DOI: 10.1534/genetics.107.086603

High Rates of "Unselected" Aneuploidy and Chromosome Rearrangements in *tel1 mec1* Haploid Yeast Strains

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Manuscript received January 2, 2008
Accepted for publication March 3, 2008

ABSTRACT

The yeast *TEL1* and *MEC1* genes (homologous to the mammalian *ATM* and *ATR* genes, respectively) serve partially redundant roles in the detection of DNA damage and in the regulation of telomere length. Haploid yeast *tel1 mec1* strains were subcultured nonselectively for ~200 cell divisions. The subcultured strains had very high rates of chromosome aberrations: duplications, deletions, and translocations. The breakpoints of the rearranged chromosomes were within retrotransposons (Ty or δ-repeats), and these chromosome aberrations nonrandomly involved chromosome III. In addition, we showed that strains with the hypomorphic *mec1-21* allele often became disomic for chromosome VIII. This property of the *mec1-21* strains is suppressed by a plasmid containing the *DNA2* gene (located on chromosome VIII) that encodes an essential nuclease/helicase involved in DNA replication and DNA repair.

IN the yeast *Saccharomyces cerevisiae*, as in other eukaryotes, the rates of mutation and changes in chromosome structure in wild-type strains are very low. The rate of deletion of the *CAN1* gene located near the end of chromosome V in a wild-type haploid strain is $\sim 4 \times 10^{-10}$/division (Myung *et al.* 2001). This low deletion rate is dramatically elevated in certain genetic backgrounds. In strains with mutations in both *TEL1* (related to the mammalian ATM gene) and *MEC1* (related to the mammalian ATR gene), this deletion rate is $\sim 10^{-5}$/division, an increase of more than four orders of magnitude (Myung *et al.* 2001; Craven *et al.* 2002). In addition, *tel1 mec1* strains have very elevated rates of chromosome loss and mitotic recombination (Craven *et al.* 2002). As discussed below, the synergistic effect of the two mutations on genome stability is likely to reflect their shared role in two processes: DNA damage checkpoints and telomere length regulation.

The *TEL1* gene was first defined as a mutant that had unusually short poly($G_{1-3}T$) telomeric tracts (Lustig and Petes 1986). *TEL1* encodes a very large kinase that is related to the human ATM gene (the gene mutated in patients with ataxia telangiectasia) and to the yeast *MEC1* gene described below (Greenwell *et al.* 1995; Morrow *et al.* 1995). Two recent articles demonstrate that Tel1p is involved in recruiting telomerase to the telomeres. Goudsouzian *et al.* (2006) showed that recruitment of Est2p (the protein subunit of telomerase) and Est1p (a telomerase cofactor) is substantially reduced in a *tel1* mutant. In addition, Tseng *et al.* (2006) demonstrated that Tel1p and Mec1p phosphorylate Cdc13p, a telomere-binding protein that interacts with Est1p. Thus, it is likely that Tel1p- and Mec1p-dependent phosphorylation of Cdc13p aids in the recruitment of telomerase and cofactors to the chromosome ends.

Although *tel1* strains have the same sensitivity to most DNA-damaging agents as wild-type strains, Usui *et al.* (2001) showed that Tel1p was involved in the repair of unprocessed double-strand DNA breaks (DSBs). In addition, the DNA damage-induced phosphorylation of various proteins involved in the DNA damage checkpoint is substantially reduced in a *tel1* strain (D'Amours and Jackson 2001; Usui *et al.* 2001). In summary, Tel1p has an important role in the regulation of telomere length and a modest role in the DNA damage checkpoint resulting from unprocessed DSBs.

In contrast to Tel1p, Mec1p has a minor role in telomere length regulation (Ritchie *et al.* 1999) and a major role in the DNA damage checkpoint (Harrison and Haber 2006). Strains with a *mec1* mutation are sensitive to both DNA-damaging agents and drugs that block DNA synthesis because the strains lack multiple checkpoint pathways. Mec1p, like Tel1p, is a large kinase and the phosphorylation of many proteins in the DNA damage checkpoint pathways is Mec1p dependent (Harrison and Haber 2006). Overproduction of Tel1p can alleviate some of the sensitivity of *mec1* strains to DNA-damaging agents (Morrow *et al.* 1995). Unlike *TEL1*, *MEC1* is an essential gene, but double-mutant *mec1 sml1* strains are viable (Zhao *et al.* 1998); the *sml1* mutation

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results in elevated dNTP pools. Desany *et al.* (1998) showed that the lethality associated with the *mec1* mutation reflects an inability of cells to complete DNA replication. Consistent with this conclusion, Cha and Kleckner (2002) showed that *mec1* strains accumulated broken chromosomes with the DSBs occurring in regions in which DNA replication forks were moving slowly. Presumably as a consequence of elevated levels of DSB formation and inefficient DNA damage checkpoints, *mec1* strains have increased rates of chromosome loss and mitotic recombination (Klein 2001; Craven *et al.* 2002).

As a generalization, the genome-destabilizing properties of the single *tel1* and *mec1* single mutations are very greatly increased in strains with the double mutation. As discussed above, the double-mutant strain has a greatly elevated rate of loss of the *CAN1* gene that is located ~32 kb from the end of chromosome V (Myung *et al.* 2001; Craven *et al.* 2002). In most of these *can1* strains, the deletion of *CAN1* includes all centromere-distal sequences and the remaining portion of chromosome V is fused to other chromosomal sequences by nonhomologous end joining (NHEJ) (Myung *et al.* 2001; Craven *et al.* 2002). In addition to fusions between different chromosomal sequences by NHEJ, which presumably requires two DSBs, the GCR assay used in the Kolodner lab also detects terminal deletions with telomere additions. Such events presumably require only a single DSB. Terminal deletions with telomere additions are not detected in the *tel1 mec1 sml1* background (Myung *et al.* 2001).

As described above, *tel1* strains have very short, but stable telomeres, and *mec1* strains have telomeres that are only slightly shorter than those of wild-type strains. In strains of the *tel1 mec1* genotype, the telomeres are unstable. Although most cells with this genotype die, a fraction survive as a consequence of recombination-dependent amplification of telomeric or subtelomeric repeats (Ritchie *et al.* 1999). In addition, high levels of telomere–telomere fusions occur in *tel1 mec1* strains, but not in either single-mutant strain (Mieczkowski *et al.* 2003).

The *tel1 mec1* strains are also more sensitive to DNA-damaging agents than either single mutant (Morrow *et al.* 1995), as a consequence of complete loss of the DNA damage checkpoint. Since some proteins involved in the DNA damage checkpoint response (for example, Rad9p and Ies4p; Emili 1998; Morrison *et al.* 2007) are substrates for both the Tel1p and the Mec1p kinases, this increased sensitivity to DNA damage is expected. In addition, Tel1p contributes to the processing of broken DNA ends that affects the efficiency of the Mec1p-dependent checkpoint response (Mantiero *et al.* 2007).

Because of the association between very high levels of genome instability and the formation of solid tumors (Lengauer *et al.* 1998), it is important to understand the nature of the chromosome rearrangements in strains with genome-destabilizing mutations. Below, we describe the use of DNA microarrays and other methods to look for chromosome rearrangements in subcultured *tel1 mec1* yeast strains in which the only selection was for cell viability. We found a very high rate of chromosome alterations. Many of these rearrangements occurred on chromosome III and involved homologous recombination between nonallelic Ty elements. Ectopic recombination between nonallelic Ty elements has been observed previously in a number of studies (Dunham *et al.* 2002; Umezu *et al.* 2002; Lemoine *et al.* 2005; Mieczkowski *et al.* 2006); Umezu *et al.* (2002) also observed ectopic recombination between the *MAT* locus and *HMR*, a rearrangement also detected in our study. In addition, we found that haploid strains of the *mec1-21* genotype develop chromosome VIII disomy. This property is related to the gene dosage of *DNA2*, a gene encoding a DNA replication-associated nuclease/helicase located on chromosome VIII.

MATERIALS AND METHODS

Strain and plasmid constructions: All strains used in this study were isogenic with W303a (Thomas and Rothstein 1989), except for alterations introduced by transformation or by crosses with isogenic strains. The progenitor W303a strain has the markers *a leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100 rad5-535*). Details of the construction of derivatives of this strain are described in supplemental materials.

In some of the haploid strains, we introduced the hypomorphic *mec1-21* allele by transformation with the plasmid pMD92. This plasmid was derived from the *URA3*-containing integrating plasmid YIp5. We used two primers (MEC1-21F 5′ GACACTATAGAGATCTTTGCTAAGATTATGTGTGATG and MEC1-21R 5′ TTCAGACAGGAGATCTACCTAATTCAGGCT TGCCTAC) to amplify an ~800-bp fragment containing the *mec1-21* mutant substitution (G to A at +2644 in the *MEC1* gene; Mallory and Petes 2000); DNA isolated from the *mec1-21*-containing yeast strain JMY303-1d (a spore derived from JMY303; Mallory and Petes 2000) was used as a substrate for the PCR amplification. This fragment was treated with *Bgl*II and inserted within the *Bam*HI site of YIp5 to generate pMD92. The restriction enzyme *Bsr*GI was used to linearize the plasmid for transformation. The *mec1-21* mutation was introduced into various strains by the standard two-step transplacement procedure (details in supplemental materials).

Genetic methods: Standard media and genetic procedures were employed. For most of the experiments, each strain was subcultured on solid rich growth medium (YPD) 10 times. Strains were grown at 30° for 2–3 days for each subculturing. For each subculturing, we used a toothpick to scrape cells from regions of relatively heavy cell growth, rather than picking individual colonies. Individual colonies were picked only after the last subculturing.

Microarray and gel analysis: Each strain was then analyzed by microarrays containing all yeast ORFs [microarrays purchased from Corning (Corning, NY)] or all ORFs and all intergenic regions (Lemoine *et al.* 2005). Following subculturing described above, we picked a single colony to begin the DNA isolation. The colony was inoculated into 5 ml of liquid growth medium and grown to stationary phase at 30°, representing ~10 additional cell generations. Protocols for isolating and labeling DNA, and the hybridization conditions for

the microarray analysis, are also described in the supplemental data section of Lemoine *et al.* (2005). The hybridization images were acquired using a GenePix 4000B scanner and were analyzed with GenePix Pro 5.0 software. Subsequent analysis was done with either Gene Spring 5.1 (Silicon Genetics) or CGH Miner (http://www-stat.stanford.edu/~wp57/CGH-Miner/). We examined chromosomal DNA using the contour-clamped homogeneous electric field (CHEF) Mapper from Bio-Rad (Hercules, CA). Details of the procedures are described in Narayanan *et al.* (2006) (supplemental material).

RESULTS

On the basis of our previous studies (Craven *et al.* 2002; Mieczkowski *et al.* 2003) and those of others (Myung *et al.* 2001), we expected that haploid strains of the *tel1 mec1* genotype would have a very high rate of genomic instability. Consequently, in the experiments described below, we subcultured *tel1 mec1* strains and analyzed the subcultured derivatives by DNA microarrays (which detect changes in gene dosage), CHEF gels (which detect alterations in chromosome size), Southern analysis, and PCR analysis.

Pilot experiments demonstrating high rates of instability in *tel1 mec1-21* strains: Our initial study was based on examining *tel1 mec1* spores derived from two independent diploid strains (strains 24 and 56) that were heterozygous for a null mutation of *tel1* and the hypomorphic *mec1-21* allele. Three *tel1 mec1-21* spores derived from diploid 24 (MV24-15, MV24-17, and MV24-18) and two *tel1 mec1-21* spores (MV56-3 and MV56-5) were examined. Colonies derived from each spore were streaked to solid rich growth medium and allowed to form new colonies. Two colonies derived from each spore were then subcultured separately by streaking to solid growth medium. After 10 subculturings (~200 cell divisions), we isolated DNA from individual colonies and examined the DNA on microarrays containing all of the yeast genes. This type of analysis (comparative genome hybridization, CGH) detects deletions and duplications with the resolution of about one gene (Dunham *et al.* 2002; Lemoine *et al.* 2005). We also did CGH analysis on DNA isolated from each of the original five spore colonies before subcloning.

The results of this analysis are summarized in supplemental Table 1. All five spores were disomic for chromosome VIII, even before subculturing. In addition, MV24-5 was disomic for chromosome II, MV24-17 was disomic for chromosome III, and MV24-18 had a duplication of a 35-kb interstitial region of chromosome VII. Upon subculturing, some additional alterations occurred and some of the alterations observed in the original spore cultures were lost (supplemental Table 1). Examples of strains disomic for II and VIII (MV24-15) and having an interstitial duplication of VIII (MV56-5-2) are shown in Figure 1, a and b, respectively.

Although we did not examine these chromosome alterations in detail in the pilot experiment, several conclusions were evident. First, the genomic differences between different subcultured isolates of the same haploid strains indicate a high rate of chromosome nondisjunction and rearrangements associated with the *tel1 mec1-21* genotype. Second, some of the rearrange-

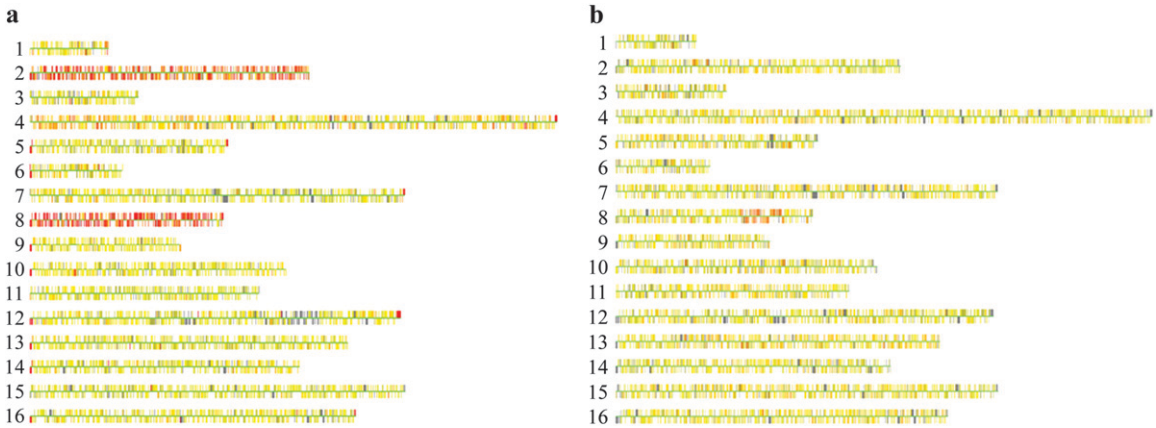

Figure 1.—Microarray analysis (CGH) of two *tel1 mec1-21* haploid strains derived from diploids MV24 and MV56. Diploids heterozygous for the *tel1* and *mec1-21* mutations were sporulated and dissected. Cultures derived from spores with the double-mutant genotype were examined before or after vegetative subculturing. DNA was isolated from each subcultured strain, labeled with a Cy5-fluorescent nucleotide, and mixed with DNA from a control strain that was labeled with a Cy3-fluorescent nucleotide. This mixture was hybridized to a microarray containing all of the yeast ORFs, and the ratio of hybridization of the two different samples to each ORF was determined (Dunham *et al.* 2002). In the Gene Spring 5.1 images used, each chromosome is shown by a series of adjacent very small rectangles representing the ORFs. In the image, yellow indicates the same dosage in the experimental and the control strain; red and blue indicate a duplication and a deletion, respectively, in the experimental strain relative to the control. The large gray region on chromosome XII is an uninformative signal on the microarray. (a) MV24-15. This strain (which was examined prior to subculturing) is disomic for chromosomes II and VIII. (b) MV56-5-2. This strain was vegetatively subcultured 10 times before the DNA was analyzed. The strain has a duplication of a 120-kb region of chromosome VIII that includes the *DNA2* gene.

ments had breakpoints near or within the repetitive Ty or δ-elements. Third, chromosome III appeared prone to rearrangements and chromosome VIII appeared prone to changes in ploidy. The rearrangements involving chromosome III occurred during the subcloning, whereas the disomy of chromosome VIII was present in the original spore cultures. Since the diploids 24 and 56 from which the MV24 and MV56 strains were derived had not been saved, we could not rule out the possibility that the observed disomy in the haploid strains reflected trisomy or tetrasomy of chromosome VIII in the diploids rather than acquired disomy in the haploids. This issue was investigated in other experiments described below.

Haploid strains of the *mec1-21* genotype often acquire an extra copy of chromosome VIII: By two independent matings of the haploids MV40-3b (*tel1::KANMX arg4::HYG CAN1*) and RCY308-7b (*MAT*α *mec1-21 RAD5;* Craven *et al.* 2002), we constructed two isogenic diploids, MV58 and MV59. By microarray analysis, we found that MV40-3b was euploid and RCY308-7b was disomic for chromosome VIII. Thus, both MV58 and MV59 were trisomic for chromosome VIII with one copy of VIII containing the *arg4::HYG* mutation and two copies containing the wild-type *ARG4* allele. Of 24 dissected tetrads derived from MV58 and MV59 that had four viable spores, we found that 16 tetrads had four Arg^+ and zero Arg^- spores, 3 had three Arg^+ and one Arg^- spores, and 5 had two Arg^+ and two Arg^- spores, as expected for the meiotic segregation of three chromosomes. In the tetrads with two Arg^+ and two Arg^- spores, the Arg^- spores should have one copy of chromosome VIII and the Arg^+ spores should be disomic.

We selected three Arg^- spores for each of three genotypes: *tel1*, *mec1-21*, and *tel1 mec1-21*. Spore colonies were streaked onto rich solid growth medium and new colonies were formed. From each of the original spore colonies, we picked seven isolates that were subcultured separately 10 times. Thus, for each of the three genotypes, we had 21 strains. After the 10th subculturing, we did a CGH microarray analysis on each of these 62 strains. Twenty of the 21 *mec1-21* strains and 18 of 21 *tel1 mec-21* strains were disomic for chromosome VIII. None of the 21 *tel1* strains was disomic for chromosome VIII.

Using an alternative approach, we introduced heterozygous *tel1* and *mec1-21* mutations into a diploid by transformation rather than by crossing mutant strains; details of the construction are in supplemental materials. The resulting diploid (MV70), when examined by CGH microarrays, was euploid and had no chromosome rearrangements resulting in deletions or duplications. We sporulated MV70 and selected two spores each of the four possible genotypes: wild type (MV70-1d, MV70-2c), *mec1-21* (MV70-6d, MV70-7b), *tel1* (MV70-1c, MV70-6c), and *tel1 mec1-21* (MV70-5a, MV70-11d). Without subculturing, we analyzed cultures of all of the strains by CGH microarrays. No aneuploidy, duplications, or deletions were detectable in any of the wild-type or *tel1* strains. All of the *mec1-21* and *tel1 mec1-21* strains were disomic for chromosome VIII, and one of the *tel1 mec1-21* strains (MV70-11d) was also disomic for chromosome III. These results demonstrate that strains with the *mec1-21* allele become disomic for chromosome VIII very rapidly.

One interpretation of these results is that the *mec1-21* mutation specifically elevates the rate of nondisjunction for chromosome VIII. Alternatively, it is possible that *mec1-21* strains with an extra copy of chromosome VIII have a selective growth advantage relative to *mec1-21* strains with only one copy of VIII. One of the *tel1 mec1-21* strains analyzed in the pilot experiment had an interstitial duplication of VIII (Figure 1b). This region of 120 kb contains ~60 ORFs. Although most of these ORFs had no obvious functional connection to DNA repair or DNA replication (two processes affected by Mec1p), one gene in this region (*DNA2*) encodes an essential DNA nuclease/helicase involved in DNA replication and DNA repair (Budd and Campbell 1997, 2000).

To determine whether extra copies of *DNA2* (a gene encoding a nuclease/helicase located on chromosome VIII) would suppress the tendency of *tel1 mec1-21* strains to become disomic for chromosome VIII, we transformed MV70 with the plasmid pRS316-DNA2, a *CEN*-containing plasmid with the wild-type *DNA2* and *URA3* genes (Lee *et al.* 2000). The resulting diploid transformant (MV71) was sporulated and tetrads were dissected. Twelve plasmid-containing spores (two wild type, two *tel1*, six *mec1-21*, and two *tel1 mec1-21*) were grown into cultures and examined by CGH analysis. All wild-type and *tel1* strains had only one copy of chromosome VIII. One of the two plasmid-containing *tel1 mec1-21* strains had one copy of VIII and one had a hybridization signal for chromosome VIII, indicating that about half of the cells in the culture had one copy of VIII and about half had two copies. Of the six plasmid-containing *mec1-21* strains, five had only one copy of chromosome VIII and one had two copies. We also isolated derivatives of two of the euploid *mec1-21* strains that had spontaneously lost the pRS316-DNA2 plasmid. These derivatives acquired disomy for chromosome VIII.

As a control, we transformed MV70 with pRS316 (the *URA3* vector without the *DNA2* insert). This diploid was sporulated and we examined by CGH the gene dosage of five different *mec1-21* haploid strains containing the vector. All five had two copies of chromosome VIII. In summary, an extra copy of *DNA2* reduces the tendency of *mec1-21* and *tel1 mec1-21* strains to acquire an extra copy of chromosome VIII.

Intrachromosomal chromosome rearrangements associated with the *tel1 mec1-21* genotype: In addition to the extra copy of chromosome VIII, the 21 *tel1 mec1-21* strains derived from MV58 and MV59 spores often had chromosome rearrangements or aneuploidy involving chromosome III. Most of the rearrangements involved homologous recombination between the 6-kb Ty retro-

transposons or the 330-bp long-terminal repeats of these elements (δ's). These rearrangements are summarized in Table 1 and discussed in detail in the supplemental materials. Our conclusions concerning the nature of the rearrangements are based on the pattern of deletions and duplications as determined by microarrays and Southern analysis of separated chromosomal DNA molecules. Some of the rearrangements were characterized in more detail by standard Southern analysis and PCR analysis.

Among the 21 *tel1 mec1-21* strains, there were 20 chromosomes that had detectable deletions or duplications resulting from intrachromosomal homologous recombination between repetitive elements in the chromosome. Two general classes were observed. One class (13 of the 20 intrachromosomal events) reflected unequal crossing over between directly oriented repeats, generating a deletion or a duplication of the sequences located between the repeats. The remaining 7 rearrangements resulted from a different type of recombination in which a break in a repeat located on one arm of the chromosome was repaired, utilizing a repeat located in inverted orientation on the opposite arm by break-induced replication (BIR) (Kraus *et al.* 2001). We describe two examples of these types of rearrangements in detail below.

Microarray analysis indicated that the *tel1 mec1-21* strain MV58-20a#1 was disomic for chromosomes VIII and III (data not shown). By CHEF gel analysis, although chromosome VIII had the same size as in the progenitor strain, MV58-20a#1 did not have a wild-type-sized chromosome III (~340 kb), but had one chromosome that was ~90 kb larger and one that was ~90 kb smaller (Figure 2a). One explanation of this pattern is that these altered chromosomes reflected an unequal crossover (Figure 2b) between the mating-type locus (*MAT*) and *HMR* (a region of homology located ~90 kb from the mating-type locus). As expected from this model, both chromosomes hybridized to the *HIS4* probe derived from the left arm of chromosome III (Figure 2a), but only the larger chromosome hybridized with a probe (*THR4*) located between *MAT* and *HMR* (data not shown). This model also predicts two novel junctions, one containing the left end of the *MAT* locus and the right end of *HMR* and a second containing the left end of *HMR* fused to the right end of *MAT*. Both junctions were detected by PCR (Figure 2c). Of the 20 chromosome rearrangements observed in the *tel1 mec1-21* strains, 7 involved recombination between *HML* or *MAT* and *HMR* (Table 1).

Thirteen of the 20 intrachromosomal rearrangements were a consequence of recombination between retrotransposons, involving two Ty elements, one Ty element and a solo δ (the long-terminal repeat associated with Ty), or two solo δ's. One strain with this type of rearrangement is MV58-20a#5. This strain had two chromosomes that hybridized to a probe derived from the left end of chromosome III, one of normal size (340 kb) and one ~260 kb (Figure 3a, lane 8). In addition, by microarray analysis, one region of chromosome III was present in one copy (FS2 to right telomere), one region in two copies (left-arm hotspot, LAHS, to FS2), and one region in three copies (LAHS to left telomere, Figure 3b). Ty elements were located at the boundaries of these regions of different gene dosages. FS1 and FS2 are direct and inverted pairs, respectively, of Ty elements that are hotspots for chromosome rearrangements in strains with low levels of α-DNA polymerase (Lemoine *et al.* 2005). The LAHS is a region defined as a hotspot for the insertion of transposable elements; in W303, there is a Ty1 element centromere distal to a Ty2 element.

To explain the microarray and CHEF data, we suggest that MV58-20a#5 was initially disomic for chromosome III. One of these two copies had a DSB near or within the centromere-proximal Ty1 element of FS2. This DSB was repaired by a BIR event utilizing the Ty1 element of the LAHS as a template (Figure 3c). The result of this mechanism would be an isochromosome of 260 kb. It should be noted that the 260 kb hybridizes to the *HIS4* gene used as hybridization probe with twice the intensity of the wild-type 340-kb chromosome (Figure 3a, lane 8), as expected since the 260-kb chromosome has two copies of the *HIS4* gene. The predicted structure was also confirmed by standard Southern analysis as described in supplemental materials.

It is striking that 15 of the 20 intrachromosomal rearrangements involve chromosome III, one of the smallest yeast chromosomes. There are two likely explanations for this result. First, there may be some physical feature of chromosome III (for example, the high density of repetitive elements) that predisposes it to rearrangements by homologous recombination. Alternatively, since many of the rearrangements result in a duplication or a triplication of sequences between the LAHS and the left telomere, it is possible that extra doses of genes in this region confer a selective growth advantage to *tel1 mec1-21* haploids. These alternatives are discussed further below.

Yeast strains lacking telomerase amplify telomeric [poly($G_{1-3}T$)] and subtelomeric (Y′) repeats by homologous recombination (Teng and Zakian 1999). Amplification of subtelomeric repeats is also observed in *tel1 mec1-21* strains (32). From our microarray analysis, amplification of Y′ elements was evident in 16 of the 21 *tel1 mec1-21* strains (Table 1). This amplification is detected as a signal of increased gene dosage at the ends of the Y′-bearing chromosomes (Figure 1a).

Interchromosomal chromosome rearrangements associated with the *tel1 mec1-21* genotype: In addition to the 20 intrachromosomal rearrangements, we observed seven interchromosomal events (translocations) (Table 1). Ty elements were observed at all breakpoints, indicating that the rearrangements were generated by ectopic homologous recombination. Four of the five types of translocations involved the Ty1 element located

TABLE 1

Genome alterations in subcultured *tel1 mec-21* haploid strains derived from diploids MV58 and MV59

Strain name	Genome alterations
MV58-20a#1	Disomy for VIII. Two copies of III, one ~100 kb smaller than wild type and one ~100 kb larger (products of unequal crossing over between *MAT* and *HMR*). No Y′ amplification.
MV58-20a#2	Disomy for VIII. Two copies of III, one wild-type chromosome and one with a deletion between *MAT* and *HMR*. Y′ amplification.
MV58-20a#3	Disomy for VIII. Interstitial 35-kb duplication of VII [breakpoints near unannotated Ty elements (A. Gabriel, personal communication) located at *YGRWδ19* and *YGRWδ21*]. Two copies of III, one wild-type chromosome and one isochromosome with duplication of sequences between LAHS and left telomere, and deletion of sequences distal to FS1. No Y′ amplification.
MV58-20a#4	Disomy for VIII. Two copies of III, one normal copy and one isochromosome with duplication of sequences between the LAHS and the left telomere, and deletion of sequences distal to *YCRCδ6*. Y′ amplification.
MV58-20a#5	Disomy for VIII (normal size). Two copies of III, one normal copy and one isochromosome with duplication between the LAHS and the left telomere, and deletion of sequences distal to FS2. No Y′ amplification.
MV58-20a#6	Intrachromosomal duplication on chromosome IV between two pairs of Ty elements (*YDRWTy2-2/YDRCTy1-2* and *YDRWTy2-3/YDRCTy1-3*). Three chromosomes with sequences from III_L: (1) normal III, (2) isochromosome with duplication between the LAHS and the left telomere and deletion of sequences distal to *YCRCδ6*, and (3) translocation between *YHRCTy1-1* on VIII and Ty at LAHS on III. No Y′ amplification.
MV58-20a#7	Disomy for VIII. Translocation between III and X with breakpoint on III near the LAHS and breakpoint on X near *YJLCδ4*, which maps near an unannotated Ty (A. Gabriel, personal communication). Two other uncharacterized chromosome rearrangements involving chromosome III. Y′ amplification.
MV59-6a#1	Disomy for VIII. Two copies of III, one isochromosome resulting from recombination between *YCLCδ6* and the LAHS and one circular chromosome resulting from recombination between *HML* and *HMR*. Y′ amplification.
MV59-6a#2	Disomy for VIII. Two copies of III, one normal and one with the Hawthorne deletion (loss of region between *MAT* and *HMR*). Y′ amplification.
MV59-6a#3	Disomy for VIII. Two copies of III, one normal and one with the Hawthorne deletion (loss of region between *MAT* and *HMR*). Y′ amplification.
MV59-6a#4	Disomy for VIII. Y′ amplification.
MV59-6a#5	Disomy for 640-kb III–VIII translocation with breakpoints near LAHS on III and *YHRCTy1-1*: 440-kb derivative of III with duplication of region between the LAHS and FS1. Y′ amplification.
MV59-6a#6	No disomy for VIII, but chromosome is ~60 kb larger than the wild type; the origin of the extra DNA is not known. Disomy for III. Y′ amplification.
MV59-6a#7	No disomy for VIII and VIII of normal size. III is ~100 kb larger than the normal chromosome as a consequence of an unequal crossover, duplicating the region between *MAT* and *HMR*. Y′ amplification.
MV59-16c#1	Disomy for VIII: 250-kb III–XII translocation with breakpoints at the LAHS and near *YLRCsigma1*, which maps at an unannotated Ty (A. Gabriel, personal communication). Interstitial 35-kb duplication of VII (same breakpoints as MV58-20a#3). Y′ amplification.
MV59-16c#2	Disomy for VIII. One VIII of normal size (565 kb) and one ~640 kb (VIII–XV translocation between *YHRCTy1-1* and *YOLWTy1-1*); 250-kb III–XII translocation with same breakpoints as MV59-16c#1. No Y′ amplification.
MV59-16c#3	Disomy for VIII. One VIII of normal size (565 kb) and one ~640 kb (VIII–XV translocation between *YHRCTy1-1* and *YOLWTy1-1*). Two copies of III, one normal III and one isochromosome resulting from recombination between *YCLCδ6* and the 5′ δ of Ty1 at the LAHS. Interstitial 35-kb duplication of VII (same breakpoints as MV58-20a#3). Y′ amplification.
MV59-16c#4	Disomy for I and VIII. Two copies of III, one normal III and one isochromosome resulting from recombination between *YCLCδ6* and the 5′ δ of Ty1 at the LAHS. Y′ amplification.
MV59-16c#5	Uncharacterized alteration duplicating the leftmost 280 kb of VIII. Extensive Y′ amplification.
MV59-16c#6	Disomy for chromosome VIII. Both VIII and III hybridized to regions extending ~50 kb beyond their normal size, probably the result of extensive Y′ amplification.
MV59-16c#7	Disomy for chromosome VIII. Interstitial 35-kb duplication of VII (same breakpoints as MV58-20a#3). No Y′ amplification.

These haploid strains were derived from sporulation of diploids heterozygous for the *tel1* and *mec1-21* mutations, as described in the text. These strains are isogenic with W303a (Thomas and Rothstein 1989). The strains were subcultured 10 times before analysis. Prior to subcloning, none of the strains had chromosome rearrangements, although all were disomic for chromosome VIII.

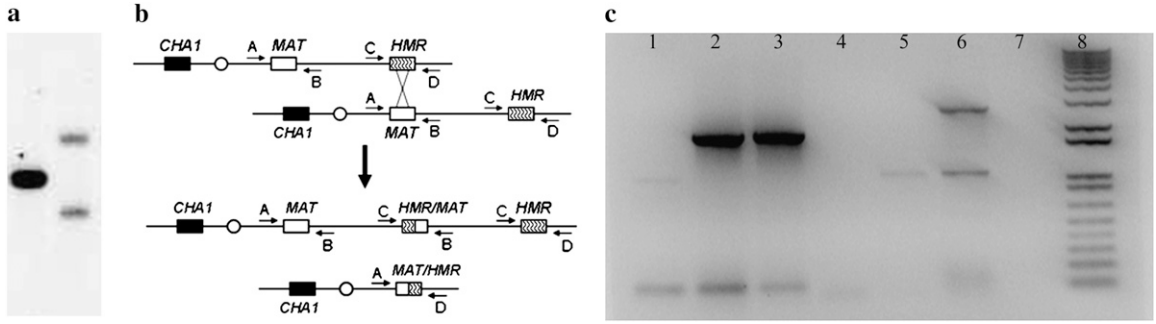

FIGURE 2.—Analysis of a *tel1 mec1-21* strain (MV58-20a#1) that has two rearranged chromosome IIIs generated by unequal crossing over. By microarray analysis, this strain was disomic for chromosomes III and VIII and had no other altered gene dosage. (a) CHEF gel analysis of chromosome III in a wild-type strain (W303, left lane) and in MV58-20a#1 (right lane). The separated chromosomal DNAs were hybridized to a probe derived from the left end of chromosome III (*CHA1*). Chromosome III in the wild-type strain was ~340 kb, whereas the two chromosome III derivatives in MV58-20a#1 were ~250 and 430 kb. (b) Depiction of the chromosome rearrangements resulting from unequal crossing over between the *MAT* and *HMR* repeats that share ~1 kb of homology. The small arrows show the positions of primers used to diagnose the chromosome rearrangements. The chromosome is not drawn to scale. (c) PCR analysis of the breakpoints resulting from unequal crossing over between *MAT* and *HMR*. In lanes 1–4, we analyzed the products of a PCR reaction performed with primers A (BUD5-5Prime-Rev) and D (ARS318-Rev) to detect the *MAT–HMR* fusion. The PCR reactions contained W303a DNA (lane 1), MV58-20a#1 DNA (lane 2), MV58-20a#3 DNA (lane 3), and water (lane 4). We also did PCR reactions with primers B (ARS317-Upstream-For) and C (TAF2-3Prime-For) to detect the *HMR–MAT* fusion. The reactions contained DNA from W303a (lane 5), WV58-20a#1 (lane 6), and water (lane 7). Lane 8 contains size standards. The expected sizes of the fusions are 1.9 kb for *MAT–HMR* and 2.8 kb for *HMR–MAT*.

at the LAHS on chromosome III. Thus, both inter- and intrachromosomal rearrangements nonrandomly involve chromosome III. We discuss only one of the translocations in detail.

In MV59-16c#2, microarray analysis indicated a duplication of sequences from chromosome III between the LAHS and the left telomere and a 168-kb duplication of sequences on chromosome XII (Figure 4a). The breakpoint on XII mapped very close to an unannotated Ty2 element identified by A. GABRIEL (personal communication). The predicted size of a translocation between the Ty elements on III and the Ty element on XII is ~250 kb. By CHEF gel analysis, we found that a probe derived from the left arm of chromosome III hybridized to two chromosomes, one normal-sized III (340 kb) and one chromosome of ~250 kb (data not shown); a probe from the right arm of III hybridized only to the longer chromosome. A more detailed Southern and PCR analysis (described in supplemental materials) demonstrated that the III–XII translocation was a consequence of a break in or near a δ-element associated with Ty2 on chromosome XII that was repaired by recombination with the δ-element of Ty1 on chromosome III (Figure 4b); although the translocation could also be generated by a DSB at or near the LAHS on III followed by a BIR event involving the Ty2 element on XII, BIR events that extend through the centromere occur inefficiently (MORROW *et al.* 1997). Other translocations in our study also occur as a consequence of homologous recombination between repetitive elements (Table 1 and supplemental materials).

Chromosome rearrangements are much less frequent in single-mutant *mec1-21* and *tel1* strains than in the double mutant: The 21 *mec1-21* strains examined in our study were derived from three *mec1-21* spores, MV58-5b (#1-7), MV58-13b (#1-7), and MV59-18c (#1-7). As described above, after subculturing the strains 10 times, all strains except one (MV58-5b#7) were disomic for chromosome VIII. The MV58-5b#7 strain was disomic for chromosome XIV. In addition, strain MV58-13b#6 was disomic for both III and VIII, and strain MV59-18c#3 was disomic for both II and VIII. Only one chromosome rearrangement was detected. In MV58-5b#2, a 35-kb region of chromosome VII located between two like-oriented δ-/Ty elements (*YGRWδ19/Ty* and *YGRWδ21/Ty*) was duplicated; this same rearrangement was observed in four of the *tel1 mec1-21* strains. No amplification of Y′ was observed for any of the *mec1-21* strains.

The 21 subcultured *tel1* strains were derived from three spore cultures, MV58-3d#1-7, MV59-2d#1-7, and MV59-10a#1-7. No aneuploidy or chromosome rearrangements were observed in any of the 21 strains. Interestingly, on the basis of the microarrays, 8 of the 21 *tel1* strains had some degree of Y′ amplification, although the level of amplification was considerably less than that observed in most *tel1 mec1-21* strains. In summary, in contrast to the *tel1 mec1-21* genotype in which chromosome rearrangements were very common, only one of 42 strains of the *mec1-21* and *tel1* genotypes had a chromosome rearrangement.

DISCUSSION

Genome stability is usually examined using selective assays that are specific for certain loci or certain chromosomal regions. This constraint exists for two reasons.

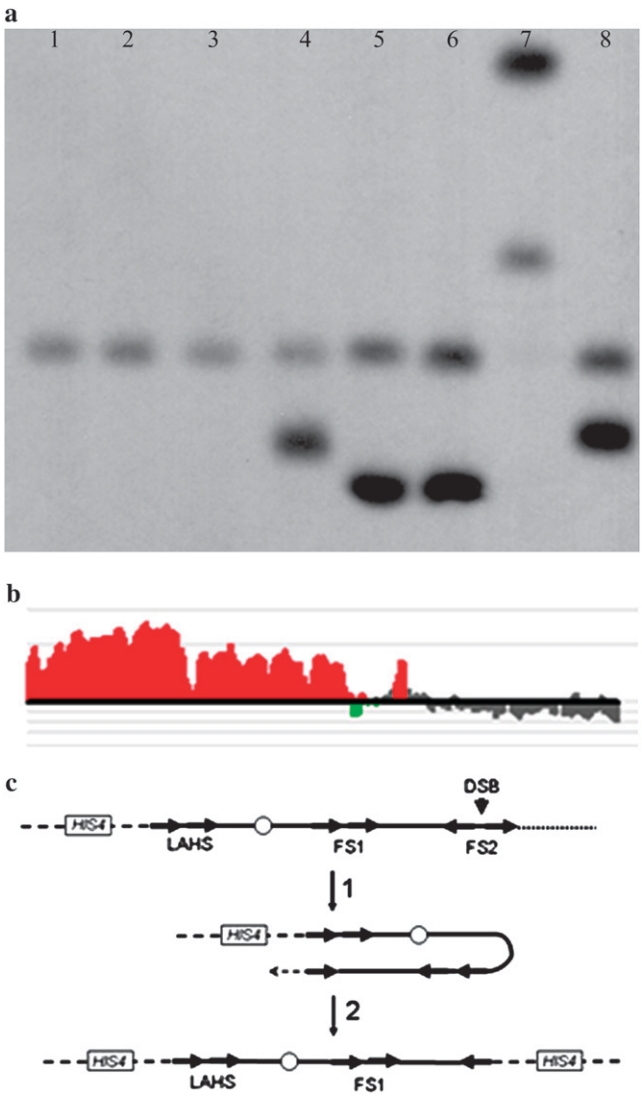

Figure 3.—Analysis of *tel1 mec1-21* strains (MV58-20a#5) with intrachromosomal rearrangements of chromosome III. (a) CHEF gel analysis of chromosome III in wild-type strains and in strains with the *tel1 mec1-21* mutations. Separated chromosomal DNAs were hybridized to *HIS4*, a probe located on the left arm of chromosome III. The normal-sized chromosome III in W303 (lane 1) and two isogenic wild-type strains (lanes 2 and 3) derived from RCY278 (described in supplemental materials) is ~340 kb. The *tel1 mec1-21* strains examined include MV58-20a#3 (lane 4), MV59-16c#3 (lane 5), MV59-16c#4 (lane 6), MV59-6a#5 (lane 7), and MV58-20a#5 (lane 8); all except MV59-6a#5 have a normal chromosome III in addition to the rearranged III. The smaller-than-normal chromosome IIIs in lanes 4, 5, 6, and 8 could reflect DSBs in repetitive elements (Ty or δ) on the right arm of III that were repaired by a BIR event using a Ty or a δ on the left arm as a template (Figure 3c). In MV59-6a#5, the 640-kb chromosome represents a translocation between chromosomes III and VIII, whereas the 440-kb chromosome has an internal duplication on III. These rearrangements are described in detail in Table 1 and in supplemental materials. (b) Level of gene duplication in different regions of chromosome III by DNA microarray analysis in MV58-20a#5. The data from the microanalysis were analyzed using CGH Miner. The region of chromosome III between the Ty elements of the LAHS and the left telomere was in three copies per genome, the region between the LAHS and FS2 was in two copies, and the region between FS2 and the right telomere was in one copy. (c) Depiction of the mechanism for the generation of an isochromosome duplicating the region between the LAHS and the left telomere (indicated by a dashed line). A double-strand break in the centromere-proximal Ty element of FS2 could be repaired by a BIR event involving a Ty element in the LAHS. Ty elements are shown as arrows.

First, in general, the level of genome instability is not high enough to be readily analyzed by nonselective methods. Second, until recently, methods did not exist to allow a whole-genome analysis of genetic instability. In our study, we have used DNA microarrays and CHEF gel analysis to study the genetic instability associated with mutations in the two related genes *TEL1* and *MEC1* in subcultured derivatives. Our conclusions are different from those predicted by previous studies in which more selective methods have been employed. Even in our experiments, however, it is possible that the types of chromosome rearrangements that were detected were influenced by selection for those that had the least deleterious consequences for cell growth.

In two previous assays of genome stability in the single mutants and the *tel1 mec1* double mutants, the rates of large deletions that include the *CAN1* locus were examined (Myung *et al.* 2001; Craven *et al.* 2002). Both studies found that haploid strains with both *tel1* and *mec1* mutations had a level of deletions that was much higher than that observed in either single-mutant strain. There are no essential genes on chromosome V that are centromere distal to *CAN1* and *PCM1* (located ~12 kb from *CAN1*) is the first essential centromere-proximal gene. In the *can1* deletions derived from the *tel1 mec1 sml1* (Myung *et al.* 2001) or the *tel1 mec1-21* (Craven *et al.* 2002) strains, deletion derivatives of chromosome V with a breakpoint between *CAN1* and *PCM1* were fused to a chromosome segment derived from a nonhomologous chromosome by nonhomologous end joining.

In contrast, in the present study, most chromosome rearrangements involve homologous recombination between dispersed repeated genes. This difference has a simple explanation. The genome instability assay used by the previous studies selects for a breakpoint in a 12-kb region that has no repetitive DNA elements. Thus, any deletion that occurs in this region will be repaired by telomere capping (which is very inefficient in strains with a *tel1* deletion; Goudsouzian *et al.* 2006; Tseng *et al.* 2006) or by nonhomologous end joining with DNA fragments derived from other chromosomes (as observed). It is likely, therefore, that the spectrum of chromosome alterations assayed by Myung *et al.* (2001) or Craven *et al.* (2002) would be dramatically altered and the rate of the events would be dramatically elevated by insertion of a Ty element between *CAN1* and *PCM1*.

Several other points should be made concerning the chromosome rearrangements that are mediated by

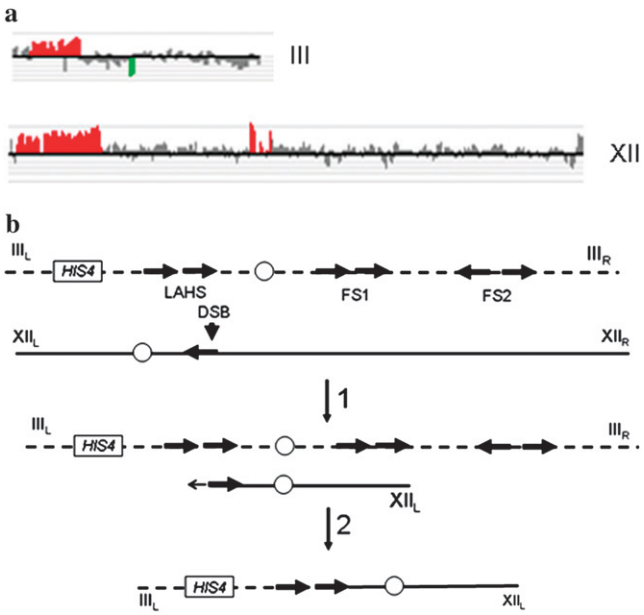

Figure 4.—Analysis of a *tel1 mec1-21* strain (MV59-16c#2) that has a III–XII translocation. (a) Gene dosage on chromosomes III and XII (based on CGH Miner analysis of microarrays). The boundaries for the gene dosage changes on III and XII are at the positions of the LAHS and an unannotated Ty element on XII, respectively. (b) Formation of a III–XII translocation by homologous recombination between Ty elements located on chromosomes III and XII.

recombination between retrotransposons. First, it is not clear whether the retrotransposons are preferred sites for DSBs in the *tel1 mec1* strains. It is possible that DSBs are random, but only the DSBs occurring within repetitive elements result in rearrangements. It is also possible that DSBs in nonrepetitive genomic regions could be processed by exonucleases to generate a chromosomal fragment with a Ty element at the end. Vanhulle *et al.* (2007) recently showed that processing of DSBs in nonrepetitive DNA on chromosome III can result in Ty–Ty-mediated rearrangements. Second, we do not know the source of the recombination-initiating DSBs in the *tel1 mec1* strains. There are at least three plausible sources: (1) breaks resulting from exonucleolytic degradation of chromosomes that lack telomere "caps" (Hackett and Greider 2003), (2) breaks resulting from processing of dicentric chromosomes reflecting telomere–telomere fusions (Mieczkowski *et al.* 2003), and (3) breaks resulting from stalled DNA replication forks (Cha and Kleckner 2002). Third, our results support previous observations that recombination between dispersed retrotransposons is important in the evolution of the yeast genome, occurring both in nature and in the lab (reviewed by Mieczkowski *et al.* 2006). Fourth, although DSBs generated in a variety of ways result in retrotransposon-mediated chromosome rearrangements, there are quantitative differences in the frequencies of the rearrangements. For example, in strains with low levels of α-DNA polymerase, a preferred site for translocations was the inverted pair of Ty elements on the right arm of chromosome III (FS2; Lemoine *et al.* 2005). Although we also found a rearrangement involving FS2 in our study, a solo δ also on the right arm of III (*YCLC*δ) was more frequently observed at rearrangement breakpoints.

Both the *tel1 mec1-21* and the *mec1-21* strains became disomic for chromosome VIII and this tendency to become disomic for chromosome VIII was reduced in strains with an extra copy of *DNA2* on a plasmid. Since Dna2p is a helicase and exo/endonuclease with many roles (Okazaki fragment processing, DNA repair, chromatin remodeling, and telomere maintenance; Budd *et al.* 2005), a simple interpretation of this effect is not yet possible. On the basis of the essential role of Mec1p in DNA replication (Desany *et al.* 1998) and the relatively small role of Mec1p in telomere length regulation (Ritchie *et al.* 1999), however, we suggest that the extra dose of Dna2p helps alleviate problems of DNA replication in the *mec1-21* strains. Strains with a *mec1* mutation also have an elevated rate of loss of chromosome V (Klein 2001; Craven *et al.* 2002), although this rate is low (5×10^{-5}/division) compared to the rate of disomy for chromosome VIII observed in the present study. Haploid strains in a different genetic background and with a different *mec1* mutation (a *mec1* deletion, in which the lethality of the deletion is suppressed by overproduction of *RNR1*) become disomic for chromosome IV, rather than chromosome VIII (Gasch *et al.* 2001). We do not know whether the difference between our observations and this previous study reflects the type of *mec1* mutation or some other feature of the genetic background.

Hughes *et al.* (2000) reported that ~8% of 300 yeast strains from the deletion collection were aneuploid for one or more chromosomes. Although most of these aneuploid strains were not analyzed in any detail, Hughes *et al.* pointed out that, in several strains, the duplicated chromosome has a gene that was structurally related to the deleted gene. For two of the strains, they demonstrate that the derivative with the extra chromosome grew better than the euploidy progenitor.

In addition to disomy of chromosome VIII, *tel1 mec1-21* strains also have a high frequency of chromosome rearrangements involving chromosome III. Since many of these rearrangements result in an elevated dosage of genes located between the LAHS and the telomere (a region of 80 kb containing ~40 genes), it is possible that an extra dose of one or more genes in this region compensates for growth defects of the *tel1 mec1-21* strain. A promising candidate gene in this region is *MRC1*, since Mrc1p is a substrate of the Mec1p kinase and is located at the replication fork (Katou *et al.* 2003; Osborn and Elledge 2003). In addition, the Mrc1p has a role in telomere capping (Tsolou and Lydall 2007). Thus far, however, we have not been able to suppress the accumulation of chromosome III rearrangements in *tel1*

mec1-21 strains with a plasmid-borne copy of *MRC1* (our unpublished data).

Cells derived from solid tumors often exhibit a very high level of aneuploidy and chromosome rearrangements, and it is likely that an early step in the development of some tumors is a mutation that results in elevated rates of genetic instability (Lengauer *et al.* 1998). Our analysis of the instability in *tel1 mec1-21* strains argues that the same mutant background can produce both elevated rates of aneuploidy and elevated rates of translocations. In addition, our results and those of Hughes *et al.* (2000) argue that the gene amplifications that occur in tumor cells may promote tumorigenesis by two different mechanisms: some amplifications ("accelerators") may directly stimulate tumor growth, whereas others ("compensators") may alleviate the negative effects of the original genome-destabilizing mutant.

We thank G. Gawel, M. Dominska, and P. Greenwell for help with the analysis of the translocations. This research was supported by National Institutes of Health grant GM52319 (T.P.) and National Science Foundation grant MCB-0417088 (K.L.).

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Communicating editor: M. D. Rose