A Protein Complex Containing Mei5 and Sae3 Promotes the Assembly of the Meiosis-Specific RecA Homolog Dmc1

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Summary

Meiotic recombination requires the meiosis-specific RecA homolog Dmc1 as well as the mitotic RecA homolog Rad51. Here, we show that the two meiosis-specific proteins Mei5 and Sae3 are necessary for the assembly of Dmc1, but not for Rad51, on chromosomes including the association of Dmc1 with a recombination hot spot. Mei5, Sae3, and Dmc1 form a ternary and evolutionary conserved complex that requires Rad51 for recruitment to chromosomes. Mei5, Sae3, and Dmc1 are mutually dependent for their chromosome association, and their absence prevents the disassembly of Rad51 filaments. Our results suggest that Mei5 and Sae3 are loading factors for the Dmc1 recombimase and that the Dmc1-Mei5-Sae3 complex is integrated onto Rad51 ensembles and, together with Rad51, plays both catalytic and structural roles in interhomolog recombination during meiosis.

Introduction

During meiosis, recombination gives rise to crossovers and noncrossovers. Only a crossover between homologous chromosomes ensures the segregation of the chromosomes during meiosis division I. Meiotic recombination is initiated by the formation of double-strand breaks (DSBs) at recombination hot spots (Keeney et al., 1997). The ends of the DSB are processed to produce 3′OH overhanging single-stranded (ss) DNAs. For the crossover-specific pathway, one of the DSB ends interacts with a homologous duplex in homologous chromosomes but not with that in sister chromosomes, resulting in the formation of a product referred to as a single-end invasion intermediate (SEI; Hunter and Kleckner, 2001). The capture of the second DSB end by the SEI, accompanied by further processing, generates an intermediate with double Holliday structures. The Holliday structures are programmed to resolve into crossovers (Allers and Lichten, 2001; Schwacha and Kleckner, 1995). The formation of SEIs is proposed as a critical commitment step for “interhomolog”-specific crossover pathways (Hunter and Kleckner, 2001; Börner et al., 2004). Interestingly, only one end participates in SEI formation, suggesting a functional difference between the DSB ends and/or the independence of the ends. In addition, all of the biochemical processes of meiotic recombination are tightly coupled with chromosome morphogenesis (for a review, see Zickler and Kleckner [1999]). The crossovers mature into a chromosome axis exchange, i.e., chiasma.

Meiotic recombination requires the two RecA homologs Rad51 and Dmc1 (Bishop et al., 1992; Shihnohara et al., 1992), which are conserved from yeast to humans. The coordinated actions of Rad51 and Dmc1 are considered to play a critical role in homology searches and strand exchange during recombination. Rad51 forms a right-handed helical filament on ssDNAs, known as the nucleoprotein filament, and promotes a robust strand exchange in vitro (Symington, 2002). However, Dmc1 shows poor in vitro strand exchange activity compared to Rad51 (Hong et al., 2001; Masson et al., 1999). Dmc1 forms an octameric ring structure, which binds to DNAs (Passy et al., 1999; Kinebuchi et al., 2004). Dmc1-ring might be an inactive form. A recent biochemical study shows that human Dmc1 promotes robust strand exchange in vitro by forming a filament on ssDNA under particular conditions (Seeho et al., 2004). Irrespective of the biochemical similarities between Rad51 and Dmc1, they play distinct roles in meiotic recombination (Shihnohara et al., 1997). Dmc1 seems to be specialized to promote recombination between homologs.

Both Rad51 and Dmc1 are detected as an immuno-staining structure on chromosomes, known as the “focus.” Genetic analyses of focus formation suggest that the foci are sites of recombination and DSB repair (Gasio et al., 1998). Indeed, we recently showed that a focus containing Rad51 marks a site of mitotic DSBs and is an intermediate during DSB repair (Miyaizaki et al., 2004). Rad51 and Dmc1 foci are readily colocalized on chromosomes. Interestingly, under some conditions, Rad51 and Dmc1 show a side-by-side staining pattern, suggesting independent complex formation of the two RecA homologs (Shihnohara et al., 2000). Immunoelectron microscopy reveals that Rad51 and Dmc1 are components of a densely stained structure on the chromosome axes in zygotene, referred to as a recombination node (RN; Anderson et al., 1997; Tarsounas et al., 1999). The presence of proteinous structures containing the RecA homologs on the chromosome axis suggests that recombination is carried out by a large protein complex, which might play both a catalytic role and a structural role in the interaction between homologous chromosomes.

The assembly pathway for Rad51 on ssDNAs is well documented based on cytological and biochemical analyses (Gasio et al., 2001; e.g., Figure 7). The assembly and activity of Rad51 is promoted by various factors, such as RPA, Rad52, Rad55-57, Rad54, and Tid1/Rhd54 (Piques and Haber, 1999; Symington, 2002). Once an ssDNA is formed, RPA binds to the DNA to remove the secondary structure of the ssDNA, which indirectly
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Introduction

Homologous recombination promotes an exchange between two homologous DNAs. The recombination is necessary for maintaining genome stability both in mitosis and meiosis. Meiosis gives rise to gametes such as eggs and sperms. During meiosis recombination ensures the segregation of the chromosomes during meiosis division I as well as generates the diversity in genome [1]. Recombination in meiosis prefers homologous chromosomes (father and mother chromosomes) for the exchange, while recombination in mitosis does sister chromosomes (identical chromosomes). Molecular mechanism to promote recombination between homologous chromosomes remains to be determined.

In the present study, we identified a new protein complex required for meiotic recombination. This complex contains two meiosis-specific proteins, Mei5 and Sae3, as well as a meiosis-specific RecA homolog, Dmc1. Our results suggest that Dmc1-Mei5-Sae3 complex is a key complex to facilitate recombination between homologous chromosomes during meiosis.

Identification of genes required for the assembly of Dmc1

Dmc1 is a meiosis-specific homolog of bacteria RecA protein, which catalyzes homology search and strand exchange between two homologous DNAs [2, 3]. To look for the genes that may function together with Dmc1 in meiotic recombination, we searched for mutations conferring a dmc1-like phenotype in baker’s yeast, Saccharomyces cerevisiae; e.g., meiotic prophase arrest. We found both mei5 and sae3 mutants show similar phenotypes to the dmc1 mutants [4, 5] and characterized them in the detail. Both mutants cannot repair double-strand breaks (DSBs) during meiosis, which initiate the recombination (Fig. 1). This suggests that Mei5 and Sae3 are required for homology search between homologous DNAs. The two mutants are defective in the formation of synaptonemal complex, which exhibits tripartite structure formed between two homologous chromosomes (Fig. 2). A key phenotype of the mei5 and sae3 mutants is that these two mutants cannot assemble Dmc1 on meiotic chromosomes (Fig. 3). Dmc1 protein can be detected on meiotic chromosome as punctate staining (called foci) by immuno-staining using anti-Dmc1 antibody. The analyses showed...
that both the mei5 and sae3 mutants are defective in the focus-formation of Dmc1 to chromosomes, but not in that of Rad51, the another RecA homolog in the budding yeast [6]. Chromatin-immuno-precipitation also showed that both Mei5 and Sae3 are necessary for the binding of Dmc1 to recombination hotspots. These indicate Mei5 and Sae3 are required for Dmc1-assembly on recombination intermediates.

Furthermore, we found that both Mei5 and Sae3 proteins are localized on chromosomes during meiosis. Both the proteins showed punctate staining on meiotic chromosomes (Fig. 4). Both Mei5 and Sae3 foci are co-localized well with Dmc1 and Rad51 foci, suggesting that Mei5 and Sae3 act together with Rad51 and Dmc1. The focus formation of Mei5 and Sae3 are dependent upon Dmc1 (Fig. 4). In addition, we found that the Mei5 is required for the recruitment of Sae3 to chromosomes and Sae3 is for that of Mei5. Taken together, Dmc1, Mei5 and Sae3 are inter-dependent for the binding to chromosomes, suggesting that these three proteins form a complex required for meiotic recombination.

**Interaction of Mei5-Sae3 complex with Dmc1**

Above results strongly suggest that Mei5 and Sae3 form a complex, which might bind to Dmc1. To test this possibility, we examined the interaction of the three proteins both in vivo and in vitro. Yeast two-hybrid experiment indicated a N-terminal half of Mei5 interacts with Dmc1. This interaction was confirmed by immuno-precipitation (IP) of meiotic extracts. Anti-Dmc1 precipitates Mei5. Furthermore, the binding of Sae3 to Mei5 was studied by IP. IP with anti-Mei5 shows the association of Sae3 with Mei5. We also found that Mei5 and Sae3 were purified as a complex when expressed in E. coli. These results showed that Mei5 and Sae3 form a stable complex, which also binds to Dmc1 through Mei5. Based on these observations, we proposed that Mei5-Sae3-Dmc1 is a complex required for inter-homolog recombination during meiosis.

![Fig. 4 Immuno-staining analysis of Mei5 and Sae3. Mei5 (A, B, D) and Sae3 (C) proteins were detected by indirect-staining. Both proteins transiently form focus on meiotic chromosomes.](image)
Mei5 and Sae3 are conserved among species

Dmc1 homolog is conserved from yeast to human. We looked for the homologs of Mei5 and Sae3 in other organisms. Initial search could not detect any homologs. However, more careful examination in database found that fission yeast Swi5 protein, which is involved in recombination, is homologous to Sae3. A Swi5 partner, Swi2, showed homology to Mei5, a Sae3 partner. Using homology between Mei5-Sae3 and Swi2-Swi5, we detected human and mouse homologs of these proteins, suggesting that these homologs might be involved in recombination in mammalian cells.

Conclusion

One of the key features of recombination in meiosis is that the recombination take place between homologous chromosomes rather than sister chromosomes. It remains to be known what kind of protein(s) catalyzes the reaction. We found that two meiosis-specific proteins, Mei5 and Sae3, form a complex, which plays a critical role in homology search and strand exchange reaction during meiotic recombination. This complex acts together with a meiosis-specific homolog of RecA, Dmc1.

The phenotypes of the mei5 and sae3 mutants suggest the presence of a biochemical step, which couples the Rad51-assembly with the Dmc1-assembly (Fig. 5). This might be a conversion step of a mitotic recombinase with Rad51 into a meiotic version with both Rad51 and Dmc1 [7]. Once Dmc1 together with Mei5-Sae3 is recruited to a recombination site covered with Rad51, the ternary complex might confer a meiosis-specificity upon the recombination reaction by promoting the recombination between homologous chromosomes or coupling the reaction with morphogenesis of meiotic chromosomes.

Our results strongly suggest that homology search and strand exchange are catalyzed by a large protein complex containing Rad51-subcomplex and Dmc1-Mei5-Sae3 complex.

References