Break-induced replication (BIR) repairs one-ended double-strand breaks in DNA similar to those formed by replication collapse or telomere erosion, and it has been implicated in the initiation of genome instability in cancer and other human diseases. Previous studies have defined the enzymes that are required for BIR, however, understanding of initial and extended BIR synthesis, and of how the migrating D-loop proceeds through known replication roadblocks, has been precluded by technical limitations. Here we use a newly developed assay to show that BIR synthesis initiates soon after strand invasion and proceeds more slowly than S-phase replication. Without primase, leading strand synthesis is initiated efficiently, but is unable to proceed beyond 30 kilobases, suggesting that primase is required for stabilization of the nascent leading strand. DNA synthesis can initiate in the absence of Pif1 or Pol32, but does not proceed efficiently. Interstitial telomeric DNA disrupts and terminates BIR progression, and BIR initiation is suppressed by transcription proportionally to the transcription level. Collisions between BIR and transcription lead to mutagenesis and chromosome rearrangements at levels that exceed instabilities induced by transcription during normal replication. Together, these results provide fundamental insights into the mechanism of BIR and how BIR contributes to genome instability.

Unlike S-phase replication, which initiates at origins, BIR begins at chromosome breaks with strand invasion of a single DNA end into a homologous sequence. Then, a migrating replication bubble is formed in which uncoupled leading and lagging strand synthesis proceeds through the telomere, leading to conservative inheritance of new DNA. The inability to discriminate between defects associated with initiation, elongation or completion of BIR has limited our knowledge of the efficiency, kinetics and genetics of different phases of this process. Moreover, it is unclear how the migrating D-loop proceeds within a template region that is known to destabilize the regular replication fork, such as repetitive DNA or sites of active transcription. We address these questions using a newly developed droplet digital (dd)PCR-based approach that allows each of the discrete phases of DNA synthesis during BIR to be studied at a high resolution.

**BIR is slower than S-phase replication**

To determine the kinetics of BIR, we developed an assay for monitoring BIR elongation rate (AMBER). AMBER combines a DNA purification technique that preserves DNA synthesis intermediates (a derivative of a recently developed DLE method that measured D-loop extension) with ddPCR for sensitive and quantitative detection of BIR synthesis along the entire template chromosome length. We assayed BIR in an established yeast system in which BIR repairs double-strand breaks (DSBs) induced by the HO endonuclease in a truncated chromosome III (Fig. 1a). DNA isolated from samples collected at specific time points following DSB induction was analysed by ddPCR (Methods). We designed pairs of primers with fluorophore probes at 0.5-kb, 20-kb, or 90-kb positions telomere-proximal to the DSB along the donor chromosome III (Fig. 1a). Evidence of DNA synthesis was defined as an increase in donor DNA copy number of at least 1.1×, normalized to the value of the ACT1 control locus. An additional pair of primers specific for EMC1 on the left arm of both copies of chromosome III constituted a control readout of two copies (Fig. 1a, P(−150 kb)).

BIR synthesis of the first 500 base pairs (bp) was detected by ddPCR 2.5 h after DSB induction (Fig. 1b), about 1 h after initial strand invasion (detected by chromatin immunoprecipitation (ChIP) of Rad51 loading onto the donor chromosome III (reflecting strand invasion), or by D-loop capture (DLC) (Extended Data Fig. 1a, b)). The longer delays in BIR synthesis previously reported probably resulted from the use of a different method of DNA preparation that does not preserve DNA synthesis intermediates (Extended Data Fig. 1c). Copy number at 0.5 kb increased at subsequent time points and reached about 1.7× by 10 h (Fig. 1b). Synthesis through the 20-kb and 90-kb positions was detected as early as 3.5 h and 5.5 h, respectively, after the addition of galactose and increased over time to about 1.5–1.7× by 10 h (Fig. 1b). Synthesis through the 20-kb and 90-kb positions was detected as early as 3.5 h and 5.5 h, respectively, after the addition of galactose and increased over time to about 1.5–1.7× by 10 h (Fig. 1b).
Fig. 1 | The dynamics, efficiency and rate of BIR synthesis determined by AMBER. a, Schematic of BIR progression in disomic chromosome III strain (AM1003) showing primer sets used for AMBER (Methods). A DSB is introduced into the recipient (top, blue) chromosome III by GAL-HO and repair occurs by copying the donor (bottom, red) chromosome III. Coloured triangles: positions of primer sets with distance from MATα inc indicated. b, DNA synthesis detected by AMBER. A Boltzmann sigmoidal was used to fit the data (here and also most figures generated from AMBER) and to determine the time at which 50% synthesis occurs and the rate of synthesis (estimated using the slope at this time ($V_{50}$), which is in this experiment for 0.5 kb, 20 kb and 90 kb, 2.2 at 3.8 h, 1.5 at 5.6 h and 0.7 at 6.5 h, respectively). c, AMBER analysis in rad51Δ and rad52Δ cells. b, c, One out of three independent biological repeats that showed similar results (Supplementary Table 5). Mean values of target to reference (ACTI) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% confidence intervals (CI) (Methods). d, Positions of primers used to characterize BIR in pol32Δ, pif1Δ, and pol32Δpif1Δ mutant strains indicated by triangles. e, The extent of DNA synthesis measured by AMBER in pol32Δ, pif1Δ, and pol32Δpif1Δ strains 10 h after addition of galactose. Mean ± s.d. (n = 3 independent biological repeats).

2× for the duration of the experiment (Fig. 1b). As expected, we did not detect BIR synthesis in rad51Δ or rad52Δ mutants (Fig. 1c, Extended Data Fig. 1d), both of which are defective for BIR3, which confirmed that the DNA copy number gain resulted from BIR repair. By comparing the time required to detect DNA synthesis 0.5 kb from the DSB (initiation; 1.1× increase at 2.5 h) and 90 kb from the DSB (completion; 1.1× increase at 5.5 h), we calculated the average rate of BIR synthesis to be 0.5 kb per minute—approximately sixfold slower than that of S-phase replication16. This BIR rate was calculated in G2/M checkpoint-arrested cells8 (Methods) and was similar to the rate of BIR in cells pre-arrested at G2/M by nocodazole before DSB induction (Extended Data Fig. 2a, b). We further determined that the rate of copy number increase when BIR synthesis reached 50% of the total for each chromosome position increased along the donor chromosome (Fig. 1b (legend), Extended Data Fig. 2c). Thus, the speed of BIR synthesis increases with distance from the position of strand invasion, consistent with prior reports suggesting a transition from less-processive to more-processive DNA synthesis during BIR4,6,33,35. Notably, in a pol3-01 mutant, which has been suggested to support higher BIR processivity owing to the lack of Pol6 exo/nuclease activity36, the kinetics of BIR was similar to that in wild-type cells (Extended Data Fig. 2d, e).

Fig. 2 | Primase is required to stabilize long leading strand ssDNA. a, Schematic of experiment in PRI2 (wild-type) and pri2-1 temperature-sensitive mutant. b, DNA synthesis in PRI2 (left) and pri2-1 (right) cells detected by AMBER. c, The extent of DNA synthesis in pri2-1 cells after 10 h compared to PRI2. Mean ± s.d. (n = 3 independent biological repeats). d, AMBER analysis of PRI2 and pri2-1 strains following treatment with S1-nuclease. a, b, One out of three independent biological repeats that showed similar results (Supplementary Table 5). Mean values of target to reference (ACTI) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% CI.

Analysis of BIR elongation in mutants

The Pif1 helicase and Pol32, a non-essential subunit of Pol6, promote BIR synthesis4,5, but the specific step for which they are required in cells remains unclear. We found that pif1Δ and pol32Δ mutant strains are proficient in strand invasion (Extended Data Fig. 3a, b) and initiate BIR synthesis (albeit somewhat less efficiently than the wild type (0.2-kb position)), but are deficient in long-range synthesis. Specifically, in the pif1Δ strain, we detected almost no synthesis at or beyond the 5-kb position; in the pol32Δ strain, we detected BIR synthesis up to 15 kb beyond the DSB (Fig. 1d, e, Extended Data Fig. 3c–f). Notably, in pol32Δpif1Δ double mutants, there was no initial synthesis (Fig. 1e), suggesting that Pol32 can promote at least some DNA synthesis in the absence of Pif1 and vice versa. In addition, there was a defect at 90 kb, but not at the beginning of synthesis, following auxin-inducible degron (AID) inactivation of Pol (Extended Data Fig. 3g), consistent with previous findings4, and with the primary role of Pol6 in BIR4,17.

Primase stabilizes the long leading strand

Unlike S-phase DNA replication, BIR synthesis is asynchronous: the leading strand primed at the 3′-OH end accumulates as single-stranded DNA (ssDNA) behind the migrating D-loop16, while the lagging strand eventually uses the leading strand as its template28. However, the extent to which lagging strand synthesis can be delayed after the leading strand is synthesized remains unknown. We analysed BIR repair by contour-clamped homogeneous electric field (CHEF) gel electrophoresis in pri2-1 cells in which primase was inactivated at 37 °C28 (Fig. 2a, Methods) and observed a notable defect in the formation of full-length BIR products (Extended Data Fig. 4a), consistent with published reports42. However, AMBER analysis of BIR progression demonstrated that BIR synthesis was successfully initiated in pri2-1 mutants, albeit...
with reduced efficiency compared to wild-type cells (Fig. 2b, c). Also, whereas the full-length BIR product was detected in wild-type cells, the furthest synthesis detected in the pri2Δ mutant cultures was at 25 kb (Fig. 2b, c). We obtained similar results in cells in which another subunit of the Polα-prime complex, Pol1, was inactivated (Extended Data Fig. 4b). Notably, treatment of DNA with SI nuclease before ddPCR eliminated all copy number increases in pri2Δ but not wild-type cells (Fig. 2d), consistent with new DNA detected upon primase inactivation being single-stranded and accumulating during leading strand synthesis. Together, our results support a model in which the uncoupling of leading and lagging strand BIR synthesis is limited to up to 20–30 kb. We propose that primase-deficient cells can use the 3′ invading strand as a primer to successfully initiate BIR leading strand synthesis; however, stable extension of the leading strand requires primase, probably for synthesis of Okazaki fragments (Extended Data Fig. 4c).

**Interstitial telomeres interrupt BIR**

Interstitial telomere sequences (ITSs) promote genetic instabilities linked to human diseases. To study the effect of ITSs on BIR, we inserted a sequence containing human telomere repeats into the donor copy of chromosome III 6 kb centromere-distal to MATα-inc (Fig. 3a). DNA was synthesized robustly before the ITS, but not downstream of it (Fig. 3b), indicating that BIR was interrupted within this region. CHEF electrophoresis analysis of DSB repair outcomes (Methods) revealed that only about 24% (7/29) of DSBs were repaired using BIR, whereas 76% (22/29) of DSBs resulted in a gross chromosome rearrangement (GCR) (Fig. 3d, e). Whole-genome sequencing of five of the GCR events demonstrated that they were all truncated at the position of the ITS and stabilized by addition of yeast telomeres (Fig. 3f), which was further confirmed by PCR (Methods). Because we engineered the ITS region with non-yeast telomeres, truncated BIR products could not be formed by template switching to natural yeast telomeres, and therefore the truncation is likely to have resulted from de novo telomere addition. When TLCI, which encodes the RNA component of telomerase, was deleted, BIR synthesis was still defective beyond the ITS for more than 10 h after DSB induction (Fig. 3c). However, after 16 h, synthesis beyond the ITS was increased compared to wild-type cells (Extended Data Fig. 5a), and eventually most cells completed BIR to the end of the chromosome (Fig. 3d, e). We conclude that ITSs stall and disrupt BIR, and that telomerase can efficiently stabilize BIR aborted at ITSs by de novo telomere addition (Extended Data Fig. 5b). The ability of ITSs to interrupt DNA synthesis showed repeat-length dependence, as AMBER analysis indicated that reducing the number of telomere repeats from about 40 to 28 alleviated BIR blockage (Extended Data Fig. 6a, b). Nevertheless, even at this reduced length, ITSs still promoted genetic instability at the level that was specific to BIR, with a higher frequency of ITS repeat size changes detected among BIR events compared to no-DSB controls (Extended Data Fig. 6c). Interruption of BIR at ITSs might be promoted either by a protein bound to the ITS or by the formation of secondary DNA structures. The former is more likely because BIR easily progressed through another, non-ITS, G4-forming sequence in either orientation at the same location, even in the presence of the G4-stabilizing agent Phen-DC321 (Extended Data Fig. 6d–f). In addition, deletion of RRM3, which unwind G4 structures, did not exacerbate BIR disruption upon encountering either 28- or 40-repeat ITSs (Extended Data Fig. 6g–i).

**Transcription blocks BIR initiation**

Collisions between the regular replication fork and transcription machinery can result in fork collapse, and BIR has been proposed as a mechanism for recovering such collapsed replication forks. To achieve this, BIR would have to be able to both successfully initiate at the site of collision between replication and transcription, and successfully traverse the highly transcribed unit. To test whether this was the case, we inserted HIS3 under the control of the unidirectional GAL1 promoter (P_{gal}29) into MATα-inc of the donor chromosome III, which is near the site of BIR strand invasion, in head-on (H-On) or co-directional (Co-D) orientation with respect to BIR progression (Fig. 4a). RNA-PolII ChIP confirmed that both orientations were transcribed with similar efficiency (Extended Data Fig. 7a, b). AMBER analysis detected no BIR-specific DNA synthesis within or beyond the H-On-orientated P_{gal}–HIS3 sequence through the entire time course (Fig. 4b, Extended Data Fig. 8a, b), whereas strand invasion was efficient (Extended Data Fig. 8c). In addition, the frequency of abnormal DSB repair products was greatly increased in these cells (Extended Data Fig. 9a, b). Thus, transcription in the H-On orientation blocks the initiation of BIR synthesis. Similarly, when we inserted H-On orientated P_{tet}–HIS3, where the level of transcription was regulated by the amount of doxycycline added, the level of BIR synthesis was inversely correlated with the amount of doxycycline (Fig. 4d). Notably, the high level of HIS3 mRNA that was detected 1 h after addition of doxycycline decreased following BIR induction and was 25-fold lower in strains with a DSB, compared to no-DSB controls, 10 h after the addition of galactose (Fig. 4e). Thus, we
The resulting ‘stuck intermediates’ may eventually be processed, as evidenced by the presence of some BIR outcomes among surviving cells (Extended Data Fig. 9a, b). $P_{\text{GAL1}}$–HIS3 inset $\text{Co-D}$-orientation also decreased initial DNA synthesis and increased the number of abnormal DSB repair outcomes when compared with the wild type, albeit not as strongly as in the H-On orientation (Extended Data Figs. 9d–f, 9b). Together, our findings suggest that initiation of BIR synthesis is strongly impaired when it occurs in close proximity to actively transcribed areas.

**Collision of BIR with transcription**

When we inserted $P_{\text{GAL1}}$–HIS3 further (6 kb) from the site of invasion in either orientation, AMBER analysis detected no defects in BIR synthesis (Fig. 4a, c, Extended Data Fig. 8g). This could be because established BIR synthesis has less trouble traversing transcription units, or because the initiation of BIR leads to a global change in transcription along the entire template. To distinguish between these possibilities, we compared rPolII distribution on the donor chromosome III before and 6 h after DSB induction in strains proficient and deficient ($\text{rad51}^\Delta$) for BIR. At 6 h, when more than half of the cells had completed the first 30 kb and few had completed 90 kb of BIR synthesis (Extended Data Fig. 9e), rPolII had accumulated in the transcription end site (TES) regions of several H-On transcription genes within the first 30 kb of BIR in wild-type cells, but not in $\text{rad51}^\Delta$ cells (Fig. 4f top, Extended Data Fig. 9d, f, g). We observed no accumulation of rPolII from 60 kb downstream of the strand invasion site to the end of donor chromosome III (Extended Data Fig. 9f, d), probably because BIR had not yet passed through this region in the majority of the cells. We also observed no accumulation of rPolII in Co-D-oriented genes (Fig. 4f bottom, Extended Data Fig. 9d, f), or in genes located on other chromosomes (Extended Data Fig. 9f). Thus, BIR appears to have only a localized effect on the regulation of transcription. Our data suggest that established BIR can progress through transcribed genes, but active transcription in the H-On orientation may promote transient stalling of BIR. In further support of this idea, $P_{\text{GAL1}}$–HIS3 at the 6-kb position increased the level of abnormal DSB repair outcomes in the H-On but not the Co-D orientation (Extended Data Fig. 9c, Supplementary Table 1). In addition, high transcription in the H-On orientation strongly increased the rate of BIR-associated mutagenesis, as measured by the $P_{\text{GAL1}}$–ura3-29 reporter (Methods) inserted at the same chromosomal position (Fig. 4g, Supplementary Table 2).

In summary, BIR is slower than normal replication and more susceptible to mutations and instability at roadblocks (Fig. 4g, Supplementary Table 1, 2). Thus, the purported role for BIR in the recovery of collapsed replication forks at fragile sites1–4,21,22 would seem paradoxical. We propose that initiation of BIR can be adjusted by extensive DNA resection, 3’-end degradation or fork reversal to initiate BIR away from the highly transcribed site. Once initiated, BIR can traverse transcribed regions, although traversing transcription units in the H-On orientation promotes mutations and chromosomal rearrangements (Extended Data Fig. 10a, b).

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-03172-w.

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**Fig. 4 | Highly transcribed units interfere with BIR.** a, $P_{\text{GAL1}}$–HIS3 or $P_{\text{TET(on)}}$–HIS3 inserted at MATα-inc or at 6-kb position in two orientations. b, c, AMBER analysis in strains with H-On $P_{\text{GAL1}}$–HIS3 at MATα-inc (b) or at 6 kb (c). d, BIR synthesis negatively correlates with transcription level at $P_{\text{TET(on)}}$–HIS3. The amount of doxycycline (DOX) added to induce transcription is indicated. Blue, BIR synthesis at 0.5 kb 10 h after DSB induction; red, mRNA levels of $P_{\text{TET(on)}}$–HIS3 at 10 h after addition of doxycycline (just before DSB induction by galactose). e–g, One out of three independent biological replicates that showed similar results (Supplementary Table S). Mean values of target to reference (ACT1) loci were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% CI, e. Fold change of mRNA level of HIS3 1 h after addition of 5 μg ml$^{-1}$ doxycycline to cells with or without DSB (as compared to the mRNA level before doxycycline addition). Mean ± s.d. (n = 3 independent biological repeats). **P = 0.0065 (two-tailed t-test), f, rPolII enrichment at TESs detected by ChiP with sequencing (ChiP-seq) for all H-On genes (top), but not in Co-D genes (bottom) located on donor chromosome III within 30 kb centromere-distal to MAT. y-axis shows transcription start site (TSS), TES, and 500-bp flanking regions, y-axis shows the mean value of rPolII depth. g, The effect of transcription on BIR-associated mutagenesis measured as Ura+ frequency resulting from BIR synthesizing across $P_{\text{GAL1}}$–ura3-29 (low transcription) or $P_{\text{GAL1}}$–ura3-29 (high transcription) in the H-On or Co-D orientation (with respect to BIR) inserted 6 kb centromere-distal to the DSB. GAL-HO cut site eliminated in the no-break control strains. Median ± 95% CI of mutation frequencies (n = 6 independent biological repeats). *P = 0.0022 (two-tailed Mann–Whitney U-test), Supplementary Table 2.
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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Media and strains

All yeast strains (Supplementary Table 3) were isogenic to AM1003, which is a chromosome III dicentric strain with the following genotype: *MATα-leu2-3,112/leu2 thr4 his3 Δ::ADE1 his3 Δ::ADE3 his3 Δ::HYG ade3Δ::GAL-HO F2 Δ::NAT/F2 Δ*. AM5658 is a pir2-1 derivative of AM1003 and was constructed by the pop-in-pop-out method using the YipA16 plasmid digested with Hpal (NEB R0105S). The pir2-1 mutation was confirmed phenotypically (temperature sensitivity) as well as by Sanger sequencing. The strains containing the AID-tagged POL1 gene, AM3701, and the strain containing the AID-tagged POL2 gene, AM3706, were constructed by inserting the AID sequence at the C terminus of POL1 and of POL2, respectively, in strain AM3067 (derivative of AM216; Supplementary Table 3) that contains ADH-OsTIR15. The insertion of the AID tag and ADH-OsTIR were confirmed by PCR and by defective growth on medium containing 2.5 mM indole-3-acetic acid (IAA). To study the effect of transcription on BIR, the *PGAL1–HIS3* unit was constructed by ligating PCR fragments containing the GAL1 promoter (a truncated version of *GAL1/GAL10* based on ref. 15) and the HIS3 coding region with the HindIII-digested pGp564 plasmid using the NEBuilder kit (NEB: E5520S). The *PGAL1–HIS3* construct was inserted into derivatives of AM1003 at different positions in two orientations using the *delitto perfetto* approach14 or CRISPR–Cas916. Specifically, two AM1003 derivatives containing the *LYS2* gene under its native promoter at *MATα-inc* in two orientations were used. We first replaced the *LYS2* gene with a *lys2–PCORE* cassette, where PCORE contains the *URA3 and G418* markers, and selected *URA*’, *G418*, and *LYS*’ transformants. Next, we deleted the native *HIS3* gene on chromosome XV by replacement with a *Bleo*’4 cassette. We then transformed the derivative strains with a PCR product containing the *PGAL1–HIS3* amplified using 80- bp-long homology primers homologous to the *lys2* gene to replace PCORE by selecting for *FOA*’ and *G418*, which allowed us to obtain two strains with two orientations of the *PGAL1–HIS3* corresponding to orientations of two original *LYS2* cassettes. To insert *PGAL1–HIS3* at the 6-kb position, a CRISPR–Cas9 plasmid targeting this position was built and co-transformed with a PCR fragment containing the *PGAL1–HIS3* construct and 80-bp-long homology to both sides of the CRISPR–Cas9 target cut site in two orientations. Successful insertion of *PGAL1–HIS3* was confirmed by PCR and phenotypically by observing growth of transformants on synthetic medium without histidine that was dependent on the presence of galactose as the only source of carbon. Similarly, the *ura3-29* reporter under the *GAL1* or *URA3* promoter was inserted into the 6-kb position in two orientations in the same way as described for *PGAL1–HIS3* at this position. To determine how transcriptional level affects BIR standing, we replaced the *GAL1* promoter in *PGAL1–HIS3* in AM5299 with the *TET* (on) promoter using a PCR product containing the *TET* promoter and activator. Transformants were selected with synthetic medium without histidine and containing 5 μg/ml doxycycline.

AM5582 is a strain containing about 250–300-bp human ITSs (ITS–)60–62, including about 40 telomere repeats from the pSP73-pSty1 plasmid62 inserted 6 kb centromere-distal from *MATα-inc*. The number of telomere repeats was estimated by PCR using two primers (one primer specific to the *KANMX* locus and another specific to the *PERI* gene located centromere-distal to the ITSs). AM6068 is a strain containing human (ITS)60–62 (28 telomere repeats confirmed by sequencing) at the same place as AM5582, and was introduced by using CRISPR–Cas9 similarly to a published method49. The deletions of *RRM3, RAD51*, and *RAD52* were constructed by transformation with DNA fragments containing about 200 bp homology to corresponding genes flanking either the *Bleo*’ or *KANMX* cassette, while transformation with a fragment containing about 200 bp homology to *TLC1* or *PIF1* flanking the BSD cassette (Invitrogen) was used to obtain the deletion of *TLC1* or *PIF1*. The sequences of all of the oligos used in this work are available upon request.

AM6026 is strain containing a G-quadruplex sequence that can form G4 structures during BIR leading strand synthesis. The G4-forming sequence is a 750-bp Sau3AI fragment from pT7-Sμ63 inserted into the BglII site of a *LYS2* gene placed 6 kb centromere-distal from *MATα-inc* by CRISPR–Cas9 targeted integration. The AM6026 strain was constructed using pop-in-pop-out allele replacement of *LYS2* with Hpal linearized plasmids containing the aforementioned lys2* G4 cassettes and the *URA3* gene as described30,31. Strain AM6081 contains a sequence that can form G4 structures during BIR lagging strand synthesis, which was inserted at the 6-kb position by co-transforming with CRISPR–Cas9 plasmid targeting at this position and with the PCR product of pT7-Sμ containing the G4 sequence and 80-bp homology to the target site.

To study GCRs caused by a highly transcribed gene during normal (S-phase) DNA replication, we generated haploid strains containing the *pCAI-HIS3* cassette at MAT or NO- *GAL1-HIS3* control strains by loss of the truncated (MATα) chromosome in AM5299, AM5301, and AM411 (see Supplementary Table 3 for genotypes). The resulting strains (AM6082–AM6084) contained full-length chromosome III, *hml::ADE3, hmr::H4P*, and a lys2 insertion at MAT with or without *PGAL1–HIS3*. These strains were crossed to the AM614 strain (*MATα-inc* that contained a normal-length chromosome III). The resulting diploids were named AM6088–AM6090. We also generated strains containing the *PGAL1–HIS3* cassette 6 kb centromere distal to MAT or NO-*PGAL1–HIS3* control by the loss of the truncated (MATα) chromosome in AM5644, AM5646, and AM1003. The resulting strains (AM6085–AM6087) contained full-length chromosome III, *MATα-inc hml::ADE3, hmr::H4P*. These strains we crossed to the SSA-repair outcome of YMV80 (hml::ADE1 mata::his3 hmr::ADE1 leu2 ade3::GAL1/HO ade1 lys5 ura3-52)64. The resulting diploids were named AM6091–AM6093 (see Supplementary Table 3 for the genotypes of these diploids).

Rich medium (yeast extract-peptone-dextrose (YPD)), synthetic complete medium, and YEP-rafineose, YEP-galactose and YEP-lac media were prepared as previously described65. Antibiotics were added to YEP medium after autoclaving at concentrations previously described65. All cultures were grown at 30°C unless specifically indicated.

Analysis of BIR efficiency

DSBs were induced by HO endonuclease in asynchronous cell populations, which led to G2/M cell cycle arrest within 2–3 h. BIR then proceeded in a synchronized culture of G2/M-arrested cells. To follow BIR dynamics, cells were grown in liquid lactate medium to log phase, and exceeded in a synchronized culture of G2/M-arrested cells. To follow BIR efficiency, cultures were grown at 30°C unless specifically indicated.

Mutagenesis assay

To determine the mutation frequency associated with BIR, yeast strains were grown from individual colonies with agitation in liquid synthetic medium lacking leucine for approximately 20 h, diluted 20-fold with fresh YEP-lac medium, and grown to logarithmic phase for approximately 16 h until cell density reached about 5 × 10^6 cells ml^-1^. To measure the frequency of Ura’ cells, samples were plated at appropriate concentrations on YEPD medium and on uracil drop-out medium where glucose was substituted for 2% galactose. The frequencies of mutagenesis were calculated in a similar way to that previously described65,66.
Analysis of GCR (chromosome loss) associated with vegetative growth (S-phase replication)
Diploid strains (AM6088–AM6090 and AM6091–AM6093) were grown in YEP-lac medium overnight, then plated onto YEP-galactose plates and incubated at 30 °C for 3–4 days. After that, plates were replicated to selective medium (YPED with 0.5g/l hygromycin) for AM6088–AM6090 to determine the frequency of chromosome loss by the frequency of hygromycin-sensitive colonies, or to synthetic medium without adenine for AM6091–AM6093 to determine the frequency of chromosome loss by the frequency of Ade⁺ colonies.

D-loop capture (DLC) assay experiments
D-loop capture (DLC) assay experiments were performed as previously described. See Supplementary Table 4 for primers.

PCR analysis of GCRs in ITS strain
PCR analysis of GCRs in the ITS strain were performed using primers specific to yeast telomere sequence (as previously described) and to the KANMX locus centromere proximal to the ITS insertion. The formation of 0.5- to approximately 1-kb PCR products was indicative of de novo telomere addition that led to the formation of truncated BIR products.

Whole-genome sequencing
Whole-genome sequencing analysis was performed using QIAGEN CLC Genomics Workbench 20.

The analysis of BIR kinetics by AMBER

Time course experiment. Yeast cells were grown overnight in synthetic leucine drop-out medium, transferred to YEP-lac and incubated for about 16 h, until cell density reached about 5 × 10⁹ cells ml⁻¹. An aliquot (50 ml) was taken for analysis of pre-DSB copy number (0 h) and galactose was added to a final concentration of 2% to induce HO endonuclease. The obtained aliquots were used to prepare DNA for AMBER and mRNA extraction.

DNA preparation. Cells were resuspended in spheroplasting buffer with 250 μg/ml 20T zymolyase and incubated at 37 °C for 10 min to digest the cell wall. Cells (2.5 × 10⁹) were then centrifuged at 3,000 rpm for 2 min and all remaining liquid removed. Cells were resuspended with 400μl 1× NEB Cutsmart buffer (B7204S) or (S1 digestion buffer for prip2-1 ssDNA digest experiment (Thermo Fisher, EN0321)). Then 4 μl of 10% SDS was added and the mixture was incubated at 60 °C for 15 min. The mixture was placed on ice and 50 μl of 10% triton X-100 was added. RNase (20 μl, 10 mg/ml) was added and incubated at 37 °C for 30 min (for the prip2-1 ssDNA digest experiment, 20 μl RNase (10 mg/ml) and 10 μl 1:10,000 diluted S1 enzyme together were incubated at 25 °C for 30 min instead). Next, 20 μl protease K (40 mg/ml) was added and incubated at 60 °C for 30 min. The mixture was placed on ice, 0.5 ml phenol-chloroform was added and the mixture was vortexed for 1 min and then centrifuged at 13,000 rpm for 15 min. The aqueous phase was taken and added to 0.5 ml phenol-chloroform, and mixed by inverting. Centrifugation of the mixture was repeated, and the aqueous phase was taken. DNA was precipitated by adding 35 μl 3 M sodium acetate (pH 5.2) and 0.35 ml isopropanol, and incubated at room temperature for 30 min. Centrifugation at 13,000 rpm for 20 min pelleted the DNA and the supernatant was removed. The pellet was resuspended in 0.2 ml NaCl solution (0.2 M). The DNA was precipitated by addition of 0.8 ml ice-cold 100% ethanol and incubated at −20 °C for 20 min followed by centrifugation at 13,000 rpm for 20 min. After removing the supernatant, the pellet was washed by adding 0.5 ml 80% ethanol and centrifuged at 13,000 rpm for 5 min. The ethanol was removed and the pellet was dried completely before resuspending in 100 μl molecular grade water for later use. The purified DNA was quantified using QuantiIT PicoGreen dsDNA Assay Kit (Invitrogen, P11496), and stored at −20 °C until use.

ddPCR. The PCR reaction mix was assembled in the following way: 10 μl 2× Supermix for probe (Biorad, 1863026), 1 μl 20 primer mix for the target locus (containing primers and fluoresce probe (IDT)), 1 μl 20 primer mix for the ACT1 locus, 1–2 μl DNA diluted to an appropriate concentration, and molecular grade water was added to a total volume of 20 μl. Next, the reaction mix and 70 μl oil (Biorad, 1863005) were added to a Droplet Generation Cartridge (Biorad, 1864008), where droplets were generated. The standard two-step Biorad ddPCR protocol was employed for ddPCR. The copy numbers for target loci were calculated by normalizing DNA concentrations to the concentration for the ACT1 locus (chromosome VI). The means and 95% CIs were calculated for individual experiments using QuantaSoft from BioRad. The copy numbers for positions located centromere-distal to MATA-locus started from 1× before BIR and were expected to increase up to 2× by the end of BIR. The copy number for EMCI located at the 45-kb position (left arm of chromosome III (positions according to Saccharomyces genome database)) was expected to stay at 2× and was used as a control. The best-fitting BIR curves were generated by the Boltzmann sigmoidal equation. The threshold for BIR synthesis was established at 0.1× increase, based on the error level of ddPCR that was calculated from an experiment where no change in copy number was expected. In particular, the copy number of the EMCI gene (which was not affected by BIR) demonstrated ±0.1× variation. In addition, the variation in copy number for BIR-specific probes in rad52Δ and rad52Δ mutants, where BIR cannot occur, following DSB induction proved to be within 0.1×.

Analysis of HIS3 mRNA level. The extraction of mRNA was conducted using Trizol reagent (Thermo Fisher 15596026), an RNeasy kit from Qiagen (74104) and RNase-Free DNase Set kit from Qiagen (79254). The cDNA was produced using iScript cDNA Synthesis Kit (BioRad, 1708890). The levels of HIS3 mRNA were measured by ddPCR using HIS3-specific primer sets (Supplementary Table 4) and normalized to ACT1 using the same primer sets as in AMBER.

ChIP–qPCR. Cells were cultured in YEP-raffinose liquid medium overnight at 30 °C to a final density of 5 × 10⁶ cells ml⁻¹. DSBs were induced as described above. Formaldehyde (1% final concentration) was used to cross-link the DNA to the proteins.
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.
Extended Data Fig. 1 | Kinetics of DSB repair. **a**, Strand invasion measured by Rad51 ChIP followed by qPCR. Forward primer is shared by donor and recipient at X region, and reverse primer is unique to the Yα region in the donor. The means ± s.d. (n = 3 independent biological repeats) are indicated. **b**, Strand invasion measured by DLC assay. DLC signal is normalized to ARG4 and then compared to 0hr by fold changes. The means ± s.d. (n = 3 independent biological repeats) are indicated. **c**, DNA synthesis detected by ddPCR using traditional DNA preparation protocol as previously described39. **d**, AMBER analysis of DNA clipping or degradation using primers located centromere proximal to MATα in wt (top), rad51Δ (middle), or rad52Δ (bottom) following DSB induction. In rad52Δ the rate of DNA degradation was >8kb/hr, much higher than in wt and rad51Δ. c and d each represents one out of three independent biological repeats that showed similar results (see Supplementary Table 6 for other repeats). Mean values of target to reference (ACT1) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95%CI.
Extended Data Fig. 2 | Kinetics of BIR synthesis. 

**a**, Schematic of primers (triangles) used for AMBER analyses (similar to Fig. 1a). 

**b**, DNA synthesis detected by AMBER in a time course designed as described in Fig. 1b, but with nocodazole addition 3 h before DSB induction (0hr). The data represent one experiment that was similar to 3 experiments including the one presented in Fig. 2b. 

**c**, Slopes of BIR at the time when 50 percent of the cells had completed BIR ($V_{50}$) at the indicated chromosomal position based on three independent experiments ($n = 3$), including the one shown in Fig. 1b. The means ± s.d. (error-bars) are indicated. Statistics is determined by two-tailed $t$-test with p value indicated. 

**d**. AMBER analysis of POL3 (wt), and **e**, pol3-01. b, d and e each represents one out of three independent biological repeats that showed similar results (see Supplementary Table 6 for other repeats). Mean values of target to reference ($ACT1$) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% CI.
Extended Data Fig. 3 | BIR synthesis in pif1Δ, pol32Δ, and pol2-AID. a, Strand invasion in pif1Δ and pol32Δ mutants 6h post DSB measured by DLC assay. DLC signal is normalized to ARG4 and compared to 0hr by fold changes. The means ± s.d. (n = 3 independent biological repeats) are indicated. b, Kinetics of Ya disappearance in wt, pif1Δ, pol32Δ and pif1Δ pol32Δ. c, Schematic of primers used for AMBER analyses. d–f, AMBER analysis of BIR synthesis in wt (PIF1, POL32) (d), in pif1Δ (e), and pol32Δ (f). d–f each represents one out of three independent biological repeats that showed similar results (see Supplementary Table 6 for other repeats). Mean values of target to reference (ACT1) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% CI. g, AMBER analysis of BIR synthesis following inactivation of Polε (pol2-degron) with schematic of analysis (top), degradation of AID-tagged Pol2 verified by Western Blot (middle) and calculation of copy number (bottom). The means ± s.d. (n = 3 independent biological repeats) are indicated. * represents significant difference (P = 0.0351) determined by t-test, two-tailed.)
**Extended Data Fig. 4 | Primase defect leads to defective long leading strand synthesis.**

**a**, CHEF gel electrophoresis analysis of DSB repair in PRI2 (wt) and temperature-sensitive pri2-1 mutant followed by Southern blot analysis using ADE1-specific probe (one representative experiment from $n = 3$ independent biological repeats). BIR: BIR repair product; HC: half-crossover resulting from fusion between fragments of recipient and donor Chromosome III (see Extended Data Fig. 9a for details); Chr I: Chromosome I; Uncut: full-length recipient chromosome.

**b**, Top: Schematic of experiment to characterize BIR progression following depletion of Pol1 ($pol1$-degron) by addition of IAA. Bottom left: Degradation of AID-tagged Pol1 verified by Western Blot. Bottom right: AMBER analysis of BIR synthesis. The means ± s.d. ($n = 3$ independent biological repeats) indicated by error bars.

**c**, Proposed model explaining the role of primase and Okazaki fragment synthesis in stabilization of leading strand.
Extended Data Fig. 5 | BIR stalls inside ITS. a, AMBER analysis of BIR synthesis in *TLC1* and *tlc1Δ* cells traversing (ITS)~40, similar to Fig. 3b, c, but with nocodazole added 6 hr post DSB. The means ± s.d. (n = 3 independent biological repeats) are indicated. **Statistical difference is determined by t-test (two-tailed), P = 0.0053. b, Proposed model of BIR interruption at ITS followed by telomerase-mediated de novo telomere addition.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | G4-forming sequence does not block BIR progression. a, A G4-forming sequence or (TTAGGG)$_{28}$ was inserted at the 6-kb position. The colours indicating positions are kept the same in panels b, d, e, g and h. b, AMBER analysis of BIR through (ITS)$_{28}$ inserted at the 6 kb position. c, Sanger sequencing analysis of repair outcomes of BIR that traversed (ITS)$_{28}$ show change in ITS copy number resulting from template switching. (Sample sizes (n) are indicated. * = statistically significant difference (P = 0.0288) determined by Chi-square test (two-sided, df = 1)). d, AMBER analysis of BIR through sequence that can form G4 structure during leading strand (Ori1) synthesis and e, during lagging strand (Ori2) synthesis. f, AMBER analysis of BIR through G4-forming sequences similar to d and e, but in the presence of G4-stabilizing drug, Phen-DC3. The means ± s.d. (n = 3 independent biological repeats) indicated by error-bars. g, h, AMBER analysis of BIR synthesis in rrm3Δ mutant through (ITS)$_{28}$ (g) and through (ITS)$_{-40}$ (h). i, genetic analysis of GCRs in (g) and (h) performed similar to Fig. 3d, e. Sample sizes (n) are indicated. b, d, e, g and h each represents one out of three independent biological repeats that showed similar results (see Supplementary Table 6 for other repeats). Mean values of target to reference (ACT1) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95%CI.
Extended Data Fig. 7 | Initiation of BIR immobilizes rPolII on H-On transcribed genes. a, Location of primer pairs homologous to GAL1 promoter (pair 1), TSS (transcription start site) (pair 2) and the middle of HIS3 gene (pair 3) used for qPCR (see Supplementary Table 4 for primer sequences). b, RNA PolII enrichment at PGAL1/HIS3 in H-On and Co-D orientations measured at indicated times following galactose addition. The means ± s.d. indicated by error-bars (n = 3 independent biological experiments). c, Transcription levels of Puer/HIS3 8 h post-DSB detected by HIS3-specific primers and normalized to ACT1 mRNA control. The means ± s.d. (n = 3 independent biological repeats), indicated by error-bars. (** = statistically significant difference (P = 0.0033) determined by t test, two tailed).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Interference between BIR and transcription.

a, Schematic of \( P_{\text{gal}}/\text{HIS3} \) inserted at \( \text{MAT} \alpha/-\text{inc} \) in H-On or Co-D orientations with respect to BIR progression. The same primer pairs were used for AMBER analysis as described in Fig. 1a, although their actual positions along the BIR track (donor chromosome) are shifted by insertion of \( P_{\text{gal}}/\text{HIS3} \). b, AMBER analysis of experiment in Fig. 4b using primers located in \( \text{HIS3} \) gene. c, Strand invasion kinetics for experiment shown in d and Fig. 4b assessed by Rad51 ChIP followed by qPCR using primers amplifying the junction region with the forward primer targeting a donor and recipient shared region while the reverse primer targeting donor-specific region. The means ± s.d. (\( n = 3 \) independent biological repeats) are indicated. d, AMBER analysis of AM1411 (the NO-\( P_{\text{gal}}/\text{HIS3} \) control strain used for experiments shown in Fig. 4b, and Extended Data Fig. 8e) containing insertion of \( \text{lys2} \) under its native promoter at \( \text{MAT} \alpha/-\text{inc} \) to match the experimental strains in mating type and in the presence of insertion at \( \text{MAT} \alpha/-\text{inc} \). e, AMBER analysis in strain with \( P_{\text{gal}}/\text{HIS3} \) inserted at \( \text{MAT} \alpha/-\text{inc} \) in Co-D orientation. f, The amount of BIR synthesis detected at 10 h using 0.5 kb primers in strains with or without \( P_{\text{gal}}/\text{HIS3} \). The means ± s.d. (\( n = 3 \) independent biological repeats) are indicated. Asterisks indicate statistically significant difference determined by two-tailed \( t \)-test (**: \( P = 0.0035 \); ***: \( P = 1.12\times10^{-4} \)). g, AMBER analysis of BIR progression in strains with \( P_{\text{gal}}/\text{HIS3} \) inserted 6 kb centromere distal from \( \text{MAT} \alpha/-\text{inc} \) in Co-D orientation. b, d, e, and g each represents one out of three independent biological repeats that showed similar results (see Supplementary Table 6 for other repeats). Mean values of target to reference (ACT1) loci ratios were calculated by Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95% CI.
Extended Data Fig. 9 | BIR is interrupted by transcription. a, Products of DSB repair as distinguished by genetic markers. b, Distribution of DSB repair outcomes for strains with and without PGAL1-HIS3 inserted at MATα-inc, the same as in b, but for strains with PGAL1-HIS3 inserted at 6kb position. In b and c, sample sizes (from 3 biological repeats) are indicated (see Methods for details). Statistical comparisons are performed using Chi-square test (two-sided, df = 1); p values are indicated. d, (i) rPolII enrichment at TES detected by ChIP-seq for PWP2 gene located 20kb centromere distal to MAT. Red rectangle: BIR-promoted accumulation of rPolII at TES; (ii) rPolII distribution for all H-On genes located on donor chromosome III between 60 and 90 kb centromere distal to MAT. Data from the same experiment as shown in Fig. 4f. The labels are similar to Fig. 4f. e, AMBER analysis of BIR synthesis from the same samples as in Fig. 4f (n = 1). Mean values of ratios between target and reference (ACT1) loci are calculated using Poisson distribution based on 10,000 droplets with error bars representing upper and lower Poisson 95%CI. f, independent repeat of experiment shown in Fig. 4f. Metagene plot showing the distribution of normalized rPolII-Chip-seq reads along chromosome III. i) At genes located within 0-30kb centromere distal of the MAT locus (n = 10) in H-On direction; ii) at genes located within 60-90kb centromere distal to the MAT locus in H-On orientation (n = 11); iii) at genes located within 0-30kb centromere distal to the MAT locus in Co-D orientation (n = 9); iv) at all the genes except genes on chromosome III (n = 2950). Transcription start site (TSS), transcription end site (TES) and 500 bp flanking regions were plotted. Y axis showing the mean value of rPolII depth. g, Example of BIR-caused rPolII re-distribution in yeast gene PWP2 located on chrIII (data from same experiment in Extended Data Fig. 9f). The panels show normalized rPolII ChIP-seq reads from RAD51 (wild type) (top) and rad51Δ (bottom). Red rectangle: BIR-promoted rPolII accumulation.
Extended Data Fig. 10 | Model of highly transcribed region interfering BIR.

a. Model of the block of BIR initiation by transcription. Left: For H-On transcription, BIR immobilizes rPolII, which leads to the block of BIR and transcription. Right: Co-D transcription also interferes with BIR initiation.

b. Established BIR can traverse transcription units in both orientations, even though it is associated with transient accumulation of rPolII at TES and leads to increased level of GCRs and mutagenesis in H-On orientation.
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

QuantaSoft BioRad

Data analysis

QIAGEN CLC Genomics Workbench 20, samtools version 1.10, fastp version 0.20.1, bowtie2 version 2.2.4, macs2 version 2.2.7.1

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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Data Availability

The Whole Genome Sequencing raw data is deposited to the NCBI Sequence Read Archive database (https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB8309966/overview). The rPolII ChIP sequencing data is deposited to the NCBI Gene Expression Omnibus (GSE159384 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE159384). All data generated and analyzed in this paper are available from corresponding author upon request.
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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | For genetic analysis, at least 100 colonies were analyzed for each experiment. The sample size was determined based on the previous similar experiments. This sample size allows to detect the change of frequency of the main DSB repair outcomes. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No exclusions were done                                                                                                                                                                           |
| Replication | Experiments were conducted with at least 3 biological repeats. All attempts of biological replications were successful.                                                                             |
| Randomization | The Molecular Biology experiments do not require randomization because the analysis is done automatically (using machines). Genetic experiments use yeast colonies, which all are similar in size and phenotypes are easy to distinguish. |
| Blinding | The Molecular Biology experiments do not require blinding because the analysis is done automatically (using machines). Genetic experiments use yeast colonies, which all are similar in size and phenotypes are easy to distinguish. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☒ | ☒ |
| Antibodies          | ChIP-seq |
| Eukaryotic cell lines  | Flow cytometry |
| Palaeontology       | MRI-based neuroimaging |
| Animals and other organisms | |
| Human research participants | |
| Clinical data        | |

Antibodies

Antibodies used

The Anti-Rad51 antibody (1:300 for dilution) used in Rad51 ChIP was a gift received from Dr. Patrick Sung, UT Health San Antonio (made in Dr. Sung laboratory). Anti-myc antibody (9B11, 1:1,000 for dilution) used for Western Blots was purchased from Cell Signaling Technology (Danvers, MA). Anti-alpha-Tubulin antibody (#4A1, 1:1,000 for dilution) was purchased from Developmental Studies Hybridoma Bank (DSHB) at the University of Iowa (Iowa City, IA). HRP-conjugated goat-anti-mouse IgG (#115-035-003, 1:10,000 for dilution) was from Jackson ImmunoResearch Laboratories (West Grove, PA). Anti-RNA Polymerase II Rpb1 antibody (BioLegend, 664912, 1:100 for dilution) was used for rPolII ChIP qPCR experiment and also for rPolII-seq experiment.

Validation

Validation: All information about antibodies and validation can be found through the following link: anti-RNA polymerase II RPB1 antibody [https://www.biolegend.com/en-us/products/go-chip-grade-purified-anti-rna-polymerase-ii-rpb1-antibody-14222]; anti-Rad51 antibody were successfully used for immunoprecipitation experiment by Dr. Patrick Sung’s group in this resulted publication (Kwon, YoungHo, et al. "ATP-dependent chromatin remodeling by the Saccharomyces cerevisiae homologous recombination factor Rdh54." Journal of Biological Chemistry 283.16 (2008): 10445-10452); anti-myc antibody (https://www.cellsignal.com/products/primary-antibodies/myc-tag-9b11-mouse-mab/2276); anti-alpha-Tubulin antibody (https://dshb.biology.uiowa.edu/4A1); HRP- conjugated goat-anti-mouse IgG (https://www.jacksonimmuno.com/catalog/products/115-035-003);
ChIP-seq

Data deposition

☑ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☑ Confirm that you have deposited or provided access to graph files (e.g., BED files) for the called peaks.

Data access links

GEO accession number GSE159384

Files in database submission

RNA_Pol2_INPUT_A0.fastq.gz
RNA_Pol2_INPUT_A6.fastq.gz
RNA_Pol2_IP_A0.fastq.gz
RNA_Pol2_IP_A6.fastq.gz
RNA_Pol2_INPUT_B0.fastq.gz
RNA_Pol2_INPUT_B6.fastq.gz
RNA_Pol2_IP_B0.fastq.gz
RNA_Pol2_IP_B6.fastq.gz
RNA_Pol2_INPUT_C0.fastq.gz
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RNA_Pol2_IP_C0_summits.bed
RNA_Pol2_IP_C6_summits.bed

Genome browser session

https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=kuaias&hgS_otherUserSessionName=sacCer3+Pol2+ChIP

Methodology

Replicates

The experiment on rPOLII distribution following BIR was repeated in two replicates, and the results obtained in these two replicates were in agreement with each other.

Sequencing depth

| Sample | Total Reads | Uniquely Mapped Reads | Reads Type |
|--------|-------------|------------------------|------------|
| RNA_Pol2_INPUT_A0 | 1837487 | 1592255 | 150 single-end |
| RNA_Pol2_INPUT_A6 | 1636195 | 1399601 | 150 single-end |
| RNA_Pol2_IP_A0 | 2006678 | 1804366 | 150 single-end |
| RNA_Pol2_IP_A6 | 2585835 | 2295992 | 150 single-end |
| RNA_Pol2_INPUT_B0 | 2143633 | 1844832 | 150 single-end |
| RNA_Pol2_INPUT_B6 | 1842631 | 1610364 | 150 single-end |
| RNA_Pol2_IP_B0 | 1523091 | 1343203 | 150 single-end |
| RNA_Pol2_IP_B6 | 1592040 | 1413987 | 150 single-end |
| RNA_Pol2_INPUT_C0 | 2441840 | 2099262 | 150 single-end |
| RNA_Pol2_INPUT_C6 | 2209187 | 1897981 | 150 single-end |
| RNA_Pol2_INPUT_C0 | 2441840 | 2099262 | 150 single-end |
| RNA_Pol2_INPUT_C6 | 2209187 | 1897981 | 150 single-end |

Antibodies

RPB1 antibody:

BioLegend,
catalog: 664912
lot: B281694

Peak calling parameters

Peaks were called using macs2 version 2.2.7.1 callpeak function with -B -g 1.2e7 -q 0.01 parameters using input as background control

Data quality

Raw reads were first trimmed using fastp version 0.20.1 to remove adaptors and low-quality reads, then mapped to the genome using bowtie2 version 2.2.4 with default parameters. Duplicates reads were removed by samtools version 1.10 markdup function with -r parameter. Peaks were called using macs2 version 2.2.7.1 callpeak function with -B -g 1.2e7 -q
0.01 parameters using input as background control. Signal tracks were generated using mac2 bdgcmp function with -m ppois parameter using input as background control. Metagene plot were generated using deeptools version 3.5.0 computeMatrix scale-regions with –upstream 500 –binSize 10 –downstream 500 –missingDataAsZero –averageTypeBins mean –skipZeros parameters.

Number of peaks with Q-value < 0.01:

| Sample          | Number of Peaks |
|-----------------|-----------------|
| RNA_Pol2_IP_A0  | 1186            |
| RNA_Pol2_IP_A6  | 1288            |
| RNA_Pol2_IP_B0  | 1194            |
| RNA_Pol2_IP_B6  | 1074            |
| RNA_Pol2_IP_C0  | 1350            |
| RNA_Pol2_IP_C6  | 1334            |
| RNA_Pol2_IP_D0  | 1318            |
| RNA_Pol2_IP_D6  | 1228            |

Software

macs2