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Abstract

We studied the relationship between recombination and segregation in the fission yeast *Schizosaccharomyces pombe*. In meiosis, chromosomes undergo two rounds of segregation to produce haploid meiotic products. Crossover meiotic recombination (chiasmata) promotes chromosome segregation during meiosis I; achiasmatic chromosomes often suffer nondisjunction in meiosis I. Rec12 protein is a topoisomerase II ortholog that introduces double-strand DNA breaks that initiate recombination. The *rec12* null (deletion) and active site (Y98F) mutants lack recombination and crossovers, and consequently suffer meiosis I nondisjunction. However, null mutants chromosomes segregate to opposite poles more frequently than predicted. Thus, fission yeast has a backup, "distributive segregation" pathway that can function in the absence of Rec12 and Rec12-dependent chiasmata. Interestingly, presence of catalytically-inactive Rec12 protein (Y98F) enhances the fidelity of distributive segregation. We hypothesize that Rec12 protein activates a checkpoint that promotes use of the distributive segregation pathway.

Introduction

Meiosis is a specialized form of cellular differentiation in which a single diploid cell gives rise to four haploid meiotic products. Subsequent post-meiotic differentiation gives rise to haploid cells such as sperm, eggs, pollen, and ascospores. The biological imperative, sex, brings together haploid gametes to generate zygotic diploids which may enter the diploid cell cycle.

In meiosis, homologous chromosomes undergo DNA replication, pair with their partners to form a "bivalent," experience a high rate of recombination, and subsequently undergo two rounds of chromosome segregation to produce haploid meiotic products. Homologous chromosomes segregate from their partners in a reductional division during meiosis I, and during meiosis II sister chromatids segregate from one-another in an equational division similar to mitosis. There is an intimate connection between meiotic recombination and meiotic chromosome segregation.

Crossover recombination (reciprocal exchange) generates physical connections, called "chiasmata," between homologs. These physical connections provide the primary mechanism to ensure proper segregation of homologs during meiosis I (Hawley, 1988). Chiasmata oppose spindle tension during meiosis I and are thought to help orient the paired homologous chromosomes (bivalent) on the metaphase I plate (Hassold and Hunt, 2001). When homologous chromosomes lack chiasmata (crossovers) they often experience nondisjunction and segregate randomly from their partners. Studies of model organisms and humans support the view that chiasmata are important for segregation of chromosomes in meiosis I (Antonarakis et al., 1986; Hawley et al., 1994; Rockmill and Roeder, 1994; Koehler et al., 1996; Hassold and Hunt, 2001; Molnar et al., 2001).

While chiasmatic segregation is widely used to partition chromosomes in meiosis I, there are a few organisms that lack crossovers (chiasmata) on chromosomes. As an alternative strategy, these organisms use achiasmatic or "distributive" mechanisms for chromosome segregation in meiosis I (Grell, 1976; Carpenter, 1991; Dernburg et al., 1996; Koehler and Hassold, 1998). Such distributive segregation systems can partially or almost fully support faithful segregation of chromosomes during meiosis I in the absence of recombination. However, it is not clear whether distributive segregation occurs in organisms that rely upon chiasmatic segregation.

It is difficult to study mutations affecting meiotic chromosome segregation because the probability of obtaining viable meiotic products is inversely proportional to the power of chromosome number. Most products of such meioses are aneuploid (*i.e.*, have the wrong number of chromosomes) and inviable. In fission yeast, however, there are only three pairs of chromosomes. As a consequence, random assortment can produce, at a relatively high frequency, meiotic products that receive at least one copy of each chromosome and are hence viable (Krawchuk et al., 1999). We have taken advantage of this feature of *S. pombe* biology to study the relationship between crossover

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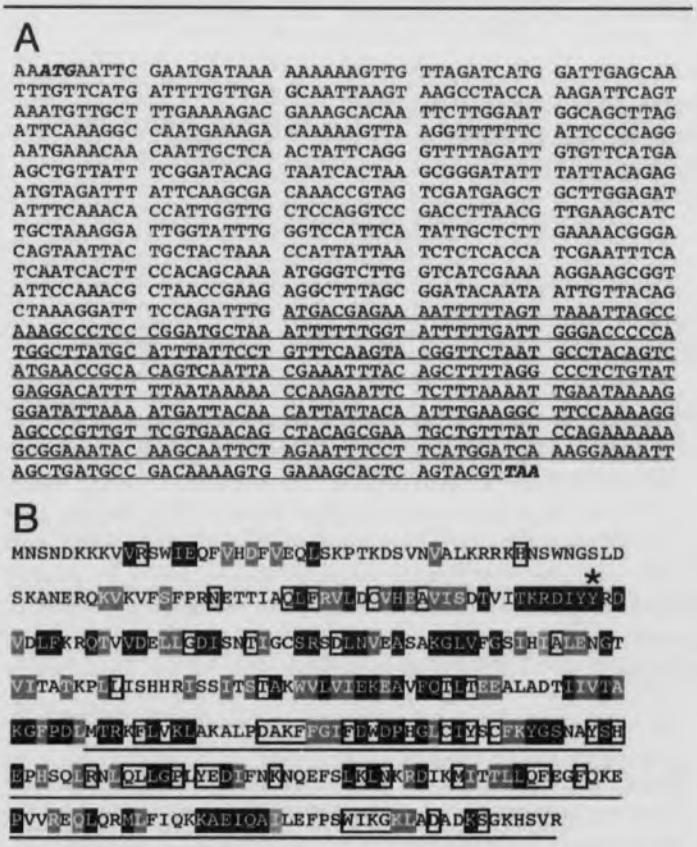

Fig. 1. Revised sequence of rec12+ open reading frame and homology of Rec12 protein to type II topoisomerases. (A) DNA sequence of complementing *rec12*$^{+}$ cDNA clone showing positions of the ATG start codon (italics), TAA stop codon (italics), and region reported previously to encode the Rec12 protein (underlined) (Lin and Smith, 1994). (B) Amino acid sequence of Rec12 protein showing residues that are identical (black boxes), conserved (gray boxes), or conserved in family members other than Rec12 (open boxes). Also shown is the position of the active site tyrosine (*) and the region reported previously to encompass Rec12 (underlined). Alignments evaluated proteins from *S. pombe* (P40384), *Neurospora crassa* (Q9P6Y7), *Coprinus cinereus* (Q9P4D2), *Homo sapiens* (Q9NQM7), *Mus musculus* (Q9QZS1), *Arabidopsis thaliana* (AAL01152), *Drosophila melanogaster* (O77205), *Caenorhabditis elegans* (Q22236), and *Saccharomyces cerevisiae* (P23179).

recombination (chiasmata) and chromosome segregation (Krawchuk et al., 1999; Sharif et al., 2002). We found that Rec12 protein is essential for recombination (chiasmata) and chiasmatic chromosome segregation, and also functions to promote an alternative, backup distributive segregation pathway in the presence of achiasmatic chromosomes.

Materials and Methods

S. pombe culture--Culture media and genetic methods were as described (Gutz et al., 1974; Krawchuk et al., 1999).

Intron mapping and sequence analysis.--Cells harboring the *pat1-114*^{ts} allele were induced to enter meiosis (Wahls and Smith, 1994) and were collected after 3 hours of meiotic induction. Genomic DNA and total RNA were prepared (Kon et al., 1998) and subject to PCR and RT-PCR using primers flanking each putative intron. Full-length *rec12*$^{+}$ cDNA was obtained using RT-PCR using primers designed to amplify the *rec12*$^{+}$ cDNA from the first ATG in exon 1 to a position +142 bp downstream of the stop codon in exon 5. RT-PCR products were cloned by blunt-end ligation into pCR-Blunt (Invitrogen Corp.) and both strands were subject to DNA sequencing (GenBank accession no. AF195027). Conceptual translation of the cDNA open reading frame was used to infer the sequence of Rec12 protein. Protein sequences homologous to that of *S. pombe* Rec12 were identified with a NCBI Blast search (Altschul et al., 1997) using matrix BLOSUM62 and were aligned using T-COFFEE (Notredame et al., 2000). Output was prepared using a 50% threshold for identical and conserved residues.

Construction of *rec12-D13::ura4*$^{+}$ and *rec12-Y98F* alleles. A PCR based gene targeting approach (Bähler et al., 1998) was used to delete the *rec12*$^{+}$ coding region and replace it with the *ura4*$^{+}$ gene (Sharif et al., 2002). Candidates were screened with a combination of PCR analysis, restriction digestion, and DNA sequencing to identify those with successful allele replacement. The *rec12-Y98F* allele was constucted by site-directed mutagenesis of plasmid-born *rec12* (Sharif et al., 2002). Replacement of the endogenous *rec12*$^{+}$ locus with the *rec12-Y98F* allele was achieved by transformation and a pop-in, pop-out approach (Francesconi et al., 1993). Transformation, forward selection, and reverse selection were as described (Grimm et al., 1988; Francesconi et al., 1993). Candidates were screened as described above.

Recombinant frequency determination. Mating, meiosis, and preparation of free spores were as previously (Kon et al., 1997). Intergenic and intragenic recombinant frequencies were determined as described (Kon et al., 1997; Krawchuk et al., 1999). Because diploid spores could contain complementing markers and be mistaken for recombinants, they were excluded from recombinant frequency determinations. Recombinant frequencies from mutltiple inervals spanning approximately 20% of the genome (Sharif et al., 2002) were used to calculate the average number of crossovers per genome per meiosis.

Diploid spore isolation and haploidization analysis. Identification of diploid spore colonies and their haploidization were as described (Krawchuk et al., 1999). Fifty haploidized colonies derived from each diploid spore

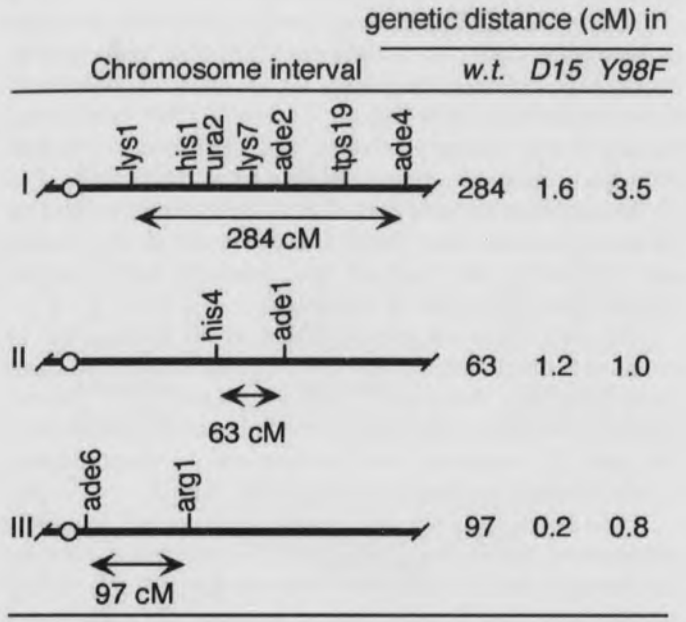

Fig. 2. Effects of Rec12 upon crossover recombination and formation of chiasmata. Intergenic recombinant frequencies were determined in *rec12* mutants (Sharif et al., 2002) and were converted to genetic map distances using the formula of Haldane (Haldane, 1919). These were compared to published map distances in wild-type cells (NCI Entrez Genome) (Munz, 1994). The genetic map distances (*i.e.*, % frequency of crossovers/chiasmata) between the most distal marker pairs are indicated. The average number of crossovers/chiasmata per meiosis (bottom panel) was extrapolated from the average values for all intervals tested (encompassing ~20% of the genome).

colony were replica plated to differentially supplemented minimal media to genotype the *lys1* and *ade6* loci (Sharif et al., 2002). The *tps13* alleles were scored by replica plating colonies onto rich (YEA) media and testing for growth at permissive (22°C) and restrictive (35°C) temperatures (Sharif et al., 2002).

Microscopy. Asci from meiotic cultures were fixed with 70% ethanol at -20°C for at least 15 minutes, washed with H_2O, and stained with 4,6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 µg/ml. Cells were examined by differential interference contrast (DIC) and fluorescence (DAPI) microscopy with a Zeiss axiophot (Carl Zeiss, Thornwood, NY). Images were analyzed using the MetaMorph software package (Universal Imaging, West Chester, PA).

Results

Rec12 is a meiotic ortholog of archaebacterial Topoisomerase VI.--The *rec12-117* mutant of fission yeast exhibits a 6-fold reduction in crossover recombination (De Veaux et al., 1992). The *rec12* mRNA is induced only during meiosis and was reported to encode an orphan protein of 139 amino acids (Lin and Smith, 1994). However, our analysis of the DNA sequence revealed the presence of consensus sequences for splicing and five potential exons, suggesting that *rec12* encodes a larger protein. PCR analysis of genomic DNA and RT-PCR analysis of mRNA from meiotic cells, using primers flanking the putative introns, confirmed the presence of four introns (Sharif et al., 2002).

The confirmed presence of introns suggested that *rec12* encodes a protein larger than originally reported. In order to confirm the intron/exon assignments, we cloned and sequenced a cDNA (Fig. 1A) and introduced it into expression vectors (Maundrell, 1993) in such a way that the first ATG in predicted exon 1 would be used for translation. This construct complemented the recombination defect of *rec12-117* mutants (Sharif et al., 2002), confirming that the cDNA encodes a functional Rec12 protein of 345 amino acids in length. The revised protein sequence shares homology with a family of eukaryotic proteins (Spo11) and belongs to the type-II topoisomerase family (Fig. 1). Rec12/Spo11 is most closely related to the Top6A (catalytic) subunit of archaebacterial topoisomerases, suggesting that Rec12/Spo11 might catalyze meiosis-specific double-strand breaks that initiate recombination.

DNA sequence analysis revealed that the *rec12-117* allele had a single missense mutation in exon 5 (our unpublished observations). We therefore used *in vitro* mutagenesis and gene replacement to construct two new alleles of *rec12* expressed from the endogenous locus (Sharif et al., 2002). The *rec12-D15* (deletion of 1.5 kbp; null) allele removed the entire *rec12* coding region. Tyrosine-98 was of particular interest because it is in the most conserved region of the Rec12 protein and it is in the same position as the active site tyrosine in type-II topoisomerases (Fig. 1B). The *rec12-Y98F* (active site) allele was designed to express full length Rec12 protein in which a single tyrosine at position 98 was replaced with phenylalanine (Fig. 1B).

Rec12 and its active site tyrosine are essential for recombination.--Fission yeast has three pairs of chromosomes. We analyzed the frequency of intergenic recombination for multiple intervals encompassing approximately 20% of the genome to determine the requirements for Rec12 in crossover (chiasmata) formation (Fig. 2). Recombinant frequencies were determined for intervals on all three chromosomes, for intervals close to a centromere, for interstitial intervals, and for distal intervals to determine whether there was any regional and/or

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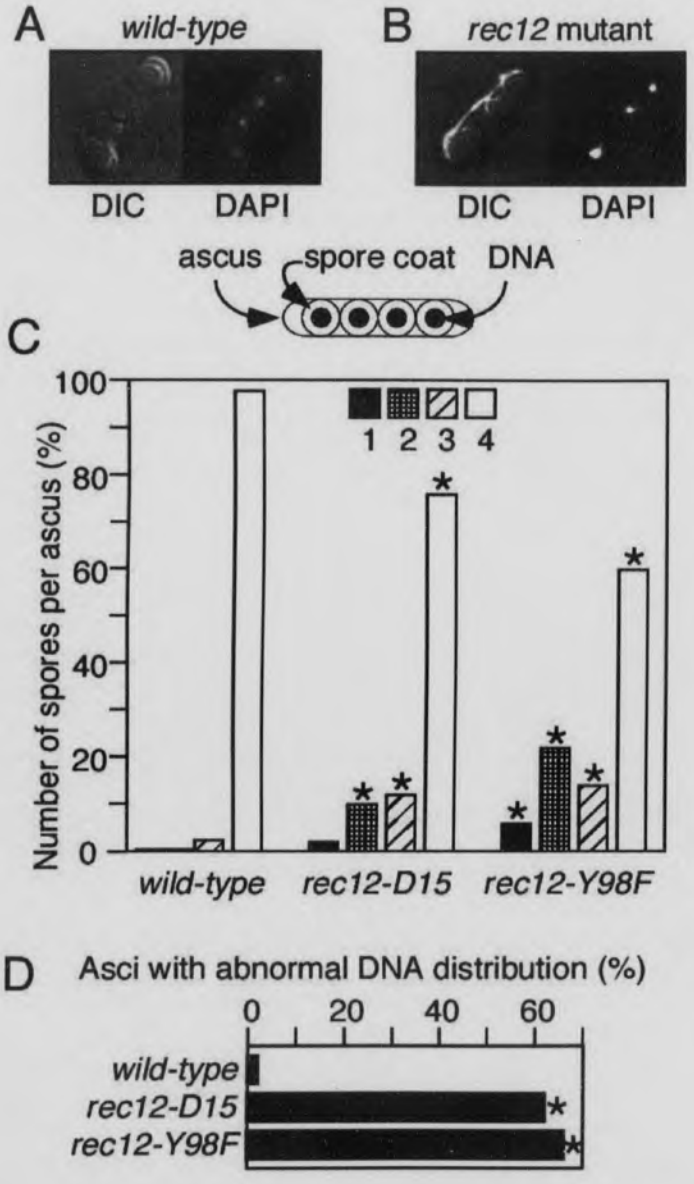

Fig. 3. Cytological phenotypes of *rec12* mutant meioses. Asci from (A) wild-type and (B) *rec12* mutant meiotic cultures were characterized by differential interference contrast (DIC) and DNA fluorescence (DAPI) microscopy (Krawchuk et al., 1999; Sharif et al., 2002). The ascus wall, spore coat, and DNA distribution can be visualized. (C) The number spores per ascus was determined as a measure of the efficiency with which the *rec12* mutants complete meiosis. (D) Frequency of aberrant DNA segregation; asci were scored for aberrant number, intensity, or distribution of DAPI fluorescent signals as in panel B. Data in panels C and D are from analysis of more than 300 asci per genotype and frequency values significantly different from those of wild-type cells (test between two proportions) are indicated (*)

chromosomal specificity (Sharif et al., 2002).

The *rec12-D15* (null) mutants were profoundly deficient in recombination–they exhibited a 256-fold reduction in average recombinant frequency for all intervals, relative to those in wild-type cells (Fig. 2). The *rec12-Y98F* (active site) mutants were similarly affected and exhibited a 263-fold reduction in recombination, relative to wild-type cells (Fig. 2). Recombination was affected in all intervals tested and on all three chromosomes. These data prove that Rec12 protein and its active site tyrosine are essential for crossover recombination (reciprocal exchange).

In each *S. pombe* meiosis there is an average of 44 crossovers distributed on the three chromosomes and those crossovers are distributed without interference (Munz, 1994). A 250-fold reduction in recombination would leave less than 0.1 crossovers per chromosome, so chromosomes in *rec12* mutant meioses are achiasmatic (Fig. 2).

Rec12 and its active site tyrosine are required for meiotic chromosome segregation.–Differential interference contrast microscopy and fluorescence microscopy using a DNA-specific dye (DAPI) were used to examine the phenotypes of asci from wild-type and *rec12* mutant meioses (Sharif et al., 2002). In wild-type cells, meioses produced asci with four well-rounded spores and equivalent DNA content in each spore (Fig. 3A). While the *rec12* mutants lacked chiasmata, they were proficient for meiosis; most cells clearly underwent two meiotic divisions, and the majority of cells formed asci with visible spores (Fig. 3B, 3C). However, gross defects in chromosome segregation and ascus morphology were apparent (Fig. 3D). The heterogeneity of phenotypes suggested that chromosome segregation was random in one or both of the meiotic divisions, as has been described for other mutations affecting meiotic chromosome segregation (Krawchuk et al., 1999). The additional defects in spore formation may be a secondary consequence of chromosome segregation errors, as ascospore formation in *S. pombe* is controlled by the spindle pole body of the meiosis II spindle (Hirata and Shimoda, 1994).

Aberrant chromosome segregation should produce meiotic products that are aneuploid (*i.e.*, have the wrong complement of chromosomes). In *S. pombe,* haploids and diploids are viable, nullisomic aneuploids (missing one or more chromosomes) are inviable, and disomic or polysomic aneuploids (having extra chromosomes) are unstable and rapidly lose the extra chromosome(s) (Niwa and Yanagida, 1985). Since *S. pombe* has only three pairs of chromosomes random assortment can, by chance, result in a relatively high frequency of meiotic products that receive at least one copy of each chromosome and hence are viable (Krawchuk et al., 1999). Similarly, some meiotic products might receive, by chance, at least two copies of each chromosome and thus produce viable diploids. We therefore determined the frequency of spore viability and of meiotic diploidy as

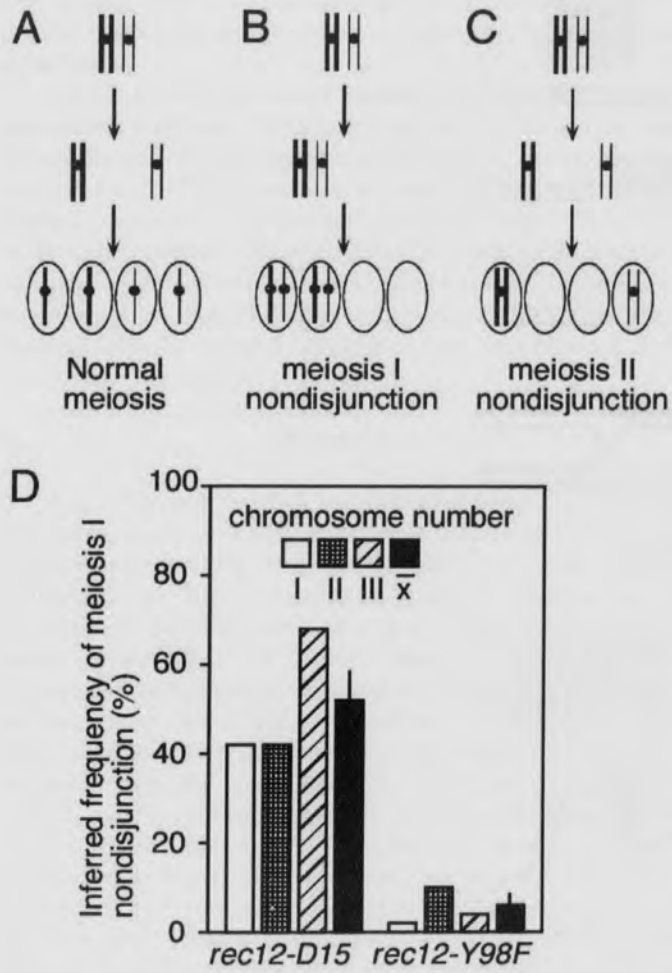

Fig. 4. Segregation patterns of chromosomes. (A) In a normal meiosis two rounds of chromosome segregation produce haploid meiotic products (B) Meiosis I nondisjunction produces disomic (or diploid) meiotic products that are heterozygous for centromere-linked markers. (C) Meiosis II nondisjunction produces disomic (or diploid) meiotic products that are homozygous for centromere-linked markers. (D) Frequency of meiosis I nondisjunction in *rec12* mutants. Diploid spore colonies were genotyped for heteroallelic, centromere-linked markers on each of the three chromosomes (Krawchuk et al., 1999; Sharif et al., 2002). The frequency of meiosis I nondisjunction was inferred from the frequency of diploid meiotic products heterozygous for centromere-linked markers (Sharif et al., 2002).

genetic measures of chromosome segregation errors (Krawchuk et al., 1999).

Most (97 ± 21%) of the spores from wild-type meioses were viable. Less than half (41 ± 5%) of the spores from *rec12-D15* (null) meioses were viable, suggesting that 59% of the products failed to receive at least one copy of each chromosome (*i.e.*, were nullisomic). This value is very close to the expected frequency of nullisomics (58%) if segregation were entirely random in one of the two meiotic divisions. Interestingly, spore viability was significantly lower (20 ± 6%) from crosses of the *rec12-Y98F* mutants than from *rec12-D15* mutant crosses, suggesting that *rec12-Y98F* may encode a separation-of-function mutation (see below).

Very few (0.3 ± 0.1%) of the spores from wild-type meioses were diploid. As predicted, the *rec12-D15* (null) and *rec12-Y98F* (active site) mutants produced an elevated frequency of diploid meiotic products (9 ± 4% and 12 ± 1%, respectively) (Sharif et al., 2002). The spore viability data, spore diploidy data, and cytological data (Fig. 3) demonstrate that chromosomes segregate aberrantly in the absence of Rec12 protein and its active site tyrosine. Importantly, the recovery of viable diploid meiotic products from *rec12* mutant meioses permitted us to infer the meiotic division in which chromosomes segregated aberrantly.

Loss of Rec12 protein causes meiosis I nondisjunction and reveals presence of a backup, distributive segregation pathway.– Meiotic diploids derived from nondisjunction of chromosomes during meiosis I should contain chromosomes that are heterozygous for centromere-linked markers (Fig. 4B). Precocious separation of sister chromatids during meiosis I would also produce diploids that are predominantly heterozygous for centromere-linked markers (not shown). Meiotic diploids that result from mis-segregation during meiosis II should contain chromosomes that are homozygous for centromere-linked markers (Fig. 4C). Since fluorescence *in situ* hybridization revealed no defects in sister chromatid cohesion in *rec12* mutants (Nabeshima et al., 2001), one can use the frequency of heterozygous diploids as a direct measure of the frequency of meiosis I nondisjunction.

Individual diploid spore colonies were haploidized, and fifty individual haploid derivatives from each diploid spore colony were scored for heteroallelic, centromere-linked markers on each of the three chromosomes (Sharif et al., 2002). Approximately 50% of the diploid spore colonies from *rec12-D15* mutant meioses were heterozygous for centromere-linked markers on each of the three chromosomes (Fig. 4D), demonstrating that these diploids arose as a consequence of meiosis I nondisjunction. However, this frequency of meiosis I nondisjunction is only half that expected for achiasmatic chromosomes. Distributive segregation occurs when achiasmatic chromosomes segregate away from their homologous partners (during meiosis I) at a frequency that is higher than the frequency of random assortment. The lower-than-expected frequency of nondisjunction in meiosis I in the *rec12-D15* mutants suggests that *S. pombe* has a backup,

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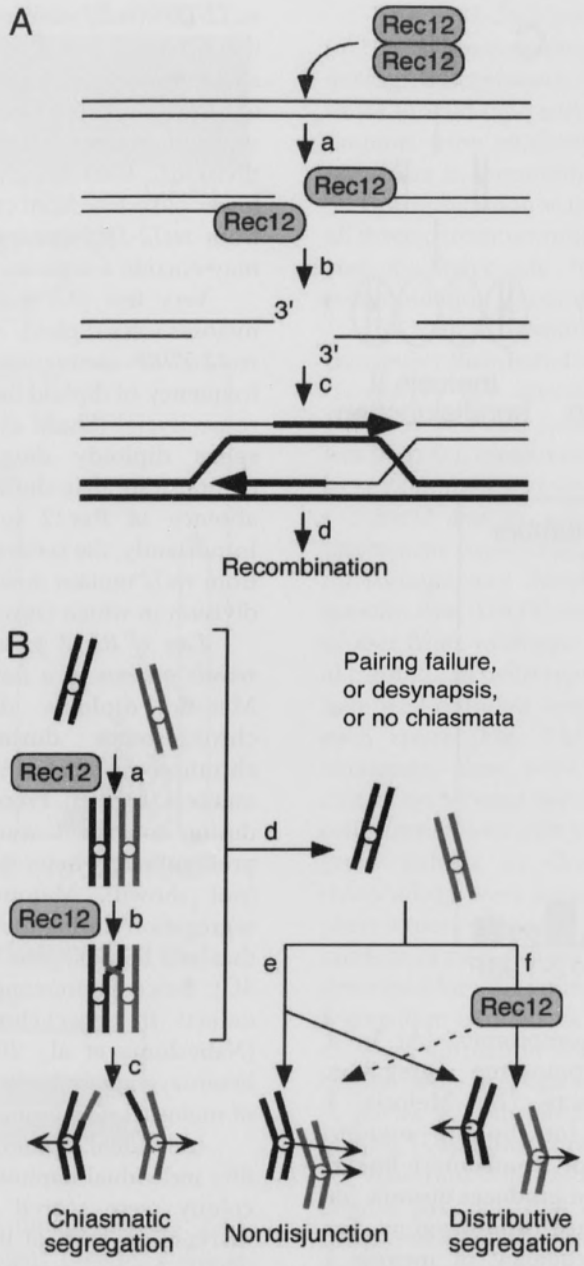

Fig. 5. Models for multiple functions of Rec12 in meiotic chromosome dynamics. (A) Initiation of recombination: Wild-type Rec12 protein is the catalytic subunit of a recombinase holoenzyme that introduces recombinogenic double-strand DNA breaks (*a*). Those breaks are processed to generate 3' single-stranded DNA tails (*b*) that can invade a homologous chromosome and prime DNA synthesis (*c*). Subsequent processing (*d*) produces recombinant products with or without reciprocal exchange (crossing over) of flanking markers, as originally proposed (Szostak et al., 1983). Reciprocal exchanges produce chiasmata which help hold paired homologs together. (B) Chiasmatic and distributive segregation: Rec12 protein may promote interhomolog pairing interactions (*a*) (Cha et al., 2000; Romanienko and Camerini-Otero, 2000). Wild-type Rec12 protein is essential to initiate recombination and crossover formation (*b*), which are in turn essential for chiasmatic segregation during meiosis I (*c*). Various defects can lead to achiasmatic chromosomes (d). In the absence of Rec12, achiasmatic chromosomes experience both meiosis I nondisjunction and distributive segregation (*e*). However, when achiasmatic chromosomes are present, catalytically-inactive Rec12 (and presumably also wild-type Rec12) promotes function of the backup, distributive segregation pathway (*f*).

distributive (achiasmatic) pathway of chromosome segregation that can function in the absence of Rec12 protein and Rec12-dependent crossover recombination (chiasmata).

Rec12 protein promotes function of the distributive segregation pathway.--While meioses lacking Rec12 protein frequently suffered meiosis I nondisjunction, the active site mutants (*rec12-Y98F*) produced meiotic diploids with nearly normal meiosis I segregation patterns (Fig. 4D). This difference is not related to crossover frequency, because the two mutants exhibit identical deficiencies in meiotic recombination (Fig. 2). Thus, the presence of catalytically-inactive Rec12 protein promotes the function of the distributive segregation pathway.

Discussion

The *rec12* gene has five exons and encodes a protein of 345 amino acids in length that shares homology with type-II topoisomerases (Fig. 1) (Keeney, 2001; Sharif et al., 2002). In eukaryotes, topoisomerase-II enzymes function as a homodimer that introduces a double-strand DNA break, passes a second DNA strand through the break, and religates the broken ends. A catalytic domain introduces the breaks, and an ATPase domain is required for conformational changes involved in strand passage and religation (Fortune and Osheroff, 2000). In prokaryotes, type-II topoisomerases function as a heterotetramer in which the catalytic and ATPase functions are in separate polypeptides. Rec12/Spo11 shares the highest homology with the Top6A subunit of archaebacterial enzymes (Fig. 1) (Keeney, 2001; Sharif et al., 2002). However, there is no obvious homolog for Top6B in most eukaryotes, including fission yeast. This makes sense in terms of models for meiotic recombination (Szostak et al., 1983) because initiation of recombination does not require DNA strand passage and religation activities like those carried out by canonical type-II topoisomerases (Fig. 5A).

Meiotically-induced double-strand DNA breaks have been demonstrated in budding yeast and in fission yeast (Sun et al., 1989; Cao et al., 1990; Zenvirth and Simchen, 2000). Rec12/Spo11 enzyme almost certainly catalyzes these breaks: First, it shares homology with topoisomerases (Fig. 1). Second, Rec12 and its active site tyrosine are essential for recombination (Fig. 2) (Sharif et al., 2002). Third, Rec12/Spo11 becomes covalently linked by a phosphotyrosine linkage to the 5' end of the meiotic double-strand DNA breaks ((De Massy et al., 1994; Keeney and Kleckner, 1995; Liu et al., 1995); our unpublished observations). Proteins homologous to Rec12 (Spo11) are found in a wide range of eukaryotes and available evidence suggests that the initiation of meiotic recombination by Rec12/Spo11-dependent double-strand DNA breaks is conserved (Dernburg et al., 1998; McKim and Hayashi-Hagihara, 1998; Baudat et al., 2000; Romanienko and Camerini-Otero, 2000). Double-strand DNA breaks initiate repair synthesis from the homologous chromosome, leading to recombination intermediates that can be resolved with or without reciprocal exchange of flanking markers (crossing over) (Fig. 5A).

Because *rec12-D15* (null) and *rec12-Y98F* (active site) mutants lack recombination (Fig. 2), their chromosomes are achiasmatic and would be expected to suffer nondisjunction during meiosis I. Indeed, the *rec12-D15* (null) mutants fail to properly segregate their chromosomes (Fig. 3), and they exhibit significant levels of meiosis I nondisjunction (Fig. 4D). However, the frequency of meiosis I nondisjunction observed is only half of that expected–in half of the instances in which homologs should suffer nondisjunction, they end up segregating to opposite poles (Fig. 4D). We conclude that fission yeast has a distributive (achiasmatic) chromosome segregation system. This distributive system partially circumvents the meiosis I segregation errors of achiasmatic chromosomes and can function in the complete absence of Rec12 protein and Rec12-dependent crossovers (chiasmata).

Distributive segregation has been reported in organisms with naturally-occurring achiasmatic chromosomes and in a few other experimental circumstances (Carpenter, 1973; Dawson et al., 1986; Hawley et al., 1992; Hawley and Theurkauf, 1993; Hawley, 1996; Molnar et al., 2001). Our results indicate that a pathway for distributive segregation exists (and is fairly robust) in an organism that normally uses chiasmatic segregation. While Rec12 protein and Rec12-dependent crossovers (chiasmata) are not required for distributive segregation, catalytically-inactive Rec12 markedly increases the fidelity of distributive segregation (Fig. 4D) (Sharif et al., 2002). Presumably wild-type Rec12 protein can do so as well, although this has not been demonstrated directly. Thus, Rec12 has two key roles in meiotic chromosome segregation. It generates chiasmata to promote chiasmatic segregation, and when things go awry, it promotes distributive segregation of achiasmatic chromosomes (Fig. 5B).

There are two non-mutually exclusive hypotheses regarding how Rec12 promotes distributive segregation. First, Rec12/Spo11 may have a structural function for meiotic interhomolog interactions prior to and after initiation of recombination (Romanienko and Camerini-Otero, 2000). For example, Rec12 may help hold bivalents together in the absence of crossovers. This would be akin to the role of heterochromatin in distributive segregation in *Drosophila* (Carpenter, 1973; Carpenter, 1991; Hawley and Theurkauf, 1993). Second, Spo11/Rec12 may activate a "meiosis I nondisjunction/distributive segregation" checkpoint that provides time for the backup, distributive

segregation pathway to be established. For example, Rec12 and other components of the recombinase complex are in the right place at the right time to sense whether or not crossovers (chiasmata) are successfully generated. Rec12-dependent signal transduction (*e.g.*, checkpoint activation) would provide a parsimonious way to promote the backup, distributive segregation pathway in the presence of achiasmatic chromosomes.

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