DOI: 10.1534/genetics.104.040253

Dynein Promotes Achiasmate Segregation in *Schizosaccharomyces pombe*

Luther Davis and Gerald R. Smith[1]

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

Manuscript received December 21, 2004
Accepted for publication February 25, 2005

ABSTRACT

Most organisms use crossovers (chiasmata) to maintain physical connections between homologous chromosomes that ensure their proper segregation at the first meiotic division. The fission yeast *Schizosaccharomyces pombe* has a residual ability to segregate homologous chromosomes in the absence of meiotic recombination (achiasmate segregation). Using cytologically tagged chromosomes, we established a role for the microtubule motor dynein in meiotic chromosome segregation. Dhc1, the motor subunit of dynein, is required for chromosome segregation in both the presence and the absence of recombination. Dlc1, a member of the Tctex-1 dynein light-chain family, preferentially affects the segregation of achiasmate chromosomes. Dlc1 is the first identified protein, outside of Drosophila, that preferentially affects achiasmate chromosome segregation. We discuss possible roles of the dynein motor in this process.

SEXUALLY reproducing organisms must produce gametes that contain precisely half the somatic number of chromosomes. This is accomplished by a specialized form of cell division, meiosis, that consists of one round of DNA replication followed by two successive rounds of chromosome segregation. After meiotic DNA replication, homologous chromosomes (homologs), each consisting of two sister chromatids, find each other, align, and recombine during an extended prophase. At the first meiotic division (MI) homologs segregate to opposite poles, halving the number of chromosomes. For this reason MI is called a reductional division. At the second meiotic division (MII) sister chromatids segregate to opposite poles, producing four haploid nuclei that differentiate into gametes.

One of the hallmarks of meiosis is an elevated level of genetic recombination. The immediate role of recombination in meiosis is to provide the physical connection between homologs generally required to ensure proper homolog segregation at MI. Meiotic recombination is initiated by a developmentally regulated program that involves the formation of DNA double-strand breaks (DSB) by Rec12, the *Schizosaccharomyces pombe* ortholog of Spo11 (Keeney *et al.* 1997; Cervantes *et al.* 2000). The DNA DSBs are then repaired via an interaction with a homolog, frequently resulting in the formation of crossovers (reviewed in Roeder 1997; Keeney 2001). Although the initiating DNA DSB is crucial, to recombine homologs must also be in close proximity. Clustering of telomeres is observed in meiotic prophase of many organisms and is thought to facilitate the initial interaction between homologs (reviewed in Scherthan 2001; Yamamoto and Hiraoka 2001). In the fission yeast *S. pombe*, telomeres cluster tightly at the spindle pole body (SPB). Telomere clustering is followed, in *S. pombe*, by a telomere-led oscillatory nuclear movement (horsetail movement) that continues throughout prophase (Chikashige *et al.* 1994). Horsetail movement depends completely on the microtubule motor, dynein (Yamamoto *et al.* 1999). Mutations that reduce or eliminate either telomere clustering or horsetail movement also reduce pairing and meiotic recombination (Shimanuki *et al.* 1997; Cooper *et al.* 1998; Nimmo *et al.* 1998; Yamamoto *et al.* 1999; Ding *et al.* 2004).

While crossovers are generally required for proper MI segregation, some organisms exhibit a robust ability to segregate recombinationless, or achiasmate, chromosomes. Male fruit flies, *Drosophila melanogaster*, complete a faithful meiosis in the absence of meiotic recombination (reviewed in Hawley 1989; McKee 1998), but female Drosophila and the budding yeast *Saccharomyces cerevisiae* require recombination to properly complete meiosis (Hall 1972; Klein *et al.* 1999). In the latter cases, however, if only one or two pairs of achiasmate chromosomes per meiosis are present, they are segregated faithfully (reviewed in Dawson *et al.* 1986; Guacci and Kaback 1991; Hawley and Theurkauf 1993). In *S. pombe*, mutants that lack meiotic recombination display a significant residual ability to segregate homologs (achiasmate segregation; Molnar *et al.* 2001a,b; Sharif *et al.* 2002; Davis and Smith 2003).

Segregation of achiasmate chromosomes in Drosophila has been extensively characterized. Drosophila females possess two distinct achiasmate segregation mechanisms (reviewed in Hawley and Theurkauf 1993). One mechanism of achiasmate segregation requires pairing of homologous centromere-proximal hetero-

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

chromatin (Dernburg *et al.* 1996; Karpen *et al.* 1996). In the second mechanism two achiasmate chromosomes, heterologous in this case, segregate from each other with high fidelity but pairing is not observed (Dernburg *et al.* 1996). Additionally, several proteins in Drosophila, including α-tubulin 67C (Matthies *et al.* 1999), the kinesin-like protein Nod (Zhang and Hawley 1990; Zhang *et al.* 1990), Axs (Whyte *et al.* 1993; Kramer and Hawley 2003), and Mtrm (Harris *et al.* 2003), are required preferentially for achiasmate segregation. Some of these proteins are conserved among diverse eukaryotes, allowing the possibility that their role in achiasmate chromosome segregation is also conserved.

In humans, decreased meiotic recombination is associated with MI missegregation events (reviewed in Hassold and Hunt 2001). The resulting aneuploid gametes are associated with ~35% of lost pregnancies (reviewed in Hassold and Hunt 2001). Additionally, aneuploidy, including trisomy 21, which results in Down syndrome, is the leading known cause of mental retardation. Down syndrome is also associated with an increased incidence of leukemia (Iselius *et al.* 1990; Lorber *et al.* 1992; Minelli *et al.* 2001). While it is not known whether humans have a system to segregate achiasmate chromosomes (reviewed in Koehler and Hassold 1998), greater knowledge of the processes that promote proper meiotic segregation may help us to understand the origins of human meiotic aneuploidy.

We thought it likely that the mechanism of achiasmate segregation would require that homologs be brought into close proximity during meiotic prophase. In *S. pombe rec12* mutants, in which meiotic recombination is essentially eliminated (DeVeaux *et al.* 1992; Davis and Smith 2003), pairing is reduced but not abolished (Nabeshima *et al.* 2001; Ding *et al.* 2004). The residual pairing in the *rec12* mutant is most pronounced at the telomeres and centromeres (Ding *et al.* 2004) and, at least at the centromeres, is dependent on Dhc1, the dynein heavy chain (Ding *et al.* 2004). We therefore assayed segregation genetically in several mutants that might, like *dhc1* mutants, have reduced recombination-independent pairing, in both *rec*$^+$ and *rec12*Δ genetic backgrounds. Most mutations had little or no effect on achiasmate segregation (see supplementary materials at http://www.genetics.org/supplemental/). However, as reported here, *dlc1*Δ and *dhc1*Δ had a strong achiasmate segregation defect when assayed cytologically. Our results with these two mutants have demonstrated that the dynein light chain, Dlc1, was required preferentially for achiasmate segregation, while the dynein heavy chain was required for meiotic chromosome segregation in both the presence and the absence of recombination.

MATERIALS AND METHODS

Yeast strains, media, and culture conditions: Strains were grown at 32° on yeast extract agar (YEA) plus histidine (50 μg/ml), leucine (100 μg/ml), lysine (100 μg/ml), and uracil (100 μg/ml) (YEA + 4S; Gutz *et al.* 1974); YEA + 4S plus adenine (100 μg/ml) (YEA + 5S); or supplemented Edinburgh minimal medium 2 (EMM2 as modified in Nurse 1975) solid media. Liquid cultures were grown at 30° in yeast extract liquid + 5S [YEL (Gutz *et al.* 1974) plus five supplements as in YEA + 5S]. Sporulation was at 25° on supplemented sporulation agar (SPA; Gutz *et al.* 1974) for 2–4 days. The yeast strains and mutant alleles used are described below or in references in Table 1.

A complete replacement of the *dhc1* coding sequence with *3HA-6His-kanMX* was performed as follows. Plasmid pFA6a-3HA-6His-kanMX6 (Davis and Smith 2003) was used as template in a polymerase chain reaction (PCR) to generate the *dhc1-D126::kanMX6* allele using the method of Bahler *et al.* (1998). The forward and reverse primers in this reaction contained homology to the 5′ and 3′ ends of *dhc1*$^+$ corresponding, respectively, to nucleotides 7022–7101 of cosmid c30C2 (GenBank accession no. AL355652) and 12826–12905 of cosmid c1093 (GenBank accession no. AL132839). The resulting PCR product was used to transform *S. pombe* strain GP3845 (*h*$^-$ *ade6-M216 ura4-D18 dhc1-D2::ura4*$^+$), which contained a *ura4*$^+$ insertion in the *dhc1* gene. The resulting G418-resistant transformants were screened for the loss of the *ura4*$^+$ marker. Deletion of *dhc1* was confirmed by a PCR.

Detecting aneuploid meiotic products: The frequency of aneuploid meiotic products was determined by random spore analysis. Spores were liberated from asci, and vegetative cells killed, by treatment with glusulase and ethanol (Ponticelli and Smith 1989). Spore suspensions were plated on YEA + 5S and replicated to supplemented SPA after 3–4 days to detect I_2-staining spore colonies (*mat1-P/mat1-M* heterozygous diploids; Bresch *et al.* 1968). Spore suspensions were also plated on YEA + 4S + guanine (100 μg/ml) (YEAG), which inhibits uptake of adenine (Cummins and Mitchison 1967), to detect adenine-prototrophic spores and on YEA + 4S to determine the total number of viable spores. Both *ade6-M210/ade6-M216* heterozygous diploids and heterozygous chromosome III (ChrIII) disomes grow on YEAG. Heterozygous diploids form large colonies after 3 days, while heterozygous ChrIII disomes form small colonies only after 4 or more days. Only the small colonies (ChrIII disomes) were counted on YEAG.

Microscopy: For detection of *lacO*-tagged chromosomes in live cells we used a previously described 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). Cells were mated on supplemented SPA and, after 18–24 hr, zygotes were examined. By this time, many zygotes had undergone both MI and MII. Only those zygotes with four nuclei, as detected by background GFP13–LacI12–NLS fluorescence, were counted. In crosses homozygous for the *lacO* array, zygotes with segregation patterns consistent with premature segregation of sister chromatids at MI were eliminated from the analysis. Stacks of images from 24 focal planes separated by 0.2 μm were captured using SoftWoRx software and a DeltaVision microscope system (Applied Precision), which included an Olympus IX70 microscope and an Olympus UPlan Apo 100× 1.35 NA objective.

RESULTS

Visualization of ChrI segregation indicates a role for both Dhc1 and Dlc1 in achiasmate segregation of homologs at MI: To determine the frequency of homolog segregation at MI, we used strains in which chromosome I

TABLE 1

S. pombe strains

Strain	Genotype
GP4	*h*$^+$ *ade6-M210*
GP2106	*h*$^-$ *ade6-M216 his2 leu1-32*
GP3714	*h*$^-$ *ade6-M210 lys1-37*
GP3716	*h*$^+$ *ade6-M216*
GP3721	*h*$^+$ *ade6-M210 lys1-37 rec12-169::kanMX6*
GP3723	*h*$^-$ *ade6-M216 rec12-169::kanMX6*
GP3845	*h*$^-$ *ade6-M216 ura4-D18 dhc1-D2::ura4*$^+$
GP3894	*h*$^+$ *ade6-M210 lys1-37 ura4-D18 dlc1::ura4*$^+$
GP3898	*h*$^-$ *ade6-M216 ura4-D18 dlc1::ura4*$^+$
GP4000	*h*$^-$ *ade6-M210 lys1-37 ura4-D18 rec12-171::ura4*$^+$
GP4002	*h*$^+$ *ade6-M216 ura4-D18 rec12-171::ura4*$^+$
GP4080	*h*$^+$ *ade6-M210 lys1-37 ura4-D18 dhc1-D126::kanMX6*
GP4081	*h*$^+$ *ade6-M210 lys1-37 ura4-D18 dhc1-D126::kanMX6 rec12-171::ura4*$^+$
GP4082	*h*$^-$ *ade6-M216 ura4-D18 dhc1-D126::kanMX6*
GP4083	*h*$^-$ *ade6-M216 ura4-D18 dhc1-D126::kanMX6 rec12-171::ura4*$^+$
GP4249	*h*$^+$ *ade6-M216 ura4-D18 dlc1::ura4*$^+$ *rec12-171::ura4*$^+$
GP4250	*h*$^-$ *ade6-M210 lys1-37 ura4-D18 dlc1::ura4*$^+$ *rec12-171::ura4*$^+$
GP4957	*h*$^+$ *leu1-32 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$
GP4958	*h*$^-$ *leu1-32 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$
GP4959	*h*$^+$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *rec12-171::ura4*$^+$
GP4960	*h*$^-$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *rec12-171::ura4*$^+$
GP4961	*h*$^+$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$
GP4962	*h*$^-$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$
GP4963	*h*$^+$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$ *rec12-171::ura4*$^+$
GP4964	*h*$^-$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$ *rec12-171::ura4*$^+$
GP5099	*h*$^+$ *leu1-32 ura4-D18 lys1-37 GFP-lacI@his7*$^+$ *rec12-171::ura4*$^+$
GP5101	*h*$^+$ *leu1-32 ura4-D18 lys1-37 GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$
GP5103	*h*$^+$ *leu1-32 ura4-D18 lys1-37 GFP-lacI@his7*$^+$ *dlc1::ura4*$^+$ *rec12-171::ura4*$^+$
GP5117	*h*$^-$ *leu1-32 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6*
GP5118	*h*$^+$ *leu1-32 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6*
GP5120	*h*$^+$ *leu1-32 lys1-37 GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6*
GP5121	*h*$^-$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6 rec12-171::ura4*$^+$
GP5122	*h*$^+$ *leu1-32 ura4-D18 lacO@lys1*$^+$ *GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6 rec12-171::ura4*$^+$
GP5124	*h*$^+$ *leu1-32 ura4-D18 lys1-37 GFP-lacI@his7*$^+$ *dhc1-D126::kanMX6 rec12-171::ura4*$^+$

The strains were derived from lab stocks by meiotic crosses. Complete genealogies are available upon request. Alleles are commonly used auxotrophic markers, the mating-type locus and *dhc1-D2::ura4*$^+$ (Yamamoto *et al.* 1999), *dhc1-D126::kanMX6* (see materials and methods), *dlc1::ura4*$^+$ (Miki *et al.* 2002), *lys1-37::lacO-lys1*$^+$ (*lacO@lys1*$^+$; Nabeshima *et al.* 1998), *rec12-169::3HA6His-kanMX6*, *rec12-171::ura4*$^+$, and *his7-366::GFP13-lacI12-NLS-his7*$^+$ (*GFP-lacI@his7*$^+$; Davis and Smith 2003).

(ChrI) was marked near the centromere with a tandem array of *lacO* DNA (Nabeshima *et al.* 1998). This array can be visualized by fluorescence microscopic observation of the GFP–LacI–NLS fusion protein that binds it (Straight *et al.* 1996). We performed crosses homozygous for the *lacO* array in both a *rec*$^+$ and *rec12*Δ background and examined zygotes that had undergone MII. Faithful MI homolog segregation would result in one *lacO*-containing chromatid in each of the four nuclei. As the nuclei in *S. pombe* asci are ordered (Kitajima *et al.* 2003), missegregation of a homolog at MI would result in four *lacO*-containing chromatids in one pair of sister nuclei (the two nuclei at one end of a zygote) and none in the other pair (see Figure 1).

In a *rec*$^+$ background, the *dlc1*Δ mutant showed high levels of proper homolog segregation (99%, Table 2) that were not significantly different from wild type ($\chi^2 = 0.20$; $P > 0.5$). In contrast, the frequency of proper homolog segregation in the *dhc1*Δ mutant (91%, Table 2) was reduced relative to that in wild type ($\chi^2 = 12.9$; $P < 0.0005$). The frequency of proper segregation in a *rec12*Δ background, 66%, was reduced significantly by both the *dlc1*Δ and *dhc1*Δ mutations (52 and 53%, Table 2; $\chi^2 = 8.32$, $P < 0.005$; $\chi^2 = 6.07$, $P < 0.02$, respectively). Segregation was not significantly different from random, 50%, in either double mutant ($\chi^2 = 0.10$, $P > 0.7$ and $\chi^2 = 0.22$, $P > 0.5$, respectively). These data indicate that, while Dlc1 played a role preferentially in achiasmate segregation, Dhc1 was required for chromosome segregation in both the presence and the absence of recombination.

We occasionally observed segregation patterns consis-

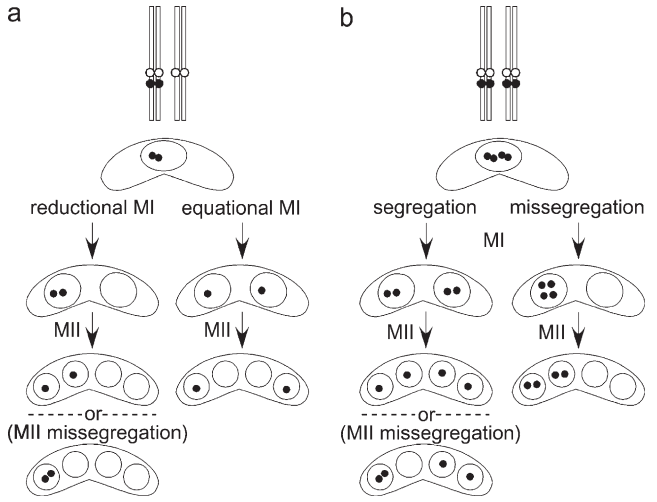

FIGURE 1.—Characteristic patterns of GFP–LacI–NLS staining in strains heterozygous (a) and homozygous (b) for a *lacO*-tagged ChrI. Solid circles represent the *lacO* array. Open circles represent the centromere of ChrI. See RESULTS for description. Not all missegregation patterns are illustrated.

TABLE 2

Dlc1 and Dhc1 are required for achiasmate segregation

		Homologous chromosomes at MI	
Parental genotype	n^a	Proper segregation (%) [b]	Missegregation (%) [b]
rec$^+$	225	99	1
*dhc1*Δ	157	91	9
*dlc1*Δ	136	99	1
*rec12*Δ	324	66	34
*dhc1*Δ *rec12*Δ	148	53	47
*dlc1*Δ *rec12*Δ	180	52	48

Crosses homozygous for the *lacO* array near centromere I were performed, and zygotes were examined by fluorescence microscopy after 18–24 hr on sporulation medium. Zygotes with four nuclei, as detected by background GFP13–LacI12–NLS fluorescence, were scored for the number and location of *lacO* signals. The segregation pattern of homologs at MI was inferred as described in Figure 1 and RESULTS.

[a] Number of zygotes counted.

[b] See Figure 1 for a description of the segregation patterns.

tent with premature segregation of sister chromatids (PSSC) in MI and also missegregation of sister chromatids at MII (data not shown). In these crosses, which were homozygous for the *lacO* array, it was not always possible to distinguish these two segregation patterns. To more precisely determine the frequency of other types of segregation errors, we examined the behavior of sister chromatids in crosses heterozygous for the *lacO* array. The results of the heterozygous crosses (below) were consistent with the observed frequency of PSSC and MII missegregation in homozygous crosses.

Visualization of ChrI segregation indicates that the absence of Rec12, Dlc1, or Dhc1 modestly affects the cosegregation of sister chromatids at MI: MI of wild-type meiosis is reductional, that is, sister chromatids move together to the same pole and homologs move to opposite poles. To determine whether sister chromatids move together to the same pole at MI (cosegregate) in *dlc1*Δ, *dhc1*Δ, and *rec12*Δ mutant meioses, we performed crosses heterozygous for the *lacO* array. We examined zygotes that had undergone MII and determined whether or not *lacO*-containing sister chromatids were in sister nuclei. If the first division is reductional-like, both sister chromatids containing the array will be in one pair of sister nuclei and none in the other pair. In contrast, one *lacO*-containing sister chromatid in each pair of sister nuclei indicates an equational first division (see Figure 1). In wild-type crosses, *lacO* cosegregated in 87% of MI divisions (Table 3). The *lacO* array is integrated at the *lys1* gene, ~4 cM from the centromere in wild-type meiosis (WATANABE and NURSE 1999). The frequency of apparent sister chromatid segregation at MI in wild type can be explained by recombination between the centromere and the *lacO* array and is consistent with previously published results (WATANABE and NURSE 1999). In *rec12*Δ, *dlc1*Δ, *dhc1*Δ, *rec12*Δ *dlc1*Δ, and *rec12*Δ *dhc1*Δ mutants, sister chromatids cosegregated at MI at least 92% of the time (Table 3). Because meiotic recombination is reduced or abolished in these mutants (DEVEAUX *et al.* 1992; YAMAMOTO *et al.* 1999; MIKI *et al.* 2002; DAVIS and SMITH 2003), recombination between the centromere and the *lacO* array is unlikely to significantly influence these results. These data indicate that Rec12, Dlc1, and Dhc1 play a small but significant role in ensuring that sister chromatids cosegregate at MI.

We were also able to detect MII errors. If sister chromatids cosegregate at MI but missegregate at MII, both *lacO*-containing sister chromatids would be contained in a single nucleus (see Figure 1). While sister chromatid segregation at MII in *dlc1*Δ or *dhc1*Δ single mutants was not different from that in wild type, the frequency of MII missegregation in *rec12*Δ and *rec12*Δ *dhc1*Δ mutants was 8 and 9%, respectively (Table 3). These data indicate that, although none of the mutants tested were severely impaired, sister chromatid missegregation at MII was elevated in *rec12*Δ relative to wild type ($\chi^2 = 5.85$, $P < 0.02$). A similar conclusion, based on genetic assays of segregation, was reached by SHARIF *et al.* (2002).

Genetic assays of segregation suggest a role for Dhc1 in the presence of recombination: In the first meiotic division, homologous chromosomes segregate to opposite poles. In the absence of recombination between a heterozygous marker and the centromere, this reductional segregation eliminates heterozygosity in the products of meiosis. However, if homologous chromosomes missegregate at MI (*i.e.*, if both homologs remain together), heterozygosity is retained. Because *S. pombe* has only three chromosomes, even mutants that segregate homologs at random are expected to produce a significant number of viable progeny. Nonetheless, if homo-

TABLE 3

Sister chromatids predominantly cosegregate at MI in *dlc1*Δ, *dhc1*Δ, and *rec12*Δ mutants

		Sister chromatids		
Parental genotype[a]	n[b]	MI cosegregation (%)[c]	MI missegregation (%)[c]	MII missegregation (%)[c]
rec$^+$	183	87	13	2
*dhc1*Δ	157	92	8	2
*dlc1*Δ	63	94	6	2
*rec12*Δ	236	94	6	8
*dhc1*Δ *rec12*Δ	121	93	7	9
*dlc1*Δ *rec12*Δ	44	98	2	<7

Crosses heterozygous for the *lacO* array near centromere I were performed, and zygotes were examined by fluorescence microscopy after 18–24 hr on sporulation medium. Zygotes with four nuclei, as detected by background GFP13–LacI12–NLS fluorescence, were scored for the number and location of *lacO* signals. The segregation pattern of sister chromatids at MI was inferred as described in Figure 1 and RESULTS.

[a] Crosses were homozygous for the indicated mutations, except the *rec*$^+$ cross was heterozygous for the *rec12*Δ allele.

[b] Number of zygotes counted.

[c] See Figure 1 for a description of the segregation patterns.

logs missegregate at MI, an elevated frequency of heterozygous meiotic products is expected (Figure 2 and DAVIS and SMITH 2003). These meiotic products include heterozygous diploids and heterozygous ChrIII disomes, the only aneuploids that have been propagated in *S. pombe* (NIWA and YANAGIDA 1985; MOLNAR and SIPICZKI 1993). These features of *S. pombe* biology allowed us to characterize meiotic segregation by genetic analysis of the products of meiosis.

Mutations that specifically abolish achiasmate segregation by definition would not alter segregation nor, therefore, the frequency of heterozygous meiotic products, in the presence of meiotic recombination. To determine if this was true of *dhc1*Δ and *dlc1*Δ mutants, we analyzed their segregation phenotype in a *rec12*$^+$ (chiasmate) background. Crosses heterozygous for the complementing *ade6* alleles, *M210* and *M216*, allowed missegregation of ChrIII to be assayed by formation of Ade$^+$ colonies (MORENO *et al.* 1991). Because formation of a heterozygous ChrIII disome requires only one missegregation event (that of ChrIII), the frequency of heterozygous ChrIII disomes among viable spores increases linearly as the frequency of MI missegregation increases (Figure 2 and DAVIS and SMITH 2003). The frequency of heterozygous ChrIII disomes is predicted to increase from 0.06 ± 0.01% of viable spores, as observed in wild-type meioses (Table 4), to 30.7% of viable spores if homolog segregation at MI is random (Figure 2 and DAVIS and SMITH 2003), an increase of 500-fold. The observed ChrIII disome frequencies of *dlc1*Δ mutant crosses were 1.4 ± 0.1%, ~25 times higher than that of wild type (Table 4). The *dhc1*Δ mutant was more severely affected, with a ChrIII disome frequency (9.1 ± 0.7%; Table 4) that, although >150 times higher than that of wild type, was lower than that of the *rec12*Δ mutant (23 ± 1.7%; Table 4). These data suggest that Dhc1 plays a significant role in MI segregation even in the presence of recombination.

Formation of a heterozygous diploid requires three missegregation events (those of ChrI, II, and III). Therefore, the frequency of heterozygous diploids among viable spores does not increase markedly until the frequency of MI missegregation is >~30% (Figure 2 and DAVIS and SMITH 2003). The frequencies of heterozygous diploids (assayed as Spo$^+$, resulting from *mat1-P/mat1-M* heterozygosity on ChrII) among viable spores in the *dhc1*Δ and *dlc1*Δ mutant crosses was low (Table 4), consistent with ChrIII disome results.

Genetic assays of segregation suggest a role for Dlc1 specific to achiasmate segregation: Mutations that specifically abolish achiasmate segregation should result in an increased frequency of heterozygous meiotic products only when combined with a mutation, such as *rec12*Δ, that eliminates meiotic recombination. To determine if *dhc1*Δ and *dlc1*Δ had a more dramatic effect on MI segregation in the absence of recombination, we analyzed their segregation phenotype in a *rec12*Δ (achiasmate) background. As above, crosses were performed heterozygous for the complementing *ade6* alleles, *M210* and *M216*. In a *rec12*Δ background the formation of heterozygous ChrIII disomes among viable spores is not predicted to be very sensitive to increased missegregation because the missegregation frequency is already high (Figure 2 and DAVIS and SMITH 2003). Accordingly, neither the *rec12*Δ *dhc1*Δ nor the *rec12*Δ *dlc1*Δ crosses had a frequency of heterozygous ChrIII disomes significantly higher than that in the *rec12*Δ single mutant (Table 4).

In contrast, the formation of heterozygous diploid spores in a *rec12*Δ background is predicted to be very sensitive to increased missegregation. The frequency of heterozygous diploids is predicted to increase from

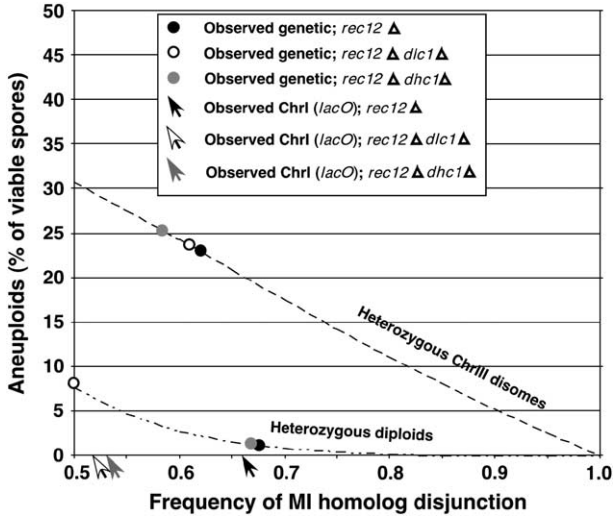

FIGURE 2.—Expected frequency of heterozygous meiotic products at homolog segregation frequencies of 50–100%. The formulas used to construct the graph have been described (DAVIS and SMITH 2003). The observed frequencies of heterozygous spore colonies characteristic of MI homolog missegregation (Table 4) are plotted on the appropriate expectation curve and are indicated by solid circles (*rec12*Δ), open circles (*rec12*Δ *dlc1*Δ), and shaded circles (*rec12*Δ *dhc1*Δ). The solid (*rec12*Δ), open (*rec12*Δ *dlc1*Δ), and shaded (*rec12*Δ *dhc1*Δ) arrows indicate the frequency of proper segregation by ChrI as observed cytologically (Table 2).

1.1 ± 0.2% of viable spores, as observed in *rec12*Δ meioses (Table 4), to 7.7% of viable spores if homolog segregation at MI is random (Figure 2 and DAVIS and SMITH 2003). In a *rec12*Δ mutant background *dhc1*Δ mutant crosses produced no more heterozygous diploid meiotic products than did the *rec12*Δ single-mutant cross (Table 4). The *dlc1*Δ *rec12*Δ double-mutant crosses resulted in 8.1 ± 0.7% heterozygous diploid meiotic products (Table 4), consistent with random segregation of homologs at MI (7.7% heterozygous diploids predicted; Figure 2). Together with the data from *rec12*$^+$ meioses, these data suggest that Dlc1 is required preferentially for achiasmate segregation. Interestingly, while *dlc1*Δ *rec12*Δ double mutants yielded elevated frequencies of heterozygous diploid spores, the *dhc1*Δ *rec12*Δ crosses did not result in more heterozygous diploid spores than the *rec12*Δ crosses (1.5 ± 0.3% and 1.1 ± 0.2%, respectively; Table 4). The discrepancy between the genetic results, which indicate no significant role for Dhc1 in achiasmate segregation, and the cytological results is discussed below.

DISCUSSION

While it appears to be generally true that reciprocal recombination is required for proper MI segregation, in many organisms recombination-independent mechanisms can promote the proper segregation of achiasmate chromosomes. Here, we provide evidence that the microtubule motor dynein promotes meiotic chromosome segregation in both the presence and the absence of recombination. Additionally, we identify *dlc1*Δ as the first mutation, outside Drosophila, that preferentially affects the segregation of achiasmate chromosomes.

The dynein light chain, Dlc1, is required for achiasmate segregation: Analysis of *dlc1*Δ meioses indicated that chromosome missegregation was only modestly elevated by the absence of Dlc1 in *rec*$^+$ (chiasmate) meioses. The frequency of ChrIII disomes (Table 4) observed in *dlc1*Δ crosses was consistent with a homolog missegregation frequency of ~3% (Figure 2). The frequency of missegregation in *dlc1*Δ measured by direct observation of *lacO*-tagged ChrI, 1% (Table 2), was not significantly different from that expectation ($\chi^2 = 0.81$; $P > 0.3$). MIKI *et al.* (2002) measured crossovers in *dlc1*Δ mutants in five nonoverlapping intervals that constitute ~4% of

TABLE 4

Genetic assays of segregation defects in *rec*$^+$ and *rec12*Δ background

Genotype	Strains crossed	Heterozygous ChrIII disomes (% of viable spores) [a]	Heterozygous diploids (% of viable spores) [b]
Wild type	[c]	0.06 ± 0.01 (8)	0.9 ± 0.3 (8)
*dhc1*Δ	GP4080 × GP4082	9.1 ± 0.7 (4)	<0.3 (4)
*dlc1*Δ	GP3894 × GP3898	1.4 ± 0.1 (3)	1.8 ± 0.4 (3)
*rec12*Δ	[d]	23.0 ± 1.7 (14)	1.1 ± 0.2 (14)
*dhc1*Δ *rec12*Δ	GP4081 × GP4083	25.1 ± 1.9 (4)	1.5 ± 0.3 (4)
*dlc1*Δ *rec12*Δ	GP4249 × GP4250	23.5 ± 2.0 (3)	8.1 ± 0.7 (3)

[a] Spores were plated on YEAG to assay ChrIII heterozygosity. Small Ade$^+$ colonies were counted as ChrIII disomes (see MATERIALS AND METHODS). Values are mean ± SEM (from *n* experiments).

[b] Spores were plated on YEA + 5S. Colonies were replicated to supplemented SPA to assay heterozygous diploids by staining with I_2 to determine the frequency of Spo$^+$ (*mat1-P/mat1-M* heterozygous) spores. Values are mean ± SEM (from *n* experiments). Where no heterozygous diploid spores were observed, the upper 95% confidence limit based on the Poisson distribution is given.

[c] Data are from five GP3714 × GP3716 and three GP4 × GP2106 crosses.

[d] Data are from six GP3721 × GP3723 and eight GP4000 × GP4002 crosses.

the genome; recombination frequencies were reduced <10-fold relative to wild type. If recombination were decreased by a factor of five uniformly throughout the genome, the mean number of crossovers per homolog would be 3.8, 3, and 2.2 for chromosomes I, II, and III, respectively (Munz 1994). Since *S. pombe* lacks crossover interference (Munz 1994), the frequency of achiasmate ChrI, -II, and -III would be expected to be the corresponding Poisson null terms of 0.02, 0.05, and 0.11, respectively. Missegregation of the infrequent achiasmate chromosomes thus appears to be sufficient to explain the segregation defect we observed in *dlc1*Δ (*rec*$^+$) meioses. Our data indicate that when crossovers are present, Dlc1 plays at most a modest role in faithful chromosome segregation at MI. In contrast, *dlc1*Δ had a more severe effect in a *rec12*Δ background. Direct observation of *lacO*-tagged ChrI showed that segregation of homologs was reduced from 66% in *rec12*Δ to 52% in *dlc1*Δ *rec12*Δ, *i.e.*, nearly random (Table 2). The frequency of proper segregation in *dlc1*Δ *rec12*Δ crosses was significantly different from that of *rec12*Δ (χ^2 = 8.32; $P < 0.005$) but was not significantly different from random (χ^2 = 0.10; $P > 0.7$). Our genetic results also are consistent with completely random segregation of homologs at MI; the level of heterozygous diploid meiotic products seen in *dlc1*Δ *rec12*Δ meioses (8.1%, Table 4) was not significantly different from that predicted for random segregation (7.7%; $\chi^2 = 0.08$, $P > 0.7$). Our data indicate that Dlc1 is required preferentially for achiasmate segregation.

There are two ways to think about the role of Dlc1 in achiasmate segregation. Dlc1 may play a novel role in achiasmate segregation that is distinct from its minor role in wild-type meiosis. For instance, in *rec12*Δ, but not *rec12*$^+$, meioses, Dlc1 may be required for homologous association of centromeres (discussed below). Alternatively, Dlc1 may play the same role as in wild-type meiotic chromosome segregation but this role becomes crucial only in the absence of recombination. For instance, the aberrant horsetail movement observed in the absence of Dlc1 (Miki *et al.* 2002) may not perturb homologous interactions that are stabilized by crossovers (*rec12*$^+$), but it may severely perturb homologous interactions that are not stabilized by crossovers (*rec12*Δ).

Cytological data indicate that the dynein heavy chain, Dhc1, is required for achiasmate segregation: Analysis of *dhc1*Δ meioses indicates that horsetail movement or some other function of Dhc1 is required for faithful MI homolog segregation in *rec*$^+$ meiosis. The observed ChrIII disome frequency (Table 4), our most sensitive genetic assay, is consistent with a homolog missegregation frequency of ~15% (Figure 2). The frequency of missegregation in *dhc1*Δ measured by direct observation of *lacO*-tagged ChrI, 9% (Table 2), is not significantly different from that expectation ($\chi^2 = 2.21$; $P > 0.10$). The level of missegregation we observed in *dhc1*Δ meioses cannot be explained by the modest decrease in recombination observed by others in *dhc1* mutants (Yamamoto *et al.* 1999; see above). In fact, meiotic recombination is reduced less in the *dhc1*Δ mutant than in the *dlc1*Δ mutant in all three genetic intervals tested in both mutants (Yamamoto *et al.* 1999; Miki *et al.* 2002). The level of missegregation that we observe in the *dhc1*Δ mutant suggests that, in addition to its essential role in horsetail movement (Yamamoto *et al.* 1999), Dhc1 may be directly involved in the segregation of homologs on the MI spindle. Alternatively, the recombination events in *dhc1*Δ meioses may not efficiently hold homologs together. Strikingly, while direct observation of *lacO*-tagged ChrI indicated that Dhc1 was required for achiasmate segregation, the genetic assays did not suggest a role for Dhc1 in achiasmate segregation (Table 2). In the genetic assay, heterozygous diploid formation in the *dhc1*Δ *rec12*Δ mutant was not significantly different from that in the *rec12*Δ mutant (Table 4). This is in contrast to direct observation using *lacO*-tagged ChrI in which proper segregation of homologs was reduced from 66% in *rec12*Δ to 53% in *dhc1*Δ *rec12*Δ, a significant difference (Table 2; $\chi^2 = 6.07$, $P < 0.02$). Segregation in *dhc1*Δ *rec12*Δ was not significantly different from random (χ^2 = 0.22; $P > 0.5$). Because, unlike the genetic assays, observation of *lacO*-tagged ChrI is a direct measure of homolog segregation, we consider the cytological data to indicate an important role for Dhc1 in achiasmate segregation.

A likely cause of the disparity between our genetic and cytological results for the *dhc1*Δ *rec12*Δ mutant (as well as the failure to measure an increase, relative to *rec12*Δ, in ChrIII disome formation in *dhc1*Δ *rec12*Δ and *dlc1*Δ *rec12*Δ mutants) is the indirect nature of the genetic assays. While genetic analysis can easily generate large data sets, it is limited to viable meiotic products. Additionally, the genetic assays (heterozygous diploid and ChrIII disomic spore formation; Figure 2, Table 4) are interpretable only if several assumptions are made (see Davis and Smith 2003). Two of these assumptions are particularly relevant:

1. The only type of segregation error is MI homolog missegregation. In the case of the mutants tested in this article by direct observation using *lacO*-tagged ChrI, we know that this is not strictly true. Sister chromatids occasionally segregated from each other at MI in the mutants tested, and *rec12*Δ mutants occasionally missegregated sister chromatids at MII (Table 3).
2. Heterozygous diploids and ChrIII disomes are as likely to form a colony as a haploid cell. This is most likely to be a problem for mutants with a mitotic phenotype. Several mutants of *S. pombe* are viable as haploids but are not stable as diploids (Bernard *et al.* 1998; Catlett and Forsburg 2003). Both Dhc1 and Dlc1 play a role in attachment of chromosomes to the mitotic spindle (referenced in Yamamoto and Hiraoka 2003). Perhaps *dhc1*Δ diploids (as well as

*dhc1*Δ and *dlc1*Δ ChrIII disomes) are less stable than wild type. While it is difficult to predict the degree to which a deviation from these assumptions might influence the results of the genetic assays, these caveats complicate interpretation of the genetic assays and may explain the difference between the *dhc1*Δ *rec12*Δ genetic and cytological results.

Because of the ease of assaying many meioses with the genetic assays, we tested other likely candidates for missegregation in the presence and absence of Rec12. These candidates include Taz1 (Cooper *et al.* 1998; Nimmo *et al.* 1998; Ding *et al.* 2004), Kms1 (Shimanuki *et al.* 1997; Niwa *et al.* 2000), Meu13 (Nabeshima *et al.* 2001), Hopl (Lorenz *et al.* 2004), and Mad2 (He *et al.* 1997). The results are provided in the supplementary materials (Tables S1–S3; http://www.genetics.org/supplemental/) and suggest that some of these proteins may play a role in achiasmate segregation.

The role of dynein in achiasmate segregation: Taken together, our results indicate that Dhc1 and Dlc1 promote the proper segregation of achiasmate chromosomes. Because both Dhc1 and Dlc1 are required for proper horsetail movement (Yamamoto *et al.* 1999; Miki *et al.* 2002), the recent examination of the role of recombination and horsetail movement in homologous association during prophase by Hiraoka and colleagues (Ding *et al.* 2004) is directly relevant. In brief, telomere clustering and horsetail movement are required to bring chromosomes into close alignment; recombination then stabilizes homologous associations. Significant levels of homologous association are retained in *rec12* mutants, especially at the centromeres and telomeres. Importantly, homologous association of centromeres is eliminated in the *dhc1 rec12* mutant (Ding *et al.* 2004), and proper segregation in the *dhc1*Δ *rec12*Δ mutant was reduced to random (Table 2). It is interesting to note that pairing of centric heterochromatin is required in one of the two distinct achiasmate segregation mechanisms possessed by Drosophila females (Dernburg *et al.* 1996; Karpen *et al.* 1996). Additionally, centromere pairing of achiasmate chromosomes has recently been observed in *S. cerevisiae* (Kemp *et al.* 2004). These data lead us to suggest that pairing of centric heterochromatin is important for achiasmate segregation in *S. pombe*. A conserved mechanism of centromere pairing may be widely important in the segregation of achiasmate chromosomes.

Other, though not necessarily mutually exclusive, mechanisms may promote achiasmate segregation in *S. pombe*. One such mechanism is suggested by the second achiasmate system in Drosophila females. As mentioned above, one mechanism of achiasmate segregation in Drosophila females requires pairing of homologous centromere-proximal heterochromatin (Dernburg *et al.* 1996; Karpen *et al.* 1996). However, in the second mechanism two achiasmate chromosomes, heterologous in this case, segregate from each other with high fidelity. Interestingly, pairing is not observed (Dernburg *et al.* 1996), and the heterologous chromosomes are thought to orient toward the less crowded pole, thus assuring that one will go to each pole. It is interesting to speculate that this type of mechanism might be especially effective in an organism, like *S. pombe*, that makes only a small number of microtubule connections per kinetechore (Ding *et al.* 1993). A mechanism, such as this, that relies on spatial or numerical constraints might be more effective if fewer chromosomes were achiasmate. This possibility has not been sufficiently tested in *S. pombe*.

This type of mechanism might be especially sensitive to perturbation of meiotic spindle function. Given the localization of Dlc1 to the spindle pole body at MI (Miki *et al.* 2002), and the function of dynein in spindle assembly and mitotic chromosome movement in metazoans (reviewed in Banks and Heald 2001), mutations in Dlc1 or Dhc1 may result in perturbation of meiotic spindle function. In fact, regardless of the underlying mechanism promoting achiasmate segregation, altered spindle function in the absence of Dlc1 or Dhc1 could lead to the observed failure of achiasmate segregation.

We are grateful to Julia Cooper, Yasushi Hiraoka, Osami Niwa, Hiroshi Nojima, Shelly Sazer, Takahiro Tougan, and Mitsuhiro Yanagida for providing strains; to Takahiro Tougan, Hiroshi Nojima, Ayumu Yamamoto, and Yasushi Hiraoka for sharing unpublished data; and to Sue Amundsen, Sue Biggins, Gareth Cromie, Joseph Farah, and Andrew Taylor for helpful comments on the manuscript. This work was supported by National Institutes of Health research grant GM32194 to G.R.S. and postdoctoral fellowship F32-GM20125 to L.D.

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Communicating editor: R. S. HAWLEY