A Genetic and Structural Study of Genome Rearrangements Mediated by High Copy Repeat Ty1 Elements

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Abstract
Ty elements are high copy number, dispersed repeated sequences in the Saccharomyces cerevisiae genome known to mediate gross chromosomal rearrangements (GCRs). Here we found that introduction of Ty912, a previously identified Ty1 element, onto the non-essential terminal region of the left arm of chromosome V led to a 380-fold increase in the rate of accumulating GCRs in a wild-type strain. A survey of 48 different mutations identified those that either increased or decreased the rate of Ty-mediated GCRs and demonstrated that suppression of Ty-mediated GCRs differs from that of both low copy repeat sequence- and single copy sequence-mediated GCRs. The majority of the Ty912-mediated GCRs observed were monocentric nonreciprocal translocations mediated by RAD52-dependent homologous recombination (HR) between Ty912 and a Ty element on another chromosome arm. The remaining Ty912-mediated GCRs appeared to involve Ty912-mediated formation of unstable dicentric translocation chromosomes that were resolved by one or more Ty-mediated breakage-fusion-bridge cycles. Overall, the results demonstrate that the Ty912-mediated GCR assay is an excellent model for understanding mechanisms and pathways that suppress genome rearrangements mediated by high copy number repeat sequences, as well as the mechanisms by which such rearrangements occur.

Introduction
Gross chromosomal rearrangements (GCRs) are associated with many different diseases. Disease-causing GCRs include translocations, deletions, and inversions that can inactivate genes, form chimeric genes encoding proteins with altered activity, or change gene copy numbers or gene expression. The human genome contains many highly duplicated elements, such as Alu and LINE elements, which collectively comprise nearly 40–50% of the human genome [1–3]. Non-allelic homologous recombination (HR) between repeated sequences can mediate rearrangements leading to segmental duplications [4], numerous human genetic diseases [5] including certain sex disorders thought to be due to HR between palindromic regions on the Y chromosome [6], and many of the GCRs present in adult solid tumors [7]. Despite the importance of suppressing non-allelic recombination between highly duplicated repeats to maintain genome stability, little is known about the genetic factors that suppress these types of rearrangements.

Studies in the yeast Saccharomyces cerevisiae have contributed greatly to our general understanding of the suppression and formation of GCRs mediated by both single-copy sequence and, more recently, low copy number segmental duplications [8–11]. These studies, however, have not generally addressed the roles of highly repeated genomic elements. The Ty1 family of retrotransposons is the most common class of retrotransposons in S. cerevisiae [12]. A full length Ty1 element is ~5.9 kb long and consists of ~5.2 kb of unique sequence (known as the epsilon sequence) flanked by one copy of a ~332 bp Long Terminal Repeat (LTR) sequence (also known as a delta sequence) at each end. The LTR sequences are both oriented in the same direction and homologous recombination between them results in the deletion of the internal Ty1 epsilon sequence and one copy of the delta sequences, giving rise to a “solo delta” element [13]. The reference S288c S. cerevisiae genome sequence contains at least 32 full length Ty1s and at least 217 solo delta sequences, comprising at least 2.1% of the genome [12]. Because Ty1-related sequences are the most repetitive components of the S. cerevisiae genome, they are the best S. cerevisiae analog to the highly repetitive human Alu sequences, which are smaller than Ty elements, and LINE sequences, which are similar in size to Ty elements.

Like highly repetitive human elements, Ty1s appear to mediate many types of chromosomal rearrangements, including inversions, deletions, and both reciprocal and non-reciprocal translocations [14–18]. Such events are believed to result from the repair of DNA double strand breaks (DSBs) at or near Ty1 sequences and indeed induction of such DSBs through fragile sites, unstable inverted
Author Summary

In this study, we developed a Saccharomyces cerevisiae gross chromosomal rearrangement (GCR) assay that measures the rate of GCRs mediated by high-copy, dispersed repeat sequences in the S. cerevisiae genome known as Ty elements. Like LINE and Alu elements found in the human genome, Ty elements are repeated throughout the yeast genome. Our results demonstrate that presence of this high-copy repetitive DNA increases the rate of GCRs. These results also show that there exist differences between the pathways that affect these genome rearrangements and those that suppress GCRs mediated by either low- or single-copy DNA sequences. The majority of the Ty-mediated GCRs observed were mononcentric nonreciprocal translocations mediated by RAD52-dependent HR between the Ty element present on the assay chromosome and Ty elements located on other chromosomes. The remaining Ty-mediated GCRs appeared to involve the Ty-mediated formation of unstable dicentric translocation chromosomes that were resolved by one or more Ty element-mediated breakage-fusion-bridge cycles. Because the human genome consists of nearly 50% repetitive DNA, our work establishing this S. cerevisiae assay as a useful tool for studying non-allelic recombination between dispersed, high-copy repeat elements can give insight into those pathways that help maintain the stability of the human genome.

Results

A Ty912 element increases GCR rates

To identify how Ty elements influence GCRs, we placed Ty912, a Ty1 retrotransposon originally isolated during a screen for spontaneous histidine auxotrophic mutants [32], in a nonessential region of the left arm of chromosome V between the NPR2 and CEN8 genes (Figure 1a). This site is between the most telomeric repeats, ionizing radiation and formation of unstable dicentric chromosomes stimulates Ty-mediated GCRs [18–27]. A number of mechanisms have been proposed to account for these Ty-mediated GCRs, including Break Induced Replication (BIR) between a Ty element on a broken chromosome and a Ty element at another site on either a broken or intact chromosome, and crossing over between two Ty elements potentially mediated by single strand annealing (SSA) and other HR mechanisms [17,22,25,27–30]. Ty1 sequences are also a target of GCR-causing rearrangements involving non-repetitive sequences [31]. Because GCRs mediated by highly repetitive genome sequences underlie a number of human diseases and because there is currently a dearth of information about which pathways prevent such GCRs, we developed a quantitative genetic assay that measures the rate of Ty-mediated GCRs. Our results demonstrate that repetitive sequences greatly contribute to genomic instability and we identify genes and pathways that suppress and promote these Ty-mediated GCRs. In addition, we characterized 88 Ty1-mediated GCRs at varying levels of detail and demonstrated that the most common Ty1-mediated GCRs appear to involve non-reciprocal HR between ectopic Ty sequences that often results in the duplication of stretches of sequences bounded by a target Ty element at one end and a telomere at the other end. In a small number of cases, we observed complex rearrangements consistent with multiple exchanges between target sequences, as well as rearrangements consistent with the formation and resolution of dicentric chromosomes initially formed by HR between Ty elements.

Diverse genes and pathways suppress Ty912-mediated GCRs

We next surveyed a series of mutations for their effects on GCR rates in both the ~Ty and +Ty912 strain backgrounds (Table 1; Table S1). The selected mutations affected many pathways, including HR, DNA replication, checkpoints, Ty transposition, mismatch repair, post replication repair, chromatin structure and assembly, transcription, accumulation of oxidative DNA damage, and telomere synthesis. To simplify the analysis of the mutations in this survey, we divided the mutations into three classes, Class I, II, and III, that caused +Ty912 GCR rates that were higher than, the same as, or lower than the wild-type +Ty912 GCR rate, respectively. Each class was then divided into two subclasses, A and B, depending on whether or not the ~Ty GCR rate was greater than, or the same or lower than, the wild-type ~Ty GCR rate.

Class IA mutations increased the GCR rate in both assays and comprised the largest proportion of the mutations tested (20 of 48; 42%). Of these, 4 caused a similar fold increase in both the ~Ty and +Ty912 rates, 13 caused a greater fold increase in the ~Ty rate, and 3 (ugu1A, mms1A, and tsa1A) caused a greater fold increase in the +Ty912 rate. Both ugu1A and tsa1A were previously identified as mutations that significantly increased the GCR rate in the segmental duplication-mediated GCR assay relative to assays that detected single copy sequence-mediated GCRs (Table S1) [11,33]. There were a smaller number of Class IB mutations that increased the +Ty912 GCR rate and had no effect on the ~Ty GCR rate. Mutations in many of the Class IB genes, including SRS2, RRM3, MRC1, MSH2 MLH1 and RTT109 increased GCRs mediated by segmental duplications (Table S1) [11], which suggests that the Class IB genes play general roles in suppressing various aspects of non-allelic HR. In addition, this class of mutations also included com3A and rtt107A, which have not been tested in the segmental duplication assay. Intriguingly, RTT107/ESCA4 and RTTI09 are both genes that affect the rate of Ty transposition [34], modulate chromatin structure [35,36], and play roles in processing stalled replication forks [37].

Three Class IIA mutations (rad9A and rad50A single mutations, and the rad51A rad39A double mutation) did not significantly increase the +Ty912 GCR rate but did increase the ~Ty GCR rate. In contrast, there were 8 Class IIB mutations that caused little to no effect in either GCR assay. These Class IIB mutations represented individual deletions of a number of genes, including SPT2, SPT4, SPT8, RTTI02, and SIR4, involved in suppressing Ty1 transposition and/or transcription [34,38–40].

The Class IIIA mutations that decreased the +Ty912 GCR rate and increased the ~Ty GCR rate were restricted to a small number of mutations in mutant backgrounds containing a deletion of RAD52 (rad52A, rad52A rad51A, rad52A rad39A, and rad52A rad51A rad59A). There were also 3 Class IIIB mutations that did

essential gene on the left arm of chromosome V (PCM1) and two counter-selectable genes (CAN1 and aux3::URA3) used in the original GCR assay [9]. We chose this integration site for the Ty912 because it allows direct analysis of the effect of a Ty element on GCRs mediated by a well characterized single copy sequence GCR breakpoint region. We determined the rate of accumulating GCRs by measuring the rate of simultaneous loss of CAN1 and URA3 by fluctuation analysis. The presence of Ty912 on chromosome V (hereafter referred to as +Ty912) in a wild-type strain resulted in a 390-fold increase in the rate of accumulating Can1+ 5FOA+ progeny compared to an isogenic wild-type strain without the Ty912 insertion (hereafter referred to as ~Ty) (Table 1). As will be demonstrated below, the Can1+ 5FOA+ progeny that accumulated in the +Ty912 strain were the result of Ty1-mediated translocations.
Ty1-Mediated Genome Instability

To understand how Ty1 increases GCR rates, we first used array Comparative Genomic Hybridization (aCGH) to analyze 7 independent GCR-containing strains isolated from the wild-type —Ty strain (11–17; Table S2). All 7 GCR-containing strains had terminal deletions of the left arm of chromosome V starting at positions between the PGM1 and CAN1 genes and extending to the left telomere TEL50L (Figure S1a). These strains contained no additional copy number changes of other chromosomal regions. The aCGH results were consistent with the smaller chromosome V identified by a combination of pulse-field gel electrophoresis (PFGE) followed by Southern blot analysis using a probe to KME7, an essential gene on the left arm of chromosome V (Figure S1b). The data suggest that GCRs from all 7 isolates from the —Ty background were formed by breakage of the left arm of chromosome V followed by healing of the chromosome end by de novo telomere addition, similar to GCRs formed in other wild-type strains lacking repetitive elements in their breakpoint regions [9,49].

Most GCRs isolated in the +Ty912 assay duplicated large chromosomal regions bordered by Ty1 and telomeres. We then performed aCGH analysis on 10 GCR-containing strains isolated from the wild-type +Ty912 strain, and 78 GCR-
## Table 1. Wild-type and mutant GCR rates.

| Grouping | Genotype | RDKY Rates | Class |
|----------|----------|------------|-------|
|          |          | −Ty  | +Ty912 | −Ty  | +Ty912 |
| HR       | mre11.1  | 6105/6106 | 6499/6500 | 6.8 × 10⁻⁸ (309) | 4.2 × 10⁻⁸ (50) | IA |
|          | rad52.1  | 6619/6620 | 6503/6504 | 5.1 × 10⁻⁹ (55) | 1.3 × 10⁻⁸ (0.2) | IIIA |
|          | rad51.1  | 6557/6558 | 6555/6556 | 2 × 10⁻⁹ (9) | 5.9 × 10⁻⁷ (7) | IB |
|          | rad59.1  | 6597/6598 | 6599/6600 | 1.6 × 10⁻⁹ (7) | 6.1 × 10⁻⁸ (0.7) | IA |
|          | sn2.1    | 555⁷  | 6539/6540 | 2.2 × 10⁻¹⁰ (1) ³ | 9.6 × 10⁻⁷ (11) | IB |
|          | sgs1.1   | 6107/6108 | 6501/6502 | 2.1 × 10⁻⁶ (10) | 3.1 × 10⁻⁶ (37) | IA |
|          | rad51.1 rad59.1 | 7081/7082 | 7083/7084 | 1.5 × 10⁻⁹ (8) | 0.9 × 5⁻⁹ (0.9) | IA |
|          | rad52.1 rad51.1 | 7185/7186 | 7187/7188 | 4.5 × 10⁻⁹ (20) | 1.9 × 10⁻⁸ (0.2) | IIIA |
|          | rad52.1 rad59.1 | 7189/7190 | 7191/7192 | 7.9 × 10⁻⁹ (36) | 6.9 × 10⁻⁹ (0.1) | IIIA |
|          | rad52.1 rad51.1 rad59.1 | 7087/7088 | 7085/7086 | 4.9 × 10⁻⁹ (22) | 1.7 × 10⁻⁸ (0.2) | IIIA |
| Replication | rfa1-t33  | 7042  | 7043/7072 | 4.5 × 10⁻⁹ (2) | 3.5 × 10⁻⁷ (4) | IA |
|          | rrn3/rtt04.1 | 555⁴  | 6527/6528 | 1.4 × 10⁻⁹ (6) ³ | 1.9 × 10⁻⁷ (2) | IB |
|          | mms1.1   | 6189/6190 | 6535/6536 | 1.1 × 10⁻⁹ (5) | 9.3 × 10⁻⁷ (11) | IA |
|          | rad27.1  | 6543/6544 | 6545/6546 | 5.8 × 10⁻⁷ (2636) | 1.4 × 10⁻⁵ (166) | IA |
|          | cdc9-1   | 7039  | 7040/7041 | 3.3 × 10⁻⁷ (1500) | 2.5 × 10⁻⁵ (298) | IA |
| Checkpoint | elg1/rtt10.1 | 6151/6152 | 6509/6510 | 7.1 × 10⁻⁸ (32) | 1.1 × 10⁻⁶ (13) | IA |
|          | rtt107/esc4.1 | 6167/6168 | 6525/6526 | <5.3 × 10⁻¹⁰ (<2) | 6.1 × 10⁻⁷ (7) | IB |
|          | sml1.1   | 6589/6590 | 7073/7074 | <6.9 × 10⁻¹⁰ (<2) | 9.2 × 10⁻⁸ (1) | IIIB |
|          | mec1.1 sml1.1 | 6583/6584 | 6581/6582 | 2.1 × 10⁻⁸ (95) | 1.1 × 10⁻⁶ (13) | IA |
|          | rad53.1 sml1.1 | 6585/6586 | 6587/6888 | 1.0 × 10⁻⁸ (45) | 1.3 × 10⁻⁶ (12) | IA |
|          | dun1.1   | 373⁹  | 6515/6516 | 7.3 × 10⁻⁸ (155) ⁹ | 5.9 × 10⁻⁷ (7) | IA |
|          | chk1.1   | 374⁵  | 6623/6624 | 1.2 × 10⁻⁹ (213) | 2.7 × 10⁻⁷ (3) | IA |
|          | tel1.1   | 6569/6570 | 6571/6572 | <5.1 × 10⁻¹⁰ (<2) | 1.4 × 10⁻⁷ (2) | IIIB |
|          | rad9.1   | 371⁹  | 6625/6626 | 2.1 × 10⁻⁹ (9) ⁹ | 8.8 × 10⁻⁸ (1) | IA |
|          | mrc1.1   | 6175/6176 | 6529/6530 | <7.4 × 10⁻¹⁰ (<3) | 1.2 × 10⁻⁶ (14) | IB |
|          | csm3.1.1 | 6149/6150 | 6517/6518 | <6.8 × 10⁻¹⁰ (<3) | 3.6 × 10⁻⁷ (4) | IA |
|          | sic1.1   | 6551/6552 | 6553/6554 | 2.1 × 10⁻⁸ (95) | 5.2 × 10⁻⁶ (62) | IA |
| Ty-related | rtt105.1 | 6191/6192 | 6537/6538 | 3.3 × 10⁻⁸ (136) | 4.4 × 10⁻⁶ (52) | IA |
|          | pmr1.1   | 7017/7018 | 7019/7020 | <3.4 × 10⁻¹⁰ (<2) | 1.5 × 10⁻⁸ (0.2) | IIIB |
| MMR      | mh1.1    | 6603/6604 | 6601/6602 | <6.7 × 10⁻¹⁰ (<3) | 1.5 × 10⁻⁷ (2) | IB |
|          | msh2.1   | 6605/6606 | 6607/6608 | 5.3 × 10⁻¹⁰ (2) | 2.2 × 10⁻⁷ (3) | IB |
| PRR      | rad6.1   | 6143/6144 | 6507/6508 | 1.2 × 10⁻⁹ (6) | 3.5 × 10⁻⁶ (36) | IB |
|          | rad5.1   | 551⁹  | 6523/6524 | 1.2 × 10⁻⁹ (7) ⁹ | 8.3 × 10⁻⁷ (10) | IA |
| Chromatin | nif2/cac1.1 | 475³  | 7183/7186 | 1.2 × 10⁻⁵ (545) ³ | 1.8 × 10⁻⁷ (2) | IA |
|          | rtt109.1 | 6792/6793 | 6513/6514 | <4.8 × 10⁻¹⁰ (<2) | 2.3 × 10⁻⁹ (27) | IB |
|          | rtt101.1 | 6117/6118 | 6505/6506 | 1.6 × 10⁻⁸ (7) | 8.1 × 10⁻⁸ (10) | IA |
|          | asf1.1   | 6621/6622 | 6519/6520 | 5.3 × 10⁻⁹ (24) | 1.6 × 10⁻⁸ (19) | IA |
|          | sp12.1   | 6549/6550 | 6547/6548 | 5.4 × 10⁻⁸ (245) | 1.1 × 10⁻⁸ (13) | IA |
|          | rtt106.1 | 6159/6160 | 6521/6522 | 1.4 × 10⁻⁹ (6) | 1.6 × 10⁻⁷ (2) | IB |
|          | rtt102.1 | 6123/6124 | 6511/6512 | <5.2 × 10⁻¹⁰ (<2) | 1.0 × 10⁻⁷ (1) | IIIB |
containing strains isolated from 11 different +Ty912 strains (I8–I94, Table S2). Unlike the wild-type –Ty GCR-containing strains, only 5 of 88 GCR-containing strains isolated from the +Ty912 GCR assay had aCGH patterns consistent with terminal deletions associated with de novo telomere additions (Class I GCRs; Table 2; Figure S2a–g). Two of these events also contained putative copy number changes of small internal regions on the terminally deleted chromosome V with no duplications or deletions on other chromosomes. Strikingly, the majority (83 of 88) of GCR-containing strains isolated using the +Ty912 GCR assay contained additional non-Ty related insertions or deletions (Table S2). Some isolates contained aneuploidy of one or more chromosomes (Table S2).

### Table 2. Summary of GCR events determined by aCGH data.

| Genotype | Assay | RDKY | GCR Class |
|----------|-------|------|-----------|
|          |       |      | I (Terminal Deletion) | II (Telomere-oriented Tys) | III (Centromere-oriented Tys) | IV (Multiple Tys) | V (Complex GCRs) |
| wild type | –Ty   | 6088 | 7 | 0 | 0 | 0 | 0 |
| All genotypes | +Ty912 | 5 | 53 | 11 | 14 | 5 |
| wild type | +Ty912 | 6076 | 0 | 7 | 2 | 0 | 1 |
| mre11.1 | +Ty912 | 6499 | 0 | 6 | 1 | 1 | 0 |
| rtt105.1 | +Ty912 | 6537 | 0 | 6 | 1 | 0 | 0 |
| mrc1.1 | +Ty912 | 6529 | 0 | 6 | 1 | 0 | 0 |
| spt2.1 | +Ty912 | 6539 | 0 | 6 | 1 | 0 | 0 |
| spt4.1 | +Ty912 | 6539 | 0 | 6 | 1 | 0 | 0 |
| mec1.1 smal1.1 | +Ty912 | 6581 | 0 | 1 | 1 | 3 | 2 |
| rtt109.1 | +Ty912 | 6513 | 0 | 5 | 0 | 1 | 1 |
| spt2.1 | +Ty912 | 6547 | 0 | 2 | 3 | 1 | 1 |
| rad53.1sml1.1 | +Ty912 | 6587 | 2 | 2 | 3 | 0 | 0 |
| rtt103.1 | +Ty912 | 6531 | 2 | 4 | 0 | 1 | 0 |
| asi1.1 | +Ty912 | 6519 | 0 | 6 | 0 | 1 | 0 |
| rad27.1 | +Ty912 | 6545 | 1 | 3 | 2 | 1 | 0 |

*Some isolates contained additional non-Ty related insertions or deletions (Table S2).
*Some isolates contained aneuploidy of one or more chromosomes (Table S2).

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and analyzed by aCGH had a deletion from Ty912 to TEL05L (Ty912-TEL05L deletion) and had one or more duplicated regions that were bounded at least on one side by a full-length Ty1 or a solo Ty1 or Ty2 delta element in the reference genome. None of these duplicated regions spanned centromeres. In addition, 5 of these duplicated regions contained the chromosome V Ty912-TEL05L deletion and additional copy number changes bounded by Ty-related sequences that are described below.

Class II GCRs contained duplications bordered by a telomere and one or more telomere-oriented Ty1s and were fused to chromosome V at Ty912.

The 53 Class II GCR-containing strains (60.2% of the total surveyed) contained both the chromosome V Ty912-TEL05L deletion (Figure 1b) and a single duplicated region of another chromosome arm extending from a Ty1 element or solo Ty1 delta element to a telomere. In this class, the Ty1 elements were transcriptionally oriented towards the telomere and away from the centromere (Figure 1c; Table 2); this is the same orientation as the Ty912 element. In spite of the fact that 121 full-length Ty1, solo Ty1 delta, and solo Ty2 delta elements are oriented appropriately to generate Class II GCRs, only 16 such elements in the S288c genome reference sequence were involved as targets in the observed Class II rearrangements as determined by the aCGH data, suggesting the distribution of Ty elements mediating the rearrangements is non-random (Figure 1d).

Several lines of evidence support the idea that a non-reciprocal HR-mediated process, such as BIR or half-crossovers [29,43,50–55], occurred between Ty912 and the Ty target to generate the observed products. First, the orientations of the Ty1 sequences at the boundaries of the duplications relative to the duplicated regions are consistent with a homology-driven translocation process. Second, PFGE and Southern blotting with a probe to MCM3 revealed that the seven analyzed GCR-containing strains in this class (I8, I10, I11, I13, I15, I16, and I17) had a single abnormally-sized chromosome V that was consistent with loss of 36 kb from the left arm of chromosome V (from the Ty912-TEL05L deletion) and gain of sequence equal to the length of the duplicated region from the target chromosome (Figure 2a; Table 3). Third, the predicted junctions from these seven isolates could be amplified by PCR using primers in unique sequences flanking the Ty-mediated junction or verified by cloning the junction and sequencing of the resulting plasmid; the sequences of these amplified junction had SNPs in the 5’ ends of the junctions that generally corresponded to the Ty912 sequence from chromosome V and had SNPs in the 3’ ends of the junctions that generally corresponded to the target Ty1 sequences (Figure 2b; Table 4). Fourth, the pattern of SNPs observed across the Ty fusion junction indicated that breakpoints occurred throughout the region of homology between Ty912 and the target Ty sequences and were not restricted to terminal delta elements.

Although all of the 7 Class II GCR fusion junctions that were amplified and analyzed featured a pattern of 5’ SNPs consistent with Ty912 sequence and a pattern of 3’ SNPs consistent with the target Ty1 sequence bordering the duplication, the fusion junctions from I10, I11, I13, I16, and I17 contained other notable features (Figure 2b). The junction sequence from isolate I10 featured an almost continuous block of SNPs at the 3’ end of its epsilon sequence that could not be attributed to the Ty912 or to an unannotated solo delta near YCRWdelta10 that bordered the duplicated region. However, this sequence region had 100% sequence identity to other Ty1s elsewhere in the genome (YHRCTy1-1, YMLWTy1-1, YPRWTy1-3, and YDRWTy1-4). This

Table 3. Observed and predicted Chr Vs from a Southern blot of isolates I8–I17.

| Isolate | Observed Size (kb) | Difference from WT ChrV (kb) | A: Region deleted from ChrV (kb) | B: Regions of Increased Copy Number (kb) | ChrV<sup>a</sup>–A+B |
|---------|--------------------|-----------------------------|---------------------------------|-------------------------------------------|---------------------|
| WT –Ty  | 617                | -                           | -                               | -                                         | -                   |
| WT +Ty912 | 624             | -                           | -                               | -                                         | -                   |
| I8      | 876                | 252                         | 36                              | 261 (ChrX-R)                              | 849                 |
| I9      | 815                | 191                         | 36                              | 190 (ChrXIII-L)                           | 778                 |
| I10     | 768                | 144                         | 36                              | 147 (ChrII-L)                             | 735                 |
| I11     | 1086               | 462                         | 36                              | 427 (ChrXII-R)                            | 1015                |
| I12     | 859                | 235                         | 36                              | 214 (ChrXIII-L)                           | 802                 |
| I13     | 815                | 191                         | 36                              | 165 (ChrII-L)                             | 753                 |
| I14     | 894                | 270                         | 36                              | 145 (ChrV-R)                              | 733                 |
| I15     | 750                | 126                         | 36                              | 128 (ChrV-R)                              | 716                 |
| I16     | 805                | 181                         | 36                              | 165 (ChrIII-R)                            | 753                 |
| I17     | 894                | 270                         | 36                              | 261 (ChrX-R)                              | 849                 |

<sup>a</sup>WT +Ty912 ChrV.
SNP pattern was consistent with a tripartite fusion in which Ty912 first recombined with one of the 4 ectopic Ty1 elements (YHRWT1-1, YMLWT1-1, YPRWT1-3, or YDRWT1-4) followed by a second recombination event between the 3’ delta sequence of the target Ty and the unannotated delta sequence next to YCRWdelta10 on chromosome III. In contrast, the amplified junction regions from I13, I16, and I17 were approximately twice the size of a full-length Ty1 element, and sequencing of the regions with primers internal to Ty1 elements revealed heterozygous SNPs consistent with the fusion junction containing more than one Ty element. In the case of I17, the chromosome X target consisted of two tandem Ty1s (containing more than one Ty element). The sequencing of the regions with primers internal to Ty1 elements allowed by a second recombination event between the 3’ target Ty and the unannotated delta sequence resulting in a junction containing two Ty1 elements.

YJRWT1-1

and a mixture of homozygous and heterozygous SNPs from YCRWdelta10 and itself, we lacked sufficient sequence information to perform full SNP analyses of the multiple Ty1 elements found in the fusion junctions.

Class III GCRs containing duplications from telomeres to centromere-oriented Ty1s were generated by complex rearrangements

The 11 Class III GCR-containing strains (12.5%) resembled the more common Class II GCRs, except that the duplicated region on the target chromosomes were bounded by Ty sequences that the reference genome suggested were transcriptionally oriented towards the centromere rather than towards the telomere (Table 2). In each of these 11 cases, homology-driven rearrangements between the telomere-oriented Ty912 and the centromere-oriented target would be expected to duplicate a region on the centromeric side of the target Ty element in contrast to duplication on the telomeric side, as was observed in all 11 cases. Such events have been previously observed [17,30], but their structure has not been investigated in detail. Eight of the 13 centromere-oriented Ty sequences present in the reference sequence bordered regions that were duplicated in the 11 Class III GCR-containing strains (Figure 1d). To understand the nature of this unexpected class of GCRs, we first examined 6 of these 8 native Ty loci; the remaining 2 loci were located in the repetitive regions of chromosome XII and could not be definitively analyzed. Southern analyses of the six target Ty loci in the wild-type parental strain (RDKY6076) revealed that only YMLWT1-1 (targeted in isolate I9) had increased size compared to the reference sequence (Table 5). The change in size of the YMLWT1-1 locus was consistent with the presence of an additional full-length Ty1 element and subsequent analysis showed that our strains contained 2 tandem Ty1s (termed YMLWT1-1A and YMLWT1-1B below) at the YMLWT1-1 locus that were oriented towards the centromere. We chose two isolates of this class (I9 and I14) to analyze further. Both isolates had a single abnormally-sized chromosome V consistent with fusion of Ty912 to the duplicated region (Figure 2a; Table 3). Details of the structure of each isolate are described below.

Isolate I9, derived from a wild-type strain, contained a GCR associated with the chromosome V Ty912-TEL05L deletion (Figure 3a) and a 184 kb chromosome XIII duplication from TEL13L to the tandem centromere-oriented Ty1s, YMLWT1-1A and YMLWT1-1B (Figure 3b); no other region of the genome was observed to be duplicated. To confirm that chromosome V was indeed fused to a copy of the left arm of chromosome XIII,
Figure 3. Analysis of isolate I9 reveals a structure consistent with breakage-fusion-bridge. A. Deletion of the region between TEL05L and Ty912 in isolate I9. B. Duplication of the genomic region between TEL13L and YMLWTy1-1A/B. The duplicated region is located upstream of the 5’ end of YMLWTy1-1A/B as indicated. C. Schematic of the integration of pRDK1564 near the fusion junction and the subsequent steps utilized to create a vector cloning the fusion junction. The linearized form of the vector measures approximately 30 kb; 25 kb of the cloned fusion vector is predicted to be Ty DNA. D. Internal primer hybridizing within the epsilon sequence of the Ty reveals a heterogeneous chromatogram, indicating presence of at least two unique Ty sequences. E. A proposed mechanism for the creation of the observed structure. Exposed Ty912 sequence invades YMLWTy1-1A, resulting in a dicentric chromosome. Formation of the dicentric results in one round of breakage-fusion-bridge (with the break at the vertical arrow) followed by a subsequent break due to the formation of a second dicentric. The new break invades a wild type copy of chromosome XIII at YMLWTy1-1A/B resulting in the observed duplication and correctly sized fusion junction.

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we cloned the fusion junction by: 1) integrating plasmid pRDK1564 into the region adjacent to the junction on chromosome V, 2) isolating genomic DNA, 3) cutting the genomic DNA with the restriction enzyme Xba I that cut once within the plasmid but not within Ty-related sequences, 4) circularizing the resulting fragments, and 5) recovering the plasmid by transformation into E. coli (Figure 3c). Partial sequencing of the cloned junction confirmed that the I9 GCR contained the duplicated region of chromosome XIII at the YMLWTy1-1A/B locus fused to chromosome V at the Ty912 locus. This analysis also verified the orientations of YMLWTy1-1A/B and Ty912. Restriction mapping of the recovered plasmid indicated that the cloned Ty fusion junction was unexpectedly large (~25 kb) and consistent with the size of approximately four...

Figure 4. The dicentric structure of isolate I14 is resolved by Ty-mediated recombination. A. Deletion of the region between TEL05L and Ty912 in isolate I14. B. Duplication of the region between YERCdelta14 and TEL05R. C. Schematic of the integration of pRDK1564 near the fusion junction and subsequent steps utilized to create a vector cloning the fusion junction. The Ty fusion junction measures approximately 6 kb, the size of one full length Ty1 element. D. Exposed Ty912 DNA invades YERCTy1-1, copying through the centromere and creating a dicentric chromosome. The unstable dicentric chromosome breaks at YERCdelta14 and invades YERWdelta20b, copying the rest of the duplicated region to TEL05R. * is a verified junction; † a predicted junction. doi:10.1371/journal.pgen.1002089.g004
Ty1 elements. Sequencing of the junction with internal Ty1-specific primers resulted in a highly heterogeneous sequencing read consistent with the simultaneous sequencing of 2 or more unique Ty1 sequences (Figure 3d). The large size of the junction, combined with the sequencing data and the fact that only chromosome XIII sequences were observed to be duplicated, suggests that the extra Ty1 sequences in the fusion junction arose as a result of a complex rearrangement mechanism, such as a breakage-fusion-bridge event driven by the formation of an initial dicentric chromosome that amplified the Ty1 sequences present on the 2 partner chromosomes (Figure 3e). Ty-mediated resolution of dicentric translocation chromosomes have been previously observed during both an analysis of the structure of dicentric GCRs [25] and an analysis of GCRs derived from endogenous inverted Ty1 repeats [27].

We also investigated isolate I14, which contained both the chromosome V Ty912-TEL05L deletion and a 145 kb duplication of the right arm of chromosome V between YERCdelta14 and TEL05R (Figure 4a, 4b). Based on the aCGH data, we originally predicted that Ty912 had fused to YERCdelta14. However, after cloning and sequencing the fusion junction containing Ty912, we found that Ty912 underwent a HR-mediated fusion with YERCTy1-1, a centromere-oriented Ty1 located approximately 17.5 kb telomeric to YERCTel1 (Figure 4c). The size of the cloned junction was consistent with a single Ty1 element fused to the unique chromosome V sequence downstream of YERCTy1-1. This cloned Ty912/ YERCTy1-1 fusion indicated the initial rearrangement could have resulted in either a dicentric chromosome if two copies of chromosome V were involved (Figure 4d) or a ring chromosome if only one copy of chromosome V was involved. The data strongly suggest that I14 initially formed a dicentric chromosome that later rearranged rather than a ring chromosome because (1) chromosome V from I14 was not trapped in a well during PFGE (Figure 2a), (2) the region telomeric to YERCTy1-1 was duplicated, (3) the region centromeric to YERCTel1 was not duplicated, and (4) dicentric chromosomes, but not ring chromosomes, are known to be unstable [25]. The aCGH data are consistent with the initial dicentric chromosome breaking at or near the centromere-oriented delta sequence YERCdelta14 allowing the chromosome end to then invade the telomere-oriented YERWdelta17 or an unannotated telomere-oriented delta we call YERWdelta20B (ChrV: 449316-449625; Figure 4c) and copy to the telomere, possibly by BIR (Figure 4d). Regardless of the final fusion event, it is clear that the GCR present in isolate I14, like the GCR present in isolate I9, was the product of multiple rearrangements.

Class IV GCRs contain duplications from telomeres to clusters of Tys in both telomeric and centromeric orientations

The 14 Class IV GCR-containing strains each had both the chromosome V Ty912-TEL05L deletion and a single duplicated region of another chromosome extending from a telomere to a region of DNA containing a cluster of Ty-related elements in both telomeric and centromeric orientations (Figure 1d; Table 2; Figure S3a–S3c). No other duplicated or deleted regions were identified. Based on the hypothesis that these rearrangements arose by the same mechanisms that gave rise to Class II and III GCRs, we successfully amplified fusion junctions for isolates I23, I65, I76, I58, and I48, indicating the telomere-oriented Ty1 loci YLRWTy1-2, YLRWTy1-2, YHLChd1, and YGRWTy1-1 mediated the respective fusion junctions (Figure S3d) and that these 5 GCRs were all Class II GCRs. Since Class IV rearrangements were unlikely to be mechanistically distinct from Class II and III GCRs and since Class IV GCRs had similar aCGH patterns to those analyzed above, we did not further analyze other GCRs of this class.

Class V GCRs contain multiple duplicated regions

A complex GCR containing a duplicated and triplicated region. Isolate I12, originally obtained from a wild-type strain, contained the chromosome V Ty912-TEL05L deletion (Figure 5a), a 6.2 kb triplicated region of chromosome XIII bordered by the centromere-oriented YMLWTy1-1A/B at one end and the centromere-oriented YMLWTy1-2 at the other end, and a 104 kb duplicated region of chromosome XIII bordered by YMLWTy1-1A/B and TEL13L (Figure 5b). The copy numbers of the amplified chromosome XIII regions were confirmed by qPCR (data not presented). PFGE analysis of this isolate revealed an abnormal chromosome V, consistent with the size of a copy of the terminally deleted chromosome V joined to 2 copies of the 6.2 kb triplicated segment and 1 copy of the 184 kb duplicated segment (Figure 2c; Table 3). All of the other chromosomes present in this isolate appeared to be of wild-type size. We therefore cloned and sequenced the fusion junction containing Ty912 and found that Ty912 was fused to the centromere-oriented YMLWTy1-1A; moreover, the size of the cloned fusion junction was ~12 kb, consistent with the presence of both a hybrid Ty912/YMLWTy1-1A Ty element and the YMLWTy1-1B element at the junction (Figure 5c). Southern blots of cloned genomic DNA using probes flanking Ty912 and YMLWTy1-1A/B were consistent with a hybrid Ty912/ YMLWTy1-1A/B junction and further indicated the presence of a wild-type copy of chromosome XIII (Figure 5d), consistent with PFGE results (data not presented). Cloning and sequencing of the second fusion junction involving YMLWTy1-2 revealed that each side of the fusion junction contained a copy of the triplicated region adjacent to YMLWTy1-2, demonstrating that the fusion junction contained two copies of YMLWTy1-2 in an inverted orientation (Figure 5e). Consistent with this, restriction mapping indicated that the fusion junction was ~11.4 kb in length, slightly shorter than the length of two full-length Ty1s. This inverted duplication is consistent with a product from breakage-fusion-bridge cycles involving a dicentric chromosome generated by an initial Ty912/YMLWTy1-1A fusion (Figure 5f). Moreover, given the fact that a Southern blot with a probe hybridizing to the triplicated region flanking YMLWTy1-1A/B revealed only two discrete bands and a Southern blot with a probe hybridizing to the duplicated region flanking YMLWTy1-1A/B revealed only one discrete band instead of the three and two bands suggested by the triplication and duplication of the regions, it is likely the inverted triplicated DNA is fused to a wild-type configuration of the YMLWTy1-1A/B locus. This is consistent with a breakage-fusion-bridge mechanism that would have fused together two inverted copies of YMLWTy1-2, as the dicentric chromosome created from the breakage-fusion-bridge cycle would have broken again and, by HR, copied from the triplicated region to the left telomere of chromosome XIII. Thus, the chromosome V-chromosome XIII translocation predicted by both junctions accounts for the duplicated and triplicated regions observed in the aCGH data.

GCRs associated with multiple duplications bordered by Ty elements. Among the GCRs studied were 3 that contained aCGH patterns indicative of the chromosome V Ty912-TEL05L deletion and duplication of two different chromosomal segments from one or two different chromosomes. In each isolate, one duplicated region was bounded on both sides by Ty sequences and
the other duplicated region was bounded by a Ty sequence and a telomere. Each isolate appears to have undergone two rounds of Ty-mediated translocations. Isolate I46 was obtained from a mex1A sml1A mutant and contained a GCR that was associated with the chromosome V Ty912-TEL05L deletion, a ~377 kb duplicated region of the right arm of chromosome XII (centromeric border YLRCdelta10, YLRTOS1-2, and YLRWdelta12; telomeric border YLRTCTy1-2-3), and a ~91 kb duplicated region of chromosome XVI (centromeric border YPRHTy2-3-1, YPdeltae22, YPRTCty1-4; telomeric border TEL16R) (Figure S4a–S4c). This isolate can be explained by two simple translocations between two different groups of Ty loci: one between Ty912 on chromosome V and YLRTOS1-2 on chromosome XII and another translocation between YLRTCTy1-2-3 on chromosome XIV and YPRHTy1-4 on chromosome XVI (Figure S4d). Similarly, isolate I47, also obtained from a mex1A sml1A mutant, contained a GCR that was associated with the chromosome V Ty912-TEL05L deletion, a ~174 kb duplication of chromosome IV (centromeric border YDRWdelta11 and YDR170W-A; telomeric border YDRWTy2-3 and YDRTCTy1-3), and another ~179 kb duplication of chromosome IV (centromeric border YDRWdelta29 and YDRWdelta30; telomeric border TEL14R) (Figure S4e, S4f). This isolate can also be explained by two simple translocations between two groups of Ty elements: an initial translocation between Ty912 on chromosome V and YDRWdelta11 on chromosome IV and a second between YDRTWTy2-3 and YDR170W-A (Figure S4g). The final isolate in this class, isolate I54, was obtained from a mno109A mutant and contained a GCR associated with the chromosome V Ty912-TEL05L deletion, a ~105 kb duplication of the right arm of chromosome IV (centromeric border YDRTWTy1-4; telomeric border YDRTWTy1-5), and a ~262 kb duplication of chromosome X (centromeric border YJRWTy1-1 and YJRWCTy1-2; telomeric border TEL13R) (Figure S4h–S4j). Like the previous two isolates, this isolate can be explained by two Ty-mediated translocations: the first between Ty912 on chromosome V and YDRTWTy1-4 on chromosome IV, and the second between YDRTWTy1-5 on chromosome IV and YJRWCTy1-1/2 on chromosome X. In all cases, the aCGH data were consistent with the GCR-containing strains also containing a complete copy of the chromosomes that were the source of the duplicated regions. We were unable to amplify the predicted translocation breakpoint junctions in these three isolates by PCR. While it was not possible to completely determine the structure of each GCR, the available data were consistent with the hypothesis that each of these 3 GCRs involved non-reciprocal translocations that joined 3 translocated segments. In the case of I47 and I54, the orientations of the Ty elements at the breakpoint junctions were only consistent with mechanisms involving template switching between 3 templates and were inconsistent with the formation of an intermediate dicentric chromosome. In contrast, in the case of I46, the orientations of the Ty elements at the breakpoint junctions were consistent with mechanisms involving switching between 3 templates but could also have been created by the formation of an intermediate dicentric translocation chromosome formed between chromosome V and chromosome XII.

A complex translocation containing a microhomology-mediated fusion. Isolate I66 was obtained from a spo21A mutant and resembled isolates I46, I47, and I54 described above except that one of the junctions involved a fusion mediated by a microhomology breakpoint. I66 contained the chromosome V Ty912-TEL05L deletion, a ~100 kb duplication of chromosome X (centromeric border YJRWCTy1-1 and YJRWCTy1-2; telomeric border part of the CSN12 gene), and a ~92 kb duplication of chromosome XVI (centromeric border part of the SKB3 gene; telomeric border TEL16R) (Figure S5a–S5c). We confirmed the fusion junction between Ty912 and YJRWCTy1-1 by PCR (Figure S5d). Amplification of CSN12 and SKB3 by PCR revealed wild-type copies of both genes and PCR amplification and sequencing confirmed the presence of a CSN12-SKB3 fusion gene mediated by a region of microhomology (5’-CTTC-3’) between the two genes (Figure S5e). The data were most consistent with the presence of a tripartite translocation consisting of chromosome V at Ty912 joined to a copy of a fragment of chromosome X at YJRWCTy1-1 that was then joined at CSN12 to a copy of a fragment of chromosome XVI at SKB3 extending to TEL16R (Figure S5f). This rearrangement was likely a product of a non-reciprocal translocation as the aCGH data were consistent with the presence of a complete copy of both chromosomes X and XVI. Furthermore, the orientations of the sequences at the breakpoint junctions were only consistent with mechanisms involving switching between the chromosome X and XVI templates and were inconsistent with the formation of an intermediate dicentric chromosome.

Discussion

In the present study, we describe the development of a quantitative genetic assay that allows for the assessment of the impact of genetic defects on the rate of Ty1-mediated GCRs and facilitates analyses of the structures of the resulting Ty1-mediated GCRs. The results described here demonstrate that presence of a telomere-oriented Ty912 on a nonessential terminal arm of chromosome V greatly increases the spontaneous rate of loss of that chromosome arm. Furthermore, this loss appears to be driven primarily by non-reciprocal translocations between Ty912 and other Ty-related elements in the genome, resulting in a broken chromosome V joined to a fragment of another chromosome that terminates with a telomere. The observed rearrangement products are consistent with HR-mediated processes, such as BIR and half crossovers [29,43,50–55], which result in translocation breakpoints occurring at regions of homology mediated by RADS2-dependent HR.

The majority of the Ty1-mediated GCRs observed (~60.2%) were simple non-reciprocal translocations likely mediated by HR between the telomere-oriented Ty912 on chromosome V and a
single telomere-oriented Ty elements located on another chromosome arm. These results are consistent with a simple model in which a resection of a spontaneous DSB on chromosome V exposes single stranded Ty912 DNA that then invades a telomere oriented Ty element on another chromosome arm and leads to the replication of DNA from this Ty element to the telomere by BIR [17,22,24,25,28,56]. The results are also consistent with another model in which spontaneous DSBs form on both chromosome V and another target chromosome during the G2 phase of the cell cycle. These DSBs are followed by HR between Ty912 on chromosome V and a Ty element on the broken target chromosome. Mitosis then occurs, and the cell containing the remaining intact copy of chromosome V is selected against in the assay selection system; such HR events could be mediated by SSA as well as other HR mechanisms [27,29,30,55,57]. Other GCRs identified, such as those that involved the duplication of telomeric regions adjacent to centromere-oriented Ty elements or those that involved the duplication of multiple chromosomal regions, appear to be the products of more complicated mechanisms, ranging from the formation and resolution of unstable dicentric translocation chromosomes [25,27] to sequential linked monocentric translocations consistent with template switching during BIR [58,59]. In all of the events observed, it is possible that the initiating DSBs occurred at the site of participating Ty elements. However, it is more likely that the initiating DSBs occurred randomly and were resected to the participating Ty elements; the selection of Ty-mediated GCR events was due to the fact that the rates of HR mediated GCRs are much higher than those of single copy sequence mediated GCRs (Table 1; [11]). Surprisingly, most of the genetic defects that increased the rate of Ty912-mediated GCRs did not appear to significantly alter the types of GCRs recovered (Table S2) in spite of the fact that Ty elements targeted in rearrangements had an apparently nonrandom distribution (Figure 1d). This observation raises the possibility that Ty-mediated repair of DNA damage may be biased to target specific locations or Ty elements in the genome. However, because our data were pooled from the results of analyses of GCRs isolated in multiple mutant backgrounds, and because we currently lack a rapid, cost efficient method to identify very large numbers of chromosome arm duplications, we were unable to determine whether this result was due to the analysis of a biased set of GCRs or was a manifestation of true repair target bias.

To gain insights into whether the pathways affecting Ty-mediated GCRs were similar to those affecting other types of GCRs, we surveyed mutations previously demonstrated to affect GCR rates as well as mutations known to affect Ty metabolism. Most of the mutations that increased rates of single copy sequence-mediated GCRs also increased GCR rates in the +Ty912 GCR assay, as well as in the segmental duplication GCR assay [11], suggesting that these increases are likely due to elevated levels of DNA damage leading to aberrant repair. In addition, all of the mutations tested that specifically increase GCRs mediated by segmental duplications (mre11A, sgs1A, srs2A, rmm3A, mit109A, and rad62J) increased the rate of Ty912-mediated GCRs, indicating an overlap between the pathways that suppress segmental duplication-mediated GCRs and the Ty912-mediated GCRs. These mutations potentially cause defects in pathways that specifically suppress non-allelic HR. Interestingly, the wild-type strain containing the Ty912 on chromosome V had a higher GCR rate than that of the wild-type strain containing the segmental duplication-mediated GCR assay. This is likely due at least partially to both the larger size of the Ty912 element and the larger number of potential alternative repair templates available in the genome. In contrast, most of the gene defects affecting Ty1 transcription and transposition seemed to have little or no specific effects on the rate of Ty912-mediated GCRs, as many of the mutations that increased the +Ty912 GCR rate also increased the GCR rates in other GCR assays that did not involve Ty1 elements. This suggests that the +Ty912 GCR assay is an excellent model for understanding mechanisms suppressing rearrangements between high copy number repeats.

Some of the mutations tested had distinct effects in the Ty1-mediated GCR assay that were surprisingly different than their effect in other GCR assays, including the segmental duplication assay. First, a rad52A mutation that decreases HR increases the rate of single copy sequence-mediated GCRs [8] and decreases the rate of Ty1-and other duplication-mediated GCRs [11]; this is expected as HR is thought to play a central role in the formation of duplication mediated GCRs, but promotes the correct repair of DNA damage that would otherwise lead to single copy sequence-mediated GCRs. Second, deletion of rad51A increased both single copy-mediated and Ty-mediated GCRs (Table 1), but had relatively little effect on low-copy segmental duplication-mediated GCRs. This was surprising, especially given the fact that products of Ty-mediated HR in a wild-type strain were most consistent with the products of BIR, a process that is highly dependent on RAD51 [50]. A previous report found that a rad51A deletion caused an increased Ty1 recombination rate that led to a rise in the formation of solo LTRs but suppression of Ty conversion events [44]. Our study also revealed an increase in Ty-mediated GCRs upon deletion of RAD51 and suggested that another HR mechanism, such as a RAD59-dependent RAD51-independent single-stranded annealing event followed by a half-crossover [29,55], could be responsible for the formation of Ty-mediated GCRs and that furthermore, the mechanism of repair was mutagenic and suppressed by RAD51. Deletion of both RAD51 and RAD59 resulted in a GCR rate equivalent to that of a wild-type strain in the +Ty912 GCR assay (Table 1), which is consistent with a view that RAD59 promotes the mutagenic repair of DNA in the presence of the Ty912 and absence of RAD51. Third, the rad51A rad59A double deletion resulted in a GCR rate that was higher than that of a rad52A strain (unpaired Wilcoxon rank sum test; p = 5.59×10^{-4}), a pattern which was not observed in the segmental duplication assay [11], but has been noted in a previous assay [46]. This difference suggests that other RAD52-dependent factors besides RAD51 and RAD59 play a larger role in the formation of Ty1-mediated GCRs than in the formation of lower copy segmental duplication-mediated GCRs. Fourth, we identified mutations that significantly reduced the +Ty912 GCR rate, but did not affect the rate of single copy sequence-mediated GCRs. These mutations include pnu1A, mt103A and gall11A, all of which alter Ty metabolism [34,41,42]. This suggests that some aspects of normal Ty metabolism may impact the formation of GCRs. Overall, the genetic analysis performed as part of the present study indicates that the +Ty912 GCR assay is a high sensitivity assay suitable for the analysis of pathways that affect the rate of GCRs mediated by repetitive DNA and provides a means to detect common pathways that suppress genome instability, novel pathways that affect repetitive DNA, and different aspects of known GCR suppression pathways not previously studied.

The work presented here indicates that dispersed repetitive elements in S. cerevisiae DNA, like the Ty elements that are analogous to human LINE and Alu elements in abundance, are chromosomal features that result in increased genomic instability. Analysis of this genomic instability has provided insights into both the HR-based mechanisms that yield Ty-mediated GCRs and the pathways that normally act to prevent such GCRs. In humans, suppression of non-allelic HR is likely important for preventing
GCRs from occurring due to the large numbers of dispersed repetitive sequences in the human genome, particularly because such GCRs have been seen to underlie genetic diseases and are found among the genome rearrangements in many cancers. Our observations on the pathways that preferentially suppress Ty-mediated GCRs and on the mechanisms that produce such GCRs suggest that the structures of GCRs observed in disease situations will provide signatures diagnostic for particular genome instability-causing genetic defects.

Methods

General methods

PCR. All PCR reactions used a mixture of Pfu from Stratagene and KlenTaq from Ab Peptides. A master mix of 16:1 KlenTaq (25 U/μl) to Pfu (2.5 U/μl) by volume was made for a total unit ratio of 160 U KlenTaq per 10 U Pfu per microliter.

DNA isolation. In general, the Gentra Puregene Kit (Qiagen) was used to isolate S. cerevisiae DNA for the microarray hybridizations and PCRs as described by the manufacturer. We modified a previous protocol [60] to isolate DNA for use in Southern blots, cloning, and amplification of Ty fusion junctions. Modifications included the use of 5 ml cultures instead of 10 ml cultures, an extra chloroform extraction step after the phenol:chloroform:isoamyl alcohol (25:24:1) extraction step, incubation at 37°C for 10 minutes instead of 5 minutes after addition of RNAse A, use of 5 mg/ml RNAse A instead of 1 mg/ml RNase A, and an additional phenol:chloroform:isoamyl-alcohol and chloroform extraction after incubation of the DNA with RNAse A.

Statistical tools. R (version≥2.9.2) was used to calculate p-values for Wilcoxon rank-sum tests. Ninety-five percent confidence intervals for the median were calculated using a two-sided nonparametric test (http://www.math.ubc.ca/~knight/utility/ MedInt95.htm).

Ty912 cassette construction

We constructed the plasmid pRDK1251 containing Ty912 [32] surrounded by chromosome V targeting sequence to integrate the Ty1 element into the NPR2-CIN8 intergenic region. The flanking targeting sequence from CIN8 (ChrV: 39724-36426) was amplified by PCR from S. cerevisiae genomic DNA with primers JCP41 and JCP42 (Table S3), which introduced flanking Sac I and Sma I restriction sites. The flanking targeting sequence from NPR2 (ChrV: 36340-33913) was also amplified with primers JCP43 and JCP44, which introduced flanking Bgl II and Bam H I restriction sites. Ty912 was amplified from plasmid B155 (FB118), a generous gift of Dr. Fred Winston (Harvard Medical School), with JCP39 and JCP40, which introduced flanking Sma I and Bam H I restriction sites. The pRDK1251 plasmid was generated by sequentially subcloning the Sac I-CIN8-Sma I, Sma I-Ty912-Xba I, and Bam H I-VPR2-Xba I fragments into pUC19 [61], such that the NPR2 fragment was joined to the Ty912-Xba I site present at genomic coordinate 35,997.

Construction of assay-containing strains

We sequentially transformed URA3 and HIS3 markers into the intergenic region between NPR2 and CIN8 in RDKY6078 (MATa lys2-10A, hom3-10, ura3-A0, leu2-A0, trp1-A63, his3-A200) to generate strain RDKY6081. We released the Ty912 cassette from pRDK1251 by Xba I digestion and then transformed the Ty912 cassette (~10 ug) into RDKY6081 using standard lithium acetate transformation protocols and plated the putative transformed colonies onto YPD plates. After one day of growth at 30°C, this YPD plate was replica-plated to a uracil dropout plate containing 1 g/L 5-fluoroorotic Acid (5FOA). After ~2 days of growth at 30°C, the resulting colonies were replica-plated from the dropout plate containing 5FOA to a separate histidine dropout plate. Colonies were screened for growth on the uracil dropout plate containing 5FOA and for non-growth on the histidine dropout plate. We verified the insertion of Ty912 between NPR2 and CIN8 on the Crick strand of RDKY6082 by PCR (primer pair JCP44 & JCP55; primer pair JCP125 & JCP349 (Table S3)). A hat1::URA3 KO cassette was amplified from RDKY3615 (MATa, ura3-52, leu2-A1, trp1-A63, his3-A200, lyp2-H, hom3-10, ade2-1, ade0, hat1::URA3) by PCR (primer pair JCP29 & JCP29 (Table S3)) and transformed into RDKY6082 to create RDKY6084. RDKY6084 was then backcrossed to RDKY6079 (MATalpha lyp2-10A, hom3-10, ura3-A0, leu2-A0, trp1-A63, his3-A200) and our +Ty912 wild type haploids RDKY6076/6077 (MATa lyp2-10A, hom3-10, ura3-A0, leu2-A0, trp1-A63, his3-A200, apr1::Ty912 hat1::URA3) were isolated. The ~Ty wild-type strains RDKY6083/6089 (MATa lyp2-10A, hom3-10, ura3-A0, leu2-A0, trp1-A63, his3-A200 hat1::URA3) were isolated by first transforming the previously isolated hat1::URA3 cassette into RDKY6078, backcrossing the resulting strain to RDKY6079, and isolating haploids. All strains used in the experiments were isogenic to either RDKY6076 (+Ty912) or RDKY6088 (~Ty), which differ only by the presence of the Ty912 insertion (Table S4).

Construction of mutant strains

Strains with kanMX4 marked deletions of interest were created using kanMX4 cassettes amplified from the systematic S. cerevisiae knockout library [62]. Strains with deletions marked with TRP1 and HIS3 cassettes were created by amplifying the cassettes from the pRS series of plasmids [63] with PCR primers that added 50 bases of the target homology of interest. These cassettes were then transformed into strains of interest using standard lithium acetate transformation protocols followed by verification of the correct insertion by PCR with flanking and internal primers. All strains used in the experiments are available upon request (Table S4), as are the primer sequences used in their construction.

GCR rate calculations

General methods, including use of YPD and synthetic dropout medias, have been described previously [9]. For each strain, we used 14 or more independent cultures in our fluctuation analyses [64] to calculate the median rates [65].

Array comparative genomic hybridization

For each strain of interest, 1 μg of DNA was labeled with either Cy3 or Cy5 and applied to one of four wells of a Nimblegen 4-plex microarray. The GeneChip Microarray Core (UC San Diego School of Medicine) performed the hybridization and scanning. Probes on the array had a median base pair spacing of ~200 bp between probes. DNA for seven independent GCR isolates and one ~Ty wild type strain (RDKY6088) were applied to each 4-plex microarray. Each microarray thus contained either a GCR isolate hybridized along with the ~Ty wild-type DNA or with DNA from another GCR isolate (Table S5). The R package Ringo (r> = 1.3.0) [66], an add-on to the Bioconductor suite (r> = 2.4.1) [67], was used in combination with the SignalMap software (r> = 1.9) from Nimblegen to visualize the aCGH data. Increased copy numbers of probes were revealed by identifying continuous regions whose log ratios deviated from 0. Deletions were identified by analyses of the raw intensity data and identification of regions with continuously low regional intensity.
Ty fusion junction amplification

PCR. Ty fusion junctions were amplified using the following protocol: 5 min 95°C initial denaturing step; 25 cycles of 12 or 20 sec 95°C denaturation, 30 sec 63.5°C annealing, 7 min 68 or 72°C extension; final 7 minute 68 or 72°C extension. The resulting amplicons were gel-purified and sequenced using an ABI3730 sequencer and standard protocols.

Construction and insertion of a plasmid for subcloning of GCR junctions. Plasmid pRDK1564 was constructed by cloning a HIS3 marker flanked by SacI and SmaI restriction sites (amplified using PCR primers JCP404 and JCP405 (Table S3)) into the multiple cloning site of plUC19. Primers with homology centromeric to Ty912 (primer pair JCP445 and JCP446 (Table S3)) were used to amplify and linearize the plasmid for insertion into the intergenic region between Ty912 and CIN8 in GCR isolates. The amplicon was transformed into the GCR isolates using the standard lithium acetate transformation protocol.

Subcloning of chromosomal GCR breakpoints. One μg of genomic DNA containing the integrated plasmid construct was digested with XbaI. The DNA was diluted to 2.5 ng/μL and ligated overnight at 16°C with 1,600 U of T4 ligase. The plasmid was then recovered by transforming the DNA into SURE electrocompetent cells (1 μL-MelzA) (A:ncrCB-KodSMR-mmr)-171 endA1 gexA96 thi-1 supE44 redA1 lac recB97 recF80 b6C amuC: Kanr uraC [F pmAB lacIQ AM15 Tn10 (Tet')] from Stratagene. The resulting plasmids were then characterized by restriction and PCR mapping, and sequencing using standard methods as described under individual experiments.

Supporting Information

Figure S1 Structural analyses of the Ty wild type GCRs consistent with healing of a broken chromosome V by de novo telomere addition. A. Absolute aCGH intensities of chromosome V left arm deletions from wild type Ty isolates 11–17. B. Southern blot of 11–17 with a probe specific to MCM3, an essential gene on chromosome V. The MCM3 probe was amplified using JCP447 & JCP448 (Table S2).

Figure S2 aCGH of the Class I +Ty912 GCRs is consistent with healing of a broken chromosome V by de novo telomere addition. A–G. Absolute aCGH values of the chromosome V left arm deletions from wild type +Ty912 Class I isolates. Deletions consistently occur between TEL05L and Ty912. Two isolates appeared to contain putative ectopic regions of duplications or triplications located in nonrepetitive DNA; however, we did not perform additional experiments to verify the existence of these duplicated regions.

Figure S3 Class IV +Ty912 GCRs are consistent with Class II and Class III GCRs. A. Example of a Class IV Ty912-TEL05L deletion. B. Example of a duplication-bounding multiple Ty locus site containing Tys in both centromeric and telomeric transcriptional orientations. C. Schematic of the 14 Class IV isolates with duplication bounded Ty loci containing Tys in multiple transcriptional orientations. Black arrows represent the layout of the primers used to amplify the fusion junctions. D. PCR amplicons of fusion junctions. All amplicons measured approximately ~6 kb, suggesting each contained only 1 Ty. Placement of PCR primers are indicated in the schematic maps above. Primers used are as follows: A: JC-P310 & JC-P670; B: JC-P310 & JC-P672; C: JC-P310 & JC-P674 (Table S2).

Figure S4 aCGH data and proposed model of 3 Class V +Ty912 GCRs. A–C. Deletion/Deletions from isolate I66. D. Proposed model explaining the genetic data of A–C. E–F. Deletion/Deletions from isolate I47. G. Proposed model explaining the genetic data of E–F. H–J. Deletion/Deletions from isolate I54. K. Proposed model for explaining the genetic data of H–J.

Figure S5 Structural analysis of I66 reveals a tripartite fusion. A. Ty912-TEL05L deletion from isolate I66. B–C. Duplications from isolate I66. D. PCR amplification of the Ty912/JSRWTy1-1 fusion using primers JCP310 (Chr V) and JCP392 (Chr X) (Table S2). E. Sequence of the CN1/2/SK3 junction. The mediating sequence is a palindromic CCTTC sequence. F. Proposed model showing two recombination events that can lead to the observed genetic data.

Table S1 GCR rates compared to segmental duplication assay rates.

Table S2 Summary of aCGH data.

Table S3 Primers used in this study.

Table S4 Strains used in this study.

Table S5 aCGH microarray setup.

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Author Contributions

Conceived and designed the experiments: JEC RDK. Performed the experiments: JEC. Analyzed the data: JEC RDK. Contributed reagents/materials/analysis tools: JEC RDK. Wrote the paper: JEC RDK.

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