Supporting Information

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SI Materials and Methods

Strains. Yeast strains are derivatives of the A364a strain described in previous studies and references therein (1–4). The starting strain, TY200, is a ChrVII disome that is MATα + *lys5 cyhr trp5 leu1 cen ade6* +/*hxk2:CAN* + *CYHS* + + *cen* + *ade3*. TY206 contains a *rad9* null mutation in addition, *rad9::ura3*. All gene disruptions were made into TY200 or TY206 by standard PCR-based gene disruption methods, and correct gene disruptions were verified by PCR and phenotypic assays (i.e., damage sensitivity) where appropriate (2). Some of these strains were generated previously. All *S. cerevisiae* strains were propagated at 30 °C except for *rfa1-t11, rfa1-t33,* and *dpb11-1* mutants, which were grown at 23 °C and 36 °C. Temperature-sensitive mutant alleles were inserted by using pop-in pop-out plasmids and verified by sequence analysis and by sensitivity of DNA damaging agents (Table S2).

Chromosome Instability Assays. Assays were performed as described previously in Admire et al. (2). Briefly, cells were grown in YEP with 2% dextrose for 2 d. Individual colonies were resuspended in water, counted, and plated to media containing canavanine (60 μg/mL) and all essential amino acids except arginine and serine. Cells were also plated to canavanine media without adenine to obtain frequencies of chromosome loss. Colonies on these plates are scored after 5 d as rounds or sectored (the mixed colonies) based on appearance. The stability of canavanine-resistant colonies were further verified by lineage and site assay (described in detail in ref. 1); each round colony contained of cells with the same genotype (>95%), whereas each mixed colony contained cells of multiple genotypes. In rare cases where this was not the case, frequencies were adjusted: for example, if three quarters of the round colonies were stabled, the frequency of allelic recombinants was multiplied by 75% to calculate the true frequency of recombinants, and the frequency of mixed colonies was increased by that amount. Frequencies reported were determined from analysis of at least six colonies per mutant isolate. Two independently derived isolates were tested for all mutants; the average and SDs are shown. Statistical tests were performed using the Kruskal-Wallis method (5).

Molecular Analysis of Altered Chromosomes. We analyzed Can^R Ade^+ allelic and mixed colonies for the presence of the D7–D11 specific translocation junction and the S2–S3 dicentric as described previously (1, 2). The SGD reference sequence coordinates for the D7–D11 translocation primers are VII 406041–406022 and VII 535582–535562. For each strain, at least six independent mixed colonies were analyzed by PCR to judge whether the translocation was present. To determine the frequency of inverted repeat fusion to form the S2–S3 dicentric chromosome, we used both qualitative and quantitative PCR assays as explained previously (2). Genomic DNA was isolated from six independent cultures grown without selection and subjected to qualitative or qPCR. The SGD reference sequence coordinates for the S2–S3 dicentric primers are VII 405579–405606 and VII 402296–402319. For qualitative PCR, we determined whether a DNA fragment was detectable on agarose gels. For the qPCR assays, we performed a qPCR reaction to a "normal" unrearranged DNA region of Chr V (SGD coordinates Chr V 410165–410189, Chr V 412617–412639) to determine the relative amount of genomic DNA in the sample. Real-time PCR quantification was performed using LightCycler 1.0 (Roche Diagnostics) to measure the level of dicentric chromosome in different mutant stains (described in detail in ref. 2). In each LightCycler run, the calibrator and unknown sample were measured in triplicate. Variability of the real-time PCRs was 5.8%. We did not determine the frequency of dicentrics in all strains; we can infer the relative frequency from the frequency of mixed colonies, which we have shown to be correlated with the formation of a specific dicentric chromosome (2).

1. Admire A, et al. (2006) Cycles of chromosome instability are associated with a fragile site and are increased by defects in DNA replication and checkpoint controls in yeast. *Genes Dev* 20:159–173.
2. Paek AL, et al. (2009) Fusion of nearby inverted repeats by a replication-based mechanism leads to formation of dicentric and acentric chromosomes that cause genome instability in budding yeast. *Genes Dev* 23:2861–2875.
3. Doksani Y, Bermejo R, Fiorani S, Haber JE, Foiani M (2009) Replicon dynamics, dormant origin firing, and terminal fork integrity after double-strand break formation. *Cell* 137: 247–258.
4. Weinert TA, Hartwell LH (1990) Characterization of RAD9 of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. *Mol Cell Biol* 10:6554–6564.
5. Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 47:583–621.

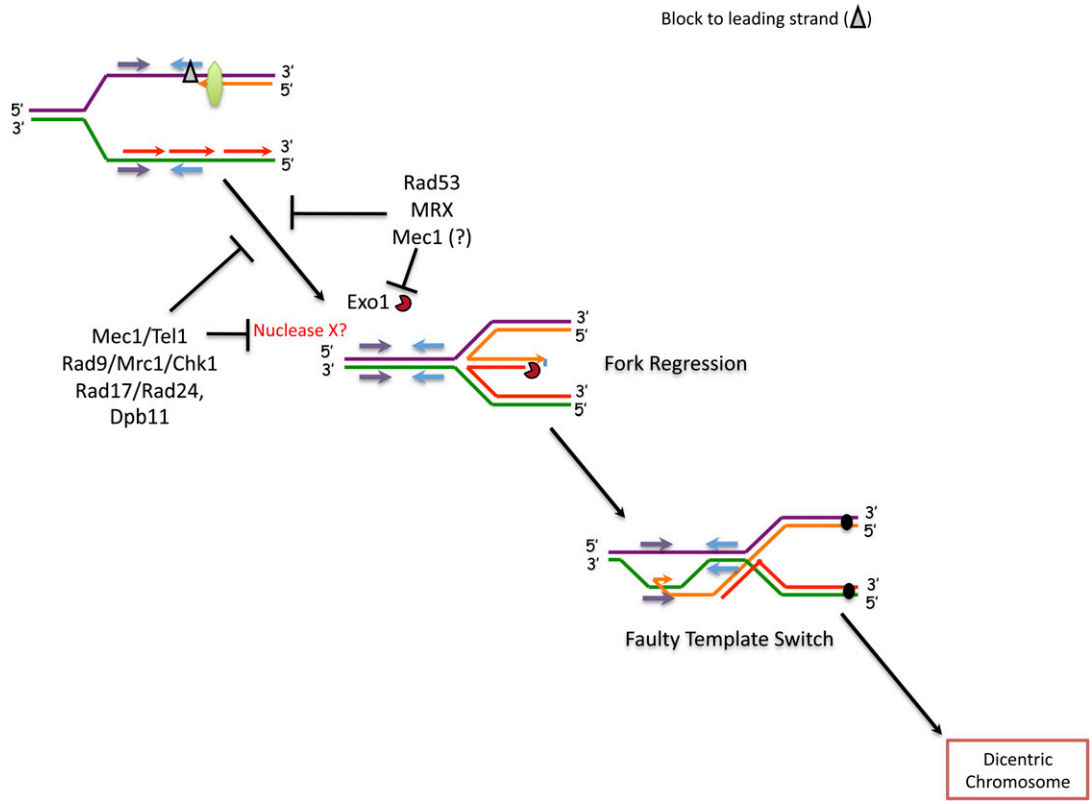

Fig. S1. A functional checkpoint may prevent replication forks from undergoing regression to a "chicken foot" structure. In the absence of Rad53 and MRX proteins, and regressed fork experiences pathological degradation by Exo1, generating a 3′ overhang. This allows for a faulty template switch event with the nearby inverted repeat sequence (which somehow becomes single-stranded). In the absence of Exo1, degradation does not occur, allowing for error-free fork reversal. With either model, we imagine that in a *mec1* mutant, perhaps another exonuclease, is left unregulated. Thus, *mec1 exo1* double mutants still degrade DNA at the fork and undergo inverted repeat fusion.

Table S1. Role checkpoint proteins in chromosome rearrangements

Genotype	Mixed colonies ($\times 10^{-5}$)	Dicentric	Translocation	Chromosome loss ($\times 10^{-5}$)	Allelic recombination ($\times 10^{-5}$)
RAD^{+} (WT)	3.3 ± 0.8 (1.0)	4/10 (1.0)	5/6	13 ± 1.2 (1.0)	11 ± 12 (1.0)
tel1Δrad9Δ	59 ± 1.4 **(18)**	4/6	6/6	940 ± 120 **(72)**	5.1 ± 0.8 (0.5)
rad9Δsml1Δ	36 ± 0.8 **(11)**	6/6	5/6	180 ± 24 **(13)**	8.7 ± 10 (0.8)
tof1Δ	2.5 ± 0.2 (0.8)	4/6	4/6	37 ± 2.7 **(2.8)**	20 ± 0.3 (1.2)
rad9Δ tof1Δ	41 ± 1.9 **(12)**	6/6	5/6	68 ± 7.7 **(5.2)**	131.1 (1.2)
mre11Δ rad9Δ	250 ± 13 **(76)**	3/4	5/6	820 ± 21 **(63)**	1.2 ± 0.67 **(1.9)**
xrs2Δ rad9Δ	281 ± 18 **(85)**	5/6	6/6	770 ± 88 **(59)**	1.2 ± 0.87 **(0.1)**
rfa1t-11 (24 °C)	1.9 ± 0.5 **(0.3)**	1/6	4/6	14 ± 0.89 (1.1)	12 ± 1.8 (1.1)
rfa1 t-11 (36 °C)	2.0 ± 0.3 (0.6)	1/6	3/6	17 ± 0.5 (1.3)	5.8 ± 0.5 (0.5)
rfa1t-33 (24 °C)	3.5 ± 0.7 (1.1)	1/6	3/6	16 ± 0.5 (1.2)	9.2 ± 0.7 (0.8)
dbp11-1Δ (24 °C)	2.1 ± 0.3 (0.7)	3/6	4/6	38 ± 0.9 **(2.9)**	8.2 ± 0.8 (0.7)

Frequencies of mixed colony, allelic, and chromosome loss were determined from 12 independent colonies. SD and fold change compared with WT are shown. Dicentric frequency was obtained from six independent cultures.

Table S2. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype*	Source
TY200	RAD$^+$ (WT)	(1)
TY202	*sml1Δ::KANMX4*	(1)
TY206	*rad9Δ::ura3*	(1)
TY216	*rad17::HisG*	(1)
TY218	*rad17::hisg sml1::KanMX4*	(1)
TY220	*mec1::URA3 sml1::KanMX4*	(1)
TY222	*mrc1::KanMX4*	(1)
TY500	*tel1::KanMX4*	This study
TY501	*rad9::ura3 tel1::KanMX4*	This study
TY502	*mec1::URA3 tel1::HPH sml1::KanMX4*	This study
TY503	*rad24::*	This study
TY504	*dpb11-1*	This study
TY505	*rad53::URA3 sml1::KanMX4*	This study
TY506	*dun1::URA3*	This study
TY507	*chk1::URA3*	This study
TY508	*rad9::ura3 mrc1::KanMX4*	This study
TY509	*mrc1-AQ::NAT*	This study
TY510	*mrc1-AQ::NAT MRC1*	This study
TY511	*mrc1-AQ NAT pRS406*	This study
TY512	*tof1::KanMX4*	This study
TY513	*rad9::ura3 tof1::KanMX4*	This study
TY514	*dun1::URA3 chk1::KanMX4*	This study
TY515	*rad9::ura3 mrc1::URA3 sml1::KanMX4*	This study
TY516	*exo1::URA3*	This study
TY517	*rad9::ura3 exo1::URA3*	This study
TY518	*rad9::ura3 xrs2::KanMX4*	This study
TY519	*rad9::ura3 rad50::KanMX4*	This study
TY520	*rad9::ura3 mre11::KanMX4*	This study
TY521	*mre11::URA3*	This study
TY522	*xrs2::KanMX4*	This study
TY523	*rad50::KanMX4*	This study
TY524	*exo1::URA3 xrs2::KanMX4*	This study
TY525	*rad9::ura3 exo1::URA3 xrs2::KanMX4*	This study
TY526	*mec1::URA3 exo1::HPH sml1::KanMX4*	This study
TY527	*rad53::URA3 exo1::HPH sml1::KanMX4*	This study
TY528	*rfa1-t11*	This study
TY529	*rad9::ura3 rfa1-t11*	This study
TY530	*rfa1-t33*	This study
TY531	*rad9::ura3 rfa1-t33*	This study
TY532	*rad53-T354A*	This study
TY533	*rad53-T354AT358A*	This study
TY534	*tel1::KanMX4 exo1::HPH*	This study
TY535	*dun1::URA3 exo1::HPH*	This study
TY536	*chk1::URA3 exo1::HPH*	This study

*All strains are disomic for ChrVII and derivatives of TY200, MATa +/hxk2::CAN1 lys5/+ cyh^r/CYH^s trp5/+ leu1/+ centromere ade6/+ +/ade3, ura3-2 (A364a genetic background), except for mutations listed (1).

1. Admire A, et al. (2006) Cycles of chromosome instability are associated with a fragile site and are increased by defects in DNA replication and checkpoint controls in yeast. *Genes Dev* 20: 159–173.