Transcript–RNA-templated DNA recombination and repair

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Homologous recombination is a molecular process that has multiple important roles in DNA metabolism, both for DNA repair and genetic variation in all forms of life. Generally, homologous recombination involves the exchange of genetic information between two identical or nearly identical DNA molecules; however, homologous recombination can also occur between RNA molecules, as shown for RNA viruses. Previous research showed that synthetic RNA oligonucleotides can act as templates for DNA double-strand break (DSB) repair in yeast and human cells, and artificial long RNA templates injected in ciliate cells can guide genomic rearrangements. Here we report that endogenous transcript RNA mediates homologous recombination with chromosomal DNA in yeast Saccharomyces cerevisiae. We developed a system to detect the events of homologous recombination initiated by transcript RNA following the repair of a chromosomal DSB occurring either in a homologous but remote locus, or in the same transcript-generating locus in reverse-transcription-defective yeast strains. We found that RNA–DNA recombination is blocked by ribonucleases H1 and H2. In the presence of H-type ribonucleases, DSB repair proceeds through a complementary DNA intermediate, whereas in their absence, it proceeds directly through RNA. The proximity of the transcript to its chromosomal DNA partner in the same locus facilitates Rad52-driven homologous recombination during DSB repair. We demonstrate that yeast and human Rad52 proteins efficiently catalyse annealing of RNA to a DSB-like DNA end in vitro. Our results reveal a novel mechanism of homologous recombination and DNA repair in which transcript RNA is used as a template for DSB repair. Thus, considering the abundance of RNA transcripts in cells, RNA may have a marked impact on genomic stability and plasticity.

To investigate the capacity of transcript RNA to recombine with genomic DNA, we sought to discover whether a chromosomal DSB could be repaired directly by endogenous RNA in yeast S. cerevisiae cells. We designed a strategy by which we could induce a DSB in the HIS3 marker gene and monitor precise repair of the DSB by a homologous transcript messenger RNA by restoration of HIS3 function resulting in histidine prototrophic (HIS+) cells (see Methods). We developed two experimental yeast cell systems, trans and cis, in strains YS–289, 290 and YS–291, 292, respectively (Extended Data Table 1). The trans system is designed to test the ability of a spliced (intron-less) antisense his3 transcript from chromosome III to repair a DSB in a different his3 allele on chromosome XV, which contains an engineered homothallic switching endonuclease cutting site (Fig. 1a and Extended Data Fig. 1a, b). The cis system is designed to test the capacity of the spliced antisense his3 transcript from chromosome III to recombine with a chromosomal DSB by transcript RNA.

Figure 1 | Repair of a chromosomal DSB by transcript RNA.

a, b, Scheme of the trans (a) and cis (b) cell systems used to detect DSB repair by transcript RNA. Al, artificial intron; HO, homothallic switching endonuclease; pGAL1, galactose-inducible promoter; RT, reverse transcriptase. Yellow triangles, cleavage activity by HO homothallic switching endonuclease; red question marks, hypothesis for transcript-RNA-templated DSB repair mechanism. c–e, Examples of replica-plating results (n = 6) from galactose medium to histidine dropout medium demonstrating the ability of various yeast strains (relevant genotypes shown) of the trans and cis systems to generate histidine prototrophic colonies in the absence of SPT3, or DBR1 function, or with phosphonoformic acid (PFA) (c), in the presence of the plasmid carrying the pGAL1-mhis3-AI cassette (BDG606) or the control (BDG283) (d), or when the artificial intron has a 23-base-pair deletion (AIΔ23) (e). WT, wild type.
to repair a homothallic-switching-endonuclease-induced DSB located inside the intron of the same his3 locus (Fig. 1b and Extended Data Fig. 1c). In both the trans and cis cell systems, the spliced antisense his3 transcript RNA can serve as a homologous template to repair the broken his3 DNA and restore its function. However, given the abundance ofTy retrotransposons in yeast cells, the spliced antisense his3 RNA could potentially be reverse transcribed by the Ty reverse transcriptase in the cytoplasm to cDNA that could then recombine with the homologous broken his3 sequence or be captured by non-homologous end joining at the homothallic switching endonuclease break site to produce His+ cells. To distinguish DSB repair mediated by the transcript RNA template from repair mediated by the cDNA template, we performed the trans and cis assays in two yeast strains that contained either a wild-type SPT3 gene or its null allele, which prevents Ty transcription and strongly reduces Ty transposition and transpositional recombination.3,8,9. In both assays, cells containing wild-type SPT3 produced numerous His+ colonies after DSB induction (Fig. 1c and Table 1a). As expected, the frequency of His+ colonies in the trans system was significantly higher than that in the cis system because the his3 transcript is continuously generated in the presence of galactose. In contrast, production of the full his3 transcript is immediately terminated upon DSB formation in the cis system. This frequency difference is not specific to the particular genomic loci in which the DSBs are induced, as transformation by DNA oligonucleotides (HIS3.F and HIS3.R) designed to repair the broken his3 gene produced the same frequency of His+ colonies in the two systems (Extended Data Tables 2a and 3), demonstrating that the homothallic switching endonuclease DSB stimulates homologous recombination in the trans and cis systems equally well. Notably, almost all the His+ colonies are dependent on SPT3 function, indicating that the DSB in his3 is repaired exclusively via the cDNA pathway (Fig. 1c and Table 1a). This finding demonstrates that if an actively transcribed gene is broken, it can be repaired using a cDNA template derived from its intact transcript. Moreover, these data also support the model in which reverse-transcribed products from any sort of RNA can be a significant source of genome modification at DSB sites.10.

For RNA to recombine with DNA, an intermediate step that is probably required is the formation of an RNA–DNA heteroduplex. We therefore deleted the genes coding for ribonuclease (RNase) H1 (RNH1) and/or the catalytic subunit of RNase H2 (RNH201), which both cleave the RNA strand of RNA–DNA hybrids.11 Remarkably, while deletion of RNH1 slightly increased the frequency of His+ colonies in the trans system, deletion of RNH201 increased the frequency of His+ colonies in both the trans and cis systems, and combined deletion of RNH1 and RNH201 resulted in an even stronger increase of His+ colonies in both systems. Moreover, we detected His+ colonies in rnh1 rnh201 cells in the absence of SPT3 (Fig. 1c and Table 1a). Notably, there were more His+ colonies in cis-system rnh1 rnh201 sp3 than in trans-system, and the frequency of His+ colonies observed in the rnh1 rnh201 sp3 relative to sp3 cells was much higher in cis (>69,000) than in trans (>6,400) (Fig. 1c and Table 1a). If DSB repair in rnh1 rnh201 sp3 cells were due to cDNA, we would expect a higher His+ frequency in the trans than in the cis system, as observed in wild-type cells. The fact that the His+ frequency is higher in the cis system suggests that DSB repair is not mediated by cDNA but instead by RNA or predominantly RNA. To further examine the possibility that residual cDNA rather than transcript RNA is responsible for his3 correction in cis-system rnh1 rnh201 sp3 cells, we introduced a trans system directly into these cells and into the control cis wild-type cells. When wild-type cells of the cis system were transformed with a low-copy-number plasmid carrying the GAL1–nhis3–AI cassette, where AI represents an artificial intron (BDG606; see Methods), they displayed a large (a factor of 4,000) increase in the His+ frequency following DSB induction in his3 compared to the same cells transformed with the control empty vector (BDG283). In contrast, BDG606 in cis-system rnh1 rnh201 sp3 cells did not significantly increase the His+ frequency (Fig. 1d and Extended Data Table 4). These results argue against the role of residual cDNA in template-dependent DSB repair in cis-system rnh1 rnh201 sp3 cells and support a predominant, direct template function of the his3-system transcript RNA in these cells. Overall, these data support the conclusion that a transcript RNA can directly repair a DSB in cis-system rnh1 rnh201 and his1 rnh1 sp3 cells. The physical proximity of the his3 transcript to its own his3 DNA during transcription could facilitate annealing of the broken DNA ends to the transcript. This possibility is consistent with the fact that closer donor sequences repair DSBs more efficiently,12,13 and that mature transcript RNAs are exported rapidly to the cytoplasm or degraded after completion of transcription.14

To confirm that inactivation of RNases H1 and H2 allows for direct transcript RNA repair of a DSB in homologous DNA, we conducted a complementation test in the cis system using a vector expressing either a catalytically inactive mutant of RNH201, rnh201(D39A),15 or wild-type RNH201. Results showed that when wild-type RNH201 was expressed from the plasmid in rnh1 rnh201 sp3 cells, there were no His+ colonies following DSB induction (Extended Data Fig. 2a). Deletion of SPT3 is a well-established and robust method to suppress reverse transcription

Table 1 | Frequencies of cDNA and transcript-RNA-templated DSB repair in trans and cis systems

| Genotype | His+ freq. | Survival | Genotype | His+ freq. | Survival |
|----------|------------|---------|----------|------------|---------|
| a | | | b | | |
| Wild type | 12,300 (10,000–14,600) | 1.1% | Wild type | 1,640 (1,200–1,850) | 1% |
| rnh1 | 33,100 (30,400–24,200) | 0.7% | rnh1 | 5,700 (4,870–7,080) | 0.1% |
| rnh1 rnh201 sp3 | 20,610 (17,100–23,900) | 0.8% | rnh1 rnh201 sp3 | 74,600 (64,900–84,000) | 0.6% |
| rnh1 rnh201 | 74,600 (64,900–84,000) | 0.6% |
| rnh1 rnh201 rad52 | 1,520 (970–2,580) | 0.1% |

*Cells with the sp3-null allele have higher survival than wild-type SPT3 cells after DSB induction because they spend more time in G2 (see Extended Data Fig. 2c).
and formation of cDNA in yeast. However, to prove that the increased frequency of His\(^{+}\) detected in the cis- relative to the trans-system rnh1 rnh201 spt3 background was not solely linked to SPT3 deletion, we impaired cDNA formation by deleting the DBR1 gene, which codes for the RNA debranching enzyme Dbr1 (refs 16, 17), or by using the reverse transcriptase inhibitor foscarnet (phosphonoformic acid). Results shown in Fig. 1c and Extended Data Table 5a support our conclusion that RNA transcripts can directly repair a DSB in chromosomal DNA without being first reverse transcribed into cDNA in rnh1 rnh201 cells.

Efficient generation of His\(^{+}\) colonies in cis wild-type, rnh1 rnh201, or rnh1 rnh201 spt3 cells requires transcription and splicing of the antisense his3 and DSB formation in the his3 gene. Deletion of pGAL1 (the galactose-inducible promoter) upstream of his3 on chromosome III, deletion of the homothallic switching endonuclease gene, or growing cells in glucose medium, in which homothallic switching endonuclease is repressed, drastically decreased His\(^{+}\) frequency (Extended Data Fig. 2b, c and Extended Data Table 5b, c). Similarly, yeast wild-type, rnh1 rnh201 and rnh1 rnh201 spt3 cells of the cis system containing a 23-base-pair truncation of the artificial intron in his3 lacking the 5′ splice site (Extended Data Table 1 and Extended Data Fig. 1c) produced no His\(^{+}\) colonies following DSB induction (Fig. 1c and Extended Data Table 5d), yet these cells were efficiently repaired by HIS3.F and HIS3.R synthetic oligonucleotides indicating that the DSB occurred in these cells (Extended Data Table 3).

Next, to examine whether DSB repair frequencies at the his3 locus in the trans and cis systems correlate with the expression level of antisense his3 transcript, we performed quantitative real-time PCR (qPCR). The qPCR data showed that with increased time of incubation in galactose medium (from 0.25 to 8 h) the trans strains had significantly more his3 RNA than the cis strains in all backgrounds, including the rnh1 rnh201 spt3 strain. Furthermore, the levels of his3 transcript dropped significantly from 0.25 to 8 h in galactose in cis but not in trans strains, except for the cis strain in which the homothallic switching endonuclease gene was deleted (Extended Data Fig. 2d). These results are expected in the cis strains because as soon as the homothallic switching endonuclease DSB is made, a full his3 transcript cannot be generated. Therefore, these data corroborate the conclusion that the higher frequency of His\(^{+}\) colonies obtained in cis- than in trans-system rnh1 rnh201 spt3 cells (Fig. 1c and Table 1a) is not due to more abundant and/or more stable transcript but rather to the proximity of the transcript to the target DNA.

PCR analysis of ten random His\(^{+}\) colonies from each of the trans- and the cis-system rnh1 rnh201 spt3 backgrounds, and Southern blot analysis of three samples from each background showed that the his3 locus that was originally disrupted by the homothallic switching endonuclease site (trans background), or by the intron with the homothallic switching endonuclease site (cis background), was indeed corrected to an intact HIS3 sequence. No integration of the RNH1 or RNH201 background revealed that all 24 clones had the same precise sequence as the spliced antisense his3 transcript and did not present a typical end joining pattern with small insertion, deletion or substitution mutations (Extended Data Fig. 1c and Extended Data Table 2b). These results, together with our observation of no His\(^{-}\) colony formation in cells unable to splice the intron in his3 (Fig. 1e and Extended Data Table 5d), strongly support a homologous recombination mechanism of DSB repair by transcript RNA in cis-system rnh1 rnh201 spt3 cells.

Figure 2  Transcripttemplated DSB repair follows a homologous recombination mechanism. a, Southern blot analysis of yeast genomic DNA derived from trans wild-type His\(^{+}\) (lane 2) or His\(^{-}\) (lane 3), rnh1 rnh201 spt3 His\(^{+}\) (lane 4) or His\(^{-}\) (lanes 5–7) cells, digested with BamHI restriction enzyme and hybridized with the HIS3 probe, or derived from cis wild-type His\(^{+}\) (lane 8) or His\(^{-}\) (lane 9), rnh1 rnh201 spt3 His\(^{+}\) (lane 10) or His\(^{-}\) (lanes 11–13) cells, digested with Nael restriction enzyme and hybridized with the HIS3 probe (Extended Data Fig. 4a, c). Lanes 1 and 14, 1-kilobase DNA ladder visible in the ethidium-bromide-stained gel (Extended Data Fig. 4b).

Previous studies showed the ability of Escherichia coli RecA to promote pairing between duplex DNA and single-strand RNA in vitro. Recent work suggests that Rad51 (the homologous protein to bacterial RecA) can promote pairing of RNA–DNA hybrids in yeast. Here we show that transcript–RNA-directed chromosomal DNA repair is stimulated by the function of Rad52 but not Rad51 recombination protein. Rad52 is important for homologous recombination both via single-strand annealing and via strand invasion. DSB repair by transcript RNA was reduced over 14-fold in cis-system rnh1 rnh201 spt3 rnh201 but was increased by a factor of 4 in cis-system rnh1 rnh201 spt3 rad51 compared to rnh1 rnh201 spt3 cells (Table 1b). Notably, our in vitro experiments demonstrate that both yeast and human Rad52 efficiently promote annealing of RNA to a DSB-like DNA end (Fig. 2b–d and Extended Data Fig. 4d–h). Importantly, Rad52 catalyses the reaction with RNA at nearly the same rate as the reaction with single-stranded DNA (ssDNA) of the same sequence. Moreover, in our experiments replication protein A (RPA), a ubiquitous ssDNA binding protein, caused a moderate inhibition of Rad52-promoted annealing between complementary ssDNA molecules, but not between ssRNA and ssDNA molecules. Thus, in the presence of RPA, the annealing between ssRNA and ssDNA proceeded with higher efficiency than the reaction between ssDNA molecules (Fig. 2b–d and Extended Data Fig. 4d–g).

In vivo, cDNA and/or RNA-dependent DSB repair may be especially important in the absence of functional Rad51 that prevents repair by the...
uncut sister chromatid via strand invasion\(^2\). Indeed, our results show that deletion of RAD51 increases the frequency of repair by cDNA and/or RNA (Table 1b). Hence, considering the bias observed for DSBR repair in cis versus trans systems when Ty reverse transcription was impaired, we propose a model that in the absence of H-type RNase function, transcript RNA mediates DSBR repair preferentially in cis systems via a Rad52-facilitated annealing mechanism. In this mechanism, the transcript may provide a template that either bridges broken DNA ends to facilitate precise re-ligation or initiate single-strand annealing via a reverse-transcriptase-dependent extension of the broken DNA ends (Fig. 3). The reverse transcriptase activity could be provided by a replicative DNA polymerase\(^2\), minimal Ty reverse transcriptase, or both. The current view in the field is that RNA–DNA hybrids formed by the annealing of transcript RNA with complementary chromosomal DNA either in cis or in trans systems are mainly a cause of DNA breaks, DNA damage and genome instability\(^2\). Here we demonstrate that under genotoxic stress, transcript RNA is recombinogenic and can efficiently and precisely template DNA repair in the absence of H-type RNase function in yeast. In the central dogma of molecular biology, the transfer of genetic information from RNA to DNA is considered to be a special condition, which has been restricted to retro-element\(^2\)s and telomeres\(^1\). Our data show that the transfer of genetic information from RNA to DNA occurs with an endogenous generic transcript (his3 antisense), and is thus a more general phenomenon than previously anticipated. In addition, in vitro RNA–DNA annealing was markedly promoted not only by yeast but also human RAD52, suggesting that transcript–RNA-templated DNA repair could occur in human cells. RNA transcripts could template DNA damage repair at highly transcribed loci, in cells that do not divide (lack sister chromatids), or have more stable RNA–DNA heteroduplexes, like those defective in RNASEH2 patients with Aicardi–Goutié`res syndrome\(^2\). Our findings lay the groundwork for future exploration of RNA-driven DNA recombination and repair in different cell types.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Experimental design to explore transcript-RNA-templated chromosomal DSB repair in yeast. In the experimental design to explore transcript-RNA-templated chromosomal DSB repair it is critical to discriminate repair of the DSB by transcript RNA from repair by the DNA region that generates the transcript. Also, translation of the repairing transcript mRNA should not produce the functional His3 protein. Moreover, it is essential that DSB repair would not restore the His3 marker sequence by simple end ligation via non-homologous end-joining (NHEJ). To satisfy these requirements, the DNA region that generates the transcript was constructed to contain a his3 allele on chromosome III consisting of a yeast HIS3 gene interrupted by an artificial intron in the antisense orientation (mhis3-AI cassette), which was previously used to study reverse transcription in yeast17,18. The antisense his3 RNA is not translated into the functional His3 protein. Moreover, after intron splicing, the transcript RNA sequence has no intron, while the DNA region that generates the transcript retains the intron; thus they are distinguishable. We developed two experimental yeast cell systems, trans and cis (Fig. 1a, b and Extended Data Fig. 1) in strains YS-289, 290 and YS-291, 292, respectively (Extended Data Table 1). In both systems, transcription of the antisense his3 RNA and expression of the homothallic switching endonuclease are regulated by the galactose-inducible promoter (pGAL1). In addition, these yeast cell systems are auxotrophic for histidine (HisΔ) and thus do not grow on media without histidine. Upon induction of the homothallic switching endonuclease by galactose, the broken his3 allele of the trans and cis cell systems can, in principle, only be repaired to a functional HIS3 allele by recombination with a homologous template. Alternative mechanism of HIS3 repair by ligation of the broken ends via NHEJ is inefficient in this system (<0.1 out of 10^7 viable cells) (data not shown), as the HIS3 gene is disrupted by a long sequence with the homothallic switching endonuclease site (trans system) or an intron and the homothallic switching endonuclease site (cis system) (Extended Data Fig. 1b, c).

To impair DSB repair by cDNA deriving from the his3 antisense, we deleted the SPT3 or the DBR1 gene. SPT3 encodes for a subunit of the SAGA and SAGA-like transcriptional regulatory complexes and its null allele reduces Ty reverse transcriptase function under 100-fold8,9. DBR1 encodes for the RNA debranching enzyme Db1 and its null allele in yeast cells impairs cDNA formation and diminishes Ty transposition up to a factor of tenfold10,11. As further proof that we can detect DSB repair by transcript RNA independently of cDNA, we performed the trans and cis assays with and without RNase H functions in the presence of foscarine (phosphonofor- matic acid, PFA), an inhibitor of the HIV reverse transcriptase, which blocks Ty reverse transcription in yeast7 (and data not shown).

Yeast strains. The yeast strains used in this work are listed in Extended Data Table 1 and derive from the FRO-767 strain7, which contains the site for the site-specific homothallic switching endonuclease in the middle of the LEU2 gene on chromosome III. A gene cassette based on plasmid pSM50 (refs 28, 29) containing the his3 gene disrupted by an artificial intron and regulated in the antisense orientation by the galactose inducible promoter pGAL1 and containing the URA3 marker gene (pGAL1-mhis3-AI-URA3) was integrated into the leu2 locus of strain FRO-767 after DSB induction at the homothallic switching endonuclease site by the gene cassette in both the trans and cis cell systems can, in principle, only be repaired to a functional HIS3 allele by recombination with a homologous template. Alternative mechanism of HIS3 repair by ligation of the broken ends via NHEJ is inefficient in this system (<0.1 out of 10^7 viable cells) (data not shown), as the HIS3 gene is disrupted by a long sequence with the homothallic switching endonuclease site (trans system) or an intron and the homothallic switching endonuclease site (cis system) (Extended Data Fig. 1b, c).

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As a control, cells were also replica-plated from the SC-Ura media onto SC-His media. The survival was calculated by dividing the number of colonies on SC-His plates by the number of cells plated on the same medium. For experiments in liquid culture, flasks with 50 ml of liquid medium containing yeast extract, peptone and 2% (w/v) galactose (YPGal) or YPGal containing phosphonoformic acid (PFA, 2.5 mg ml^-1) to turn on transcription of the his3 antisense on chromosome III and expression of the homothallic switching endonuclease. As a control, cells were also replica-plated from the YPD medium on synthetic complete medium plates lacking histidine (SC-HisΔ) and grown for 3 days at 30 °C. We never detected a single HisΔ colony from any of the trans and cis strains used in this study following replica-plating from the YPD medium on SC-HisΔ (not shown). After 2 days' incubation on YPGal medium, these cells were replica-plated onto SC-HisΔ and grown for 3 days at 30 °C to form visible colonies. At this stage, plates were replica-streaked and plated on Southern blot experiments using the BDG606 and BDG283 plasmids, cells were replica-plated from SC-Ura onto SC-Ura-Gal medium, and were then replica-plated onto SC-Ura-HisΔ. As a control, cells were also replica-plated from the SC-Ura-Gal medium onto SC-Ura-HisΔ and grown for 3 days at 30 °C. For the experiments in liquid culture, flasks with 50 ml of liquid medium containing yeast extract, peptone and 2.7% (v/v) lactic acid (YPLat) were inoculated with yeast cells of the chosen strains and incubated in a 30 °C shaker for 24 h. The density of the cultures was determined by counting cells using a hemocytometer and counting under a microscope. Generally, 10^6 or, in rare cases, 10^7 cells (we note that survival is very low on galactose medium) were then plated on YPGal medium, or 5 μL containing 10^5 cells for experiments using PFA to obtain colonies from 1 to 500 HisΔ colonies per plate after the replica-plating on HisΔ medium, and grown for 2 days at 30 °C. Two aliquots of 10^5 cells were plated, each on one YPGal medium plate, or YPGal medium containing PFA (2.5 mg ml^-1) for experiments using PFA to plate, to measure the cell survival after galactose treatment. After 2 days' incubation on YPGal medium, cells were replica-plated on HisΔ plates and grown for 3 days at 30 °C. The frequency of DSB repair was calculated by dividing the number of HisΔ colonies on SC-HisΔ by the number of colonies on YPGal medium. The survival was calculated by dividing the number of colonies on YPGal medium by the number of cells plated on the same medium. For experiments using the BDG606 and BDG283 plasmids, cells were treated as described above except that they were plated on YPLac on SC-Ura-Gal medium in different dilutions, and were then replica-plated on SC-Ura-HisΔ. The frequency of HisΔ colonies was calculated by dividing the number of HisΔ colonies on SC-Ura-HisΔ by the number of colonies on SC-Ura-Gal medium. The survival was calculated by dividing the number of colonies on SC-Ura-Gal medium by the number of cells plated on the same medium.
Oligonucleotide transformation. Transformation by oligonucleotides (1 nmol) was performed as described. Induction of the homothallic switching endonuclease DSB was done by incubating cells in 2% galactose medium for 3 h.

Transposition assay. Yeast cells of the chosen strains transformed with BDG102 (empty plasmid) or BDG598 (pGTy-H3mhis3-AI) plasmid (containing a Ty transposon fused to the his3 gene, which is in the antisense orientation and disrupted by an artificial intron; both Ty and the his3 antisense are regulated by the galactose-inducible promoter) were patched on SC-Ura and grown overnight at 30 °C. Cells were then replica-plated on synthetic medium lacking uracil with 2% (w/v) galactose (SC-Ura–Gal) and grown for 48 or 96 h at 30 °C or 22 °C, respectively. As control, cells were also replica-plated on SC-His to determine the background of His clones. After the incubation in galactose, cells were replica-plated on SC-His–Gal liquid medium or in 10 ml of YPLac liquid medium in a 30 °C shaker for 24 h. Then, 1 × 10^6 cells were transferred from the SC-Ura–liquid medium into 5 ml SC-Ura–Gal liquid medium and incubated for 48 or 96 h at 30 °C or 22 °C, respectively. After 24 h, YPlac cultures were split in half. One-half was kept growing for additional 48 h at 30 °C, while galactose was directly added to the other half to reach 2% and cells were then incubated for 48 h at 30 °C. From glucose and YPlac cultures grown at 22 °C or 30 °C, 10^6 or 10^8 cells were plated on SC-His–Ura medium, respectively, and were grown for 2 days at 30 °C. From galactose cultures grown at 22 °C or 30 °C, 10^8 or 10^10 cells were plated on SC-His–Ura medium, respectively, and were grown for 2 days at 30 °C. Two aliquots of 5 × 10^6 cells were plated each on one SC-Ura–medium plate, to measure the cell survival after glucose, YPlac or galactose treatment. The rate of formation of His+ cells was calculated using the maximum-likelihood method described in ref. 37.

Quantitative real-time PCR. RNA was isolated from the chosen yeast strains of the trans and cis systems using a protocol adapted from a method described previously. RNA was converted to cDNA using QuantiTect Reverse Transcription Kit (Qiagen). SYBR Green qPCR Mix (BioRad) was used for analysing RNA expression in 96-well plates (Applied Biosystems). The total volume in each well was 20 μl, which consisted of 10 μl of SYBR Green qPCR Mix, 4 μl of nuclease-free water, 2 μl of primers and 4 μl of cDNA. The cDNA levels were determined using an ABI Prism 7000 RT–PCR machine (Applied Biosystems). ACT1.F and ACT1.R, HIS3.F2 and HIS3.R2 primers were used in this study (Extended Data Table 2a). ACT1 primers were used for normalization. Values for each sample were normalized with ACT1, and then a second normalization was performed by subtracting normalized values of each time point from the control normalized value per each gene. As a negative control, CEN16.F and CEN16.R primers were used to show that there is minimal or no qPCR product derived from a chromosomal region that is not transcribed (A. El Hage, personal communication) (data not shown).

Rad52 in vitro annealing assay. In vitro assays using yeast or human Rad52 were performed as described (31) (and references therein), with all DNA and RNA concentrations expressed in moles of molecules. All oligonucleotide sequences (no. 211, no. 501, no. 508 and no. 509) are shown in Extended Data Table 2a. A single nucleotide mismatch was incorporated into the dsDNA (relative to ssDNA or RNA) to reduce the spontaneous Rad52-independent annealing. Tailed dsDNA (no. 508 and no. 509) (0.4 nm) was incubated in the absence or presence of yeast or human RPA (2 nm) in a buffer containing 25 mM Tris acetate, pH 7.5, 100 μg ml⁻¹ BSA, and 1 mM DTT (dithiothreitol) for 5 min at 37 °C. Then yeast or human Rad52 (1.35 nm) was added to the mixture containing either yeast or human RPA, respectively, and incubation continued for 10 min. Annealing reactions were initiated by adding 32P-labelled ssRNA (no. 501) or ssDNA (no. 211) (0.3 nM). Aliquots were withdrawn at indicated time points and deproteinized by incubating samples in stop solution containing 1.5% SDS, 1.4 mg ml⁻¹ proteinase K, 7% glycerol and 0.1% bromophenol blue for 15 min at 37 °C. Samples were analysed by electrophoresis in 10% (17:1 acrylamide:bisacrylamide) polyacrylamide gels in 1X TBE (90 mM Tris-borate, pH 8.0, 2 mM EDTA) at 150 V for 1 h and were quantified using a Storm 840 Phosphorimager and ImageQuant 5.2 software (GE Healthcare).

Data presentation and statistics. Graphs were made using GraphPad Prism 5 (Graphpad Software). The results are each expressed as a median and 95% confidence interval (in brackets), or alternatively the range when number of repeated experiments was <6. Statistically significant differences between the His+ frequencies were calculated using the nonparametric two-tailed Mann–Whitney U-test. All P values obtained using the Mann–Whitney U-test were then adjusted by applying the false discovery rate method to correct for multiple hypothesis testing (Supplementary Table 1).

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Extended Data Figure 1 | DNA sequence of the his3 loci in the trans and cis systems. 

a, Trans system on chromosome (Chr) III. HIS3 ATG and STOP codons are boxed. The HIS3 gene is disrupted by an insert (orange) carrying the artificial intron (AI). The consensus sequences of the AI are boxed.

b, Trans system on chromosome XV. HIS3 ATG and STOP codons are boxed. The HIS3 gene is disrupted by an insert (yellow) containing the 124-base-pair homothallic switching endonuclease site (marked by lines).

c, Cis system on Chr III. HIS3 ATG and STOP codons are shown. The HIS3 gene is disrupted by an insert (orange) carrying the AI, which contains the 124 base pairs of the homothallic switching endonuclease site (yellow and marked by lines). The consensus sequences of the AI are boxed. *23-base-pair deletion of the AI, including the 5' splice site, made in some strains.
a) WT

b) WT

| Genotype          | cis 0 h GAL | cis 8 h GAL|
|-------------------|-------------|-------------|
|                   | G1 | S | G2 | G1 | S | G2 |
| WT                | 75 | 13 | 12 | 24 | 11 | 65 |
| rnh1 mh201        | 74 | 14 | 12 | 23 | 11 | 66 |
| rnh1 mh201 spt3   | 66 | 5  | 29 | 35 | 7  | 58 |
| pGAL1Δ            | 83 | 7  | 10 | 27 | 7  | 66 |
| rnh1 mh201 pGAL1Δ | 79 | 6  | 15 | 31 | 6  | 63 |
| rnh1 mh201 spt3 pGAL1Δ | 73 | 5  | 22 | 28 | 7  | 65 |
| hoΔ               | 90 | 5  | 5  | 69 | 14 | 16 |
| rnh1 mh201 hoΔ    | 91 | 4  | 5  | 66 | 19 | 15 |
| rnh1 mh201 spt3 hoΔ | 70 | 9  | 21 | 59 | 14 | 27 |

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(d) Relative amount of his3 RNA (fold change)
Extended Data Figure 2 | Efficient transcript-RNA-directed gene modification is inhibited by RNH201, requires transcription of the template RNA and formation of a DSB in the target gene. a, Complementation of rnh201 defect suppresses transcript-RNA-templated DSB repair in cis-system rnh1 rnh201 spt3 cells. Wild-type (WT), spt3, rnh1 rnh201, rnh1 rnh201 spt3 strains of the cis system were transformed by a control empty vector (YEp195spGAL-EMPTY), a vector expressing catalytically inactive form of RNase H2 (YEp195spGAL-rnh201-D39A) or a wild-type form of RNase H2 (YEp195spGAL-RNH201). All the vectors have the galactose-inducible promoter. Shown is an example of replica-plating results (n = 6) from galactose medium to histidine dropout for the indicated strains and plasmids. b, Example of replica-plating results (n = 6) from galactose medium to histidine dropout for the indicated strains of the cis system, which have functional pGAL1 promoter and homothallic switching endonuclease (HO) gene, or have deleted pGAL1 promoter (pGAL1Δ), or deleted HO gene (hoΔ). c, Table with percentages of cells in the G1, S or G2 stage of the cell cycle out of 200 random cells counted for the indicated strains of the cis system after 0 h and 8 h from galactose induction. If a homothallic switching endonuclease DSB is made in his3, yeast cells arrest in G2, thus a high percentage of G2-arrested cells indicates occurrence of the homothallic switching endonuclease DSB. We also note that strains with spt3 mutation have a higher percentage of G2 cells than strains with wild-type SPT3 before DSB induction (0 h GAL). d, Results of qPCR of his3 RNA. Cells were grown in YPLac liquid medium O/N, and were collected and prepared for qPCR at 0, 0.25 or 8 h after adding galactose to the medium. Trans, blue bars; cis, red bars. Data are represented as a fold change value with respect to mRNA expression at time zero, as median and range of 6–8 repeats. The significance of comparisons between fold changes obtained at 0.25 h versus those obtained at 8 h, fold changes of different strains of the trans and cis systems, and between fold changes obtained in the trans versus cis system for the same strains at the same time point was calculated using the Mann–Whitney U-test and P-values are presented in Supplementary Table 1j, II and III, respectively. We note that an apparent higher level of his3 RNA is detected at 8 h in galactose in both trans- and cis-system rnh1 rnh201 cells relative to the other tested genetic backgrounds. Our interpretation of these results is that his3 RNA could be more stable in rnh1 rnh201 cells if present in the form of RNA–DNA heteroduplexes, and this may explain the increased frequency of His+ colonies observed in both trans and cis in the rnh1 rnh201 cells (Fig. 1c and Table 1a).
**Extended Data Figure 3 | Verification of his3 repair in trans- and cis-system rnh1 rnh201 spt3 cells via a homologous recombination mechanism using colony PCR.**

a. Scheme of the trans system before DSB induction (BDI, groups of lanes 1 and 7) and after DSB repair (ADR, groups of lanes 2–6 and 8–12) with the primers used in colony PCR shown as small black arrows and named with roman numerals: I, HIS3.5; II, HIS3.2; III, INTRON.F; IV, HO.F. The primer pairs used for colony PCR are named A (I + II), B (I + III) and C (I + IV), and base-pair sizes of the expected PCR products are shown in brackets. b. Photos of agarose gels with results of colony PCR reactions. M, 2-log DNA ladder marker; the 100-, 300- and 500-base-pair band sizes are indicated by arrows. Groups of lanes 1 and 7, two isolates of trans-system rnh1 rnh201 spt3 mutants before DSB induction, each tested with primer pairs A, B and C. Groups of lanes 2–6 and 8–12, ten isolates of trans-system rnh1 rnh201 spt3 mutants after DSB repair, each tested with primer pairs A, B and C.

d. Scheme of the cis system before DSB induction (BDI, groups of lanes 1 and 7) and after DSB repair (ADR, groups of lanes 2–6 and 8–12) with the primers used in colony PCR shown as small black arrows and named with roman numerals: I, HIS3.5; II, HIS3.2; III, INTRON.F; IV, HO.F. The primer pairs used for colony PCR are named A (I + II), B (I + III) and C (I + IV), and base-pair sizes of the expected PCR products are shown in brackets. d. Photos of agarose gels with results of colony PCR reactions. M, 2-log DNA ladder marker; the 100-, 300- and 500-base-pair band sizes are indicated by arrows. Groups of lanes 1 and 7, two isolates of cis-system rnh1 rnh201 spt3 mutants before DSB induction, each tested with primer pairs A, B and C. Groups of lanes 2–6 and 8–12, ten isolates of cis-system rnh1 rnh201 spt3 mutants after DSB repair, each tested with primer pairs A, B and C.
Yeast Rad52

DNA-DNA annealing

| Time, min | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Annealed product | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + |
| ssDNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

RNA-DNA annealing

| Time, min | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Annealed product | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + |
| ssRNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Truncated RNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Human RAD52

DNA-DNA annealing

| Time, min | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Annealed product | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ssDNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

RNA-DNA annealing

| Time, min | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|-----------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Annealed product | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| ssRNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Truncated RNA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

RNase - RNase +

ssDNA/ssRNA RNA fragment Degraded RNA

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Extended Data Figure 4 | RNA-templated DNA repair occurs via homologous recombination and requires Rad52. a, Scheme of the trans and cis his3/HIS3 loci in His− (before DSB induction) and His+ (after DSB repair) cells. The size of the BamHI (trans) or NalI (cis) restriction digestion products and the position of the HIS3 probe are shown. b, Photo of a ruler next to ethidium-bromide-stained agarose gel with marker and genomic DNA samples visible before Southern blot analysis. Lanes 1 and 14, 1-kilobase (kb) DNA ladder; 500-base-pair, 1-kb, 1.5-kb, 2-kb, 3-kb and 4-kb bands are indicated by arrows. Trans wild-type His− (lane 2) or His+ (lane 3), rnh1 rnh201 spt3 His− (lane 4) or His+ (lanes 5–7) cells, digested with BamHI restriction enzyme. Cis wild-type His− (lane 8) or His+ (lane 9), rnh1 rnh201 spt3 His− (lane 10) or His+ (lanes 11–13) cells, digested with NalI restriction enzyme. c, Southern blot analysis (same as in Fig. 2a, but displaying the entire picture of the exposed membrane) of yeast genomic DNA derived from trans wild-type His− (lane 2) or His+ (lane 3), rnh1 rnh201 spt3 His− (lane 4) or His+ (lanes 5–7) cells, digested with BamHI restriction enzyme and hybridized with the HIS3 probe, or derived from cis wild-type His− (lane 8) or His+ (lane 9), rnh1 rnh201 spt3 His− (lane 10) or His+ (lanes 11–13) cells, digested with NalI restriction enzyme and hybridized with the HIS3 probe. Lanes 1 and 14, 1-kb DNA ladder visible in the ethidium-bromide-stained gel (b). Sizes of digested DNA bands are indicated. The annealing reactions were promoted by either yeast Rad52 (d, e) or human RAD52 (f, g) (1.35 nM) in the presence or absence of RPA (2 nM) (yeast or human RPA was used in the reaction with yeast or human Rad52, respectively). In control protein-free reactions, protein dilution buffers were added instead of the respective proteins. dsDNA containing a protruding ssDNA tail (no. 508 and no. 509) was incubated with RPA (when indicated) and then Rad52 was added to the mixture. To initiate the annealing reactions, 0.3 nM 32P-labelled ssDNA (no. 211) or ssRNA (no. 501) were added. The reactions were carried out for the indicated periods of time, and the products of annealing reactions were deproteinized and analysed by electrophoresis in 10% polyacrylamide gels in 1× TBE at 150 V for 1 h. Visualization and quantification was accomplished using a Storm 840 Phosphorimager and ImageQuant 5.2 software (GE Healthcare). e, Treatment of RNA and DNA oligonucleotides with RNase. ssDNA (no. 211) or RNA (no. 501) (3 μM) was incubated with 100 μg ml−1 (or 7 U ml−1) RNase (Qiagen) in buffer containing 50 mM Hepes, pH 7.5 for 30 min at 37 °C, then 7% glycerol and 0.1% bromophenol blue were added to the samples and incubation continued for another 15 min at 37 °C before the samples were analysed by electrophoresis in a 10% (17:1 acrylamide: bisacrylamide) polyacrylamide gel at 150 V for 1 h in 1× TBE buffer. The gel was quantified using a Storm 840 Phosphorimager. The RNA oligonucleotide, but not the DNA oligonucleotide, is completely degraded by RNase.
## Extended Data Table 1 | Yeast strains used in this study

| Strain       | Relevant genotype                                                                 | Source                  |
|--------------|-----------------------------------------------------------------------------------|-------------------------|
| **YS-164, 165** | FRO-1075, 1080 (YS3::HOCs)            | this study              |
| **YS-166, 167** | FRO-1073 (YS3::CORE)       | this study              |
| **YS-172, 174 (cis)** | FRO-1075, 1080, YCLWTY2-1: CORE   | this study              |
| **YS-278, 281** | YS-172, 174 YCLWTY2-1: CORE       | this study              |
| **YS-289, 290 (trans) WT** | YS-275, 276 YCLWTY2-1: WT    | this study              |
| **YS-291, 292 (cis) WT** | YS-278, 281 YCLWTY2-1: WT | this study              |
| **YS-414, 415 (cis)** | YS-291, 292 YEp195S::KanMX4 | this study              |
| **YS-416, 417 (cis)** | YS-291, 292 YEp195SpGAL-1::KanMX4 | this study              |
| **YS-420, 421 (trans)** | YS-291, 292 YEp195SpGAL-1::KanMX4 | this study              |
| **YS-422, 423 (trans)** | YS-291, 292 YEp195SpGAL::KanMX4   | this study              |
| **YS-444, 445 (trans)** | YS-291, 292 YEp195SpGAL::KanMX4   | this study              |
| **YS-446, 447 (trans)** | YS-291, 292 YEp195SpGAL::KanMX4   | this study              |
| **HK-76, 77 (trans)** | YS-291, 292 YEp195SpGAL-1::KanMX4 | this study              |
| **HK-72, 73 (cis)** | YS-291, 292 YEp195SpGAL-1::KanMX4 | this study              |
| **YS-502, 503 (cis)** | YS-414, 415 YEp195SpGAL::KanMX4 | this study              |
| **YS-522, 524 (cis)** | YS-416, 417 YEp195SpGAL::KanMX4 | this study              |
| **YS-542, 543 (trans)** | YS-410, 411 YEp195SpGAL::KanMX4 | this study              |
| **YS-464, 465 (cis)** | YS-412, 413 YEp195SpGAL::KanMX4 | this study              |
| **YS-486, 487 (cis)** | YS-412, 413 YEp195SpGAL::KanMX4 | this study              |
| **YS-490, 491 (cis)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **YS-492, 493 (cis)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **HK-78, 79 (trans)** | YS-422, 423 YEp195SpGAL::KanMX4 | this study              |
| **HK-74, 75 (cis)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **HK-213, 215 (trans)** | YS-422, 423 YEp195SpGAL::KanMX4 | this study              |
| **HK-217, 219 (trans)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **HK-136, 137 (trans)** | YS-422, 423 YEp195SpGAL::KanMX4 | this study              |
| **HK-138, 139 (trans)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **HK-194, 197 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-180, 184 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-112, 113 (trans)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **YS-526, 527 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **YS-528, 529 (cis)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **YS-530, 531 (cis)** | YS-486, 487 YEp195SpGAL::KanMX4 | this study              |
| **YS-532, 533 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **YS-534, 535 (cis)** | YS-424, 426 YEp195SpGAL::KanMX4 | this study              |
| **YS-536, 537 (cis)** | YS-486, 487 YEp195SpGAL::KanMX4 | this study              |
| **HK-9, 10 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-11, 12 (trans)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **YS-13, 14 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **YS-15, 16 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-17, 18 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-19, 20 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-21, 22 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-23, 24 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-25, 26 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-27, 28 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-29, 30 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-31, 32 (cis)** | YS-440, 441 YEp195SpGAL::KanMX4 | this study              |
| **YS-301** | MATa his3::Leu2-3,520 lys2-800 ura3-520 trp1-1::GAL1001-2: G1017::A4 | this study              |
| **YS-305** | MATa his3::Leu2-3,520 lys2-800 ura3-520 trp1-1::GAL1001-2: G1017::A4 | this study              |
| **KK-72** | YS-305 YEp195SpGAL::KanMX4 | this study              |
| **TY-32, 52** | YS-301 + YEp195SpGAL::KanMX4 | this study              |
| **TY-17, 53** | YS-301 + YEp195SpGAL::KanMX4 | this study              |
| **TY-36, 66** | YS-301 + YEp195SpGAL::KanMX4 | this study              |
| **TY-22, 67** | YS-301 + YEp195SpGAL::KanMX4 | this study              |
| **HK-366, 388 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-382, 384 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-396, 400 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |
| **HK-391, 394 (cis)** | YS-291, 292 YEp195SpGAL::KanMX4 | this study              |

*S. cerevisiae* strains used in this study. Strains specifically used for the trans or cis assay are indicated. Ref. 44 is cited in this table.
Extended Data Table 2 | Oligonucleotides used in this study and sequence patterns of the HIS3 region repaired by transcript RNA or via non-homologous end-joining

| Name       | Size | Sequence                                                                 | Experiment                |
|------------|------|--------------------------------------------------------------------------|---------------------------|
| HIS3.F     | 80   | 5’ACCAATGCACTCAACGATTAGGACCCAGCGGGAATGCTTG GCGAGACTATGTACATATGCTGCCCAGAAGATCCTAG | Transformation            |
| HIS3.R     | 80   | 5’CAGGGTATAGGGTTTCTGGACCATATGATACATGCTCTGGC CAAAGCATTCGGCTGCTGATAATGAGATGACATTTGGAACAG | Transformation            |
| His3.F2    | 20   | 5’ CCTGTTCTGTCTCTGCCAAGCATTCCGGCTGGTCGCTAATCGTTGAGTGCATTTGGAACAG | qRT-PCR                  |
| His3.R2    | 20   | 5’ CGATCTCTTTTTAAAGGTTGGT | qRT-PCR                  |
| ACT1.F     | 20   | 5’ TGGGATTCGTTGCTGTTG | qRT-PCR                  |
| ACT1.R     | 20   | 5’ CGGCCAAATCGATTCTCAA | qRT-PCR                  |
| CEN16.F    | 20   | 5’ TGAGGAAAATTTGAACAG | qRT-PCR                  |
| CEN16.R    | 18   | 5’ CCGATTTCGCTTTTAGAAC | qRT-PCR                  |
| His3.2     | 20   | 5’ GAGAGCAATCCGCAGCTTTT | Colony PCR               |
| His3.5     | 20   | 5’ ATGACAGAGAGAGAGCCCT | Colony PCR               |
| HO.F       | 20   | 5’ AACCAGCTCTACAAACCAAA | Colony PCR               |
| INTRON.F   | 20   | 5’ GTATGTTTAATAGGACTAA | Colony PCR               |
| S3.1       | 20   | 5’ TTTATGGCCTTTTGATGAGGC | Southern blot           |
| S3.2       | 20   | 5’ CTACATGAAGACACCCTTG | Southern blot           |
| S3.3       | 20   | 5’ TTTGGCCCTTTTGATGAGGC | Southern blot           |
| S3.4       | 20   | 5’ TGGGCAAGGCTGCTTTTCTC | Southern blot           |
| 211        | 48   | 5’ GAAGCATTTATACGAGGTTTATGTCTCATGAGCAGCTACATATTGGAAT | Rad52 Annealing |
| 501        | 48   | 5’ GAAGCAUUUAAUCGGGUAAGGCAUGACAGCCGGAUAC AUUUUUGAAU | Rad52 Annealing |
| 508        | 53   | 5’ ATTCCATATGTTACACGCTTATGAGACAAATAATCCGCTGAAATGCTTTCATAG | Rad52 Annealing |
| 509        | 32   | 5’ TTATGTTCATCTGATACGGGATACATATTGGAAT | Rad52 Annealing |

A–C, patterns of the HIS3 region from spontaneous His
1− revertants. Among the 29 sequenced HIS3 regions, 25 displayed pattern A, 3 displayed pattern B and 1 displayed pattern C. The four bases inconsistent with the wild-type HIS3 affected two codons, causing a silent mutation (GCC → GCG: Ala → Ala) and a missense mutation (AAG → GTA: GTC or GTG: Lys → Val).

a, Name, size and sequence of the oligonucleotides used in this study are described. The specific experiments in which the oligonucleotides were used are indicated. b, Sequence patterns of the HIS3 region repaired by transcript RNA or via non-homologous end-joining. All 24 His− cis-system rnh1 rnh201 spt3 clons that were sequenced perfectly matched the wild-type HIS3 sequence. In contrast, when we examined the sequence of the rare His− clones that we could obtain (∼ 10 out of 107 viable cells) from a strain that had the homothallic switching endonuclease site in his3 on chromosome (Chr) XV (the construct is identical to that described in Extended Data Fig. 1b) and was rad52−/− (FDR-1092, 1093), 29 out of 29 His− samples had replaced 4 nucleotides (CAAG) of his3 next to the homothallic switching endonuclease site with a new sequence. Differences from the wild-type HIS3 gene are in bold. A–C, patterns of the HIS3 region from spontaneous His− revertants. Among the 29 sequenced HIS3 regions, 25 displayed pattern A, 3 displayed pattern B and 1 displayed pattern C. The four bases inconsistent with the wild-type HIS3 affected two codons, causing a silent mutation (GCC → GCG: Ala → Ala) and a missense mutation (AAG → GTA: GTC or GTG: Lys → Val).
Extended Data Table 3  | His\(^{+}\) frequency in the trans and cis systems following transformation by HIS3.F and HIS3.R oligonucleotides

| Genotype | trans | | cis |
|----------|-------|-------|-------|
|          | No Oligo | HIS3.F + HIS3.R | No Oligo | HIS3.F + HIS3.R |
| WT       | 2.3 (0-8) | 1.6x10\(^6\) (1.4x10\(^6\) -1.9x10\(^6\)) | <0.1 (0-0) | 1.5x10\(^6\) (946,000-2.3x10\(^6\)) |
| mh1 mh201 | 8 (0-56) | 1x10\(^6\) (1.1x10\(^5\) -1.9x10\(^6\)) | 165\(^*\) (63-275) | 845,500 (669,000-1x10\(^6\)) |
| mh1 mh201 spt3 | 1.7 (1-2) | 215,480 (196,000-235,000) | 49\(^*\) (25-78) | 225,300 (156,000-326,700) |
| mh1 mh201 spt3 AlΔ23 | ND | ND | <0.1 (0-0) | 798,370 (610,100-1x10\(^6\)) |

Frequency of His\(^{+}\) transformant colonies per 10\(^7\) viable cells for wild-type (WT), mh1 mh201, and mh1 mh201 spt3 mutant strains after transformation with HIS3.F and HIS3.R oligonucleotides in both trans and cis systems is shown as median and 95% confidence interval (in brackets). There were four or eight repeats for each of the strains transformed with these oligonucleotides. The significance of comparisons between the strains in the trans and the cis systems, and between different strains of the trans or cis system, that is between-group and within-group analysis, were calculated using the Mann–Whitney U-test (Supplementary Table 1d). The strains used in this experiment were YS-289, YS-290, YS-291, YS-292, YS-422, YS-423, YS-424, YS-426, YS-476, YS-477, YS-486, YS-487 and HK-404, HK-407. ND, not determined.

\(^*\) We note that when the trans- and cis-system mh1 mh201 or mh1 mh201 spt3 strains were transformed using exogenous HIS3.F and HIS3.R synthetic oligonucleotides following DSB induction, the frequencies of His\(^{+}\) colonies were similar to each other in the trans- and cis-system mh1 mh201 or mh1 mh201 spt3 cells. In contrast, when no oligonucleotides were added, the few His\(^{+}\) colonies were 20- to 28-fold more numerous in cis- than in trans-system mh1 mh201 or mh1 mh201 spt3 cells, respectively, probably originating from DSB repair by the his3 antisense transcript.
### Extended Data Table 4 | His⁺ frequencies in the presence of plasmid BDG283 or BDG606 in cis strains

**Galactose**

| Genotype                        | Ura⁺His⁺ freq. | Survival |
|---------------------------------|----------------|----------|
| WT + BDG283                     | 36 (27-45)     | 9%       |
| WT + BDG606                     | 157,000 (143,020-193,000) | 9%       |
| *mnh1 mnh201 spt3 + BDG283*     | 820 (720-900)  | 25%      |
| *mnh1 mnh201 spt3 + BDG606*     | 815 (680-900)  | 25%      |

**Glucose**

| Genotype                        | Ura⁺His⁺ freq. | Survival |
|---------------------------------|----------------|----------|
| WT + BDG283                     | <0.01 (0-0)    | 56%      |
| WT + BDG606                     | <0.01 (0-0)    | 50%      |
| *mnh1 mnh201 spt3 + BDG283*     | 0.28 (0.04-0.45) | 93%      |
| *mnh1 mnh201 spt3 + BDG606*     | 8 (0-24)       | 80%      |

Frequencies of Ura⁺His⁺ colonies per 10⁷ viable cells for yeast strains of the cis cell system transformed with plasmid BDG283 or BDG606 following 48 h galactose or glucose treatment are shown as median and 95% confidence interval (in brackets). Percentage of cell survival after incubation in galactose or glucose is shown. There were six repeats for all the strains. The significance of comparisons between strains was calculated using the Mann–Whitney U-test (Supplementary Table 1e).
## Extended Data Table 5 | His⁺ frequencies for strains with dbr1-null, grown in the presence of PFA, with and without the pGAL1 promoter, grown in glucose, or containing the AIΔ23 intron truncation

**a** Frequencies of His⁺ colonies per 10⁷ viable cells for yeast strains of the trans and cis cell systems following 48 h galactose treatment are shown as median and 95% confidence interval (in brackets). Percentage of cell survival after incubation in galactose is also shown. Eighteen repeats for dbr1 (in trans), 6 repeats for dbr1; 6 repeats for mh201 dbr1, mh1 dbr1; 24 repeats for mh1 rh201 dbr1 and 4 repeats for PFA data. The significance of comparisons between strains was calculated using the Mann–Whitney U-test (Supplementary Table 1a).

| Genotype          | trans | cis |
|-------------------|-------|-----|
|                   | Hist⁺ freq. | Survival | Hist⁺ freq. | Survival |
| dbr1              | 1,330 (1,030-1,660) | 1.6% | 23 (0-47) | 2% |
| mh201 dbr1        | 2,130 (1,150-3,620) | 2.6% | 322 (122-453) | 3% |
| mh1 dbr1          | 2,455 (1,500-3,250) | 1.2% | 18 (0-78) | 2.5% |
| mh1 mh201 dbr1    | 7,420 (7,400-11,300) | 1.7% | 29,900 (26,900-33,200) | 1.2% |
| WT + PFA          | 519 (400-1,300) | 1.7% | 112 (94-380) | 0.9% |
| mh1 mh201 + PFA   | 4,120 (3,100-5,340) | 0.9% | 9,400 (7,290-20,800) | 0.7% |

**b** Frequencies of His⁺ colonies per 10⁷ viable cells for yeast strains of the cis cell system following 48 h galactose treatment are shown as median and 95% confidence interval (in brackets). Percentage of cell survival after incubation in galactose is also shown. There were 6 repeats for all the strains. The significance of comparisons between strains was calculated using the Mann–Whitney U-test (Supplementary Table 1f).

| Genotype          | cis |
|-------------------|-----|
|                   | Hist⁺ freq. | Survival |
| WT                | 1,050 (600-1,460) | 1% |
| mh1 rh201         | 62,100 (52,900-68,900) | 0.7% |
| mh1 mh201 spt3    | 5,100 (3,660-6,660) | 11% |
| pGAL1Δ            | <1 (0-0) | 0.4% |
| mh1 mh201 pGAL1Δ  | 540 (270-1,300) | 0.4% |
| mh1 mh201 spt3 pGAL1Δ | 630 (500-920) | 0.8% |

**c** Frequencies of His⁺ colonies per 10⁷ viable cells for the indicated yeast strains following 24-h glucose treatment in both the trans and the cis cell systems are shown as median and 95% confidence interval (in brackets). Percentage of cell survival after growth in glucose is also shown. There were 8 repeats for each of the strains. The significance of comparisons between the strains in the trans and cis cell systems was calculated using the Mann–Whitney U-test (Supplementary Table 1g).

| Genotype          | trans | cis |
|-------------------|-------|-----|
|                   | Hist⁺ freq. | Survival | Hist⁺ freq. | Survival |
| WT                | <0.01 (0-0) | 16% | <0.01 (0-0) | 19% |
| spt3              | <0.01 (0-0) | 96% | <0.01 (0-0) | 93% |
| dbr1              | <0.01 (0-0) | 33% | <0.01 (0-0) | 54% |
| rad52             | ND | ND | <0.01 (0-0) | 6% |
| rad51             | ND | ND | <0.01 (0-0) | 24% |
| pGAL1Δ            | ND | ND | <0.01 (0-0) | 67% |
| mh1 mh201         | 11 (5-25) | 18% | 21 (17-31) | 11% |
| mh1 mh201 spt3    | 4 (2-14) | 92% | 9 (0.3-16) | 76% |
| mh1 mh201 dbr1    | <0.01 (0-0) | 28% | 1.5 (0-6) | 34% |
| mh1 mh201 rad52   | ND | ND | <0.01 (0-0) | 23% |
| mh1 mh201 rad51   | ND | ND | <0.01 (0-0) | 17% |
| mh1 mh201 pGAL1Δ  | ND | ND | 0.9 (0-2) | 45% |
| mh1 mh201 spt3 rad52 | ND | 2 (0-5) | 26% |
| mh1 mh201 spt3 rad51 | ND | 2 (0-4) | 50% |
| mh1 mh201 spt3 pGAL1Δ | ND | ND | <0.01 (0-0) | 85% |

**d** Frequencies of His⁺ colonies per 10⁷ viable cells for yeast strains of the cis cell system following 48 h galactose treatment are shown as median and 95% confidence interval (in brackets). Percentage of cell survival after incubation in galactose is also shown. There were six repeats for all the strains. The significance of comparisons between strains was calculated using the Mann–Whitney U-test (Supplementary Table 1h).

| Genotype          | cis |
|-------------------|-----|
|                   | Hist⁺ freq. | Survival |
| WT                | 1,000 (840-1,240) | 2% |
| mh1 rh201         | 43,100 (37,500-47,000) | 1.7% |
| mh1 mh201 spt3   | 4,180 (3,310-5,550) | 21% |
| AIΔ23             | <0.1 (0-0) | 1.7% |
| mh1 mh201 AIΔ23  | <0.1 (0-0) | 1.7% |
| mh1 mh201 spt3 AIΔ23 | <0.1 (0-0) | 15% |
Extended Data Table 6 | His\(^+\) rates in wild-type and \textit{rnh1 rnh201} cells resulting from the transposition assay at 22 °C or 30 °C

| Temperature | Genotype                  | His\(^+\) rate (x10\(^{-7}\)) | Survival | His\(^+\) rate (x10\(^{-3}\)) | Survival |
|-------------|---------------------------|-------------------------------|----------|-------------------------------|----------|
| 22 °C       | **WT + BDG598**           | 5.28 (0 – 141)                | 26%      | 2.68 (2.55 – 3.06)            | 15%      |
|             | \textit{rnh1 rnh201} + BDG598 | 15.3 (16.3 – 42.4)           | 34%      | 0.78 (0.54 – 0.92)            | 27%      |
| 30 °C       | **WT + BDG598**           | 2.8* (0 – 7.37)               | 26%      | 0.58 (0.46 – 0.72)            | 15%      |
|             | \textit{rnh1 rnh201} + BDG598 | 16.1 (5.31 – 24.2)           | 34%      | 0.04 (0.03 – 0.06)            | 27%      |
| 30 °C       | **WT + BDG598**           | <0.1 (0 – 0)                  | 26%      | 1.38 (0.52 – 2.38)            | 15%      |
|             | \textit{rnh1 rnh201} + BDG598 | 15.1 (4.90 – 26.4)           | 34%      | 0.4 (0.30 – 0.60)             | 27%      |

Shown are rates of His\(^+\) colonies for wild-type (WT) and \textit{rnh1 rnh201} yeast strains containing BDG598 following growth with no galactose with plasmid selection (Ura\(^-\) medium) and without plasmid selection (YPLac medium) or galactose with plasmid selection (Ura\(^-\)Gal medium) and without plasmid selection (YPLac + gal medium) for 96 h at 22 °C, or for 48 h at 30 °C. Data are presented as median and 95% confidence interval (in brackets). Percentages of cell survival after growth without or with galactose are also shown. There were 15 repeats for the strains incubated at 22 °C and 6 repeats for those incubated at 30 °C. The significance of comparisons between strains was calculated using the Mann-Whitney U-test (Supplementary Table 1). The strains used in this experiment were TY-17, 53 and TY-22, 67.

*Average.