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Recombination Proteins Mediate Meiotic Spatial Chromosome Organization and Pairing

Aurora Storlazzi,[1,2] Silvana Gargano,[2] Gwenael Ruprich-Robert,[1] Matthieu Falque,[3] Michelle David,[1] Nancy Kleckner,[4] and Denise Zickler[1,*]
[1]Institut de Génétique et Microbiologie, UMR 8621, Université Paris-Sud, 91405 Orsay, France
[2]Istituto di Genetica e Biofisica A. Buzzati Traverso, CNR, 80131 Naples, Italy
[3]UMR de Génétique Végétale du Moulon, 91190 Gif sur Yvette, France
[4]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02139, USA
*Correspondence: denise.zickler@igmors.u-psud.fr
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SUMMARY

Meiotic chromosome pairing involves not only recognition of homology but also juxtaposition of entire chromosomes in a topologically regular way. Analysis of filamentous fungus *Sordaria macrospora* reveals that recombination proteins Mer3, Msh4, and Mlh1 play direct roles in all of these aspects, in advance of their known roles in recombination. Absence of Mer3 helicase results in interwoven chromosomes, thereby revealing the existence of features that specifically ensure "entanglement avoidance." Entanglements that remain at zygotene, i.e., "interlockings," require Mlh1 for resolution, likely to eliminate constraining recombinational connections. Patterns of Mer3 and Msh4 foci along aligned chromosomes show that the double-strand breaks mediating homologous alignment have spatially separated ends, one localized to each partner axis, and that pairing involves interference among developing interhomolog interactions. We propose that Mer3, Msh4, and Mlh1 execute all of these roles during pairing by modulating the state of nascent double-strand break/partner DNA contacts within axis-associated recombination complexes.

INTRODUCTION

A central unique feature of meiosis is pairing of homologous chromosomes ("homologs"). Pairing involves recognition of homology plus coming together of homologs in space. Additionally, juxtaposition must be achieved without high levels of entanglement (Kleckner and Weiner, 1993; von Wettstein et al., 1984; Wang, C.R. et al., 2009).

In most organisms, pairing involves juxtaposition of homolog structural axes, dependent upon, and mediated via, the biochemical process of DNA recombination. The two aspects are integrated by physical localization of protein/DNA recombination complexes ("recombinosomes") to their underlying axes (e.g., Henderson and Keeney, 2005; Borner et al., 2004; Moens et al., 2007; Anderson and Stack, 2005; Franklin et al., 2006; Oliver-Bonet et al., 2007; Wang, K. et al., 2009 and references therein). Recombinosome/axis association arises before, or just after, initiation of recombination via programmed double-strand breaks (DSBs) (Blat et al., 2002; Tesse et al., 2003). Thereafter, DNA recombination and recombination-mediated juxtaposition of homolog axes progress in close temporal and functional coordination.

Homologs first become aligned at a distance of ~400 nm during leptotene. Except in worm and *Drosophila*, this process requires DSB formation and is accompanied by appearance of axis-associated foci of RecA homolog(s) (review in Henderson and Keeney, 2005). At the DNA level, alignment is inferred to involve nascent DNA/DNA interactions between a 3′ single-stranded tail at one DSB end and the homologous region on a homolog partner duplex (Hunter and Kleckner, 2001; Hunter, 2006). Then, at zygotene, "synapsis" occurs: axes become progressively linked at a distance of ~100 nm by transverse filaments, which, with other proteins, comprise the synaptonemal complex (SC) (review in Page and Hawley, 2004). The leptotene/zygotene transition is also a crucial point for recombination: nascent interactions are differentiated into crossover (CO)-fated and noncrossover (NCO)-fated products, with concomitant onset of stable strand exchange (review in Hunter, 2006). In several organisms, SC formation is nucleated preferentially at sites of CO-designated interactions (Henderson and Keeney, 2005). After full SC has formed, mature CO and NCO products appear at midpachytene (e.g., Guillon et al., 2005).

This study analyzes the relationship between recombination and homolog juxtaposition in the filamentous fungus *Sordaria macrospora* which is a very powerful system for such analysis: continuous axes appear at early leptotene such that spatial relationships among chromosomes, and positions of axis-associated recombination-complexes, can be followed from S-phase onward (e.g., Storlazzi et al., 2003, 2008; Tesse et al., 2003; Zickler, 2006). Additionally, overall progression of meiosis can be followed by progressive increases in the sizes of both the

nucleus and its surrounding ascus (meiocyte), thus permitting the detection of temporal delays in the progression of chromosomal events in mutant backgrounds comparatively to wild-type (e.g., Storlazzi et al., 2003, 2008).

Interplay between homolog pairing and recombination is probed by analysis of mutants defective in three proteins previously shown to be direct participants in the DNA events of recombination: Mer3, Msh4 and Mlh1. Mer3 is a meiosis-specific 3′-5′ helicase that stimulates Rad51-mediated DNA heteroduplex extension and stabilizes recombinational interactions (Nakagawa and Ogawa, 1999; Nakagawa and Kolodner, 2002; Mazina et al., 2004). Msh4, as part of the Msh4/Msh5 heterodimer, a meiosis-specific homolog of the bacterial mismatch repair protein MutS, encircles and stabilizes branched DNA structures (Snowden et al., 2004). Mlh1/3 are eukaryotic homologs of the bacterial mismatch repair protein MutL and, by analogy, may mediate release of Msh4/5 (Snowden et al., 2004), among other possible roles (Hunter, 2006). Mer3 and Msh4 are implicated in ensuring that the fates of CO-designated interactions are faithfully maintained and efficiently implemented during and after the leptotene/zygotene "CO control" transition. In the absence of either gene product, CO formation is specifically abrogated while NCOs form at high levels (e.g., Borner et al., 2004). Mhl1 is implicated at a later stage, in finalization of CO recombinational interactions during pachytene (Hunter, 2006; Franklin et al., 2006). In correspondence with their times of action during recombination, foci of Mer3 and Msh4/5 are observed prior to and during SC formation, whereas foci of Mlh1 are not visible until after completion of synapsis (e.g., de Boer et al., 2006; Oliver-Bonet et al., 2007; Moens et al., 2007; Franklin et al., 2006; Higgins et al., 2004; Jackson et al., 2006; Wang, K. et al., 2009 and references therein).

Our results reveal that each of these three proteins plays a central role in homolog juxtaposition one stage prior its established role in promoting recombination. Mer3 and Msh4 are implicated in leptotene alignment (in advance of the leptotene/zygotene CO control transition) while Mlh1 is implicated in resolution of interlocks at zygotene (in advance of midpachytene CO finalization). Mer3 mutant phenotypes suggest the existence of an "entanglement avoidance" process. Further, the patterns and dynamics of Mer3 and Msh4 foci provide new information about the nature of the alignment process and, by implication, the positioning of DSB end(s) during alignment and the CO control transition. Integration of these findings with previous molecular and biochemical information leads to a model for how Mer3 and Msh4 could mediate alignment by stabilizing nascent DSB/partner interactions within axis-associated recombinosomes while Mlh1 mediates interlock resolution by removing Msh4/5 stabilization of these contacts.

RESULTS

Sordaria macrospora Mer3, Msh4, and Mlh1 were cloned and completely null deletion mutations constructed, found to be recessive in heterozygous crosses, and further analyzed. Cytological localization was determined for Msh4-GFP and Mer3-GFP fusion proteins, expressed from their respective promoters at ectopic locations. Both fusion genes complement all meiotic defects of cognate null mutants. See Supplemental Information (SI) for details.

Mer3, Msh4, and Mlh1 Are Not Required for Axis Formation but Are Essential for Normal Pairing, Bouquet Dynamics, and Recombination

Chromosomes of the three null alleles were analyzed in leptotene through pachytene nuclei (n = 300 each), by DAPI staining of chromatin and with two previously characterized axis markers: Spo76/Pds5-GFP (van Heemst et al., 1999) and meiosis-specific cohesin Rec8-GFP (Storlazzi et al., 2008). In all three mutants, chromosome axes are indistinguishable from wild-type (WT) axes: Spo76 and Rec8 load at S phase and form complete lines along chromosomes from early leptotene to end pachytene (below). Thus, effects of mutations on spatial relationships can be analyzed without complications from defects in axis development.

All three mutants progress through meiosis and sporulation with the same progressive increase in ascus size as in WT. However, chromosomal progression from leptotene to pachytene is prolonged in all three mutants: by ~10 hr in *mer3Δ* or *msh4Δ* and ~12 hr in *mlh1Δ* (24–26 hr instead of 12 in WT). Analogous prolongations occur in *Arabidopsis Atmlh3* and *Atmsh4* (Higgins et al., 2004; Jackson et al., 2006).

All three mutants exhibit defects in pairing and synapsis (below). They show also defects in the bouquet dynamics. As in WT, chromosome ends transiently cluster in one area of the nuclear periphery at late leptotene and then redisperse at pachytene onset, but exit from cluster is significantly delayed (Figures S1A–1H available online).

Recombination is also defective in all three mutants (Figures S2A–S2F and details in legends). In *mer3Δ* and *msh4Δ* mutants, chiasmata are drastically reduced (4–7) compared to WT (21 ± 3). *mlh1Δ* confers a milder defect: genetic analysis reveals a ~40% reduction in COs with no reduction in NCOs (see details in legend of Figure S2B). As similar defects were found in other organisms (review in Hunter, 2006), the corresponding proteins likely play the same roles for recombination in Sordaria.

Mlh1 Is Required for Interlock Resolution

In WT meiosis, unrelated chromosomes are occasionally seen entangled with one another, usually at zygotene. In such "interlocks," either one chromosome or a pair of chromosomes is located between two aligned homologs, held in place by regular synapsis to either side (Figures 1A and 1B). For Sordaria, interlocked chromosomes are seen in ~20% of WT serially sectioned zygotene nuclei (n = 121; DZ, unpublished data) and in ~15% (n = 300) of nuclei by immunofluorescence using Spo76-GFP as axis marker (Figure 1A). Further, by early pachytene, no interlocks are seen by either method. Thus WT meiosis must include a mechanism for their resolution during zygotene. Resolution of interlocks has been suggested previously (review in von Wettstein et al., 1984; Wang, C.R. et al., 2009); however, conclusions were often limited by the small number of nuclei examined and by the inability to exclude the possibility that interlocks were absent at pachytene because the corresponding nuclei were absent from the experimental population (e.g., via apoptosis in mammals; reviewed in von Wettstein et al., 1984; Zickler and