N Saccharomyces cerevisiae, Mre11p, Rad50p, and Xrs2p form a complex (Johzuka and Ogawa 1995; U sui et al. 1998), which we term the “MRX complex,” required for several types of DNA repair and recombination (Kannar and Hoejmakers 1997; Haber 1998). Null mutations in MRE11, RAD50, or XR2 lead to: (1) sensitivity to DNA-damaging agents, reflecting failure to repair double-stranded DNA breaks (DSBs) by homologous or nonhomologous recombination; (2) slow growth; (3) short telomeres; (4) defective meiotic DSB formation; and (5) elevated levels of spontaneous mitotic recombination (H aber 1998).

Although the exact biochemical role of the MRX complex is not clear, human Mre11p has nuclease activity that is increased by the addition of hRad50p (Paul l and Gellert 1998); pRad50p exhibits ATP-dependent binding to DNA (Raymond and K leckner 1993). Addition of hNbs1p (the probable human functional equivalent of Xrs2p) to the hMre11p/hRad50p complex results in several activities (unwinding of the DNA duplex and cleavage of fully paired hairpins) not observed in the absence of hNbs1p (Paul l and Gellert 1999).

Mutations in the TEL1 gene shorten telomeres, but do not result in a senescent phenotype (Lustig and Pet es 1986). The closest structural homologue of TEL1 is the human ATM gene, which is mutated in patients with the disease ataxia telangiectasia (Green well et al. 1995; Mormor et al. 1995). One conserved region between Tel1p and ATM is a C-terminal domain required for ATM kinase activity (Khanna et al. 1998). Although there is no direct evidence that Tel1p is a kinase, mutations of this region result in short telomeres (Green well et al. 1995), and Tel1p-dependent phosphorylation of Rad53p, RPA, and Rad9p in response to DNA damage has been observed (Brush et al. 1996; Sanchez et al. 1996; Emili 1998). The closest homologue of Tel1p in the S. cerevisiae genome is Mec1p (Green well et al. 1995; Mormor et al. 1995). Strains with a mec1 mutation are sensitive to DNA-damaging agents (Weinert 1998) and have slightly shortened telomeres (Ritchie et al. 1999).

One method of attempting to define the functions of the various genes affecting telomere length is epistasis analysis, comparison of the phenotype of doubly mutant strains to the individual single mutations. By this type of analysis, Tel1p and Yku70p function in separate pathways (Porter et al. 1996), as do Tel1p and Mec1p (Ritchie et al. 1999).

To examine genetic interactions between TEL1 and the genes encoding the MRX complex, we constructed diploids heterozygous for the td1 mutation and rad50 (KRY274), mre11 (KRY277), or xrs2 (KRY282) (Table 1). These strains were sporulated and the resulting tetrads were dissected. Since mutations affecting telomere length often exhibit phenotypic lag (Lustig and Pet es 1986), we examined telomere lengths after subculturing the haploid strains derived from the spores. As shown in Figure 1, a–c, single mutations in TEL1, RAD50, MRE11, or XRS2 all shorten telomeres to the same extent, as expected from previous studies (Kironmai and Muniyappa 1999; Boul ton and Jackson 1998). Strains with the td1 mre11, td1 rad50, or td1 xrs2 genotypes have telomeres of the same length as observed for strains with the single mutations. This result indicates that all four genes are involved in a single pathway of telomere length regulation.

Strains with mre11, rad50, or xrs2 mutations have sub-
After repeated subculturings, fast-growing “survivors” than cells of either single-mutant strain (data not shown). These results demonstrate that RAD50 (like TEL1) functions in a different pathway of telomere length regulation than YKU70, supporting the previous conclusion of Nugent et al. (1998).

**TABLE 1**

| Strain name | Strain construction or reference | Relevant genotype
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>W303a</td>
<td>Thomas and Rothstein (1989)</td>
<td>Wild-type, a mating type</td>
</tr>
<tr>
<td>W303x</td>
<td>Thomas and Rothstein (1989)</td>
<td>Wild-type, α mating type</td>
</tr>
<tr>
<td>W303</td>
<td>Cross of W303a and W303x</td>
<td>Wild-type diploid</td>
</tr>
<tr>
<td>W303aU</td>
<td>Porter et al. (1996)</td>
<td>α tel1::URA3</td>
</tr>
<tr>
<td>SPY40</td>
<td>Porter et al. (1996)</td>
<td>α tel1::URA3</td>
</tr>
<tr>
<td>Y604</td>
<td>Provided by Y. Sanchez and S. Elledge</td>
<td>α mec1-21</td>
</tr>
<tr>
<td>KRY77</td>
<td>Spore derivative of KRY254</td>
<td>α rad50::hisG</td>
</tr>
<tr>
<td>KRY78</td>
<td>Spore derivative of KRY254</td>
<td>α xrs2::kanMX</td>
</tr>
<tr>
<td>KRY88</td>
<td>W303α transformed with mre11::kanMX cassette</td>
<td>α mre1::kanMX</td>
</tr>
<tr>
<td>KRY97</td>
<td>W303α transformed with xrs2::kanMX cassette</td>
<td>α xrs2::kanMX</td>
</tr>
<tr>
<td>KRY254</td>
<td>Transformation of W303 with BglII- and EcoRI-digested pNKY83 b, followed by isolation of 5FOA a derivative</td>
<td>α/α rad50::hisG/ RAD50</td>
</tr>
<tr>
<td>KRY272</td>
<td>Cross of KRY78 and W303aU</td>
<td>α/α yku70::URA3/ YKU70 rad50::hisG/ RAD50</td>
</tr>
<tr>
<td>KRY274</td>
<td>Cross of KRY78 and SPY40</td>
<td>α/α tel1::URA3/ TEL1 rad50::hisG/ RAD50</td>
</tr>
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<td>KRY275</td>
<td>Cross of KRY78 and Y604</td>
<td>α/α mre1-21/ MEC1 rad50::hisG/ RAD50</td>
</tr>
<tr>
<td>KRY277</td>
<td>Cross of KRY88 and SPY40</td>
<td>α/α mre1::kanMX/ MRE11 tel1::URA3/ TEL1</td>
</tr>
<tr>
<td>KRY278</td>
<td>Cross of KRY88 and Y604</td>
<td>α/α mre1::kanMX/ MRE11 mec1-21/ MEC1</td>
</tr>
<tr>
<td>KRY282</td>
<td>Cross of KRY97 and SPY40</td>
<td>α/α xrs2::kanMX/ XRS2 tel1::URA3/ TEL1</td>
</tr>
<tr>
<td>KRY283</td>
<td>Cross of KRY97 and Y604</td>
<td>α/α xrs2::kanMX/ XRS2 mec1-21/ MEC1</td>
</tr>
</tbody>
</table>

* All strains in the study are isogenic (except for changes introduced by transformation) with W303a (a leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100) (Thomas and Rothstein 1989).

* The MRE11 gene was disrupted with the kanMX cassette by using the PCR-based method described by Wach et al. (1994). The oligonucleotide sequences were 5’ GACAAATTGACGAGTTGTCAGCAGTTCCGAGATAAATCGACTCGTCACGTACGGAGTGCAG and 5’ GTTATATTAGTATATAATATATAGGGATCAAGTAACATGGAATTTGAGAAGCTCGACTCGTCAGGCTGAC and 5’ GCACAAATTTAATATAGGAAATTGGAATTTGAGAAGCTCGACTCGTCAGGCTGAC and 5’ GCACAAATTTAATATAGGAAATTGGAATTTGAGAAGCTCGACTCGTCAGGCTGAC.

**a** Alani et al. (1989).

**b** The MRX complex functions in a different pathway of telomere length regulation than those observed for the single mutant mre1, rad50, and xrs2 strains (Figure 1d). In addition, plating efficiencies (relative to a normalized value of 100% for wild type) were similar for rad50 (64% with 95% confidence limits ±5%) and tel1 rad50 (67% ± 7%) strains; tel1 strains had approximately the same plating efficiency as wild type (96% ± 5%).

We previously showed that tel1 mec1 strains had a senescent phenotype and telomeres that were slightly shorter than those of the tel1 single-mutant strains (Ritchie et al. 1999). We constructed diploids that were heterozygous for mec1-21 and rad50 (KRY275), mre11 (KRY278), or xrs2 (KRY283). We found that spores with the mec1-21 rad50, mec1-21 mre11, or mec1-21 xrs2 genotypes had a senescence phenotype that was indistinguishable from the tel1 mec1-21 spores analyzed previously (Figure 2). After repeated subculturings, fast-growing “survivors” appeared in the mec1-21 rad50 cultures; survivors also occur in telomerase-defective (Lundblad and Blackburn 1993) or tel1 mec1-21 (Ritchie et al. 1999) strains. We also examined telomere lengths in wild-type, rad50, mec1-21, and mec1-21 rad50 derivatives of KRY275. After 25 cell generations, the telomeres in the mec1-21 rad50 strains were shorter than those in the rad50 strain (data not shown). We conclude that the MRX complex functions in a different pathway from Mec1p, consistent with our previous conclusion that Tel1p and Mec1p represent different pathways.

Since the Tel1p and MRX complex are in a single pathway, but Tel1p and Yku70p are in separate pathways (Porter et al. 1996), Rad50p and Yku70p should function in separate pathways. In previous epistasis studies of the relationship between rad50 and yku70, different conclusions were reached by different groups (Boulton and Jackson 1998; Nugent et al. 1998). We reexamined this issue by analyzing telomere lengths in spore cultures derived from a diploid (KRY272) that was heterozygous for rad50 and yku70 mutations. Telomere lengths in the double-mutant strain were shorter than those in either single mutant (Figure 3). In addition, spores of the double-mutant genotype grew more slowly than cells of either single-mutant strain (data not shown).
Figure 1.—Telomere lengths and growth rates in wild-type, tel1, rad50, mre11, xrs2, tel1 rad50, tel1 mre11, and tel1 xrs2 strains. Diploids heterozygous for tel1 and rad50, mre11, or xrs2 were sporulated and dissected. Spore cultures were vegetatively subcloned four times (~80 cell divisions) on rich growth medium (YPD) at 30°C. Genomic DNA was isolated from each strain and treated with PstI. The resulting fragments were examined by Southern analysis, using a telomere-specific probe (Ritchie et al. 1999). The diffuse band below the 1-kb marker represents telomeric sequences, whereas the two bands at 3.5 and 4.8 kb represent tandem subtelomeric Y′ elements (Ritchie et al. 1999). Growth rates were qualitatively examined by streaking strains of various genotypes on rich growth medium (YPD), followed by growth at 30°C for 2 days. All strains derived from spores were subcultured at least four times before analysis. Strain names and genotypes are as follows: (a) KRY274-3a (wild type), KRY274-3b (tel1), KRY274-3c (tel1 rad50), and KRY274-3d (rad50); (b) KRY277-2a (wild type), KRY277-2b (mre11), KRY277-2c (tel1 mre11), and KRY277-2d (tel1); (c) KRY282-3a (wild type), KRY282-3b (tel1), KRY282-3c (xrs2), and KRY282-3d (tel1 xrs2); and (d) W303a (wild type), SPY40 (tel1), KRY77 (rad50), KRY274-3c (tel1 rad50), KRY88 (mre11), KRY277-2c (tel1 mre11), KRY97 (xrs2), and KRY282-3d (tel1 xrs2).

Figure 2.—Growth rates in wild-type, mec1-21, rad50, and mec1-21 rad50 strains. The diploid strain KRY275 is heterozygous for mec1-21 and rad50. This strain was sporulated and dissected. After spore colonies had formed, they were subcloned by repeated streaking (nine times) on YPD medium (Ritchie et al. 1999). The strain names and genotypes are as follows: KRY275-11a (wild type), KRY275-11b (mec1-21), KRY275-11c (mec1-21 rad50), and KRY275-11d (rad50). Plates from subclonings (SC) 1, 2, 4, and 9 were photographed. The mec1-21 rad50 strain underwent senescence, with very poor growth by SC4. Survivors were generated by SC9. It should be noted that plates were usually incubated for 30°C for 3 days before they were photographed. Under these conditions, the slower growth rate of rad50 strains relative to wild-type strains is subtle.

What is the function of Tel1p and the MRX proteins in regulating telomere length? One obvious possibility is that these proteins directly activate telomerase catalytic activity. An argument against this possibility is that tel1 tlc1 strains and rad50 tlc1 strains have synthetic phenotypes different from those of the single mutants: tel1 tlc1 strains senesce more slowly than tlc1 strains (Ritchie et al. 1999), and rad50 tlc1 strains accumulate postsenescence survivors more slowly than tlc1 strains (Le et al. 1999). Thus, as described below, we prefer models in
which the Tel1p and the MRX proteins promote the activity of telomerase indirectly.

Since Tel1p has kinase motifs, one model is that Tel1p is required to phosphorylate one or more proteins of the MRX complex and that this phosphorylation is required for the role of the complex in telomere elongation. The role of the complex could be to "open" the telomere chromatin, allowing telomerase to interact with telomeric DNA. A related possibility is that the single-stranded poly G₃T telomeric sequences could form a hairpin-like structure, and cleavage of this structure by a Tel1p-dependent phosphorylated MRX complex could increase accessibility of telomeric DNA to telomerase. Two further points should be mentioned. First, Tel1p and the MRX complex could affect accessibility of the telomere to cellular exonucleases as well as telomerase. Thus, in the absence of telomerase, tel1 strains might have delayed senescence relative to strains with only a telomerase mutation (Ritchie et al. 1999). Second, since tel1 mutants do not exhibit the growth deficiency or DNA repair defects shared by mre11, rad50, and xrs2 strains, lack of phosphorylation by Tel1p does not affect all of the functions of the MRX complex.

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LITERATURE CITED
Le, S., J. K. Moore, J. E. Haber and C. W. Greider, 1999 RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. Genetics 152: 143-152.
Nugent, C. I., G. Bosco, L. O. Ross, S. K. Evans, A. P. Salinger...


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