Spatiotemporal regulation of meiotic recombination by Liaisonin

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Sexual reproduction involves diversification of genetic information in successive generations. Meiotic recombination, which substantially contributes to the increase in genetic diversity, is initiated by programmed DNA double-strand breaks (DSBs) catalyzed by the evolutionarily conserved Spo11 protein. Spo11 requires additional partner proteins for its DNA cleavage reaction. DSBs are preferentially introduced at defined chromosomal sites called "recombination hotspots." Recent studies have revealed that meiotically established higher-order chromosome structures, such as chromosome axes and loops, are also crucial in the control of DSB formation. Most of the DSB sites are located within chromatin loop regions, while many of the proteins involved in DSB formation reside on chromosomal axes. Hence, DSB proteins and DSB sites seem to be distantly located.

To resolve this paradox, we conducted comprehensive proteomics and ChIP-chip analyses on Spo11 partners in *Schizosaccharomyces pombe*, in combination with mutant studies. We identified two distinct DSB complexes, the "DSBC (DSB Catalytic core)" and "SFT (Seven-Fifteen-Twenty four; Rec7-Rec15-Rec24)" subcomplexes. The DSBC subcomplex contains Spo11 and functions as the catalytic core for the DNA cleavage reaction. The SFT subcomplex is assumed to execute regulatory functions. To activate the DSBC subcomplex, the SFT subcomplex tethers hotspots to axes via its interaction with Mde2, which can interact with proteins in both DSBC and SFT subcomplexes. Thus, Mde2 is likely to bridge these two subcomplexes,

forming a "tethered loop-axis complex." It should be noted that Mde2 expression is strictly regulated by S phase checkpoint monitoring of the completion of DNA replication. From these observations, we proposed that Mde2 is a central coupler for meiotic recombination initiation to establish a tethered loop-axis complex in liaison with the S phase checkpoint.

Spo11 and its Partner Proteins: Conservation of Meiotic DSB Proteins

Activated recombination between homologous chromosomes in meiosis provides diversification of genetic information in descendant generations, which confers robustness and sustainability of species against brutal environmental fluctuation. It also ensures accurate chromosome segregation into haploid gametes.

Before entering into meiosis, premeiotic DNA synthesis takes place in the germ cell precursor. This DNA replication is then followed by formation of meiotic DSB formation, recombination, and two successive rounds of chromosome segregation to generate haploid gametes. During the first meiotic division, homologs should be physically connected by chiasmata, which are transiently formed by reciprocal recombination between homologs (crossing over). Failure of such meiotic recombination processes leads to chromosome aberration or infertility due to formation of inviable gametes. Therefore, integrity of regulation systems for meiotic recombination is pivotal for sexually reproducing organisms. In particular, the spatiotemporal regulation of DSB formation should be coordinated with other meiotic events
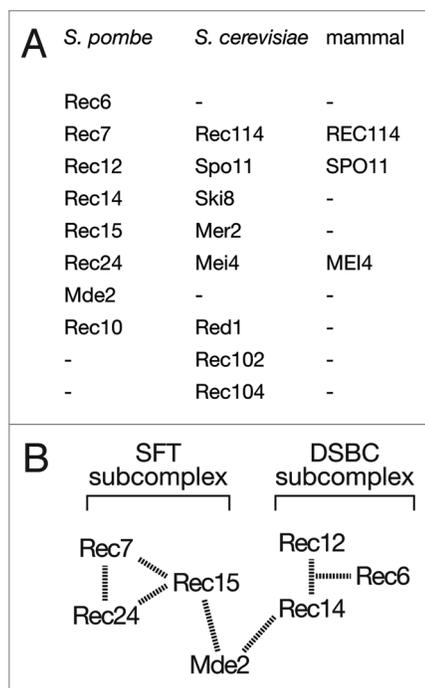
Figure 1. (**A**) DSB proteins in *S. pombe*, *S. cerevisiae* and mammals. (**B**) The two DSB subcomplexes and the network for protein-protein interactions among DSB proteins.

such as premeiotic DNA synthesis, formation of higher-order chromosome architectures, and sister chromatid cohesion.

The highly conserved Spo11 protein encodes a type II topoisomerase-like protein and catalyzes meiotic DSB formation.[1,2] The DNA cleavage activity of Spo11 requires additional partners.[3] In *S. cerevisiae*, the nine DSB proteins are known as the Spo11 accessory proteins, and form four subcomplexes: Spo11-Ski8, Rec102-Rec104, Rec114-Mer2-Mei4 (RMM), and Mre11-Rad50-Xrs2 (MRX). The Spo11-Ski8 complex is the catalytic core of DSB formation. The Rec102-Rec104 complex associates with the catalytic core complex and the RMM complex that further interacts with the MRX complex, suggesting the existence of the multimeric DSB complex.[4-6] Several DSB proteins are highly conserved among eukaryotes (**Fig. 1**). In *S. pombe*, Rec12, Rec14, Rec7, and Rec24 are the homologs of *S. cerevisiae* Spo11, Ski8, Rec114 and Mei4, respectively.[2,7-10] Our study identified Rec15 as the functional and structural homolog of Mer2.[11] The MRX complex is conserved in eukaryotes, but its role in DSB formation seems to vary from species

to species.[12] Importantly, in addition to SPO11, REC114 and MEI4 have been recently identified in mice.[9] They are suggested to form a complex like their homologs in *S. cerevisiae*. Further biochemical analysis or extensive homology search may enable identification of other orthologs.

To investigate *S. pombe* DSB complexes, we employed two approaches: mass spectrometry analysis of the immunoprecipitated DSB protein complexes and comprehensive yeast two-hybrid assays. These analyses enabled us to identify two major DSB subcomplexes, which are quite similar to the DSB complexes in *S. cerevisiae* (**Fig. 1**). One type of subcomplex consists of Rec6, Rec12 and Rec14. This subcomplex contains Rec12 (a Spo11 homolog). Thus, it is very likely to function as the catalytic core complex: hence we referred to this subcomplex as the "DSBC (DSB Catalytic core)" subcomplex. The second type of subcomplex comprises Rec7, Rec15 and Rec24. This subcomplex is named the "SFT (Seven-Fifteen-Twenty four)" subcomplex. We also found that Mde2 can interact with Rec14 and Rec15 in each subcomplex, suggesting that Mde2 bridges the two subcomplexes. Moreover, Mde2 turned out to be essential for the assembly and stabilization of the SFT subcomplex. These observations suggest that Mde2 plays critical roles in DSB control.

"Liaisonin" Mde2 Links Higher-Order Chromosome Architecture to the S Phase Checkpoint to Initiate DSB Formation

As DNA synthesis proceeds, cohesion of the meiotic sister chromatid occurs along the entire length of the chromosome to form chromosomal "axes." Concomitantly, chromatin "loops" emanate from the axes. The axis structure then develops into a "synaptonemal complex (SC)" involved in crossover control of meiotic recombination. Although *S. pombe* does not have an SC, some axial proteins are well conserved.[13] For example, Rec8, a subunit of meiotic cohesin, and other axial elements (Red1 and Hop1) are highly conserved and enriched in axes.[14-16] Early cytological observations suggest that axes provide a scaffold for the assemble of meiotic recombination

proteins (including Rad51, Msh2 or RecA homolog etc.).[17] Indeed, Rad51 foci are found in axis regions in *S. pombe*.[18]

On the other hand, chromatin immunoprecipitation analyses using a genome tiling array (ChIP-chip) indicated that DSB sites in *S. cerevisiae* exhibit a strong inverse correlation with potential axis sites revealed as Rec8 or Red1 binding sites.[14,15,19] Therefore, DSB sites are seemingly apart from the binding sites of some axial proteins.

The most likely explanation for this paradox is the "tethered loop-axis complex" model, in which a DSB hotspot located in a loop physically interacts with an axis site.[14,17] This model is supported by the observation that the *S. cerevisiae* RMM complex interacts with chromosome axes.[16] It should be also pointed out that the *S. pombe* axial protein Rec10, a homolog of *S. cerevisiae* Red1 is a prerequisite for DSB formation.[20] These observations suggested that higher-order chromosomal architecture controls DSB formation. However, the precise molecular mechanism has not been elucidated.

To explore this model at a molecular level, we investigated interactions between all known DSB and axial proteins. This analysis revealed that Rec15 (a component of the SFT subcomplex) directly binds to axial Rec10, suggesting that the SFT subcomplex is recruited to axes by the Rec15-Rec10 interaction. This notion can explain why nuclear localization of Rec7 (another component of the SFT subcomplex) exhibits a dispersed distribution in cells lacking Rec10.[18]

We then examined the genome-wide distribution of some of these DSB and axial proteins by ChIP-chip. We found that Rec10 is enriched not only in axis sites (revealed by Rec8 binding sites) but also in many of the DSB hotspots. Such an unexpected Rec10 distribution can be explained by transient association of DSB sites to axes where Rec10 is intrinsically enriched. The conventional ChIP analysis using mutants for DSB proteins revealed that Mde2 and all SFT proteins are necessary for Rec10 binding to DSB hotspots, whereas the other DSB proteins (Rec6, Rec12 and Rec14) were not. This result suggests that the SFT-Mde2

complex, but not the DSBC subcomplex mediates the loop-axis interaction.

ChIP-chip analysis of the Rec15 distribution revealed that it is highly enriched at both axis and DSB sites like Rec10. Importantly, Rec15 is no longer observed in axis sites in the *rec10Δ* strain, but still persists on many DSB hotspots. These results indicate the presence of two distinct modes for Rec15 binding to the chromosome: Rec10-dependent (axes) and Rec10-independent (DSB hotspots). Further ChIP experiments revealed that Rec15 binding to DSB sites requires Rec7, Rec24 and Mde2, and vice versa. Therefore, facilitation and stabilization of the SFT subcomplex formation by Mde2 is essential for the binding of SFT-Mde2 to DSB hotspots. All these data support the notion of a "tethered loop-axis" model. Possibly, axis and loop regions are bridged by the protein-protein interactions via Rec10 and the SFT-Mde2 complex.

Then, how can the SFT-Mde2 complex recruit DSB hotspots to axes? Given that SFT proteins still bind to DSB sites even in the *rec10Δ* strain, sequence-specific DNA binding proteins and/or chromatin configuration are seemingly important for their recognition of DSB sites. Indeed, an *S. pombe* DSB hotspot called *ade6-M26* is activated by the sequence-specific binding of CREB/ATF type transcription factor Atf1-Pcr1.[21] In addition, the binding of Atf1-Pcr1 to the hotspot leads to establishment of local modification of histones distinctive for DSB hotspots (see below).[22,23] An alternative idea is the involvement of non-coding RNA (ncRNA) transcription in DSB hotspot activation. It should be noted that ncRNAs are often transcribed around DSB hotspots in *S. pombe*,[24] though the functional relevance of such ncRNAs to DSB formation is yet to be uncovered. With regards to this aspect, we previously reported that long ncRNAs (lncRNAs) are transcribed from Atf1-Pcr1 binding sites where the Rec12-binding site exists.[25] Such lncRNA transcription participates in local chromatin remodeling and finally leads to massive gene activation of the *fbp1+* gene in response to glucose starvation. It would be interesting to examine roles of lncRNA transcription in DSB hotspot activation.

The finding on the roles of Mde2 in DSB control further sheds light into a mechanism for temporal control of DSB formation. In normal yeast meiosis, DSB formation must occur strictly after pre-meiotic DNA replication.[26,27] Most of the DSB proteins are upregulated at the onset of meiosis. Among them, Mde2 is solely upregulated by the meiosis-specific transcriptional activator Mei4 (Mde2 is named after Mei4-dependent expression. Note that *S. pombe* Mei4 is not the homolog of *S. cerevisiae* Mei4).[28] Ogino and Masai suggested that the S phase checkpoint prevents DSB formation by altering the transcriptional profile of the Mei4-dependent regulon until DNA replication is completed.[29] We also found that Mde2 is the sole DSB protein whose expression is repressed in the presence of the DNA replication inhibitor hydroxyl urea (HU). These observations suggest that the S phase checkpoint controls the timing of the loop-axis interaction through Mde2 expression, though other regulation mechanisms such as posttranslational modifications on DSB proteins by checkpoint kinases are not excluded. As described above, Mde2 was suggested to mediate the interaction between the two DSB subcomplexes, DSBC and SFT. Therefore, we hypothesized that the DSBC subcomplex is recruited to DSB hotspots by interacting with Mde2. This idea is consistent with the observation that Rec12 does not bind to DSB hotspots in *mde2Δ*. Taken together, we propose that Mde2 acts as a liaison factor ("Liaisonin") that links S phase checkpoint, higher-order chromosome structures, and assembly of DSB machinery (**Fig. 2**).

Histone Modification Links DSB Sites to Axes

Recently, the connection between histone modification and DSB hotspots has been demonstrated. Histone modification influences local chromatin states to modify activity of a large number of DNA activities, such as DNA repair, replication or transcription. For instance, we previously reported that histone acetyltransferase Gcn5 is involved in histone acetylation around the *ade6-M26* hotspot and its DSB formation.[22] In addition, H3 lysine4 trimethylation (H3K4me3), which is correlated with a transcriptionally active promoter, has been demonstrated to be enriched in *S. cerevisiae* DSB hotspots.[30,31] Deletion of the gene for Set1, the unique H3K4me3 methyltransferase in *S. cerevisiae*, caused a perturbation of DSB frequency and distribution.[30,31] Buard et al. and Smagulova et al. also found H3K4me3 enriched at recombination sites in the mouse genome.[32,33] It should be noted that a mouse histone methyltransferase PRDM9 with multiple zinc-finger motifs is assumed to determine a large portion of meiotic recombination hotspots in mammals.[34-36] We recently found that DSB hotspots in *S. pombe* exhibit relatively lower H3K4me3 levels, while they show higher levels of H3K9 acetylation.[23] Alanine substitution of H3K9 resulted in overall reduction in DSB frequency, while deletion of *S. pombe* Set1 (the unique H3K4me3 methyltransferase) affected the DSB frequency at each location. However, DSB formation per se is still detected at many DSB hotspots in these mutants. From these, we hypothesize that recombination hotspots are determined by the combination of redundant histone modification marks.

Then, how are DSB proteins targeted to specific sites with such histone marks? Two recent papers suggest that Spp1, a subunit of the COMPASS complex containing Set1, tethers chromatin with H3K4 methylation to axes, allowing Spo11 activation in *S. cerevisiae*.[37,38] Considering that Spp1 associates with axes by interacting with Mer2, a Rec15 homolog, the central players of the loop-axis interaction appear to be common in these two yeasts. It will be interesting to search for the mammalian counterpart of Spp1 harboring a PHD motif that binds to H3K4me2 and H3K4me3, or to investigate whether PRDM9 or proteins interacting with PRDM9 participate in the loop-axis interaction.

It should be noted that, even though the location or frequency of DSBs can be influenced in the *set1Δ* (or *spp1Δ*) yeast strain or in mice lacking PRDM9, DSBs are still actively formed in these mutants.[39,40] This suggests that DSB formation is spatiotemporally controlled at multi-layers, such as histone modifications, higher-order chromosome structures, and DSB proteins.
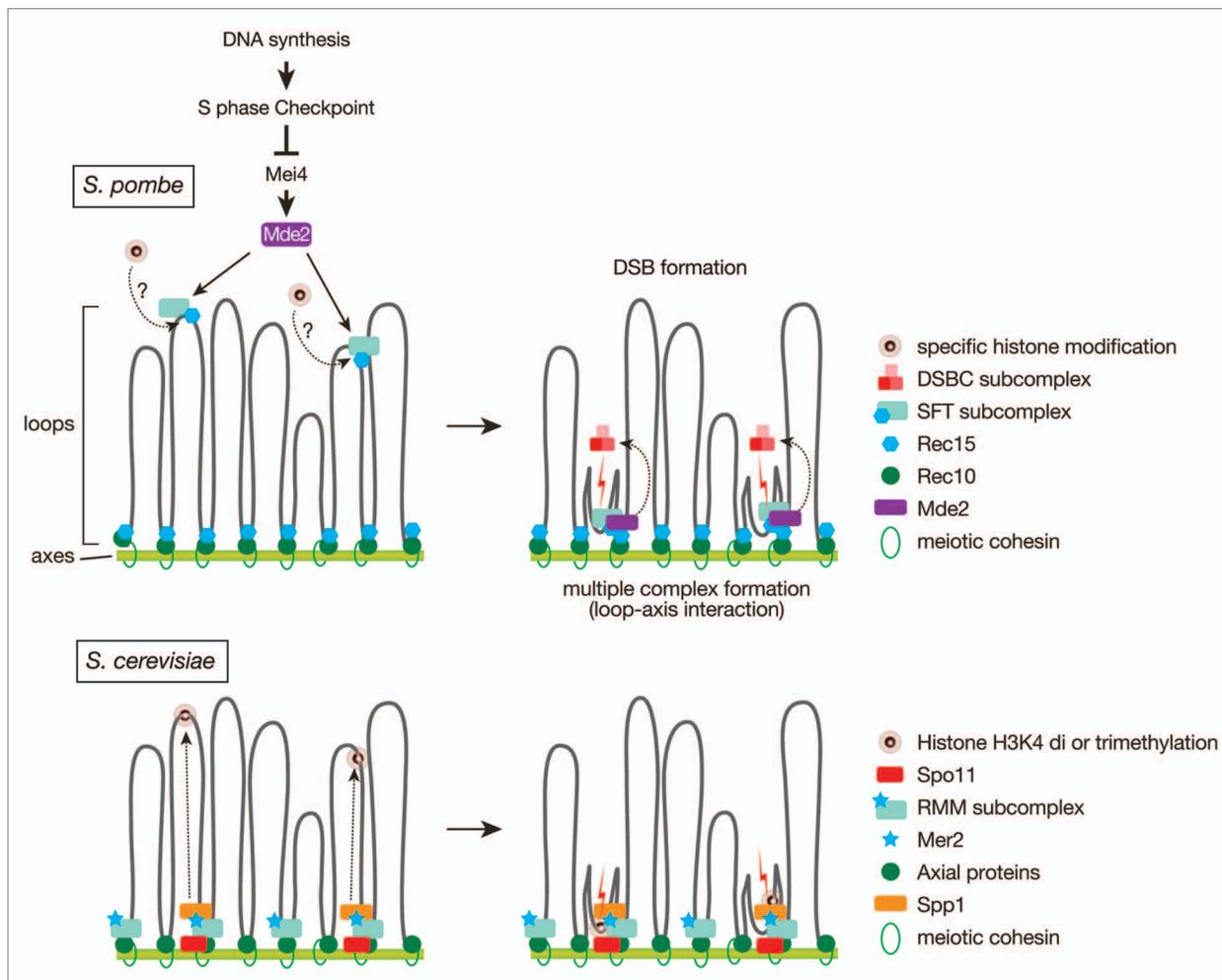
Figure 2. We propose a model for the step-wise and cooperative assembly of DSB subcomplexes on DSB hotspots in *S. pombe*. During premeiotic DNA replication, chromosomal axes are established by the meiotic cohesin, the major axial protein Rec10, and those associated proteins along meiotic chromosomes. Rec15 is recruited to Rec10 on axis sites. At this point, Mde2 expression is inhibited by the active S phase checkpoint through the regulation of Mei4. After DNA replication, the SFT subcomplex and Mde2 are recruited cooperatively to DSB hotspots located in loop regions. This allows for stabilization of the SFT subcomplex at DSB sites as well as the interaction between axes and DSB sites on loops through the multi-protein complex formation. In such circumstances (stabilized SFT subcomplex in close proximity to axis-components), the SFT subcomplex and Mde2 can recruit the active DSBC subcomplex to DSB sites. Such DSBC recruitment is possibly mediated by the interaction between Rec14 and Mde2. On the other hand, in *S. cerevisiae*, axis-bound Mer2 recruits Spp1, which recognizes a specific histone modification such as H3K4 di or trimethylation around DSB hotspots, and participates in tethering DSB hotspots to axes, allowing Spo11 activation. These models explain how DSB hotspots can interact with chromosome axes and why axis-bound proteins influence DSB formation.

Future Perspective

Our study provides a new insight into how cells spatiotemporally regulate meiotic recombination. In this model, Mde2 functions as "liaisonin" between the timing and the position of DSB formation. However, many questions still remain to be solved. The first one is the conservation of the Mde2 function in other eukaryotes. We failed to identify the Mde2 homolog in distantly related organisms, but it would be intriguing to test whether the gene expression (or protein amount) of each DSB protein is under control of the S phase checkpoint in other species, which may be helpful to discover a functional homolog of Mde2.

Another important issue is regulation of Mde2 expression. Nutrient depletion triggers inactivation of the exosome to stabilize some meiosis-specific mRNAs.[41] Intriguingly, the S phase checkpoint suppresses the *mei4*+ transcript that is regulated by the nuclear exosome.[29] It would be interesting to study whether the S phase checkpoint directly controls the exosome activity or its localization.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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