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Meiotic Recombination in *Schizosaccharomyces pombe*: A Paradigm for Genetic and Molecular Analysis

Gareth Cromie · Gerald R. Smith (✉)

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
gsmith@fhcrc.org

Abstract The fission yeast *Schizosaccharomyces pombe* is well-suited for both genetic and biochemical analysis of meiotic recombination. Recent studies have revealed ~50 gene products and two DNA intermediates central to recombination, which we place into a pathway from parental to recombinant DNA. We divide recombination into three stages – chromosome alignment accompanying nuclear "horsetail" movement, formation of DNA breaks, and repair of those breaks – and we discuss the roles of the identified gene products and DNA intermediates in these stages. Although some aspects of recombination are similar to those in the distantly related budding yeast *Saccharomyces cerevisiae*, other aspects are distinctly different. In particular, many proteins required for recombination in one species have no clear ortholog in the other, and the roles of identified orthologs in regulating recombination often differ. Furthermore, in *S. pombe* the dominant joint DNA molecule intermediates contain single Holliday junctions, and intersister joint molecules are more frequent than interhomolog types, whereas in *S. cerevisiae* interhomolog double Holliday junctions predominate. We speculate that meiotic recombination in other organisms shares features of each of these yeasts.

Abbreviations

DSB	double-strand break
HJ	Holliday junction
LinE	linear element
MCM	mini-chromosome maintenance
MI	first meiotic division
MMR	mismatch repair
MRN	Mre11-Rad50-Nbs1 complex
NER	nucleotide excision repair
SC	synaptonemal complex
SDSA	synthesis-dependent strand annealing
SPB	spindle-pole body
ss	single-stranded
SSB	ss DNA binding protein

1
S. pombe: An Excellent Model Organism for Studying Meiotic Recombination

Homologous genetic recombination plays two important roles during meiosis, the special nuclear divisions during which chromosome number is reduced from two (diploid) to one (haploid). First, recombination provides the physical connection between homologs that aids their pairing and proper segregation at the first meiotic division (MI), and second, it increases the genetic diversity that aids evolution (see Lankenau, this book). Elucidating the molecular mechanism of meiotic recombination requires a combination of genetic and biochemical analysis. Fungi, such as yeasts, have been particularly useful in this regard, for they have the essential features of meiosis found in complex organisms yet are more tractable for genetics and biochemistry. Notably, in many fungi the haploid products (spores) from each meiosis are enclosed in an ascus. Analysis of the haploid progeny from one ascus reveals all of the products of a single meiotic recombination event at each locus analyzed.

Meiosis has been especially well-studied in the budding yeast *Saccharomyces cerevisiae* (see Keeney, this SERIES; see chapters by Heyer; Lichten; Hunter, this book) and the distantly related fission yeast *Schizosaccharomyces pombe* discussed here (see also Ding & Hiraoka, this book; see further Pérez-Hidalgo, Moreno and Martín-Castellanos; or Tanaka and Watanabe, this SERIES). Both haploid and diploid cells of these yeasts can be grown indefinitely by mitotic division; genetic analysis that uses recessive markers is simpler in haploids. Large cultures of cells can be synchronously induced for meiosis, facilitating biochemical analysis. The nucleotide sequences of their relatively small genomes, ~14 Mb, are essentially complete, permitting comprehensive genomic studies. In addition, *S. pombe* offers special advantages. The strongest meiotic recombination-deficient (Rec^-) mutants of *S. pombe* produce many viable spores in part because this species has only three chromosomes, which, in the absence of recombination, would still be expected to segregate correctly and produce viable spores 12.5% of the time (2^{-3}). *S. pombe* also has a mechanism for actively segregating non-recombinant (achiasmate) chromosomes at MI (Molnar et al. 2001; Davis and Smith 2005). Consequently, strong Rec^- mutants that cannot initiate recombination are nevertheless able to produce ~ 10–25% as many viable spores as the wild type, an outcome that greatly aids analysis of such mutants (Ponticelli and Smith 1989; Young et al. 2004; Gregan et al. 2005). All commonly used strains are derived from a single culture (Munz et al. 1989): their near isogenicity simplifies the use of strains and comparisons of results among labs. The *M26* and closely related hotspots of recombination are exceptionally strong and are the best-defined meiotic hotspots in terms of nucleotide sequence (see Sect. 6.1).

Some aspects of the molecular biology of *S. pombe* are more similar to those of humans than are those of *S. cerevisiae*. These aspects of *S. pombe* include more complex centromeres and origins of replication, the presence of RNAi and certain histone modifications, and the specifics of cell-cycle control. In contrast, *S. pombe* is unusual in not having a fully developed synaptonemal complex (SC; Olson et al. 1978; Bähler et al. 1993), a large meiosis-specific structure joining paired homologs (see chapters by Suja and Julio S. Rufus, or Mehrotra, Hawley and McKim, this SERIES). The role of the SC is not clear, but its absence from *S. pombe* indicates that it is not essential for meiosis or recombination. In addition, *S. pombe* does not have crossover interference, the regulation of the number of crossovers and their distribution along chromosomes (Munz 1994). These characteristics allow in *S. pombe* a study of the essential features of recombination without the complexities of the SC or interference. Comparison of results among different species, such as *S. pombe* and *S. cerevisiae*, has revealed both conserved and diverged aspects of meiosis. In this regard, comparison of *S. pombe* and *S. cerevisiae* may help deduce the evolution of meiosis.

2
Overview: A Pathway for *S. pombe* Meiotic Recombination

In our current understanding of *S. pombe* meiotic recombination there are three stages, the first of which is concurrent with the other two: 1) the overall alignment and then intimate pairing of homologs, 2) the programmed formation of DNA double-strand breaks (DSBs), and 3) the repair of DSBs (Fig. 1). Stages 1 and 2 are meiosis-specific, whereas stage 3 shares many functions with mitotic DNA repair. Stage 1, homolog alignment, involves the clustering of telomeres ("bouquet" formation) and the movement of the nucleus back and forth in the cell ("horsetail" formation) (see Ding & Hiraoka, this book). These features are found in most organisms but are exaggerated in *S. pombe*. Homolog alignment reduces recombination between non-allelic loci with similar sequences (Niwa et al. 2000; Davis and Smith 2006); ectopic recombination between such loci could generate deleterious translocations.

During the horsetail stage chromosomes are replicated and sister chromatid cohesion is modified to allow the unique segregation of homologs at MI (see Tanaka & Watanabe, this SERIES). The meiosis-specific cohesin subunits Rec8 and Rec11 are important for the formation of linear elements, which are reminiscent of the axial elements of the SC (Lorenz et al. 2004). Linear elements, in turn, appear to be important for the assembly onto the chromosomes (or activation) of the proteins that make DSBs, including the active-site protein Rec12 (Spo11 homolog).

DSBs are made by Rec12 in concert with other proteins (stage 2) and are repaired by interaction with homologous DNA of either the sister chromatid

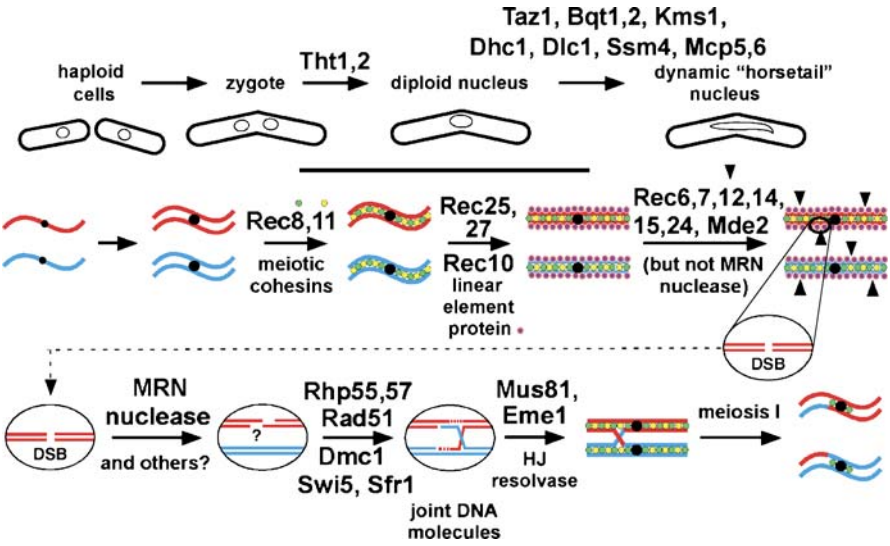

Fig. 1 A pathway for meiotic recombination in *S. pombe*. The upper panels portray the fusion of cells and nuclei and the formation of the "horsetail" nucleus. The middle and lower panels portray the chromosome and DNA events that occur during the horsetail stage, which ceases shortly before meiosis I. Identified gene products required at each stage are indicated above the arrow leading to that stage. Additional proteins required for meiotic recombination, but whose points of action are not clear, include *rec13*, *rec17 – 21*, *mug1*, *mug5*, *pds5*, *rqh1*, and *meu13* (see sections 2, 3, and 8). MRN, Rad32 (Mre11)-Rad50-Nbs1 complex. Modified from Ellermeier and Smith (2005)

or the homolog (stage 3). Only interhomolog interaction gives rise to the physical connections (chiasmata) that aid homolog segregation at MI, but sister chromatid exchange does occur. The regulation of these two types of repair is an intriguing problem not yet solved. DSB repair occurs in steps. First is the formation of hybrid DNA, which has one strand from each parental DNA, and one or two Holliday junctions, an intermediate with two crossed, single strands connecting the parental duplexes. Second is the resolution of the Holliday junction(s) into linear duplexes, which may occur in either the crossover or non-crossover configuration. Regulation of this outcome is also an intriguing, largely unsolved problem.

In the following sections we discuss each of these stages of meiotic recombination. Tables 1–3 list the known *S. pombe* gene products required for wild-type levels of meiotic recombination. (Most of the primary references are given in these tables rather than in the text.) These gene products are listed according to the stage at which they play the most prominent role; however, some may act at more than one stage. Mutations in additional genes (*rec13* and *rec17 – rec21*) reduce meiotic recombination frequencies (DeVeaux

et al. 1992), but these mutations have not been placed in genes identified otherwise.

S. pombe typically grows as haploid cells. When cells of opposite mating type meet under starvation conditions, the cells and then their nuclei fuse to form a diploid. Unless nutrients are supplied, the diploid immediately undergoes meiosis (see Pérez-Hidalgo, Moreno & Martín-Castellanos, this SERIES). Two mutants, *tht1* and *tht2*, are deficient in nuclear fusion and produce essentially no interhomolog recombinants among spores. Although not reported, intersister recombination in these mutants is expected to be high, since DSBs are formed and repaired as in wild-type. Each nucleus undergoes an aberrant meiosis, sometimes with only one nuclear division.

3
Nuclear Movement Promotes Chromosome Alignment: "Bouquet" and "Horsetail" Formation

Before the outset of meiosis the centromeres are clustered at the spindle-pole body (SPB), the fungal equivalent of the centrosome. As meiosis proceeds, the telomeres cluster and replace the centromeres at the SPB, to form the meiotic "bouquet" arrangement of chromosomes (Chikashige et al. 1994). The SPB leads the nucleus back and forth in movement across the cell, and the nucleus becomes elongated and curved, like a horse's tail. Meiotic recombination is reduced by a factor of ~5 in all of the tested mutants deficient in bouquet or horsetail formation (Table 1). In these mutants DSBs are formed and, where tested, repaired with nearly wild-type frequency and kinetics. Presumably, repair occurs most frequently by interaction with the sister chromatid, with some residual interhomolog interaction accounting for the observed recombinants.

Bouquet formation requires two meiosis-specific gene products, Bqt1 and Bqt2. These small proteins appear to act as a complex, gluing the telomeres to the SPB. Throughout the life cycle, Taz1 binds to telomeres and to Rap1. The Bqt1-Bqt2 complex forms a meiosis-specific bridge between Rap1 and Sad1, a component of the SPB, thereby joining the telomeres to the SPB. In the absence of Taz1, Rap1, Bqt1, or Bqt2 the nucleus moves but, since the telomeres are not attached to the SPB, the nucleus does not assume the characteristic horsetail shape, and chromosomes are not properly aligned.

The bouquet restricts ectopic recombination, which can cause deleterious genome rearrangements. In *S. pombe*, ectopic recombination occurs 10–1000 times less frequently than allelic recombination (Virgin and Bailey 1998). Mutations in *kms1* and *bqt2* affect bouquet formation and increase the frequency of meiotic ectopic recombination up to 20-fold. Attachment of telomeres to the SPB during bouquet formation may restrict recombination to sequences

Table 1 Genes required for recombination and nuclear fusion, nuclear movement, bouquet formation, or chromosome alignment

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs. for role in recombination
tht1	63	–[a]	Kar5 (19)	Nuclear fusion	Tange et al. 1998
tht2	23	1000[a]	–[b]	Nuclear fusion	Martín-Castellanos et al. 2005
taz1	75	5	Tbf1 (30)	Binds telomere repeats	Cooper et al. 1998; Niwa et al. 2000
rap1	80	–[c]	Rap1 (22)	Binds Taz1	Chikashige and Hiraoka 2001; Kanoh and Ishikawa 2001
bqt1	12	5	–	Connects telomeres and SPB	Martín-Castellanos et al. 2005; Chikashige et al. 2006
bqt2	14	5	–	Connects telomeres and SPB	Martín-Castellanos et al. 2005; Chikashige et al. 2006; Davis and Smith 2006
sad1	58	–[c]	Mps3 (17)	SPB component	Hagan and Yanagida 1995
kms1	69	5	–	SPB component	Shimanuki et al. 1997; Miki et al. 2002
mcp6	38	5	–	SPB component	Saito et al. 2005
dhc1	484	5	Dyn1 (25)	Dynein motor protein	Yamamoto et al. 1999
dlc1	12	10	YER071C (21)	Dynein accessory factor	Miki et al. 2002
ssm4	77	10	Nip100 (24)	Binds Dhc1	Niccoli et al. 2004
mcp5 (*num1*)	111	5	Num1 (24)	Binds dynein to microtubules	Saito et al. 2006; Yamashita and Yamamoto 2006; C. Ellermeier, pers. comm.

Table 1 (continued)

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs.
pds5[d]	139	5	Pds5 (24)	Sister chromatid cohesin partner	Tanaka et al. 2001; Wang et al. 2002; Ding et al. 2006; unpublished data
rqh1 (*rec9*)[d]	150	5	Sgs1 (37)	RecQ family helicase	Stewart et al. 1997; J. Young, pers. comm.
mug1[d]	41	5	Uso1 (23) Sla2 (23)		Martín-Castellanos et al. 2005; C. Ellermeier, pers. comm.
mug5[d]	21	5	–		Martín-Castellanos et al. 2005; C. Ellermeier, pers. comm.

[a] *tht1* is recombination-proficient in azygotic meiosis (that of an established diploid); *tht2* was not tested. *tht1* is assumed to be as deficient in zygotic meiosis (that immediately following mating) as *tht2*
[b] No ortholog is obvious
[c] Not determined. Requirement for meiotic recombination is assumed, based on the protein being required for telomere clustering during meiosis
[d] Role in recombination is uncertain. See section 3

equivalent distances from the anchored telomeres. This spatial constraint would favor allelic over ectopic recombination.

Horsetail movement requires the dynein components Dhc1 (heavy chain) and Dlc1 (light chain), the dynactin component Ssm4, and the SPB components Mcp6 (meiotic coiled-coil protein) and Kms1. During meiosis the SPB is linked to the dynein motor complex via Kms1 and perhaps the dynactin complex. Dynein is the motor that moves the nucleus, led by the SPB, along the microtubule arrays in the cell. In *dhc1*, *dlc1*, and *ssm4* mutants, the nucleus does not move and the homologs do not align, although the telomeres become attached to the SPB. Attachment of dynein to microtubules at the cell cortex, which generates the force for horsetail movement, requires Mcp5 (Num1).

Additional genes are placed at the bottom of Table 1 because the corresponding mutants produce recombinant frequencies ~5 times lower than that of wild type and, where tested, make and repair DSBs with nearly wild-type kinetics and frequencies, as is the case for the bouquet- and horsetail-defective mutants previously discussed. Pds5 aids loading of the Rec8 cohesin subunit (see sections 4 and 5.2); in *pds5* mutants the chromosomes are hypercompacted, and horsetail shape is aberrant. Rqh1 is a homolog of the *E. coli* RecQ and *S. cerevisiae* Sgs1 helicases; *rqh1* was identified (as *rec9*) in the initial screen for *S. pombe* meiotic Rec$^-$ mutants (Ponticelli and Smith 1989). *mug1* and *mug5* are meiotic up-regulated genes; the mutants make aberrant asci indicative of chromosome missegregation. Further analyses are required to determine the stage at which these proteins promote recombination.

4
Meiosis-specific Sister Chromatid Cohesins: Behavior Change

During or shortly after meiotic replication, the meiosis-specific cohesin subunits Rec8 and Rec11 are recruited to the chromosomes, where they largely replace the mitotic cohesin subunits Rad21 and Psc3 (see Tanaka & Watanabe, this SERIES). During mitotic division Rad21 is cleaved by separase (Cut1) to allow sister chromatid segregation. During the first meiotic division, Rec8 located in the chromosome arms is, like Rad21, cleaved by separase. However, unlike Rad21, Rec8 at the centromeres is protected from separase by Sgo1 (Kitajima et al. 2004). This differential cleavage allows sisters to separate distal to the crossovers that hold homologs together but maintains cohesion between sisters at the centromeres (Fig. 1). Thus, the change in cohesins permits segregation of homologs, rather than sisters, at MI. The role of Rec11 is less clear; its location primarily in the arms suggests involvement in arm cohesion, but, as noted in Sect. 5.3, Rec11 and Rec8 are also required for recombination.

5
DSB Formation by Rec12: Preparation and Partnership

5.1
S. pombe: A Second Eukaryote with Directly Observed Meiotic DSBs

DSBs were postulated to initiate meiotic recombination (Resnick 1976; Szostak et al. 1983) many years before their demonstration in *S. cerevisiae* at hotspots of recombination (Sun et al. 1989; Cao et al. 1990). Searches for DSBs in *S. pombe* were first successful when whole chromosomes and large restriction fragments were examined (Cervantes et al. 2000). Aided by a mutant, *rad50S* (see Sect. 7.1), DSBs were later found at the genetically well-characterized hotspot *M26* (Steiner et al. 2002; see Sect. 6.1). Meiotic DSBs have not, to our knowledge, been directly observed in other organisms. They have been inferred, however, from the requirement for Spo11 homologs for successful meiosis or recombination, from the Spo11-dependent fragmentation of chromosomes in DSB repair-deficient mutants (Pasierbek et al. 2001; Puizina et al. 2004), or from the appearance on meiotic chromosomes of foci of a particular form of histone H2 that is thought to be a signal of DSBs (see Lichten, this book). The direct detection of DSBs in *S. pombe* opened the way for the discovery of natural *S. pombe* hotspots, discussed below, and the study of other intermediates of recombination.

5.2
Modification of Sister Chromatid Cohesion: A Foundation for Meiosis-specific DSB Formation

As noted above, the substitution of the Rec8 and Rec11 cohesin subunits for their Rad21 and Psc3 mitotic counterparts dramatically modifies the segregation behavior of chromosomes during meiosis. Rec8 and Rec11 also initiate a series of events that lead to meiotic DSBs. Current evidence indicates that, after Rec8 and Rec11 are placed on chromosomes, the Rec25 and Rec27 proteins, perhaps as a complex, form foci on the chromosomes. In turn, these proteins allow the loading of Rec10, a major component of linear elements (Lorenz et al. 2004; see Sect. 5.3). Finally, Rec7 and presumably the other proteins required for DSB formation, including Rec12, are recruited to the chromosomes (Lorenz et al. 2006). Thus, the modification of chromosomes both for their unique segregation and for high-level recombination appears to be initiated at the time of meiotic replication (see Sect. 4).

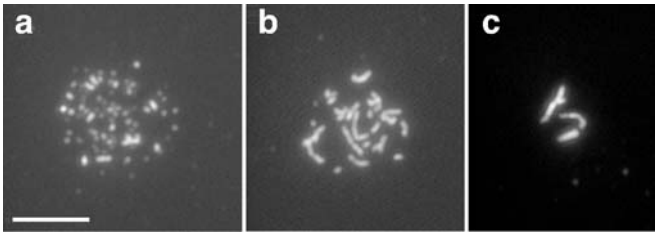

Fig. 2 Linear elements containing Rec10 protein. Spreads of meiotic nuclei were stained using an antibody to Rec10 protein. Dots (**a**), filaments (**b**), and bundles (**c**) appear to occur in that order during meiosis. The filaments and bundles appear to reflect the linear elements seen by electron microscopy (see text). Bar indicates 5 μm. Figure supplied by J. Loidl

5.3 Formation of Linear Elements: Structures Reminiscent of the Synaptonemal Complex

Electron microscopy of thin sections of *S. pombe* meiotic cells or of spreads of their nuclear contents fail to reveal the synaptonemal complexes (SC) common to most organisms (Olson et al. 1978; Bähler et al. 1993; Loidl 2006). Structures similar to one part of the SC, however, are observed and are designated linear elements (LinEs; Bähler et al. 1993; Lorenz et al. 2004). The classical SC is composed of a central element between two parallel lateral elements connected by transverse filaments when homologs are fully aligned and intimately paired. Before this pairing, the lateral elements are called chromatid cores or axial elements; they encase the bases of the chromatin loops of each sister chromatid pair (see chapters by Suja and Julio S. Rufus, or Mehrotra, Hawley and McKim, this SERIES). The LinEs of *S. pombe* appear similar to axial elements, but LinEs do not show the parallel alignment of lateral elements in paired chromosomes and do not extend the full length of the chromosomes as do the axial elements of the SC (Bähler et al. 1993). The role of LinEs, like that of the SC, is not clear, but *rec8* and *rec10* mutants have aberrant or no LinEs, respectively, and are recombination-deficient (Molnar et al. 1995, 2003; Lorenz et al. 2004; Loidl 2006).

Fluorescence microscopy of meiotic cells or nuclear spreads reveals structures likely identical to the LinEs, and this analysis confirms the close connection between LinEs and recombination. During meiosis the LinE component Rec10 first forms discrete nuclear foci and then filaments (Fig. 2), whose numbers and morphological classes approximate those of the LinEs seen by electron microscopy. Formation of Rec10 filaments requires Rec8, Rec11, Rec25, and Rec27 (Lorenz et al. 2004; C. Martín-Castellanos, personal communication). All of these proteins are required for full levels of recombi-

nation, presumably by allowing the loading or activation of the DSB-forming complex.

5.4
Rec12: The Active Site Protein for DSB Formation

Meiotic DSBs are formed by Rec12, the *S. pombe* homolog of Spo11, in conjunction with other proteins. *S. cerevisiae* Spo11 becomes covalently linked to the 5′ ends of the DNA at a DSB, presumably via a tyrosine residue that is essential for DSB formation and recombination (Keeney 2001). The corresponding tyrosine in Rec12 is also required for DSB formation and recombination (Cervantes et al. 2000), and covalent linkage of Rec12 to DNA has been inferred from chromatin-immunoprecipitation studies using an epitope-tagged version of Rec12 (R. Hyppa, pers. comm.). Spo11 homologs from a wide variety of organisms, including *S. pombe* Rec12, have amino acid sequences similar to that of an archeal DNA topoisomerase, whose crystal structure reveals a dimer with the two active-site tyrosine residues pointed into a cleft plausibly holding DNA during catalysis of DSB formation (Nichols et al. 1999). Thus, the mechanism of meiotic DSB formation appears to be highly conserved and closely related to that of type II topoisomerases (see Keeney, this SERIES).

5.5
Other Proteins Essential for DSB Formation: Potential Rec12 Partners and Regulators

Rec12 does not make DSBs on its own but requires numerous other proteins. The cascade of proteins noted in Sections 5.2 and 5.3 appear to be needed for the proper localization of Rec12, and other proteins are needed for Rec12 activity. *rec12* mutants have no detectable meiotic recombination above the level in mitotic cells and no detectable DSBs (Young et al. 2002). This is also true for *rec6* and *mde2* mutants (Table 2), indicating that their gene products are essential for Rec12 action. The corresponding proteins may be partners for Rec12, perhaps in a complex with it, much as several proteins activate *S. cerevisiae* Spo11 by forming a complex with it (see Keeney, this SERIES). Loading of Rec7 requires Rec10 (Lorenz et al. 2006) and presumably also the proteins needed for Rec10 loading (Rec8, Rec11, Rec25, and Rec27; see above). It is noteworthy that the MRN complex (see Sect. 7.1) is not required for DSB formation in *S. pombe*, although its homolog MRX in *S. cerevisiae* is required (Cao et al. 1990; Young et al. 2004); in both organisms the complex is required for DSB repair. Other differences in the control of DSB formation and repair are discussed in Sect. 13.

In *S. cerevisiae* meiotic replication is essential for DSB formation (Borde et al. 2000; Smith et al. 2001), but in *S. pombe* the situation is less clear. As in *S. cerevisiae*, DSBs appear after replication (Cervantes et al. 2000), but repli-

Table 2 Genes required for recombination and meiosis-specific sister chromatid cohesion, linear element formation, or DSB formation

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs. for role in recombination
rec8	64	5–500[a]	Rec8 (20)	Sister chromatid cohesion	Ponticelli and Smith 1989; Parisi et al. 1999; Ellermeier and Smith 2005
rec11	107	5–500[a]	Irr1(Scc3) (20)	Sister chromatid cohesion	Ponticelli and Smith 1989; Ellermeier and Smith 2005
rec25	17	15	–[b]	Aids linear element formation	Martín-Castellanos et al. 2005
rec27	16	15	–	Aids linear element formation	Martín-Castellanos et al. 2005
rec10	90	1000	Red1 (v. limited)[c]	Linear element component	Ponticelli and Smith 1989; Lorenz et al. 2004; Ellermeier and Smith 2005
rec12	39	1000	Spo11 (30)	Makes DSBs	DeVeaux et al. 1992; Cervantes et al. 2000
rec6	21	1000	–	Putative Rec12 partner	Ponticelli and Smith 1989; Cervantes et al. 2000
rec7	38	1000	Rec114 (9)	Putative Rec12 partner	Ponticelli and Smith 1989; Cervantes et al. 2000; Molnar et al. 2001
rec14	33	1000	Ski8 (25)	Putative Rec12 partner	DeVeaux et al. 1992; Cervantes et al. 2000

Table 2 (continued)

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs. for role in recombination
rec15	21	1000	–	Putative Rec12 partner	DeVeaux et al. 1992; Cervantes et al. 2000
rec24	40	1000	–	Putative Rec12 partner	Martín-Castellanos et al. 2005
mde2	23	300	–	Putative Rec12 partner	Gregan et al. 2005
hsk1	58	10	Cdc7 (37)	Protein kinase; substrate unknown	Ogino et al. 2006
atf1	60	15[d]	Sko1 (25)	Stress response transcription factor	Kon et al. 1997; Fox et al. 2000
pcr1	19	15[d]	–	Stress response transcription factor	Kon et al. 1997; Fox et al. 2000

[a] The extent of reduction depends on the interval measured. See section 6.3.
[b] No ortholog is obvious.
[c] A region of 64 amino acids has ~27% identity.
[d] Reduction only at *M26* and related hotspots.

cation can be severely inhibited by mutations or by hydroxyurea with only slight diminution of DSB formation, provided the replication checkpoint is inactivated (Tonami et al. 2005). Hydroxyurea blocks transcription of several meiotic genes required for DSB formation, including *mde2*, and replication checkpoint mutations relieve this block (Ogino and Masai 2006). Conversely, a particular *hsk1* mutant does not form detectable DSBs under conditions in which meiotic replication appears normal (Ogino et al. 2006). Hsk1 protein kinase is required for mitotic replication, via phosphorylation of the MCM (mini-chromosome maintenance) complex; the *hsk1* mutant tested may lack a second function needed for DSB formation, such as phosphorylation of Rec12 or one or more of its putative partners. Meiotic replication and DSB formation in *S. pombe* may be normally coupled by a checkpoint mechanism but not obligatorily coupled as appears to be the case in *S. cerevisiae*.

6
DSB Hotspots and Coldspots: Regulating Where Recombination Occurs

Rec12 does not make DSBs uniformly across chromosomes; rather, there are sites or regions with DSBs at above-average frequency (hotspots) and below-average frequency (coldspots). Hotspots and coldspots were first identified genetically as chromosomal intervals with higher or lower than average intensity of recombination (Gutz 1971; see May, Slingsby and Jeffreys, this SERIES). Wild-type chromosomes in all organisms tested have such hot and cold intervals, but the *S. pombe* mutation *ade6-M26*, which creates a hotspot, has been especially informative.

6.1
M26: A Eukaryotic Sequence-specific Hotspot

The *ade6-M26* mutation recombines with other *ade6* mutations ~10 times more frequently than does the closely linked *M375* mutation (Gutz 1971). By tetrad analysis *M26* also converts ~10 times more frequently than does *M375*, and it converts preferentially to *ade6*$^{+}$. *M26* is a single bp mutation G → T that creates the sequence 5′ ATGACGT 3′, each nucleotide of which is important for hotspot activity (Ponticelli et al. 1988; Szankasi et al. 1988; Schuchert et al. 1991). This sequence is bound by the Atf1-Pcr1 "stress response" transcription factor, which is essential for *M26* hotspot activity (Wahls and Smith 1994; Kon et al. 1997). An iterative binding and PCR-amplification scheme identified 5′ GNVTATGACGTCATNBNC 3′ as a consensus sequence for Atf1-Pcr1 binding to DNA, and mutations creating this sequence in *ade6* have hotspot activity greater than that of *M26* itself (Steiner and Smith 2005b). Sequences closely related to this consensus occur in the wild-type *S. pombe* genome, and the majority of 15 such loci tested are hotspots of DSB for-

mation (Steiner and Smith 2005a). In the one case tested, in the *cds1* gene, this sequence is also a hotspot of recombination. This appears to be the first case of meiotic recombination hotspots being successfully predicted from a genome's sequence. [In *S. cerevisiae*, sites bound by the Bas1 transcription factor are hot- or coldspots (Mieczkowski et al. 2006).] Collectively, the *M26*-like sequences may account for a few percent of all of the meiotic DSBs and recombination. Other transcription factors may account for additional DSBs and recombination.

The molecular basis of the *M26* hotspot is partially understood. During meiosis the chromatin at the *M26* site becomes more sensitive ("open") to exogenous micrococcal nuclease in an *M26* sequence- and Atf1-Pcr1 factor-dependent manner (Mizuno et al. 1997, 2001; Yamada et al. 2004). DSB formation at and around *M26* depends on Rec12 and Pcr1 (Steiner et al. 2002). Among *M26*-like sequences, there is a strong correlation between DSB frequency and hotspot activity, leaving no doubt that these DSBs are causally related to recombination. The *M26* sequence is not sufficient, however, for hotspot activity. Most transplacements of the *ade6-M26* gene, with >1 kb of DNA to each side of *M26*, to a distant site do not manifest hotspot activity (Ponticelli and Smith 1992). Presumably, the chromatin structure is influenced by nucleotide sequences > 1 kb away from *M26* and is more "open" at the endogenous *ade6* locus. The features of "open" chromatin that permit DSB-formation are currently unknown but may involve binding of Rec12 or its putative partners to proteins that "open" the chromatin (see Lichten, this book).

6.2 Hotspots in Large Intergenic Regions: Another Role for "Junk" DNA?

Surveys for DSBs across large regions of wild-type *S. pombe* chromosomes reveal prominent DSB hotspots roughly 50–100 kb apart separated by regions with few, if any, DSBs (Young et al. 2002). Each of these hotspots appears to be a cluster of DSB sites spread over ~1–3 kb. Among 24 such prominent DSB hotspots examined, 21 fall in intergenic intervals markedly larger than the mode of 0.4 kb (Wood et al. 2002): 15 of these 21 DSB hotspots are in intergenic intervals > 4 kb, and the smallest of these 21 intervals is 1.9 kb (unpublished data). The nucleotide sequences responsible for these prominent hotspots have not been determined. They may be collections of transcription factor binding sites exemplified by *M26*, as previously discussed. Alternatively, the primary role of this apparently "junk" intergenic DNA may be to promote meiotic recombination.

6.3
Region-specific Activation by Cohesins: Megabase-scale Control of DSB Formation

Early studies showed that *rec8*, *rec10*, and *rec11* mutants are far more deficient for recombination at the *ade6* locus, the basis for their isolation (Ponticelli and Smith 1989), than in several other intervals tested (DeVeaux and Smith 1994). For example, in *rec8Δ* and *rec11Δ* mutants, *ade6* recombination is reduced by a factor of ~500, whereas recombination in many other intervals is reduced by a factor of 10 or less. In *rec10Δ* mutants recombination is strongly reduced throughout the genome, although the initial mutant *rec10-109*, a double missense, behaves much like the *rec8Δ* and *rec11Δ* mutants (Ellermeier and Smith 2005). The intervals with the least reduction in *rec8* and *rec11* mutants appear to be toward the ends of the chromosomes, although no clear pattern has been established (Parisi et al. 1999). Nevertheless, the strongly affected intervals are large – up to a few Mb.

The basis for this remarkable regional specificity is not entirely clear. It is noteworthy that in a *rec8Δ* mutant, Rec10 forms short patches that may correspond to short LinEs seen by electron microscopy (Molnar et al. 1995; Lorenz et al. 2004). Rec8 and Rec11 meiosis-specific cohesin subunits are required for the cascade resulting in the loading of Rec12 (see Sect. 5). These cohesin subunits do not entirely replace the mitotic cohesin subunit Rad21, residual levels of which remain along meiotic chromosomes (Yokobayashi et al. 2003). In the absence of Rec8 or Rec11, Rec10 may be able to load onto Rad21-bound intervals and lead to DSBs in those intervals; the Rec10-109 mutant protein may be active with Rad21 but not with Rec8 or Rec11.

6.4
Recombination in DSB-poor Intervals: Action at a Distance or Novel Lesions?

Between the prominent hotspots noted in Sect. 6.2 are regions of 50–100 kb with few, if any, DSBs. Nevertheless, in the intervals tested crossovers occur at an intensity (cM per kb) close to that in intervals with prominent DSB hotspots (Young et al. 2002). The origin of these crossovers is currently unclear. The hypothesis that crossovers are generated by distant DSBs (Smith 2001; Young et al. 2002) was not supported by direct and indirect tests (Cromie et al. 2005). Perhaps there are DNA lesions other than DSBs that occur in the DSB-poor regions and lead to crossovers. If so, these lesions must depend on Rec12 and the tyrosine at its putative active site, for a mutant lacking this tyrosine is completely deficient for meiotic recombination (Cervantes et al. 2000). Rec12 may generate recombinogenic single-strand lesions, such as nicks and gaps, in some intervals and DSBs in others. Alternatively, some Rec12-dependent DSBs may not be detectable by the methods used.

6.5
Coldspots: Forbidden Regions for Recombination

Reciprocal recombination (crossing-over) occurs throughout most of the genome with nearly uniform intensity of 0.16 cM/kb (Young et al. 2002). Two regions, however, appear to have essentially no recombination. The first recognized is the 15 kb "K region" between the two silent mating-type loci, *mat2* and *mat3*, which are separated by < 0.002 cM, indicating a recombination intensity < 0.1% of the genome average (Egel 1984). Recombination also appears to be rare within centromeres: loci flanking *cenII* and *cenIII* recombine with an intensity (cM/kb) <3% of the genome average (Nakaseko et al. 1986; C. Ellermeier, V. Tseng, and G. Smith, unpublished data).

The reduced recombination appears to be due to heterochromatin at these loci. There are multiple copies of several different repeats in the centromeres (Wood et al. 2002), and ~4.3 kb of the *mat* K region shares 96% identity with parts of two of these repeats (Grewal and Klar 1996). In the K region and at centromeres, heterochromatin blocks expression of inserted genes, and mutations that alter chromatin structure, such as *swi6* (HP-1 homolog) or *rik1* (putative partner for the Clr4 histone methyltransferase), allow both gene expression and recombination in these intervals (Egel et al. 1989; Klar and Bonaduce 1991; C. Ellermeier and G. Smith, unpublished data). Presumably, wild-type "closed" chromatin in these intervals prevents DSB formation by Rec12. The biological advantage of such cold regions is not clear, but recombination in these intervals may interfere with normal mating-type switching (K region) or chromosome segregation (centromeres) (see chapters by Kokotas, Grigoriadoa and Petersen, or Tanaka & Watanabe, this SERIES).

7
Processing of Rec12-generated DSBs: Converting a Lesion into a Recombinogenic DNA-Protein Complex

Rec12-generated DSBs must be processed into a DNA-protein complex capable of initiating strand exchange with a homologous duplex. The major steps are thought to be removal of bound Rec12 from the 5′ strand termini of the DSB, resection of the 5′ strands to give long 3′ single-strand (ss) DNA overhangs, and loading of strand exchange proteins onto the single-stranded (ss) DNA (Table 3).

Table 3 Genes required for recombination and DSB or mismatch repair

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs. for role in recombination
rad32	74	15	Mre11 (42)	MRN component; nuclease	Tavassoli et al. 1995
rad50	150	–[a]	Rad50 (34)	MRN component; ATPase	–
nbs1	69	–	Xrs2 (limited)[b]	MRN component	–
rad51[c]	40	15	Rad51 (71)	Strand exchange protein	Grishchuk and Kohli 2003
dmc1	36	3	Dmc1 (63)	Strand exchange protein	Grishchuk and Kohli 2003
rad55	39	5	Rad55 (32)	Rad51 accessory protein	Grishchuk and Kohli 2003
rad57	40	3	Rad57 (37)	Rad51 accessory protein	Grishchuk and Kohli 2003
swi5	10	5	Sae3 (24)	Rad51 and Dmc1 accessory protein	Ellermeier et al. 2004
sfr1	34	10	Mei5 (limited)[d]	Rad51 and Dmc1 accessory protein	Unpublished data
rlp1	42	2 (xo only)[e]	hXRCC2 (31)	Rad51 accessory protein	Grishchuk and Kohli 2003
mcp7	24	10	Mnd1 (26)	Acts with Dmc1	Saito et al. 2004
meu13	25	10	Hop2 (27)	Acts with Dmc1	Nabeshima et al. 2001; Saito et al. 2004
rad54	97	1[f]	Rad54 (54)	Chromatin remodeling	Catlett and Forsburg 2003
rdh54	93	3[f]	Rdh54 (33)	Chromatin remodeling	Catlett and Forsburg 2003
mus81	69	50 (xo only)[g]	Mus81 (28)	Holliday junction resolvase	Osman et al. 2003; Smith et al. 2003
eme1	83	–	Mms4 (10)	Holliday junction resolvase	Boddy et al. 2001
rad22	52	3[h]	Rad52 (32)	Rad51 accessory protein?	van den Bosch et al. 2002
rti1	42	0.8[h]	Rad52 (32)	Rad51 accessory protein?	van den Bosch et al. 2002

Table 3 (continued)

Gene	Protein size (kDa)	Approx. extent of reduction by mutation	Putative *S. cerevisiae* ortholog (~% identity)	Inferred primary activity	Refs. for role in recombination
swi4	115	2–3	Msh3 (33)		Tornier et al. 2001
pms1	88	gc effects[i]	Pms1 (33)	Mismatch repair (MMR)[j]	Fleck et al. 1999
msh2	110	gc effects[i]	Msh2 (42)	Mismatch repair (MMR)	Fleck et al. 1999
msh6	141	gc effects[i]	Msh6 (39)	Mismatch repair (MMR)	Tornier et al. 2001
swi10	29	gc effects[i]	Rad10 (37)	Mismatch repair (NER)[k]	Fleck et al. 1999
rad13	126	gc effects[i]	Rad2 (35)	Mismatch repair (NER)	Kunz and Fleck 2001
exo1	64	gc effects[i]	Exo1 (29)	5′ → 3′ ds DNA exonuclease	Szankasi and Smith 1995

[a] Not determined. Requirement for meiotic recombination is assumed, based on other phenotypes similar in *rad32* mutants (for *rad50* and *nbs1*) or in *mus81* mutants (for *eme1*)
[b] Regions of 79 and 27 amino acids show ~22% and 37% identity, respectively
[c] Also called *rhp51*
[d] A region of 160 amino acids shows ~21% identity
[e] Reduction affects crossovers (xo) only; gene conversions are modestly increased in frequency
[f] The *rad54 rdh54* double mutant has greatly reduced spore viability and is severely defective in DSB repair
[g] Crossovers are reduced by factors of 10–90; gene conversions are reduced by a factor of <2
[h] The *rad22 rti1* double mutant has greatly reduced spore viability (van den Bosch et al. 2002) and is defective in recombinational repair of meiotic DSBs (Young et al. 2004)
[i] Frequency of intragenic recombinants (gene convertants) is decreased or increased by factors of up to 35, but generally less, depending on the markers tested for recombination
[j] Mismatch repair
[k] Nucleotide excision repair

7.1
The MRN Complex Is Needed for Removing Rec12 from DSBs But Not for DSB Formation

The Mre11-Rad50-Nbs1 complex (MRN) is a widely conserved eukaryotic protein complex with both exo- and endonuclease activities and is essential for meiotic recombination in all tested organisms. Consistent with this conserved phenotype, *S. pombe rad32* (*MRE11* ortholog) and *rad50* mutants have strongly reduced spore viability and meiotic recombination and fail to repair meiotic DSBs (Tavassoli et al. 1995; Young et al. 2004). In contrast, *S. cerevisiae MRN* null mutants fail to make breaks (see Sect. 5.5 and Sect. 13).

The three components of the MRN complex have distinct roles. Rad50, an ATPase, is a member of the structural-maintenance-of-chromosomes (SMC) family of proteins, which have a long coiled-coil hairpin-like structure. This structure may allow Rad50 to co-ordinate events at the two sides of a meiotic DSB. The nuclease domain of Rad32 is required for processing DSBs; Rad50 appears to regulate this nuclease, as the *rad50S* (K81I) mutant accumulates Rec12-DNA complexes (Young et al. 2002; R. Hyppa, pers. comm.). The Nbs1 subunit is also believed to be regulatory.

S. pombe MRN nuclease-deficient mutants, like *MRN* null mutants, accumulate meiotic DSBs (Young et al. 2002, 2004; J. Farah, pers. comm.). Despite the 3′ to 5′ exonuclease polarity of the human and *S. cerevisiae* enzymes (Paull and Gellert 1998; Usui et al. 1998), it has been suggested that the MRN exonuclease is required for 5′ end resection. However, *S. cerevisiae* MRN appears to cleave DNA ~10–40 nucleotides from the covalently linked Spo11 (Neale et al. 2005). *S. pombe MRN* nuclease mutants are also unable to remove Rec12 from the sites of DSBs (E. Hartsuiker, pers. comm.), and they can carry out meiotic recombination initiated by the I-*Sce*I endonuclease (J. Farah, pers. comm.), which, unlike Rec12, does not remain covalently linked to the DNA. These data suggest that the sole nucleolytic role of MRN is to remove Rec12 from the ends of meiotic DSBs, with 5′ resection being carried out by another enzyme. The responsible exonuclease is unknown. Exonuclease I has the appropriate specificity but seems to play little role other than mismatch correction in *S. pombe* meiotic recombination (Szankasi and Smith 1995; see Sect. 10).

7.2
Loading Strand-Exchange Proteins: Many Actors with Overlapping Roles

Strand-exchange proteins, bound to ss DNA, generate the joint molecule intermediates of recombination. However, these proteins alone are unable to compete with ss DNA binding (SSB) proteins for DNA. Consequently, from bacteriophages to humans, accessory proteins are needed to assist in their loading. *S. pombe* possesses several such accessory proteins: Rad22, Rti1, the

Rhp55-Rhp57 and Swi5-Sfr1 complexes, and possibly the Rlp1 protein. Rad22 and Rti1 (also called Rad22B) appear to have redundant functions, but a suppressor mutation commonly found in *rad22* strains has complicated their analysis (Doe et al. 2004). The *S. pombe* Rhp55, Rhp57, and Rlp1 proteins are paralogs of the strand exchange protein Rad51 (also called Rhp51; Grishchuk and Kohli 2003). The Rhp55-Rhp57 and Swi5-Sfr1 complexes promote Rad51 and Dmc1 loading onto ss DNA (Haruta et al. 2006). Mutations in *rlp1*, *rhp55*, *rhp57* or the double *rhp55-rhp57* mutation have relatively mild effects on meiotic recombination frequencies and spore viability (Grishchuk and Kohli 2003). Mutations in *swi5* or *sfr1* also show a moderate reduction in meiotic recombination and slightly lowered spore viability (Young et al. 2004; unpublished data). In contrast to the single mutants, double mutants affecting both the Rhp55-Rhp57 and the Swi5-Sfr1 complexes have severe defects in spore viability and recombination, similar in magnitude to a *dmc1 rad51* double mutant (Ellermeier et al. 2004), suggesting that the two complexes possess redundant functions. However, the exact roles of these proteins, and the distinctions between them, remain unclear.

8
Strand Invasion and Partner Choice

By analogy with recombination in other organisms, the action of Rad22, Rti1, Rlp1, Rhp55-57 and Swi5-Sfr1 is believed to produce DNA ends with 3′ overhangs coated in strand exchange proteins. Through the process of strand invasion, this nucleoprotein complex generates joint molecules between homologous DNA duplexes that can then be processed to give recombinants.

8.1
The Dmc1 and Rad51 Strand Exchange Proteins: Finding a Homologous Partner for Recombination

The archetypal DNA strand exchange protein is *Escherichia coli* RecA, which is loaded onto ss DNA by the RecBCD or the RecFOR proteins to form a nucleoprotein filament capable of strand exchange (see Prévost, this book). The filament pairs with the complementary strand of a homologous duplex, displacing the other strand to form a displacement loop (D-loop). This joint molecule is held together by a region of hybrid DNA, having one strand from each parent. Finally, RecA cycles off the DNA after hydrolysis of ATP.

S. pombe possesses two RecA structural and functional homologs – Rad51, which is expressed in all cells of all studied eukaryotes, and Dmc1, which is meiosis-specific but absent from some species. Mutations in *rad51* and *dmc1* have quite distinct effects on meiotic recombination. Although both mutations show an approximately 5-fold reduction in crossing-over, the ef-

fect of the *rad51* mutation on gene conversion (non-reciprocal recombination) is much stronger. The spore viability of a *rad51* mutant is very low, while that of a *dmc1* mutant is close to the wild-type level (Grishchuk and Kohli 2003). The relative spore viabilities can be explained by the observation that in *dmc1* mutants meiotic DSBs are repaired, but in *rad51* mutants, they are not repaired (Young et al. 2004; see Sect. 13). The recombination and spore viability phenotypes of the double mutant are much more severe than those of the single mutants, suggesting some redundancy in the function of the two proteins (Grishchuk and Kohli 2003). Double mutant analyses suggest that Dmc1, Swi5, and Sfr1 function in one branch of a pathway and Rhp55 and Rhp57 in another (Ellermeier et al. 2004; Fig. 1). In addition, the Mcp7-Meu13 complex, which is homologous to *S. cerevisiae* Mnd1-Hop2, also appears to act specifically with Dmc1. Mutations in either *mcp7* or *meu13* have mild spore-viability defects and substantial recombination defects (Saito et al. 2004), but the exact function of this complex is unclear. Further studies are needed to elucidate these aspects of *S. pombe* meiotic recombination.

8.2
The Rhp54 and Rdh54 Proteins: Enabling Strand Exchange in a Chromatin Context?

S. pombe Rhp54 and its paralog Rdh54 are members of the Swi2 (Snf2) family of proteins, many of which remodel chromatin. Rhp54 is expressed both mitotically and meiotically, while Rdh54 is meiosis-specific (Catlett and Forsburg 2003). *rhp54* and *rdh54* mutants show mild defects in recombination, spore viability, and meiotic DSB repair, but the double mutant is severely defective. The *S. cerevisiae* orthologs of *rhp54* and *rdh54* appear to alter chromatin structure to facilitate Rad51 and Dmc1 strand exchange (Heyer et al. 2006). The *S. pombe* proteins may act similarly, since Rhp54 interacts with Rad51, and Rdh54 interacts with both Rad51 and Dmc1 (Catlett and Forsburg 2003). Mutations in *S. pombe rdh54* have meiotic phenotypes similar to those of *dmc1* mutations (high spore viability and successful repair of DSBs but reduced recombination), suggesting that these two meiosis-specific proteins may act in the same pathway.

8.3
Intersister vs. Interhomolog Recombination: Any Partner Will Do?

In meiosis there are almost always three homologous DNA targets with which a recombinogenic DNA end can interact – the sister chromatid or either of the two chromatids of the homolog. Interhomolog recombination might be expected to be favored over intersister recombination because meiotic recombination must produce crossovers between homologs for their proper segregation (see Mehrotra, Hawley and McKim, this SERIES). Results from

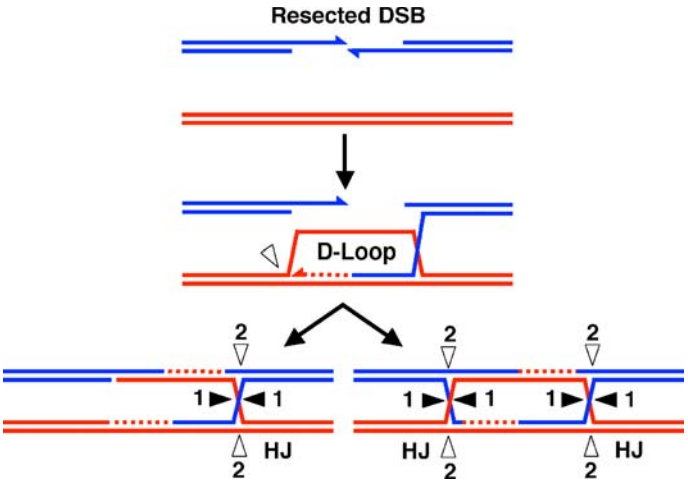

Fig. 3 A scheme that produces either crossover or non-crossover recombinants from single or double Holliday junctions. Resection of a DSB produces two 3′ single strand overhangs, one of which invades a homologous duplex, producing a D-loop. A single HJ results if this D-loop is cut before second end capture (*left*). A double HJ results if the D-loop remains uncut before second end capture (*right*). In both cases resolution of the HJ(s) results in crossover or non-crossover products, depending on the strands cleaved

S. cerevisiae support this idea (see Sect. 12). However, genetic studies and physical analysis of joint molecules in *S. pombe* have demonstrated that intersister recombination does occur and is actually more frequent than interhomolog recombination (Cromie et al. 2006). How this is reconciled with the necessity of producing interhomolog crossovers is discussed in Sect. 12. Nevertheless, *S. pombe* does appear to have mechanisms that specifically promote interhomolog recombination (see Sect. 3). As discussed above, *dmc1* mutants have reduced recombination frequencies, but they repair meiotic DSBs and have high spore viability. This suggests that Dmc1 promotes the repair of DSBs using homologs, but, if Dmc1 is absent, breaks are repaired using sister chromatids. The distinct phenotypes of *S. cerevisiae* vs. *S. pombe dmc1* mutants may result from a mechanism, present in *S. cerevisiae* but not *S. pombe*, that inhibits intersister recombination (see Sect. 12 and 13).

9 Joint Molecule Resolution

In the current canonical model of meiotic crossing over (Szostak et al. 1983; Sun et al. 1989), after strand invasion and D-loop formation the second end of the initiating DSB anneals to the D-loop (Fig. 3). Branch migration at each

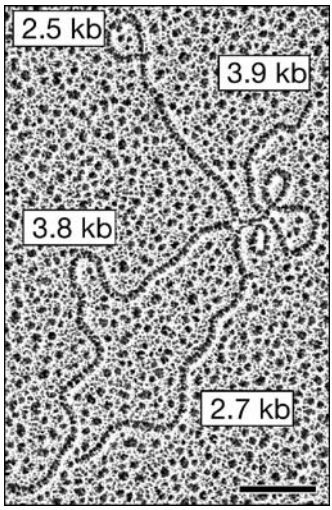

Fig. 4 Single Holliday junction intermediates in *S. pombe* meiotic recombination. DNA from meiotic *mus81* mutant cells was separated by two-dimensional gel electrophoresis, and DNA in the joint molecule region was visualized with an electron microscope. The junction of the four DNA duplex segments is splayed out in a "traffic circle" due to formamide in the spreading mixture. Bar indicates 0.2 μm. From Cromie et al. (2006)

side of the D-loop then generates two Holliday junctions (double HJs, Fig. 3 right) connecting the homologous duplexes. Cleavage and rejoining of appropriate pairs of strands in the two HJs can then generate a crossover.

9.1
Single Holliday Junctions: An Unexpected Recombination Intermediate

Electron microscope studies of meiotic joint molecules in *S. pombe* reveal DNA structures different from those predicted by the current canonical model and previously observed in *S. cerevisiae* (Cromie et al. 2006). Instead of double HJs, the majority of molecules contain single HJs (Fig. 4). Two dimensional gel electrophoretic analyses are also consistent with a majority of single HJs. The single HJ and double HJ mechanisms may differ only by the timing of cleavage of the strand forming the D-loop: cleavage before second end capture produces a single HJ (Fig. 3, left), whereas cleavage after second end capture allows formation of a double HJ (Fig. 3, right).

9.2
Mus81-Eme1: The Meiotic Holliday Junction Resolvase of *S. pombe*

To generate crossovers from joint molecules, the HJs must be cleaved and the broken strands ligated. The *S. pombe* Mus81-Eme1 endonuclease can re-

solve HJs and closely related structures (Boddy et al. 2001). *mus81* and *eme1* mutants have, as far as tested, indistinguishable phenotypes which are those expected of nuclear HJ resolvase mutants. Most notably, HJs accumulate in *mus81* mutant meiosis (Cromie et al. 2006). Physical and genetic assays show that meiotic crossovers, which are expected to depend on an HJ resolvase, are greatly reduced in *mus81* mutants, whereas genetic assays show that gene conversions without crossovers, which are not necessarily resolvase-dependent, are essentially unaffected (Osman et al. 2003; Smith et al. 2003). In *mus81* mutants the asci are aberrantly shaped, spore viability is very low, and there is a single mass of apparently entangled DNA. These phenotypes are indicative of chromosome segregation failure, as expected from the chromosomes remaining held together by unresolved HJs. The phenotypes of *mus81* mutants can be relieved by expression of a bacterial HJ resolvase. As expected, in meiosis *rec12Δ* is epistatic to *mus81Δ* and *eme1Δ*, indicating that Mus81-Eme1 is required for meiotic DSB repair. Amino acid substitutions affecting the Mus81 nuclease active site have a phenotype indistinguishable from that of a complete deletion. Collectively, these observations indicate that the complex's role in crossing-over is endonucleolytic resolution (Boddy et al. 2001; Osman et al. 2003; Smith et al. 2003).

The archetypal HJ resolvase, *E. coli* RuvC, cleaves intact HJs by symmetrical cleavages of two strands; the cleaved product is a good substrate for DNA ligase. Mus81-Eme1 purified from *S. pombe* and human cells cleaves intact HJs, although not symmetrically. All reported preparations of Mus81-Eme1 show higher activity on three-strand junctions than on intact HJs, but nicked HJs are preferred over the 3-strand junctions, with cleavage occurring at the site on the HJ strand opposite the nick (Doe et al. 2002; Gaillard et al. 2003; Osman et al. 2003). Mus81-Eme1 may readily cleave 3-strand junctions because they resemble nicked HJs. Osman et al. (2003) have proposed a model of recombination involving Mus81-Eme1 cleavage of nicked HJs. However, Mus81-Eme1 purified from *S. pombe* and human cells slowly cleaves one strand of an intact HJ and then rapidly cleaves the nicked product (Gaillard et al. 2003); this may be the relevant in vivo activity, perhaps stimulated by other factors. Asymmetric HJ cleavage would prevent immediate ligation of the cut strands, but repair DNA synthesis and single-strand flap-processing would allow the required ligation.

10
Mismatch Correction

Gene conversion is a form of recombination involving the non-reciprocal transfer of sequence information between homologous DNA sequences. It is observed as heterozygous marker segregation other than the normal Mendelian 2:2 among the four spores in an ascus and is often produced as

an outcome of mismatch correction. Hybrid DNA is generated between homologous loci undergoing strand exchange, and, if the loci are non-identical in sequence, the hybrid DNA will contain mismatches. Repair of such mismatches can lead to gene conversion, seen as 3:1 segregation among the four spores. Unrepaired mismatches segregate at the first round of DNA replication after meiosis and, thus, can be identified as post-meiotic segregation (PMS) events, detectable as a sectored colony arising from one spore.

In *S. pombe*, both mismatch repair (MMR) and nucleotide excision repair (NER) function in the repair of mismatches arising during meiotic recombination. Presumably, after mismatch recognition by MMR proteins and strand incision by unknown factors, exonuclease I removes part of a strand with one of the mismatched nucleotides and repair DNA synthesis restores complementarity. MMR appears to repair efficiently small insertion or deletion loops and all base mismatches other than C/C. The C/C mismatch is repaired, but inefficiently, by the NER system, which can also repair other base mismatches in the absence of the MMR pathway (Fleck et al. 1999). The MMR and NER gene products demonstrably required for these processes are shown in Table 3. In meiotic crosses involving the *ade6-M26* hotspot, essentially all mismatch correction (identified as 3:1 marker segregation) is abolished in the absence of both the MMR and NER pathways, and only PMS events are seen, at elevated frequency. One class of PMS event has hybrid DNA in one spore (~70% of all asci with non-2:2 segregation), and another class has hybrid DNA in two spores (~30%). The first class may reflect a mismatch in the region resected adjacent to a meiotic DSB (asymmetric heteroduplex), and the second class may reflect a mismatch in a more distant region where branch migration formed an HJ (symmetric heteroduplex). Thus, both symmetric and asymmetric hybrid DNA forms appear to be frequent in *S. pombe* meiosis.

The fission yeast *swi4* gene encodes a homolog of the budding yeast MMR protein Msh3. However, rather than a meiotic MMR defect, mutations in *swi4* exhibit a mild deficiency in both intragenic and intergenic recombination. The function of Swi4 in meiotic recombination is unclear.

11
Relation of Gene Conversion and Crossing-over

Gene conversion indicates hybrid DNA at a marked locus and, thus, strand exchange. Markers flanking this locus may or may not undergo crossing-over. Until recently, it was generally believed that there was a single pathway of homologous recombination with an HJ intermediate containing hybrid DNA. At the HJ resolution stage, if the pairs of complementary strands cleaved in the HJs are chosen at random, crossovers and non-crossovers would be equally frequent (Fig. 3). Recent work in both prokaryotes and eukaryotes

has thrown doubt on this model and has suggested that crossovers and non-crossovers are generated by different pathways (Cromie and Leach 2000; Allers and Lichten 2001). The mechanism that generates non-crossovers is unclear but may involve sequential DNA synthesis, DNA unwinding, and annealing, termed "synthesis-dependent strand annealing" (SDSA; see Haber, this book; Lankenau, 2007, this SERIES). Gene conversion could occur in the hybrid DNA envisaged as a part of the SDSA model.

In *S. pombe*, crossovers accompany gene conversions ~75% of the time rather than 50%, as predicted by the random HJ resolution model, or ~40%, as observed in *S. cerevisiae* (Grimm et al. 1994; Cromie et al. 2005). In addition, as noted in Sect. 9.2, *mus81* resolvase mutations have very little effect on the frequency of gene conversions that lack an associated crossover (Osman et al. 2003; Smith et al. 2003). This suggests that these non-crossovers do not result from HJ resolution. Therefore, it appears that in *S. pombe* crossovers result from HJ resolution and non-crossovers from a second mechanism, such as SDSA, although HJ resolution may contribute to some non-crossover events.

12
Species-specific Strategies for Ensuring, With or Without Interference, the Crossovers Required for Chromosome Segregation

Meiotic crossovers generate new combinations of alleles that increase genetic diversity in the population, and in most organisms, crossovers also aid the correct segregation of homologs at the first meiotic division. Intersister recombination achieves neither of these aims. Why then does *S. pombe* show a bias towards intersister events, while *S. cerevisiae* shows a bias towards interhomolog events (Cromie et al. 2006)? Similarly, why is crossover interference, which reduces the probability of crossovers occurring close together, present in *S. cerevisiae* and not in *S. pombe* (Munz 1994)?

The bias to interhomolog recombination seen in *S. cerevisiae* appears to result from a barrier to intersister recombination events (Niu et al. 2005), i.e., it is a form of regulation of recombination, as is crossover interference. Some of the 16 chromosomes of *S. cerevisiae* are very small, as short as 230 kb, and all are smaller than the smallest of *S. pombe* (3500 kb). If the total number of crossovers in *S. cerevisiae* were distributed randomly across the DNA (i.e., if there were no interference), then these small chromosomes would receive no crossover ~10% of the time and would frequently missegregate. Interference may ensure that crossovers are distributed so that small chromosomes always receive at least one. Interhomolog bias may be a further adaptation to ensure enough interhomolog crossovers on small chromosomes without increasing the number of DSBs.

In contrast to *S. cerevisiae*, *S. pombe* has only three, large chromosomes. Random (Poisson) distribution of crossovers suffices to ensure that each chromosome receives at least one interhomolog crossover, since there is an average of 10, 15, and 20 interhomolog crossovers per meiotic cell (Munz et al. 1989). The average number of crossovers on the smallest chromosome is high enough to prevent missegregation without needing to regulate recombination through crossover interference (Munz et al. 1989) or a barrier to intersister recombination. In this view, *S. pombe* uses a meiotic recombination system "pared down" to its fundamentals. The obvious success of this scheme raises the question of why other organisms regulate crossovers rather than alter the size and number of chromosomes or increase the number of DSBs so that unregulated recombination would allow successful chromosome segregation.

13
Differences Between *S. pombe* and *S. cerevisiae* Meiotic Recombination: A Reprise

At all steps of meiotic recombination, significant differences are seen between *S. pombe* and *S. cerevisiae* – in the production and processing of DSBs, in the loading of strand exchange proteins, in choice of partner for the strand exchange reaction, in the structure of joint molecules, and in the processing of joint molecules into recombinant products. These differences may account for the differences, noted in Sect. 1, 3, and 11 and below, in the occurrence of interference, the frequency of ectopic recombination, and the frequency of crossovers associated with gene conversion.

Several components for the formation and processing of meiotic DSBs differ markedly in *S. pombe* and *S. cerevisiae*. Each species has proteins essential for DSB formation that have no obvious ortholog in the other species (Table 2). Furthermore, the Rec8 cohesin subunit is required for DSB formation in *S. pombe* but not in *S. cerevisiae*; it is required for DSB repair in *S. cerevisiae* but perhaps not in *S. pombe* (Klein et al. 1999; Ellermeier and Smith 2005). Conversely, the MRN nuclease complex is required for DSB formation in *S. cerevisiae* but not in *S. pombe*; it is required for DSB repair in both species (Cao et al. 1990; Young et al. 2004). Presumably, Rec8 and MRN are required indirectly and differentially in the two species for the assembly of the Rec12 (Spo11) complex at sites of DSB formation. DSB repair in both species may require the MRN nuclease to remove Rec12 (Spo11) linked to the DNA (see Sect. 7.1). The role of Rec8 in DSB repair in *S. cerevisiae* is unclear.

There are several differences between *S. cerevisiae* and *S. pombe* relating to Rad51 accessory proteins. First, the function of the *S. cerevisiae* Rad52 protein appears to be carried out by two partially redundant Rad52 homologs in *S. pombe*. Interestingly, *rad52*$^{-/-}$knockout mice are viable and fertile (Rijkers et al. 1998), suggesting that mammals may be more similar in this regard

to *S. pombe* than to *S. cerevisiae*. Second, mutations in the *rad55*, *rad57* and *rdh54* genes have very severe recombination defects in *S. cerevisiae*, but the *S. pombe* ortholog mutants have only mild defects (Petes et al. 1991; Catlett and Forsburg 2003; Grishchuk and Kohli 2003). Third, the *S. pombe* Swi5-Sfr1 complex appears to function in both mitosis and meiosis, unlike the *S. cerevisiae* meiosis-specific Sae3-Mei5 ortholog. This is consistent with the ability of Swi5-Sfr1 to promote both Rad51 and Dmc1 activity (Haruta et al. 2006), whereas Sae3-Mei5 is believed to interact only with Dmc1 (Hayase et al. 2004). Fourth, there appears to be no Rlp1 ortholog in *S. cerevisiae*, but mammals have an ortholog, Xrcc2 (Khasanov et al. 2004).

The mechanics of strand invasion are not known to differ between *S. pombe* and *S. cerevisiae*. However, significant differences are seen in the choice of DNA used as the target of strand invasion; i.e., in *S. pombe* intersister events are preferred, while in *S. cerevisiae* interhomolog events predominate (see Sect. 8.3 and 12). These differences appear to be related to differences in the phenotypes of *dmc1* mutants. *S. pombe dmc1* mutants successfully repair meiotic DSBs, presumably using sister chromatids. In contrast, in *dmc1* mutants of *S. cerevisiae* strain SK1, DSBs remain unrepaired (Bishop et al. 1992), apparently due to a barrier to intersister recombination that involves the Hop1, Mek1 and Red1 proteins (Niu et al. 2005). The apparent absence of this barrier in *S. pombe* may explain why intersister events are more frequent in *S. pombe* than in *S. cerevisiae*.

The structures of recombination joint molecules in *S. pombe* and *S. cerevisiae* are distinctly different. As discussed in Sect. 9.1, most recombination joint molecules in *S. pombe* contain single HJs rather than the double HJs seen in most *S. cerevisiae* joint molecules and predicted by a current recombination model (Szostak et al. 1983). A variation on this model can account for either a single HJ or a double HJ arising from a D-loop and producing recombinants (Fig. 3).

In *S. pombe* the Mus81-Eme1 complex appears to be the only meiotic nuclear HJ resolvase. However, despite the wide conservation of these two proteins among eukaryotes and the similar mitotic phenotypes of the mutants, the importance of these proteins in meiosis appears to vary among organisms. In *S. cerevisiae* crossovers are only mildly reduced by *mus81* or *mms4* (*eme1*) mutations, and in mice *mus81* mutants are viable and fertile (de los Santos et al. 2003; McPherson et al. 2004). *S. cerevisiae* and mice may have a second crossover pathway that requires the Msh4 and Msh5 proteins, which are apparently absent from *S. pombe*. Interestingly, *mus81*-dependent crossovers in *S. cerevisiae*, like those of *S. pombe*, are not subject to crossover interference (de los Santos et al. 2003).

The similarities of meiotic recombination in *S. pombe* and *S. cerevisiae* indicate that the basic process of DSB formation and repair is widely conserved and perhaps universal. But the multiple differences suggest that the regulation of meiotic recombination is variable among species and may have arisen

independently during evolution. Further understanding of these similarities and differences may provide insight into how meiotic recombination arose, presumably from a mitotic precursor.

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