Whence Meiosis?

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Sexual reproduction predominates among eukaryotic organisms on our planet. While debate continues over *why* this should be so, burgeoning genomic and functional information now allows us to begin to think reasonably about some of the events that may have occurred to make sex possible in the first place.

At the heart of sexual reproduction is meiosis, the specialized cell division program whereby diploid organisms reduce their chromosome number in half to generate haploid progeny cells. This reduction in chromosome number is essential so that the union of two gametes will regenerate the diploid chromosome complement in the subsequent generation, thereby ensuring continuity of the species. Meiosis can occur in a wide variety of cellular and physiological milieux, but in nearly all cases, correct segregation at the meiosis I division depends on crossover recombination events between the DNA molecules of homologous chromosomes (Moore and Orr-Weaver, 1998). Crossovers at the DNA level collaborate with cohesion between sister chromatids to form temporary connections (chiasmata) between homologs that allow them to orient toward opposite poles of the meiosis I spindle (Figure 1). Thus, the very essence of sex is meiotic recombination.

Here, we will attempt to reconstruct some of the events that occurred to permit the development of sexual reproduction in an ancient eukaryotic ancestor. We now have enough genomic and functional information about meiosis from several different experimental systems to allow us to define a "core meiotic recombination machinery" (Table 1). This "core" is comprised of components that exhibit strong conservation across eukaryotic kingdom boundaries, indicating that they were all present in a common ancestor of animals, plants, and fungi. Based either on direct empirical data or inference from their membership in larger protein families, these components are all assumed to be involved rather directly in DNA metabolism. By focusing on the roles of meiosis-specific components of the core meiotic recombination machinery, we will develop a case for three key landmark evolutionary events on the road toward sex—acquisition of the means to (1) initiate recombination at high frequency, (2) use the homologous chromosome as a recombination partner, and (3) promote completion of recombination events as crossovers. Further, we will discuss insights about meiotic recombination that emerge from integrating information about the meiotic programs in different organisms with an inventory of core components in those organisms.

A Means to Generate Ends

Recombination occurs at a much higher frequency during meiosis than during vegetative/somatic growth (Pâques and Haber, 1999). This elevation is essential to ensure that each homolog pair will enjoy at least one crossover in every meiosis. This, in turn, suggests that an important step in the development of meiosis was the generation of a means to greatly stimulate the frequency of recombination. In *S. cerevisiae*, stimulation of recombination during meiotic prophase is accomplished by deliberate introduction of double-strand breaks (DSBs) in DNA (Keeney, 2001; Figure 2). The culprit most directly responsible for making these breaks is the Spo11 enzyme. The presence of Spo11 orthologs in virtually all eukaryotes, coupled with functional experiments in all organisms listed in Table 1, indicates that Spo11-generated DSBs are the initiating lesions for most, if not all, meiotic recombination (Keeney, 2001).

Spo11 proteins of eukaryotic organisms are homologs of the A subunit of topoisomerase VI, a type II topoisomerase from Archaebacteria (Keeney, 2001). Type II topoisomerases provide a means of topological disentanglement of DNA, acting to pass one DNA molecule through another by generating a transient DSB in one of the two molecules. Topo VI is a heterotetramer of A and B subunits; Top6A is likely responsible for DNA cleavage, while Top6B likely acts to coordinate breakage with strand passage and rejoining. Most eukaryotes have a single Spo11 homolog and lack a recognizable Top6B homolog (Keeney, 2001). Recently, however, *Arabidopsis thaliana* was found to have three Spo11 homologs (which appear to be ancient paralogs and not the result of a recent duplication), as well as a homolog of Top6B (Hartung and Puchta, 2001). Two of the three Spo11 homologs interact with AtTop6B in a two-hybrid assay, suggesting that they may form complexes with Topo VI-like activity. The third Spo11 homolog (Spo11-1) does not interact with AtTop6B in this assay. Moreover, Spo11-1 is responsible for most meiotic recombination, indicating that it is a bona fide Spo11 ortholog (Grelon et al., 2001).

Identification of Top6B in plants suggests that Top6B homologs in other eukaryotes would be recognizable, and thus, that their absence is significant. As Top6B is likely important for coordination of cleavage and rejoining, liberation of a Top6A paralog (Spo11) from Top6B may have freed it from the restricted functions of topoisomerases and allowed it to become an endonuclease. Since Spo11 orthologs in most eukaryotes do not have a Top6B partner in evidence and plants have a Spo11 ortholog that appears not to interact with Top6B, we suggest that this emancipation occurred before divergence of the extant eukaryotic lineages. Indeed, liberation of Spo11 from Top6B may have been a crucial defining event in the development of meiotic recombination and thus, sex.

In many eukaryotes, Spo11 is essential not only for

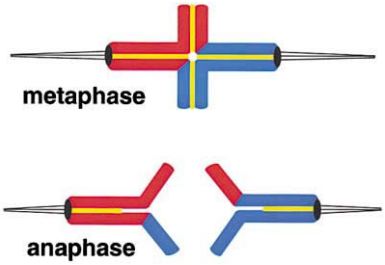

Figure 1. Crossovers Promote Meiosis I Segregation of Homologous Chromosomes

A meiotic bivalent, consisting of a pair of attached homologous chromosomes (one blue, one red), at metaphase of meiosis I. Spindle microtubules and centromeres are indicated in black; sister chromatid cohesion proteins in yellow. Because cohesion proteins provide connections along the lengths of sister chromatids, a reciprocal exchange between the DNA molecules of one chromatid from each homolog results in a cross-shaped connection (chiasma) at the position of the crossover. The chiasma provides a point of attachment between homologs that persists until the metaphase-anaphase (M-A) transition of meiosis I and allows homologs to orient toward opposite spindle poles. At the M-A transition, cohesion is lost distal to the chiasma but is maintained at centromeres, allowing homologs to disjoin and separate to opposite poles at anaphase.

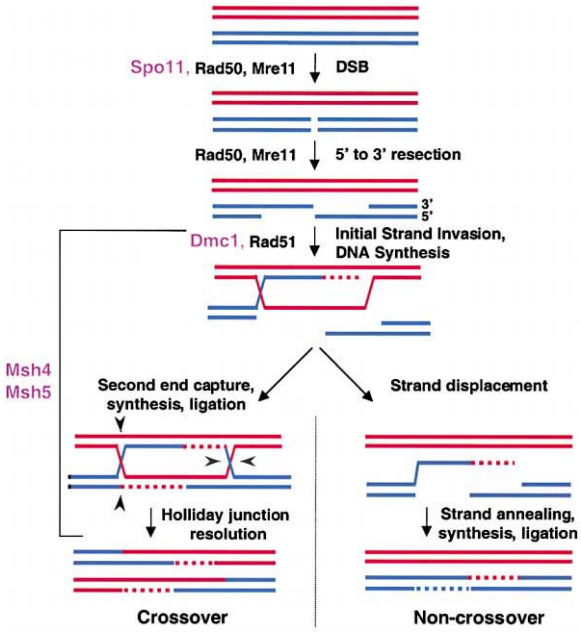

Figure 2. Meiotic Recombination Primer

A model for meiotic recombination that incorporates conclusions based on the recent work of Allers and Lichten (2001) and Hunter and Kleckner (2001). In contrast to earlier incarnations of the DSBR model for meiotic recombination (reviewed in Pâques and Haber, 1999), in which both crossover and noncrossover products were proposed to arise via a double Holliday junction (DHJ) intermediate, the current model invokes earlier divergence of the majority crossover and noncrossover pathways, prior to formation of DHJs. In this model, most noncrossovers arise as a result of synthesis-dependent strand annealing (SDSA, right pathway; Pâques and Haber, 1999). Crossovers, and some noncrossovers, arise from a pathway involving double-Holliday junction (DHJ) resolution (left pathway). DNA duplexes from the two homologous chromosomes are indicated in red and blue (sister chromatid duplexes not involved in the recombination event are not shown); newly synthesized DNA is indicated by dashed lines. Meiosis-specific recombination machinery components are indicated in purple; components that also function in DSBR during mitotic growth are indicated in black.

DSB formation but also for proper assembly of the synaptonemal complex (SC), a proteinaceous structure that forms between the axes of aligned homologous chromosomes during meiotic prophase (Keeney, 2001). It has been inferred that DSBs and other early recombination intermediates are required to promote SC assembly (synapsis) between homologs. This dependence is not universal, however—in some organisms (e.g., *D. melanogaster* and *C. elegans*), homologous synapsis occurs in the absence of functional Spo11 protein (Dernburg et al., 1998; McKim et al., 1998). Interestingly, there is independent evidence that both of these organisms have developed alternate means of stabilizing pairing interactions and/or promoting SC formation. Genetic studies have identified *cis*-acting chromosomal domains (known as pairing centers or sites) that govern the formation of crossovers over large chromosome segments, presumably through roles in stabilizing interhomolog associations and/or promoting synapsis (Albertson et al., 1997; Hawley, 1980).

Using the Homolog as the Recombination Partner

Once meiosis-specific DSBs have been generated, the strands ending 5′ at the break are degraded to expose 3′-ending single-stranded DNA (ssDNA) tails. These tails then invade an intact homologous DNA duplex and prime DNA synthesis, initiating repair of the DSB (Pâques and Haber, 1999). Invasion of an intact duplex by a single strand is promoted by members of the RecA family of DNA strand exchange proteins (Figure 2).

A gene duplication prior to the divergence of the eukaryotes gave rise to Rad51 and Dmc1, the two closest eukaryotic relatives of bacterial RecA (Masson and

Table 1. Inventory of Core Meiotic Recombination Machinery

	S. cerevisiae	*S. pombe*	*A. thaliana*	*C. elegans*	*D. melanogaster*	mammals
Spo11	+	+	+(3)	+	+	+
Rad50/Mre11	+	+	+	+	+	+
Dmc1	+	+	+	−	−	+
Rad51	+	+	+	+	+	+
Msh4/Msh5	+	−	+	+	−	+
Mlh1	+	+	+	+	+	+
SC?	yes	no	yes	yes	yes	yes
Interference?	yes	no	yes	yes	yes	yes
Spo11-dependent synapsis?	yes	N/A	yes	no	no	yes

West, 2001). Rad51 is used not only in meiotic recombination but also in double-strand break repair (DSBR) in vegetative or somatic cells. Dmc1, in contrast, is expressed and used exclusively in meiosis. Both proteins promote limited strand exchange in vitro (Masson and West, 2001). In vivo, Rad51 and Dmc1 exhibit extensive colocalization early in meiotic prophase (e.g., Shinohara et al., 2000), dependent on initiation of recombination by Spo11. Both are required for normal progression from the DSB stage of meiotic recombination to the formation of double Holiday junction (DHJ) intermediates and mature recombinant products (Schwacha and Kleckner, 1997). Further, Hunter and Kleckner (2001) recently showed that Dmc1 is required in vivo for the formation of stable strand invasion intermediates (see below).

During meiosis, broken ends are directed to use the homologous chromosome, rather than the sister chromatid, as a partner at the strand invasion step. This is in contrast to the situation in vegetative cells, where the sister chromatid is the preferred partner for Rad51-promoted DSBR (Pâques and Haber, 1999). This switch in recombination partner preference was a crucial event in the development of sex, since crossover events must occur between homologs, rather than between sisters, if they are to afford a connection between homologs that will allow them to orient toward opposite spindle poles. Meiosis-specific differentiation of chromosome structure appears to play a primary role in conferring preference for the homolog, both by stimulating recombination along an interhomolog-only pathway and by insulating against use of sister chromatids as recombination partners (Schwacha and Kleckner, 1997; Thompson and Stahl, 1999); in contrast to the recombination machinery, however, meiotic structural proteins are notoriously poorly conserved. The Dmc1 protein also appears to have become specialized for promoting interhomolog strand exchange during meiosis (Schwacha and Kleckner, 1997). It is unlikely that the ability to discriminate between homologs versus sisters resides in the strand exchange protein itself, however. More likely, Dmc1 has acquired a special ability to allow strand invasion to occur in the context of constraints imposed by meiotic chromosome structure.

While widespread occurrence of the Rad51/Dmc1 gene pair suggests that this represents the ancestral state, Dmc1 genes are absent from the genomes of both *C. elegans* and *D. melanogaster*. Interestingly, these organisms also do not rely on nascent recombination events to achieve synapsis between homologs. This correlation suggests that as other mechanisms for promoting homolog synapsis became more prominent in these organisms, a role for Dmc1 became dispensable. Perhaps if proper synapsis can be achieved by a recombination-independent means, then interhomolog strand exchange can occur without the assistance of Dmc1. It may be the case that not all of Dmc1's specialized functions have been jettisoned, however. They may instead have been "consolidated" in a modified version of Rad51 that retains some Dmc1-like character. Although the surviving Rad51 homolog in both *C. elegans* and *D. melanogaster* is clearly more similar overall to Rad51 than to Dmc1, there are positions in each protein where a Dmc1 signature residue has been substituted for the residue normally found in Rad51 orthologs.

Promoting the Crossover Outcome of Initiated Recombination Events

DSBR by homologous recombination can result in either crossover or noncrossover products (Figure 2), but only crossovers between homologs ensure correct segregation at meiosis I. Thus, it is not sufficient to promote use of the homolog as the recombination partner: a meiotic cell must also ensure that recombination between homologs results in crossing over. In vegetative/somatic cells, the proportion of recombination events associated with crossing over is low (e.g., 5% to 20% of interhomolog gene conversions are associated with crossing over of flanking markers in *S. cerevisiae*). In contrast, a significantly higher fraction of meiotic recombination events (30%–50% in *S. cerevisiae*) are associated with crossing over (Pâques and Haber, 1999). This suggests that meiotic cells may actively promote the crossover outcome of recombination, and that development of a means to do so was another significant milestone on the road to sex. The early solution apparently involved the enlistment of Msh4 and Msh5, a duo of proteins from the MutS DNA mismatch-repair (MMR) family.

Msh4 and Msh5 comprise a heterodimer that plays no apparent role in MMR and instead has become specialized to function in meiotic recombination (Pochart et al., 1997; Roeder, 1997; Zalevsky, et al., 1999). Msh4/Msh5 acts after DSB formation, specifically to promote the formation of crossover products. By analogy to its well-studied paralogs in MMR, which recognize and bind to single base-pair or small insertional mismatches in DNA, Msh4/Msh5 likely recognizes and binds to some specific perturbation(s) of DNA duplex structure. Binding may lead to stabilization of a key recombination intermediate and/or recruitment of additional factors to allow or direct completion of the recombination event as a crossover rather than a noncrossover. At what point in the recombination process might this effect be exerted? Our thinking has been informed by recent studies providing evidence for an early bifurcation in the pathway for repairing meiotic DSBs (Figure 2). Hunter and Kleckner (2001) demonstrated the existence of stable strand-exchange intermediates involving only one of the two DSB ends and the corresponding unbroken homologous duplex; these single-end invasion (SEI) species are likely precursors to the later-arising DHJ intermediate. This provided support for the notion that ssDNA tails from two sides of a DSB engage a homologous duplex in a sequential rather than contemporaneous fashion (see also Pâques and Haber, 1999). Further, elegant experiments of Allers and Lichten (2001) examining the kinetics of formation of crossover and noncrossover products built a strong case that the canonical DHJ intermediate gives rise mainly to crossover products, whereas most noncrossover recombinants arise earlier via a different pathway. These and previous results have been synthesized in the model depicted in Figure 2, in which the crossover and noncrossover pathways diverge soon after single-ended strand invasion and initial repair synthesis. According to the model, events designated to become crossovers capture the ssDNA tail from the other side of the DSB and form the canonical DHJ intermediate, which will eventually be resolved by an HJ resolvase to give predominately crossover products. Recombination events relegated to the noncrossover fate fail to capture the second

end, and instead, the invading strand is displaced. The displaced strand then anneals with the second end, such that further repair synthesis and ligation result in recombinational repair without crossing over.

In the framework of this model, the Msh4/Msh5 complex could act to promote crossing over at a number of stages. It could act as early as the strand invasion step, by promoting conversion of a nascent unstable joint into a more stable and extensive strand exchange intermediate. It might act to prevent strand displacement and/or to promote second end capture. Alternatively, it might act after DHJ formation to promote resolution with the appropriate geometry to yield a crossover. Application of biochemical assays for the above-described recombination intermediates in *msh4* and *msh5* mutants should help "resolve" this issue in the near future.

Msh4/Msh5-dependent crossovers are subject to regulatory mechanisms that govern their distribution along chromosomes (e.g., Novak et al. 2001). One prominent manifestation of meiotic crossover control is crossover interference, the capacity of a (nascent) crossover in one region of a chromosome to discourage formation of other crossovers nearby (Roeder, 1997). Interference is another widespread feature of meiotic recombination that has been observed across kingdoms, suggesting that it coevolved along with the crossover recombination mechanism itself. While understanding the mechanism of interference remains an unrequited passion of many meiosis aficionados, several theories currently under discussion argue that interference is conferred by properties of the continuous axial structures that develop along the lengths of meiotic chromosomes (Zickler and Kleckner, 1998) and/or the SC that joins the axes of two homologs (Roeder, 1997). It has been suggested that the action of Msh4/Msh5 in promoting formation of crossovers may, in fact, be dependent on, as well as constrained by, this meiotically specialized organization of chromosomes (Zalevsky et al., 1999).

S. pombe, which does not form continuous axial structures or SCs, also lacks both interference and Msh4/Msh5 (Roeder, 1997). The coordinate absence of these features further supports the notion that meiosis-specific chromosome organization, crossover interference, and the Msh4/Msh5-dependent crossover pathway are functionally interconnected. *S. pombe* does make an ample supply of crossovers, however. Perhaps during the evolution of *S. pombe* a release from constraints imposed by axial or SC structures removed a requirement for Msh4/Msh5 in promoting crossovers; alternatively, development of an alternate pathway for ensuring crossovers may have rendered these structures expendable. An informative "fly in the ointment" of this tidy correlation is the fact that *Drosophila* females, which make crossovers in the context of SC that are subject to crossover interference, nevertheless lack Msh4/Msh5. This suggests that although Msh4/Msh5-dependent crossovers in other organisms are subject to interference, Msh4 and Msh5 may not themselves be integral components of the interference mechanism per se, and further that the interference mechanism can operate even when crossing over is facilitated by another means.

Concluding Remarks

We have defined and discussed a "core meiotic recombination machinery" whose components and functions are widely conserved among eukaryotes that engage in sexual reproduction. This core machinery has apparently been adapted from vegetative DNA metabolism functions to promote a high frequency of crossover recombination between homologous chromosomes during prophase of meiosis I. This stimulation of interhomolog crossing over was critical for the emergence and evolutionary success of sex. In some organisms, reliance on certain core components has apparently been lost or supplanted by the development of alternative strategies for ensuring crossover recombination between homologs. Successful variations are interesting not only on their own merits, but also because they provide insights into the roles of conserved components as well.

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