Supplementary Information

Fluorescent tagging of the Swi5, Sfr1, Swi2, Swi6 and Rhp51 proteins

An NdeI site was created by site-directed mutagenesis at the swi5, swi6 and rhp51 termination codons and at the sfr1 and swi2 initiation codons after the respective genomic DNA fragments were cloned into appropriate vectors. The EGFP cassette from pGEM-T-EGFP (Craven et al., 1998) was inserted at the NdeI sites of swi5, sfr1 and swi2. To create the ECFP cassette, an ECFP fragment was amplified from pECFP (Clontech) by PCR with the primer set ECFPfNde (5'-GGCGGCCATATGGTGAGCAAGGGCGAGGAGC-3') and ECFPrNde (5'-CCTCCTCATATGCTTGTACAGCTCGTCCCATGC-3') and inserted into pBluescript II SK (+) at the SmaI site. The ECFP cassette was verified by DNA sequencing and inserted at the swi6 and rhp51 NdeI site. The ura4+ marker was integrated into these plasmids. The plasmids were linearized at a unique restriction site in the cloned genomic DNA and transformed into S. pombe, and targeted integrants were selected. Strains in which ura4+ was looped out were isolated based on their resistance to 5-fluoroorotic acid (5-FOA). The correct EGFP- or ECFP-tagging of endogenous genes was confirmed by PCR and Southern blot analyses. For YA990 and YA1083 encoding direct repeats of rhp51-ecfp and rhp51+, the selection of 5-FOA resistant cells was omitted.

Fluorescence microscopy

Cells grown to exponential phase in a minimum media (EMM) containing appropriate supplements at 30 °C were observed. For observation of damage-induced foci, cells were washed twice with H2O before UV irradiation (100 J/m²). Cells were observed 3 h post irradiation with a Nikon Eclipse E800 microscope with a VFM epi-fluorescence attachment. A Nikon filter block, GFP (R)-BP, was used to detect the EGFP signal. To analyze co-localization, a yellow GFP HYQ filter (Nikon) was used to detect Swi5-EGFP, EGFP-Swi2 and EGFP-Sfr1, and a CYAN GFP filter (Nikon) was used to detect Swi6-ECFP and Rhp51-ECFP. For three-dimensional images, cells were grown to late log phase in EMM2 supplemented with appropriate amino acids and 0.1X YE, and washed with water. Cells were immobilized with lectin as described previously (Yamashita et al., 2005) and observed with a DeltaVision microscope system (Applied Precision Inc., Issaquah, WA) attached to an Olympus IX-70 fluorescence microscope equipped with a PlanApo x100 oil
objective lens (Olympus, Tokyo, Japan). YFP (excitation 500 ± 20nm; emission 535 ± 30nm) and CFP
(excitation 430 ± 25nm; emission 470 ± 30nm) filters were used for the EGFP and ECFP signals, respectively.
Images of 15 different focal planes at 0.3 μm were corrected, deconvolution was performed using
SoftWoRx™ 3.4 software, and projection images were reconstituted.

Supplementary references

the expression of tagged proteins in Schizosaccharomyces pombe. Gene, 221, 59-68.
mitotic cell division, meiotic nuclear oscillation, and cytokinesis checkpoint signaling. Mol Biol Cell, 16, 1378-1395.
Legends for Supplemental Figures

Supplemental Figure S1.

An rhp51-ecfp allele is recessive with respect to the repair of UV-induced damage. Wild type (YA119), crosses; rhp51-ECFP, rhp51+ (YA990), solid circles; rhp51Δ (T3), solid triangles; rhp51-ECFP (YA1023), open triangles.

Supplemental Figure S2.

Schema of genetic assay for HO-induced DSB repair. The Ch16-MG minichromosome, Chromosome III, centromeric regions (circles) and the complementary ade6-M216 and ade6-M210 heteroalleles (small vertical bars) are indicated. The cut site (black box) and kanMX6 (light grey) sequences are on Ch16-MG. Induction of HO endonuclease expression results in a DSB. Three phenotypes (ade+ G418-resistant (G418'), ade+ G418-sensitive (G418'), and ade+ G418') can arise following DSB repair. Most segregants with the ade+ G418' phenotype are predicted to arise by NHEJ. The DSB repair products of G418' segregants were determined by analyzing the chromosomes with pulse-field gel electrophoresis (PFGE). Among the ade+ G418' segregants, those in which the two chromosomes maintained their original length are classified as GC, those that have two 2 Mb chromosomes (visualized as two thick bands in gels) are classified as CO, and those have a fragment of the length expected for Chromosome III (3.5 Mb) and a larger minichromosome (2 Mb) are classified as non-reciprocal exchange type 1 (probably a product of break-induced replication (BIR)).

Among ade+ G418' segregants, those that have lost the minichromosome (minichromosome loss) are classified as DSB repair negative, those in which the two chromosomes maintained their original length are classified as long-tract gene conversion (LTGC), those that have two 2 Mb chromosomes (two thick bands in gels) are classified as two-marker gene conversion (GC) with crossing over (CO) and those that have a fragment of the length of Chromosome III (3.5 Mb) and a larger minichromosome (2 Mb) are classified as non-reciprocal exchange type 2 (probably a product of BIR).
Supplemental Figure S3.

Representative pulse-field gel images.

Supplemental Figure S4.

Sequence analysis reveals that LTGC segregants are ade6-M210 homozygous.

(A) Schematic diagram of ade6-M216 and ade6-M210 mutation sites. Mutations confirmed as described in (B) are represented in red. The sequences of primers used here were as follows: F, 5’-TTGCATTTCACAATGCTTGG-3’; R, 5’-TTTAACAGTTATGTCTATGG-3; r1, 5’-GAATAATTTTTCCAACCAAC-3; r2, 5’-GTCTTGAATGCATCGCAGAG-3.

(B) Identification of the ade6 mutations. Genomic DNA containing the ade6 region from each strain was amplified by PCR using F and R primers. DNA sequences were determined using with r1 and r2 as sequence primers. The mutation site of ade6-M216 was nucleotide at 46 (G to A), which is consistent with a previous report (Szankasi et al. (1988) JMB 204, 917-925). Since the precise mutation site of ade6-M210 was unknown, we first determined the whole nucleotide sequence of the allele and found that the mutation site is nucleotide 1466 (C to T). Sequence profiles of the diploid strain, which contains ade6-M210 and ade6-M216, exhibit mixed peaks both at nucleotides 46 and 1466 (right panel).

(C) Sequence analysis of strains containing Ch16-MG. The sequence profiles of HO-repressed YA1095, as well as its parental strain TH805, exhibit the mixed peaks at both ade6-M210 and ade6-M216 sites, indicating that they are ade6 heterozygous diploid (left two panels). On the other hand, LTGC candidates obtained from HO-induced YA1095 contained only the ade6-M210 mutation, but no ade6-M216 mutation. All of 10 LTGC candidates analyzed in this study gave the same results. Since PFGE revealed the existence of Ch16-MG without obvious size reduction (Supplemental Figure S3), these results suggest that the ade6 locus of Ch16-MG in ade- G418 segregation with 0.5 Mb and 3.5 Mb chromosome bands has been changed into ade6-M210 by the LTGC event.

Supplemental Figure S5.
A working model for the three mediators.

Three mediators (Rhp55/57, Swi5/Sfr1, or Swi5/Swi2) promote independently the formation of active Rhp51 nucleoprotein filaments, leading to the invasion of a single 3’-tail into a homologous double-strand DNA and the formation of a D-loop or single end invasion (SEI) intermediate. Rhp54 facilitates all three of the SEI reactions. Rhp51/55/57-mediated SEI occurs via one of two alternative pathways, sDSBR or SDSA. The SEI intermediate captures the second end by strand invasion or annealing (second end capture; SEC), which is mediated by Rhp51 and the Rhp55/57 heterodimer, and a double Holliday junction (dHJ) is formed. The directions of dHJ resolution determine the GC/CO production (sDSBR pathway), although mitotic HR repair is biased to GC over CO. If the 3’ end in the SEI intermediate is extended by DNA synthesis, the SDSA pathway is initiated to produce only GOs. Rhp51/Swi5/Sfr1-mediated SEI also occurs via one of two alternative pathways, sDSBR or SDSA. In the sDSBR pathway, SEC is mediated by Rhp51 and Rhp55/57, but not by Rhp51/Swi5/Sfr1. In the rhp57Δ mutant, the sDSBR pathway initiated from Swi5/Sfr1-SEI is “shut off”, leading to production of GC only. In MT switching, the initial unstable Rhp51 filament is stabilized when it encounters the Swi5/Sw2 mediator that localizes on donor loci. The Swi5/Sw2-mediated SEI is processed via an SDSA-like pathway to produce GC during the mating-type switching reaction. In the swi5Δ mutant, Swi5/Sw2-mediated SEI cannot operate, resulting in MT switching defect. However, in the swi5Δ mutant, Rhp51/Rhp55/Rhp57 pathway is fully functional, although Rhp51/Swi5/Sfr1 pathway is completely inactive, resulting the reduced levels of both GC and CO.