Centromere Mapping Functions for Aneuploid Meiotic Products: Analysis of *rec8*, *rec10* and *rec11* Mutants of the Fission Yeast *Schizosaccharomyces pombe*

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ABSTRACT

Recent evidence suggests that the position of reciprocal recombination events (crossovers) is important for the segregation of homologous chromosomes during meiosis I and sister chromatids during meiosis II. We developed genetic mapping functions that permit the simultaneous analysis of centromere-proximal crossover recombination and the type of segregation error leading to aneuploidy. The mapping functions were tested in a study of the *rec8*, *rec10*, and *rec11* mutants of fission yeast. In each mutant we monitored each of the three chromosome pairs. Between 38 and 100% of the chromosome segregation errors in the *rec8* mutants were due to meiosis I nondisjunction of homologous chromosomes. The remaining segregation errors were likely the result of precocious separation of sister chromatids, a previously described defect in the *rec8* mutants. Between 47 and 100% of segregation errors in the *rec10* and *rec11* mutants were due to nondisjunction of sister chromatids during meiosis II. In addition, centromere-proximal recombination was reduced as much as 14-fold or more on chromosomes that had experienced nondisjunction. These results demonstrate the utility of the new mapping functions and support models in which sister chromatid cohesion and crossover position are important determinants for proper chromosome segregation in each meiotic division.

DURING meiosis each chromosome undergoes one round of DNA replication; homologous chromosomes then pair and undergo recombination. Subsequently, homologous chromosomes segregate from one another during meiosis I (MI) and then sister chromatids segregate from one another during meiosis II (MII). In most organisms, reciprocal recombination events (crossovers) help ensure proper MI segregation (Roeder 1997).

Recent evidence suggests that the position of crossovers has an important role in chromosome segregation during both MI and MII. In several organisms, chromosomes that suffer spontaneous MI nondisjunction (MI NDJ) exhibit a preferential reduction of crossovers near the centromeres (Gethmann 1984; Rasooly *et al.* 1991; Ross *et al.* 1992; MacDonald *et al.* 1994; Moore *et al.* 1994; Sherman *et al.* 1994; Hassold *et al.* 1995). In contrast, chromosomes that suffer spontaneous MII nondisjunction (MII NDJ) exhibit an elevated frequency of crossovers near their centromeres (MacDonald *et al.* 1994; Fisher *et al.* 1995; Koehler *et al.* 1996a,b; Lamb *et al.* 1996). Models invoking a synergistic interaction between crossover position and sister chromatid cohesion can best explain these observations (Moore and Orr-Weaver 1998; Krawchuk *et al.* 1999).

The *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes of the fission yeast *Schizosaccharomyces pombe* were identified in a screen for mutations that decrease meiotic recombination (Ponticelli and Smith 1989; Lin *et al.* 1992; Lin and Smith 1995; Li *et al.* 1997). In each mutant, crossover recombination is preferentially reduced toward the centers of each of the three chromosomes and, intriguingly, the mutations differentially affect chromosome segregation during MI and MII (Molnar *et al.* 1995; Krawchuk *et al.* 1999; Parisi *et al.* 1999). The *rec8* mutants suffer aberrant segregation of homologous chromosomes during MI, at least part of which is attributable to the precocious separation of sister chromatids (PSS). The *rec10* and *rec11* mutants suffer aberrant segregation of sister chromatids during MII. The three genes are proposed to define a pathway of "meiotic chromatid cohesion" important for proper chromosome segregation in both meiotic divisions (Krawchuk *et al.* 1999). We therefore developed mapping functions to simultaneously measure centromere-proximal recombination and to determine the segregation patterns of each of the three *S. pombe* chromosomes in aneuploid meiotic products from each of the *rec8*, *rec10*, and *rec11* mutants.

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MATERIALS AND METHODS

***S. pombe* strains and culture media:** The *S. pombe* strains used for this study are listed in Table 1. Rich medium was yeast extract agar (YEA) or liquid (YEL); sporulation media was synthetic sporulation agar (SPA); minimal media was nitrogen base agar (NBA) or liquid (NBL). All media formulations and culture techniques were as described (Gutz *et al.* 1974). YEA-B was YEA containing 2.5 μg/ml of Phloxin-B (Sigma,

TABLE 1

***S. pombe* strains**

Strain[a]	Genotype
GP 424	*h*⁻ *ade6-52 ura4-595 pro2-1 rec10-109*
GP 426	*h*⁺ *ade6-52 ura4-595 pro2-1 rec8-110*
GP 428	*h*⁻ *ade6-52 ura4-595 pro2-1 rec11-111*
GP 434	*h*⁺ *ade6-M26 ura4-294 arg3-124 rec10-109*
GP 436	*h*⁻ *ade6-M26 ura4-294 arg3-124 rec8-110*
GP 437	*h*⁺ *ade6-M26 ura4-294 arg3-124 rec11-111*

[a] Strains were as described (DeVeaux *et al.* 1992).

St. Louis) and 100 μg/ml of adenine. SPA, NBA, and NBL were supplemented with required amino acids, adenine, and uracil at 100 μg/ml. The growth factor requirement of *pro2* mutants is partially satisfied by arginine. Therefore, citrulline (50 μg/ml), which does not satisfy the growth factor requirement of the *pro2* mutants, was used to satisfy the growth factor requirement of the *arg3* mutants.

Mating and meiosis: Strains to be crossed were grown at 32° in 5 ml of YEL to a density of 1×10^7 cells/ml. The cultures were combined, the cells were harvested by centrifugation, washed with water, resuspended in 100 μl of water, and spotted on SPA. The mating mixtures were incubated at room temperature for 3–5 days. Asci and vegetative cells were harvested into 0.5 ml of 0.5% glusulase and incubated at room temperature for 8 hr to liberate spores and kill vegetative cells. The suspensions were then treated with an equal volume of 60% ethanol for 30 min to kill any remaining vegetative cells. Spore suspensions were washed once with water, diluted, and plated on the appropriate media.

Diploid spore isolation and haploidization analysis: Spores were plated on YEA-B and incubated for 3 days at 32° to distinguish the diploid spore colonies from the haploid spore colonies. On Phloxin-B-containing plates, haploid cells produce light pink colonies whereas diploid cells produce dark pink colonies (Gutz *et al.* 1974). The ploidy was confirmed by microscopic examination of the cells. Diploid cells are both longer and wider than haploid cells and can be readily distinguished under the microscope.

The parental haploid strains that were crossed had different alleles at several loci on all three chromosomes. The genotypes of the resulting diploid spore colonies were determined by analysis of haploidized colonies on diagnostic plates. Diploid spore colonies were haploidized with *m*-fluorophenylalanine (*m*-FPA; Kohli *et al.* 1977). After 2 days incubation at 32° in supplemented NBL containing 0.4–0.8% *m*-FPA, the liquid cultures were diluted and plated on YEA-B to determine haploidization efficiency, which ranged from 40 to 95%. Fifty haploidized colonies derived from each individual diploid spore colony were patched onto YEA to score for the two different *ade6* alleles. On media with limiting amounts of adenine, such as YEA, *S. pombe* strains carrying the *ade6-M26* allele form dark red colonies while strains carrying the *ade6-52* allele form pink colonies (Gutz 1971; Gutz *et al.* 1974). After genotyping the *ade6* locus, the patches were replica plated to various differentially supplemented minimal media to genotype the *pro2* and *arg3* loci. The mating-type locus on chromosome *II* was scored for allele type by analysis of the diploid spore colonies. Diploid spore colonies were patched onto SPA and incubated at room temperature for 2–3 days. Iodine vapor staining was used to distinguish the heterozygous diploids (h^+/h^-, sporulate and stain with iodine) from the homozygous diploids (h^+/h^+ or h^-/h^-, do not sporulate and stain with iodine; Gutz 1971; Gutz *et al.* 1974).

Development of mapping functions: In a meiosis that generates four haploid products the centromeres undergo first division segregation (FDS). Loci unlinked to the centromere undergo second division segregation (SDS) 67% of the time. This is due to the statistical probability of the various patterns by which the unlinked alleles can enter the ascus. By extension, disomics or diploids arising from MI NDJ will be 100% heterozygous for the centromere and 67% heterozygous for unlinked loci. Likewise, disomics or diploids arising from MII NDJ will be 0% heterozygous for the centromere and 67% heterozygous for unlinked loci. The Haldane (1919) mapping function describes the relationship between the frequency of recombinants and genetic distance in haploid meiotic products,

$$\mathrm{RF} = \tfrac{1}{2}(1 - e^{-2M}), \tag{1}$$

where M is the genetic distance in Morgans ($1M = 1$ exchange) between two markers and RF is the fraction of recombinants. In the Haldane mapping function the RF reaches a theoretical maximum of $\frac{1}{2}$ because of multiple recombination events: the second exchange event in a genetic interval restores the parental linkage of markers. As the genetic distance becomes large the probability of even numbers of crossovers (that remove recombinants) equals the probability of odd numbers of crossovers (that create recombinants). Hence, for two unlinked markers and a sufficiently large sample size the intermarker RF will reach a plateau of ($\frac{1}{2}$), which is the limiting multiplier in the Haldane equation.

In a similar fashion, the following equation relates diploid heterozygosity to genetic distance in those diploids arising from MII NDJ:

$$\mathrm{RF}_{\mathrm{heterozygous}} = \tfrac{2}{3}(1 - e^{-2m}). \tag{2}$$

In this case, the $\mathrm{RF}_{\mathrm{heterozygous}}$ is the fraction of diploids that are heterozygous for the locus being studied and m is the genetic distance between the marker being studied and the centromere. (We apply a lowercase m for the genetic distance between a marker and the centromere to distinguish from the uppercase M used for the genetic distance between two markers.) Because of the way that alleles assort into the diploids, the heterozygosity resulting from MII NDJ can range from 0 to 67%, so the Haldane multiplier of $\frac{1}{2}$ becomes $\frac{2}{3}$, but the remainder of the equation is unchanged. By solving for m we can determine the genetic map distance of the locus from the centromere as a function of the frequency of heterozygosity in the MII NDJ diploids:

$$m = -\tfrac{1}{2}[\mathrm{Ln}(1 - 1.5\mathrm{RF}_{\mathrm{heterozygous}})]. \tag{3}$$

A similar equation describes the relationship between frequency of heterozygosity and genetic distance in those diploids arising from MI NDJ:

$$1 - \mathrm{RF}_{\mathrm{heterozygous}} = \tfrac{1}{3}(1 - e^{-2m}). \tag{4}$$

In this case, $1 - \mathrm{RF}_{\mathrm{heterozygous}}$ is the fraction of diploids that are homozygous for the locus being studied. Because of the way that alleles assort into the diploids, the homozygosity values for MI NDJ diploids can range from 0% for the centromere to 33% for an unlinked marker. Thus, the Haldane multiplier of $\frac{1}{2}$ becomes $\frac{1}{3}$. Solving for m gives the MI NDJ diploid mapping function

$$m = -\tfrac{1}{2}[\mathrm{Ln}(3\mathrm{RF}_{\mathrm{heterozygous}} - 2)]. \tag{5}$$

For PSS during MI, followed by random assortment of all the chromatids in both meiotic divisions, 67% of the diploid meiotic products are expected to be heterozygous for the centromere. This is because the centromeres become essentially nonfunctional for segregation and thus partition into the diploids with the same statistical probability as the various

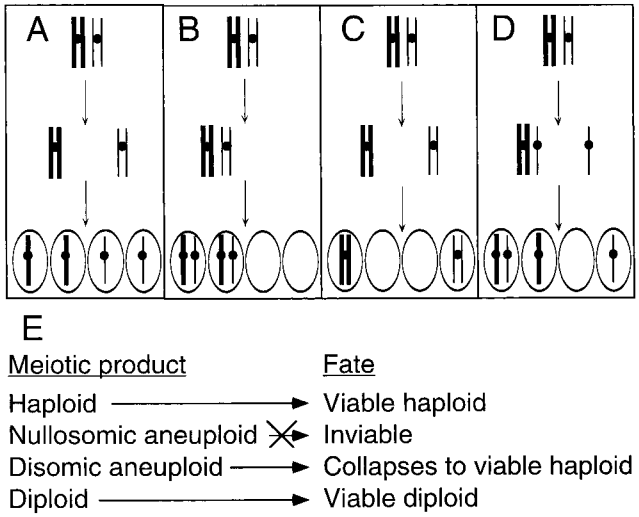

Figure 1.—Types of chromosome segregation in meiosis based upon inheritance of centromere-linked markers. Only a single homologous chromosome pair is shown for the sake of clarity. The lines represent individual chromatids within homologous chromosomes. (A) Normal meiosis results in four haploid products. (B) Meiosis I nondisjunction: homologous chromosomes fail to segregate during the reductional division, resulting in meiotic products that contain two heterozygous chromosomes. (C) Meiosis II nondisjunction: sister chromatids fail to segregate during the equational division, resulting in meiotic products that contain two homozygous chromosomes. (D) Precocious separation of sister chromatids: if both sets of sister chromatids separate precociously, and individual chromatids distribute at random during both MI and MII, 67% of disomic or diploid spores will be heterozygous (materials and methods). (E) Fates of meiotic products.

patterns by which the unlinked alleles can enter the ascus. Therefore, no diploid mapping function can be derived for PSS events, because markers linked to the centromere and markers unlinked to the centromere will each yield the same fraction (67%) of heterozygous diploids.

RESULTS AND DISCUSSION

Centromere mapping functions for aberrantly segregated chromosomes: In a normal (wild-type) meiosis, two rounds of chromosome segregation produce haploid meiotic products (Figure 1A). MI NDJ (Figure 1B), MII NDJ (Figure 1C), and PSS (Figure 1D) can be inferred from the segregation patterns of centromere-linked genetic markers. In *S. pombe*, most disomic aneuploids are unstable and cannot be readily studied (Figure 1E; Niwa and Yanagida 1985). However, because fission yeast contains only three pairs of chromosomes, aberrant chromosome segregation leads to a relatively high frequency of stable diploid meiotic products, thus permitting segregation analyses of all three pairs of chromosomes within individual meioses (Krawchuk *et al.* 1999).

The principle of centromere mapping (Figure 2, A and B), coupled with the genetic mapping function of

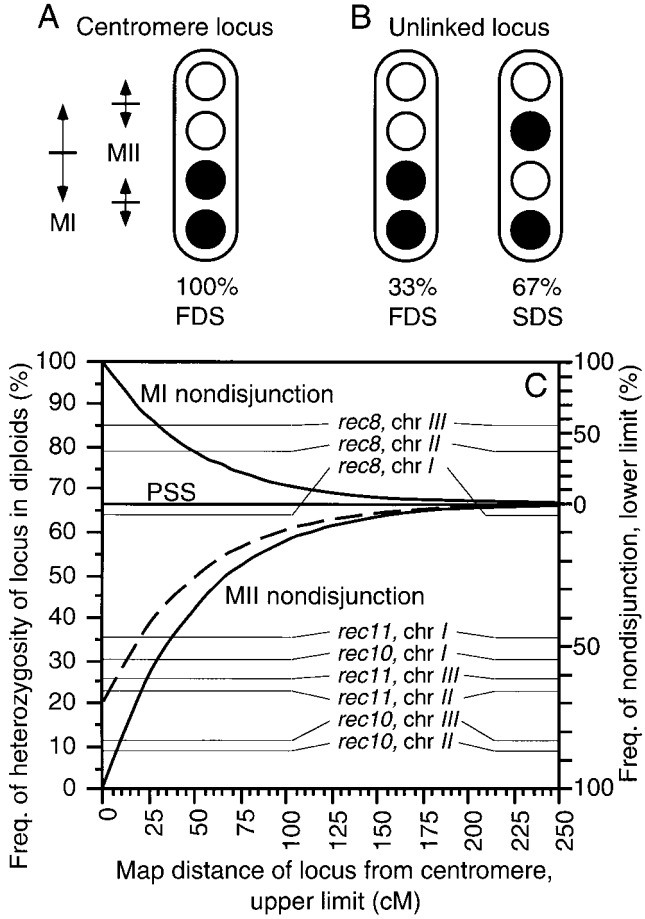

Figure 2.—Centromere mapping principles and practice. Loci can be mapped relative to the centromere based upon the frequencies of first division segregation (FDS; markers segregate during MI) and second division segregation (SDS; markers segregate during MII). (A) The centromere exhibits 100% FDS. (B) Recombination between the centromere and an unlinked locus generates 33% FDS and 67% SDS of alleles at that locus. Any deviation from 67% SDS of alleles indicates that the locus is linked to the centromere and the genetic distance can be calculated with a mathematical equation derived from the mapping function of Haldane (1919). This principle can be applied to the meiotic diploid (or disomic) spores to reveal both the segregational and recombinational history of loci relative to the centromere. (C) Mapping functions showing the relationship between the frequency of heterozygous diploids, the type of chromosome segregation error, and genetic distance. By definition, MI NDJ generates diploids that are all heterozygous at the centromere, while MII NDJ generates diploids that are all homozygous at the centromere. Departures from these diploid heterozygosity values are due to the genetic distance of the locus from the centromere (*i.e.*, recombination, thick lines), or NDJ events of the opposite type (*e.g.*, dashed line), or the presence of PSS, or some combination of the three. The diploid heterozygosity mapping function thus sets the *upper limit* for genetic distance and the *lower limit* for the type of NDJ. Diploid heterozygosity values from Table 2 are superimposed upon the mapping functions (thin lines)—these are further described in the text.

Haldane (1919), allowed us to develop mathematical functions that describe the relationship between the type of meiotic segregation error, the frequency of het-

erozygosity of any given locus in the meiotic diploids (or disomics), and the genetic distance of that locus from the centromere (Figure 2C). For alleles of loci unlinked to the centromere, 67% of the diploids will be heterozygous regardless of the type of missegregation that occurs. PSS will also produce 67% heterozygous diploids regardless of whether or not the marker is linked to the centromere. In contrast, any significant departure from 67% heterozygosity demonstrates that the locus in question is genetically linked to its centromere. Furthermore, heterozygosity values of >67% reveal MI NDJ events, while heterozygosity values of <67% reveal MII NDJ events (Figure 2C).

The assay system: As a test of our mathematical functions, we examined chromosome segregation patterns in diploid meiotic products from one mutant (*rec8*) with a known defect in MI segregation and two mutants (*rec10* and *rec11*) with known defects in MII segregation (Molnar *et al.* 1995; Krawchuk *et al.* 1999). We used strains that allowed us to monitor simultaneously the segregation of markers on all three chromosomes. As an internal control we used alleles of the *ade6* locus, which is linked (12 cM) to the centromere of chromosome *III*. For chromosome *II*, we selected the *mat1* locus, which maps ~100 cM from centromere *II* in wild-type cells. We also monitored the segregation of alleles of the *pro2* and *arg3* genes, which map close together ~300 cM from the centromere of chromosome *I*. If centromere-proximal recombination on chromosomes *I* and *II* were normal in the *rec8*, *rec10*, and *rec11* mutants, we would expect the chromosome *I* and *II* marker alleles to assort independently of their centromeres. In other words, the markers on chromosomes *I* and *II* would be heterozygous in ~67% of the diploid meiotic products. Conversely, if centromere-proximal recombination were sufficiently reduced in the *rec* mutants, the markers would become linked to their respective centromeres and would therefore exhibit missegregation patterns characteristic of centromere-linked markers (Molnar *et al.* 1995; Krawchuk *et al.* 1999).

MI NDJ in *rec8* mutants: In the *rec8* mutants, the *ade6* alleles on chromosome *III* were heterozygous in 85% of the meiotic diploids (Table 2). This value falls on the MI NDJ curve (Figure 2C) and is significantly higher than the frequency (67%) of heterozygosity expected as a result of PSS. Thus, the chromosome *III* segregation defect is unlikely the result of PSS alone, and some MI NDJ must have occurred. But how much? It is not possible to determine the precise value, but the mapping functions allow us to set the absolute limits. Three possible models are considered.

Model 1, elevated recombination and 100% MI NDJ: Exclusively MI NDJ accompanied by some level of recombination might account for the data. Examination of the mapping functions (Figure 2C) or Equation 5 (materials and methods) reveals that a genetic distance of 30 cM would produce the 85% heterozygosity that was observed (Table 2). In other words, a 2.5-fold increase in recombination relative to wild-type cells (Table 3) and 100% MI NDJ can explain the data. Recombination cannot have been increased by >2.5-fold, because this would further reduce the genetic linkage between centromere *III* and *ade6*, thereby producing a frequency of heterozygosity <85% (Figure 2C). However, 30 cM only defines the upper limit to the amount of recombination because some fraction of the homozygous diploids could have been generated by PSS and/or MII NDJ (Figure 1).

Model 2, normal recombination and 70–90% MI NDJ: In wild-type cells, *ade6* is 12 cM from centromere *III*. Examination of the mapping functions (Figure 2C) or Equations 2 and 4 (materials and methods) can convert this into the frequency of heterozygosity at *ade6* expected for each type of segregation error. Exclusively MI NDJ would produce 93% heterozygosity, exclusively MII NDJ would produce 14% heterozygosity, and exclusively PSS would produce 67% heterozygosity. One can determine algebraically what proportions of each would generate the observed 85% heterozygosity. Segregation errors of 70% MI NDJ and 30% PSS, or of 90% MI NDJ and 10% MII NDJ, would fit the data if recombination in the *rec8* mutants occurred at wild-type levels. If both PSS and MII NDJ contribute to the segregation errors, then, depending upon their relative contributions, the frequency of MI NDJ would be somewhere between 70 and 90%.

Model 3, reduced recombination and 55–85% MI NDJ: In the absence of recombination, *ade6* would be genetically inseparable from centromere *III*. Exclusively MI NDJ would produce 100% heterozygosity, exclusively MII NDJ would produce 0% heterozygosity, and exclusively PSS would produce 67% heterozygosity. Thus, segregation errors of 55% MI NDJ and 45% PSS, or of 85% MI NDJ and 15% MII NDJ, would fit the data if centromere *III*-proximal recombination were absent in the *rec8* mutants.

In summary, the mapping functions demonstrate that between 55 and 100% of the *rec8* mutant meioses suffered MI NDJ of chromosome *III*. While the mapping functions cannot provide the precise frequency, we favor a combination composed predominantly of MI NDJ and PSS. Centromere-proximal recombination is reduced in the *rec8* mutant meioses (Krawchuk *et al.* 1999; Parisi *et al.* 1999), and 4.4% (genetic assay)–20% (cytological assay) of *rec8* mutant meioses exhibit unambiguous PSS (Molnar *et al.* 1995), thus supporting *model 3*.

Similar results were obtained for the markers on chromosome *II*. In the *rec8* mutants, the *mat1* locus was heterozygous in 79% of the meiotic diploids (Table 2). This value falls on the MI NDJ curve (Figure 2C) and is significantly higher than the frequency (67%) of heterozygosity expected as a result of PSS. Exclusively MI NDJ accompanied by wild-type levels of recombination could account for the data (just on the edge of the 95%

TABLE 2

Segregation patterns of markers in aneuploid meiotic products

Relevant genotype	Diploids genotyped	Heterozygosity of marker in diploids (%)[a]		
		pro2-arg3 Chromosome *I*, 300 cM	*mat1* Chromosome *II*, 100 cM	*ade6* Chromosome *III*, 12 cM
rec8$^-$	92	63 (53–73)	79 (71–87)	85 (78–92)
rec10$^-$	35	31 (16–46)	9 (0–18)	12 (1–23)
rec11$^-$	45	36 (22–50)	23 (11–35)	26 (13–39)

[a] Meiotic crosses between pairs of haploid strains were heterozygous for the indicated marker loci; the genetic distances of those markers from their respective centromeres in wild-type cells (Munz 1994) are also shown. Diploid (aberrant chromosome segregation) spore colonies were identified and a clonal population of each was haploidized. Fifty haploid derivatives from each diploid spore colony were genotyped for each locus. Data are the frequency of diploid spore colonies heterozygous for the indicated markers on each of the chromosomes. Values in parentheses indicate the 95% confidence interval for each frequency.

confidence interval). However, the mapping functions (Figure 2C) indicate that recombination is reduced twofold or more between centromere *II* and *mat1* (Table 3). Thus, the *rec8* mutants suffer missegregation of chromosome *II* as a result of MI NDJ events accompanied by some level of PSS, MII NDJ, recombination, or some combination of the three. By the same rationale and types of calculations applied to the chromosome *I* data, the frequency of MI NDJ for chromosome *II* in the *rec8* mutants must fall between 38 and 100%.

The chromosome *I* marker loci were heterozygous in 63% of the *rec8* meiotic diploids (Table 2). The 95% confidence interval of the data encompasses an uninformative area at which the MI NDJ, PSS, and MII NDJ mapping functions converge. Exclusively MI NDJ accompanied by recombination, PSS, and exclusively MII NDJ accompanied by recombination would each produce ~67% heterozygosity (Figure 2C).

MII NDJ in *rec10* mutants: In the *rec10* mutants the *ade6* markers were heterozygous in 12% of the meiotic diploids (Table 2). This value falls on the MII NDJ curve (Figure 2C) and is significantly lower than the frequency (67%) of heterozygosity expected as a result of PSS. Thus, the *rec10* mutants suffer some level of MII NDJ of centromere *III*. Exclusively MII NDJ accompanied by normal levels of recombination would produce 14% heterozygosity at *ade6* (Figure 2C; materials and methods, Equation 2), close to the observed value. The mapping functions also revealed an upper limit to the crossover frequency near that of wild-type meioses (Table 3), consistent with such a model. However, centromere-proximal recombination might be reduced much further, perhaps even eliminated, in the *rec10* mutants. In that case, the observed 12% heterozygosity would be due to a low level of MI NDJ and/or PSS. Segregation errors of 82% MII NDJ and 18% PSS, or of 88% MII NDJ and 12% MI NDJ, would fit the data if centromere *III*-proximal recombination were absent. Therefore, the mapping functions demonstrate that between 82 and 100% of the *rec10* mutant meioses suffer MII NDJ of

TABLE 3

Genetic map distances in meioses with aberrant chromosome segregation

Relevant genotype	Genetic distance between marker and centromere (cM)[a]		
	cen1-pro2/arg3 Chromosome *I*	*cen2-mat1* Chromosome *II*	*cen3-ade6* Chromosome *III*
Wild type	300	100	12
rec8$^-$	NI[b]	≤50[c]	≤30[c]
rec10$^-$	≤31[d]	≤7.3[d]	≤9.9[d]
rec11$^-$	≤39[d]	≤21[d]	≤25[d]

[a] Wild-type genetic distances were as reported (Munz 1994). The maximum possible genetic distances in meioses suffering aberrant segregation were calculated from the heterozygosity values in Table 2 using the mapping functions (Figure 2C; materials and methods, Equations 3 and 5).

[b] Not informative. The heterozygosity value (63%) is too close to the value (67%) at which the various mapping functions converge (Figure 2C) to permit meaningful calculation of the genetic distance.

[c] Based on the assumption of 100% MI NDJ of centromeres. The genetic distances would be smaller if any of the segregation errors resulted from MII NDJ or PSS.

[d] Based on the assumption of 100% MII NDJ of centromeres. The genetic distances would be smaller if any of the segregation errors resulted from MI NDJ or PSS.

chromosome *III*, and the precise value is dependent upon the relative additional contributions from recombination, MI NDJ, and PSS.

Nine percent of the *rec10* mutant meiotic diploids were heterozygous for the *mat1* locus on chromosome *II* (Table 2). Thus, the *rec10* mutants suffer predominantly MII NDJ of chromosome *II* (Figure 2C). Interestingly, the maximum possible genetic distance in those meioses leading to aberrant segregation was 7.3 cM, which is 14-fold (or more) below that of wild-type cells (Table 3). In other words, centromere-proximal crossover recombination on chromosome *II* was reduced in those meioses with aberrant segregation. As discussed previously, the heterozygous diploids (9%) could be due to this residual recombination, to a low level of MI NDJ, to a low level of PSS, or to some combination of the three. By any of the models, between 87 and 100% of the *rec10* mutant meioses suffer MII NDJ of chromosome *II*.

Similar observations and conclusions apply to the segregation patterns of chromosome *I* in the *rec10* mutants (Tables 2 and 3; Figure 2). A reduction of 10-fold (or more) in centromere-proximal recombination was observed in those meioses with aberrant segregation, and between 54 and 100% of the segregation errors are due to MII NDJ.

MII NDJ in *rec11* mutants: The results and conclusions for the *rec11* mutants parallel those of the *rec10* mutants (Tables 2 and 3; Figure 2C). Each of the three chromosomes suffered MII NDJ of sister chromatids and centromere-proximal recombination was reduced at least eightfold and at least fivefold on chromosomes *I* and *II*, respectively, in those meioses with aberrant segregation. Between 47 and 100% (chromosome *I*), 66 and 100% (chromosome *II*), and 61 and 100% (chromosome *III*) of the segregation errors are due to MII NDJ.

Reduced centromere-proximal recombination in the *rec8*, *rec10*, and *rec11* mutants: Reduced centromere-proximal recombination was demonstrated for *rec8* (chromosome *II*), *rec10* (chromosomes *I*, *II*, and *III*), and *rec11* (chromosomes *I* and *II*) mutant meioses with aberrant chromosome segregation (Table 3). Two studies of crossing over between pairs of markers have shown that recombination is reduced markedly near the centromeres of each of the three chromosomes in each of the *rec8*, *rec10*, and *rec11* mutants, but recombination toward the telomeres occurs at nearly wild-type levels (Krawchuk *et al.* 1999; Parisi *et al.* 1999). Because those studies looked at the majority of meioses, and this study examined only those meioses in which the chromosome missegregation could be scored genetically, it is not possible to make a direct comparison. However, centromere-proximal recombination may actually be reduced on each chromosome in each of those *rec8*, *rec10*, and *rec11* mutant meioses leading to diploidy. The mapping functions reveal the maximum possible genetic distance between a marker and its centromere and therefore probably underestimate the magnitude of the centromere-proximal recombination defects. If, for example, some fraction of *rec10* or *rec11* mutant meioses suffered MI NDJ, then the amount of centromere-proximal recombination that occurred could actually be considerably lower than calculated based upon the assumption of 100% MII NDJ at the centromeres (*e.g.*, dashed line in Figure 2C). A similar rationale applies to the *rec8* mutant meioses, which have predominantly MI NDJ errors.

Cohesion, crossovers, and segregation: It has been reported that the *rec8*$^+$, *rec10*$^+$, and *rec11*$^+$ genes encode chromosome *III*-specific activators of recombination (DeVeaux and Smith 1994; Fox and Smith 1998). That claim seems indefensible in light of the results of this study and others (Molnar *et al.* 1995; Krawchuk *et al.* 1999; Parisi *et al.* 1999). The *rec8*$^+$ gene actually encodes a sister chromatid cohesion (cohesin) protein required for normal sister chromatid cohesion, homologous chromosome pairing, and MI chromosome segregation. The *rec8-110* mutation is epistatic to *rec10-109* and to *rec11-111*, thereby defining a genetic pathway. And the *rec10*$^+$ and *rec11*$^+$ genes are required for proper segregation of sister chromatids during MII. These genes are proposed to encode components of a pathway required to establish, maintain, and appropriately release meiotic interactions between homologous chromosomes (Krawchuk *et al.* 1999). Hypotheses of how sister chromatid cohesion and crossover position work synergistically to ensure the fidelity of chromosome segregation in each of the two meiotic divisions can be found in that study.

The utility of the mapping functions: The new mapping functions permit the simultaneous analysis of centromere-proximal recombination and chromosome segregation defects. There are three principal advantages of these functions. First, it is not necessary for the marker to be tightly linked to the centromere in wild-type cells, nor is it even necessary to know the map distance between the marker and its centromere in wild-type cells. Any significant deviation from 67% heterozygosity in the meiotic aneuploids proves that the locus is linked to its centromere. Thus, one can readily map loci relative to the centromeres in mutant meioses or even in otherwise wild-type meioses with spontaneous nondisjunction events. Second, the frequency of heterozygosity provides a direct readout of the type of meiotic missegregation. Any significant deviation from 67% heterozygosity must be due to either MI NDJ or MII NDJ; the type of error and the lower limit to its magnitude can be read directly from the mapping functions (Figure 2C). And third, these functions may be applied to any organism in which diploid or disomic meiotic products can be studied. While it is easiest to visualize the derivation of the mapping functions for organisms with ordered tetrads (Figure 2), the functions will work equally well for organisms without ordered tetrads.

There are also three limitations to the new mapping functions. First, they only reveal the upper limit to the

genetic distance between any given marker and its centromere (Figure 2C). Second, they only reveal the lower limit to the type of NDJ (Figure 2C). And third, it is not possible to map the distance between a marker and the centromere in those meioses that undergo predominantly PSS. Nevertheless, these functions can provide a better understanding of the relationship between meiotic recombination and chromosome segregation.

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