The repair of chromosomal double strand breaks (DSBs) is crucial for the maintenance of genomic integrity. However, the repair of DSBs can also destabilize the genome by causing mutations and chromosomal rearrangements, the driving forces for carcinogenesis and hereditary diseases. Break-induced replication (BIR) is one of the DSB repair pathways that is highly prone to genetic instability1–3. BIR proceeds by invasion of one broken end into a homologous DNA sequence followed by replication that can copy hundreds of kilobases of DNA from a donor molecule all the way through its telomere4–5. The resulting repaired chromosome comes at a great cost to the cell, as BIR promotes mutagenesis, loss of heterozygosity, translocations, and copy number variations, all hallmarks of carcinogenesis6–9.

We propose that the BIR mode of synthesis presents a powerful mechanism that can initiate bursts of genetic instability in eukaryotes, including humans.

In theory, BIR may constitute a unidirectional, bona fide replication fork producing two semiconservatively replicated molecules10,11 (Fig. 1A, a). Alternatively, a D-loop (displacement loop) formed by invasion of the broken chromosome may persist throughout BIR, migrating down the length of the chromosome, creating an unusual condition of conservative inheritance of newly synthesized DNA12,13 (Fig. 1A, b–d).

To distinguish between these models, we used a disomic yeast system (Fig. 1B, a) containing a second, truncated copy of chromosome III, cleaved by HO endonuclease under control of a galactose-inducible promoter2. The HO-induced DSB possesses only one efficiently repairable end that invades the second copy of chromosome III, and initiates BIR that copies over 100 kilobases (_kb) of the distal part of the chromosome. Using this system, we recently demonstrated that BIR stimulates mutagenesis along the path of DNA synthesis at a series of lys2 frameshift reporters9. Here we examined these Lys− mutations to determine whether errors during BIR were acquired semiconservatively (inherited by either the donor or recipient molecule; Fig. 1B, b) or conservatively (inherited only by the recipient molecule; Fig. 1B, c).

Pulse-field gel electrophoresis (PFGE) was used to separate donor and recipient molecules from Lys− reporter strains with reporters at 16 and 36 kb, respectively) were inherited by the recipient molecule, whereas the donor sequence remained unchanged (see also Supplementary Discussion). Overall, the mutation pattern supports a conservative replication mechanism for BIR. However, because

Figure 1 | The mode of DNA synthesis during BIR. A. The models of BIR. a. Replication fork proceeds semiconservatively. b–d, Migrating bubble leads to conservative inheritance of newly synthesized DNA. B. Synchronous synthesis of leading and lagging strands. C. Asynchronous synthesis of leading and lagging strands.
were illuminated by >30 kb BrdU tracts (Fig. 3d, e and Extended Data Fig. 2a). These data confirm a strong bias (P < 0.0001) towards BrdU tracts present only in the recipient chromosome. The four cases of BrdU incorporation in the donor could result from rare semiconservative synthesis or from BIR initiated >30 kb proximal to the DSB site, which would result in a donor-like size and hybridization pattern due to copying of regions unique to the donor molecule. On the basis of these data, we estimate that, even if semiconservative synthesis occurs, it can account for no more than 8% of the BIR events that we analysed (see Supplementary Discussion and Extended Data Fig. 4 for the results of another series of experiments supporting this conclusion).

The unusual mode of replication prompted us to characterize the structure of BIR molecular intermediates at LYS2 inserted ~16 kb from the point of strand invasion. Genomic DNA extracted from nocodazole-arrested cells undergoing BIR was digested with PstI and analysed by two-dimensional (2D) gel electrophoresis using a LYS2-specific probe (Fig. 4a, top panel). We detected bubble-like structures between 3 and 7 h after DSB induction (Fig. 4b–d), but not at 13 h, consistent with the timing of BIR progression (Extended Data Fig. 5).

All bubble-like intermediates were markedly different from the Y structures indicative of S-phase replication forks observed before addition of nocodazole and induction of the break (Fig. 4c, 0 h). Furthermore, no bubble-like structures were observed in control strains in which HO endonuclease cannot initiate a DSB (Fig. 4d, no-cut), thus linking these structures to BIR exclusively. The bubble-like structures observed in BIR were reminiscent of bubbles routinely detected at replication origins, with one important difference: the BIR bubbles included a long, high-molecular-mass tail that extended well beyond the size expected for complete replication (arrows in Fig. 4c, d). We proposed that initiation of BIR lagging-strand synthesis is often delayed compared to leading strand, resulting in accumulation of single-stranded DNA (ssDNA) behind the BIR bubble, which makes the region around LYS2 refractory to PstI digestion. Indeed, pre-incubation of genomic DNA with oligonucleotides (PstO3 and PstO4; Fig. 4a, middle and bottom panels) complimentary to the Watson strand of two PstI sites flanking the LYS2 gene eliminated the tail and resulted in a second arc that probably corresponds to molecular intermediates with bubbles consisting of one double-stranded branch (leading-strand synthesis) and one single-stranded branch (lagging-strand synthesis) (Fig. 4a, b, d and Extended Data Fig. 6). Similar results were also obtained using BglII digestion (Extended Data Fig. 7). Notably, whereas simultaneous addition of oligonucleotides BglO3 and BglO4, complimentary to the Watson strand of two BglII sites, eliminated the ssDNA tail, the addition of each of these oligonucleotides individually failed to eliminate the tail. This confirms that two types of DNA intermediates contribute to the observed ssDNA tail: those containing ssDNA centromere proximal to LYS2 and those with ssDNA distal to LYS2 (Fig. 4a and Extended Data Fig. 7a, panels ii, iii). Addition of oligonucleotides complimentary to the Crick strand did not have any effect (data not shown). Bubble migration intermediates were also detected with an HPH-specific probe that hybridizes to the end of the donor chromosome (Fig. 4a, e). These data strongly support a migrating D-loop type of DNA replication.

We proposed that ssDNA accumulated behind the migrating BIR bubble is the cause of BIR-associated mutagenesis because of the propensity of ssDNA to accumulate unrepairred DNA lesions. This was tested by using methyl methanesulphonate (MMS), a DNA damaging agent that predominantly creates mutagenic lesions in cytosines of ssDNA. In addition, a ura3-29 reporter, which can revert to Ura+ via three different base substitutions at one C+G pair (Fig. 2c), was inserted in the donor chromosome in two different orientations (Or1 and Or2). We expected that MMS will specifically elevate the level of BIR-associated mutagenesis in Or2, where cytosine is located in the mutant position of the leading (ssDNA) strand, but not in Or1, which contains guanine instead (Fig. 2d). Indeed, we observed that even though BIR markedly stimulated base substitutions in ura3-29 irrespective of its orientations, the effect of MMS was orientation...
dependent (Fig. 2e and Extended Data Table 1). Specifically, MMS highly amplified BIR-induced mutagenesis in cells containing ura3-29 in Ori2, whereas its effect on BIR mutagenesis in Ori1 was relatively modest. This observation supports the conjecture that ssDNA accumulated behind the BIR bubble is the cause of BIR-associated mutagenesis. Additionally, the spectrum of BIR-induced mutations was also orientation dependent, supporting our conclusion (Extended Data Fig. 2b).

Because the Pif1 helicase is a key component of the BIR machinery24 (see also accompanying paper25), we proposed that Pif1 is essential for long-range BIR. We observed that even though BIR-sized products...
were involved in BIR and associated mutagenesis\textsuperscript{2,9,10,15}. Therefore, Pif1 can be added to the list of other previously identified factors except those specific for pre-RC assembly. Genes Dev. 24, 1133–1144 (2010).

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Author Contributions S.R., N.S., K.S.L. and A.M. designed experiments. S.R. and A.D. carried out 2D experiments. S.R. and R.E. constructed the experimental system. N.S., S.R. and R.E. carried out 2D experiments. N.S. and S.R. and R.E. carried out 2D experiments involving sequencing of BIR-induced mutations. R.E., R.S. and S.A. carried out experiments aimed to determine the effect of BIR on base substitutions. J.E.H. provided key expertise. G.I. contributed to the studies of the role of Pif1 in BIR. S.R., N.S., A.D., J.E.H., K.S.L. and A.M. wrote the paper. N.S. and S.R. contributed equally to this work.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.M. (amalkova@iupui.edu or amalkova@uiowa.edu) or K.S.L. (krilllobache@biology.gatech.edu).
METHODS

Media, strains and plasmids. All yeast strains (Extended Data Table 3) were isogenic to AM1003 (ref 2), which is a chromosome III disome with the following genotype: hmr::ADE1/hmr::ADE3 MATa-LEU2-TEL/MATa- inc hmr::HPH FS2A::NAT/FS2 leu2-3,112 thr4 ura3-52 ade3::GAL::HO ade1 met13. AM1291 and AM1482 are derivatives of AM1003 and were created by deleting LYS2 from its native location, and inserting yts-2-IncA(yts-2) at different positions of chromosome III. AM2191 and AM 2198 were constructed from AM1291 and AM1482 by replacement of PIFI with KANMX module22. Control strains AM1449, AM1649, AM2247 and AM2257, which contained no HO cut site in the recipient chromosome III, were obtained from AM1291, AM1482, AM2191 and AM2198 as previously described23. AM2439 and AM2438 were created by integrating three and two copies of TEF1/BSD-snt1 into SNT1 of AM1291 and AM1482, respectively. The TEF1/BSD-snt1 plasmid was constructed by cloning of a PCR-amplified 1-kb region of SNT1 (from 185626 to 186589 positions of chromosome III) into the BamHI/HindIII fragment of TEF1/BSD (Invitrogen). The resulting plasmid was linearized by SnaBI and integrated at SNT1 to introduce a donor-specific region into the MATa-inc containing copy of chromosome III. The selection of transformants with integration of multiple copies of the plasmid was achieved by PFGE followed by Southern hybridization with TEF1/BSD used as a probe. AM2118 was isogenic to AM2147 (ref 9), but contained KANMX module to replace chromosome III between PTCA and TPSI.

AM2110 is a derivative of AM1003, and was created by deleting URA3 (using delitto perfeito approach) and replacing hmr::HPH with hmr::KANMX. In addition, it contains yts-2-IncA(yts-2) integrated into SED4 (36 kb centromere distal to MATa-inc). AM2161 and AM2820 were derivatives of AM2110 where ura3-29-HPH fragments (Orl and Or2 respectively) were inserted 16 kb centromere distal to MATa-inc between RSC6 and THR4. The ura3-29-HPH cassettes containing ura3-29-allele24 in two orientations were a gift from Y. Pavlov. The insertion of ura3-29-HPH 16 kb centromere distal to MATa-inc was achieved by transformation of AM2110 with DNA fragments generated by PCR amplification of ura3-29-HPH using the following primers with targeting tails (upperspace) and ura3-29-HPH amplification sequence (lowercase): 5'-TCTTCTTGCAGATTTGCGACCTCCTCGTGTAGTGGCAGCCGAACAAAGTCATCATAAAACGAAGTAGAAGtactggtagaggac-3' and 5'-ATATTATGCGTACATCTACAAAGTGAAAATAATAGATACAAATATGGTAAATAACACGACGTGATGTTATTGCTGTCAGCC-3'. Control strains AM2242, AM2259 and AM2824, which did not have NO HO cut site in the recipient chromosome III, were obtained from AM2118, AM2161 and AM2820 as previously described22.

AM2406 is a derivative of AM1003 that was constructed by inserting BrdU cassette (with the human equilibrative nucleoside transporter (hENT1) and the herpes simplex virus thymidine kinase)25 into URA3 to facilitate efficient BrdU incorporation in yeast. In particular, the p306-BrdU plasmid26 was linearized with Stul and inserted by transformation into the URA3 gene (chromosome V). In addition, AM2406 contained insertion of three tandem arrays of the TEF1/BSD-snt1 at SNT1, and replacement of TPS1 with a KANMX module. TPS1 was deleted to reduce accumulation of trehalose, which interfered with DNA purification.

Rich medium (yeast extract–peptone–dextrose (YEPD)) and synthetic complete media, strains and plasmids. AM2110 is a derivative of AM1003, and was created by deleting

Alternatively, to guarantee that the observed intermediates do not result from mechanical stress during genomic DNA preparation, we conducted 2D-gel electrophoresis using chromosomal DNA embedded in agarose plugs. In particular, cells collected at different intervals after induction of BIR were treated with psoralen as described previously29. The cells were then re-suspended in 750 μl solution of 1 M sorbitol, 0.5 M EDTA (pH 7.8) and treated with 0.2 mg ml-1 5′-hydantoin at 1 h for 37 °C. The speroplasts were washed in a solution of 50 mM Tris, 50 mM EDTA and 100 mM NaCl. The speroplasts were then embedded in 0.8% low melt agarose and incubated at 4 °C for 1 h. The DNA embedded in agarose was digested with BglII, and 2D-gel electrophoresis was carried out as described in the 2D analysis of PstI-digested chromosomal DNA.

To identify regions of single-stranded DNA, a Psrl or BglIII digest was preceded by pre-incubation of genomic DNA with oligonucleotides that were complimentary to the Psrl or BglIII sites flanking the LYS2 gene and had the following sequences: 5′-GGTCGCCCTTGCAGACAAAGC-3′ (PstO3), 5′-GTCCTTCCATGGCTGCA-3′ (PstO2), 5′-GCTTGTGTGCGAGGGCCC-3′ (PstO5), 5′-AAGTGGCAGATCTGAAAGCG-3′ (PstO4), 5′-ACTGCTTCTGCAGATCATA-3′ (PstO6), 5′-CAAGTGTGCTCAGACATCACT-3′ (BglO6), where ‘O4’ and ‘O6’ indicate oligonucleotides that are complimentary to the Watson and Crick strands at the centromere-proximal site, respectively, and 5′-TAGATGGCTGCGAGGG-3′ (PstO4), 5′-TAGATGGCTGCGAGGG-3′ (BglO4), 5′-ACTGCTTCTGCAGATCATA-3′ (PstO6), 5′-CAAGTGTGCTCAGACATCACT-3′ (BglO6), where ‘O4’ and ‘O6’ indicate oligonucleotides that are complimentary to the Watson and Crick strands at the telomere-proximal site, respectively.

Southern hybridization was performed using LYS2 fragment obtained by PCR amplification of a 0.6-kb region of LYS2 (from 471835 to 472443 kb positions of chromosome II) or using HPH-hybridizing fragment obtained by PCR amplification of HPH from the pAG32 plasmid27. Along with analysis of BIR intermediates, cell cycle distribution was analysed by flow cytometry30 and BIR kinetics were analysed by PFGE. For PFGE, chromosome plugs were prepared28 with genomic DNA embedded in plugs of 1% melting agarose and separated at 6 °C for 40 h using the CHEF DRII apparatus. PFGE was followed by Southern analysis with an ADE1-specific probe labelled with P32. Images were analysed using a Molecular Dynamics PhosphorImager.

DNA combing and fluorescence in situ hybridization. Cells were grown overnight in synthetic sucrose drop-out media, transferred to YEP-lactate, and incubated for ~20 h, until cell density reached ~1 × 107 cells ml-1. Cells were arrested by nocodazole added to 0.015 mg ml-1, and DSBs were induced 2.5 h later by addition of galactose to the final concentration of 2%. When experiments were performed according to this protocol, the efficiency of BIR was 50 ± 9.8%, as determined by PFGE analysis31 1 h after DSB induction (Extended Data Fig. 1a, b). BrdU was added to the culture 3.5 h after DSB induction by galactose to the final concentration of 0.4 mg ml-1 after all normal DNA replication was completed but was removed before the beginning of BIR. Aliquots were removed to embed cells into agarose plugs before and 11 h after induction of DSBs with galactose. In experiments involving pif1A strains, the analysis was performed 13 h after DSB induction due to slower kinetics of DSB repair in pif1A (data not shown). The uniform arrest of cells at G2/M was confirmed by the absence of BrdU incorporation in any chromosomes other than chromosome III, which was assayed by PFGE analysis of yeast chromosomes extracted from samples taken before the addition of BrdU and 11 or 13 h after DSB induction and probing with anti-BrdU antibodies.

Genomic DNA preparation and molecular combing were performed as described31. Colour hybridization of chromosome III molecules was performed using three fluorescent probes. P1 probe was prepared using the TEF1/BSD plasmid (Invitrogen) and hybridized to the 15-kb region containing three tandem repeats of the TEF1/BSD-snt1 plasmid inserted into the donor copy of chromosome III at position 186535. P2 probe marked the position close to strand invasion during BIR and was comprised of a set of four 5-kb fragments that corresponded to the following positions on the donor chromosome III: 200205 to 205140, 205117 to 210385, 211130 to 216030 and 215360 to 220105. The P1 probe hybridized close to the telomeric end of chromosome III and is made up of three 5-kb fragments corresponding to the following positions on the donor chromosome: 274778 to 279801, 279778 to 284814 and 284791 to 289782. The probes were made by PCR amplification of genomic DNA from AM2406. Nucleotide sequences of the primers used to generate fragments for labelling are available upon request. Probes were labelled with biotin-DUTP. Hybridization and fluorescent detection of combed DNA molecules were achieved according to protocols described31 with a few modifications. Successive layers of fluorophore-conjugated antibodies diluted in 1× PBS (1× PBS + 0.05% Tween) were used. For the biotin-conjugated probes, a secondary antibody was used at a dilution of 14,000: (1) Alexa–488-Streptavidin (Molecular Probes; Life Technologies, catalogue no. BA-0500); (2) biotinylated anti-streptavidin (from Vector Lab, catalogue no. BA-0500); (3) Alexa–488-Streptavidin; (4) biotinylated anti-streptavidin; and (5) Alexa–488-Streptavidin. To detect BrdU incorporation, the following series were used at the indicated dilutions: 1:1 20

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dilution of mouse anti-BrdU (BD Biosciences, catalogue no. 347580); (2) 1:50 dilution of Cy3-coupled rat anti-mouse (Jackson ImmunoResearch Lab, catalogue no. 415-165-166); and (3) 1:50 dilution of Cy3-mouse anti-rat (Jackson ImmunoResearch Lab, catalogue no. 212-165-168). All images were acquired using the Zeiss LSM 510 Confocal Microscope with 100× objective. The lengths of the fluorescent stretches were calculated by comparison with the length of P1, P2 and P3 hybridization signals.

The statistical comparison between donor and recipient chromosomes in respect to BrdU incorporation was performed using the Chi-squared test. For each experiment, the frequency of semiconservative BIR (F) was calculated as follows: \( F = A/N \times f/b \), where A represents the number of donor molecules with long BrdU tracts; \( N \) represents the total number of analysed donor molecules; \( f \) represents the efficiency of BIR in the experiment (calculated by physical analysis as a percentage of the truncated chromosome III converted in the BIR product\(^{1} \)); and \( b \) represents the fraction of recipient molecules containing full and long interrupted BIR tracts.

**Mutagenesis associated with DSB repair.** To determine mutation frequency associated with BIR, yeast strains were grown from individual colonies with agitation in liquid synthetic media lacking leucine for approximately 20 h, diluted 20-fold with fresh YEP-Lac, and grown to logarithmic phase for approximately 16 h. Next, 20% galactose was added to the culture to a final concentration of 2%, and samples from each culture were plated at appropriate concentrations on adenine drop-out media and on media omitting lysine and adenine before (0 h) and 7 h after the addition of galactose (7 h) to measure the frequency of Lys\(^{-} \) cells. To measure the frequency of Ura\(^{-} \) cells, samples were plated at appropriate concentrations on adenine drop-out media and on media omitting uracil and adenine before (0 h) and 7 h after the addition of galactose (7 h). To determine spontaneous mutation frequencies, no-DSB strains were grown similarly to the DSB-containing strains. Because spontaneous mutation frequencies were calculated based on the number of mutations accumulated during many cell generations, the rate of spontaneous mutagenesis in no-DSB control strains was calculated using the following modification of Drake equation: \( \mu = 0.4343 \frac{f}{N} \log(Nb) \), where \( \mu \) is the rate of spontaneous mutagenesis, \( f \) is mutation frequency, and \( N \) is the number of cells in yeast culture. The rate of mutations after galactose treatment (\( \mu_f \)) was determined using a simplified version of the Drake equation: \( \mu_f = (f_f - f_b) \), where \( f_f \) and \( f_b \) are the mutation frequencies among Ade\(^{-} \) cells at times 7 h (following MMS treatment) and 0 h, respectively. This modification was necessary because experimental strains did not divide or underwent \( \leq 1 \) division between 0 h and 7 h in the presence of MMS.

Rates are reported as the median value (Fig. 2b, e and Extended Data Tables 1 and 2), and the 95% confidence limits for the median are calculated for the strains with a minimum of six individual experiments. For strains with four–five individual experiments, the range of the median was calculated. Statistical comparisons between median mutation rates were performed using the Mann–Whitney U-test\(^{14} \).

**Analysis of BIR-induced Lys\(^{-} \) mutants.** Lys\(^{-} \) revertants were obtained in BIR mutagenesis experiments\(^{4} \). After phenotypic examination, cultures were grown from mutants for chromosome analysis by PFGE using 1% low-melting agarose at 6 V cm\(^{-1} \) for 48 h. DNA bands corresponding to the donor and repaired recipient chromosome III were excised, equilibrated in β-agarase buffer (NEB), melted at 65 °C, and subjected to β-agarase treatment for 1 h at 40 °C. The obtained DNA was PCR amplified using LYS2-specific DNA primers\(^{4} \), followed by sequencing analysis.

**Analysis of mutation spectra of ura3-29 Ura\(^{+} \) reversions.** To determine the spectrum of Ura\(^{+} \) in individual experiments, a portion of the URA3 gene from independent Ura\(^{+} \) was PCR-amplified using URA3-specific primers: 5′-GTGTG CTTCATGGATGTTCGTA-3′ and 5′-AAAAAGCTCTTAGTTCCCTGTTG-3′ followed by sequencing analysis using 5′-CTGGAGTTAGTTGAAGCATTAGG-3′ as a primer.

For experimental strains undergoing BIR repair, 7 h Ura\(^{+} \) BIR events (confirmed as Ade ‘Leu’ on selective media) were sequenced. Because these cells underwent \( \leq 1 \) division between the 0 h and 7 h time points and the Ura\(^{-} \) frequency at 7 h significantly exceeded that at 0 h, all Ura\(^{-} \) events resulting from DSB repair were considered independent.

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Extended Data Figure 1 | BIR efficiency during molecular combing analysis of molecular intermediates of BIR. a, BIR efficiency was analysed by PFGE from samples used for dynamic molecular combing analysis (Fig. 3d). DNA was prepared from cells containing truncated chromosome III (Trunc Chr III) before DSB induction and 11 h or 13 h after DSB induction from wild-type (PIF1) and pif1Δ cells, respectively. In pif1Δ, a later time point (13 h) was analysed owing to slower kinetics of DSB repair in pif1Δ as compared to PIF1.

Chromosomes were separated by PFGE followed by Southern hybridization with an ADE1-specific probe. b, Quantification of DSB repair efficiency (BIR, or other recombination pathways) based on the results of 3–5 individual experiments and presented as average ± s.d. c, Schematic of the BIR assay. Interruption of BIR leads to the resolution of BIR intermediates resulting in half-crossover formation.
Extended Data Figure 2 | Analysis of molecular mechanism and mutagenesis associated with BIR. a, The summary of molecular combing analysis presented in Fig. 3 and in Extended Data Fig. 3 is shown. A strong bias towards BrdU tracts present only in the recipient chromosome was also observed in three additional independent experiments. b, Mutation spectra of BIR-induced base substitutions in *ura3-29* in the presence or absence of 1.5 mM MMS is shown.

### a

| Relevant Genotype | Molecule analyzed | Full BIR (<100 kb BrdU) | Long BIR (>30 kb BrdU) | Short BrdU* patches (<20 kb BrdU) | No BrdU | Total |
|------------------|-------------------|-------------------------|------------------------|-----------------------------------|---------|-------|
| *wt* (*Pif1*)    | Donor             | 4                       | ---                    | 14 [4] (10)                       | 85      | 103   |
|                  | Recipient         | 70                      | 14                     | ---                               | 14      | 98    |
| *pif1Δ*          | Donor             | 0                       | 0                      | 12 [5] (7)                        | 80      | 92    |
|                  | Recipient         | 0                       | 0                      | 31 [23] (8)                       | 72      | 103   |

* - BrdU patches between P2 and P3 are included. [] - overlaps with P2; () - overlaps with P3

### b

| Orientation of *ura3-29* | MMS* | BIR Ura* mutations |
|--------------------------|------|---------------------|
|                          | C→A  | C→T    | C→G    |
| **Cri1**                 |      |        |       |
| No                       | 79 (80%) | 18 (18%) | 2 (2%) |
| Yes                      | 31 (66%) | 12 (25%) | 4 (9%) |
| **Cri2**                 |      |        |       |
| No                       | 13 (28%) | 24 (51%) | 10 (21%) |
| Yes                      | 21 (36%) | 26 (45%) | 11 (19%) |

* - Cells exposed to 1.5 mM MMS; * and ** - Statistically different from Cri1 (P<0.0001 and P=0.04 respectively)
Extended Data Figure 3 | Molecular outcomes of BIR. a, Left: interrupted BrdU tract in recipient may result from half-crossover. Right: an example of wild-type (PIF1) recipient with interrupted BrdU tract hybridized to P1, P2, P3 probes (green) and treated with anti-BrdU antibody (red). b, Left: BIR initiated by strand invasion between FS2 (inverted repeat of Ty1 located 30 kb centromere proximal to MAT) and P1 results in formation of recipients hybridizing to P1, P2, P3 and BrdU. Right: an example of wild-type (PIF1) recipient. Top: hybridization to P1, P2, P3. Middle: treatment with anti-BrdU antibody. Bottom: merge. c, Left: BrdU incorporation in the recipient resulting from BIR (red) and from filling-in synthesis (pink) following extensive resection. Right: an example of wild-type (PIF1) recipient. Top: hybridization to P1, P2, P3. Middle: treatment with anti-BrdU antibody. Bottom: merge. d, Left: HJ resolution at the end of BIR progression leads to switch from conservative to semiconservative BIR resulting in a short patch of BrdU overlapping with P3 in the donor. Right: an example of BrdU incorporation in the donor from wild-type (PIF1) strain hybridized to P1, P2, P3 and treated with anti-BrdU antibody.
Extended Data Figure 4 | Conservative DNA synthesis associated with BIR. Results from a series of three experiments where only P1, P2 and anti-BrdU antibody were used. a, BrdU incorporation in the recipient is expected from conservative BIR (i; red) and from filling-in synthesis (pink) following extensive resection (ii). b, c, Analysis of the donor (D) and repaired recipient (R) chromosomes extracted after PFGE (b) and hybridization with probes (green tract) and treatment with anti-BrdU antibodies (red tract) (c). No BrdU tracts are visible in more than 97% of donors. The repaired recipient contains long stretches of BrdU overlapping with the P2 region.
Extended Data Figure 5  |  BIR kinetics during 2D analysis of molecular intermediates of BIR.  
a, BIR kinetics was analysed by PFGE from samples used to determine the structure of BIR intermediates by 2D electrophoresis (Fig. 4c, d). DNA was prepared for PFGE at intervals after induction of DSBs at MATa and separated by PFGE (a) followed by Southern hybridization with an ADE1-specific probe (b). c, BIR efficiency quantified based on the results of four individual experiments including the one shown in Fig. 4 presented as average ± s.d.  
d, Flow cytometry of DNA analysis of cells undergoing BIR repair.
Extended Data Figure 6 | The structure of molecular intermediates of BIR.

a, The structure of the chromosome III region with LYS2 inserted 16 kb centromere distal to MATx-inc. P1, P2, P3, and so on designate positions of PstI sites flanking LYS2. b, The structure of replication bubbles migrating through LYS2 (with black rectangle designating LYS2-specific probe). i, Replication bubble with synchronous leading and lagging strands (double-stranded). ii, Replication bubble with delayed initiation of the lagging strand with respect to the leading strand (partially single-stranded bubble). iii, A partially single-stranded bubble with one or several PstI sites behind the bubble inactivated due to accumulation of single-stranded DNA. Red and pink rectangles represent oligonucleotides PstO3 and PstO4, respectively. iv, A single-stranded bubble that has passed beyond the P3–P4 region.

c, Theoretical bubble-migration curves for the intermediates shown in b. d, Calculation of parameters of the bubble-like structures for the intermediates shown in b.
Extended Data Figure 7 | Molecular intermediates of BIR. BIR intermediates were analysed by 2D gel electrophoresis of BglII-digested intact chromosomal DNA embedded in agarose plugs. **a**, D-loop migration in 2D gels (hybridized to LYS2, black rectangle) during coordinated (i) and uncoordinated (ii, iii) leading- and lagging-strand synthesis. **b**, Schematic of 2D gel separation of replication and BIR intermediates. Annealing to oligonucleotides (BglO3 and BglO4) restores BglII sites (B) in ssDNA (see **a**, ii) and changes migration of the intermediate as shown by 2' (red). **c**, 2D analysis of Y-arc during normal replication (0 Hr) and bubble-like structures at time points after BIR induction. Similar bubble structures were observed in nine additional independent experiments (see the legend to Fig. 4). **d**, High-molecular-mass tails (arrows) disappear after simultaneous addition of BglO3 and BglO4 (BIR/BglO3 + BglO4). The addition of each of these oligonucleotides individually (BIR/BglO3 or BIR/BglO4) failed to eliminate the tail.
**Extended Data Table 1 | The rate of spontaneous and DSB-associated Ura<sup>+</sup> mutations**

| Orientation of ura3-29 | HO site | CI or range | 0 h frequency | CI or range | 7 h frequency | Fold above no-damage | Ade<sup>+</sup> | Ade<sup>+</sup>Ura<sup>+</sup> | Ade<sup>+</sup> | Ade<sup>+</sup>Ura<sup>+</sup> |
|------------------------|---------|-------------|---------------|-------------|---------------|----------------------|------------|----------------|------------|----------------|
| **Before galactose (0 h)** |         |             |               |             |               |                      |            |                |            |                |
| Ori1                   | DSB     | 28          | (10 - 41) [7] | 3.846       | (2.305 - 4.159) [7] | 9.415 (4.911 - 11.061) [7] | 2.6 (P<0.0006) | 86 ± 8 | 94 ± 8 | 84 ± 10 | 98 ± 0.4 |
| Ori2                   | DSB     | 6           | (5 - 17) [7]  | 1.903       | (0.979 - 2.941) [7] | 41.835 (34.830 - 79.488) [7] | 22 (P=0.0006) | 74 ± 9 | 94 ± 2 | 78 ± 14 | 92 ± 2 |
| Ori1                   | No      | 8           | (5 - 10) [17] | 0           | (0 - 11) [10] | 198 (49 - 358) [7] | 24.8       | N/A   | N/A   | N/A   | N/A   |
| Ori2                   | No      | 12          | (5 - 19) [13] | 0           | (0 - 7) [7]  | 157 (101 - 245) [7] | 13.1       | N/A   | N/A   | N/A   | N/A   |
| **After galactose (frequency (7 h – 0 h))** |         |             |               |             |               |                      |            |                |            |                |
| **No MMS**             |         |             |               |             |               |                      |            |                |            |                |
| **1.5 mM MMS**         |         |             |               |             |               |                      |            |                |            |                |

* Rates calculated at 0 h based on 0 h frequencies using the Drake equation (see Methods for details). At 7 h, rates were calculated as (7 h frequency – 0 h frequency); differences < 0 are reported as ‘0’.
† For strains with ≥6 experiments, the 95% CI of the median is given.
‡ Statistically significant elevation of 7 h mutation rate in strains in the presence of MMS over 7 h mutation rate in the absence of MMS.
§ Per cent of BIR (average ± s.d.) calculated based on 3-6 experiments among DSB repair outcomes collected at 7 h on either adenine dropout media (Ade<sup>+</sup>) or on adenine/uracil dropout media (Ade<sup>+</sup>Ura<sup>+</sup>).
## Extended Data Table 2 | The rate of DSB-associated Lys<sup>+</sup> mutations (top), and the rate of spontaneous Lys<sup>+</sup> mutations (bottom)

| Position | Construct | HO site | Relevant Genotype | Median | CI or range<sup>†</sup> [# of repeats] | Median | CI or range<sup>†</sup> [# of repeats] | Fold below WT<sup>‡</sup> (P-value) | BiR efficiency (%)<sup>§</sup> | Ade<sup>*</sup> | Ade<sup>+</sup>Lys<sup>+</sup> |
|----------|-----------|---------|-------------------|--------|--------------------------------------|--------|--------------------------------------|----------------------------------|------------------------|----------------|--------------------------|
| 16 kb    | A<sub>4</sub> | DSB     | wt                | 40     | (12.7 - 64.3) [13]                  | 2.690  | (1,073.0 - 4,361) [13]               | NA                              | 77 ± 12                | 99.7 ± 0.5         |
| 16 kb    | A<sub>4</sub> | DSB     | pif1Δ             | 6      | (4.0 - 10.4) [14]                  | 134.7  | (104 - 1,580) [14]                   | 20 (0.0001)                    | 73 ± 11                | 99 ± 3             |
| 36 kb    | A<sub>4</sub> | DSB     | wt                | 5.3    | (2.7 - 15.2) [8]                    | 1,248.10 | (660 - 1,552) [8]                  | NA                              | 80 ± 1                 | 99 ± 1             |
| 36 kb    | A<sub>4</sub> | DSB     | pif1Δ             | 1      | (0.5 - 12) [13]                    | 1.4    | (0 - 4.7) [13]                      | 892 (0.0003)                  | 91 ± 4                 | 100*            |

* Rates calculated at 0 h based on 0 h frequencies using the Drake equation (see Methods for details). At 7 h, rates were calculated as (7 h frequency – 0 h frequency); differences < 0 are reported as '0'.
† For strains with ≥6 experiments, the 95% CI of the median is given. For the strains with < 6 experiments, the median range is given.
‡ Statistically significant decrease of median rate at 7 h in pif1Δ compared to wild type.
§ Per cent of BiR (average ± s.d.) calculated based on 4–8 experiments among DSB repair outcomes collected at 7 h on either adenine dropout media (Ade<sup>*</sup>) or on adenine/lysine dropout media (Ade<sup>+</sup>Lys<sup>+</sup>).
* No s.d. could be calculated because of a very low number of Lys<sup>+</sup> (between 1 and 5) in each experiment.

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## Extended Data Table 3 | Strain list

| Strain name     | Genotype                                                                 | Reference |
|-----------------|--------------------------------------------------------------------------|-----------|
| AM1003          | MATa-LEU2-tef/MATa-inc ade1 met13 ura3                                    | 2         |
|                 | leu2-3,112/leu2 thr4 lys5 hml·ADE1/hml·ADE3                              |           |
|                 | hmr·HPH ade3::GAL·HIS3 F52::MATa::FS2                                    |           |
| AM1291          | AM1003, but lys2Δ thr4·lys2-Ins(A2)                                      | 9         |
| AM1449          | AM1291, but MATa-inc-LEU2-tef                                           | 9         |
| AM1482          | AM1003, but lys2Δ sed4·lys2-Ins(A2)                                      | 9         |
| AM1649          | AM1482, but MATa-inc-LEU2-tef                                           | 9         |
| AM2191          | AM1291, but pif1::KANMX                                                  | this study|
| AM2247          | AM2191, but MATa-inc-LEU2-tef                                           | this study|
| AM2298          | AM1482, but pif1::KANMX                                                  | this study|
| AM2357          | AM2196, but MATa-inc-LEU2-tef                                           | this study|
| AM2417          | AM1003, but lys2Δ thr4·LYS2                                              | 9         |
| AM2439          | AM1291, but snf1:(TEF1/BSO)                                              | this study|
| AM2438          | AM1482, but snf1:(TEF1/BSO)                                              | this study|
| AM2118          | AM1247, Chr ii::KANMX                                                   | this study|
| AM2442          | AM2118, but MATa-inc-LEU2-tef                                           | this study|
| AM2406          | AM1003, but ura3∷p306-BrdU tps1∷KANMX                                    | this study|
|                 | snf1:(TEF1/BSO)                                                        |           |
| AM2806          | AM2406, but tps1∷BLEO pif1∷KANMX                                       | this study|
| AM2110          | AM1003, but lys2Δ ura3Δ sed4·lys2-Ins(A2)                                | this study|
|                 | hmr·KANMX                                                               |           |
| AM2161          | AM2110, but thr4∷ura3-29 (Gri1)                                        | this study|
| AM2259          | AM2161, but MATa-inc-LEU2-tef                                           | this study|
| AM2820          | AM2110, but thr4∷ura3-29 (Gri2)                                        | this study|
| AM2842          | AM2820, but MATa-inc-LEU2-tef                                           | this study|