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Note

Cooperative Interactions Between Pairs of Homologous Chromatids During Meiosis in *Saccharomyces cerevisiae*

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ABSTRACT

We report a novel instance of negative interference during *Saccharomyces cerevisiae* meiosis, where Cre-mediated recombination between pairs of allelic *loxP* sites is more frequent than expected. We suggest that endogenous crossover recombination mediates cooperative pairing interactions between all four chromatids of a meiotic bivalent.

HOMOLOGOUS chromosomes pair in early meiotic prophase to ensure accurate segregation during anaphase I. Meiotic recombination plays an important role in homolog pairing for many organisms, including the budding yeast *Saccharomyces cerevisiae* (Page and Hawley 2003). The Cre/*loxP* site-specific recombination system provides a quantitative genetic probe of meiotic homolog pairing in living yeast cells (Peoples *et al.* 2002; Peoples-Holst and Burgess 2005; Lui *et al.* 2006). In an accompanying article, we show that crossover-associated meiotic recombination increases the probability of nearby Cre-mediated *loxP* recombination events ("collisions") between homologous chromatids (Mell *et al.* 2008, this issue). These data suggest that formation of stable joint molecule recombination intermediates between homologs brings *cis* allelic sequences into close proximity.

We asked whether Cre-mediated *loxP* recombination between allelic sites on homologous chromatids affects the frequency of *loxP* recombination between the two remaining chromatids. To analyze the segregation pattern of Cre/*loxP* crossovers in tetrads without resorting to tetrad dissection, we used the gene for green fluorescent protein (*GFP*) as a reporter of allelic *loxP* recombinants (Figure 1). One homologous chromosome bears a promoterless *loxP:gfp* contruct, while the other carries a reporterless *GPD1:loxP* contruct, bearing a promoter that is repressed during meiosis but constitutively expressed at other times. Cre-mediated recombination between allelic *loxP* sites places the *GFP* reporter behind the *GPD1* promoter for one chromatid. Expression from the recombined *GPD1:loxP:GFP* reporter is sufficiently robust to allow visualization of GFP in single cells by fluorescence microscopy. When diploids carrying the *GPD1:loxP:GFP* reporter were sporulated, the Gfp^+ phenotype was serendipitously spore autonomous: a *GPD1:loxP:GFP* heterozygote generated all 2:2 Gfp^+ asci ($n = 500$ asci), while a Gfp^+ homozygote produced all 4:0 Gfp^+ asci ($n = 500$ asci).

We analyzed four-spore asci from synchronized meiotic cultures of *GPD1:loxP/loxP:gfp* heterozygotes, in which Cre expression was induced at various time points after the start of sporulation (Figure 2; Table 1). The appearance of Gfp^+ spores was used to measure the frequency of Cre-mediated *loxP* recombination between homologous chromatids. We interpreted 0:4, 1:3, and 2:2 Gfp^+ asci as analogous to parental ditypes (P), tetratypes (T), and nonparental ditypes (N) as in a typical two-factor cross. We calculated the expected frequencies of 1:3 Gfp^+ (T) and 2:2 Gfp^+ (N) tetrads under the assumptions of Papazian (1952), using the "better" method to detect interference described in the Stahl Lab Online Tools at http://www.molbio.uoregon.edu/~fstahl/, which assumes a Poisson-distributed number of events per meiotic cell, and a random distribution of two-, three-, and four-strand double events (*i.e.*, "no chromatid interference"). Specifically, the appearance of 2:2 Gfp^+ asci (N) indicates the occurrence of either a prereplicative Cre/*loxP* collision or a four-strand double-*loxP* collision involving all four chromatids of a meiotic bivalent (Figure 1).

When Cre recombinase was induced early in meiotic prophase I, we observed an approximately fourfold

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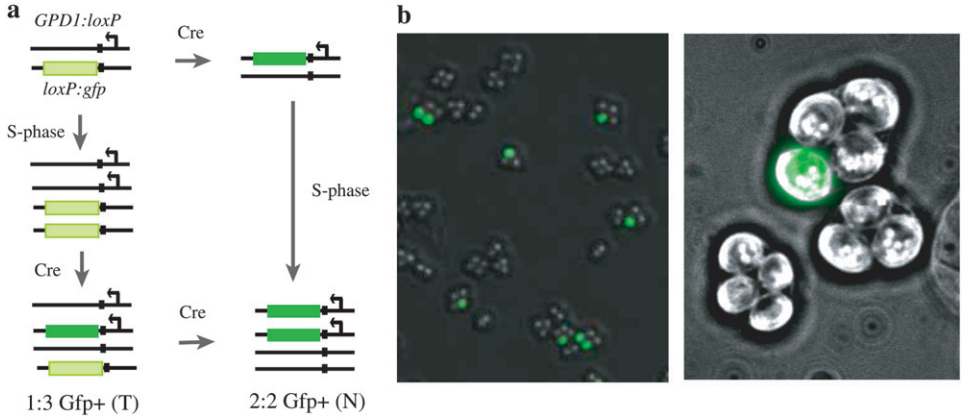

Figure 1.—Fluorescent tetrad analysis of allelic *loxP* collisions. (a) A schematic is shown for the Cre-mediated allelic *loxP* recombination system, where the frequency of reporter activation ("collision") is proportional to the physical proximity of the two interacting *loxP* sites (Hildebrandt and Cozzarelli 1995; Burgess and Kleckner 1999). The *loxP* site has been incorporated into the *ACT1* intron to allow for sufficient expression of the GFP protein to visualize the Gfp^+ phenotype by fluorescence microscopy (plasmid and strain construction details available upon request). Presumably, expression from the *GPD1* promoter in spores and the removal of the inverted repeat *loxP* site from the 5′-UTR of *GFP* mRNA by splicing allow for robust expression of *GFP* (K. Komachi and O. Hughes, unpublished data). Two paths are shown for generating 2:2 Gfp^+ (N) tetrads, with Cre-mediated recombination occurring either before or after meiotic S-phase. (b) Two fields of asci sporulated with 0.03% galactose (which activates expression of Cre recombinase) added at $t = 1$ hr are shown. On the left, 1:3 Gfp^+ (T) and 2:2 Gfp^+ (N) four-spore asci are visible. On the right, a single tetrad showing 1:3 Gfp^+ (T) segregation is shown at higher magnification.

excess of 2:2 Gfp^+ tetrads, indicative of "negative interference" (Figure 2; Table 1). When Cre was induced later in meiotic prophase I, there was an even stronger excess of doublet Gfp^+ tetrads above that expected (Figure 2; Table 1). These results rule out the possibility that the excess four-strand double-*loxP* recombinants are due to Cre-mediated *loxP* recombination occurring at the two-strand stage (prior to DNA replication of the *loxP* sequences), since in this case we would expect negative interference to disappear when Cre is induced at later time points, as more of the culture transits meiotic S-phase.

These data show that the sister chromatids behave cooperatively, such that allelic *loxP* double crossovers involving all four chromatids occur more often than expected during late prophase I. This is in contrast to positive interference observed for Spo11p-initiated crossovers, where the frequency of nearby double crossovers is less than random expectations in wild-type yeast (Bishop and Zickler 2004).

Using a combination of endogenous and Cre/*loxP* recombination reporters, we have found that crossover-bound meiotic recombination intermediates increase interactions between *loxP* sites located on homologous chromatids (Mell *et al.* 2008). The results of the tetrad analysis presented here further suggest a constrained geometry of all four chromatids in a bivalent around these sites. We speculate that "synapsis initiation complexes" (Fung *et al.* 2004) stabilize local homologous chromatid interactions and also constrain interactions between the remaining two chromatids. Endogenous meiotic crossing over at the DNA level is coordinated with exchange between "homologous" axial elements, which involves both sister chromatids of a chromosome (Blat *et al.* 2002); thus the negative interference observed in Cre/*loxP* recombination may reflect this

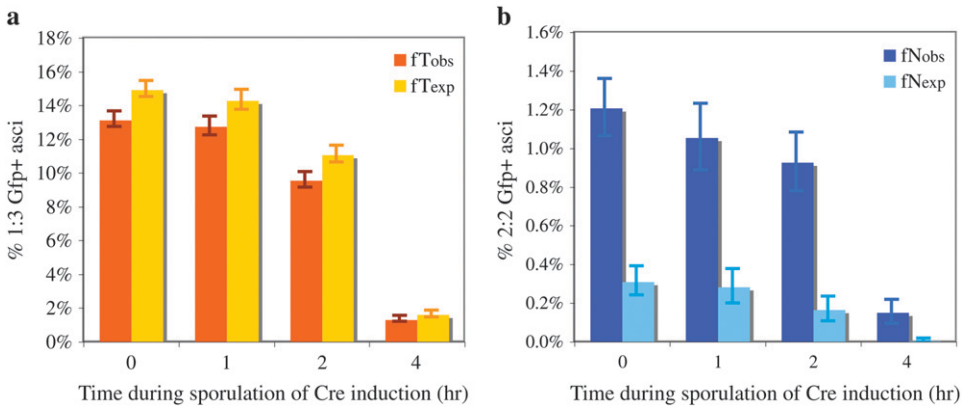

Figure 2.—Nonrandom segregation of Gfp^+ in tetrads. The observed and expected frequencies of (a) 1:3 Gfp^+ and (b) 2:2 Gfp^+ are shown (indicating fT and fN, respectively). For expected values, the assumptions of Papazian (1952) and the method described at the Stahl Lab Online Tools were used: $fT_{exp} = \frac{2}{3}(1 - e^{-3X})$ and $fN_{exp} = \frac{1}{2}[(1 - e^{-2X}) - fT_{exp}]$, where $X = \frac{1}{2}\ln(1 - 2R)$ and $R = fN_{obs} + fT_{obs}/2$. (a) The observed and expected frequency of 1:3 Gfp^+ tetrads (fT_{obs} and fT_{exp}) for different Cre induction times is illustrated. (b) The observed and expected frequency of 2:2 Gfp^+ tetrads (fN_{obs} and fN_{exp}) is illustrated. Estimated standard error was used to size the error bars.

TABLE 1

Segregation pattern of Gfp$^+$ allelic *loxP* recombinants in tetrads

Gal+ (hr)	Count of tetrad classes				
	0:4 Gfp$^+$	1:3 Gfp$^+$	2:2 Gfp$^+$	Total	N_{obs}/N_{exp}
0	4683	718	66	5467	3.9
1	3025	447	37	3509	3.7
2	3574	381	37	3992	5.6
4	3915	52	6	3973	46.4

Cre recombinase was induced with 0.03% galactose in synchronized meiotic cultures at the time point indicated, as described in Peoples *et al.* (2002). Unambiguous four-spore tetrads were evaluated for the number of Gfp$^+$ spores by fluorescence light microscopy (Figure 1). The N_{obs}/N_{exp} ratio was determined as the ratio of observed to expected 2:2 Gfp$^+$ tetrads under the assumptions of Papazian (1952) as described in Figure 2. Using the χ^2 method found at the Stahl Lab Online Tools, *P*-values for all time points were $\ll 0.001$, indicating a significant deviation from random expectations.

coupling between the sisters. Finally, the spore-autonomous segregation of Gfp$^+$ using the reporter constructs described here may offer a useful tool for studies of meiotic recombination. Adding different fluorescent markers to linked positions in the genome will provide a rapid method of tetrad analysis that does not require tetrad dissection, similar to the visual tetrad analysis assay developed for Arabidopsis reported by Francis *et al.* (2007).

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