File S1

Supporting text

Details of the microarray analysis: Details of microarray analysis including sample preparation, hybridization conditions, and data analysis were described previously (St. Charles *et al.,* 2012; St. Charles and Petes, 2013). In summary, genomic DNA from strains with a gene conversion event and a control parental strain EY7 were extracted from purified colonies. The extracted DNA was sheared to 200-400 bp fragments. The DNA from the derivatives containing a gene conversion was labeled with the Cy5-dUTP fluorescent dye, while the control DNA was labeled with Cy3-dUTP. Experimental and control samples were mixed and competitively hybridized to SNP probes on a microarray with oligonucleotides derived from chromosome IV. The microarray was then washed and scanned at wavelengths of 635 and 532 nm, specific to Cy5-dUTP and Cy3-dUTP respectively, with a GenePix scanner.

For each probe at each SNP, the relative fluorescence hybridization ratio of experimental to control genomic DNA was measured. A ratio of one indicates heterozygosity (equal representation of both SNP alleles). If the normalized ratio of W303-1A to the control signal is below 0.3, and the ratio of YJM789 to the control is greater than 1.6, we interpret the SNP as homozygous for the YJM789-derived allele. The pattern will be reversed if the SNP is homozygous for W303-1A-derived allele.

Mapping conversion tract lengths by an alternative method, SPA (single-nucleotide polymorphism PCR analysis): The microarrays used for mapping do not contain all of the SNPs that distinguish the two homologs. For conversion events that had a breakpoint in a region sparsely represented by oligonucleotides on the microarray, we refined the mapping using a different method (Lee *et al.*, 2009). For this method, we identified SNPs that altered a restriction site in the region of interest. For example, at SGD coordinate 1028509, W303-1A strain has a T and YJM789 strain has a C. This alteration results in a *Ear*I site that is present in the YJM789 homolog that is absent in W303-1A. To monitor LOH at this position, we amplified the region from genomic DNA samples (usually derived from EY7 spore cultures) with primers that produced a fragment of about 580 bp, and treated the resulting product with *Ear*I. The digest was analyzed by electrophoresis to determine whether the strain contained the W303-1A-specific SNP (resulting in one fragment of about 580 bp) or the YJM789-specific SNP (resulting in two fragments of about 440 and 140 bp). In heterozygous diploid EY7 strains, we expect to see three fragments of about 580, 440, and 140 bp. The coordinates of the polymorphisms used in this analysis, the sequence of the primers, and the diagnostic restriction enzyme are in Table S3.

In our analysis, we found that a small fraction of the microarrays (about 10%) had no detectable LOH events. Subsequently, we first used SPA analysis for the polymorphic sites IV1013924 and IV 1011936 before doing microarrays. If both of these sites were heterozygous, we did not examine the samples by microarray. There were five such samples in the total collection of 59 conversion events.

Gene conversion tract measurements

Conversion tract lengths were determined using the transition coordinates summarized in Table S4. The *URA3* gene on chromosome IV was inserted as a 1140 bp fragment between coordinates 1013217 and 1013218. The SNPs that most closely flanked the insertion were at coordinates 1012642 and 1013370. The mutant substitution in *ura3-e* was located at base 170, about 1 kb from 1012642 and about 870 bp from 1013370. In most of the conversion events, both of these markers underwent LOH. In addition, most conversion events had only two transitions: transition "a" marks the centromere-proximal transition between heterozygous (left column) and homozygous (right column) SNPs, and transition "b" marks the centromere-distal transition between homozygous (left column) and heterozygous (right column) SNPs. For these derivatives, the homozygous region duplicates YJM789-derived SNPs. For these classes of conversion, we calculated the tract length by subtracting the mid-point of transition “a” from the mid-point of transition “b”, and adding 1140 bp (the length of the *URA3* insertion).

In conversion events in which one or more of the flanking markers 1012642 and 1013370 did not undergo LOH, the *ura3-e* mutation represented the SNP that had undergone LOH. For example, in EY7-8, neither of the flanking markers had lost heterozygosity. For this event, therefore, we calculated the conversion tract length as distance between the mid-points of the transition between *ura3-e* and 1012642 (transition “a”) and the transition between *ura3-e* and 1013370 (transition “b”).

The conversion events in EY7-63 and EY7-69 had more than two transitions. In EY7-63, there were four transitions: “a” (heterozygous SNPs to SNPs homozygous for YJM789-derived SNPs), “b” (homozygous for YJM789-derived SNPs to heterozygous SNPs), “c” (heterozygous SNPs to homozygous for YJM789-derived SNPs), and “d” (homozygous for YJM789-derived SNPs to heterozygous). The conversion tract length for this event was calculated as the distance from the mid-point of transition “a” to the mid-point between *ura3*-e and coordinate 1013370 (transition “d”). In EY7-69, there were also four transitions: “a” (heterozygous SNPs to SNPs homozygous for W303-1A-derived SNPs), “b” (homozygous for W303-1A-derived SNPs to heterozygous), “c” (heterozygous to homozygous for YJM789-derived SNPs), and “d” (homozygous for YJM789-derived SNPs to heterozygous). For this conversion event, we calculated the distance between the mid-points of transitions “a” and “d”, and added 1140 bp (the length of the *URA3* insertion).

In our previous studies of mitotic gene conversion, we found many other examples of complex conversion events similar to EY7-63 and EY7-69 (St. Charles *et al.*, 2012; St. Charles and Petes, 2013; Yin and Petes, 2013). Conversion events in which a conversion tract is interrupted by a heterozygous region (similar to EY7-63) can be explained by “patchy” repair of mismatches in a heteroduplex. More specifically, if mismatches in a middle region of a heteroduplex are repaired in the restoration mode and mismatches in the flanking region are repaired in the conversion mode, the observed pattern would be detected (Text S1 of St. Charles and Petes, 2013). Similarly, complex events in which LOH regions contributed by two different donating chromosomes (similar to EY7-69) have been observed previously. Although such events can reflect several different mechanisms, one possibility is that they reflect branch migration of the

Holliday junction resulting in symmetric heteroduplexes (Supporting Information in St. Charles *et al.*, 2012). Whatever the mechanisms that give rise to the complex conversion events, in the current study, these events are a small fraction of the total.

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