *Eco*RI/*Sal*I fragment of pAD-UCA. Constructions were confirmed by Southern analysis.

The relevant genotypes for all haploid strains are shown in Table 1. Many of these haploids were spores derived from diploids. These diploids, constructed by crossing the strains listed in parentheses, are as follows: RCY61 (RCY28 [8] × RCY56 [8]), RCY78 (RCY61-1a × JMY303-15c [30]), RCY84 (RCY11 [8] × RCY28 [8]), RCY106 (RCY78-3b × RCY50), RCY109 (RCY50 × JMY303-2d), RCY110 (RCY77 × JMY303-2d), RCY112 (LPY253 × JMY303-15c), RCY123 (Y301 × RCY109-4d), RCY124 (RCY84-2c × LBY253), RCY126 (YS148 × RCY109-4d), RCY143 (DLY298 × RCY109-4d), RCY144 (Y286 × RCY109-4d), RCY155 (Y286 × RCY109-2b), RCY160 (DLY298 × RCY109-25c), RCY161 (DLY339 × RCY109-25c), RCY175 (RCY144-4b × JMY303-3b), RCY203 (W1973-6b × RCY109-9b), and RCY204 (W1974-6d × RCY109-9b).

To determine the mutant substitutions in the *mec1-21* and *rad53-21* alleles, we sequenced PCR fragments derived from the strains JMY303-3c (30) and RCY123-1b (**a** *rad53-21* spore derived from RCY123), respectively.

Measurement of telomere lengths. Yeast DNA was isolated by standard methods (15) and treated with *Pst*I. The resulting fragments were separated by agarose gel electrophoresis and transferred to Hybond N+ nylon membranes by

TABLE 1. Haploid strains used in this study[a]

Strain	Relevant genotype	Progenitor strain or reference
W303a	Wild type	36
RCY50	*TELXV$_L$::URA3*	W303a
JMY303-2d	α *mec1-21 sml1::HIS3*	30
JMY303-3b	α *sml1::HIS3*	30
RCY61-1a	*rif1 rif2*	RCY61
RCY78-3b	α *rif1 mec1-21*	RCY78
RCY106-1d	*rif1 TELXV$_L$::URA3*	RCY106
RCY106-3d	*mec1-21 TELXV$_L$::URA3*	RCY106
RCY106-4d	*TELXV$_L$::URA3*	RCY106
RCY106-14a	*rif1 mec1-21 TELXV$_L$::URA3*	RCY106
RCY109-1b	*sml1::HIS3 TELXV$_L$::URA3*	RCY109
RCY109-1c	*mec1-21 TELXV$_L$::URA3*	RCY109
RCY109-2b	*TELXV$_L$::URA3*	RCY109
RCY109-3a	*mec1-21 sml1::HIS3 TELXV$_L$::URA3*	RCY109
RCY109-4d	α *TELXV$_L$::URA3*	RCY109
RCY109-9b	α *TELXV$_L$::URA3*	RCY109
RCY109-25c	α *mec1-21 TELXV$_L$::URA3*	RCY109
RCY77	*TELVII$_L$Δ::URA3*	W303a
RCY110-1b	α *mec1-21 TELVII$_L$Δ::URA3*	RCY110
RCY110-5d	α *TELVII$_L$Δ::URA3*	RCY110
LPY253	*hml::TRP1*	29
JMY303-15c	α *mec1-21*	30
RCY112-5c	*hml::TRP1 mec1-21*	RCY112
RCY112-13b	*hml::TRP1 mec1-21*	RCY112
RCY84-2c	α *rap1-17*	RCY84
RCY124-2a	*hml::TRP1 rap1-17*	RCY124
YS148	*chk1Δ::HIS3 rad53-21*	Y. Sanchez and S. Elledge
W1973-6b	*rad53-1*	X. Zhao and R. Rothstein
W1974-6d	*rad53-17*	X. Zhao and R. Rothstein
RCY126-3c	*rad53-21 TELXV$_L$::URA3*	RCY126
RCY126-1d	*chk1Δ::HIS3 TELXV$_L$::URA3*	RCY126
DLY298	*rad9Δ::HIS3*	T. Weinert
RCY143-2c	*rad9Δ::HIS3 TELXV$_L$::URA3*	RCY143
Y286	α *dun1-Δ100::HIS3*	43
RCY144-4b	*dun1-Δ100::HIS3 TELXV$_L$::URA3*	RCY144
DLY339	*mec3::HIS3*	T. Weinert
RCY160-1b	α *rad9Δ::HIS3 TELXV$_L$::URA3*	RCY160
RCY160-3a	α *mec1-21 rad9Δ::HIS3 TELXV$_L$::URA3*	RCY160
RCY160-4b	α *mec1-21 TELXV$_L$::URA3*	RCY160
RCY160-4d	α *TELXV$_L$::URA3*	RCY160
RCY175-4c	*dun1-Δ100::HIS3 sml1::HIS3 TELXV$_L$::URA3*	RCY175

[a] All strains are isogenic with W303a (**a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100*), except for the markers indicated. The construction of the diploids is described in Materials and Methods.

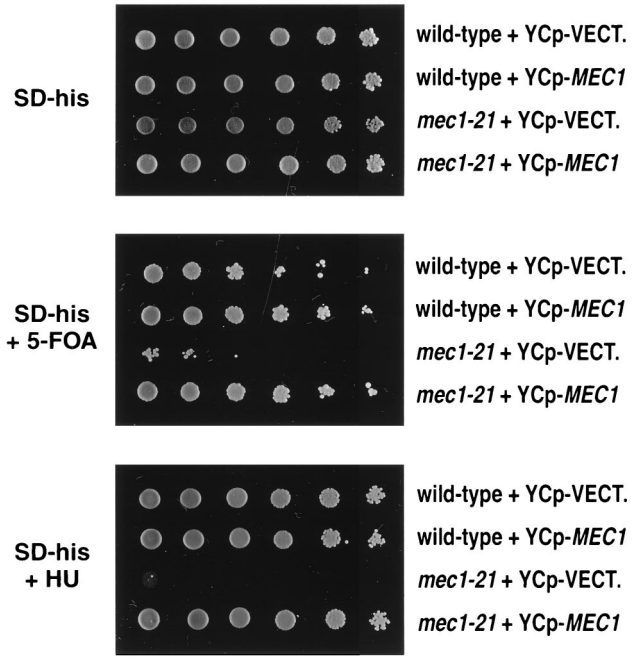

FIG. 2. Complementation of the *mec1-21* silencing defect by the centromere-containing *MEC1* plasmid. TPE was monitored by plating serial dilutions of various strains onto media lacking or containing 5-FOA; in some strains, sensitivity to HU, an inhibitor of ribonucleotide reductase, was also measured. Since the plasmids were marked with *HIS3*, the strains were grown in SD−his to force retention of the plasmid. All strains had the $TELXV_L$*::URA3* substitution. Top rows, RCY109-2b + pRS313 (YCp-VECT.); second rows, RCY109-2b + pRC4 (YCp-*MEC1*); third rows, RCY109-1c + pRS313; fourth rows, RCY109-1c + pRC4.

standard procedures. The transferred fragments were hybridized to a probe derived from the Y′ element located centromere distal to the *Pst*I site (30).

Genetic methods and silencing assays. Methods of transformation, sporulation, tetrad analysis, and medium preparation were standard (15). Telomeric silencing assays were done as described by Gottschling et al. (14). Cells were grown on standard rich growth medium (yeast extract-peptone-dextrose) for 2 days at 30°C. Individual colonies were resuspended in water, and serial dilutions of 1:10 ($TELVII_L\Delta$*::URA3* strains) or 1:5 ($TELVII_L$*::URA3* strains) were performed. Five microliters of each dilution was plated onto yeast extract-peptone-dextrose (YPD) and synthetic complete medium containing 1 mg of 5-fluoroorotate (5-FOA)/ml. In experiments involving strains with *HIS3*-containing plasmids, we used synthetic medium lacking histidine (SD−his). Sensitivity to hydroxyurea (HU) was monitored using medium containing 50 mM HU. To detect silencing of the *TRP1* gene integrated at *HML* (*hml::TRP1*), we streaked cells onto medium lacking tryptophan (29).

RESULTS

Loss of TPE in *mec1-21* strains. In order to assay silencing of genes near the telomere, we inserted *URA3* near the left end of chromosome XV or near the left telomere of chromosome VII (Fig. 1a and b). In strains with this insertion, inactivation of *URA3* as a consequence of TPE can be detected by plating the cells onto medium containing 5-FOA (14). To examine the effects of Mec1p on TPE, we used the *mec1-21* allele. This mutation results in a defect in the checkpoint pathway for DNA damage but, unlike null alleles of *MEC1*, is haploid viable (32). As shown in Fig. 1c, wild-type strains with *URA3* inserted at the telomere of either chromosome XV or chromosome VII had high levels of 5-FOA^r cells, indicating substantial TPE. Derivatives of these strains with the *mec1-21* mutation had greatly reduced levels of TPE. Thus, the silencing of gene expression at two different chromosomal telomeres is greatly reduced by the *mec1* mutation. We observed that the silencing of gene expression of a *URA3* insertion at the right telomere of chromosome V is also Mec1p dependent (data not shown).

The silencing defect conferred by the *mec1-21* mutation was complemented by the *CEN*-containing plasmid (YCp-*MEC1*; also called pRC4) that carries the wild-type *MEC1* gene (Fig. 2, middle panel). This plasmid also complements the sensitivity of *mec1* strains to the drug HU (Fig. 2, bottom panel). The phenotypes of the *mec1-21* strain were unaffected by transformation with the vector plasmid (YCp-VECT.; also called pRS313). Transformation of the wild-type strain with pRC4 resulted in a slight increase in telomere silencing (compare top two rows in Fig. 2, middle panel).

Although *MEC1* is required for telomeric silencing, the *mec1-21* mutation had no substantial effect on silencing at the *HML* locus. For this analysis, we used strains in which the *TRP1* gene was integrated at *HML* (29). In wild-type (LPY253) and *mec1-21* strains with this construction, expression of *TRP1* was efficiently silenced (Fig. 3). In a strain with the *rap1-17* mutation, shown previously to be defective in silencing at the telomere and at the silent mating-type loci (20), the same *TRP1* gene was efficiently expressed.

Loss of TPE in strains overexpressing Mec1p. Telomere silencing can be disrupted by the overproduction of several different classes of yeast gene products, including Sir4p and the RNA component of telomerase (34). We transformed the wild-type strain containing *TELXVL::URA3* with plasmid pRS423 (YEp-VECT.), pRC5 (YEp-*MEC1*), pRS313 (YCp-VECT.), or pRC4 (YCp-*MEC1*). The strain with pRC5 lost telomeric silencing, and silencing was slightly enhanced in the strain with pRC4 (Fig. 4, middle panel). Interestingly, although pRC5 caused a loss of TPE, this plasmid did not increase the sensitivity of the strain to HU (Fig. 4, bottom panel). This result argues that the defective telomeric silencing observed in *mec1* strains is unrelated to functions involved in the checkpoint defect associated with the *mec1* mutation.

Roles of other checkpoint proteins in TPE. In response to DNA damage or delays in DNA replication, yeast cells arrest the cell cycle and induce the transcription of several genes involved in DNA repair (11, 40), as discussed in the introduction. We examined the effects of various checkpoint genes

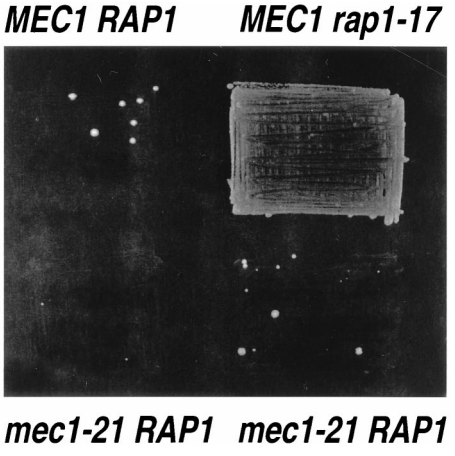

FIG. 3. Effects of *mec1-21* and *rap1-17* on silencing at *HML*. Four isogenic strains were constructed in which the *TRP1* gene was inserted at *HML*: LPY253 (*MEC1 RAP1* [29]), RCY112-5c and RCY112-13b (both strains are *mec1-21 RAP1*), and RCY124-2a (*MEC1 rap1-17*). These strains were then plated onto medium lacking tryptophan. The *rap1-17* mutation, but not the *mec1-21* mutation, resulted in a silencing defect.

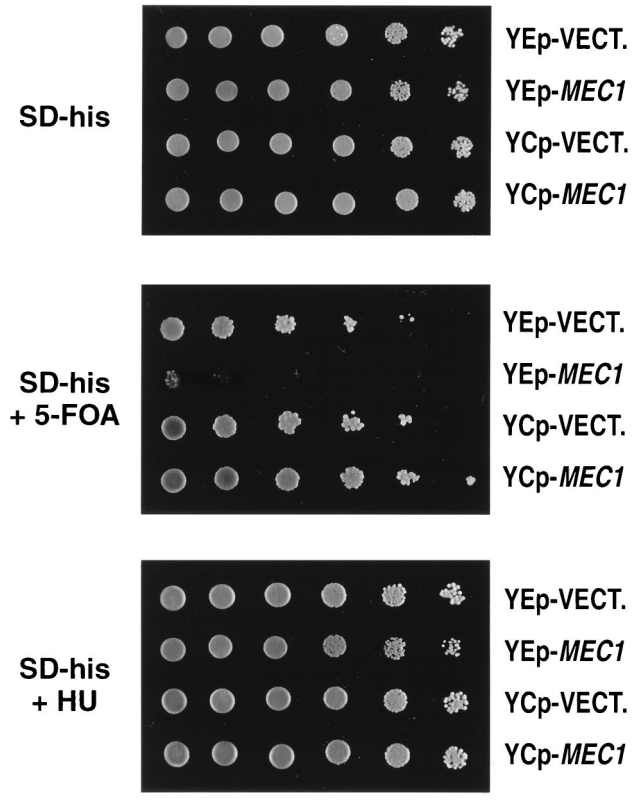

FIG. 4. Loss of TPE by overproduction of *MEC1*. TPE was monitored by plating serial dilutions of various strains onto media lacking or containing 5-FOA; in some strains, sensitivity to HU, an inhibitor of ribonucleotide reductase, was also measured. Since the strains contained plasmids marked with *HIS3*, the strains were grown in SD−his to force retention of the plasmid. All strains had the *TELXV$_L$::URA3* substitution and the wild-type *MEC1* gene. Top rows, RCY109-2b + pRS423 (YEp-VECT.); second rows, RCY109-2b + pRC5 (YEp-*MEC1*); third rows, RCY109-2b + pRS313 (YCp-VECT.); fourth rows, RCY109-2b + pRC4 (YCp-*MEC1*).

(*RAD9*, *RAD53*, *DUN1*, and *CHK1*) by crossing W303a-derived strains with mutations in these genes to an isogenic strain with the *TELXVL::URA3* substitution. From each cross, we monitored TPE in at least four pairs of wild-type and mutant spores. Figure 5a shows examples of the results. Wild-type TPE was observed for strains with *rad53-21* or null mutations of *CHK1* and *RAD9*. In contrast, spores with a null mutation of *DUN1* had greatly reduced TPE.

To determine the types of alterations in the hypomorphic *mec1-21* and *rad53-21* alleles, we PCR amplified and sequenced these alleles. The *rad53-21* allele had a G-to-A alteration at position 1093, resulting in an E365K substitution. This alteration is within the kinase domain of the protein. The *mec1-21* allele contained a change of G to A at position 2644 (G882S) (J. Mallory and T. Petes, unpublished data).

We also examined the effects of two additional hypomorphic alleles of *RAD53*, *rad53-1* and *rad53-17* (provided by X. Zhao and R. Rothstein). Diploids heterozygous for *rad53-1* (RCY203) or *rad53-17* (RCY204) and the *TELXVL::URA3* substitution were sporulated and dissected. Neither *rad53-1* nor *rad53-17* affected telomeric silencing, confirming our observations with *rad53-21* (data not shown). Since all of the *rad53* alleles examined in our study are hypomorphic, we cannot exclude the possibility that other *rad53* mutant alleles might affect TPE.

Since double-mutant *rad53 chk1* strains are more sensitive to DNA-damaging agents than either single-mutant strain (31), we also examined telomeric silencing in the double-mutant strains. Such strains were constructed by sporulating a diploid (RCY126) that was heterozygous for *rad53-21*, *chk1*, and *TELXVL::URA3*. No telomere silencing defect was observed in the *rad53-21 chk1* spores (data not shown).

Epistasis analysis of *mec1* and other mutations affecting checkpoints. In response to DNA damage, Rad9p exhibits Mec1p-dependent phosphorylation (12, 38), as discussed above. Rad9p is also required for the translocation of the silencing proteins Sir3p and Rap1p from the telomere following DNA damage (24, 26, 28). We found that *mec1-21 rad9* double-mutant strains had the same silencing defect as the *mec1-21* single-mutant strains (Fig. 5b).

We attempted to construct haploid strains with both *mec1-21* and *dun1Δ-100* mutations by sporulating a diploid strain (RCY155) that was heterozygous for both mutations. Of 18

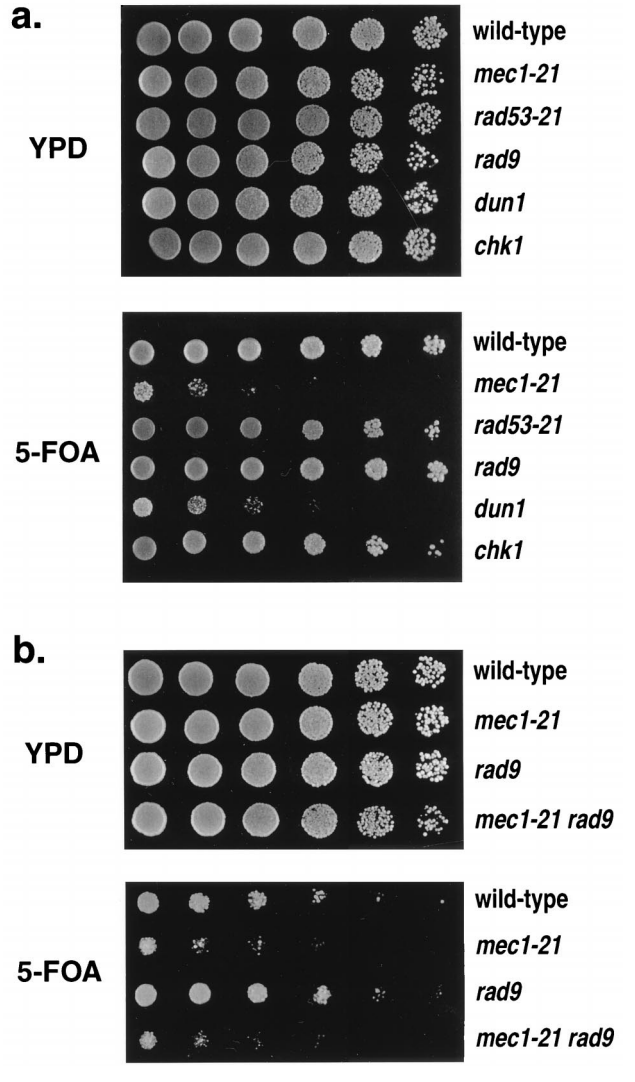

FIG. 5. Effects of *mec1-21*, *rad53-21*, *rad9*, *dun1*, *chk1*, and *sml1* on TPE. As for Fig. 1, TPE was monitored in a series of isogenic strains with *TELXV$_L$::URA3*. (a) TPE in strains with single mutations affecting checkpoints. Top rows, RCY109-2b; second rows, RCY109-1c; third rows, RCY126-3c; fourth rows, RCY143-2c; fifth rows, RCY144-4b; sixth rows, RCY126-1d. (b) Epistasis analysis of *mec1-21* and *rad9* mutations. Top rows, RCY160-4d; second rows, RCY160-4b; third rows, RCY160-1b; fourth rows, RCY160-3a.

tetrads examined, the numbers with four, three, and two viable spores were 3, 9, and 6, respectively. Of the spores analyzed, 19 were wild type, 16 were *mec1-21*, 16 were *dun1*Δ*-100*, and none contained the double mutation. Thus, *mec1-21* and *dun1*Δ*-100* are synthetically lethal. We also analyzed interactions between the *mec3*Δ mutation, which causes an increase in telomere length and telomeric silencing (7), and *mec1-21*. A diploid heterozygous for these mutations (RCY161) was sporulated and dissected. Of 15 tetrads examined, the numbers with four, three, and two viable spores were 1, 12, and 2, respectively. Of the spores analyzed, 20 were wild type, 12 were *mec1-21*, 12 were *mec3*Δ, and none contained the double mutation.

Although *mec1-21* strains are haploid viable, null mutations of *MEC1* result in haploid lethality. This lethality is suppressed in strains with an *sml1* mutation (42) or in strains overexpressing *RNR1* (10); both of these alterations are likely to result in elevated nucleotide pools. As shown in Fig. 6a, although the *sml1* mutation has no obvious effect on TPE in otherwise wild-type strains, this mutation suppressed the silencing defects in both the *mec1-21* and the *dun1*Δ*-100* strains. In addition, overexpression of *RNR1* suppressed the silencing defects of both strains (Fig. 6b).

The silencing defect caused by *mec1-21* is not reverted by increased telomere length. Cells harboring the *mec1-21* mutation have slightly shortened telomeres, which elongate to wild-type lengths in the presence of the *sml1* mutation (30). Telomere tract shortening is frequently correlated with loss of telomeric silencing, while elongated telomeres cause silencing to increase (20). Thus, it is possible that the loss of telomeric silencing in *mec1-21* cells and the restoration of silencing in *mec1-21 sml1* cells reflect changes in telomere length. To test this possibility, we constructed a strain (RCY106-11b) containing both the *mec1-21* and *rif1* mutations. Cells with *rif1* mutations have increased levels of telomeric silencing and elongated telomeres (16, 20, 41). We found that strains with both *mec1-21* and *rif1* mutations had the same deficiency in TPE as that observed in *mec1-21* strains (Fig. 7a). Telomeres in the double-mutant strain were longer than those in wild-type strains but shorter than those in *rif1* strains (Fig. 7b). We conclude that telomeric tract size is not the sole determinant of silencing in *mec1-21* cells.

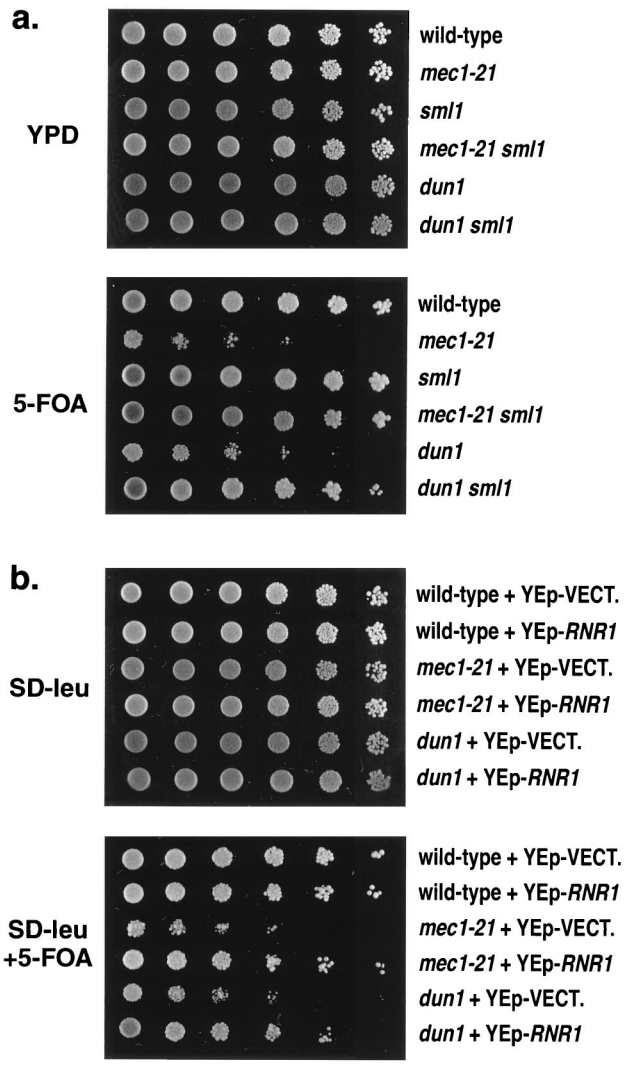

FIG. 6. Effects of *sml1* and overproduction of *RNR1* on TPE in *mec1-21* and *dun1* strains. As for Fig. 1, TPE was monitored in a series of isogenic strains with $TELXV_L$*::URA3*. (a) Effect of *sml1* on TPE. Top rows, RCY109-2b; second rows, RCY109-1c; third rows, RCY109-1b; fourth rows, RCY109-3a; fifth rows, RCY144-4b; sixth rows, RCY175-4c. (b) Effect of overproduction of *RNR1* on TPE. Wild-type (RCY109-2b), *mec1-21* (RCY109-1c), and *dun1* (RCY144-44b) strains were transformed with either a high-copy-number vector plasmid (YEplac181 [YEp-VECT.]) or a high-copy-number plasmid containing the *RNR1* gene (pRC11 [YEp-*RNR1*]).

DISCUSSION

Our main conclusions are that (i) reduction or overexpression of Mec1p results in decreased telomeric silencing; (ii) the *mec1-21* mutation does not substantially affect silencing at the *HML* locus; (iii) *dun1* strains, but not strains with the *rad53*, *chk1*, or *rad9* mutation, have a defect in TPE; (iv) the defects in TPE observed in *mec1-21* and *dun1* strains are alleviated by the *sml1* mutation or overexpression of *RNR1*; and (v) the decreased telomeric silencing observed in *mec1-21* strains is not a direct consequence of decreased telomere length.

Strains with mutations in most of the checkpoint genes have no obvious phenotype in the absence of DNA damage. Since null mutations of *MEC1* and *RAD53* are haploid lethal, these two genes are an exception to this generalization. It is likely that null mutations of *MEC1* and *RAD53* result in low nucleotide pools, since both *MEC1* and *RAD53* are required to activate Dun1p, and Dun1p is a positive activator of transcription of the *RNR* genes (11). The viability of strains with null mutations in *MEC1* can be rescued by mutations in the *sml1* gene (42), by overexpression of *RNR1* (encoding ribonucleotide reductase) (10), and by mutations in the cyclin genes *CLN1* and *CLN2* (37). Strains with the *sml1* mutation have elevated nucleotide pools (42), and it is likely that strains in which Rnr1p is elevated or Cln1 and Cln2 are reduced also have elevated nucleotide pools. Overproduction of *RNR1* also suppresses the lethal effects of a null mutation in *RAD53* (10).

The reasons for the lethality of null mutations of *RAD53* and *MEC1* and the suppression of this lethality by elevated nucleotide pools are not clear. One possibility is that strains with null *mec1* and *rad53* mutations die as a consequence of elevated levels of DNA damage (reflecting attempts to replicate DNA with low nucleotide pools) coupled with a defect in the DNA damage-sensitive checkpoint. Alternatively, the lethality observed in *mec1* and *rad53* strains may reflect an inability to complete chromosome replication (10, 42).

Why should the *mec1-21* mutation lead to the loss of telomeric silencing? The most straightforward explanation of the loss of silencing is that one or more of the telomere-associated

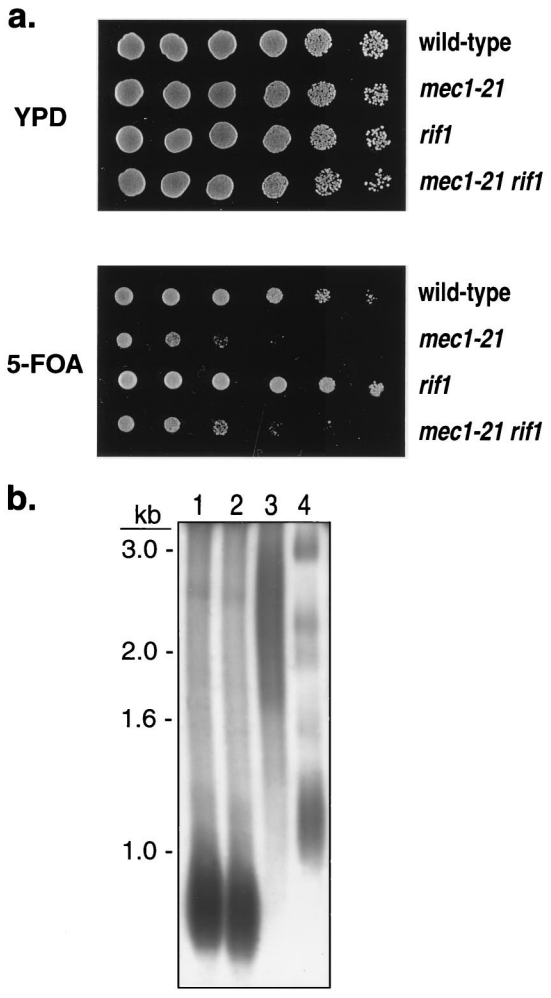

FIG. 7. Effect of telomere length on TPE. As for Fig. 1, TPE was monitored in a series of isogenic strains with *TELXV$_L$::URA3*. (a) TPE in wild-type, *mec1-21*, *rif1*, and *mec1-21 rif1* strains. Top rows, RCY106-4d; second rows, RCY106-3d; third rows, RCY106-1d; fourth rows, RCY106-14a. (b) Telomere lengths in wild-type, *mec1-21*, *rif1*, and *mec1-21 rif1* strains. Purified genomic DNA was digested with *Pst*I and examined by Southern analysis using a hybridization probe derived from the Y′ subtelomeric repeats (30). The positions of size standards are shown on the right. Lane 1, RCY106-4d (wild type); lane 2, RCY106-3d (*mec1-21*); lane 3, RCY106-1d (*rif1*); lane 4, RCY106-11b (*mec1-21 rif1*).

proteins involved in silencing (Sir2-4p, the Ku proteins, or Rap1p [2, 4, 20]) are depleted from the telomeres in *mec1-21* strains. We will consider two models to explain this loss.

The first model is that Mec1p, acting as part of a protein complex, directly affects telomeric silencing by regulating the structure of the telomere. If Mec1p is part of a complex, then either mutations of *MEC1* or overexpression of Mec1p could disrupt the function of this complex, leading to the loss of TPE; dominant negative effects caused by an overexpression of one component of a protein complex in yeast are quite common (15). The simplest form of this model is that the Mec1p complex phosphorylates one or more of the silencing proteins and that this phosphorylation is necessary for the telomeric silencing activity of these proteins. It should be pointed out that although the *mec1-21* mutation is not located in the C-terminal putative kinase region, mutant substitutions located outside of the C-terminal region eliminate the kinase activity of the related Rad3 protein of *S. pombe* (5). An alternative form of this model is that the Mec1p complex acts to control the accessibility of telomeric DNA to the silencing proteins. In our previous study of the effects of Tel1p and Mec1p on telomere length, we suggested that Mec1p might affect the accessibility of the telomeric DNA to enzymes involved in telomere length regulation (30). This same activity could affect the binding of the silencing proteins.

One observation that is not explained by the simplest form of this model is that the elevation of nucleotide pools eliminates the telomeric silencing defect of *mec1-21* strains. One possibility is that low levels of nucleotides in wild-type cells might affect the stability or enzymatic activity of the mutant Mec1-21p complex. Alternatively, low levels of nucleotides could affect the stability or function of the silencing proteins. At present, it is unclear whether the restoration of telomeric silencing caused by elevating nucleotide pools is directly related to the original defect or is caused by the superimposition of a different type of mechanism.

An alternative model is based on the observation that in yeast strains with double-stranded DNA breaks, several silencing proteins (Sir3p, Sir4p, yKu80p, and Rap1p) become redistributed from the telomeres to the sites of these DNA breaks (24, 26, 28). This redistribution is associated with the loss of telomeric silencing (26). One interpretation of our results is that a low level of DNA damage occurring in the *mec1-21* and *dun1* strains results in a redistribution of silencing proteins from the telomere, resulting in decreased TPE. Elevation of nucleotide pools by the *sml1* mutation or by the overproduction of *RNR1* would be expected to reduce the level of damage in *mec1-21* strains, allowing the restoration of telomeric silencing. One argument against this model is that Mills et al. (28) and Martin et al. (24) reported that Mec1p and Rad9p were required for the redistribution of Sir3p. Although the protein encoded by *mec1-21* could still be proficient in directing the redistribution of Sir3p, we found that a null mutation of *RAD9* did not affect the silencing defect of *mec1-21* (Fig. 5b). For this reason, we favor the first model. Finally, we point out that although the discussion above emphasizes the role of Mec1p in telomeric silencing, similar models could be proposed for the effects of the *dun1* mutation.

Other studies support the conclusion that the pathways of telomeric silencing and DNA damage repair have functional overlaps. For example, the proteins yKu70p, Sir3p, and Mec3p affect both telomeric silencing and the repair of DNA damage (4, 7). Furthermore, the roles of Mec1p in telomere length regulation and telomeric silencing are evolutionarily conserved, because mutations of the *rad3*$^+$ gene of *S. pombe*, a *MEC1* homologue, result in short telomeres and the loss of TPE (9, 25). Thus, in two very different yeast species, similar proteins have multiple roles in checkpoint function, telomere length regulation, and TPE.

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