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The Meiotic Bouquet Promotes Homolog Interactions and Restricts Ectopic Recombination in *Schizosaccharomyces pombe*

Luther Davis and Gerald R. Smith[1]

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024

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ABSTRACT

Chromosome architecture undergoes extensive, programmed changes as cells enter meiosis. A highly conserved change is the clustering of telomeres at the nuclear periphery to form the "bouquet" configuration. In the fission yeast *Schizosaccharomyces pombe* the bouquet and associated nuclear movement facilitate initial interactions between homologs. We show that Bqt2, a meiosis-specific protein required for bouquet formation, is required for wild-type levels of homolog pairing and meiotic allelic recombination. Both gene conversion and crossing over are reduced and exhibit negative interference in *bqt2*Δ mutants, reflecting reduced homolog pairing. While both the bouquet and nuclear movement promote pairing, only the bouquet restricts ectopic recombination (that between dispersed repetitive DNA). We discuss mechanisms by which the bouquet may prevent deleterious translocations by restricting ectopic recombination.

MEIOSIS, the specialized form of nuclear division that reduces the diploid number of chromosomes by half, consists of one round of DNA replication followed by two successive nuclear divisions. At the first meiotic division (MI) homologous chromosomes (homologs), each consisting of two sister chromatids, are segregated to opposite poles, reducing the chromosome number by half. The second meiotic division (MII), like mitosis, segregates sister chromosomes to opposite poles, producing four haploid nuclei.

The elevated rate of recombination characteristic of meiosis is due to a programmed set of meiosis-specific events that includes the formation of DNA double-strand breaks (DSBs) by Spo11, called Rec12 in *Schizosaccharomyces pombe* (Keeney *et al.* 1997; Cervantes *et al.* 2000). The DSBs are then repaired via interaction with an intact duplex, giving rise to gene conversions and crossovers (reviewed in Roeder 1997; Keeney 2001). At least one crossover per homolog pair is required to promote the proper attachment of each homolog in a pair to spindle microtubules from opposite poles, ensuring segregation of homologs at MI (reviewed in Page and Hawley 2003; Petronczki *et al.* 2003). In addition to its critical role in segregation, recombination generates diversity in subsequent generations by creating new combinations of alleles.

An elevated frequency of recombination is not sufficient to promote proper meiotic chromosome segregation; recombination must involve homologs rather than sister chromatids and must frequently produce a crossover. In meiosis, unlike mitosis, gene conversion is frequently associated with crossing over (Grimm *et al.* 1994; Virgin *et al.* 2001; Cromie *et al.* 2005 and references therein). Consequently, the recombination events that occasionally occur between dispersed repetitive DNA, such as transposons, genes for tRNAs, subtelomeric sequences, and multigene families, may frequently involve crossovers. Such crossovers can produce deleterious chromosomal rearrangements (reviewed in Shaffer and Lupski 2000). However, in both *S. pombe* and the budding yeast *Saccharomyces cerevisiae*, recombination between dispersed repetitive DNA is significantly lower than allelic recombination (Munz *et al.* 1982; Kohli *et al.* 1984; Kupiec and Petes 1988a,b; Goldman and Lichten 1996, 2000; Virgin and Bailey 1998; Schlecht *et al.* 2004). The restriction of ectopic recombination may, in some cases, result from an insufficient length of sequence identity. The restriction of ectopic recombination may also reflect the recombination-independent propensity of chromosomes to align, in register, along their entire length. In the latter case, if homologs were unable to align, ectopic recombination would not be restricted and dispersed repetitive elements might recombine as efficiently as allelic sequences.

Despite the elevated frequency of recombination in meiosis, in many organisms the number of crossovers per genome is not much larger than the number of chromosomes (reviewed in Hillers 2004). If these crossovers were placed randomly throughout the genome, a large fraction of meioses would contain at least one homolog pair with no crossovers and therefore be

[1]*Corresponding author:* Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. North, A1-162, P.O. Box 19024, Seattle, WA 98109-1024. E-mail: gsmith@fhcrc.org

prone to missegregation at MI. However, in many organisms the placement of crossovers is regulated; for instance, the presence of one crossover reduces the likelihood of a second nearby crossover (reviewed in van Veen and Hawley 2003; Hillers 2004). This phenomenon, crossover interference, is manifest as a frequency of double crossovers lower than that expected for two independent events. In *S. pombe*, which does not have crossover interference, each of its three chromosomes receives 10–20 randomly placed crossovers, resulting in a very low frequency of chromosomes without a crossover (Munz 1994).

Before recombination can take place, two DNA molecules must first be in close proximity. Thus, a critical step in meiotic recombination is the juxtaposition of homologous chromosomes. Homologs are brought together in a stepwise process (reviewed in Gerton and Hawley 2005). In this process we define homologs as being "aligned" when they are in register along their entire length. Homologs are subsequently defined as being "paired" when they are intimately associated along their entire length. Clustering of telomeres at the nuclear periphery, the bouquet configuration, is a conserved feature of meiosis that is thought to facilitate the alignment of homologs (reviewed in Scherthan 2001; Yamamoto and Hiraoka 2001). In *S. pombe* the bouquet consists of a tight cluster of all telomeres at the spindle-pole body (SPB). This clustering requires the telomere-binding protein Taz1, the Taz1-binding protein Rap1, and the heterochromatin protein Rik1 (Cooper *et al.* 1997, 1998; Nimmo *et al.* 1998; Chikashige and Hiraoka 2001; Kanoh and Ishikawa 2001; Tuzon *et al.* 2004). Telomere- and SPB-led oscillatory nuclear movement ("horsetail" movement) occurs throughout meiotic prophase (Chikashige *et al.* 1994) and depends on Dhc1, the microtubule motor protein dynein (Ding *et al.* 1998; Yamamoto *et al.* 1999). Perturbing either the bouquet or horsetail movement reduces pairing and meiotic recombination (Shimanuki *et al.* 1997; Cooper *et al.* 1998; Nimmo *et al.* 1998; Yamamoto *et al.* 1999; Niwa *et al.* 2000; Miki *et al.* 2002; Ding *et al.* 2004; Saito *et al.* 2005). By providing a physical linkage between chromosomes, recombination stabilizes their initial alignment and pairing promoted by the bouquet and horsetail movement (Nabeshima *et al.* 2001; Ding *et al.* 2004). Thus, the initial alignment and pairing of homologs are required for wild-type levels of meiotic recombination, which in turn is required for stable homolog pairing.

The Bqt2 protein of *S. pombe* is a meiosis-specific SPB component and is required for telomere clustering (Martin-Castellanos *et al.* 2005; Chikashige *et al.* 2006). In *bqt2*Δ mutant meioses SPB movement still occurs but telomeres are dispersed throughout the nucleus and chromosome movement is diminished. Here we show that, as expected, pairing of homologs in meiotic prophase and allelic recombination are reduced in *bqt2*Δ mutants. Furthermore, recombination events in both *bqt2*Δ and *dhc1*Δ mutants display *negative* interference: the presence of one recombination event *increases* the likelihood of a second nearby event. Bqt2, but not Dhc1, restricts ectopic recombination. We suggest a model in which ectopic recombination is restricted by the position of the two repetitive DNA elements relative to their nearest telomere, rather than pairing *per se*.

MATERIALS AND METHODS

Yeast strains, media, and culture conditions: Solid media were YEA + 4S, YEA + 5S, YEAG, or supplemented EMM2 used at 32° as described previously (Davis and Smith 2003). Liquid cultures were grown at 30° in YEL + 5S. Sporulation was at 25° on supplemented SPA (Gutz *et al.* 1974) for 2–4 days. The yeast strains and mutant alleles used are described below or in references in supplemental Table S1 at http://www.genetics.org/supplemental/.

Genetic screen for meiotic segregation mutants: In the absence of recombination (*e.g.*, in *rec12*Δ mutants), *S. pombe* possesses a residual ability to segregate homologs at MI (Davis and Smith 2003 and references therein). Missegregation of homologs at MI results in an elevated frequency of heterozygous diploid spores. Random segregation at MI is expected to produce ~10-fold more heterozygous diploid spores than that observed in *rec12* mutant meioses (Davis and Smith 2003, 2005). Heterozygous diploid spore formation in *rec12* mutants is thus expected to be increased by loss of the recombination-independent MI homolog segregation ability. To identify gene products required for this process, we enriched for mutants of strain GP2640 (*h*^{90} *ade6-52 leu1-32 ura4-294 his3-D1 fus1::LEU2 rec12-152::LEU2*) as follows. Haploid *fus1* mutant cells are unable to mate and therefore cannot sporulate; however, *fus1* mutant diploids, if heterozygous at the mating-type locus, are able to properly complete meiosis and sporulation (Petersen *et al.* 1995). By coupling the ability of *fus1*Δ to prevent sporulation of haploid cells with the ability to selectively kill non-sporulated cells using glusulase treatment (Ponticelli and Smith 1989), the meiotic progeny of diploid cells can be efficiently selected.

Strain GP2640 carrying plasmid pDW220 (*ura4*$^+$ *fus1*$^+$; Petersen *et al.* 1995) was mutagenized by random integration of linearized plasmid pAF1 (*his3*$^+$; Ohi *et al.* 1996) into the genome. Pools of mutagenized cells were sporulated, and the vegetative cells were killed and spores liberated from asci by treatment with glusulase. The spore suspensions were allowed to germinate in EMM2 medium, and cells that lost pDW220 were selected on plates containing 5-fluoroorotic acid. The remaining cells, a mixture of haploids and diploids, were phenotypically Fus1$^-$, and therefore only the diploids could sporulate. Each subsequent round of sporulation of these diploid spores coupled with killing of haploid cells results in a theoretical 10-fold enrichment for mutants with random segregation at MI. Nine pools of His$^+$ transformants (totaling ~10,000 individual colonies) were carried through two rounds of enrichment. The resulting mutants were screened by testing ~25 individual colonies from each pool for those that produced an elevated level of diploid spores (*i.e.*, I_2-staining spore colonies on EMM2 medium), and two mutants were identified. One mutant contained an insertion within the *klp6* (SPBC649.01C) coding sequence. Klp6 is a kinesin-like protein that belongs to the kinesin-8 family of microtubule-destabilizing proteins (Lawrence *et al.* 2004; Miki *et al.* 2005). The role of Klp6 in mitosis and meiosis has been

described by others (West *et al.* 2001, 2002; Garcia *et al.* 2002a,b; Li and Chang 2003; Sanchez-Perez *et al.* 2005). The other mutant contained an insertion within the coding sequence of SPAC1002.06C. This gene, recently named *bqt2*, is required for meiotic bouquet formation (Martin-Castellanos *et al.* 2005; Chikashige *et al.* 2006) and is the subject of this study.

Microscopy: To assay pairing, strains were used in which *lacO* was integrated near the centromere of chromosome I (ChrI) (Nabeshima *et al.* 1998) and bound by a variant of the green fluorescent protein-LacI-nuclear localization signal fusion (GFP13-LacI12-NLS; Straight *et al.* 1998), adapted for *S. pombe* (Davis and Smith 2003). Approximately 10^7 cells were mated on supplemented SPA and collected after 16–24 hr. Live zygotes, arrested in prophase by the *mei4* mutation (Shimoda *et al.* 1985; Hiraoka *et al.* 2000), were examined by fluorescence microscopy performed on a Nikon Eclipse 600 microscope using a Nikon 60× 1.40 NA Plan Apo objective (Nikon, Melville, NY). Images were captured using MetaMorph software (Molecular Devices, Sunnyvale, CA) and a Cascade 512B CCD camera (Photometrics, Tucson, AZ). GFP foci were counted in two experiments and statistical significance was calculated for each experiment independently using a χ^2-test.

Deletion constructs: A complete replacement of the *bqt2* coding sequence with *3HA-6His-kanMX6* was constructed using the method of Bahler *et al.* (1998). A PCR was performed using as template plasmid pFA6a-3HA-6His-kanMX6 (Davis and Smith 2003). The forward and reverse primers in this reaction contained nucleotides corresponding to the 5′ and 3′ ends of *bqt2*$^+$ (nucleotides 10,147–10,226 and 9555–9634, respectively, of cosmid SPAC1002; GenBank accession no. AL136078). The resulting PCR product was used to transform *S. pombe* strain GP363 (*h*$^+$ *ade6-M26 ura4-294 arg3-124*) to G418 resistance, conferred by *kanMX*. Deletion of *bqt2* (*bqt2-168::kanMX*) was confirmed by a PCR.

Recombinant frequencies: Intergenic recombinant frequencies among Ade$^+$ viable spores were determined by plating spore suspensions on YEAG to select Ade$^+$ spores and, after 3–5 days, colonies were toothpicked to grids on YEAG. After growth overnight, the segregants were replicated to the appropriate test media. Otherwise, recombinant frequencies were determined as previously described (Young *et al.* 2002). Statistical significance of Ade$^+$ recombinant frequencies was calculated using Student's *t*-test. χ^2-tests were used for statistical analysis of genetic interference data. For crossover interference in the *ura4-aim–tps16–arg1* intervals we determined whether or not the observed frequency of double crossovers was greater than that expected for two independent events. For interference between a conversion and a crossover we determined whether or not the frequency of crossovers was greater among the Ade$^+$ spores than among total spores.

Unequal sister-chromatid exchange (SCE) frequencies were determined as follows. Appropriately diluted mitotic cultures of the *ade6-Dup*-containing strain were plated on YEA + 4S to determine the total number of viable cells and on YEAG to determine the frequency of Ade$^+$ recombinants. The *ade6-Dup* strain and the appropriate *ade6-D19* strain were then mated on supplemented SPA. Spores were harvested and spore suspensions were plated on YEA + 4S to determine the total number of viable cells and on YEAG to determine the frequency of Ade$^+$ recombinants. The mitotic frequency, which was typically 5–10% of the meiotic frequency and never >27%, was subtracted from the meiotic frequency to give the final meiotic SCE frequency. Ten crosses were performed for each genotype and the statistical significance was calculated using Student's *t*-test.

RESULTS

Bqt2 is required for homolog pairing in meiotic prophase: We isolated a mutation in the *S. pombe* gene SPAC1002.06c in a screen for mutations that increase MI chromosome missegregation in a *rec12* background (see materials and methods for details). SPAC1002.06c was subsequently named *bqt2* and reported to encode a meiosis-specific spindle-pole body protein required for telomere clustering and wild-type levels of meiotic recombination (Martin-Castellanos *et al.* 2005; Chikashige *et al.* 2006). To determine whether, as expected, the telomere-clustering defect of *bqt2*Δ mutants resulted in defective homolog pairing, we examined both wild-type and *bqt2*Δ mutants marked with a tandem array of *lacO* DNA near the centromere of ChrI (Nabeshima *et al.* 1998). As a control, we also examined pairing in the absence of Dhc1, the heavy chain of the microtubule motor dynein, which is required for meiotic horsetail movement and efficient homolog pairing (Yamamoto *et al.* 1999; Ding *et al.* 2004). Pairing was visualized by fluorescence microscopy of the GFP-LacI-NLS fusion protein, which binds to the *lacO* array.

Homolog pairing in *S. pombe* is a dynamic process (Ding *et al.* 2004). To aid the analysis, we used the *mei4* mutation, which arrests cells in meiotic prophase, after horsetail nuclear movement, with paired homologs (Yokobayashi and Watanabe 2005). To the best of our knowledge, the precise position of the *mei4* arrest point relative to the dynamics of pairing is unknown. A single-GFP focus indicates pairing of the *lacO* array, while unpaired arrays generate two GFP foci in a single nucleus. Three or four GFP foci in a single nucleus indicate a defect in sister-chromatid cohesion. Two experiments, each with wild type and mutants on the same SPA plate, were performed, one ∼16 hr and the other ∼24 hr after the cells were mated. A single-GFP focus was found in 66, 39, and 43% of prophase nuclei in wild-type, *bqt2*Δ, and *dhc1*Δ cells, respectively, in the first experiment, and 96, 51, and 53% in the second (Table 1). The difference in the absolute level of pairing observed in the two experiments may be due to the degree to which the cells reached the *mei4* arrest point in each experiment. The fraction of paired homologs for both *bqt2*Δ and *dhc1*Δ was statistically different from that in wild type in each experiment ($P < 0.0005$). This indicates that pairing of the *lacO* array was significantly reduced in both *bqt2*Δ and *dhc1*Δ mutants. In all of the strains examined, ≤2% prophase nuclei contained three or four GFP foci, indicating that sister-chromatid cohesion is not significantly altered in *bqt2*Δ and *dhc1*Δ mutants.

Telomere clustering is required for wild-type levels of homologous intergenic recombination: In *bqt2*Δ mutants meiotic DSB formation and repair is nearly wild type but recombination is reduced by factors of ∼3–7 in the three intervals examined (Martin-Castellanos *et al.* 2005; Chikashige *et al.* 2006). To better understand

TABLE 1

Bqt2 is required for pairing of homologous chromosomes in meiotic prophase

	Homologous chromosomes				Sister chromatids:	
	% paired		% unpaired		% separated	
Parental genotypes	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
mei4-B2 (GP4101)	66	96	31	4	2	0
*bqt2*Δ *mei4-B2* (GP5764)	39	51	60	49	1	0
*dhc1*Δ *mei4-B2* (GP5733)	43	53	56	45	2	2

Cells were induced to mate and arrested in meiotic prophase. GFP dots, reflecting LacI-GFP bound to the *lacO* array at *lys1*, were counted in two experiments. In the first experiment 131, 99, and 129 zygotes were counted in wild-type, *bqt2*Δ, and *dhc1*Δ cells, respectively, and in the second 98, 102, and 113 were counted. One and two dots indicate paired and unpaired homologs, respectively. Three or four dots indicate separation of sister chromatids. The numbers are the percentage of zygotes in indicated classes for both experiments. In each experiment the fraction of paired homologs for both *bqt2*Δ and *dhc1*Δ was statistically different from that of wild type ($P < 0.0005$, contingency χ^2-tests).

this apparent discrepancy between reduced recombination and normal formation and repair of DSBs, which are expected to produce recombinants, we extended the analysis of meiotic recombination. We measured intergenic recombination (crossovers) in four intervals: *lys3–met5* on ChrI, *pat1–leu1* on ChrII, and *ura4-aim–tps16–arg1* on ChrIII (Figure 1A). Recombinant frequency in the *bqt2*Δ mutants was reduced by a factor of 2–4 in the regions tested, a reduction similar to that seen in the *dhc1*Δ mutant (Tables 2 and 3). The *lys3–met5* interval encompasses a region with DSB frequency and kinetics of repair that are nearly wild type in *bqt2*Δ mutant meioses (Martin-Castellanos *et al.* 2005). Significantly, recombination in this interval was reduced in the *bqt2*Δ mutant by a factor of ~4 (Table 2). These data suggest that repair of meiotic DSBs in the *bqt2*Δ mutant, and perhaps all pairing mutants, frequently involves the use of either sister chromatids or homologous nonallelic sequences as a template.

Recombination in pairing mutants displays negative interference: In many organisms the presence of one crossover reduces the likelihood of a second nearby crossover (reviewed in Hillers 2004). This phenomenon, called crossover interference, is manifest as a lower-than-expected frequency of double crossovers. Interference is defined as $I = 1 - C$, where C (coefficient of coincidence) $= R_d/(R_1 \cdot R_2)$ and R_1, R_2, and R_d are the frequencies of crossovers in interval one, interval two, and double crossovers, respectively. When $I = 0$, double crossovers occur at the frequency expected for two independent events; when $I = 1$, no double crossovers are observed. In rare instances the opposite situation has been reported whereby the presence of one crossover increases the likelihood of a second nearby crossover: $I < 0$, a situation called negative interference. In wild-type *S. pombe* there is no meiotic crossover interference, either positive or negative (Munz 1994).

If recombination is limited by inefficient pairing, the presence of a crossover may select for cells in which adjacent chromosomal intervals are necessarily in close proximity and may also stabilize the interaction between homologs. This may increase the likelihood of a second event, resulting in negative interference. We calculated the coefficient of coincidence (C) for the two adjacent intervals, *ura4-aim–tps16* and *tps16–arg1*, on ChrIII (see Figure 1A) using the recombination data from Table 3. In wild-type crosses $C = 1.2$, not significantly different from 1 ($P > 0.3$). In *bqt2*Δ mutant crosses $C = 2.5$,

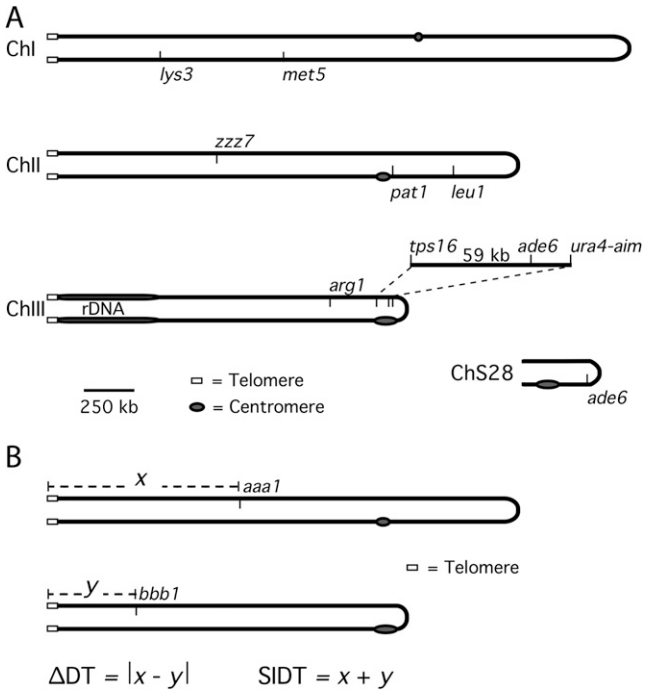

Figure 1.—*S. pombe* chromosomes. (A) Centromeres, telomeres, rDNA, and relevant genetic markers are indicated. ChS28 is a deletion derivative of ChrIII (Niwa *et al.* 1989). Drawn to scale. (B) Graphical representation of the *s*um of *i*nsert *d*istances to their nearest *t*elomere (SIDT) and the *d*ifference between the *d*istances of each locus from its nearest *t*elomere (ΔDT) for two loci, *aaa1* and *bbb1*, on heterologous chromosomes.

TABLE 2

Pairing mutants demonstrate reduced allelic recombination and negative interference between a conversion and a crossover

	Recombinant frequency					
	Wild type		*bqt2*Δ		*dhc1*Δ	
Interval (Chr)	Among total	Among Ade$^+$ [a]	Among total[b]	Among Ade$^+$ [a]	Among total[b]	Among Ade$^+$ [a]
lys3–met5 (I)	0.30	0.27	0.08**	0.10	0.14**	0.14
$C_{lys3–met5}$[c]	0.92		1.18		1.02	
leu1–pat1 (II)	0.37	0.43	0.16**	0.22*	0.14**	0.21*
$C_{leu1–pat1}$[c]	1.15		1.31*		1.46***	
tps16–arg1 (III)	0.43	0.40	0.14**	0.31**	0.14**	0.34**
$C_{tps16–arg1}$[c]	0.93		2.29**		2.49**	

Three or more independent crosses were performed for each interval. For the *lys3–met5* interval the strains crossed were: wild type, GP13 × GP5570 (four crosses); *bqt2*Δ, GP5478 × GP5644 (four crosses); *dhc1*Δ, GP5572 × GP5642 (four crosses). For the *leu1–pat1* interval: wild type, GP13 × GP5585 (three crosses); *bqt2*Δ, GP5478 × GP5584 (three crosses); *dhc1*Δ, GP5571 × GP5583 (three crosses). For the *tps16–arg1* interval: wild type, GP5217 × GP5224 (five crosses) and GP5216 × GP5223 (two crosses); *bqt2*Δ, GP5219 × GP5226 (five crosses) and GP5218 × GP5225 (two crosses); *dhc1*Δ, GP5221 × GP5279 (three crosses) and GP5220 × GP5280 (one cross). Each frequency is based on the cumulative number of spore colonies, >500 colonies in each case.

[a] Statistical significance relative to frequency among totals: $*P < 0.02$; $**P < 0.0005$.

[b] Statistical significance relative to wild type: $**P < 0.0005$.

[c] The coefficient of coincidence, *C*, equals the observed frequency of crossovers among Ade$^+$ spores divided by the frequency of crossovers among total spores. Statistically significant difference from $C = 1$: $*P < 0.02$; $***P < 0.0025$; $**P < 0.0005$.

significantly >1 ($P < 0.05$). Similarly, in *dhc1*Δ mutant crosses $C = 2.9$. Although 2.9 was not significantly >1 ($0.05 < P < 0.1$), we suspect that this reflects the limited number of observed double crossovers (12). These data indicate that *bqt2*Δ, and perhaps *dhc1*Δ, mutants exhibit negative crossover interference.

The negative interference described above was between two crossovers. Similarly, positive interference is typically observed between two crossovers; in both *S. cerevisiae* and the filamentous fungus *Neurospora crassa* a gene conversion without an associated crossover does not exhibit interference with an adjacent interval (Fogel and Hurst 1967; Stadler and Towe 1968; Malkova *et al.* 2004). However, if one allelic interaction promotes the interaction of nearby chromosomal regions, negative interference might be observed between a conversion and a crossover. The crosses used to measure homologous intergenic recombination, above, also contained heteroalleles of *ade6*, allowing us to address this possibility. We measured intergenic recombination among selected *ade6*$^+$ gene convertants in these crosses and calculated *C*. Here $C = R_{Ade^+}/R_{\text{total}}$, where R_{Ade^+} and R_{total} are the frequencies of crossovers among *ade6*$^+$ convertants and among total spores, respectively. For gene convertants at *ade6* and crossovers in the *tps16–arg1* interval, which is ~60–300 kb from *ade6* on ChrIII (Figure 1A), $C = 0.93$ in wild-type crosses, not significantly different from 1 ($P > 0.25$; Table 2). In *bqt2*Δ and *dhc1*Δ mutant crosses $C = 2.29$ and 2.49, respectively, and is significantly >1 ($P < 0.0005$; Table 2). The data in Table 2 include *ade6*$^+$ gene convertants with and without an associated crossover between *ura4*$^+$*-aim* and *tps16*. When only those convertants without an associated crossover are considered, $C = 2.45$ and 2.51 in *bqt2*Δ and *dhc1*Δ mutant crosses, respectively, and is significantly >1 ($P < 0.0005$). These data indicate that both *bqt2*Δ and *dhc1*Δ mutants exhibit negative interference between a conversion and a crossover.

An alternative explanation for apparent negative interference is a subpopulation of "hot" meiotic cells that are recombinationally more competent than the bulk population. To address this possibility, we determined the recombinant frequency in the *lys3–met5* and *pat1–leu1* intervals, on ChrI and -II, respectively, among *ade6*$^+$ (ChrIII) convertants. If there is a significant subpopulation of hot cells, then the recombinant frequency in both intervals would be higher among *ade6*$^+$ convertants than among total cells ($C > 1$), despite the intervals

TABLE 3

Pairing mutants demonstrate negative crossover interference

	Recombinant frequency		
Interval (Chr)	Wild type	*bqt2*Δ[a]	*dhc1*Δ[a]
aim–tps16 (III)	0.11	0.04**	0.04**
tps16–arg1 (III)	0.43	0.14**	0.14**
$C_{aim–tps16–arg1}$[b]	1.2	2.5***	2.9

Three or more independent experiments were performed for each interval. Each frequency is based on >500 colonies analyzed. *tps16–arg1* data are from Table 2.

[a] Statistical significance relative to wild type: $**P < 0.0005$.

[b] The coefficient of coincidence, *C*, equals the observed frequency of double crossovers divided by the product of the frequencies of the respective single crossovers. The numbers of double crossovers observed for wild type, *bqt2*Δ, and *dhc1*Δ were 62, 18, and 12, respectively. Statistically significant difference from $C = 1$: $***P < 0.05$.

being on different chromosomes. The recombinant frequency in the *lys3–met5* interval was not significantly higher among *ade6*$^+$ convertants than among total cells in wild type, *bqt2*Δ, or *dhc1*Δ ($C = 0.92$, $P > 0.3$; $C = 1.18$, $P > 0.3$; $C = 1.02$, $P > 0.9$, respectively; Table 2). These data argue against a subpopulation of hot cells.

In the *pat1–leu1* interval the recombinant frequency was modestly higher among *ade6*$^+$ convertants than among total spores in wild type, *bqt2*Δ, and *dhc1*Δ (Table 2). In wild-type crosses $C = 1.15$, not significantly different from 1 ($P > 0.05$), but in *bqt2*Δ and *dhc1*Δ mutant crosses $C = 1.31$ and 1.46, respectively, and was significantly >1 ($P < 0.02$ and $P < 0.0025$, respectively). To determine if the increase in recombinant frequency among *ade6*$^+$ convertants observed in *bqt2*Δ and *dhc1*Δ mutants was significantly different from the increase observed in wild type, we determined whether or not the observed frequency of crossovers was greater than that expected if $C = 1.15$, the wild-type value. The increase in recombinant frequency among *ade6*$^+$ convertants observed in the *bqt2*Δ mutant was not significantly different from the increase observed in wild type ($P > 0.25$), but that in the *dhc1*Δ mutant was significantly different ($P < 0.05$). These results indicate that the observed increase of recombinant frequencies among *ade6*$^+$ convertants in the *pat1–leu1* interval (ChrII) is independent of the *bqt2*Δ mutation but partially dependent on the *dhc1*Δ mutation. The reason for this result is unknown, but in light of the results with the *lys3–met5* interval (ChrI), it does not indicate that the negative interference observed at the *ura4-aim–ade6–tps16–arg1* region of ChrIII in *bqt2*Δ and *dhc1*Δ mutants is due to hot cells specifically present in the mutant population.

Telomere clustering, but not horsetail movement, restricts ectopic recombination: To determine whether reduced pairing resulted in excess ectopic (nonallelic) recombination, we first determined the recombinant frequency between the *ade6-M26* allele on ChrIII and the *ade6-M210* allele on an artificial minichromosome (ChS28; Niwa *et al.* 1989 and see Figure 1A). In this assay, the frequency of Ade$^+$ recombinants was increased, relative to that in wild type, by a factor of 18 in *bqt2*Δ mutants but was not affected in *dhc1*Δ mutants (Table 4). We infer that the minichromosome is released from its position near the telomeres of ChrI, -II, and -III in the *bqt2*Δ mutant and can more readily come into proximity to the *ade6* locus on ChrIII and therefore recombine with it.

We next determined the recombinant frequency between the *ade6-M26* allele on ChrIII and the *ade6-469* allele ectopically transplaced on ChrII (*zzz7*; Virgin and Bailey 1998 and see Figure 1A). In this assay, the frequency of Ade$^+$ recombinants was increased, relative to that in wild type, by a factor of 3.5 in *bqt2*Δ mutants but was not significantly affected in *dhc1*Δ mutants ($P > 0.05$; Table 4). In *bqt2*Δ mutants, recombinant frequencies increased in both assays of ectopic recombination but decreased by a factor of 4.4 for allelic recombination (at the endogenous locus) between the *ade6-M26* and *ade6-M210* alleles (Table 4). These data indicate that Bqt2, but not Dhc1, restricts the interaction of ectopic sequences.

TABLE 4

Telomere clustering, but not horsetail movement, limits ectopic recombination

		Recombinant frequency ($\times 10^5$)		
Loci[a]	ΔDT[b]	wt	*dhc1*Δ	*bqt2*Δ
ade6 (allelic)	0	530 ± 55	140 ± 8	120 ± 15
ade6 × ChS28	~1.34 Mb	2.2 ± 0.5	2.4 ± 0.4	40 ± 6.2
ade6 × *zzz7*	~0.85 Mb	8.9 ± 0.8	13 ± 1.5	32 ± 3.3
ade6-Dup (SCE)	~5 kb	1250 ± 160	1230 ± 120	1910 ± 160

Recombinant frequencies are the mean ± SEM for at least four experiments. wt, wild type.

[a] The *ade6-M26* and *ade6-M210* alleles were used in the *ade6* (allelic) and *ade6* × ChS28 experiments. The *ade6-M26* and *ade6-469* alleles were used in the *ade6* × *zzz7* and *ade6-Dup* (SCE) experiments. The *M210* and *469* alleles are 3 bp apart (Szankasi *et al.* 1988; G. Freyer, personal communication).

[b] ΔDT is the difference between the distances of each locus from its nearest telomere (Figure 1B).

The normal frequency of DSB formation and repair (Martin-Castellanos *et al.* 2005), but reduced homolog recombination (Tables 2 and 3), suggested that the *bqt2*Δ mutation might increase the frequency of meiotic sister-chromatid exchange. To address this possibility, we determined the frequency of recombination between tandemly duplicated copies of the *ade6* gene, one marked with the *M26* allele and the other with the *469* allele, flanking *ura4*$^+$ at the endogenous *ade6* locus (Schuchert and Kohli 1988). In this assay, recombination must use the sister as a template, since *ade6* is deleted from the homologous chromosome. In *bqt2*Δ mutant meioses the Ade$^+$ recombinant frequency was modestly but significantly greater than that in wild type (Table 4; $P = 0.009$). The Ade$^+$ recombinant frequency in *dhc1*Δ mutant meioses was not significantly different from that in wild type (Table 4; $P > 0.9$). These data indicate that Bqt2, but not Dhc1, restricts unequal sister-chromatid exchange.

DISCUSSION

In *S. pombe*, telomere clustering and horsetail nuclear movement promote the pairing of homologous chromosomes during meiotic prophase (reviewed in Burgess 2004), perhaps by limiting the space that must be searched for a homologous sequence. Recombination requires homolog proximity but also promotes pairing, perhaps by stabilizing the initial alignment of homologs. To understand the role that each of these processes

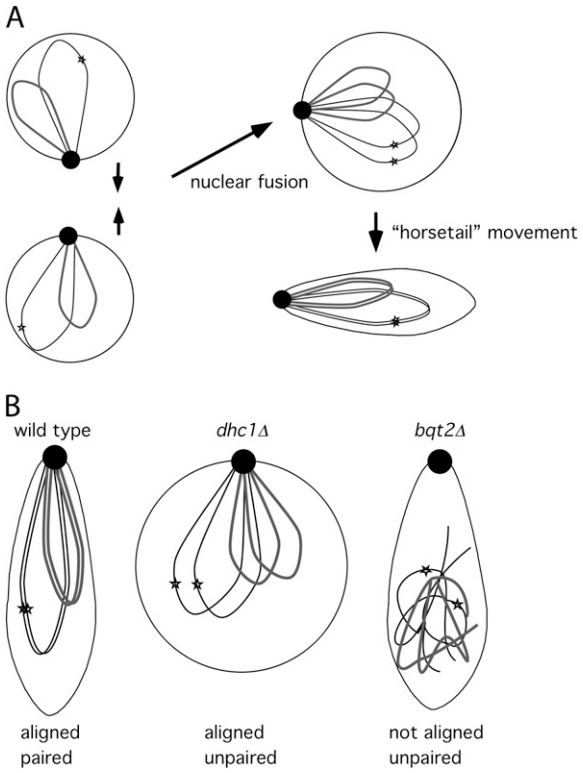

FIGURE 2.—The role of telomere clustering and "horsetail" nuclear movement in meiotic homolog pairing. (A) Prior to nuclear fusion, telomeres cluster at the SPB (solid circle). In the prophase nucleus, the bouquet promotes alignment of homologs, and horsetail movement facilitates the transition to full pairing. (B) Proposed chromosome configuration in wild-type, *dhc1*Δ, and *bqt2*Δ mutant prophase nuclei. Only two of the three chromosome pairs are shown. The stars represent homologous sites on a pair of homologs.

plays in homolog pairing, we have analyzed mutations that specifically abolish telomere clustering (*bqt2*Δ; MARTIN-CASTELLANOS *et al.* 2005; CHIKASHIGE *et al.* 2006) and horsetail nuclear movement (*dhc1*Δ; YAMAMOTO *et al.* 1999). Bqt2 is a meiosis-specific SPB component that, together with Bqt1, tethers the telomere protein Rap1 to the SPB protein Sad1 (MARTIN-CASTELLANOS *et al.* 2005; CHIKASHIGE *et al.* 2006). Dhc1, the heavy chain of the microtubule motor dynein, is required for horsetail nuclear movement and, although expression of Dhc1 is not meiosis specific, it has no detected mitotic phenotype (YAMAMOTO *et al.* 1999). We first showed that Bqt2, as predicted, is required for efficient pairing of homologs in meiotic prophase (Table 1). We then extended the analysis of meiotic recombinant frequencies in *bqt2*Δ and *dhc1*Δ mutants. In light of our results, we propose that (1) the bouquet promotes the alignment of homologs but not their full pairing, (2) horsetail movement facilitates the transition from alignment to full pairing (Figure 2A), and (3) ectopic recombination is restricted by the alignment of homologs, rather than pairing *per se*.

Recombination in homolog-pairing mutants displays negative interference: Positive crossover interference is widespread in meiotic recombination (reviewed in HILLERS 2004). Negative crossover interference (higher than expected frequency of double crossovers) is less common and has been associated with special chromosomal regions—centromeres in *Drosophila melonogaster* and several species of plants (GREEN 1975; SINCLAIR 1975; DENELL and KEPPY 1979; PENG *et al.* 2000; BOYKO *et al.* 2002; ESCH and WEBER 2002; ESCH 2005) and heterozygous translocations in the mosquito *Aedes aegypti* and several species of plants (AUGER and SHERIDAN 2001, and references therein). Several proposals have been put forth to explain these cases of negative interference. Apparent negative crossover interference has been attributed to gene conversion of the central marker, *i.e.*, one, not two, events (GREEN 1975). DENELL and KEPPY (1979) suggested that negative interference may be characteristic of chromosomal regions, such as centromeres, that have a low density of recombination events (per unit physical length). AUGER and SHERIDAN (2001) suggested that negative interference is a result of reduced competence for crossover formation near translocation breakpoints. Just as hot cells result in negative interference (GROSSENBACHER-GRUNDER 1985), so would hot regions of chromosomes—the chromosomal regions that do pair in a pairing-deficient mutant. After submission of this article, negative crossover interference was reported in a *zip4*Δ mutant of *S. cerevisiae* using a single-interval assay (nonparental ditype ratio; TSUBOUCHI *et al.* 2006). Our results suggest that inefficient homolog pairing leads to negative interference in *S. pombe*.

*bqt2*Δ and *dhc1*Δ mutants display negative interference, both between two crossovers and between a conversion and a crossover (Tables 2 and 3). This is in contrast to wild-type *S. pombe* where there is no interference (Tables 2 and 3; KOHLI and BAHLER 1994; MUNZ 1994). The negative crossover interference in the *ura4-aim–tps16–arg1* intervals is unlikely to result from gene conversion of the central marker for the following reason. The frequency of conversion at *tps16* would have to be 0.9%, the frequency of apparent double crossovers in excess of the expected frequency, in the *ura4-aim–tps16–arg1* intervals in *bqt2*Δ and *dhc1*Δ mutants. This is more than twice the frequency of conversion observed in wild type (0.4%; ZAHN-ZABAL *et al.* 1995). Given that both mutations reduce all allelic recombination examined by more than a factor of 2 (Tables 2–4), it is unlikely that conversion at *tps16* could explain these results. Additionally, to explain the negative interference between *ade6*$^+$ convertants and *tps16–arg1* crossovers (Table 2), the frequency of conversion at *tps16* would have to be at least 17%, but only among *ade6*$^+$ spores.

Gene conversion in *S. pombe* meiosis is frequently associated with crossing over between flanking markers (GRIMM *et al.* 1994; CROMIE *et al.* 2005). Negative

interference between a conversion and a crossover could be explained if the conversion and crossover were not separate events. However, to explain the negative interference between *ade6*$^+$ convertants and apparent *tps16–arg1* crossovers, a single recombination event would frequently have to cover more than the 59 kb between *ade6* and *tps16*. While we cannot formally rule out this possibility, it seems unlikely that the frequency of this type of event would be increased in *bqt2*Δ and *dhc1*Δ mutants.

We propose that negative interference in *bqt2*Δ and *dhc1*Δ mutants is the consequence of inefficient homolog pairing and an otherwise wild-type ability to repair meiotic DSBs and reflects a propensity for localized proximity of homologs to extend to larger regions—at least 59 kb, the distance between *ade6* and *tps1* (Figure 1A). The propensity for extended proximity may be related to recombination in two distinct ways. First, an initial recombination event may stabilize the interaction between homologs. This would limit the space that an adjacent chromosomal interval must search for a homologous sequence with which to recombine, thus increasing the likelihood of a second nearby recombination event. In this model, recombination is required to extend localized proximity. Second, when, by chance, one locus is close enough to its homolog to recombine, adjacent chromosomal intervals are necessarily in close proximity, thus increasing the likelihood of a second nearby recombination event. In this model, the first recombination event does not cause local proximity to be extended; rather, extended proximity reflects simply the physical properties of the chromosome. Our genetic data do not differentiate between these models, although a cytological assay for local alignment in a Rec$^-$ mutant may be able to do so.

Telomere clustering, but not horsetail movement, restricts ectopic recombination: Crossovers between dispersed repetitive DNA such as transposons, genes for tRNA, subtelomeric sequences, and multigene families can produce deleterious chromosomal rearrangements. Endogenous repetitive sequences are unlikely to be identical. At least in *S. cerevisiae*, the mismatch repair (MMR) pathway, which can detect regions of sequence divergence, restricts recombination between diverged sequences (reviewed in Borts *et al.* 2000). Despite this restriction, ectopic recombination (that between nonallelic sequences) does occur. In humans, a significant number of diseases and syndromes are due to chromosomal translocations, duplications, or deletions generated by meiotic recombination between repetitive DNA (reviewed in Stankiewicz and Lupski 2002). This emphasizes the importance of restricting ectopic recombination. In fact, processes other than MMR must restrict ectopic recombination since the frequency of recombination between nearly identical repeats is significantly lower than that of allelic recombination in both *S. cerevisiae* and *S. pombe* (Goldman and Lichten 1996, 2000; Virgin and Bailey 1998; Schlecht *et al.* 2004). The mechanisms that limit ectopic recombination have not been well characterized. Our data indicate that the meiotic bouquet plays a critical role in *S. pombe*, perhaps by promoting the alignment of chromosomes, in register, along their entire length.

We have shown that, in *S. pombe*, ectopic recombination is predominantly constrained by telomere clustering, not homolog pairing *per se*. Both *bqt2*Δ and *dhc1*Δ mutants reduce pairing and allelic recombination, although the meiotic DSB frequency is nearly wild type (Tables 1–4; Yamamoto *et al.* 1999; Ding *et al.* 2004; Martin-Castellanos *et al.* 2005; Chikashige *et al.* 2006; C. Ellermeier and G. R. Smith, unpublished data). The high viable spore yield in *bqt2*Δ and *dhc1*Δ mutants (~50% of wild type; our unpublished data) indicates that the meiotic DSBs are repaired. We had initially inferred that DSB repair in both mutants frequently involves the use of either sister chromatids or homologous nonallelic sequences as a template. Instead, we found that *unequal* sister-chromatid exchange and ectopic recombination were unaffected in the *dhc1*Δ mutant (Table 4). Perhaps in *dhc1*Δ mutant meioses DSBs are repaired by *equal* sister-chromatid exchange or by nonhomologous end joining. In *bqt2*Δ mutant meioses *unequal* sister-chromatid exchange was elevated 1.5-fold, relative to wild type (Table 4). If sister-chromatid exchange is more frequent than exchange between homologs in wild-type *S. pombe*, a reduced ability to repair DSBs using the homolog as template (*e.g.*, in pairing mutants) would result in only a small increase in SCE. Ectopic recombination, measured in two different assays, was elevated 3.5- and 18-fold in *bqt2*Δ mutant meioses (Table 4). Both *bqt2*Δ and *dhc1*Δ mutants reduce homolog pairing and allelic recombination to approximately the same extent (Tables 1–4) while the restriction of meiotic ectopic recombination is eased in *bqt2*Δ mutants and maintained in *dhc1*Δ mutants. This indicates that the restriction is not dependent on homolog pairing *per se*. Instead, we suggest that ectopic recombination is restricted predominantly by chromosomal position.

We propose that ectopic recombination between two dispersed repetitive sequences is restricted by their relative positions in the prophase nucleus. Because of telomere clustering, the distance, in base pairs, from the nearest telomere determines the position of a locus within the nucleus (Ding *et al.* 2004). As a result, the frequency of ectopic recombination is inversely proportional to the *d*ifference between the *d*istances of each locus from its nearest *t*elomere (ΔDT, Figure 1B). This proposal is supported by the following data. First, in wild type, the frequency of recombinants increases as ΔDT decreases: the frequency of recombinants is highest when ΔDT = 0 (*i.e.*, in allelic recombination) and decreases as ΔDT increases (Table 4). When ΔDT is the highest, the ratio of allelic to ectopic recombinant

frequencies is 240 (*ade6* × ChS28, Table 4). Second, in *bqt2*Δ mutant meioses the effect of ΔDT was nearly eliminated: the ratio of allelic to ectopic recombinant frequencies was never >4 (Table 4). Together, these data suggest that the effect of ΔDT on ectopic recombination reflects the role of telomere clustering in restricting ectopic recombination. Determination of recombinant frequencies at additional pairs of loci is required to establish the generality of the effect of ΔDT on ectopic recombination.

We interpret the different effects of *bqt2*Δ and *dhc1*Δ mutants on ectopic recombination as follows. Alignment and subsequent pairing of homologs along their entire length are required to ensure wild-type levels of meiotic recombination. Bqt2 (telomere clustering) and Dhc1 (horsetail movement) contribute to this in different ways (Figure 2). Prior to mating, chromosomes in wild-type *S. pombe* are in the "Rabl" orientation; *i.e.*, the centromeres are clustered at the SPB and telomeres are dispersed (Funabiki *et al.* 1993). When mating is induced, centromeres are released and telomeres move to the SPB (Chikashige *et al.* 1994, 1997). After nuclear fusion, the bouquet promotes alignment but not full pairing of homologs. The Dhc1-dependent horsetail movement facilitates the transition to full pairing but is dependent on telomere clustering for this effect. In a *dhc1*Δ mutant meiosis telomeres still cluster at the SPB and the bouquet is sufficient to promote alignment of homologs but not full pairing (Figure 2B). In the absence of telomere clustering (*bqt2*Δ), chromosomes are not able to align (Figure 2B). In this view, both *bqt2*Δ and *dhc1*Δ mutants are scored as pairing defective, measured cytologically at a single locus, but have distinctly different configurations of homologous chromosomes. One prediction of this model is that meiotic ectopic recombination should be elevated to the same extent in the *bqt2*Δ *dhc1*Δ double mutant as in *bqt2*Δ.

We expect that like *bqt2*Δ, other mutations that disrupt telomere clustering such as *bqt1*Δ, *taz1*Δ, *rap1*Δ, and *rik1*Δ would result in elevated meiotic ectopic recombination. Additionally, we expect that mutations that disrupt horsetail nuclear movement without disrupting telomere clustering would have no effect on meiotic ectopic recombination. Like Bqt2, Mcp6 is a meiosis-specific SPB protein in *S. pombe*. In *mcp6*Δ mutant meioses telomere clustering appears normal but horsetail nuclear movement is reduced (Saito *et al.* 2005; Tanaka *et al.* 2005). In contrast to our view above, the frequency of ectopic recombinants is increased modestly in *mcp6*Δ mutant meioses (Saito *et al.* 2005). Additional experiments may determine whether telomere clustering is fully wild type in *mcp6*Δ mutants.

In an extensive analysis of ectopic recombination in *S. cerevisiae*, the authors concluded that for loci on heterologous chromosomes the efficiency of ectopic recombination is negatively correlated with the *s*um of *i*nsert *d*istances to their nearest *t*elomeres (SIDT) (see Figure 1B; Goldman and Lichten 1996; Schlecht *et al.* 2004). In *S. cerevisiae*, Ndj1 is required for bouquet formation and pairing (Conrad *et al.* 1997; Trelles-Sticken *et al.* 2000). The negative correlation of ectopic recombination efficiency with SIDT does not depend on Ndj1 (Schlecht *et al.* 2004). Additionally, ectopic recombination efficiencies are only modestly increased in *ndj1*Δ mutants (Schlecht *et al.* 2004). In *S. cerevisiae*, unlike in *S. pombe*, the bouquet does not play a major role in restricting ectopic recombination. Perhaps in *S. cerevisiae* the bouquet is not required for alignment, and the synaptonemal complex promotes alignment and restricts ectopic recombination.

Pairing functions and the distribution of meiotic recombination events: Our data have several important implications. First, we suggest that by promoting homolog pairing, the bouquet and horsetail movement prevent negative interference in *S. pombe*. Similarly, in *S. cerevisiae*, Ndj1 and presumably the bouquet contribute to positive interference (Chua and Roeder 1997). In both of these highly diverged species, the bouquet affects the distribution of recombination events. Second, the bouquet functions to restrict ectopic recombination in *S. pombe*. This function is critical for successful completion of meiosis. Ectopic recombination not only results in deleterious chromosomal rearrangements (reviewed in Stankiewicz and Lupski 2002), but also disturbs meiotic chromosome segregation in *S. cerevisiae* (Jinks-Robertson *et al.* 1997). Understanding the mechanism by which the bouquet functions in *S. pombe* should contribute to our understanding of how chromosome rearrangements are formed in humans.

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LITERATURE CITED

Auger, D. L., and W. F. Sheridan, 2001 Negative crossover interference in maize translocation heterozygotes. Genetics **159:** 1717–1726.

Bahler, J., J. Q. Wu, M. S. Longtine, N. G. Shah, A. McKenzie, 3rd *et al.*, 1998 Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast **14:** 943–951.

Borts, R. H., S. R. Chambers and M. F. Abdullah, 2000 The many faces of mismatch repair in meiosis. Mutat. Res. **451:** 129–150.

Boyko, E., R. Kalendar, V. Korzun, J. Fellers, A. Korol *et al.*, 2002 A high-density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense-related genes: insights into cereal chromosome structure and function. Plant Mol. Biol. **48:** 767–790.

Burgess, S. M., 2004 Homolog pairing in *S. pombe*: the ends are the means. Mol. Cell **13:** 766–768.

Cervantes, M. D., J. A. Farah and G. R. Smith, 2000 Meiotic DNA breaks associated with recombination in *S. pombe*. Mol. Cell **5:** 883–888.

Chikashige, Y., and Y. Hiraoka, 2001 Telomere binding of the Rap1 protein is required for meiosis in fission yeast. Curr. Biol. **11:** 1618–1623.

Chikashige, Y., D. Q. Ding, H. Funabiki, T. Haraguchi, S. Mashiko *et al.*, 1994 Telomere-led premeiotic chromosome movement in fission yeast. Science **264:** 270–273.

Chikashige, Y., D. Q. Ding, Y. Imai, M. Yamamoto, T. Haraguchi *et al.*, 1997 Meiotic nuclear reorganization: switching the position of centromeres and telomeres in the fission yeast *Schizosaccharomyces pombe*. EMBO J. **16:** 193–202.

Chikashige, Y., C. Tsutsumi, M. Yamane, K. Okamasa, T. Haraguchi *et al.*, 2006 Meiotic proteins Bqt1 and Bqt2 tether telomeres to form the bouquet arrangement of chromosomes. Cell **125:** 59–69.

Chua, P. R., and G. S. Roeder, 1997 Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev. **11:** 1786–1800.

Conrad, M. N., A. M. Dominguez and M. E. Dresser, 1997 Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science **276:** 1252–1255.

Cooper, J. P., E. R. Nimmo, R. C. Allshire and T. R. Cech, 1997 Regulation of telomere length and function by a Myb-domain protein in fission yeast. Nature **385:** 744–747.

Cooper, J. P., Y. Watanabe and P. Nurse, 1998 Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. Nature **392:** 828–831.

Cromie, G. A., C. A. Rubio, R. W. Hyppa and G. R. Smith, 2005 A natural meiotic DNA break site in *Schizosaccharomyces pombe* is a hotspot of gene conversion, highly associated with crossing over. Genetics **169:** 595–605.

Davis, L., and G. R. Smith, 2003 Nonrandom homolog segregation at meiosis I in *Schizosaccharomyces pombe* mutants lacking recombination. Genetics **163:** 857–874.

Davis, L., and G. R. Smith, 2005 Dynein promotes achiasmate segregation in *Schizosaccharomyces pombe*. Genetics **170:** 581–590.

Denell, R. E., and D. O. Keppy, 1979 The nature of genetic recombination near the third chromosome centromere of *Drosophila melanogaster*. Genetics **93:** 117–130.

Ding, D. Q., Y. Chikashige, T. Haraguchi and Y. Hiraoka, 1998 Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. J. Cell Sci. **111:** 701–712.

Ding, D. Q., A. Yamamoto, T. Haraguchi and Y. Hiraoka, 2004 Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. Dev. Cell **6:** 329–341.

Esch, E., 2005 Estimation of gametic frequencies from F2 populations using the EM algorithm and its application in the analysis of crossover interference in rice. Theor. Appl. Genet. **111:** 100–109.

Esch, E., and E. Weber, 2002 Investigation of crossover interference in barley (Hordeum vulgare L.) using the coefficient of coincidence. Theor. Appl. Genet. **104:** 786–796.

Fogel, S., and D. D. Hurst, 1967 Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics **57:** 455–481.

Funabiki, H., I. Hagan, S. Uzawa and M. Yanagida, 1993 Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J. Cell Biol. **121:** 961–976.

Garcia, M. A., N. Koonrugsa and T. Toda, 2002a Spindle-kinetochore attachment requires the combined action of Kin I-like Klp5/6 and Alp14/Dis1-MAPs in fission yeast. EMBO J. **21:** 6015–6024.

Garcia, M. A., N. Koonrugsa and T. Toda, 2002b Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A. Curr. Biol. **12:** 610–621.

Gerton, J. L., and R. S. Hawley, 2005 Homologous chromosome interactions in meiosis: diversity amidst conservation. Nat. Rev. Genet. **6:** 477–487.

Goldman, A. S., and M. Lichten, 1996 The efficiency of meiotic recombination between dispersed sequences in *Saccharomyces cerevisiae* depends upon their chromosomal location. Genetics **144:** 43–55.

Goldman, A. S., and M. Lichten, 2000 Restriction of ectopic recombination by interhomolog interactions during *Saccharomyces cerevisiae* meiosis. Proc. Natl. Acad. Sci. USA **97:** 9537–9542.

Green, M. M., 1975 Conversion as a possible mechanism of high coincidence values in the centromere region of Drosophila. Mol. Gen. Genet. **139:** 57–66.

Grimm, C., J. Bahler and J. Kohli, 1994 *M26* recombinational hotspot and physical conversion tract analysis in the *ade6* gene of *Schizosaccharomyces pombe*. Genetics **136:** 41–51.

Grossenbacher-Grunder, A.-M., 1985 Spontaneous mitotic recombination in *Schizosaccharomyces pombe*. Curr. Genet. **10:** 95–101.

Gutz, H., H. Heslot, U. Leupold and N. Loprieno, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics*, edited by R. C. King. Plenum, New York.

Hillers, K. J., 2004 Crossover interference. Curr. Biol. **14:** R1036–R1037.

Hiraoka, Y., D. Q. Ding, A. Yamamoto, C. Tsutsumi and Y. Chikashige, 2000 Characterization of fission yeast meiotic mutants based on live observation of meiotic prophase nuclear movement. Chromosoma **109:** 103–109.

Jinks-Robertson, S., S. Sayeed and T. Murphy, 1997 Meiotic crossing over between nonhomologous chromosomes affects chromosome segregation in yeast. Genetics **146:** 69–78.

Kanoh, J., and F. Ishikawa, 2001 spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast. Curr. Biol. **11:** 1624–1630.

Keeney, S., 2001 Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. **52:** 1–53.

Keeney, S., C. N. Giroux and N. Kleckner, 1997 Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384.

Kohli, J., and J. Bahler, 1994 Homologous recombination in fission yeast: absence of crossover interference and synaptonemal complex. Experientia **50:** 295–306.

Kohli, J., P. Munz, R. Aebi, H. Amstutz, C. Gysler *et al.*, 1984 Interallelic and intergenic conversion in three serine tRNA genes of *Schizosaccharomyces pombe*. Cold Spring Harbor Symp. Quant. Biol. **49:** 31–40.

Kupiec, M., and T. D. Petes, 1988a Allelic and ectopic recombination between Ty elements in yeast. Genetics **119:** 549–559.

Kupiec, M., and T. D. Petes, 1988b Meiotic recombination between repeated transposable elements in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 2942–2954.

Lawrence, C. J., R. K. Dawe, K. R. Christie, D. W. Cleveland, S. C. Dawson *et al.*, 2004 A standardized kinesin nomenclature. J. Cell Biol. **167:** 19–22.

Li, Y., and E. C. Chang, 2003 *Schizosaccharomyces pombe* Ras1 effector, Scd1, interacts with Klp5 and Klp6 kinesins to mediate cytokinesis. Genetics **165:** 477–488.

Malkova, A., J. Swanson, M. German, J. H. McCusker, E. A. Housworth *et al.*, 2004 Gene conversion and crossing over along the 405-kb left arm of *Saccharomyces cerevisiae* chromosome VII. Genetics **168:** 49–63.

Martin-Castellanos, C., M. Blanco, A. E. Rozalen, L. Perez-Hidalgo, A. I. Garcia *et al.*, 2005 A large-scale screen in *S. pombe* identifies seven novel genes required for critical meiotic events. Curr. Biol. **15:** 2056–2062.

Miki, F., K. Okazaki, M. Shimanuki, A. Yamamoto, Y. Hiraoka *et al.*, 2002 The 14-kDa dynein light chain-family protein Dlc1 is required for regular oscillatory nuclear movement and efficient recombination during meiotic prophase in fission yeast. Mol. Biol. Cell **13:** 930–946.

Miki, H., Y. Okada and N. Hirokawa, 2005 Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol. **15:** 467–476.

Munz, P., 1994 An analysis of interference in the fission yeast *Schizosaccharomyces pombe*. Genetics **137:** 701–707.

Munz, P., H. Amstutz, J. Kohli and U. Leupold, 1982 Recombination between dispersed serine tRNA genes in *Schizosaccharomyces pombe*. Nature **300:** 225–231.

Nabeshima, K., T. Nakagawa, A. F. Straight, A. Murray, Y. Chikashige *et al.*, 1998 Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle. Mol. Biol. Cell **9:** 3211–3225.

Nabeshima, K., Y. Kakihara, Y. Hiraoka and H. Nojima, 2001 A novel meiosis-specific protein of fission yeast, Meu13p, promotes homologous pairing independently of homologous recombination. EMBO J. **20:** 3871–3881.

Nimmo, E. R., A. L. Pidoux, P. E. Perry and R. C. Allshire, 1998 Defective meiosis in telomere-silencing mutants of *Schizosaccharomyces pombe*. Nature **392:** 825–828.

Niwa, O., T. Matsumoto, Y. Chikashige and M. Yanagida, 1989 Characterization of *Schizosaccharomyces pombe* minichromosome deletion derivatives and a functional allocation of their centromere. EMBO J. **8:** 3045–3052.

Niwa, O., M. Shimanuki and F. Miki, 2000 Telomere-led bouquet formation facilitates homologous chromosome pairing and restricts ectopic interaction in fission yeast meiosis. EMBO J. **19:** 3831–3840.

Ohi, R., A. Feoktistova and K. L. Gould, 1996 Construction of vectors and a genomic library for use with the *his3*-deficient strains of *Schizosaccharomyces pombe*. Gene **174:** 315–318.

Page, S. L., and R. S. Hawley, 2003 Chromosome choreography: the meiotic ballet. Science **301:** 785–789.

Peng, J., A. B. Korol, T. Fahima, M. S. Roder, Y. I. Ronin *et al.*, 2000 Molecular genetic maps in wild emmer wheat, Triticum dicoccoides: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res. **10:** 1509–1531.

Petersen, J., D. Weilguny, R. Egel and O. Nielsen, 1995 Characterization of *fus1* of *Schizosaccharomyces pombe*: a developmentally controlled function needed for conjugation. Mol. Cell. Biol. **15:** 3697–3707.

Petronczki, M., M. F. Siomos and K. Nasmyth, 2003 Un menage a quatre: the molecular biology of chromosome segregation in meiosis. Cell **112:** 423–440.

Ponticelli, A. S., and G. R. Smith, 1989 Meiotic recombination-deficient mutants of *Schizosaccharomyces pombe*. Genetics **123:** 45–54.

Roeder, G. S., 1997 Meiotic chromosomes: it takes two to tango. Genes Dev. **11:** 2600–2621.

Saito, T. T., T. Tougan, D. Okuzaki, T. Kasama and H. Nojima, 2005 Mcp6, a meiosis-specific coiled-coil protein of *Schizosaccharomyces pombe*, localizes to the spindle pole body and is required for horsetail movement and recombination. J. Cell Sci. **118:** 447–459.

Sanchez-Perez, I., S. J. Renwick, K. Crawley, I. Karig, V. Buck *et al.*, 2005 The DASH complex and Klp5/Klp6 kinesin coordinate bipolar chromosome attachment in fission yeast. EMBO J. **24:** 2931–2943.

Scherthan, H., 2001 A bouquet makes ends meet. Nat. Rev. Mol. Cell. Biol. **2:** 621–627.

Schlecht, H. B., M. Lichten and A. S. Goldman, 2004 Compartmentalization of the yeast meiotic nucleus revealed by analysis of ectopic recombination. Genetics **168:** 1189–1203.

Schuchert, P., and J. Kohli, 1988 The *ade6–M26* mutation of *Schizosaccharomyces pombe* increases the frequency of crossing over. Genetics **119:** 507–515.

Shaffer, L. G., and J. R. Lupski, 2000 Molecular mechanisms for constitutional chromosomal rearrangements in humans. Annu. Rev. Genet. **34:** 297–329.

Shimanuki, M., F. Miki, D. Q. Ding, Y. Chikashige, Y. Hiraoka *et al.*, 1997 A novel fission yeast gene, *kms1*$^+$, is required for the formation of meiotic prophase-specific nuclear architecture. Mol. Gen. Genet. **254:** 238–249.

Shimoda, C., A. Hirata, M. Kishida, T. Hashida and K. Tanaka, 1985 Characterization of meiosis-deficient mutants by electron microscopy and mapping of four essential genes in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **200:** 252–257.

Sinclair, D. A., 1975 Crossing over between closely linked markers spanning the centromere of chromosome 3 in *Drosophila melanogaster*. Genet. Res. **26:** 173–185.

Stadler, D. R., and A. M. Towe, 1968 A test of coincident recombination in closely linked genes of Neurospora. Genetics **58:** 327–336.

Stankiewicz, P., and J. R. Lupski, 2002 Genome architecture, rearrangements and genomic disorders. Trends Genet. **18:** 74–82.

Straight, A. F., J. W. Sedat and A. W. Murray, 1998 Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. J. Cell Biol. **143:** 687–694.

Szankasi, P., W. D. Heyer, P. Schuchert and J. Kohli, 1988 DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*. Wild-type and mutant alleles including the recombination hot spot allele *ade6–M26*. J. Mol. Biol. **204:** 917–925.

Tanaka, K., T. Kohda, A. Yamashita, N. Nonaka and M. Yamamoto, 2005 Hrs1p/Mcp6p on the meiotic SPB organizes astral microtubule arrays for oscillatory nuclear movement. Curr. Biol. **15:** 1479–1486.

Trelles-Sticken, E., M. E. Dresser and H. Scherthan, 2000 Meiotic telomere protein Ndj1p is required for meiosis-specific telomere distribution, bouquet formation and efficient homologue pairing. J. Cell Biol. **151:** 95–106.

Tsubouchi, T., H. Zhao and G. S. Roeder, 2006 The meiosis-specific Zip4 protein regulates crossover distribution by promoting synaptonemal complex formation together with Zip2. Dev. Cell **10:** 809–819.

Tuzon, C. T., B. Borgstrom, D. Weilguny, R. Egel, J. P. Cooper *et al.*, 2004 The fission yeast heterochromatin protein Rik1 is required for telomere clustering during meiosis. J. Cell Biol. **165:** 759–765.

van Veen, J. E., and R. S. Hawley, 2003 Meiosis: when even two is a crowd. Curr. Biol. **13:** R831–R833.

Virgin, J. B., and J. P. Bailey, 1998 The *M26* hotspot of *Schizosaccharomyces pombe* stimulates meiotic ectopic recombination and chromosomal rearrangements. Genetics **149:** 1191–1204.

Virgin, J. B., J. P. Bailey, F. Hasteh, J. Neville, A. Cole *et al.*, 2001 Crossing over is rarely associated with mitotic intragenic recombination in *Schizosaccharomyces pombe*. Genetics **157:** 63–77.

West, R. R., T. Malmstrom, C. L. Troxell and J. R. McIntosh, 2001 Two related kinesins, *klp5*$^+$ and *klp6*$^+$, foster microtubule disassembly and are required for meiosis in fission yeast. Mol. Biol. Cell **12:** 3919–3932.

West, R. R., T. Malmstrom and J. R. McIntosh, 2002 Kinesins *klp5*$^+$ and *klp6*$^+$ are required for normal chromosome movement in mitosis. J. Cell Sci. **115:** 931–940.

Yamamoto, A., and Y. Hiraoka, 2001 How do meiotic chromosomes meet their homologous partners?: lessons from fission yeast. BioEssays **23:** 526–533.

Yamamoto, A., R. R. West, J. R. McIntosh and Y. Hiraoka, 1999 A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. J. Cell Biol. **145:** 1233–1249.

Yokobayashi, S., and Y. Watanabe, 2005 The kinetochore protein Moa1 enables cohesion-mediated monopolar attachment at meiosis I. Cell **123:** 803–817.

Young, J. A., R. W. Schreckhise, W. W. Steiner and G. R. Smith, 2002 Meiotic recombination remote from prominent DNA break sites in *S. pombe*. Mol. Cell **9:** 253–263.

Zahn-Zabal, M., E. Lehmann and J. Kohli, 1995 Hot spots of recombination in fission yeast: inactivation of the *M26* hot spot by deletion of the *ade6* promoter and the novel hotspot *ura4-aim*. Genetics **140:** 469–478.

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