Chromosome rearrangements and aneuploidy in yeast strains lacking both Tel1p and Mec1p reflect deficiencies in two different mechanisms

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The human ATM and ATR proteins participate in the DNA damage and DNA replication checkpoint pathways and are critical to maintaining genome stability. The *Saccharomyces cerevisiae* homologs of ATM and ATR are Tel1p and Mec1p, respectively. Haploid *tel1 mec1* strains have very short telomeres and very high rates of chromosomal aberrations. Here, we examine genetic stability in *tel1 mec1* diploid cells. In the absence of induced DNA damage, these yeast strains had very high frequencies of aneuploidy (both trisomy and monosomy) in addition to elevated rates of chromosome rearrangements. Although we found the aneuploidy in the *tel1 mec1* diploids mimicked that observed in *bub1* diploids, the *tel1 mec1* diploids had a functional spindle assembly checkpoint. Restoration of wild-type telomere lengths in the *tel1 mec1* strain substantially reduced the rate of chromosome rearrangements but had no effect on the frequency of aneuploidy.

chromosome nondisjunction | spindle assembly checkpoint | telomeres

T wo types of genetic instability are associated with cells derived from solid tumors (1): elevated rates of single-base substitutions/microsatellite alterations and high frequencies of chromosome rearrangements and aneuploidy. The first type of instability is associated with tumors deficient in nucleotide excision repair and DNA mismatch repair. Tumors with elevated rates of chromosome aberrations (deletions, duplications, and translocations) and/or aneuploidy have been termed "chromosome instability" (CIN) tumors (2). Although some CIN tumors have mutations in genes affecting the spindle assembly checkpoint (SAC) (3) or chromatid cohesion (4), the relevant genome-destabilizing mutations have not been identified in most CIN tumors.

Many yeast mutants have been identified that have elevated rates of chromosome alterations and/or aneuploidy. One assay designed to detect gross chromosomal rearrangements (GCRs) involves selecting for the deletion of two closely linked markers near the end of chromosome V (5). Mutations that elevate GCRs are found in genes controlling S-phase DNA damage checkpoints (6), DNA replication (7), telomere length regulation (8, 9), and chromatin assembly (10) as well as other pathways. Importantly, strains with mutations in the SAC (which result in elevated levels of chromosome nondisjunction) do not necessarily have elevated rates of GCRs (11), indicating that these two phenotypes are not intrinsically linked.

In *Saccharomyces cerevisiae*, the related kinases Tel1p (orthologous to the mammalian ATM gene) and Mec1p (orthologous to the mammalian ATR gene) have overlapping roles in the DNA damage/S-phase checkpoint and in telomere length regulation (12). In response to DNA damage such as a double-strand break (DSB), Tel1p and Mec1p are recruited to the site of the break, where they initiate a signal transduction cascade that results in phosphorylation and activation of effector proteins, transcription of DNA repair genes, and cell cycle arrest. The *tel1 mec1 s*trains are more sensitive to DNA damaging agents than either single-mutant strain (13). Single-mutant *tel1* and *mec1* strains have short but stable telomeres, whereas double-mutant strains have a phenotype similar to a telomerase-negative strain (14). Mec1p (but

not Tel1p) is an essential protein, but the lethality of the *mec1* mutation can be suppressed by a mutation in *SML1* (15). In addition, *mec1* strains have elevated levels of DSBs in hard-to-replicate regions of the genome (16). These and other results suggest that the essential role of Mec1p may be to stabilize stalled replication forks (17).

Haploid yeast strains lacking both Tel1p and Mec1p have very high rates of chromosome rearrangements (6, 9). In haploid strains, the rate of deletions of *CAN1* is elevated about 10,000-fold in the *tel1 mec1* strain relative to WT. In some *can1* strains, the deletion derivative of chromosome V (the location of *CAN1*) was fused to the telomere of a nonhomologous chromosome by a nonhomologous end-joining (NHEJ) event (9). In addition, the *tel1 mec1* strains had very high rates of telomere–telomere fusions (TTFs) relative to *tel1* or *mec1* strains (18). Expression of a Cdc13-Est2 fusion protein allows extension of telomeres to WT length in a *tel1 mec1* strain (19). This extension reduces the rate of *CAN1* deletions about 20-fold, arguing that about 95% of the deletions/ chromosome rearrangements are likely to reflect breakage of dicentric chromosomes formed by TTFs (18). It should be noted, however, that *tel1 mec1* strains with the Cdc13-Est2 fusion protein have a deletion rate that is several hundred times higher than the very low rate ($<10^{-9}$) observed in WT cells (18), possibly reflecting DSBs formed at stalled replication forks (16). We also observed a high frequency of unselected chromosome rearrangements in haploid *tel1 mec1* strains (20), reflecting homologous recombination between nonallelic Ty elements.

As described above, CIN tumors have elevated rates of aneuploidy in addition to high rates of chromosome rearrangements. Yeast strains with high rates of aneuploidy often have a defect in the SAC. This checkpoint ensures that chromosomes establish bipolar orientation on the spindle before anaphase. There are two types of kinetochore defects that activate the checkpoint: lack of kinetochore occupancy by microtubules and lack of tension among sister kinetochores (21, 22). Although most SAC proteins (Mad1p–Mad3p, Bub1p, and Bub3p) are required to respond to both types of defects, separation-of-function alleles of *bub1* have been obtained; strains that lack the kinase domain of Bub1p (*bub1-ΔK*) are defective only in the tension-sensing function of the SAC (23).

Although mutants affecting the DNA damage response usually have elevated levels of GCRs and mutants affecting the SAC have elevated levels of aneuploidy, connections between these two pathways exist. The elimination of the delay of the cell cycle

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in response to DNA damaging agents in yeast requires mutations in both the DNA damage checkpoint pathway and the SAC (24–27). Also, the DNA damage checkpoint and the SAC are both activated by defective telomeres (28) and nocodazole (29).

The connections between the DNA damage checkpoint and the SAC previously observed are in the presence of DNA damage. Below, we show that *tel1 mec1* diploid strains have a very high rate of aneuploidy and chromosome rearrangements in the absence of induced DNA damage but that the SAC remains functional in these strains. We also show that these two phenotypes reflect two different cellular defects because the frequency of chromosome rearrangements (but not the frequency of aneuploidy) is reduced by correcting the telomere defect of the *tel1 mec1* mutant strain.

Results

Rationale. Previous studies of *tel1 mec1* haploid yeast strains found high rates of chromosome aberrations. Because some genetic alterations are haploid-lethal, in this study, we examined genomic alterations in *tel1 mec1 sml1* diploids. The *sml1* mutation in necessary to suppress the lethality of *mec1* null alleles (15); in our subsequent discussion, the *tel1 mec1 sml1* genotype will be abbreviated to *tel1 mec1*. The *tel1 mec1* diploids initially had a plasmid-borne *MEC1* gene (details are provided in Tables S1–S3). The strains JLMy80–JLMy83 are isogenic *tel1 mec1* diploids. We then selected derivatives that lost the *MEC1* plasmid, subcultured these derivatives for 100 cell divisions, and retransformed them with the *MEC1*-containing plasmid (details provided in *Materials and*

Table 1. Number of trisomic and monosomic (in parentheses) chromosomes in strains with mutations in the DNA damage checkpoint after five cycles of subculturing*

Chromosome no.	*tel1 mec1* diploids	WT diploids	*tel1* diploids	*mec1* diploids	*tel1 mec1* haploids	*tel1 mec1* + pVL1107-*URA3* diploids
I	4 (4)	0	1	0	0	4 (1)
II	9	0	0	1	3	4
III	0	0	0	0	1	0 (1)
IV	1	0	0	0	0	1
V	1	0	0	0	0	2
VI	0 (7)	0	0 (1)	0	0	0
VII	1	0	0	1	0	5
VIII	9	0	1	1	1	8
IX	3	0	0	1	0	4
X	8	0	0	2	0	2
XI	2	0	0	0	0	2
XII	9	0	0	0	2	5
XIII	0	0	0	0	0	3
XIV	0	0	0	1	0	0
XV	0	0	0	0	0	0
XVI	1	0	0	1	0	2
Total no. aneuploid chromosomes	48 (11)	0 (0)	2 (1)	8 (0)	7 (0)	42 (2)
Total no. chromosomes analyzed[†]	320	64	64	160	144	192
Aneuploid/euploid chromosomes	59/261	0/64[‡]	3/61[§]	8/152[¶]	7/137[⊥]	44/148

*After five cycles of subculturing, we examined aneuploidy using CGH microarrays. The numbers outside parentheses represent the number of strains in which trisomy was observed; the numbers inside parentheses represent the number of times we observed monosomy. The strain names for each genotype are provided in Table S4.

[†]Total number of chromosomes was calculated by multiplying the number of independent strains examined by 16, the number of homologs in S. cerevisiae.

[‡]Significant (P < 0.001) reduction in aneuploidy compared with the *tel1 mec1* diploid (Fisher exact test).

[§]Significant (P < 0.05) reduction in aneuploidy compared with the *tel1 mec1* diploid.

[¶]Significant (P < 0.0001) reduction in aneuploidy compared with the *tel1 mec1* diploid.

[⊥]Significant (P < 0.005) reduction in disomy compared with trisomy in the *tel1 mec1* diploid.
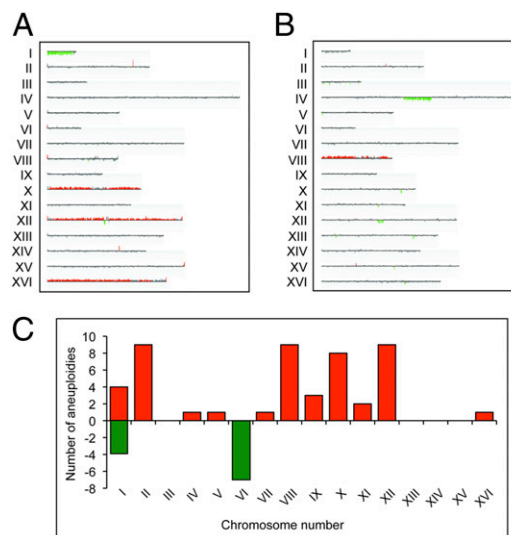

Fig. 1. Microarray analysis of aneuploidy and chromosome rearrangements in *tel1 mec1* diploids. Representative microarrays of two subcultured strains are shown (CGH Miner depiction). Each chromosome is shown as a single line with losses and gains of chromosomes or chromosome segments indicated in green and red, respectively. (*A*) The *tel1 mec1* diploid JLMy80-1s is monosomic for chromosome I and trisomic for chromosomes X, XII, and XVI. (*B*) Strain JLMy81-1L is trisomic for chromosome VIII and contains an interstitial deletion on chromosome IV. (*C*) Distribution of trisomies (red) and monosomies (green) in 20 independently subcultured *tel1 mec1* strains. Chromosomes II, VIII, X, and XII became trisomic with the highest frequencies.

Methods). We examined the frequency of aneuploidy and other changes in gene dosage in these strains by comparative genome hybridization (CGH) microarrays and alterations in the sizes of chromosomal DNA molecules by contour-clamped homogeneous electrical field (CHEF) gel analysis. No alterations were present in the mutant diploids before subculturing.

Aneuploidy in *tel1 mec1* Diploids. From our CGH analysis, it was clear that the *tel1 mec1* subcultured diploids had very high frequencies of aneuploidy and other changes in gene dosage that reflected chromosome rearrangements. For example, strain JLMy80-1s (Fig. 1*A*) was monosomic for chromosome I and trisomic for chromosomes X, XII, and XVI, whereas strain JLMy81-1L (Fig. 1*B*) was trisomic for chromosome VIII and had an interstitial deletion on chromosome IV. Of the 20 subcultured diploids analyzed, 18 were trisomic for one or more chromosomes and half were monosomic for chromosomes I and/or VI (Table S4).

Chromosomes II, VIII, X, and XII accounted for three-quarters of the trisomies, and chromosomes I and VI accounted for all the monosomies (Fig. 1*C*). By χ^2 goodness-of-fit tests (with Yates' correction), the distributions of trisomies and monosomies were very significantly (P < 0.001) different from random. Because chromosomes I and VI are the smallest chromosomes, it is likely that strains monosomic for other chromosomes have a selective growth disadvantage or that the small chromosomes have a lower rate of reduplication. Haploid strains that are disomic for chromosome VI are difficult to generate because of the imbalance created in the level of β-tubulin (encoded by a gene on chromosome VI) and α-tubulin (encoded by a gene on chromosome XIII) (30). Our results suggest that diploids may more readily tolerate this imbalance, a conclusion also supported by observations of Reid et al. (31).

We also examined the frequencies of aneuploid chromosomes in subcultured WT, *tel1*, and *mec1* diploids and in *tel1 mec1* haploids (Table 1 and Table S4). The *tel1 mec1* diploid strain had significantly more aneuploidy than any of these other strains.

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Thus, as for a number of other phenotypes, the *tel1* and *mec1* mutations have a synergistic or additive interaction for the aneuploidy phenotype. It is likely that the higher frequency of trisomy in the diploid *tel1 mec1* strains compared with the frequency of disomy in the haploid *tel1 mec1* strain reflects an ability of the cell to tolerate a 1.5-fold imbalance in gene expression of chromosomes more readily than a 2-fold imbalance.

Chromosome Rearrangements in *tel1 mec1* Diploids. We also diagnosed chromosome rearrangements using microarrays (*SI Text*). Not including aneuploidy or changes involving the ribosomal RNA, *CUP1*, or Y′ genes, we found 20 large (>10 kb) genomic deletions or duplications among the 20 subcultured strains (Tables S4 and S5).

Some of the chromosome rearrangements were characterized in detail (*SI Text*), and a few of these are indicated by arrows in Fig. 2. The strain JMY80-5L had a unique chromosome of about 1,350 kb. By both band microarray analysis (excision of the chromosome from a CHEF gel, followed by hybridization to a microarray; Fig. 3*B*) and Southern blot analysis of the CHEF gel (Fig. 3*C*), we showed that this chromosome had all the DNA of chromosome II, a portion of the left arm of chromosome III, and an interstitial segment of chromosome XII. We used standard Southern blot analysis to determine the orientation and connectivity of the chromosome fragments and PCR analysis to amplify the fusion junctions (*SI Text*). Sequence analysis of fusion junctions (Fig. 3*D*) showed that the breakpoint on chromosome II is at the telomere. The sequence junctions share no homology, indicating that the tripartite chromosome was a consequence of two NHEJ events. Although NHEJ events are suppressed in *MAT*a/*MAT*α diploids (32), JLMy80-5L is hemizygous at the mating type locus (Fig. 3*A*).

With the exception of JLMy80-5L, most chromosome rearrangements reflected changes in the lengths of tandemly repeated genes (e.g., the *CUP1* gene array; Fig. S1) or intrachromosomal or interchromosomal exchanges in dispersed Ty retrotransposons (Fig. S2). These rearrangements are characterized in detail in *SI Text*. Deletions and duplications of chromosomal sequences were observed more frequently in the *tel1 mec1* diploids than in the WT, *tel1*, or *mec1* diploids (Tables S4 and S5).

Restoring WT-Length Telomeres to *tel1 mec1* Strains Reduces the Frequency of Chromosome Rearrangements but Does Not Reduce the Frequency of Aneuploidy. To examine the relationship between telomere length and the increased levels of aneuploidy and chromosome rearrangements in *tel1 mec1* diploid cells, we introduced a plasmid (pVL1107-*URA3*) encoding a Cdc13-Est2 fusion protein (33) that allows telomere elongation independent of Tel1p and Mec1p (19). This plasmid restored WT-length telomeres to the *tel1 mec1* diploid (Fig. S3*A*) and substantially reduced the level of TTFs (Fig. S3*B*). The *tel1 mec1* strains with WT-length telomeres had the same frequency of aneuploidy as the *tel1 mec1* strains with the short telomeres (Table 1). In contrast, only 1 of 12 *tel1 mec1* diploids with WT-length telomeres had a chromosome alteration compared with 10 of 20 *tel1 mec1* strains with short telomeres (Tables S4 and S5). In addition, there were a total of 20 chromosomes with deletions/duplications in the *tel1 mec1* strains with short telomeres vs. a total of 2 chromosomes in the *tel1 mec1* strains with long telomeres.

These results demonstrate two important points. First, the high frequencies of aneuploidy and chromosome rearrangements are caused by two different types of mechanisms. Second, in *tel1 mec1* diploids, as in haploids (18), it is likely that many chromosome rearrangements are initiated by telomere fusions. This conclusion is somewhat surprising, because TTFs are a type of NHEJ, a recombination pathway that is suppressed in *MAT*a/*MAT*α diploids. Another observation that TTF formation and fusions between nontelomeric broken ends are regulated dif-
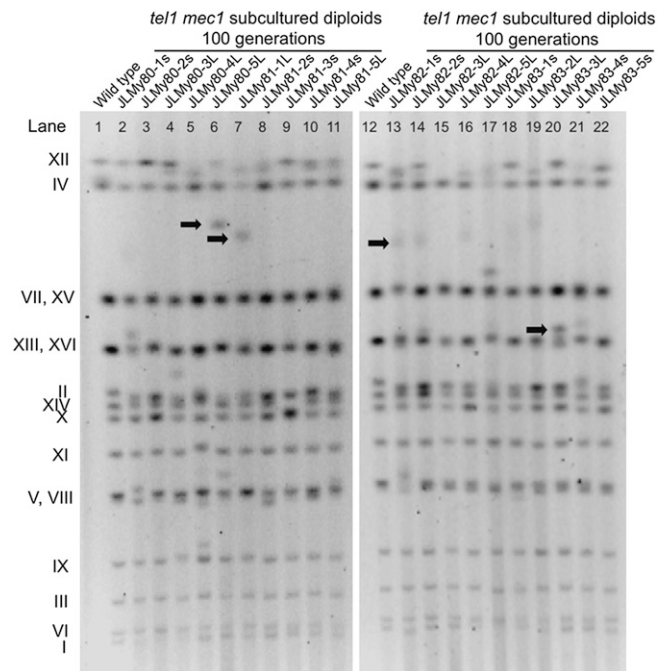

Fig. 2. CHEF gel analysis of DNA derived from subcultured *tel1 mec1* diploids. Separated yeast chromosomes were visualized by staining of the gel with ethidium bromide and exposure to UV light. Relative to the WT strains (lanes 1 and 12), *tel1 mec1* diploids often had chromosomes of altered size. Chromosomes indicated with arrows are discussed in the main text or in *SI Text*.

ferently is that the Nej1 protein, which promotes NHEJ events among nontelomeric broken ends, prevents telomere fusions in diploid yeast strains (34).

Investigation of the Relationship Between the Elevated Levels of Aneuploidy in *tel1 mec1* Diploids and the SAC. One large class of genes involved in chromosome disjunction in both yeast and mammalian cells is that involved in the SAC. In addition, in re-
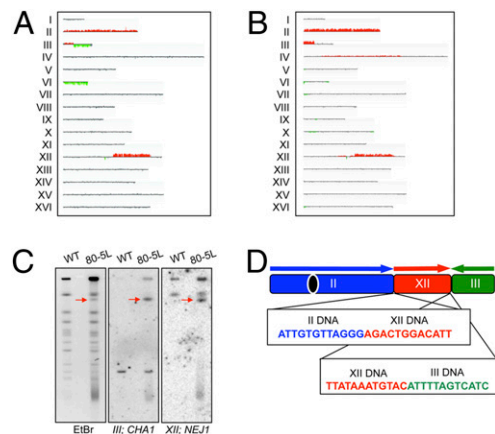

Fig. 3. Mapping of a tripartite chromosome rearrangement. (*A*) Genomic microarray analysis showed that the strain JLMy80-5L had an interstitial duplication on chromosome XII, a tandem deletion/duplication on chromosome III, trisomy for chromosome II, and monosomy for chromosome VI. (*B*) The 1,350-kb altered chromosome was excised from the CHEF gel (indicated by an arrow in lane 6, Fig. 2) and analyzed by a microarray, delimiting the regions derived from chromosomes II, III, and XII. (*C*) The unique chromosome hybridized to probes derived from chromosomes III (*CHA1*) and XII (*NEJ1*). (*D*) PCR amplification and sequencing of the breakpoint junctions revealed that the tripartite chromosome was formed through two NHEJ events.

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sponse to DNA damage, Tel1p and Mec1p activate the SAC. To examine the relationship between Tel1p, Mec1p, and the SAC, we first examined the aneuploidy associated with mutations in the known SAC genes *BUB1* and *MAD2* using microarrays. In diploids with the *bub1-Δ* mutation, eight of eight independent unselected *bub1-Δ* diploids examined by microarrays were trisomic and/or monosomic for ≥1 chromosome (Table S4). Interestingly, 16 of the 25 trisomic chromosomes observed were chromosomes II, VIII, and X (Table 2); these 3 chromosomes plus chromosome XII were the most commonly observed trisomes in the *tel1 mec1* diploid. Strains monosomic for chromosomes III, IX, XI, and XIV were observed, whereas only monosomy for chromosomes I and VI was seen in the *tel1 mec1* diploids.

We extended our analysis to examine diploids with the *bub1-ΔK* allele. This deletion of the kinase domain of Bub1p results in a strain that has a functional kinetochore-occupancy SAC but a defective tension-sensing SAC (23). After ≈100 cell divisions, 12 of 12 independent isolates were trisomic and/or monosomic for multiple chromosomes (Table S4). Both the frequencies of aneuploidy and the types of chromosomes that become trisomic are similar to the observations with the *tel1 mec1* diploids, except that the *bub1-ΔK* strains have higher frequencies of trisomy for chromosome XVI and lower levels of trisomy for chromosome XII (Tables 1 and 2).

In contrast, subcultured *mad2* diploids had much lower frequencies of aneuploidy (Table 2). Because *mad2* diploids lack all SAC function, these observations argue that aneuploidy in *tel1 mec1* and *bub1* diploids reflects a defect in chromosome segregation that is, at least in part, independent of the SAC. We also examined aneuploidy in triple-mutant *tel1 mec1 bub1-ΔK* diploids. The frequency of aneuploidy exceeded the frequencies observed in *tel1 mec1* and *bub1-ΔK* strains.

Because yeast strains with a defective SAC are sensitive to the microtubule-destabilizing drug benomyl (35, 36), we investigated the benomyl sensitivity of the *tel1 mec1* strain. As shown in Fig. 4A, the *tel1 mec1* strain grew poorly on all plates, including the plate lacking benomyl. The same strain with the pVL1107-*URA3* plasmid, which restores WT-length telomeres to the *tel1 mec1* strain, had better growth on all plates and did not exhibit obvious sensitivity to benomyl.

We also tested whether *tel1 mec1* strains arrest the cell cycle in response to benomyl by examining individual cells in the presence of the drug. We treated haploid *MAT*a WT, *tel1 mec1*, and *mad2* strains with α-factor to arrest the cells in G1 phase. We then removed α-factor and plated the cells on rich solid growth medium (YPD) containing benomyl or lacking benomyl. We photographed cells that were unbudded (>80% after α-factor treatment) and reexamined the same cells after 8 h at room temperature. In the absence of benomyl, about 80% of the WT and *mad2* cells budded more than once. In the presence of benomyl, 97% of the WT cells arrested as doublets, as expected for cells with a functional SAC (Fig. 4B and Table S6). In contrast, most (78%) of the *mad2* cells continued to divide. Less than half of the *tel1 mec1* cells produced a bud in the presence or absence of benomyl, as expected, because *tel1 mec1* cultures contain many cells incapable of division (14). Of those cells producing a bud, however, most (97%) arrested as doublets in the presence of benomyl. Thus, *tel1 mec1* strains have a functional SAC.

Discussion

As discussed in the introductory section, CIN tumors have very elevated rates of chromosome rearrangements and aneuploidy. Our observations of diploid *tel1 mec1* strains show that both of these phenotypes can be produced by a single genotype. The main conclusions from our study are as follows: (*i*) the elevated frequencies of chromosome rearrangements and aneuploidy in *tel1 mec1* strains reflect two different mechanisms, (*ii*) the patterns of aneuploidy in the *tel1 mec1* and *bub1* strains are similar, and (*iii*)

Table 2. Number of trisomic/tetrasomic and monosomic (in parentheses) chromosomes in strains with mutations in the SAC genes and/or DNA damage checkpoint genes*

Chromosome no.	bub1-Δ diploid (SC 0)	bub1-ΔK diploid (SC 5)‡	tel1 mec1 bub1-ΔK diploid (SC 5)‡	mad2 diploid (SC 5)
I	1	1	8	1 (1)
II	3	5	9	0
III	2 (1)	4	2	0
IV	0	0	1	0
V	3	1	4	0
VI	0	2	0	0
VII	0	0	0	0
VIII	8	8	11	2
IX	0 (1)	0	1 (1)	0
X	5	5	11	0
XI	0 (2)	0 (2)	1	1
XII	0	0	4	0
XIII	0	1	1	0
XIV	1 (1)	2	1	0
XV	0	0	0	0
XVI	2	6	7	0
Total no. trisomic (monosomic) chromosomes	25 (5)	35 (2)	61 (1)	4 (1)
Total no. chromosomes analyzed†	128	192	192	128
Aneuploid/euploid chromosomes	30/98	37/155	62/130	5/123

*We examined aneuploidy using microarrays. Strains with the null mutation of *BUB1* (*bub1-Δ*) had very high frequencies of aneuploidy without subculturing (indicated as SC 0). Strains of the other three genotypes were subcultured five times (SC 5). The numbers outside parentheses represent the number of strains in which trisomy (or, rarely, tetrasomy) was observed for a given chromosome; the numbers inside parentheses represent the number of times we observed monosomy.

†Total number of chromosomes was calculated by multiplying the number of independent strains examined by 16, the number of homologs in *S. cerevisiae*.

‡Genotypes of *bub1-ΔK* and *tel1 mec1 bub1-ΔK* were not free of aneuploidy at SC 0; the data show all genetic alterations at SC 5 regardless of whether they existed at SC 0 or not. The complete spectra of aneuploidy for these strains at SC 0 and SC 5 are shown in Table S4.

the aneuploidy in *tel1 mec1* strains is not a consequence of a defective SAC.

Chromosome Rearrangements. As in our previous study of haploids (20), most of the chromosome rearrangements in the diploid *tel1 mec1* strain are a consequence of homologous recombination among Ty elements. The frequency of chromosome rearrangements was substantially reduced in *tel1 mec1* strains that expressed the Cdc13-Est2 protein. Because these strains have fewer TTFs than strains without the Cdc13-Est2 fusion protein (Fig. S3B), one simple interpretation of this result is that most chromosome rearrangements in *tel1 mec1* strains are initiated as a consequence of breakage of dicentric chromosomes resulting from telomere fusions. DSB formation in a Ty element could be repaired by recombination with an ectopic Ty element, leading to chromosome rearrangements (20). An alternative model is that chromosomes in the *tel1 mec1* background are degraded from the ends until a Ty element is rendered single-stranded and recombinogenic (37).

Frequencies of Aneuploidy in *tel1 mec1* and in SAC-Deficient Strains. Strains with both *tel1* and *mec1* mutations had significantly elevated frequencies of aneuploidy compared with either single-mutant strain, although the *mec1* strain also had a high frequency of aneuploidy, as expected (9, 38). We interpret this result as indicating that Tel1p and Mec1p have functionally redundant roles in chromosome disjunction similar to their functionally redundant roles in DNA damage checkpoints and telomere length regulation.
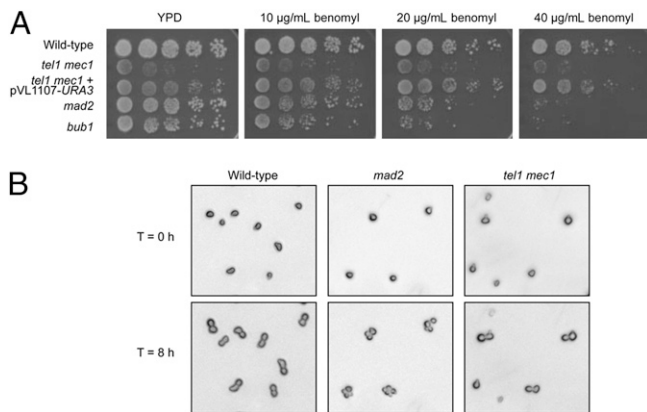
Fig. 4. The *tel1 mec1* cells have a functional SAC in response to the microtubule-destabilizing drug benomyl. (*A*) Ten-fold serial dilutions of cells from diploid WT (JLMy101), *tel1 mec1* (JLMy80-Trp⁻), *tel1 mec1* + pVL1107-*URA3* (JLMy1262-Trp⁻), *mad2* (JLMy366), and *bub1* (JLMy156) strains were spotted onto solid medium containing various amounts of benomyl. (*B*) Haploid WT (JLMy62-7c), *tel1 mec1* (JLMy62-18c-Trp⁻), and *mad2* (JLMy309) strains were arrested in α-factor, sonicated, and released onto plates containing 70 μg/mL benomyl. The cell cycle progression of individual cells was followed for 8 h. Representative pictures show that whereas *mad2* cells fail to arrest at G2/M following exposure to benomyl, WT and *tel1 mec1* strains demonstrate robust cell cycle arrest. By microscopic examination, we determined that the small protuberances seen in the WT and *tel1 mec1* cells represent "shmoos" (cell shapes produced by long incubation of cells in α-pheromone) rather than buds.

Because there is considerable heterogeneity in the growth rates of various aneuploid strains and many *tel1 mec1* cells fail to give rise to viable colonies, it is difficult to calculate an accurate rate of chromosome nondisjunction from the frequency data. As a crude estimate, we calculate that the probability of detecting aneuploidy in 100 cell divisions, if the rate of nondisjunction per cell division is 10^{-3}, is $1 - (0.999)^{100}$ or about 0.1; if the rate of nondisjunction in 100 divisions is 10^{-2}, the probability of detecting aneuploidy in 100 divisions is $1 - (0.99)^{100}$ or about 0.7. Thus, the rate of nondisjunction per chromosome per cell division is about 10^{-2} to 10^{-3} for the most frequently lost chromosomes.

The frequency of nondisjunction was also very high in the *bub1-Δ* and *bub1-ΔK* strains but not in the *mad2* strain. Warren et al. (39) previously observed that *bub1* mutants had about 10-fold higher chromosome loss rates than *mad2* strains and suggested that Bub1p (but not Mad2p) had a role in chromosome segregation in the absence of induced spindle damage. A variety of experiments have indicated that Bub1p is important in establishing the correct inner and outer kinetochore structures (40), and cells with low levels or no Bub1p have defective associations between the kinetochore and microtubules, decreased levels of chromosomes that are bioriented at the spindle, and decreased sister chromatid cohesion at the centromere (41). Thus, loss of Bub1p (unlike the loss of Mad2p) leads to defects in the mechanics of chromosome segregation in addition to defects in the SAC, resulting in very high levels of aneuploidy.

Nonrandom Recovery of Trisomic Chromosomes in *tel1 mec1* and in SAC-Deficient Strains. There is a preference for the recovery of chromosomes II, VIII, X, and XII as trisomes in the *tel1 mec1* strain (Table 1). If we perform a χ^2 goodness-of-fit test for these same four chromosomes in the *bub1-Δ* and *bub1-ΔK* strains, there is a very significant ($P < 0.001$) nonrandomness in their recovery (Table 2). There are three plausible explanations for the preferential recovery of certain chromosomes as trisomes. First, certain chromosomes, when present in three copies in a diploid, may greatly reduce cell growth rates. This explanation is unlikely for two reasons: in nine strains with trisomic chro-
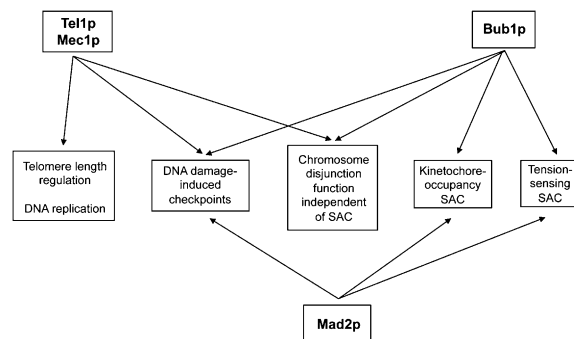

Fig. 5. Pathways involving Tel1p, Mec1p, and SAC proteins. The indicated associations of proteins and pathways are based on information provided in the main text.

mosomes resulting from γ-irradiation of diploids, a broad spectrum of trisomies was observed that did not include chromosome II or X (42) and Torres et al. (30) observed that all yeast chromosomes, except chromosome VI, were recoverable as disomes in haploids. Second, the presence of certain trisomic chromosomes may alleviate some of the negative effects of *tel1 mec1* and/or *bub1* mutations. For example, we previously showed that chromosome VIII disomy in *tel1 mec1-21* haploids was suppressed by *DNA2*, a gene located on chromosome VIII (20). A third possibility is that the defect in chromosome segregation observed in the *tel1 mec1* and *bub1* strains affects some chromosomes more than others. We suggest that the nonrandom recovery of trisomes is likely to reflect the two latter explanations, possibly arguing a similarity in the mechanistic chromosome disjunction defects in the *tel1 mec1* and *bub1* strains.

Cellular Roles of Tel1p and Mec1p in Regulating Aneuploidy. Our analysis demonstrates that Tel1p and Mec1p have an important role in chromosome segregation in addition to their previously demonstrated roles in DNA repair and telomere length regulation. Elevated rates of chromosome loss have been observed in mutants affecting kinetochores, microtubules, the spindle pole body, sister chromatid cohesion, DNA replication/repair, and the SAC. Our results rule out an involvement of Tel1p and Mec1p in the SAC, although we cannot define what other role these proteins have in chromosome segregation.

One possibility is that Tel1p and Mec1p affect chromosome segregation in the same pathways affected by Bub1p related to kinetochore function (Fig. 5). An argument in favor of this possibility is the similar patterns of trisomies observed in *tel1 mec1* and *bub1* strains. An argument against this possibility is that the chromosome segregation defect of the triple-mutant *tel1 mec1 bub1-ΔK* strain is more extreme than the defects of the *tel1 mec1* or *bub1-ΔK* strain. Although this result could be interpreted as indicating that Tel1p/Mec1p and Bub1p affect different chromosome disjunction mechanisms, it is also possible that the Tel1p/Mec1p effects are partially redundant with those of Bub1p, acting in the same pathway. Finally, we point out that direct investigations of kinetochore function are very difficult in *tel1 mec1* strains because this genotype results in a high rate of nonviable cells and heterogeneity in growth rates among the viable cells (14).

Materials and Methods

Strain Construction and Subculturing of Yeast Strains. All strains were constructed in the MS71 background (MATα *ade5-1 leu2-3 trp1-289 ura3-52 his7-2 LEU2*) (43) using standard techniques (44). Because the lethality of a *mec1* null allele can be rescued by a concurrent deletion of *SML1* (15), all *mec1::NAT* strains in our study had a *sml1::HYG* deletion as well. Details of the constructions are provided in *SI Materials and Methods* and in Tables S1–S3. The plasmid pVL1107 contains *LEU2* and a gene encoding a Cdc13-Est2 fu-

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sion protein (33). In the related plasmid (pVL1107-*URA3*), the *LEU2* gene was replaced by *URA3* (*SI Materials and Methods*).

Because *tel1 mec1* strains are genomically unstable, the diploid strains were constructed with a WT copy of *MEC1* on a *TRP1*-marked plasmid (pSAD3-3b/*MEC1*) (45). Four isogenic diploids (JLMy80–83) were constructed by mating spores derived from JLMy62 (details are provided in Table S2). To examine genomic instability, we identified derivatives of each diploid that had lost the plasmid (subculture 0) and subcultured these derivatives by streaking the cells on YPD (46), allowing the cells to grow for 2 to 3 days at 30 °C (subculture 1). Five individual colonies were picked from subculture 1 of these Trp⁻ derivatives of JLMy80–83 and separately subcultured four more times on plates containing YPD, picking cells from the heavy growth region of the plate. Each subculture equals ≈20 cell divisions. The 20 subcultured derivatives were retransformed with pSAD3-3b/*MEC1* before analysis.

Physical Analysis of Chromosome Rearrangements and Aneuploidy. The sizes of rearranged chromosomes were determined by CHEF gel analysis and Southern blotting techniques, as previously described (47). We also used CGH microarrays to diagnose the breakpoints of chromosome rearrangements and to examine changes in chromosome number. The details of the gel and microarray analyses are given in *SI Text*.

Statistical Analysis of the Frequency of Aneuploidy in *tel1 mec1* and Related Strains. For independently subcultured strains of each genotype, we calculated the number of aneuploid and euploid chromosomes. These numbers were compared in strains of different genotypes using the Fisher exact probability test or χ^2 analysis tool on the VassarStats Web site (http://faculty.vassar.edu/lowry/VassarStats.html).

Assay of Benomyl Sensitivity and Assay of the SAC in Response to Benomyl. We examined the ability of WT, *tel1*, *mec1*, and *tel1 mec1* cells to grow on YPD plates containing concentrations of benomyl (Sigma) of 10, 20, or 40 μg/mL (details provided in *SI Text*). We also determined the fraction of cells (unbudded at the start of treatment with 70 μg/mL benomyl) that arrested as doublets after 8 h of treatment.

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