Genome-Wide Analysis of Rad52 Foci Reveals Diverse Mechanisms Impacting Recombination

David Alvaro¹, Michael Lisby², Rodney Rothstein¹*

¹Department of Genetics and Development, Columbia University Medical Center, New York, New York, United States of America, ²Department of Molecular Biology, University of Copenhagen, Copenhagen, Denmark

To investigate the DNA damage response, we undertook a genome-wide study in Saccharomyces cerevisiae and identified 86 gene deletions that lead to increased levels of spontaneous Rad52 foci in proliferating diploid cells. More than half of the genes are conserved across species ranging from yeast to humans. Along with genes involved in DNA replication, repair, and chromatin remodeling, we found 22 previously uncharacterized open reading frames. Analysis of recombination rates and synthetic genetic interactions with rad52Δ suggests that multiple mechanisms are responsible for elevated levels of spontaneous Rad52 foci, including increased production of recombinogenic lesions, sister chromatid recombination defects, and improper focus assembly/disassembly. Our cell biological approach demonstrates the diversity of processes that converge on homologous recombination, protect against spontaneous DNA damage, and facilitate efficient repair.

Introduction

Homologous recombination (HR), a repair mechanism that depends on DNA sequence homology, underlies a number of important DNA processes that act to both stabilize and diversify a genome. In mitotic cells, HR functions to maintain the integrity of the genome through such processes as the repair of DNA double-strand breaks (DSBs), the maintenance of rDNA copy number, and the rescue of collapsed replication forks. HR is entirely conservative when it occurs following DNA replication where a sister chromatid is available as a template. However, utilization of sequences on a homologous chromosome can lead to crossovers and potential loss of heterozygosity (LOH), while recombination at ectopic or repeated sequences may lead to genomic rearrangements such as deletions, duplications, and translocations (reviewed in [1]).

In Saccharomyces cerevisiae, Rad52 is the defining member of an epistasis group that includes: RecA homologs Rad51, Rad55, Rad57, and Dmc1; putative SWI/SNF family ATPase Rad54; Rad52 homolog Rad59 and Mre11, Xrs2, and Rad50. The Rad52 epistasis group is essential for HR. Rad52 binds single-stranded DNA in vitro and has been shown to stimulate DNA annealing and to enhance Rad51-catalyzed strand invasion [2–5].

In response to DNA damage, proteins involved in HR relocalize into discrete subnuclear foci. Fluorescently tagged repair and checkpoint proteins have been used to explore the composition and dynamics of these foci, which are gigadalton-sized assemblies of proteins [6]. Repair foci colocalize with fluorescently tagged inducible DSB sites, regions of single-stranded DNA, and sites of unscheduled DNA synthesis [7–9]. Multiple DSBs often colocalize at a single focus showing that foci reflect recombination centers capable of the simultaneous repair of more than one lesion. The assembly of proteins into repair foci is a coordinated process beginning with detection of damage by the Mre11/Rad50/Xrs2 complex. Next, checkpoint proteins are bound and activated to arrest cell cycle progression until completion of repair. The lesion is repaired through HR performed by the Rad52 epistasis group proteins and finally the repair apparatus is disassembled [10]. From a cell biology perspective, Rad52 focus formation is an excellent marker for HR, since it is required for the recruitment of all other HR proteins into repair foci.

While exogenous DNA damage greatly stimulates the formation of Rad52 foci, foci also form spontaneously in S phase cells, likely reflecting the repair of spontaneous DNA lesions such as DSBs, nicks, and single-stranded gaps [6,11]. Time-lapse microscopy indicates that foci form in approximately 50% of cells during S phase and most spontaneous foci persist for less than 10 min [7]. Since spontaneous foci generally last for only a fraction of S phase, they are observed in 20% of S phase cells in a population of logarithmically growing cells (5% of the total population). Mutants defective in various aspects of DNA metabolism, including damage checkpoints (mecl sml1), HR (rad53Δ), and DNA replication (pol12–100) exhibit elevated levels of spontaneous foci [6]. This elevation may be the consequence of an increased incidence of focus formation reflecting the generation of more DNA lesions, or the consequence of foci that persist
Homologous recombination (HR) is a cellular process that permits efficient repair of both endogenous and exogenous DNA damage. Although the principal players in HR have been well characterized, the interplay of diverse processes with the HR pathway remains mysterious. Traditionally, genetic screens investigating HR have utilized genetic assays, such as survival following exposure to DNA damaging agents or alterations in the rate of the generation of recombinant products. In this work, we instead utilize a cell biology phenotype, the relocation of the central HR protein Rad52 into subnuclear foci reflecting repair centers actively engaged in HR. This approach allows us to identify mutants that affect the kinetics of HR repair center assembly and disassembly regardless of the outcome of recombination. We identified 86 gene deletions that lead to increases in the levels of spontaneous foci in proliferating diploid cells, 22 of which were deletions of previously uncharacterized ORFs (designated IRC2–11, 13–16, 18–25). Genetic characterization of the mutants revealed a diversity of mechanisms that underlie the focus phenotype. These include increasing the generation of DNA lesions, blocking the completion of HR, and altering the kinetics of genetic recombination and the assembly/disassembly of the HR protein complexes.

**Results**

A Genome-Wide Screen to Identify New Genes Affecting DNA Metabolism

Rad52, a central recombination protein, relocates to form sub-nuclear foci in response to spontaneous and induced DNA damage. Therefore, Rad52 focus levels can be used as a sensitive indicator for processes that impinge on the genome. An initial screen to identify gene deletions affecting the levels of spontaneous Rad52 foci was performed by transforming a plasmid containing a Rad52-YFP fusion gene directly into library haploid strains. However, this approach yielded an excessive number of false-positive results caused by additional recessive factors in the individual library strains. We therefore developed a method that permits the systematic creation of hybrid diploids that are homozygous for the gene deletion from each library strain (Figure 1A), while simultaneously facilitating the introduction of plasmid or chromosomal constructs into the gene deletion strains [12].

Using the systematic hybrid LOH method, we screened 4,805 nonessential gene deletions for levels of spontaneous foci (Figure 1B). Twenty gene deletions could not be constructed as hybrid homozygous diploids, including 11 that are deficient in mating. The distribution of focus levels is shown in Figure 2. To evaluate the reliability of the mutant screen, we partitioned the genes into four sub-sets shown (A, B, C, and D). Sub-set B consists of the strains that exhibit focus levels within the range of variation seen in a wild-type strain. These account for approximately 90% of the deletions screened. For 233 gene deletions (sub-set A), no foci were observed. Upon retest, most of these strains formed foci; however, the distribution was shifted slightly to the left compared to the entire deletion library (Figures 2 and S1A). Furthermore, for the 96 strains that showed the lowest levels upon retest (less than 1% foci), all gave rise to foci after ionizing radiation, indicating that no fundamental aspect of Rad52 focus formation was disrupted in these deletion strains (Figure S1B). Further study of this group of genes may shed some light on processes that generate spontaneous DNA damage.

We next examined those mutants that exhibit foci in more...
than 20% of all cells (sub-set D), a 4-fold elevation over average wild-type levels. These 108 gene deletions (2.2% of the library) fall into a number of broad functional groups involved in various aspects of DNA and chromatin metabolism. Following two independent retests, 80 mutants (75% of the 108) consistently exhibited elevated focus levels including deletions of 16 previously uncharacterized ORFs (Tables 1 and S1D).

Subsequently, we examined the 144 deletions from the genome-wide screen that exhibited foci in 15%–20% of cells (sub-set C). We suspected that interesting candidate genes would be found in this sub-set since it includes deletions of several genes directly associated with genomic integrity, including replication (CTF18, RAD27), homologous repair (MRE11, RAD50), chromatin remodeling (HPA1, SET2, SNT1, SW16), and cell cycle control (CLB5, RAD17). To decide how much further to study this sub-set, we retested all 41 deletions of 16 previously uncharacterized ORFs concurrently with the gene deletions during the screen.

Lastly, we recognize that the screen produces some false negatives since several gene deletions expected to exhibit elevated focus levels (e.g., mre11Δ, xrs2Δ, rad50Δ, rad27Δ) failed the cutoff for significance (16%, 13%, 9%, and 16%, respectively). In addition, the screen identified some gene deletions (e.g., dde1Δ, rad57Δ, sld8Δ, mms1Δ, and rtt109Δ), but failed to identify other members of the same complexes (e.g., mec3Δ and rad17Δ, rad55Δ, sld5Δ, asf1Δ, and mms22Δ) that were expected to be phenotypically equivalent. We then retested the other members individually and they consistently demonstrated elevated levels of spontaneous foci (Table S1E) indicating that the focus phenotype was shared among all members of each complex and that the mutants not identified in the initial screen were false negatives. We did not include these mutants in our subsequent analysis, as we studied only those gene deletions identified by our original screening criteria.

**IRC Genes Are Assigned to 22 Uncharacterized ORFs**

Among the 89 deletions that exhibit elevated levels of spontaneous Rad52 foci were 25 previously uncharacterized ORFs. Eight of these IRC genes are listed as dubious ORFs on the *Saccharomyces* Genome Database due to the small size of the coding region. In addition, three IRC ORFs overlap other genes identified in the screen (IRC1 and BDF2, IRC15 and CTF19, IRC17 and RTT103), while several others overlap genes not identified in the screen. To verify that the focus phenotype observed in each IRC mutant was the result of disruption of that ORF, we performed a complementation test. Twenty-two of the 25 ircΔs were complemented by their corresponding ORF. However, a wild-type copy of the IRC ORF was unable to complement irc1Δ (ydr071wΔ), irc12Δ (yor024wΔ), and irc17Δ (ydr290wΔ) (Figure S2A). Of these three, irc1Δ and irc17Δ remove sequences from overlapping genes that were also identified in the screen and their focus phenotype was complemented by a plasmid containing the neighboring gene (Figures S2B and S2D). For irc12Δ the adjacent non-overlapping gene HST3 complemented the phenotype (Figure S2C). We conclude that the elevated focus phenotype in these three strains was likely a consequence of mutation of the neighboring genes or regulatory sequences, thus reducing the number of mutant strains to 86.

**Defining Genetic Interaction Sub-Networks**

Increased levels of Rad52 foci may indicate a dependency on RAD52 gene function. In fact, 14 of the 86 mutants previously showed a synthetic fitness defect or lethality when combined with a rad52Δ allele [13–19]. Synthetic genetic interactions between all 86 gene deletions and rad52Δ were assayed through a comprehensive quantitative analysis (see Materials and Methods). The analysis revealed 27 synergistic interactions, 15 of which had not been previously described. All interactions observed resulted in synthetic growth defects, with no double mutants exhibiting improved growth compared to the single mutants. One gene deletion, *mupl6060Δ*, exhibited synthetic lethality with the *rad52Δ* allele. To distinguish between strong and weaker interactions, the remaining 26 were parsed into synergistic and additive growth defects (see Materials and Methods). Growth differences revealed 17 synergistic and 10 additive interactions (Table 2). Finally, we are unable to reproduce the previously reported synthetic interactions of *mus81Δ* and *rtt107Δ* with
Screen for Mutants Affecting Rad52 Foci

Table 1. Genes Identified in the Screen for Increased Spontaneous Rad52-YFP Foci

| Gene       | % Foci | Cellular Role | Class | Gene       | % Foci | Cellular Role | Class |
|------------|--------|---------------|-------|------------|--------|---------------|-------|
| AHC1       | 23     | Chromatin remodeling | III   | IRC25 /YLR021W | 19     | Undetermined | IV    |
| ATR1       | 20     | Membrane transport | IV    | IZH2       | 22     | Zinc/phosphate homeostasis | IV    |
| BCK1       | 20     | Stress response | IV    | LAG2       | 22     | Cell aging | IV    |
| BDF1       | 23     | Chromatin remodeling | IV    | LCBS       | 25     | Lipid metabolism | IV    |
| BDF2       | 20     | Chromatin remodeling | IV    | LRS4       | 39     | Chromatin remodeling | III   |
| BUB2       | 24     | Mitotic checkpoint | IV    | MAD1       | 21     | Mitotic checkpoint | IV    |
| BUD27      | 23     | Stress response | III   | MAD2       | 23     | Mitotic checkpoint | IV    |
| CBT1       | 29     | Mitochondrial function | IV    | MAD3       | 28     | Mitotic checkpoint | IV    |
| CCK16      | 22     | Mitochondrial function | II    | MDM2O      | 32     | Mitochondrial inheritance | IV    |
| CTF4       | 42     | DNA replication/cohesion | III   | MEDI       | 24     | Transcription | IV    |
| CTF19      | 37     | Chromosome segregation | II    | MLH1       | 24     | DNA repair | II    |
| DAK2       | 20     | Stress response | III   | MMS1       | 35     | Chromatin remodeling | III   |
| DDC1       | 22     | DNA damage checkpoint | III   | MRP1       | 21     | Mitochondrial function | II    |
| DDR2       | 28     | Stress response | II    | MRP11      | 22     | Mitochondrial function | IV    |
| ECM11      | 32     | Undetermined | III   | MRP516     | 23     | Mitochondrial function | IV    |
| ELG1       | 41     | DNA replication | II    | MUS81      | 24     | DNA repair | III   |
| ESC2       | 27     | Chromatin remodeling | III   | NUP60      | 24     | Nuclear pore | II    |
| GDH2       | 20     | Amino acid metabolism | III   | NUP133     | 29     | Nuclear pore | II    |
| GH92       | 20     | Stress response | IV    | PAC10      | 23     | Protein folding | III   |
| HPR1       | 21     | Transcription | II    | PDR10      | 22     | Membrane transport | III   |
| HRT2 /YMR027W | 24 | Undetermined | IV    | POM152     | 20     | Nuclear pore | II    |
| HST3       | 30     | Chromatin remodeling | III   | RAD51      | 21     | DNA repair | IV    |
| IRC2 /YDR112W | 24 | Undetermined | IV    | RAD54      | 24     | DNA repair | I     |
| IRC3 /YDR332W | 31 | Undetermined | III   | RAD57      | 24     | DNA repair | I     |
| IRC4 /YDR540C | 23 | Undetermined | III   | RAD59      | 34     | DNA repair | III   |
| IRC5 /YFR038W | 21 | Undetermined | III   | RC01       | 20     | Chromatin remodeling | IV    |
| IRC6 /YFR043C | 36 | Undetermined | IV    | RIM9       | 20     | Sporulation | I     |
| IRC7 /YFR055W | 29 | Undetermined | III   | RM1        | 39     | DNA repair | II    |
| IRC8 /YJL505W | 23 | Undetermined | II    | RM3        | 25     | DNA replication and repair | III   |
| IRC9 /YJL142C | 20 | Undetermined | III   | RRT101     | 44     | DNA replication and repair | III   |
| IRC10 /YOL015W | 23 | Undetermined | IV    | RRT103     | 22     | Ty1 transposition | II    |
| IRC11 /YOR013W | 20 | Undetermined | IV    | RRT107     | 28     | DNA silencing | IV    |
| IRC13 /YOR235W | 26 | Undetermined | IV    | RRT109     | 54     | Chromatin remodeling | III   |
| IRC14 /YOR135C | 27 | Undetermined | III   | SAE2       | 24     | DNA repair | III   |
| IRC15 /YPL017C | 27 | Undetermined | II    | SGO1       | 23     | Chromosome segregation | IV    |
| IRC16 /YPR038W | 20 | Undetermined | II    | SG51       | 22     | DNA repair | II    |
| IRC18 /YOL037W | 25 | Undetermined | IV    | SLX8       | 39     | Genome stability | II    |
| IRC19 /YLL033W | 19 | Undetermined | III   | TOF2       | 23     | Chromatin remodeling | IV    |
| IRC20 /YLR247C | 21 | Undetermined | IV    | TRF4       | 21     | RNA poly(A) polymerase | IV    |
| IRC21 /YMR073C | 21 | Undetermined | IV    | VPS71      | 21     | Chromatin remodeling | III   |
| IRC22 /YEL011C | 17 | Undetermined | II    | VPS72      | 30     | Chromatin remodeling | III   |
| IRC23 /YOR044C | 18 | Undetermined | IV    | WSS1       | 26     | Undetermined | III   |
| IRC24 /YHR036C | 19 | Undetermined | IV    | YMR31      | 20     | Mitochondrial function | III   |

This list includes gene deletions identified with consistently elevated levels of Rad52-YFP foci, with listed values reflecting the observed levels of foci for each gene deletion in the initial screen (wild type = 5%). Descriptions of cellular role were derived from the YPD database (http://www.proteome.com) and consensus from the available literature. The set of 86 mutants is significantly enriched for proteins localized to the nucleus (41 genes; p-value = 3.2 × 10⁻⁵), and for genes associated with DNA metabolism (33 genes; p-value = 1 × 10⁻⁴) and stress response (24 genes; p-value = 6.0 × 10⁻⁴). Recombination classes are defined based on the results of two recombination assays at the LEU2 locus. Class I represents gene deletions leading to decreased levels of sister chromatid and interhomolog recombination, while Class II contains deletions that elevate levels of recombination in both assays. Class III mutants are hyper-recombinant between homologous chromosomes but not between sisters. Class IV mutants are wild type for recombination at LEU2.

The rad52Δ allele [18]. The 27 interactions described here suggest that the absence of these genes intensifies the requirement for HR in cell survival and growth.

Mitotic Recombination Rates Define Four Mutant Classes

The mutants were parsed into functional classes based on their effect on homologous recombination. The increase in spontaneous Rad52 foci observed in the mutant strains may correspond to altered levels of spontaneous recombination. This hypothesis is underscored by the observation that a number of these mutants have been previously demonstrated to exhibit either elevated (hpr1Δ) or reduced (rad51Δ) mitotic recombination [20,21]. Each focus mutant was subjected to two heteroallelic recombination assays to sort them into

Table 2. Mutants with rad52Δ Synthetic Interactions

| Synergistic Interactors | Additive Interactors |
|-------------------------|----------------------|
| bub2Δ                  | irc19Δ               |
| irc4Δ                  | rmt3 Δ              |
| bck1Δ                  | mih1Δ               |
| mgs13Δ                 |                          |
| elg1Δ                  | mms1Δ               |
| mpr11Δ                 | rtt101Δ             |
| ddc1Δ                  | irc3Δ               |
| prp1Δ                  | pac101Δ             |
| hpr1Δ                  | nup60Δ              |
| trf4Δ                  | irc14Δ              |
| slx8Δ                  | rtt103Δ             |
| irc5A                   | nup133Δ             |
| irc8Δ                   | wss1Δ               |

*Previously described interaction.  
**Synthetic lethal interaction.  

doi:10.1371/journal.pgen.0030228.t002
alleles is one underlying cause of LOH. Interhomolog recombination between heteroalleles, specifically those recombination events that do not involve a single strand annealing, or a functional LEU2 allele from two leu2 heteroalleles. The URA3 marker between the heteroalleles permits discrimination between sister chromatid conversion events (Leu⁺ Ura⁻ recombinants) and SSA (Leu⁻ Ura⁻ recombinants). (B) The interhomolog recombination assay measures recombination between leu2 heteroalleles on homologous chromosomes in diploid cells. Chromosomes are shown following DNA synthesis and reflect a pair of sister chromatids for each chromosomes homolog. A recombinogenic lesion occurring at the LEU2 locus is most often repaired using the template on the sister chromatid, which is a conservative event resulting in no loss or gain of genetic information (left). However, a gene conversion event or reciprocal exchange between two homologous chromosomes can generate a functional LEU2 allele (right). doi:10.1371/journal.pgen.0030228.g003

functional classes corresponding to the mechanisms that lead to the accumulation of Rad52 foci (Figure 3). The direct repeat assay measures the rate of sister chromatid gene conversion and intrachromosomal single-strand annealing (SSA) events, while the interhomolog assay measures recombination between alleles on homologous chromosomes in diploids, specifically those recombination events that do not utilize the sister chromatid, which is the preferred template for HR [22]. Interhomolog recombination between heteroalleles is one underlying cause of LOH.

Table 3. Direct Repeat Recombination Rates

| Gene Deletion | Gene Conversion (Leu⁺ Ura⁻) \(10^{-5}\) | SSA (Leu⁻ Ura⁻) \(10^{-5}\) |
|---------------|---------------------------------|-----------------|
| Wild type     | 2.2 ± 0.5                       | 2.3 ± 0.5       |
| cx16Δ         | 4.2 ± 0.8                       | 2.1 ± 0.4       |
| ct19Δ         | 5.3 ± 1.0                       | 1.2 ± 0.3*      |
| elg1Δ         | 4.4 ± 1.0                       | 3.2 ± 0.8*      |
| hpr1Δ         | 135 ± 27                        | 100 ± 20        |
| irc8Δ         | 5.6 ± 1.2                       | 2.1 ± 0.5*      |
| irc15Δ        | 5.0 ± 0.9                       | 1.5 ± 0.3*      |
| mih1Δ         | 7.4 ± 1.5                       | 5.8 ± 1.2       |
| mmp17Δ        | 6.5 ± 1.3                       | 3.0 ± 0.7*      |
| nup133Δ       | 19 ± 3.3                        | 9.5 ± 1.8       |
| nup60Δ        | 26 ± 4.9                        | 11 ± 2.1        |
| pom152Δ       | 4.6 ± 0.2                       | 3.7 ± 0.5       |
| rmi1Δ         | 5.9 ± 1.2                       | 4.9 ± 1.0       |
| sgi1Δ         | 4.4 ± 1.0                       | 3.2 ± 0.7*      |
| stkΔ          | 5.4 ± 1.2                       | 6.0 ± 1.3       |

Rates of direct repeat recombination were measured using the assay illustrated in Figure 3A. Only the gene deletions exhibiting gene conversion rates significantly greater than wild type are shown (Class II). *Does not reflect a significant change from wild type. doi:10.1371/journal.pgen.0030228.t003

The recombination rates for the two assays divide the set of mutants into four classes (Tables 1, 3, and 4). The three Class I mutants are in the RAD52 epistasis group and exhibit significantly reduced levels of gene conversion between both sister chromatids and homologous chromosomes but wild-type levels of SSA, consistent with previous studies [21,23].

Table 4. Interhomolog Recombination Rates

| Gene Deletion | Recombination Rate \(10^{-5}\) | Gene Deletion | Recombination Rate \(10^{-5}\) |
|---------------|--------------------------------|---------------|--------------------------------|
| Wild type     | 8.8 ± 1.4                       | irs4Δ         | 22 ± 4.0                       |
| ach1Δ         | 40 ± 6.8                        | mhl1Δ         | 309 ± 40                       |
| leu2Δ         | 118 ± 20                        | mss1Δ         | 88 ± 15                        |
| cox16Δ        | 35 ± 5.7                        | mnt17Δ        | 26 ± 4.2                       |
| ct19Δ         | 18 ± 3.8                        | mus81Δ        | 46 ± 7.2                       |
| ct4Δ          | 172 ± 29                        | nup133Δ       | 232 ± 38                       |
| dakΔ          | 23 ± 3.9                        | nup60Δ        | 33 ± 6.9                       |
| ddc1Δ         | 44 ± 7.4                        | pac10Δ        | 20 ± 3.7                       |
| ddr2Δ         | 22 ± 4                          | pdr10Δ        | 28 ± 4.9                       |
| ecm11Δ        | 29 ± 4.8                        | pom152Δ       | 16 ± 3.1                       |
| elg1Δ         | 20 ± 3.3                        | rad59Δ        | 30 ± 5.2                       |
| esc2Δ         | 57 ± 8.6                        | rmi1Δ         | 268 ± 44                       |
| hpr1Δ         | 122 ± 30                        | rmt3Δ         | 94 ± 14                        |
| hst3Δ         | 60 ± 9.0                        | rtt101Δ       | 90 ± 15                        |
| irc4Δ         | 53 ± 8.6                        | rtt109Δ       | 48 ± 7.6                       |
| irc5Δ         | 59 ± 8.8                        | sde2Δ         | 51 ± 9.0                       |
| irc7Δ         | 21 ± 3.7                        | sgi1Δ         | 222 ± 34                       |
| irc8Δ         | 29 ± 5.4                        | stkΔ          | 105 ± 18                       |
| irc9Δ         | 18 ± 3.5                        | wps21Δ        | 25 ± 4.8                       |
| irc11Δ        | 59 ± 8.9                        | wps72Δ        | 21 ± 3.8                       |
| irc14Δ        | 22 ± 4.4                        | wss1Δ         | 35 ± 6.9                       |
| irc16Δ        | 17 ± 3.1                        | ymr31Δ        | 18 ± 3.6                       |
| irc19Δ        | 27 ± 4.5                        | ymr32Δ        | ...                            |

The interhomolog recombination assay used here is shown in Figure 3B. Only the gene deletions that exhibited recombination rates significantly greater than wild type are shown, including all those shown in Table 3 (Class II) and others that are hyper-recombinant specifically in this assay (Class III). doi:10.1371/journal.pgen.0030228.t004

Figure 3. Recombination Assays Used in This Study

(A) The direct repeat recombination assay measures intra-chromosomal and sister chromatid recombination events that generate a functional LEU2 allele from two leu2 heteroalleles. The URA3 marker between the heteroalleles permits discrimination between sister chromatid conversion events (Leu⁺ Ura⁻ recombinants) and SSA (Leu⁻ Ura⁻ recombinants). (B) The interhomolog recombination assay measures recombination between leu2 heteroalleles on homologous chromosomes in diploid cells. Chromosomes are shown following DNA synthesis and reflect a pair of sister chromatids for each chromosomes homolog. A recombinogenic lesion occurring at the LEU2 locus is most often repaired using the template on the sister chromatid, which is a conservative event resulting in no loss or gain of genetic information (left). However, a gene conversion event or reciprocal exchange between two homologous chromosomes can generate a functional LEU2 allele (right). doi:10.1371/journal.pgen.0030228.g003
The 14 gene deletions that exhibit increased rates of both direct repeat and interhomolog recombination define Class II. The 32 mutants in Class III exhibit increased rates of recombination specifically between homologous chromosomes, while sister chromatid recombination (SCR) in the direct repeat assay is unaffected. Surprisingly, 37 Class IV gene deletions fail to demonstrate any alteration in the rate of recombination with either assay. It is possible that these mutants affect recombination only at specific genomic regions. To begin to examine this notion, we tested 33 of the Class IV deletion strains using an assay that measures recombination in the multiple tandem rDNA array [24]. Elevated frequencies of recombination in the rDNA array compared to wild type (1.7 × 10⁻² ± 0.7) are observed in four gene deletion strains (bek1Δ 18 × 10⁻² ± 2.2, bdf1Δ 7.4 × 10⁻² ± 2.9, rtt107Δ 5.8 × 10⁻² ± 2.3, and tfd4Δ 9.3 × 10⁻² ± 3.7), demonstrating that the focus phenotype may be triggered by events at specific loci.

Discussion

To identify pathways involved in the maintenance of genomic integrity, we used a newly developed method, systematic hybrid loss of heterozygosity, to introduce a cell biological assay into over 4,800 nonessential gene deletion strains. The levels of spontaneous relocational of Rad52-YFP protein into subnuclear foci were analyzed in individual gene deletion strains. Our screen differs from previous genomic studies where changes in DNA metabolism phenotypes were identified using the output of DNA repair or recombination assays, such as survival following exposure to exogenous DNA damage or the gain or loss of genetic markers following recombination. Our cell biological screen permits the identification of alterations in the HR pathway in living cells regardless of the outcome of these events. In addition to mutations affecting recombination that ultimately block the appearance of recombinants (Class I) and mutations that increase the formation of spontaneous DNA damage (Class II), our screen uncovered mutations that alter the kinetics rather than the frequency of events (Class III) along with those that do not affect recombination globally (Class IV).

The majority of the genes identified in this study fall into functional groups associated with DNA metabolism and chromosome dynamics, including replication and repair, transcription, and chromatin remodeling (Table 1). In addition, we found genes involved in nuclear pore complexes and mitochondrial function as well as diverse cellular processes such as the spindle assembly checkpoint not previously associated with HR. Finally, one quarter of the genes identified in our screen (22) were uncharacterized. It is notable that many of these IRC genes are very small ORFs, which would have reduced the likelihood of identifying them in traditional mutagenesis screens. Indeed, most have either no apparent impact on the outcome of recombination (Class IV) or a subtle effect below the sensitivity of other assays (Class III).

Both direct repeat and interhomolog recombination assays were used to parse the complete set of mutants into a handful of classes that indicate the mechanisms leading to the focus phenotype. The four classes of mutants were based upon their recombination phenotype: Class I: decreased Rad51-depend-

ent HR; Class II: increased HR between sister chromatids and between homologous chromosomes; Class III: increased HR specifically between homologous chromosomes; and Class IV: wild-type levels of HR (Figure 4). The quantitative analysis of synthetic genetic interactions between all 86 mutants and rad52A provide further insight into the subdivisions within the classes.

Class I: Hypo-Recombination

Three members of the RAD52 epistasis group, rad51Δ, rad54Δ, and rad57Δ display elevated levels of Rad52-YFP foci and decreased levels of Rad51-dependent recombination (this study and [25]). Rad51, Rad54, and Rad57 are recruited to a DNA lesion subsequent to Rad52 focus formation and their recruitment is dependent on Rad52 [25]. Deletion of genes that encode proteins that function downstream of Rad52 in HR leads to a failure to complete the recombination process. Thus, the increased focus levels in these mutants likely reflect the persistence of Rad52-YFP foci. Since SSA is dependent upon Rad52, but not Rad51 or other later HR proteins, the rate of that process is not affected by these gene deletions [21,26]. Class I gene deletions do not exhibit synthetic interactions with rad52A, since they are in the same epistasis group. It is noteworthy that none of the newly defined IRC gene deletions fall into this class, indicating that these genes are not required for Rad51-dependent HR.

Class II: Hyper-Recombination

Class II mutants exhibit elevated levels of recombination between sister chromatids and between homologous chromosomes likely reflecting an increase in the generation of spontaneous DNA lesions requiring repair via HR. Thus, the Class II focus phenotype reflects an increase in the overall frequency of formation of Rad52-YFP foci in these mutants. This class includes a number of genes with well-characterized roles in the maintenance of genomic integrity and the suppression of spontaneous DNA damage (SGS1, RMI1, ELG1, MLH1, HPR1, and SLX8), as well as all three nuclear pore genes identified in the study (NUP60, NUP133, and POM152) [27–35]. The Class II recombination phenotype is exhibited by two mitochondrial genes (COX16 and MRPI17), which suggests that an increase in oxidative damage (e.g., reactive oxygen species) stimulates spontaneous DNA lesions in these mutants [36–38]. Among the Class II mutants, ten of 14, including the previously uncharacterized gene IRC8, sensitize cells to the absence of a functional RAD52 allele. Such a synthetic defect is consistent with an increased requirement for Rad52-mediated HR in these gene deletion strains as a result of the increased generation of spontaneous lesions.

Class III: Interhomolog recombination

Class III mutants exhibit elevated levels of recombination between homologous chromosomes in diploid cells, but wild-type levels of SCR in haploids. It is unlikely that the Rad52 focus phenotype observed in this class reflects a general increase in endogenous DNA damage, but rather that spontaneous lesions that do arise are processed differently in these mutants. In diploid cells, lesions are preferentially repaired using the sister chromatid as template, effectively preventing LOH that can result from repair from the homologous chromosome. In Class III mutants, the 2- to 20-
fold elevation in interhomolog recombination occurs without a concomitant increase in SCR. We suggest that the increased utilization of the homolog for repair reflects a defect in the efficiency of SCR. However, reduced SCR in these mutants is not a result of a defect in sister chromatid cohesion since only one Class III mutant, *ctf4*Δ, exhibited precocious sister chromatid separation ([39] and unpublished data).

The increase in interhomolog recombination observed in Class III mutants may reflect a defect in the recombination center itself. Null mutants of members of the Mre11-Rad50-Xrs2 complex and certain separation-of-function alleles of *rad52* exhibit elevated levels of spontaneous Rad52-YFP foci and increased interhomolog recombination, identical to the phenotype of our Class III ([11,40]. Class III includes three genes (*DDC1*, *RAD59*, and *SAE2*) that encode proteins that themselves localize to repair foci in response to DNA damage [25]. In addition, *Mus81* protein functions downstream of Rad52 in the resolution of recombination intermediates [41,42]. Similar to Class I, these four mutants do not exhibit synthetic growth interactions with *rad52*Δ as they function in the same pathway.

Class III also includes deletions of a large number of genes with roles in chromatin modification and remodeling (*AHC1*, *ESC2*, *LSR4*, *RRM3*, *VPS71-VPS72*, *MMR1-RTT101-RTT109*, and *HST3*) [20,43–49]. Modified chromatin at the site of damage may itself function as a scaffold for the recruitment and assembly of the repair machinery. Remodeling tightly condensed chromatin is critical to allow repair proteins access to both the damaged DNA and the homologous template [50,51]. Perhaps chromatin defects delay use of the sister chromatid as the repair template. The delay, observed here as persistent Rad52 foci, may increase the use of the homologous chromosome for repair.

Since the majority of the previously characterized Class III genes function in DNA replication, repair, and chromatin dynamics, it is likely that the six newly identified *IRC* genes in this class (*IRC4*, *IRC5*, *IRC7*, *IRC9*, *IRC14*, and *IRC19*) are also involved in DNA metabolism. Like six of the 12 Class III genes implicated in chromatin metabolism described above, *irc5*Δ, *irc14*Δ, and *irc19*Δ exhibit synthetic growth defects with *rad52*Δ, suggesting related roles for these genes. The *irc19*Δ mutant also exhibits synthetic defects with other genes associated with the maintenance of genome integrity including genes involved in replication and HR [18]. *IRC5* encodes a putative Snf2 family DNA helicase with homology to the mammalian lymphoid-specific helicase HELLS, supporting a role in chromatin remodeling [46,52]. Genetic interactions of the *irc5*Δ mutant correlate strongly with those of replicative proteins (*rad27*Δ, *els1*Δ, *rnh201Δ*, *pol30–79*, *rfe4-DAMP*, *rfe5-DAMP*), suggesting that Irc5 protein remodeling activity may be involved in DNA replication (N. Krogan, personal communication).

**Figure 4.** Pathways Leading to Increased Rad52 Foci Are Revealed by This Study

Class I mutants block HR subsequent to the formation of Rad52 foci. Class II mutants stimulate the formation of DNA lesions, which is reflected as increased focus formation and results in increased sister chromatid and interhomolog recombination. Class III mutants decrease the efficiency of sister chromatid recombination through effects on cohesion, chromatin architecture, HR, and other mechanisms, thereby increasing the duration of Rad52 foci (indicated by the meandering line). Class IV mutants do not have a global effect on homologous recombination. However, the focus phenotype in some mutants reflects recombination at specific sites such as rDNA or telomeres. Other mutants may cause the division of cells prior to resolution of HR foci, or lead to the generation of lesions that cannot be repaired, resulting in lethality. For all classes, mutations with an asterisk and highlighted in yellow, sensitize cells to the absence of Rad52 (see Table 2).

doi:10.1371/journal.pgen.0030228.g004
Class IV: Mutants without Recombination Phenotype

Class IV gene deletions do not affect recombination specifically at the LEU2 locus. Since it is possible that recombination is affected in other regions of the genome, we measured recombination rates within the rDNA multiple tandem array. We chose to examine this array next because it is a highly organized region where recombination is tightly regulated [53]. We identified four Class IV mutants (bck1Δ, bdf1Δ, rtt107Δ, and trf4Δ) that exhibit rDNA hyper-recombination. Similar to most of the mutants in Class II, three of the four (bck1Δ, bdf1Δ, and trf4Δ) show synthetic interactions with rad52Δ that is consistent with their potential roles in the suppression of genetic instability within this array. It is tempting to speculate that IRC13, BUB2, and RTT103, the three other Class IV genes that show synthetic interactions with rad52Δ may also be involved in the suppression of region-specific damage. Other regions of the genome that require specific factors for maintenance are telomerases. For example, defects in telomere capping may lead to increased recognition of telomerases as DSB ends that would recruit Rad52. Interestingly, irc6Δ may have such a defect (W. Zhang and D. Durocher, personal communication).

Alternatively, increased levels of Rad52 foci may occur without measurable effects on the products of recombination. For example, the focus phenotype may be due to slowing of the HR process or delaying the disassembly of foci without changing the outcome. In addition, a gene deletion may generate unreparable lesions that could result in the accumulation of repair proteins into foci but lead to cell lethality. For the spindle assembly checkpoint mutants, mad1Δ, mad2Δ, mad3Δ, and bub2Δ, an increase in the number of cells with foci in G1 was observed (unpublished data). Mitotic division before resolution of a repair focus formed during the previous round of DNA replication could result in two G1 cells with potentially unresolvable foci [54,55]. The unique phenotype of the Class IV mutants underscores the utility of taking a cell biological approach to investigate HR. Examination of intermediate steps in the process permits the identification of genes that would not have been found using assays requiring a measurable recombination product.

The large number of chromatin remodeling, mitochondrial, and spindle checkpoint genes found in Class IV suggests that some of the 12 IRC genes in this class may be involved in these processes. For example, IRC3 encodes a putative DEAD/DEAH box helicase that localizes to mitochondria and exhibits synthetic lethal interactions with deletions of spindle assembly checkpoint proteins, histones, and proteins associated with sister chromatid cohesion. Furthermore, IRC20 encodes a putative Snf2/Swi2 family helicase, which localizes to nuclei and mitochondria and is implicated in transcriptional regulation, while IRC21 is predicted to function in chromatin remodeling [14,38,46,56–58] (G. Prelich, personal communication).

Evolutionary Conservation

Overall, the 86 genes identified in the Rad52-YFP focus screen are largely conserved throughout eukaryotic evolution, with 49 having homologs in nearly every sequenced eukaryotic species (Table S4). Homologs for another 15 have been found in evolutionarily divergent yeast species including Schizosaccharomyces pombe and Candida albicans, but not in mammals, Drosophila melanogaster, or Caenorhabditis elegans. The remaining 22 are found in the closely related sensu stricto yeast species. Seven IRC genes have homologs identified across eukaryota. This includes three putative helicases, IRC3, IRC5, and IRC20. Other IRC homologs have been linked to human diseases, including the IRC7 homolog CTH, which encodes a cystathionase implicated in premature births and cancers, and the IRC24 homolog SPR, which contains polymorphisms associated with Parkinson disease [59–61]. IRC21 has homology to NADPH cytochrome B5 oxidoreductase, which is linked to insulin-dependent diabetes in mice, while IRC15 resembles mammalian mitochondrial dihydrolipoamide dehydrogenase, associated with a number of human diseases including Alzheimer disease [62,63].

Summary

The Rad52-YFP focus screen described here applies systematic hybrid LOH in a genome-wide cell biological search to identify proteins involved in diverse pathways contributing to genomic integrity. This assay permitted the identification of a large set of gene deletions that affect the incidence and dynamics of HR foci in living cells regardless of the genetic outcome. In particular, we uncovered 22 previously uncharacterized ORFs, many having only subtle effects on the process of recombination, which prevented their identification in other screens. Since recombination is a multi-step process, it will be of interest to examine the dynamics of other HR factors within our mutant set and the genome as a whole. Additionally, it will be important to show that the conserved genes in other organisms play similar roles in this process.

Materials and Methods

Strains and plasmids. Individual deletions of nonessential genes made in the BY4742 and BY4739 MATα strains were obtained from the Saccharomyces Gene Deletion Project [64]. The conditional chromosome strains used to create the hybrid LOH strains and the method of inducing LOH in these strains have been described [12]. Briefly, the systematic hybrid LOH method utilizes a set of 16 strains, each containing a conditional centromere construct on one of the 16 yeast chromosomes. Mating each gene deletion strain to the appropriate conditional centromere strain creates hybrid diploids that are transiently heterozygous for the gene deletion. After chromosome loss is induced for the conditional chromosome, homozygosis of the marked gene deletion occurs more than 95% of the time as a result of endoduplication of the monosomic chromosome. This method allows the introduction of any plasmid or chromosomal construct into individual mutants of the gene deletion library through mating rather than individual transformations.

Strain BY4742 was used as the wild-type control for the library background. Gene deletions from the library were backcrossed into the W303 background to create congenic strains. Gene deletion strains assayed for direct repeat recombination are congenic (minimum of four backcrosses) to W3880-3A (MATα ade2-leu2-AEC0R::URA3-len2-ABstEI TRPI lys2A RAD5). Homozygous diploid deletion strains tested for interhomolog recombination are congenic (minimum of five backcrosses) to W5309 (MATα ade2-leu2-AEC0R::TRP1 trp1-1 LYS2 lys2A ade2-EC0R::ABstEI). Deletion strains assayed for marker loss in the rDNA array are congenic (minimum of six backcrosses) to W6921-5A (MATα ade2-1 can1-100 TRP1 LYS2 rDNA::ADE2-CAN1).

The pWJ1314 plasmid, which expresses Rad52-YFP from the native RAD52 promoter, was used for all focus measurements performed using library gene deletion strains [12]. Plasmids used for complementation analysis of ir mutants were built utilizing plasmid pWJ1250, designed to facilitate cloning via HR in yeast. To construct plasmid pWJ1250, overlapping primers C-D-TOP (AGCGAGGTCGACTAGGGATAAAGGGTAATG- CAGGGTAATCCGCTGCTAGGCGCGCCGTGGTTAACGTCAG) and C-D-BOTTOM (CAGCTGGAGCTC TAGGGATAACAGGGTAATG-CAGGGTAATCCGCTGCTAGGCGCGCCGTGGTTAACGTCAG) were fused and ampli-
fied by PCR with primers C-D-Forward (AGGCAAGTTGACTAGG) and C-D-Reverse (CAGCTGGACGCTTAGG). This reaction resulted in a synthetic DNA containing common adaptor sequences C and D separated by the restriction site for the I-SceI meganuclease, and then flanked by SacI and SalI sites. The amplified DNA was end-repaired, cut with SacI and SalI, and transformed into the gene deletion strains congenic (minimum of six backcrosses) to W3749-14C (MATa ADE2 bar1::LEU2 trp1–1 LYS2 RAD52-YFP) along with plasmid pWJ1250 as a control. Transformation was performed by comparing levels of spontaneous Rad52-YFP foci in each gene deletion strain when transformed with the empty vector as a control (Figure S2).

In the focus screen described, 108 mutants were initially identified with Rad52-YFP foci in greater than 20% of cells examined. Of these 108, all deleted maintained the focus phenotype following repeat experiments and were selected for further analysis. After restating all uncharacterized ORFs with focus levels between 15%–20% (41), nine consistently exhibited elevated foci and were added to the set of deletions. Three hypothetical ORFs were removed from the set after expression of the wild-type gene failed to complement the focus phenotype in the gene deletion. The remaining 95 deletions were prepared for the additional assays described below.

Microscopy. Examination of Rad52-YFP focus levels by microscopy was performed as previously described [66]. Briefly, cells were grown overnight in SC-Leu media at 23°C and exponentially growing cultures were prepared for microscopy. A single DIC image and 12 YFP images obtained at 0.3-μm intervals along the z-axis were captured for each frame, and Rad52-YFP foci were counted by inspecting all focal planes intersecting each cell. For each gene deletion strain in the screen, 200–400 cells were scored for Rad52-YFP foci. All Rad52-YFP focus data are presented in Figures 2, S1, and S2, and Table S1.

GO enrichment analysis. We took advantage of the existing Gene Ontology (GO) annotations to determine whether our set of 86 mutants was enriched for any particular categories of genes. We utilized the Fisher’s Exact Test to compute statistically significant enrichment of GO categories within the 86 mutants compared to those among the complete set of 4,805 mutants assayed for Rad52-YFP foci. The set of focus mutants exhibited enrichment in the GO component category for proteins localized to the nucleus (p-value = 3.2 × 10–3), as well as the GO biological process categories for DNA metabolism and response to stress (p-value = 1.3 × 10–3 and 6.0 × 10–5). The Bonferroni corrected threshold for significance among GO components was 2.9 × 10–3 and among GO processes was 1.5 × 10–3.

Synthetic interactions. Synthetic interactions between library gene deletions and rad52Δ were determined on the basis of spore colony size following tetrad dissection. In the initial analysis, all 86 library gene deletion strains were mated to W303 background strain W3777-17A (MATa Ade2 bar1::LEU2 trp1–1 LYS2 rad52::HIS3 RAD5) and sporulated. Twenty-four tetrads were dissected for each cross, and dissection plans were scanned using Adobe Photoshop (Adobe Systems). Colony size was measured for each cross using a macro (Y. Deng, unpublished data) written for ImageJ software (W. Rasband, National Institutes of Health), followed by genotyping for segregating alleles. Average colony size and standard deviation were derived in each cross from the wild-type, gene deletion, rad52Δ, and the double mutants, and normalized to the mean value for the wild-type segregants in the cross. For this study, we defined two different classes of synthetic interactions for growth. Additive interactions are defined when the average colony size for the double mutant is significantly less than the colony size for either single mutant. Synergistic interactions are defined when the average colony size of the double mutant is significantly less than the product of the normalized colony size values of either single mutant. The synthetic interactions reported here were verified in a more closely related W303 background strain, and by performing a second trial with congenic deletion strains (minimum four backcrosses) and W3777-17A (rad52Δ). All data for synthetic interactions are presented in Table S2.

Quantitation of mitotic recombination. Spontaneous mitotic recombination between leu2-Δ100R1 and leu2-Δ100R1 heteroalleles was measured between sister chromatids in haploid strains and between homologous chromosomes in diploid strains as previously described [67,68] (Figure 3). Rates of mitotic recombination were calculated as described by Lea and Coulson [69]. For each gene deletion mutant, eight independent trials were performed for each assay. Two tailed t-tests were applied to determine significant changes in recombination rates in gene deletion strains. Recombination rates measured for all mutants in both assays are presented in Table S3. Frequencies of marker loss in the rDNA array was determined using a modification of a described method [21].

Supporting Information
Figure S1. Repeat Analysis of Strains Scored with Zero Rad52 Foci from the Initial Screen
(A) Distribution of Rad52-YFP focus levels observed after a second trial for all 233 strains initially scored with zero foci. The resulting distribution is a slightly left-shifted version of the distribution for the entire library (Figure 1), demonstrating that most of these zero focus strains are false positives.

(B) Induced Rad52-YFP focus levels following 40 Gy of ionizing radiation observed for 96 strains with the lowest spontaneous focus levels from (A). The dashed line indicates the range of focus levels observed for ten wild-type strains screened following IR.

Found at doi:10.1371/journal.pgen.0030228.s001 (59 KB PDF).

Figure S2. Complementation of the Rad52 Focus Phenotype in IRC Mutants
(A) 25 IRC gene deletion strains containing a genomically integrated RAD52-YFP fusion gene were analyzed following the introduction of either a single copy empty vector (black bars) or a vector containing a full-length copy of the IRC ORF corresponding to the one deleted in the strain (white bars). Rad52-YFP levels were scored as in the initial screen.

Twenty-two of 25 IRC genes complemented the focus phenotype, while the ORFs designated IRC1, IRC12, and IRC17 failed to complement.

(B) The irc1Δ removes a portion of the BDF2 gene, and introduction of a vector containing BDF2 (which also contains the full-length copy of IRC1) complements the focus phenotype in an irc1Δ strain.

(C) The IRC12 ORF is situated immediately between HST3 and AHCI, two other genes that exhibit elevated Rad52-YFP foci when deleted, but does not overlap either gene. Introduction of a vector containing a full-length copy of HST3 complements the focus phenotype in an irc12Δ strain, while IRC12 and AHCI do not.

(D) The irc17Δ removes a portion of the RR103 gene, and introduction of a vector containing RR103 (which also contains the full-length copy of IRC17) complements the focus phenotype in an irc17Δ strain. Since the focus phenotype observed in irc17Δ, irc12Δ, and irc17Δ is complemented by an adjacent gene, the gene names were withdrawn from the Saccharomyces Genome Database.

Found at doi:10.1371/journal.pgen.0030228.s002 (263 KB PDF).

Table S1. Rad52 Focus Screen Data
Found at doi:10.1371/journal.pgen.0030228.s001 (1.2 MB XLS).

Table S2. Rad52 Synthetic Interaction Data
Found at doi:10.1371/journal.pgen.0030228.s002 (62 KB XLS).

Table S3. Recombination Rates
Found at doi:10.1371/journal.pgen.0030228.s003 (27 KB XLS).

Table S4. Eukaryotic Homologs
Found at doi:10.1371/journal.pgen.0030228.s004 (41 KB XLS).

Acknowledgments
We thank members of the Rothstein laboratory, in particular Robert Reid, Peter Thorpe, Jacqueline Barlow, Adriana Antúnez de Mayolo, and Kara Bernstein. We also thank Nevan Krogan, Lorraine Symington, and Michael Snyder for helpful discussions concerning this work. We would also like to acknowledge the technical assistance provided by John Dittmar and Yan Deng. We are grateful to Nevan Krogan, Daniel Durocher, and Greg Prelich for sharing unpublished observations.

Author contributions. DA, ML, and RR conceived and designed the experiments, DA performed the experiments, analyzed the data, and wrote the paper.
References

1. Lisby M, Rothstein R (2004) DNA damage checkpoint and repair centers. Curr Opin Cell Biol 16: 328–334.
2. Sung P (1997) Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombination. J Biol Chem 272: 28194–28197.
3. New HD, Sugiyama T, Zaietsva E, Kowalczykowski SC (1998) Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature 391: 407–410.
4. Song B, Sung P (2000) Functional interactions among yeast Rad51 recombination, Rad52 mediator, and replication protein A in DNA strand exchange. J Biol Chem 275: 15895–15904.
5. Stasiak A, Zaruguet E, Stasiak A, Muller S, Engel A, et al. (2000) The human Rad52 protein exists as a heptameric ring. Curr Biol 10: 337–340.
6. Lisby M, Rothstein R, Mortensen UH (2001) Rad52 forms DNA repair and recombination centers during S phase. Proc Natl Acad Sci U S A 98: 8276-8282.
7. Lisby M, Mortensen UH, Rothstein R (2003) Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat Cell Biol 5: 572–577.
8. Raderschall E, Golub EL, Haaf T (1999) Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. Proc Natl Acad Sci U S A 96: 1921–1926.
9. Haaf T, Raderschall E, Reddy G, Ward DC, Radding CM, et al. (1999) Sequestration of mammalian Rad51-recombination protein into micro-nuclei. J Cell Biol 144: 11–20.
10. Lisby M, Rothstein R (2005) Localization of checkpoint and repair proteins in eukaryotes. Biochimie 87: 579–586.
11. Lehto G, Feng Q, Moyano AA, Erdenzin N, Reid RJ, et al. (2006) The role of DNA double-strand breaks in spontaneous homologous recombination in Saccharomyces cerevisiae. PLoS Genet 2: e194. doi:10.1371/journal.pgen.0020194
12. Alvaro D, Sunjevaric I, Reid RJ, Lisby M, Stillman DJ, et al. (2006) Systematic hybrid LOH: a new method to reduce false positives and negatives during screening of yeast gene deletion libraries. Yeast 23: 1097–1106.
13. Loeillet S, Palancade B, Cattron M, Thierry A, Richard GF, et al. (2005) Genetic networks orchestrations among replication, repair and nuclear pore deficiencies in yeast. DNA Repair (Amst) 4: 459–468.
14. Tong AH, Lesage G, Bader GD, Ding H, Xu H, et al. (2004) Global mapping of the yeast genetic interaction network. Science 303: 808–813.
15. Kanellis P, Aygi S, Durocher D (2003) Elg1 forms an alternative PCNA-interacting RFC complex required to maintain genome stability. Curr Biol 13: 1583–1595.
16. Bellaloui M, Chang M, Ou J, Xu H, Boone C, et al. (2003) Elg1 forms a novel RFC complex important for DNA replication and genome integrity. EMBO J 22: 3401–3413.
17. Torres JZ, Schnakenberg SL, Zakian VA (2004) Saccharomyces cerevisiae is decreased in recombinase, Rad52 mediator, and replication protein A. EMBO J 23: 7681–7687.
18. Pan X, Ye P, Yuan DS, Wang X, Bader JS, et al. (2006) A DNA integrity checkpoint affects sumoylation of DNA repair proteins and negatively regulates repair. Mol Cell Biol 26: 6153–6162.
19. Roux MP, Atchison JD, Suprapto A, Hjertaas K, Zhao Y, et al. (2006) The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 170: 635–651.
20. Carlson CG, Barrientos A, Tzagoloff A, Glerum DM (2003) The mechanism of Bloom's and Werner's syndrome genes, is required for maintenance of genomic instability. EMBO J 16: 1133–1147.
21. Collins SR, Miller KM, Maas NL, Roguev A, Fillingham J, et al. (2007) The Smc5-Smc6 complex and SUMO modification of Rad52 is an important guardian of genome stability. DNA Repair (Amst) 6: 945–955.
22. Bastin-Shanower SA, Fricke WM, Mullen JR, Brill SJ (2003) The mechanism of yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 170: 635–651.
23. Carbono GG, Barrientos A, Tragalkoff A, Lerner DM (2003) COX16 encodes a novel protein required for the assembly of mitochondrial oxidative damage in Saccharomyces cerevisiae. J Biol Chem 278: 3770–3775.
24. Cadet J, Berger M, Douki T, Ravanat JL (1997) Oxidative damage to DNA: formation, measurement, and biological significance. Rev Physiol Biochem Pharmacol 131: 1–87.
25. Sickmann A, Reinders J, Wagner Y, Joppich C, Zahedi R, et al. (2003) The proteome of Saccharomyces cerevisiae mitochondria. Proc Natl Acad Sci U S A 100: 13207–13212.
26. Hanna JS, Kroll ES, Lundblad V, Spencer FA (2001) Saccharomyces cerevisiae CTF78 and CTF11 are required for sister chromatid cohesion. Mol Cell Biol 21: 3144–3158.
27. Bressan DA, Baxter BK, Petrimi JH (1999) The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in Saccharomyces cerevisiae. Mol Cell Biol 19: 6621–6631.
28. Calvin CG, Barrientos A, Tzagoloff A, Glerum DM (2003) The mechanism of Bloom's and Werner's syndrome genes, is required for maintenance of genomic instability. EMBO J 16: 1133–1147.
29. McCarroll SA, Murphy CT, Zou S, Fletcher SD, Chin CS, et al. (2004) Comparing genomic expression patterns across species identifies shared transcriptional and processing signatures. Nat Genet 36: 197–204.
30. Schneider J, Rajca P, Johnson FC, Bhaumik SR, Shariatfard A (2006) Rtt109 is required for proper HS56 acetylation: a chroatin mark associated with the elongating RNA polymerase II. J Biol Chem 281: 37270–37274.
31. Gontijo AM, Green CM, Almouzni G (2003) Repairing DNA damage in Saccharomyces cerevisiae. Biochimie 85: 935–945.
32. Green CM, Almouzni G (2002) When repair meets chromatin. First in series on chromatin dynamics. EMBO Rep 3: 28–33.
33. Collins SR, Miller KM, Maas NL, Roguett E, Fillingham J, et al. (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature 448: 806–810.
34. Collins SR, Miller KM, Maas NL, Roguett E, Fillingham J, et al. (2007) Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature 448: 806–810.
55. Cleveland DW, Mao Y, Sullivan KF (2003) Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell 112: 407–421.
56. de la Cruz J, Kressler D, Linder P (1999) Unwinding RNA in \textit{Saccharomyces cerevisiae}: DEAD-box proteins and related families. Trends Biochem Sci 24: 192–198.
57. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, et al. (2003) Global analysis of protein localization in budding yeast. Nature 425: 686–691.
58. Tanay A, Sharan R, Kupiec M, Shamir R (2004) Revealing modularity and organization in the yeast molecular network by integrated analysis of highly heterogeneous genomewide data. Proc Natl Acad Sci U S A 101: 2981–2986.
59. Vina J, Vento M, Garcia-Sala F, Puertes IR, Gasco E, et al. (1995) L-cysteine and glutathione metabolism are impaired in premature infants due to cystathionase deficiency. Am J Clin Nutr 61: 1067–1069.
60. Klein CE, Roberts B, Holenberg J, Glode LM (1988) Cystathionine metabolism in neuroblastoma. Cancer 62: 291–298.
61. Sharma M, Mueller JC, Zimprich A, Lichtner P, Hofer A, et al. (2006) The sepiapterin reductase gene region reveals association in the PARK3 locus: analysis of familial and sporadic Parkinson disease in European populations. J Med Genet 43: 557–562.
62. Xie J, Zhu H, Ladade K, Ladoux A, Seguritan A, et al. (2004) Absence of a reductase, NCB5OR, causes insulin-deficient diabetes. Proc Natl Acad Sci U S A 101: 10750–10755.
63. Mastrogiacoma F, Lindsay JG, Bettendorff L, Rice J, Kish SJ (1996) Brain protein and alpha-ketoglutarate dehydrogenase complex activity in Alzheimer disease. Ann Neurol 39: 592–598.
64. Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, et al. (1998) Designer deletion strains derived from \textit{Saccharomyces cerevisiae} S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14: 115–132.
65. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in \textit{Saccharomyces cerevisiae}. Genetics 122: 19–27.
66. Lisby M, Antunez de Mayolo A, Mortensen UH, Rothstein R (2003) Cell cycle-regulated centers of DNA double-strand break repair. Cell Cycle 2: 479–483.
67. Smith J, Rothstein R (1999) An allele of \textit{RFA1} suppresses \textit{RAD52}-dependent double-strand break repair in \textit{Saccharomyces cerevisiae}. Genetics 151: 447–458.
68. McDonald JP, Levine AS, Woodgate R (1997) The \textit{Saccharomyces cerevisiae RAD30} gene, a homologue of \textit{Escherichia coli} dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism. Genetics 147: 1557–1568.
69. Lea DE, Coulson CA (1949) The distribution in the numbers of mutants in bacterial populations. J Genet 49: 264–285.