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Suppression of Spontaneous Chromosomal Rearrangements by S Phase Checkpoint Functions in *Saccharomyces cerevisiae*

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Summary

Cancer cells show increased genome rearrangements, although it is unclear what defects cause these rearrangements. Mutations in *Saccharomyces cerevisiae RFC5*, *DPB11*, *MEC1*, *DDC2 MEC3*, *RAD53*, *CHK1*, *PDS1*, and *DUN1* increased the rate of genome rearrangements up to 200-fold whereas mutations in *RAD9*, *RAD17*, *RAD24*, *BUB3*, and *MAD3* had little effect. The rearrangements were primarily deletion of a portion of a chromosome arm along with *TEL1*-dependent addition of a new telomere. *tel1* mutations increased the proportion of translocations observed, and in some cases showed synergistic interactions when combined with mutations that increased the genome rearrangement rate. These data suggest that one role of S phase checkpoint functions in normal cells is to suppress spontaneous genome rearrangements resulting from DNA replication errors.

Introduction

Genome instability is a characteristic of cancer cells (Kolodner, 1996; Jiricny, 1998; Kinzler and Vogelstein, 1998; Lengauer et al., 1998; Coleman and Tsongalis, 1999; Vessey et al., 1999). The genetic changes that occur in cancer cells include mutations such as base substitution and frameshift mutations, as well as deletions and translocations found to inactivate tumor suppressor genes and activate proto-oncogenes. Other types of changes include chromosomal rearrangements identified in loss of heterozygosity studies, the large changes of chromosome region copy number seen in comparative genome hybridization studies, aneuploidy, and gene amplification (Ponder and Cavenee, 1995; Hermsen et al., 1996; Klein et al., 1999). It is generally accepted that the accumulation of genetic changes is required for the development and progression of cancer cells (Loeb, 1991). Genetic changes can have dramatic effects with even a single genetic alteration, causing significantly increased cancer predisposition or progression, whereas in other cases, genetic changes are more likely to cause modest effects during the evolution of a cancer cell (Ilyas et al., 1999).

Comparatively less is known about the origin of genetic changes seen in cancer cells. Cancer cells may require the acquisition of some type of mutator phenotype to drive the accumulation of genetic changes (Loeb, 1991). Alternately, it has been suggested that selection can account for the accumulation of genetic changes (Tomlinson et al., 1996). In this latter case, the selected genetic changes are presumably due to rare errors in DNA metabolism, possibly in combination with occasional DNA damage. In the case of inherited and acquired mismatch repair (MMR) defects, the resulting high mutation rate appears to drive cancer progression, possibly in combination with defects in apoptotic responses caused by loss of MMR (Kolodner and Marsischky, 1999; Li, 1999). In the case of a number of rare inherited cancer susceptibility syndromes, chromosome spreads from lymphoblasts of patients show increased numbers of chromosomes with breaks and other types of rearrangements (Shiloh, 1997; Coleman and Tsongalis, 1999; Vessey et al., 1999). Tumors from BRCA1 and BRCA2 patients and BRCA1 and BRCA2 mutant mouse cells appear to have increased numbers of broken and abnormal chromosomes (Sharan et al., 1997; Gretarsdottir et al., 1998; Patel et al., 1998; Tutt et al., 1999; Xu et al., 1999). Finally, cells containing p53 mutations exhibit increased genome instability in response to treatment with DNA-damaging agents (Hartwell, 1992; Levine, 1997). These examples suggest that the integrity of chromosome structure may be genetically controlled, much as in the case of MMR. However, it is unclear whether this increased chromosomal instability is due to single gene defects, how much the rates of genome instability are increased, and what metabolic defects underlie the observed chromosome abnormalities.

Recently, a new class of mutator mutants has been described in *Saccharomyces cerevisiae* (Chen et al., 1998; Chen and Kolodner, 1999). The rate of accumulation of three classes of genome rearrangements, called gross chromosomal rearrangements (GCRs), was observed to be increased: interstitial deletions; deletion of a chromosome arm combined with addition of a new telomere (referred to as telomere additions); and nonreciprocal translocations with either microhomology or nonhomology at the rearrangement breakpoint. Increased rates of accumulation of GCRs were caused by *rfa* mutations that resulted in repair and recombination defects, by *rad27* mutations that cause repair and replication defects, and by mutations in some genes required for repair of double-strand-breaks (DSBs) like *MRE11*, *RAD50*, and *XRS2*, but not by mutations in other genes required for repair of DSBs like *RAD51*, *RAD54*, and *RAD57*. The results of double mutant and rearrangement breakpoint sequence analysis suggested the existence of multiple pathways for the suppression of GCRs. However, it is unclear whether mutations in these genes result in increased damage that leads to GCR formation or defects in the nonmutagenic repair of some type of naturally occurring DNA metabolic error.

Relatively little is known about the molecular mechanisms that give rise to GCRs, nor is it clear what type(s) of spontaneous DNA damage underlie GCRs. Interestingly,

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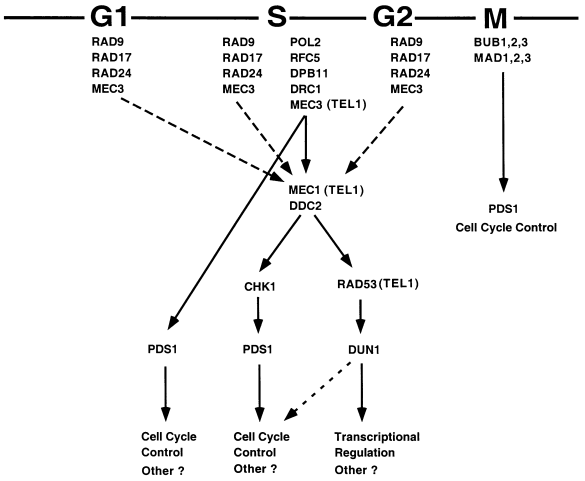

Figure 1. Genes and Checkpoint Pathways in *S. cerevisiae*

The horizontal line indicates the cell cycle. The genes known to play a role in each checkpoint are listed below each phase of the cell cycle. The central signal transduction pathway involving *MEC1*, *DDC2*, *RAD53*, *CHK1*, *PDS1*, and *DUN1* that processes signals from the G1, S, and G2 checkpoints is indicated below all three pathways. The dashed lines indicate sensor responses that are specific to treatment with DNA damaging agents. Note that *PDS1* is known to function both below *CHK1* in response to DNA damage, independently of *MEC1* in response to replication blocks and in the mitotic (M) spindle checkpoint. The dotted line indicates *DUN1* is known to be required for cell cycle arrest in response to DNA damage but not in response to all types of replication blocks. *TEL1* is positioned beside *MEC3*, *MEC1*, *DDC2*, and *RAD53* because it genetically interacts with these genes in the studies described here and it also has been shown, in other studies, to interact with many of the genes listed in the S phase checkpoint and downstream signal transduction pathway.

some of the homologs of the proteins encoded by GCR mutator genes are phosphorylated in response to DNA damage in human cells (Brush et al., 1996; Freid et al., 1996; Dong et al., 1999; Kim et al., 1999; Gatei et al., 2000; Lim et al., 2000), others may play a role in detecting DNA damage (Maser et al., 1997; Lee et al., 1998), and *S. cerevisiae* XRS2 is a substrate for phosphorylation by MEC1 and TEL1 (personal communication from J. Mallory, K. Trujillo, P. Sung, and T. Petes) suggesting a possible role of checkpoints in suppressing spontaneous GCRs. Cell cycle checkpoints are surveillance mechanisms designed to ensure correct transmission of genetic information during the cell division cycle (Weinert, 1998a). Cell cycle checkpoints detect both DNA damage and failure to properly assemble the mitotic spindle, and through phosphorylation of key proteins, promote both cell cycle arrest, to allow time for repair to occur, as well as trancriptional and other changes that may effect DNA repair more directly (Elledge, 1996; Paulovich et al., 1997; Weinert, 1998a; Amon, 1999; Bashkirov et al., 2000). Thus, cell cycle checkpoints are thought to be crucial in maintaining genome stability in response to damage to DNA and/or the mitotic apparatus. Here, we demonstrate that defects in *S. cerevisiae* S phase checkpoint genes cause increased GCR rates in the absence of exogenous sources of DNA damage. This indicates that one of the normal roles of S phase checkpoint functions is to suppress GCRs that result from the aberrant repair of DNA damage that normally occurs during DNA replication or from the misfiring of origins of replication (Santocanale and Diffley, 1998; Shirahige et al., 1998).

Table 1. Effect of Mutations in Sensor Genes on the Gross Chromosomal Rearrangement Rate

Strain	Relevant Genotype	GCR Rate (Can^r-5FOA^r)
RDKY3615	wild type*	3.5×10^{-10} (1)
RDKY3717	*bub3*Δ	3.9[2.0–5.9] $\times 10^{-10}$ (1.1)
RDKY3715	*mad3*Δ	2.4[1.0–3.8] $\times 10^{-10}$ (0.7)
RDKY3719	*rad9*Δ	2.0[1.0–2.3] $\times 10^{-9}$ (6)
RDKY3721	*rad17*Δ	3.0[2.2–3.5] $\times 10^{-9}$ (9)
RDKY3723	*rad24*Δ	4.0[2.3–6.1] $\times 10^{-9}$ (11)
RDKY3727	*rfc5-1**	6.6[3.1–10.1] $\times 10^{-8}$ (189)
RDKY4538	*dpb11-1*	9.0[8.2–9.8] $\times 10^{-8}$ (257)
RDKY3725	*mec3*Δ	1.9[1.3–2.5] $\times 10^{-8}$ (54)

All strains are isogenic with the wild-type strain, RDKY3615 [*MATa, ura3-52, leu2*Δ*1, trp1*Δ*63, his3*Δ*200, lys2*Δ*Bgl, hom3-10, ade2*Δ*1, ade8, hxt13::URA3*] with the exception of the indicated mutations, which are described under "Experimental Procedures." ( ) indicates rate relative to the wild-type rate. [ ] indicates the highest and lowest rates observed in the different fluctuation tests.

* The GCR rates in the chromosome VII assay using RDKY4479 and RDKY4481 (*rfc5-1*) to measure the rate of production of 5FOA^r, His$^-$ cells were 1.5[1.0–2.0] $\times 10^{-9}$ (1) and 4.7[3.8–5.6] $\times 10^{-7}$ (313), respectively.

Results

Sensors of S Phase DNA Damage Suppress GCR Formation

In *S. cerevisiae*, at least three different groups of checkpoint proteins play a role in sensing damage during the cell cycle (Figure 1). POL2, RFC5, DPB11, DRC1, and MEC3 have been proposed to sense DNA damage and replication blocks that occur during S phase (Araki et al., 1995; Navas et al., 1995; Longhese et al., 1996; Sugimoto et al., 1997; Wang and Elledge, 1999). RAD9, RAD17, RAD24, and MEC3 are required to sense DNA damage in the G1 and G2/M phases of the cell cycle and these gene products are also thought to promote S phase arrest in response to DNA damage (Elledge, 1996; Paulovich et al., 1997; Weinert, 1998a). BUB1, BUB2, BUB3, MAD1, MAD2, and MAD3 are thought to sense spindle defects during mitosis (Hoyt et al., 1991; Roberts et al., 1994; Hardwick and Murray, 1995; Wang and Burke, 1995; Amon, 1999; Taylor, 1999). To determine if any of these checkpoints normally function to suppress GCRs, mutations in selected genes from each group were tested for their effect on GCR rates (Table 1). The predominant assay used here measures the rate of accumulation of GCRs that simultaneously delete a region of chromosome V containing *CAN1* and *URA3* integrated in *HXT13* 7.5 kb telomeric to *CAN1* resulting in the production of Can^r, 5-FOA^r cells (Figure 2A). In addition, selected mutations were tested in a GCR assay that similarly measures deletion of a nonessential arm of chromosome VII containing *URA3* integrated into *ZRT1* and *HIS3* integrated into *ADH4*, ~21 kb and ~15 kb from the telomere, respectively, resulting in the production of 5-FOA^r, His$^-$ cells. Mutations in *BUB3* and *MAD3* had no effect on the GCR rate. Mutations in *RAD9*, *RAD17*,

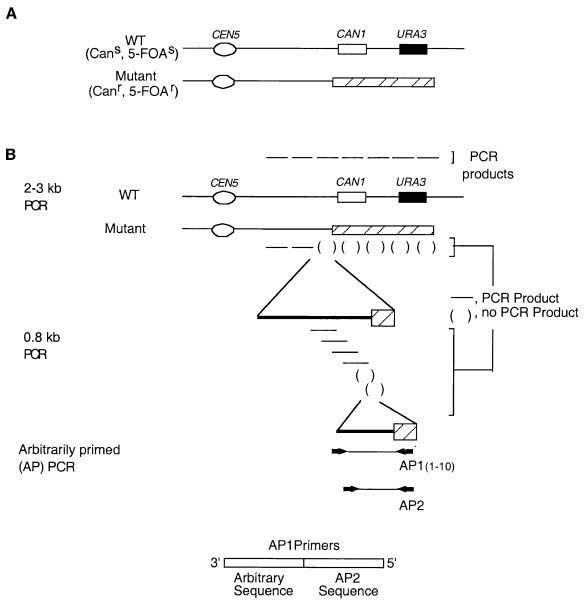

Figure 2. Assays for Characterizing GCR Rates and Breakpoint Sequences

(A) A schematic representation of chromosome V is presented showing *CAN1* and the *URA3* insertion into *HXT13*. Selection of Can^r 5-FOA^r cells results in the growth of cells in which both *CAN1* and *URA3* have been inactivated. Since the rate of independent mutation of both genes is calculated to be 10^{-12} to 10^{-14} per generation, this selects for rearrangements in which the *CAN1 hxt13::URA3* region has been lost due to genome rearrangements resulting in a new arrangement of DNA sequences on chromosome V as indicated by the hatched segment.

(B) Breakpoint sequences were mapped to single ORF resolution by PCR analysis of DNA from mutants using ORF-specific primer pairs for YEL059 through YEL063; this located the breakpoint to the first ORF in the telomeric direction that did not yield a PCR product. The breakpoint was further refined by PCR analysis of the relevant region using 5 to 8 pairs of PCR primers that amplified overlapping ~800 bp segments that were progressively shifted by ~200 bp; by analysis of the primer pairs that did and did not amplify fragments from the mutant DNA, it was possible to locate the breakpoint to ~400 bp. A fragment containing the breakpoint was then amplified using a unique PCR primer complementary to sequences centromeric to the breakpoint and one of 10 arbitrary primers (AP1 primers, sequences under Experimental Procedures). The resulting fragment was reamplified using a unique primer internal to the first stage arbitrary PCR unique primer and a primer (AP2 primer, sequence under Experimental Procedures) complementary to the AP1 primer. The resulting PCR fragment, which was generated only from Can^r 5-FOA^r cells, was sequenced. In the case of translocations, the breakpoint was independently verified by PCR with primers designed to be located in unique sequences on each side of the breakpoint followed by sequencing the resulting PCR product.

and *RAD24* caused small, 10-fold or less increases in the rate of GCR formation. The *rfc5-1* mutation, which causes a defect in the S phase checkpoint that senses DNA replication defects, caused 190-fold and 313-fold increases in the GCR rate in the chromosome V and chromosome VII assays, respectively. The *dpb11-1* mutation caused a 257-fold increase in the GCR rate. A mutation in *MEC3* caused a 54-fold increase in the rate of GCR formation. Because MEC3 plays a role in the G1, S, and G2/M DNA damage checkpoints and the S phase checkpoint that senses DNA replication defects

Table 2. Effect of Mutations in Central Signal Transduction Pathway Genes on the Gross Chromosomal Rearrangement Rate

Strain	Relevant Genotype	GCR Rate (Car^r-5FOA^r)
RDKY3615	wild type*	3.5×10^{-10} (1)
RDKY3733	*sml1*Δ	$3.1[3.0–3.13] \times 10^{-10}$ (0.9)
RDKY3735	*mec1*Δ *sml1*Δ†	$6.8[6.5–7.2] \times 10^{-8}$ (194)
RDKY4494	*ddc2*Δ *sml1*Δ	$5.7[1.4–8.6] \times 10^{-8}$ (163)
RDKY3739	*dun1*Δ*	$7.3[4.1–10.2] \times 10^{-8}$ (208)
RDKY3741	*dun1*Δ *sml1*Δ	$7.4[6.4–8.4] \times 10^{-8}$ (211)
RDKY3749	*rad53*Δ *sml1*Δ	$9.5[8.0–11] \times 10^{-9}$ (27)
RDKY3745	*chk1*Δ	$1.3[0.9–1.6] \times 10^{-8}$ (37)
RDKY3747	*chk1*Δ *sml1*Δ	$2.0[2.0–3.0] \times 10^{-8}$ (57)
RDKY3751	*rad53*Δ *chk1*Δ *sml1*Δ	$2.3[2.1–2.6] \times 10^{-8}$ (64)
RDKY3753	*mec1*Δ *rad53*Δ *sml1*Δ†	$1.0[0.8–1.6] \times 10^{-7}$ (286)
RDKY3755	*mec1*Δ *chk1*Δ *sml1*Δ	$6.6[6.0–7.5] \times 10^{-8}$ (189)
RDKY3757	*mec1*Δ *dun1*Δ *sml1*Δ	$5.3[4.8–6.4] \times 10^{-8}$ (151)
RDKY4496	*mec1*Δ *ddc2*Δ *sml1*Δ	$5.2[5.1–5.3] \times 10^{-8}$ (149)
RDKY4500	*rfc5-1 mec1*Δ *sml1*Δ	$1.0[0.95–1.1] \times 10^{-7}$ (286)
RDKY4475	*rfc5-1 dun1*Δ	$5.9[5.8–6.2] \times 10^{-8}$ (168)
RDKY3729	*pds1*Δ	$6.7[6.0–7.2] \times 10^{-8}$ (190)
RDKY3823	*pds1*Δ *mec1*Δ *sml1*Δ	$3.2[2.8–4.2] \times 10^{-7}$ (914)

All strains are isogenic with the wild-type strain, RDKY3615 [*MATa, ura3-52, leu2*Δ*1, trp1*Δ*63, his3*Δ*200, lys2*Δ*Bgl, hom3-10, ade2*Δ*1, ade8, hxt13::URA3*] except for the indicated mutations, which are described under "Experimental Procedures." ( ) indicates rate relative to the wild-type rate. [ ] indicates the highest and lowest rates observed in the different fluctuation tests.

* The GCR rates in the chromosome VII assay using RDKY4479 and RDKY4483 (*dun1*) to measure the rate of production of 5FOA^r, His$^-$ cells were $1.5[1.0–2.0] \times 10^{-9}$ (1) and $2.7[2.08–3.33] \times 10^{-7}$ (313), respectively.

† There is no significant difference between these two values based on Student's t test at the 95% confidence interval.

(Longhese et al., 1996; Weinert, 1998a), this effect of a *mec3* mutation could reflect the role of MEC3 in the S phase replication checkpoint, although it could also reflect a role of MEC3 in telomere maintenance (Corda et al., 1999). These data suggest that some type of DNA damage that can lead to GCR formation normally occurs during DNA replication and that a normal role of S phase checkpoint functions is to suppress such GCR formation.

The Central DNA Damage Signal Transduction Pathway Suppresses GCR Formation

The observation that *rfc5-1*, *dpb11-1*, and *mec3* mutations result in increased GCR rates suggested that the DNA damage signal transduction pathway might also be required for the suppression of GCRs. To investigate this possibility, mutations in the transducer genes *MEC1* and *DDC2* (Kato and Ogawa, 1994; Weinert et al., 1994; Paciotti et al., 2000) and the genes *RAD53*, *CHK1*, *PDS1*, and *DUN1* that function downstream of *MEC1* (Zhou and Elledge, 1993; Allen et al., 1994; Sanchez et al., 1996, 1999; Sun et al., 1996; Gardner et al., 1999; Liu et al., 2000) were tested for their effect on the rate of GCR formation (Table 2). Because MEC1, DDC2, and RAD53 are essential for cell viability, it was necessary to analyze *mec1 sml1*, *ddc2 sml1*, and *rad53 sml1* double mutants (Zhao et al., 1998). However, the *sml1* suppressor mutation did not appear to affect the GCR rates or breakpoints sequences by itself or in combination with other mutations such as *dun1* and *chk1* (See Tables 2 and 5).

Table 3. Effect of *tel1* Mutations on the Rate of Gross Chromosomal Rearrangements

Strain	Relevant Genotype	GCR Rate (Can^r-5FOA^r)	Fold Change Relative to		
			Wild type	*Tel1*$^+$	*mec1sml1*
RDKY3615	wild type*	3.5×10^{-10}			
RDKY3731	*tel1*Δ	2.0[1.8–2.2] $\times 10^{-10}$	0.6	0.6	
RDKY3733	*sml1*Δ	3.1[3.0–3.13] $\times 10^{-10}$	0.9	0.9	
RDKY3743	*mec1*Δ *tel1*Δ *sml1*Δ	4.5[4.4–4.8] $\times 10^{-6}$	12857	66	66
RDKY4498	*ddc2*Δ *tel1*Δ *sml1*Δ	4.3[2.6–8.3] $\times 10^{-6}$	12286	75	75
RDKY3773	*mec3*Δ *tel1*Δ	4.2[3.8–4.4] $\times 10^{-7}$	1200	22	
RDKY3767	*rad53*Δ *tel1*Δ *sml1*Δ	7.5[4.9–10.0] $\times 10^{-8}$	214	8	
RDKY3775	*rfc5-1 tel1*Δ	4.0[3.5–4.5] $\times 10^{-8}$	114	0.6	
RDKY3765	*chk1*Δ *tel1*Δ *sml1*Δ	1.2[1.0–1.3] $\times 10^{-8}$	34	1.6	
RDKY3763	*dun1*Δ *tel1*Δ *sml1*Δ	6.1[4.5–6.8] $\times 10^{-8}$	174	0.8	
RDKY3633	*mre11*Δ*	2.2[2.0–2.4] $\times 10^{-7}$	629		
RDKY3761	*mec1*Δ *mre11*Δ *sml1*Δ	2.9[2.8–3.0] $\times 10^{-6}$	8286		43
RDKY3759	*mre11*Δ *tel1*Δ	2.2[2.2–2.2] $\times 10^{-7}$	629	1	
RDKY4224	*tlc1*Δ	3.1[2.0–4.5] $\times 10^{-10}$	0.9	0.9	
RDKY4130	*mec1*Δ *tlc1*Δ *sml1*Δ	6.3[6.1–6.6] $\times 10^{-8}$	180		0.9

All strains are isogenic with the wild-type strain, RDKY3615 [*MATa, ura3-52, leu2*Δ*1, trp1*Δ*63, his3*Δ*200, lys2*Δ*Bgl, hom3-10, ade2*Δ*1, ade8, hxt13::URA3*], with the exception of the indicated mutations, which are described under "Experimental Procedures." Mutation rates for the *TEL1*$^+$ strains and the *mec1sml1* double mutant are from Tables 1 and 2. [ ] indicates the highest and lowest rates observed in the different fluctuation tests.

* The GCR rates in the chromosome VII assay using RDKY4479 and RDKY4485 (*mre11*) to measure the rate of production of 5FOA^r, His$^-$ cells were 1.5[1.0–2.0] $\times 10^{-9}$ (1) and 1.9[1.5–2.3] $\times 10^{-7}$ (127), respectively.

Mutations in *MEC1*, *DDC2*, and *DUN1* caused ~200-fold increases in the rate of GCR formation, which were comparable to those caused by the *rfc5-1* and *dpb11-1* mutations; a *dun1* mutation was also found to cause a 180-fold increase in the GCR rate in the chromosome VII assay. Mutations in *RAD53* and *CHK1*, which are thought to function parallel to each other downstream of *MEC1*, caused intermediate ~30- to 40-fold increases in the rate of GCR formation. The rate of GCR formation in the *rad53 chk1* double mutant was approximately additive compared to the effects of the respective single mutants, but was not as large as that caused by mutations in *MEC1* or *DUN1*. The *rfc5-1 dun1*, *mec1 dun1*, *ddc2 mec1*, or *rfc5-1 mec1* double mutant combinations and the double mutation combination of *mec1* with either *rad53* or *chk1* mutations caused increases in the GCR rate that were not significantly different from that caused by *rfc5-1*, *dpb11-1*, *mec1*, *ddc2*, and *dun1* single mutations. These data are consistent with previously published results indicating that RAD53, CHK1, and DUN1 function downstream of MEC1 and DUN1 functioning downstream of RFC5 (Sugimoto et al., 1997; Weinert, 1998b). The fact that the *rad53 chk1* double mutant does not have as high a GCR rate as a *mec1* or *dun1* single mutant suggests that not all of the signal from MEC1 goes through RAD53 and CHK1 or may reflect differences in the roles of MEC1 and RAD53. Overall, the effect of mutations in *MEC1*, *DDC2*, *RAD53*, *CHK1*, and *DUN1* is consistent with the idea that the central DNA damage signal transduction pathway functions in the suppression of GCRs in normal cells.

PDS1 appears to play multiple roles in checkpoints and cell cycle regulation. PDS1 was identified as an inhibitor of the initiation of anaphase (Yamamoto et al., 1996). Other studies have suggested that PDS1 also functions in the S phase and DNA damage checkpoints independently of MEC1 (Clarke et al., 1999), and also downstream from MEC1 and CHK1 because it is phosphorylated in a CHK1-dependent manner in response to DNA damage (Sanchez et al., 1999). A mutation in *PDS1* caused a strong increase in the rate of accumulation of GCRs comparable to those caused by mutations in *MEC1* and *DUN1*. This large increase in GCR rate is inconsistent with PDS1 just functioning downstream of CHK1 in suppression of GCRs because the *chk1* mutation caused a much smaller increase in the GCR rate than the *pds1* mutation. Consistent with this, the *pds1 mec1* double mutant had a 5-fold higher GCR rate than either respective single mutant (Table 2). These data suggest that while PDS1 may function downstream of CHK1 in suppression of GCRs, it likely plays other roles that could function in the suppression of GCRs, such as its role in the anaphase-metaphase transition and its MEC1-independent role in the S phase checkpoint.

TEL1 Plays a Role in the Suppression of GCRs

TEL1 was first identified as a gene in which mutations caused telomere shortening (Lustig and Petes, 1986; Greenwell et al., 1995; Morrow et al., 1995). TEL1, which is related to MEC1, is the closest *S. cerevisiae* homolog of the Ataxia Telangiectasia mutated (ATM) gene product and has been implicated in the DNA damage response (Sanchez et al., 1996; Vialard et al., 1998). A mutation in *TEL1* had no significant effect on the rate of accumulation of GCRs (Table 3). When the mutation in *TEL1* was combined with mutations in *MEC1*, *DDC2*, *MEC3*, and *RAD53*, a synergistic increase in the GCR rate of 66-fold, 75-fold, 22-fold, and 8-fold was observed relative to the respective *mec1*, *mec3*, and *rad53* single mutants. The *tel1* mutation had no effect on the GCR rate when combined with the *rfc5-1*, *chk1*, and *dun1* mutations. These results suggest that TEL1 plays a role in suppression of spontaneously occurring GCRs.

Rearrangement Breakpoint Analysis Suggests a Common Major Mechanism for GCR Formation in Checkpoint Mutants

Previous studies using the assay method described here detected 3 classes of GCRs: deletion of an arm of chro-

Table 4. Representative Examples of Telomere Additions and Translocations Breakpoints

Relevant Genotype	Breakpoint Type	Representative Breakpoint Sequences
*mec1*Δ *sml1*Δ *dun1*Δ	Deletion/ telomere addition	AGCACAAATTAGCAGAAAGAAGAGTGGgtgtggtgtgtgtgtgggtgtgg [33684] TTATCTGATCAAATGTTTTCGTTTTCGTGTGTGgtgtgggtgtgggtgtggttggtg [39765]
*mec1*Δ *tel1*Δ *sml1*Δ	Nonhomology translocations	V33941 33870 TAAAGTGTCCGAATTTTCAATAGGGCGAACT:TGAAGAATAACCAAGGTCAATAATATATCTTTTAGTATAAC CCCTTTTGCAATACTGGGATCTATTGTCCAT:GCTAATCACTGACATTTCGTTTGTGCTAATTGTCTTAGGCT V326258 326187
*mec1*Δ *tel1*Δ *sml1*Δ		V32675 32616 AAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAA:GGATAAAAACGAAGGGAGGTTCTT CAAACTCCCCACCTGACAATGTCTTCAACCCGGATC:AGCCCCGAATGGGACCTTGAATGC XII452569 452628
*tel1*Δ	Microhomology translocations	V40834 40752 AAAAATGCCAACGTTGTTGCGGTGAAAGTCTTGAG:ATCAAACGGGTCTGGTACCATGTCTGATGTCGTCAAAGGTGTCGAATA GGCATTTATGAACTACTTTTAGATAATCTAATGAG:CGAAAATTGGGAAATAAGGCATGGAGCTGCGTTGGGTCTTCGAGAATT XVI403202 403120
*pds1*Δ		V35240 35162 TTTGGCAATGCTGTTTAAGCCATCAATGTAAGGGACTA:TACTCATCATGGTTGGATCCCAGTTGACATCAATAATCTTC TGACCAACTTAGCTTATAATAAGAATGATAATCGATTA:GTCTAAATAGCATTCTTTCATTTGCGGCATCTTATAACCCA XII95092 950270
*mec3*Δ		V40969 40882 AACATCAACCACAAGGACTTCGAAAAGAGAGCCATTTGGGGGAAAACC:ATCCCACTTAACGACGAAGATCTCGACGGTAACGGCCA TTGGTATTTTCTTCTCCGCTACTTTGGCCGTGCTGGCCTTTTGAAAAT:ATCCCACTTAATGACGAAGAAGGTGCTTCACCGAAAAG V740201 740286
*mec3*Δ		V33283 33199 GAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAG:GAGAAGTACAGAACGCTGAAGTGAAGAGAGAGCTTAAGC GAAGAAGAAGAAGAAAATGATGATGAAGAAGATGAAGAAGATGAAG:AAGATGATGAAGATGATGAAGAAGAAGAAAAAAGAA XVI252317 252231

The structure of rearrangement breakpoints was determined as described under "Experimental Procedures." A representative example of each rearrangement breakpoint class is presented and the complete data set is available on request. For translocations, the numbers given above and below the sequences are the standard SGD nucleotide coordinates for the first and last nucleotide listed with the roman numerical indicating the chromosome number. The underlined nucleotides indicate those present in the translocation chromosome. The nucleotides in bold indicate identities. In the case of telomere additions, the number in [ ] indicates the SGC nucleotide coordinate of the last recognizable nucleotide of chromosome V before the added telomere sequences.

mosome V combined with addition of a new telomere referred to as telomere additions; nonreciprocal translocations with microhomology at the rearrangement breakpoint; and nonreciprocal translocations with nonhomology at the rearrangement breakpoint (Chen and Kolodner, 1999). In order to obtain insights into the rearrangement mechanisms that underlie the increased GCR rates observed in the mutator mutants discussed above, the breakpoint sequences of approximately 10 independent breakpoints per mutator strain were determined and classified. This analysis took advantage of the fact that one of the resulting breakpoints was always observed in *CAN1* or in the region containing four adjacent nonessential ORFs, YEL059 to YEL062, centromeric to *CAN1*. This observation allowed the development of a more rapid method for mapping and sequencing of the breakpoints (Figure 2B). The breakpoint junctions were then verified by amplification with junction-specific primers and the rearrangements were classified as translocations/deletions with nonhomology or microhomology breakpoints or deletions with an associated telomere addition (Chen et al., 1998; Chen and Kolodner, 1999). Representative breakpoint sequences from this analysis are presented in Table 4 and a summary of the different breakpoints observed is presented in Table 5; a database of all rearrangement sequences is available on request.

The rearrangements observed in single mutant strains in which the S phase checkpoint sensor or downstream signal transduction pathway were disrupted were predominantly telomere additions. In *rfc5-1*, *mec1*, and *dun1* single mutants, which have the largest increase in GCR rate, 100% of the GCRs were telomere additions. In the *mec3*, *rad53*, *chk1*, and *rad53 chk1* mutants, the majority of the GCRs were telomere additions, although translocations and a small proportion of deletions were also observed. The one exception was the *pds1* mutant strain where translocations predominated. However, as discussed above, PDS1 plays a role in other processes besides the S phase/DNA damage checkpoints, so it may not be surprising that the GCRs seen in a *pds1* mutant differ from those seen in the other mutant strains examined. An interesting feature of these data is that longer regions of imperfect homology were seen at the translocation breakpoints in *mec3* mutants as compared to the microhomology breakpoints seen in other mutant strains (Table 4) (Myung et al., 2001).

TEL1 Regulates Telomere Addition during GCR Formation

TEL1 is known to play an important role in telomere maintenance (Lustig and Petes, 1986; Greenwell et al., 1995; Morrow et al., 1995; Ritchie et al., 1999; Ritchie

Table 5. Summary of Telomere Addition and Translocation Breakpoint Events

Strain	Relevant Genotype	Breakpoint Type		
		Nonhomology	Microhomology	Telomere Addition
RDKY3615	wild type	1 (7.0×10^{-11})	0	5 (2.8×10^{-10})
RDKY3727	*rfc5-1*	0	0	10 (6.6×10^{-8})
RDKY3735	*mec1Δ sml1Δ*	0	0	9 (6.8×10^{-8})
RDKY3739	*dun1Δ*	0	0	10 (7.3×10^{-8})
RDKY3725	*mec3Δ*	0	2 (4.0×10^{-9})	8 (1.5×10^{-8})
RDKY3749	*rad53Δ sml1Δ*	0	4 (4.0×10^{-9})	5 (5.0×10^{-9})
RDKY3745	*chk1Δ*	0	2 (3.0×10^{-9})	7 (1.0×10^{-8})
RDKY3751	*rad53Δ chk1Δ sml1Δ*	1 (3.0×10^{-9})	1 (3.0×10^{-9})	6 (1.7×10^{-8})
RDKY3729	*pds1Δ*	0	5 (4.2×10^{-8})	3 (2.5×10^{-8})
RDKY3731	*tel1Δ*	0	6 (2.0×10^{-10})	0
RDKY3743	*mec1Δ tel1Δ sml1Δ*	9 (4.5×10^{-6})	0	0
RDKY3763	*dun1Δ tel1Δ sml1Δ*	3 (2.0×10^{-8})	6 (4.1×10^{-8})	0
RDKY3767	*rad53Δ tel1Δ sml1Δ*	0	8 (7.5×10^{-8})	0
RDKY3775	*rfc5-1 tel1Δ*	4 (1.6×10^{-8})	4 (1.6×10^{-8})	2 (8.0×10^{-9})
RDKY3773	*mec3Δ tel1Δ*	1 (4.6×10^{-8})	4 (1.9×10^{-7})	4 (1.9×10^{-7})
RDKY4130	*mec1Δ tlc1Δ sml1Δ*	0	11 (6.3×10^{-8})	0
RDKY3633	*mre11Δ*	5 (1.1×10^{-7})	2 (4.4×10^{-8})	3 (6.6×10^{-8})

Breakpoint analysis was performed as presented in Table 4 and the results are summarized here. Five breakpoints from a *sml1Δ dun1Δ* strain were all telomere additions but are not listed in the table. The *mre11Δ* data are from Chen and Kolodner, 1999. Nonhomology and microhomology refer to the two different translocation breakpoints types observed (Chen and Kolodner, 1999). The numbers given are the numbers of each breakpoint type observed. ( ) indicates the rate for each rearrangement type. A small number of the GCRs seen were interstitial deletions. These included 2 from RDKY3763 (*dun1 tel1 sml1*) and 1 each from RDKY3749 (*rad53 sml1*), RDKY3751 (*rad53 chk1 sml1*), RDKY3767 (*rad53 tel1 sml1*), and RDKY3773 (*mec3 tel1*).

and Petes, 2000). To gain further insight into the possible role that TEL1 plays in suppression of GCRs, the breakpoint sequences were determined for GCRs formed in *tel1* single and double mutant strains (Tables 3 and 5). In all cases, the *tel1* mutation reduced the proportion of GCRs that were telomere additions and increased the proportion of GCRs that were translocations/deletions. The most striking cases were the *tel1* single mutant and the *tel1 mec1*, *tel1 dun1*, and *tel1 rad53* double mutant strains, where the telomere addition class was completely eliminated and replaced by translocations and a small proportion of deletions. Even in the case of the *tel1 rfc5-1* and *tel1 mec3* combinations, there was a significant reduction in the proportion of GCRs that were telomere additions. These data suggest that the vast majority of telomere additions were TEL1-dependent. In some cases (*rfc5-1*, *chk1*, and *dun1*), mutation of *TEL1* simply resulted in a reduction of the proportion of GCRs that were telomere additions suggesting that in the absence of TEL1, the mutagenic lesions were channeled to a different outcome. In other cases (*mec3*, *mec1*, and *rad53*), mutation of *TEL1* resulted in both a reduction of telomere additions and an overall increase in GCR rate. In these cases, the results suggest that in addition to a loss of telomere addition in combination with a checkpoint defect, the combination of *tel1* with these mutations likely resulted in other repair defects.

Do MEC1 and TEL1 Regulate MRE11?

An interesting feature of the data presented here is that addition of a *tel1* mutation to a *mec1* mutant strain results in an increased GCR rate and high levels of GCRs that are all translocations with nonhomology breakpoints exactly like those seen in *mre11*, *rad50*, and *xrs2* single mutants (Chen and Kolodner, 1999). This could result if MEC1 and TEL1 regulate MRE11-RAD50-XRS2, an idea suggested by the observation that MEC1 and TEL1 function in the DNA damage response and that MRE11-RAD50-XRS2 (NBS) is modified in response to DNA damage. Thus, the *mec1 tel1* defect could be due to a combination of a checkpoint defect, a telomere addition defect (Boulton and Jackson, 1998; Ritchie and Petes, 2000), and at least a partial loss of MRE11-RAD50-XRS2-mediated repair resulting in high rates of translocations (Chen and Kolodner, 1999). Similarly, the *mre11/rad50/xrs2* defect could be due to a combination of a telomere addition defect (Boulton and Jackson, 1998; Ritchie and Petes, 2000) and loss of MRE11-RAD50-XRS2-mediated repair, and this would also result in high rates of translocations. Consistent with this, a *tel1* mutation did not change the GCR rate when combined with a *mre11* mutation (Table 3). In addition, the combination of *mec1* and *mre11* mutations showed a synergistic interaction with regard to GCR rate, resulting in a GCR rate that was essentially the same as that of the *mec1 tel1* double mutant (Table 3).

Discussion

In the present study, mutations that cause defects in S phase checkpoint functions and downstream signal transduction pathways were found to cause increased rates of GCRs similar to that seen in mutants containing defects in recombination, replication, and repair genes (Chen et al., 1998; Chen and Kolodner, 1999). These results suggest that normally DNA damage occurs during DNA replication and one normal role of S phase checkpoint functions is to facilitate nonmutagenic repair of this damage. It is unclear what this replicative damage is, but it could be stalled or collapsed replication forks that result when replication forks encounter DNA dam-

age or another replication fork, or when the replication complex occasionally disassembles (Kuzminov, 1995). It is unclear how such stalled or collapsed replication forks are repaired in *S. cerevisiae*, however, in bacteria such structures can be converted to DSBs and then recombination acts on these DSBs to reform a replication fork (Michel, 2000). That recombination can act to form a replication fork in *S. cerevisiae* is evidenced by the fact that break-induced replication has been observed (Malkova et al., 1996). The major class of rearrangement observed in S phase checkpoint mutants was deletion of part of a chromosome arm combined with de novo addition of a telomere. Possible explanations for this restricted diversity of GCRs are that in the absence of S phase checkpoint functions, there is increased de novo telomere addition activity, decreased nonmutagenic repair such as that requiring the MRE11-RAD50-XRS2 complex, the damaged structures formed may not be substrates for repair or possibly repair now occurs in a portion of the cell cycle such as during M phase or after mitosis, where other types of repair cannot occur efficiently and telomere addition is the only type of repair available (Diede and Gottschling, 1999; Marcand et al., 2000).

The analysis of the checkpoint genes examined here is consistent with the postulated roles of these genes in the S phase checkpoint that senses DNA replication damage (Elledge, 1996; Paulovich et al., 1997; Weinert, 1998a). The largest increases in GCR rates were caused by the *rfc5-1 and dpb11-1* mutations, which cause sensor defects and mutations in *MEC1*, *DDC2*, and *DUN1* that play important roles in the signal transduction pathway downstream of the checkpoint sensors (Zhou and Elledge, 1993; Kato and Ogawa, 1994; Sanchez et al., 1996; Sugimoto et al., 1997). The *rfc5-1* mutation has been suggested to cause a defect in the DNA damage checkpoints under some circumstances (Naiki et al., 2000), however, the lack of an effect on *rad9*, *rad17*, and *rad24* mutations here supports the view that the *rfc5-1* defect observed in the GCR assays is an S phase defect. The single and double mutant analysis of *DUN1* indicate that all of the increased GCR rate caused by mutations in *RFC5* and *MEC1* can be accounted for by a defect in *DUN1*. Mutations in *DUN1* do not cause a defect in replication block (hydroxyurea)-induced cell cycle arrest (Gardner et al., 1999), although a *dun1* mutation does eliminate the S phase progression defect caused by the *pol3-01* mutation, which causes increased replication errors (Datta et al., 2000). This raises the possibility that the increased GCR rate caused by checkpoint function defective mutations could be due to misregulation of proteins that function in processing of DNA replication errors (see below) rather than the lack of cell cycle arrest per se, depending on the type of replication error that underlies increased GCR rates.

Our analysis of other checkpoint functions parallels what has been observed in other systems but also suggests some differences. MEC3 has been suggested to function in the S phase checkpoint that senses DNA replication damage in addition to the checkpoints that sense DNA damage (Longhese et al., 1996). Similar to the case of *rfc5-1*, the lack of effect of mutations in *RAD9*, *RAD17*, and *RAD24* suggests the *mec3* defect seen here could be due to an S phase checkpoint function defect. If this is the case, the data presented here indicate MEC3 is not absolutely required for the S phase replication defect checkpoint. This is consistent with the fact that *mec3* mutants are not as sensitive to hydroxyurea as other S phase checkpoint mutants (Longhese et al., 1996). Alternately, the *mec3* effect could reflect a role of MEC3 in telomere maintenance since *mec3* mutants have longer than normal telomeres, suggestive of increased telomerase activity (Corda et al., 1999). *CHK1* and *RAD53* have been suggested to play parallel roles downstream of *MEC1* (Sanchez et al., 1999; Sanchez et al., 1996) and the results presented here are consistent with this. However, the fact that the *chk1 rad53* double mutant does not have as strong a mutator phenotype as either a *mec1* or *dun1* single mutant suggests that some of the signal from MEC1 to DUN1 does not involve CHK1 or RAD53; indeed, other studies have suggested differences in the roles of MEC1 and RAD53 (Desany et al., 1998; McAinsh et al., 1999; Craven and Petes, 2000). The most complex case encountered was that of PDS1, where *pds1* and *mec1* mutations showed a synergistic interaction. This suggests there could be multiple ways in which PDS1 functions in response to S phase damage. One would be its role downstream of CHK1 (Sanchez et al., 1999). A second could be related to the role of PDS1 in a MEC1-independent response to S phase blocks (Clarke et al., 1999). Finally, it is possible that loss of PDS1 function results in misregulation of repair proteins that normally function to suppress GCRs; this could explain the high levels of translocations seen in *pds1* mutants.

An interesting feature of the data presented here is the interaction between a *tel1* mutation and the S phase checkpoint mutations, which seems to be the result of TEL1 playing a role in the regulation of both telomerase and different DNA repair proteins (Sanchez et al., 1996; Vialard et al., 1998; Ritchie et al., 1999; Ritchie and Petes, 2000). While a *tel1* mutation alone did not affect the GCR rate, when a *tel1* mutation was combined with a checkpoint mutation, the GCR spectrum shifted from telomere additions to primarily translocations. In some cases, there was also a synergistic increase in GCR rate. It seems likely that the shift in GCR spectrum is the consequence of down regulation of telomerase activity by *tel1* reducing de novo telomere addition and allowing other mutagenic repair pathways to act (Boulton and Jackson, 1998; Ritchie and Petes, 2000). In support of this, *tlc1* mutations that inactivate telomerase (Singer and Gottschling, 1994) did not change the GCR rate or show synergistic interactions when combined with checkpoint mutations like *mec1*, but did cause an identical shift in GCR spectrum from telomere additions to translocations (Tables 3 and 5). It seems likely that, in the cases where synergistic effects of *tel1* mutations on the GCR rate were seen, they result from a combination of a checkpoint defect with down regulation of one or more repair proteins that function in the suppression of GCRs. The clearest case of this is that of MEC1, TEL1, and the MRE11-RAD50-XRS2 complex. A combination of *mec1* and *tel1* mutations resulted in the same GCR rate as seen in a *mec1 mre11* double mutant and resulted in the same types of GCRs (translocations with nonhomology breakpoints) as seen in *mre11*, *rad50*, and *xrs2* mutants (Chen and Kolodner, 1999). It is known

that MEC1 and TEL1, and their human homologs, play roles in the DNA damage response that modifies MRE11-RAD50-XRS2 (NBS) (Maser et al., 1997; Kim et al., 1999; Gatei et al., 2000; Lim et al., 2000). In addition, *mre11*, *rad50*, and *xrs2* mutations all cause a telomere shortening defect like that caused by *tel1* mutations, and these mutations are all in the same epistasis group with regards to telomere shortening (Boulton and Jackson, 1998; Ritchie and Petes, 2000). Thus, it seems possible that a combination of *tel1* and *mec1* mutations results in sufficient down regulation of MRE11-RAD50-XRS2 to produce the equivalent of a combination of checkpoint, telomere addition, and MRE11-RAD50-XRS2 defects. Similar to the *mec1 tel1* double mutant, down regulation of a repair function in combination with a checkpoint defect could underlie other cases where a synergistic interaction with *tel1* was seen (*rad53* and *mec3*). However, the different breakpoint sequence spectrum seen in these cases suggests that down regulation of other repair proteins besides MRE11-RAD50-XRS2 could be involved. It is also possible that TEL1 defines some other pathway that is partially redundant with the pathway involving MEC3, MEC1, and RAD53 (Sanchez et al., 1996).

The GCRs observed to occur at high rates in the checkpoint mutants studied here are similar to GCRs seen in cancer cells (Canning and Dryja, 1989; Zhang et al., 1995; Kohno et al., 1996; Chen et al., 1998). Interestingly, the human homologs of a number of the mutator genes characterized here are mutated in cancer susceptibility syndromes (Kinzler and Vogelstein, 1998; Coleman and Tsongalis, 1999; Vessey et al., 1999). Mutations in the *ATM* gene, which encodes a homolog of MEC1 and TEL1, cause Ataxia Telangiectasia, a cancer susceptibility syndrome where lymphoblasts from affected individuals show increased chromosome aberrations and a defective DNA damage response (Shiloh, 1997; Lavin and Khanna, 1999). In addition, mutations in the mouse *ATR* gene, which encodes a second homolog of MEC1 and TEL1, cause embryonic lethality and cells from $ATR^{-/-}$ embryos show high levels of chromosome abnormalities (Brown and Baltimore, 2000). Inherited mutations in human *CHK2*, which encodes the human homolog of RAD53, have been found to underlie a small number of Li-Fraumini families, although little is known about the occurrence of genome instability in these patients (Bell et al., 1999). Nijmegen Breakage Syndrome and a variant syndrome ATLD are cancer susceptibility syndromes in which lymphoblasts from affected individuals show increased chromosome aberrations and a defective DNA damage response (Carney et al., 1998; Varon et al., 1998; Stewart et al., 1999). These two syndromes are caused by inherited mutations in the human *NBS* and *MRE11* genes, which encode an ortholog and homolog of *S. cerevisiae* XRS2 and MRE11, respectively. Intriguingly, phosphorylation of BRCA1 in response to DNA damage requires ATM, and DNA damage induces the interaction of BRCA1 with the RAD50-MRE11-NBS (XRS2) complex (Zhong et al., 1999). Furthermore, BRCA1, BRCA2, and RAD51 interact, linking BRCA2 to BRCA1 and the DNA damage response (Sharan and Bradley, 1998). These observations suggest that the chromosomal abnormalities seen in *BRCA1* and *BRCA2* mutant cells could be linked to the types of checkpoint defects seen here. An implication of the studies described here is that all of these cancer susceptibility syndromes may be associated with defects in the response to spontaneous DNA replication errors. It is possible that the resulting genome instability may be a direct consequence of the types of defects characterized here, and that this plays an important role in the subsequent development and progression of cancer associated with these syndromes.

Experimental Procedures

General Genetic Methods

Yeast extract-peptone-dextrose (YPD) and synthetic drop-out (SD) media for propagating yeast strains and FC plates containing both 5-fluoroorotic acid (5-FOA) and Canavanine (Can) were as previously described (Chen et al., 1998). All yeast strains were propagated at 30°C except for strains containing temperature sensitive mutations such as *rfc5-1*, *dpb11-1*, and *pds1*, which were propagated at 23°C. Yeast transformations were as described previously (Tishkoff et al., 1997). Yeast chromosomal DNA for use as template in PCR was purified using Puregene kits (Gentra). PCR was performed in 25 μl volumes containing 0.25 units of Klentaq DNA polymerase (Ab peptides), 10 ng genomic DNA, 5 pmol each primer, and 0.1 mM each dNTP and 1 × PCII buffer (Ab Peptides).

Strains

The strains used in this study for analysis of chromosome V rearrangements were all isogenic to RDKY3615 (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-Bgl, hom3-10, ade2Δ1, ade8, hxt13::URA3*) (Chen and Kolodner, 1999) and were generated using gene disruption methods. Similarly, the strains used for the chromosome VII instability assay were isogenic with RDKY4479 (*MATa, ura3::KAN, leu2Δ1, trp1Δ63, his3Δ200, lys2-Bgl, hom3-10, ade2Δ1, ade8, zrt1::URA3, adh4::HIS3*). PCR-generated *URA3*, *HIS3*, and *KAN* disruption cassettes were prepared as described (Wach et al., 1994; Chen et al., 1998) and *TRP1* and *LEU2* disruption cassettes were generated by PCR using YEplac112 (Gietz and Sugino, 1988) and pRS425 (Christianson et al., 1992) plasmid DNAs as template, respectively. The sequences of primers used to generate disruption cassettes and confirm disruptions are available upon request. The *dpb11-1* mutation was introduced using the plasmid Yiplac211dpb11-1 and the resulting strain was verified by testing for methyl methanesulfonate (MMS) and temperature sensitivity (Araki et al., 1995) and sequencing the DPB11 gene. The *rfc5-1* mutation was introduced as described below. The strains for the chromosome V assay were: RDKY3715 *mad3::TRP1*; RDKY3717 *bub3::TRP1*; RDKY3719 *rad9::HIS3*; RDKY3721 *rad17::HIS3*; RDKY3723 *rad24::HIS3*; RDKY3725 *mec3::HIS3*; RDKY3727 *rfc5-1*; RDKY3729 *pds1::TRP1*; RDKY3731 *tel1::HIS3*; RDKY3733 *sml1::KAN*; RDKY3735 *sml1::KAN, mec1::HIS3*; RDKY3739 *dun1::HIS3*; RDKY3743 *sml1::KAN, mec1::HIS3, tel1::LEU2*; RDKY3745 *chk1::HIS3*; RDKY3749 *sml1::KAN, rad53::HIS3*; RDKY3751 *sml1::KAN, rad53::HIS3, chk1::TRP1*; RDKY3753 *sml1::KAN, mec1::HIS3, rad53::TRP1*; RDKY3755 *sml1::KAN, mec1::HIS3, chk1::TRP1*; RDKY3757 *sml1::KAN, mec1::HIS3, dun1::TRP1*; RDKY3759 *tel1::HIS3, mre11::TRP1*; RDKY3761 *sml1::KAN, mec1::HIS3, mre11::TRP1*; RDKY3763 *sml1::KAN, dun1::HIS3, tel1::TRP1*; RDKY3765 *sml1::KAN, chk1::HIS3, tel1::TRP1*; RDKY3767 *sml1::KAN, rad53::HIS3, tel1::TRP1*; RDKY3773 *tel1::HIS3, mec3::TRP1*; RDKY3775 *rfc5-1, tel1::HIS3*; RDKY4494 *ddc2::HIS3, sml1::KAN*; RDKY4496 *mec1::TRP1, ddc2::HIS3, sml1::KAN*; RDKY4475 *rfc5-1 dun1::HIS3*; RDKY4500 *rfc5-1, mec1::HIS3, sml1::KAN*; RDKY3823 *pds1::TRP1, mec1::HIS3, sml1::KAN*; RDKY4498 *ddc2::TRP1, tel1::HIS3, sml1::KAN*; RDKY4224 *tlc1::TRP1*; RDKY4130 *mec1::HIS3, tlc1::TRP1, sml1::KAN*; and RDKY4538 *dpb11-1*. The strains for the chromosome VII assay were RDKY4481 *rfc5-1*, RDKY4483 *dun1::TRP1*, and RDKY4485 *mre11::TRP1*. The *sml1* mutation was present in selected strains to suppress the lethality caused by *mec1*, *ddc2*, and *rad53* mutations, however, *sml1* did not appear to alter the mutation rate or rearrangement type when it was present.

Construction of an *rfc5-1* Substitution Cassette

Strains containing the *rfc5-1* mutation (Sugimoto et al., 1997) were made using a substitution cassette that was constructed as follows: A 618 bp PCR product spanning N terminus of *RFC5* gene was amplified using two primers: rfc5-1 (5′) 5′-GAAAGTGCTTATTTAT CAGAAAGCT and rfc5-1 (3′) 5′-ACAACGCGTTTTCTTACCTGTAC CATTTGG**TT**CATA and cloned into the plasmid pCR2.1 (Invitrogen, Inc.) to yield plasmid pRDK812. rfc5-1(5′) contains sequences −460 bp to −435 bp upstream of start codon of the *RFC5* gene and rfc5-1(3′) contains a base substitution mutation (bold) that changes amino acid 43 from glycine (GGA) to glutamic acid (GAA) and an MluI restriction site (underlined) present in the *RFC5* coding sequence. A second PCR fragment containing *TRP1* and additional *RFC5* upstream sequence was produced by amplifying the *TRP1* gene with primers TRP-5′ (KpnI) and TRP-3′ (BamHI) primers and cloned into the plasmid pCR2.1 to yield plasmid pRDK813. TRP-5 (KpnI) 5′-GGGGTACC*AACGGGGAAAAAAGGGATATTTCTTAATGAA GTCAATAATAGCTGCAAA*CGATCCGATGATAAGCTGTCAAAC contains sequences from −540 bp to −490 bp upstream of start codon of *RFC5* gene (italics), 5′ sequences for amplification of the *TRP1* cassette (the 25 bp 3′ to the *RFC5* sequences), and sequences for a KpnI site (underlined). TRP-3 (BamHI) 5′-CGCGGATCCGCGTCT CATGAGCGGATACATATTTG, contains 3′ sequences for amplification of the *TRP1* cassette (25 bp at the 3′ end sequences) and sequences for a BamHI site (underline). The KpnI to BamHI fragment from pRDK813 was excised and inserted between the KpnI and BamHI sites of the polylinker of pRDK812 to yield plasmid pRDK811 that contains in order *RFC5* sequences –540 to –490, *TRP1*, and *RFC5* sequences –460 through the *rfc5-1* mutant site and the *RFC5* MluI site. The insert from pRDK811 was excised by digestion with KpnI and MluI and used to transform *trp1* strains. The resulting Trp^+ *rfc5-1* strains were tested for characteristic MMS and temperature sensitivities and the presence of the mutation was confirmed by PCR amplification and sequencing of the *RFC5* gene.

Mutation Rates

Mutation rates were calculated by fluctuation analysis using the method of the median as previously described (Lea and Coulson, 1948; Chen et al., 1999). The reported mutation rates are the average of the values obtained in two or more independent experiments using sets of five or eleven independent cultures.

Determination of Rearrangement Breakpoint Sequences

Rearrangement breakpoints were mapped and sequenced using PCR and arbitrarily primed (AP) PCR-based methods as previously reported using modifications described below or under Results and in Figure 2. Sequences for the 21 primer sets used to locate the rearrangement breakpoints are available upon request. Sequences for the primary arbitrary primers used to then amplify the rearrangement breakpoint, where N is a mixture of all 4 nucleotides, are as follows: ARB1 5′-GGCCACGCGTCGACTAGTACNNNNNNN NNNGATAT; ARB4 5′-GGCCACGCGTCGACTAGTACNNNNNNNNN NTATAG; ARB5 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNN TATAC; ARB6 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNNT ATAA; ARBG 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNNGG GGG; ARBA 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNNAAA AA; ARBT 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNTTTTT; ARBC 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNCCCCC; AR BT1 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNTGTGT; and ARBT2 5′-GGCCACGCGTCGACTAGTACNNNNNNNNNACACA. PCR products generated using primary AP PCR were then reamplified using a specific primer located internal to the primary stage unique primer and the ARB2 primer 5′-GGCCACGCGTCGACTAG TAC. The sequences of the breakpoint junctions were determined by sequencing AP PCR products after digesting them with 2 units of shrimp alkaline phosphatase (SAP; USB Co.) and 10 units of exonuclease I (USB Co.) followed by sequencing with Perkin Elmer/Applied Biosystems 377 and 3700 DNA sequencers. Sequences were analyzed by BLAST searches against the SGD data base (http://genome-www.stanford.edu/cgi-bin/SGD/search). Some of the translocation junctions were then confirmed by PCR with junction-specific primers to amplify across the breakpoint.

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