Cohesins are required for meiotic DNA breakage and recombination in *Schizosaccharomyces pombe*

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In preparation for the unique segregation of homologs at the first meiotic division, chromosomes undergo dramatic changes. The meiosis-specific sister chromatid cohesins Rec8 and Rec11 of *Schizosaccharomyces pombe* are recruited around the time of premeiotic replication, and Rec10, a component of meiosis-specific linear elements, is subsequently added. Here we report that Rec10 is essential for meiosis-specific DNA breakage by Rec12 (Spo11 homolog) and for meiotic recombination. DNA breakage and recombination also depend on the Rec8 and Rec11 cohesins, strictly in some genomic intervals but less so in others. Thus, in addition to their previously recognized role in meiotic chromosome segregation, cohesins have a direct role, as do linear element components, in meiotic recombination by enabling double-strand DNA break formation by Rec12. Our results reveal a pathway, whose regulation is significantly different from that in the distantly related yeast *Saccharomyces cerevisiae*, for meiosis-specific chromosome differentiation and high-frequency recombination.

linear elements | meiotic recombination | regional specificity

The cardinal feature of meiosis is the reduction of chromosome number from diploid to haploid. Although chromosomes are replicated only once in meiosis, there are two nuclear divisions. In the first division (meiosis I, or MI) homologs, rather than sister chromatids, segregate from each other, a process that requires mutual recognition of homologs. Specific recognition is provided by high-frequency meiotic recombination, normally between allelic positions on homologs rather than sister chromatids. Recombination provides physical connections between homologs, in the form of one or more crossovers, which allow tension to form when homologs are properly positioned to segregate to opposite poles of the cell at MI. At the second division (meiosis II) sister centromeres segregate from each other, as in a normal mitotic division.

The unique behavior of chromosomes during meiosis requires certain meiosis-specific proteins, collectively called chromosomal core proteins, that modify sister chromatid cohesion, homolog juxtaposition and segregation, recombination, and perhaps replication. In the fission yeast *Schizosaccharomyces pombe*, the meiosis-specific cohesins Rec8 and Rec11 are recruited to chromosomes at or about the time of premeiotic replication (1, 2). Rec8 and Rec11 share sequence similarity with the *S. pombe* mitotic cohesins Rad21 and Psc3, respectively; *rec8* and *rec11* mutants manifest defects in meiotic sister chromatid cohesion and homolog segregation (1–3). Many organisms express a meiosis-specific Rec8 protein; like *S. pombe,* mice and humans express in addition a meiosis-specific Rec11-like protein, called STAG3, although the distantly related budding yeast *Saccharomyces cerevisiae* does not (4). Despite their important roles in chromosome segregation, the *rec8* and *rec11* genes were first identified by mutations that strongly reduce meiotic recombination at *ade6* (5). These observations indicate a close relation between cohesion and recombination in meiosis, but the basis of this relation has been obscure.

In most organisms, a large proteinaceous structure, the synaptonemal complex (SC), forms between homologs around the time of crossing over and disappears before meiosis I. *S. pombe* lacks a full-fledged SC but does form linear elements, which by electron microscopy resemble the axial element precursors to lateral elements of the SC in other organisms (6, 7). Linear elements are absent in *rec10* mutants (8), which were also first identified as mutants deficient in meiotic recombination (5). In *rec8* and *rec11* mutants, linear elements are present but are shorter and much less frequent than in wild type (8). As assayed by indirect fluorescence microscopy, Rec10 is recruited to chromosomes at only low levels in *rec8* and *rec11* mutants (9). Thus, cohesins are important for both cohesion and linear element formation and recombination. Why sister chromatid cohesins should be required for recombination between homologs has been a puzzle. Cohesins and linear element components might force recombination to occur between homologs by an unknown mechanism; alternatively, they might have a more direct role in recombination, as we report here.

Rec8, Rec10, and Rec11 have novel roles in meiotic recombination. Mutations in the genes encoding these proteins reduce recombination less in some regions of the genome than in other regions, including *ade6*, the locus assayed for their isolation (10–12). This region-specific behavior is unlike that of *rec12* mutants, which lack the protein with the putative active site for DNA double-strand breakage during meiosis (13); Rec12 is a homolog of Spo11, the *S. cerevisiae* double-strand break (DSB)-forming protein (14). *S. pombe rec12* mutations abolish meiotic recombination and DSB formation in all genomic intervals tested (15–17).

We have investigated the regional specificity of recombination in *rec8*, *rec10*, and *rec11* mutants by using complete deletion mutations rather than the original nitrosoguanidine-induced mutations or the partial *rec8* deletion used previously (10–12). These complete deletion mutations show that Rec10 and perhaps linear elements themselves are essential for recombination and DSB formation throughout the genome, and that Rec8 and Rec11 cohesins are required for DSB formation and recombination in substantial regions of the genome. Our results reveal that these chromosomal core proteins have multiple roles in meiotic chromosome behavior and lead us to discuss a pathway for the differentiation of mitotic chromosomes into specialized meiotic chromosomes competent for high-frequency recombination and reductional segregation. The regulation of this pathway differs significantly from that in *S. cerevisiae*, the only other species in which meiotic DSBs have been directly assayed.

Materials and Methods

***S. pombe* Strains.** Strains were constructed by standard meiotic crosses with one exception, the *rec10-175*::*kanMX6* allele, which was constructed by the method of Bähler *et al.* (18). A PCR used primers containing 80 nucleotides flanking the *rec10* coding sequence (base pairs 6807–6886 and 9263–9342 on cosmid SPAC25G10; GenBank accession no. Z70691) plus 20 nucleo-

Abbreviations: SC, synaptonemal complex; DSB, double-strand break; Chr. I/II/III, chromosome I/II/III.

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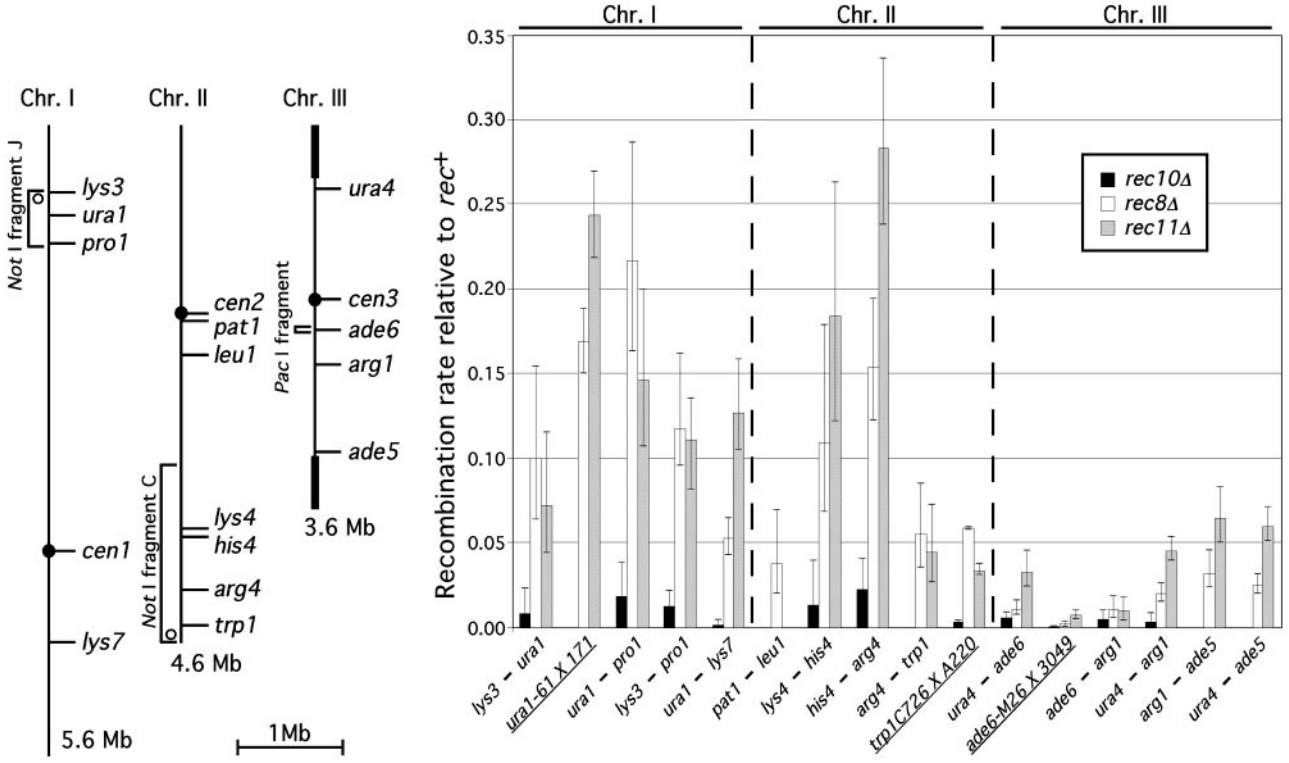

Fig. 1. Recombination is strongly reduced in *rec10*Δ mutants throughout the genome and differentially reduced in *rec8*Δ and *rec11*Δ mutants. (*Left*) *S. pombe* chromosomes and genetic loci. The chromosomes and the positions of the loci used here are drawn to scale, based on the genome sequence (23). Thick lines indicate rDNA (≈0.5 Mb) at each end of Chr. III. Brackets indicate restriction fragments analyzed in Fig. 2 using radioactive probes at the positions of the open circles. (*Right*) Relative recombination rates in *rec10*Δ (black), *rec8*Δ (white), and *rec11*Δ (gray) mutants. Intragenic (gene conversion) intervals are underlined. The genetic distance in cM at the indicated locus or interval in each mutant is expressed relative to that of wild type. Data are from Tables 1, 2, and 4. Error bar, standard deviation based on the binomial distribution of the numbers of recombinants observed and total spore colonies tested.

tides of plasmid pFA6a-kanMX6 and this plasmid as template. After purification by extraction with phenol and chloroform and precipitation with ethanol, the PCR product was used to transform strain GP3718 (*h*$^+$ *ade6-3049 rad50S pat1-114 end1-458*) to G418 resistance. The resulting strain was confirmed by nucleotide sequencing to contain the expected replacement. Additional alleles used but not listed in Tables 1–3 and Table 4, which is published as supporting information on the PNAS web site, were *ade5-36*, *arg1-14*, *arg4-55*, *his4-239*, *lys3-37*, *lys4-95*, *lys7-1*, *pro1-1*, *rad32*::*ura4*$^+$, *rec8*::*kanMX*, *rec11*::*kanMX*, *rec12-152*::*LEU2*, *ura4-D18*, and *ura4-294.* Complete genotypes and genealogies are available on request.

Meiotic Crosses and DNA Break Determination. Meiotic crosses were done and analyzed for recombinants as described (10), except that sporulations were at 25°C. Viable spore yields were determined as described (19). DNA from synchronous meiotic cultures of *pat1-114 rad50S* strains was analyzed for meiotic DNA breakage as described (16, 20).

Results and Discussion

Linear Element Component Rec10 Is Essential for Recombination Throughout the Genome. Using the *rec10-109* missense mutation (see *Supporting Text*, which is published as supporting information on the PNAS web site), DeVeaux and Smith (10) found that meiotic recombination is strongly reduced at *ade6* but occurs at essentially wild-type levels in certain other intervals, such as *lys3–ura1*. To determine whether this regional specificity is an inherent property of *rec10* mutants, we assayed recombination in the complete deletion mutant *rec10-175*::*kanMX6* (*rec10*Δ). This was done by random spore analysis: *S. pombe* has only three chromosomes, and abundant viable spores are obtained even in mutants defective in recombination and chromosome segregation (21). We assayed crossing over as intergenic recombination, a valid measure, because gene conversion in *S. pombe* occurs at low frequency for markers other than hotspots such as *ade6-M26* (on average <0.1% convertants per chromatid; P. Munz, personal communication cited in ref. 16). Gene conversion was measured as intragenic recombination, because where tested, >98% of intragenic recombinants in *S. pombe* are nonreciprocal (22). Remarkably, both types of recombination were strongly reduced in all intervals tested in the *rec10*Δ mutant. This simple outcome contrasts with the more complex results of *rec8*Δ and *rec11*Δ mutants, discussed below.

Crossing over in *rec10*Δ mutants was measured in nine intervals, representing each of the three chromosomes (Fig. 1; Tables 1 and 4). In the *lys3–ura1* interval, crossing over was reduced by a factor of >100, from 25 cM in *rec10*$^+$ crosses to ≈0.2 cM in *rec10*Δ. In the other intervals tested, crossing over was also strongly reduced by factors ranging from ≈40 to ≈600. The low frequencies of crossing over in the *rec10*Δ mutant (<1.3%) were similar to those found with *rec12* mutants (15–17) and are consistent with meiotic crossing over being essentially eliminated throughout the genome in *rec10*Δ mutants, as it appears to be in *rec12*Δ mutants.

Gene conversion was measured at loci on two chromosomes (Fig. 1; Table 2) and also appeared to be essentially eliminated by the *rec10*Δ mutation. At *ade6* (chromosome III, Chr. III), recombination was reduced by a factor of >500 in crosses between the hotspot *ade6-M26* and a nonhotspot allele *ade6-52* and by a factor of ≈2,000 in crosses between *ade6-M26* and the hyperhotspot *ade6-3049* (20). The residual level, a few Ade$^+$ per

Table 1. Crossing over requires linear element component Rec10 and sister chromatid cohesins Rec8 and Rec11

Chr.	Interval	Nominal cM*	*rec*$^+$ cM†	*rec10*Δ cM	*rec10*Δ Red'n‡	*rec8*Δ cM	*rec8*Δ Red'n	*rec11*Δ cM	*rec11*Δ Red'n
I	*lys3–ura1*	34	25	0.2	120	2.5	10	1.8	14
I	*ura1–pro1*	42	27	0.5	50	5.9	4.6	4.0	6.8
I	*lys3–pro1*	76	57	0.7	80	6.7	8.5	6.3	9.0
I	*ura1–lys7*	601	—§	1.1	600	31	20	76	7.9
II	*pat1–leu1*	47	58	ND	ND	2.2	26	ND	ND
II	*lys4–his4*	12	15	0.2	75	1.6	9.4	2.7	5.6
II	*his4–arg4*	74	53	1.2	44	8.2	6.5	15	3.5
II	*arg4–trp1*	54	47	ND	ND	2.6	18	2.1	22
III	*ura4–ade6*	197	—	1.1	180	2.2	90	6.5	30
III	*ade6–arg1*	47	73	<0.4	>180	0.8	90	0.7	100
III	*arg1–ade5*	128	—	ND	ND	4.1	31	8.2	16
III	*ura4–arg1*	244	—	0.8	>300	5.0	49	11.1	22
III	*ura4–ade5*	372	—	ND	ND	9.4	40	22.2	17

ND, not determined; Red'n, reduction.

*Calculated from the physical distance (23) and the genome average of 0.16 cM/kb (16).

†Calculated from the observed recombinant frequency (Table 4) and Haldane's mapping function: $x = -50\ \ln(1-2R)$, where x = genetic distance in centimorgans (cM) and R = fraction of recombinant spores among total spores analyzed.

‡Factor by which the observed genetic distance in the indicated mutant is less than that observed in wild type or, for values >75 cM, the nominal distance.

§Observed recombinant frequency too close to 50% for reliable calculation of genetic distance.

million viable spores, is similar to that in *rec12* mutants and reflects residual mitotic recombination potential (15). At *trp1* (Chr. II) recombination was reduced by a factor of >200 to a level below reliable detection. Thus, Rec10 appears to be required for meiotic recombination throughout the genome.

The genome-wide elimination of recombination in *rec10*Δ is starkly different from the behavior of *rec10-109*, which is strongly deficient in some intervals but essentially wild type in others (10). As detailed in *Supporting Text*, we found that *rec10-109* has two closely linked mutations and encodes a partially active protein. Below, we suggest an explanation for the regional specificity of *rec10-109.*

Rec10 Is Essential for Meiotic DNA Breakage. Meiotic recombination is initiated by programmed DSBs in both *S. cerevisiae* and *S. pombe* (21, 24). The near absence of recombination in *rec10*Δ mutants prompted us to measure meiotic DNA breakage in this mutant. To maximize the sensitivity of detecting DSBs, we used *rad50S* strains, in which meiotic DSB-processing is blocked and DSBs accumulate (16, 25). Although DSBs were readily detected at hotspots on Chr. I and II and at the *ade6-3049* hotspot on Chr. III in *rec*$^+$ *rad50S* cells, such DSBs were not detectable in the *rec10*Δ *rad50S* mutant (Fig. 2 *A–C* and Fig. 4, which is published as supporting information on the PNAS web site). Consistent with this finding, the three chromosomal DNAs remained largely intact in the *rec10*Δ *rad50S* mutant but were nearly totally broken in *rec*$^+$ *rad50S* cells (Figs. 2*D* and 4); chromosomal DNAs also remain largely intact in a *rad50*$^+$ *rec10-109* strain (unpublished data cited in ref. 13). The behavior of the *rec10*Δ mutant is thus indistinguishable from that of *rec12*Δ mutants (13, 16). We conclude that Rec10, like Rec12, is essential for meiotic DNA breakage and recombination (see additional evidence below).

Because linear elements are absent in *rec10* mutants (9), linear elements may be essential for meiotic DSB formation. If so, this role of linear elements differs from that of axial and central element components of the SC in *S. cerevisiae*, which are not essential for meiotic DSB formation, although some components facilitate DSB formation (e.g., ref. 26). We cannot exclude, however, the possibility that Rec10 has two functions, one for linear element formation and another for DSB formation; in this case linear elements *per se* may not be required for DSB formation. Nevertheless, the Rec10 protein, a major core component of meiotic chromosomes (9), is essential for DSB formation and recombination.

Meiotic Sister Chromatid Cohesins Rec8 and Rec11 Are Required for Meiotic Recombination and DNA Breakage in a Region-Specific Manner. The meiosis-specific cohesins Rec8 and Rec11 are also major components of meiotic chromosomes (1, 2). Putative point mutations in *rec8* and *rec11* reduce meiotic recombination in a region-specific manner (10–12). We tested complete deletion mutations for this behavior and found reductions whose extent depended on the interval measured, similar to the differential reductions previously observed with the putative point mutations. Thus, the regional specificity is an inherent property of

Table 2. Gene conversion requires Rec10, Rec8, and Rec11

Chr.	Alleles crossed	Recombinant frequency (prototrophs per 10^6 viable spores)* *rec*†	*rec10*Δ	*rec12*Δ	*rec8*Δ	*rec11*Δ	*rec8*Δ *rec11*Δ
I	*ura1-61* × *ural-171*	160 ± 12 (6)	ND	6.0 ± 1.7 (3)	27 ± 3 (4)	39 ± 4 (4)	23 ± 3 (4)
II	*trp1A-220* × *trp1C-726*	650, 1,700	<3.5	ND	68, 69	36, 44	ND
III	*ade6-M26* × *ade6-52*	3,800 ± 700 (5)	<5, <8	<3, <5†	5 ± 0.3 (4)	7 ± 1.7 (4)	ND
III	*ade6-M26* × *ade6-3049*	24,700 ± 640 (3)	12 ± 5 (3)	ND	55 ± 20 (3)	190 ± 47 (3)	47 ± 5 (6)

ND, not determined.

*Data are the means ± SEM from the number of independent determinations in parentheses or data from two experiments.

†Data from ref. 43.

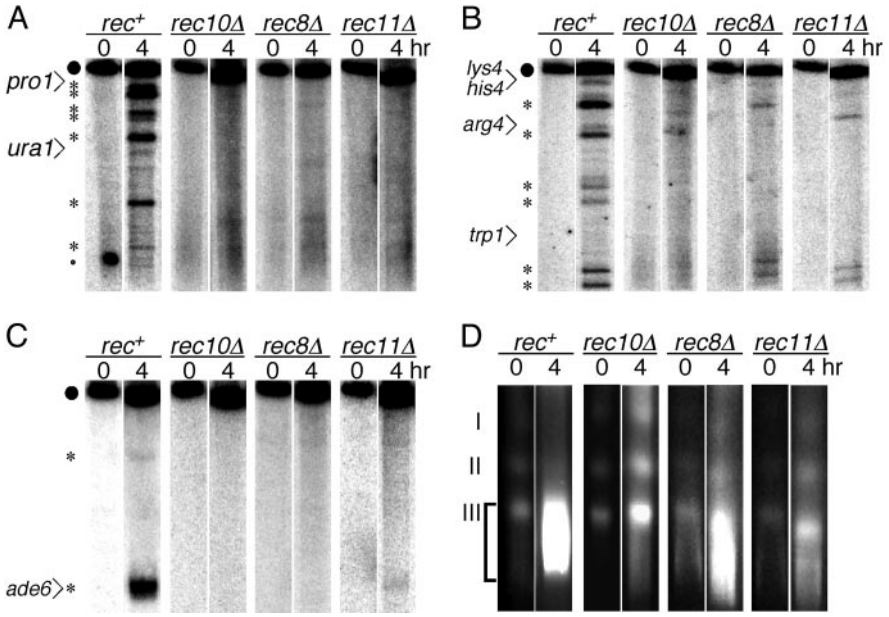

Fig. 2. Meiotic DNA breakage is strongly reduced or eliminated in *rec10Δ, rec8Δ,* and *rec11*Δ mutants. (*A*) DNA was prepared at the indicated time (hr) after meiotic induction of *rad50S* strains, digested with NotI, electrophoresed, and hybridized with a 1-kb probe from the left (top) end of the 501-kb NotI fragment J on Chr. I (see Fig. 1). A bullet (●) indicates the intact fragment, and asterisks (*) indicate DNA from wild type (*rec*$^+$) cells broken at meiosis-specific hotspots. Small open circle (o), irreproducible spot (see Fig. 4*A*). The approximate positions of genetic loci used to measure recombination (Fig. 1) are indicated. (*B*) DNA was digested with NotI, electrophoresed, and hybridized with a 1-kb probe from the right (bottom) end of Chr. II. (*C*) DNA was digested with PacI, electrophoresed, and hybridized with a 1-kb probe from the right (bottom) end of the 41-kb PacI fragment containing the *ade6-3049* hotspot on Chr. III. (*D*) Whole chromosomal DNA was analyzed by pulsed-field gel electrophoresis and staining with ethidium bromide. Meiotically broken DNA is a smear (bracket) that migrates with or more rapidly than the three intact chromosomes (Chr. I–III). For details and complete time courses, see Fig. 4.

Rec8 and Rec11, not a reflection of the particular mutations used previously (10–12).

Crossing over was differentially reduced in the mutants. The *rec8*Δ mutation reduced crossing over by factors ranging from ≈5 (*ura1–pro1*; Chr. I) to ≈90 (*ura4–ade6–arg1*; Chr. III) (Fig. 1; Tables 1 and 4). Similarly, *rec11*Δ reduced crossing over by factors of ≈4 to ≈100, depending on the interval tested. Thus, unlike the situation in *rec10*Δ mutants, some intervals, but not all, retained significant levels of recombination (Fig. 1).

Gene conversion was also differentially reduced (Fig. 1; Table 2). For example, *ura1* recombination was reduced by factors of ≈6 in *rec8*Δ and ≈4 in *rec11*Δ, whereas *ade6* recombination was reduced by factors of >100 in both mutants. The *rec8*Δ *rec11*Δ double mutant had about the same frequency of recombination at *ura1* and *ade6* as *rec8*Δ, the marginally stronger mutation (Table 4). This result and the similar reductions by *rec8*Δ and *rec11*Δ single mutations suggest that Rec8 and Rec11 act at the same step, or closely related steps, of a pathway of events leading to recombination discussed later.

In *rec8*Δ and *rec11*Δ *rad50S* mutants, we measured meiotic DNA breakage in several intervals of the genome but found substantial DSBs in only one region, near *his4–trp1* on the right end of Chr. II (compare Fig. 2 *B* with *A* and *C* and Fig. 4; additional unpublished data). These breaks occurred at 15–20% (upper band in Fig. 2*B*; *his4–arg4* interval) and 30–50% (lowest two bands; distal to *trp1*) of the frequency in wild type, consistent with the reduction but not elimination of recombination in the *his4–arg4* interval by *rec8*Δ and *rec11*Δ mutations (Fig. 1; Tables 1, 2, and 4). In other regions of the genome examined, DSB levels were reduced to near background (premeiotic) levels (Figs. 2 and 4; additional unpublished data). It is thus unclear whether recombination, whose assay is more sensitive, is reduced by factors significantly different from those of DSBs. One should note that in *rec*$^+$ cells, DSB levels and crossover levels are not strictly correlated (16). The three chromosomal DNAs were broken less extensively in the *rec8*Δ and *rec11*Δ *rad50S* mutants than in wild type (*rec*$^+$ *rad50S*; Figs. 2*D* and 4); chromosomal DNAs also remain largely intact in a *rad50*$^+$ strain with the nitrosoguanidine-induced *rec8-110* mutation (unpublished data cited in ref. 13). Our results suggest that breaks occur in *rec8*Δ and *rec11*Δ mutants but at a significantly reduced frequency throughout the genome, consistent with the surveys by recombination (Fig. 1).

These results indicate that Rec8 and Rec11 play greater roles in meiotic recombination in certain regions of the genome than in others. We propose that, although the majority of recombination depends on Rec8 and Rec11, a minor pathway indepen-

Table 3. Loss of Rec10, but not Rec8 or Rec11, abolishes DNA breakage: Genetic evidence from suppression of *rad32*Δ

Genotype	Relative viable spore yield, %*	
	rad32$^+$	*rad32*Δ
rec$^+$	100	0.05 ± 0.014
*rec10*Δ	18 ± 0.45	8.8 ± 1.1
*rec12*Δ	26 ± 1.6	3.6 ± 0.20
*rec8*Δ	27 ± 2.2	0.11 ± 0.018
*rec11*Δ	124 ± 6.2†	0.16 ± 0.021

*Viable spore yields (mean ± SEM; *n* = four crosses) are expressed as the number of viable spores produced divided by the number of cells of the less-numerous parent in the mating mixture, relative to wild type (*rad*$^+$ *rec*$^+$) set at 100. The wild type produced 9.8 ± 0.8 viable spores per less numerous parental cell (mean ± SEM; *n* = four crosses); this number is greater than the theoretical yield of four because of slight residual mitotic growth of the cells before sporulation. The data above were obtained in one experiment. In several independent experiments on different days, comparable results were obtained, except as noted in †.

†In 11 additional crosses on 4 different days, *rec11*Δ *rad32*$^+$ produced the expected lower value of 39 ± 0.8% of the wild-type yield (5).

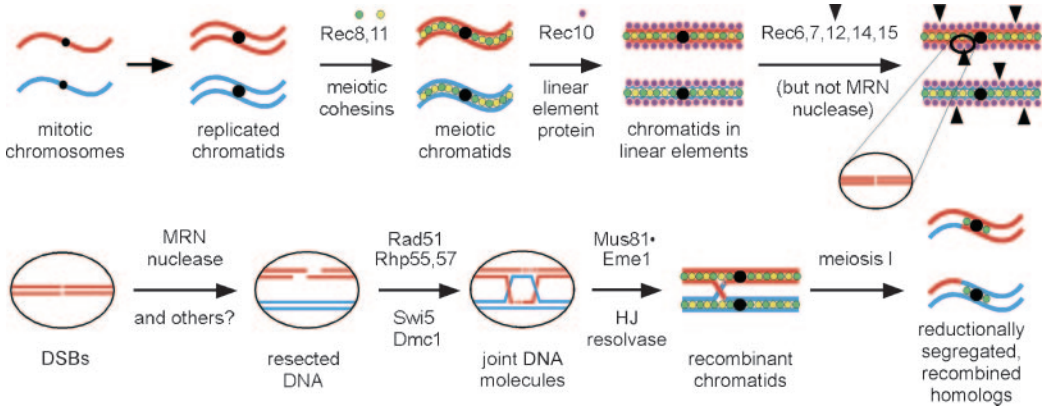

Fig. 3. Proposed pathway for *S. pombe* meiotic chromosome differentiation, DSB formation, and recombination. During or shortly after premeiotic replication, the sister chromatid cohesins Rec8 and Rec11 are recruited to the chromosomes. Largely dependent upon their presence, the linear element component Rec10 is recruited, which in turn recruits or activates Rec12 and its partner proteins, Rec6, Rec7, Rec14, and Rec15. The Rec12 complex makes DNA breaks, which are resected by the Rad32•Rad50•Nbs1 nuclease complex or other proteins. DNA strand exchange between the resected DNA and an intact homolog is promoted by a combination of Rad51, Rhp55, Rhp57, Swi5, and Dmc1 proteins. Joint molecules include Holliday junctions (HJ), but it is not known whether these have double HJ, as illustrated, or a different structure; these molecules are resolved by the Mus81•Eme1 complex into crossover molecules. The crossovers facilitate reductional segregation of homologs at the first meiotic division. Red and blue lines are duplex DNA, except within the magnified oval, where they are single DNA strands. See text for references and further discussion.

dent of Rec8 and Rec11 functions in some but not other genomic intervals. This minor pathway may depend on the residual mitotic cohesin Rad21 observed on meiotic chromosomes (1). Low-level loading of Rec10 observed in the complete absence of Rec8 or Rec11 (9) may occur by this minor pathway and account for the regional specificity of *rec8*Δ and *rec11*Δ mutations. The *rec10-109* mutant may be deficient specifically in the Rec8, Rec11-dependent pathway; this proposal accounts for the similar patterns of recombination in *rec10-109*, *rec8*Δ and *rec11*Δ mutants (Fig. 1; ref. 10). It is important to note, however, that because all intervals tested have significantly less recombination in *rec8*Δ and *rec11*Δ mutants than in wild type, these sister chromatid cohesins play an important role in meiotic DSB formation and recombination throughout the genome. Cohesins could restrain recombination between sisters, thereby favoring recombination between homologs. But the strong reduction of DSB formation in *rec8*Δ and *rec11*Δ mutants indicates a more direct active role for cohesins in recombination between homologs, as discussed below.

Genetic Evidence That Rec10 Is Essential for DNA Breakage. We next tested for meiotic DNA breakage in *rec8*Δ, *rec10*Δ, and *rec11*Δ mutants with a more sensitive genetic assay. This was done by studying the interaction of these mutations with *rad32*Δ, which eliminates the Rad32·Rad50·Nbs1 nuclease complex. This complex is essential for the repair of meiotic DSBs but, in *S. pombe*, is not required for their formation (19). Consequently, *rad32*Δ mutants form very few viable spores; in a *rec12*Δ background, in which DSBs are not formed, the viable spore yield is ≈50- to 70-fold higher (Table 3; ref. 19). The viable spore yield of *rad32*Δ *rec10*Δ was nearly the same as that of *rec10*Δ, >100 times higher than that of *rad32*Δ (Table 1). This strong suppression of *rad32*Δ by *rec10*Δ is similar to that by *rec12*Δ and indicates that few if any DSBs are formed in the *rec10*Δ mutants. Thus, these genetic results are consistent with the physical analysis of DNA (Figs. 2 and 4) and the near absence of meiotic recombination in *rec10*Δ mutants (Fig. 1; Tables 1, 2, and 4).

In contrast to *rec10*Δ, the *rec8*Δ and *rec11*Δ mutations suppressed *rad32*Δ only weakly. These mutations increased the viable spore yield of *rad32*Δ by a factor of only 2 or 3, to a level <1/100 of that in the *rec8*Δ and *rec11*Δ single mutants (Table 3). This slight suppression is consistent with the low but detectable levels of DSBs and recombination in these mutants (Figs. 1 and 2; Tables 1, 2, and 4). Even a low level of DSBs would yield dead spores in the absence of repair in *rad32*Δ strains. We conclude that Rec10 is essential for meiotic DSB formation, and Rec8 and Rec11 for the great majority of DSB formation as assayed physically.

A Pathway for Meiotic Chromosome Differentiation: Regulation of DSB Formation and Repair. The results presented above shed additional light on the pathway for differentiation of mitotic chromosomes into meiotic recombinants and properly segregated homologs in *S. pombe* (Fig. 3). This pathway can be divided into two parts, the formation of DSBs and their repair.

During or shortly after premeiotic replication, the meiosis-specific sister chromatid cohesins Rec8 and Rec11 largely replace the mitotic cohesins Rad21 and Psc3; Rec8 is bound throughout the chromatids, whereas Rec11 is largely confined to the arm regions (1, 2). These cohesins are required for normal loading of Rec10, a major component of linear elements, which resemble the axial element precursors of the SC of other organisms (7, 9). We propose that Rec10, and indirectly Rec8 and Rec11, are required for the loading or activity of a putative complex of Rec12 and other recombination-promoting proteins (Rec6, Rec7, Rec14, and Rec15). As expected from this view, Rec7 focus formation on meiotic chromosomes requires Rec10 (J. Loidl, personal communication). Rec12 is a homolog of *S. cerevisiae* Spo11, which forms DSBs with a covalent link between the 5′ DNA end and a critical active-site Tyr in the protein; as in *SPO11*, mutation of this Tyr codon in *rec12* abolishes DSB formation and meiotic recombination (13, 14, 27). By analogy with *S. cerevisiae*, we infer that Rec6, Rec7, Rec14, and Rec15 form a complex with Rec12 and activate it, because mutations in the corresponding genes have phenotypes indistinguishable from those of *rec12* mutations (reviewed in refs. 21 and 28). The Mre11·Rad50·Xrs2 (MRX) complex is essential for meiotic DSB formation in *S. cerevisiae* (24, 28). In contrast, however, the corresponding Rad32·Rad50·Nbs1 complex is not required for DSB formation in *S. pombe* (19), *Arabidopsis* (29), or *Coprinus* (S. Acharya and M. Zolan, personal communication cited in ref. 19). Conversely, Rec8 cohesin is essential for the majority of DSB formation in *S. pombe* (Fig. 2) but is not required in *S. cerevisiae* (30). Furthermore, in *S. pombe*, the linear element protein Rec10 is essential for DSB formation (Fig. 2; Table 3), but in *S. cerevisiae*, the axial and central element

components of the SC are not essential, although Hop1 and Red1, an axial element protein with very slight amino acid sequence similarity to Rec10 (9), are required for full levels of DSB formation (e.g., refs. 26 and 31). The occurrence of Rec11 and related meiosis-specific cohesins in *S. pombe* and mammals, but not in *S. cerevisiae*, indicates an additional similarity between *S. pombe* and other organisms. In summary, the chemical mechanism of DSB formation by Spo11 or its homolog appears to be the same in these two distantly related yeasts and other organisms, but the regulation of Spo11 (Rec12) localization or activity is markedly different.

The regulation of DSB repair also differs in the two yeasts. In *S. cerevisiae*, the repair of DSBs begins with the removal of the Spo11 protein and the resection of the 5′ end of the DNA, to produce single-stranded DNA with a 3′ end (28). These steps require the Mre11·Rad50·Xrs2 complex in *S. cerevisiae* (25) and perhaps the Rad32·Rad50·Nbs1 complex in *S. pombe,* too, because DSBs accumulate in *rad32*Δ and *rad50*Δ mutants (19). Joint DNA molecules are formed by invasion of the 3′ end into homologous DNA, a step promoted by Rad51, a homolog of the bacterial strand-exchange protein RecA (31). This step appears to be aided by several proteins, including the *S. pombe* proteins Rad55, Rad57, Swi5, and Dmc1 (19, 32, 33). Notably, DSBs accumulate in *dmc1* mutants in the widely studied SK1 strain of *S. cerevisiae* but not in *S. pombe* or another strain of *S. cerevisiae* (19, 24); Dmc1 is required, however, for successful meiosis in mice and *Arabidopsis thaliana* (34, 35). *S. cerevisiae* Rec8 is required for DSB repair (30), and indirect evidence suggests that Rec8 is also required for DSB repair in mice and *Caenorhabditis elegans* (36, 37). In *S. pombe*, Rec8 and Rec11 do not appear to be required for DSB repair: *rec8*Δ *rad32*Δ and *rec11*Δ *rad32*Δ mutants have low viable spore yields (Table 3), indicating that sufficient DSBs are made in *rec8*Δ and *rec11*Δ mutants to block high viable spore yields, but *rec8*Δ and *rec11*Δ (*rad32*$^{+}$) mutants have relatively high viable spore yields, indicating that these mutants can repair at least the few DSBs made. Joint molecules include Holliday junctions, which in *S. pombe* are resolved into linear molecules by the Mus81·Eme1 Holliday junction resolvase (refs. 38–41; G. Cromie, R. Hyppa, and G.R.S., unpublished observations); the resolving protein(s) in *S. cerevisiae* are not yet identified. In *S. pombe*, resolution is strongly biased toward the crossover configuration (42) and results in the physical connections between homologs that aid their reductional segregation into different nuclei at meiosis I. In summary, the repair of DSBs may occur by similar mechanisms in the two yeasts but, as with their formation, the regulation of their repair differs.

The differentiation of mitotic chromosomes into their appropriate meiotic forms begins at an early stage, the loading of sister chromatid cohesins during or shortly after premeiotic replication. This early crucial step appears to orchestrate the subsequent steps for successful recombination and homolog segregation, the essential features of meiosis.

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