Initiation of meiotic recombination by formation of DNA double-strand breaks: mechanism and regulation

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Abstract

Homologous recombination is essential for accurate chromosome segregation during meiosis in most sexual organisms. Meiotic recombination is initiated by the formation of DSBs (DNA double-strand breaks) made by the Spo11 protein. We review here recent findings pertaining to protein–protein interactions important for DSB formation, the mechanism of an early step in the processing of Spo11-generated DSBs, and regulation of DSB formation by protein kinases.

Introduction

During meiosis, recombination plays an essential role in chromosome segregation by forming physical connections between homologous chromosomes that allow them to orient properly on the spindle and to segregate accurately at the first division [1]. If recombination fails, chromosome disjunction also frequently fails, with disastrous consequences for gamete formation. Meiotic recombination has at its heart the formation and subsequent repair of DSBs (DNA double-strand breaks) formed by the Spo11 protein [2]. Spo11 is widely conserved, but it does not act alone: in *Saccharomyces cerevisiae*, at least nine other proteins are also required for DSB formation. The roles of these factors are as yet poorly understood. Recombination is also co-ordinated with progression through meiotic prophase such that specific steps in the recombination pathway occur at the proper time. For example, DSB formation occurs only after local DNA replication has occurred. How this timing is controlled is also not yet well understood. We review here several recent studies that address these aspects of the mechanism and regulation of meiotic recombination initiation.

Endonucleolytic processing of Spo11-mediated DSBs

Spo11 shares sequence similarity with the type II topoisomerase from archaea, TopoVI, and catalyses DSB formation by a similar mechanism [3,4]. A Spo11 dimer co-ordinately breaks both strands of a DNA molecule, creating a DSB with covalent linkages between the newly created 5′ DNA strand ends and the catalytic tyrosine residue in each Spo11 monomer (reviewed in [2]). In order for the DSB to be repaired, the Spo11 protein must be removed. Certain non-null alleles of the Mre11–Rad50–Xrs2 complex, and mutation of the Sae2 protein, cause covalent Spo11–DSB complexes to accumulate unrepaired [5–8]. Mre11 possesses nuclease activity (reviewed in [9]), leading to the idea that Spo11 could be removed from DSB ends by Mre11-catalysed endonucleolytic cleavage 3′ to the attachment [2] (Figure 1).

We tested this hypothesis by developing an assay for Spo11 release from DSB ends [10]. Briefly, Spo11 complexes were immunoprecipitated from yeast and mouse meiotic cell lysates, end-labelled with terminal deoxynucleotidyl transferase plus radioactive nucleotides, and separated by SDS/PAGE. Specific radiolabelled species indicative of Spo11 covalently attached to short oligonucleotides were detected in extracts from both yeast and mouse, proving the endonucleolytic release hypothesis to be both correct and evolutionarily conserved. Interestingly, the radiolabelled species formed two discrete populations that differed in the lengths of the attached oligonucleotides (either 21–37 nt or $\leqslant$12 nt). Furthermore, regardless of their combined abundance in sequential time points taken from a synchronous meiotic culture, the two populations were always found in equal quantities. Although there are numerous ways to explain the equal ratio of short and long oligonucleotide attachments (see [10] for discussion), we favour a simple model in which Spo11 is released by asymmetrically placed endonuclease cleavages flanking each DSB (Figure 1). Such a release may establish asymmetry in subsequent steps of the meiotic recombination pathway, a feature that has existed in DSB repair models since their inception [11,12] (Figure 1).

Surprisingly, the lifespan of Spo11–oligonucleotide species closely matched that of resected Spo11–DSBs [10]. Resected DSBs disappear upon transition into stable strand-exchange intermediates, whereas Spo11–oligonucleotide complexes presumably disappear upon degradation. Thus the matched lifespan supports the idea that degradation of the Spo11–oligonucleotide complex is mechanistically linked to DSB

Key words: cyclin-dependent kinase, DNA double-strand break, homologous recombination, meiosis, Mre11–Rad50–Xrs2 complex, Spo11.

Abbreviations used: Clb, B-type cyclin; DSB, DNA double-strand break.

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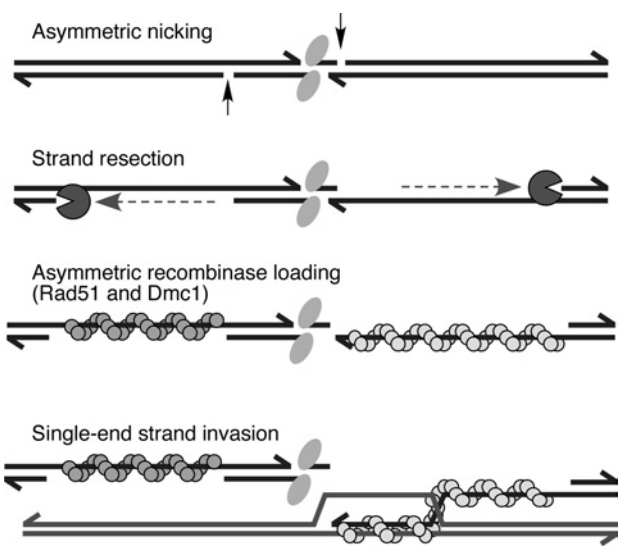
Figure 1 | Endonucleolytic processing of Spo11-mediated DSBs and asymmetric steps in meiotic recombination

Several steps in recombination are shown schematically. A Spo11 dimer (ellipses) creates a DSB with Spo11 protein covalently attached to the 5′ termini on each side of the break. The covalent protein–DNA complexes are then processed by a pair of single-strand nicks, presumably catalysed by the Mre11–Rad50–Xrs2 complex. We have proposed that these nicks are spaced asymmetrically such that Spo11 is released with either a long or a short oligonucleotide covalently attached. It is thought that exonuclease digestion of the 5′ terminal strands then initiates at these nicks and yields single-stranded gaps. Strand-exchange proteins Rad51 and Dmc1 then form helical nucleoprotein filaments on opposite sides of the DSB [41], and asymmetric strand invasion by one DSB end yields a stable strand-exchange intermediate [11]. We hypothesize that Spo11–oligonucleotide complexes, stabilized by base-pairing and protein–protein interactions, remain associated with DSB ends up to the point of strand exchange. Reproduced from [10], with permission.

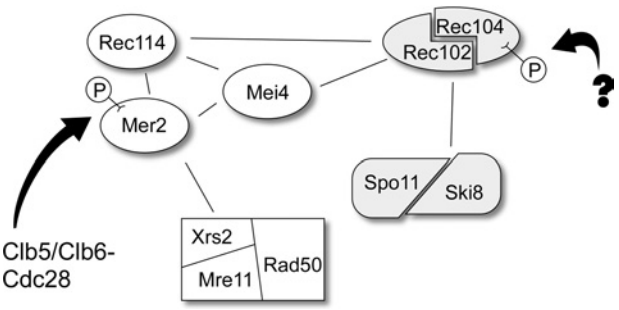


repair. One way that this might occur is if the Spo11–oligonucleotide remains associated with the 3′-end of resected DSBs, and is only degraded during DNA strand exchange (Figure 1).

Type II topoisomerases catalyse DNA breakage and resealing activities that are essential to resolve topological entanglements that arise during cellular DNA metabolism. However, malfunction mid-cleavage can result in a stable protein–DSB covalent complex that requires removal prior to repair [13]. Indeed, the toxicity of such damage is exploited in anticancer chemotherapy, where compounds act as type II topoisomerase poisons (e.g. etoposide) [14]. Due to the similarity of the Spo11–DSB reaction to type II topoisomerases, we investigated whether such topoisomerase II covalent complexes were also removed by an endonucleolytic cleavage reaction. Using similar methods to the meiotic Spo11 assay, we detected type II topoisomerase–oligonucleotide complexes, supporting the idea that endonucleolytic release is a general DNA repair pathway specific for processing covalent protein–DNA linkages [10].

Figure 2 | Protein–protein interactions and post-translational modifications important for meiotic DSB formation

Individual proteins and known or inferred multiprotein subcomplexes are represented schematically. The lines show interactions revealed by two-hybrid and/or other functional analyses (see [24] for further discussion). Mer2 is phosphorylated by cyclin–Cdc28 complexes, whereas Rec104 is phosphorylated by an as yet unknown kinase.



Protein–protein interactions important for DSB formation in budding yeast

Spo11 is the catalytic centre of the DSB-forming machinery, but its activity requires the presence of other proteins as well. A number of genes in various organisms from fungi to mammals have been identified which, when mutated, confer recombination defects similar to those of *spo11* nulls [15–23]. To date, *S. cerevisiae* provides the most complete list of known candidates for these potential Spo11 partners, with at least nine other proteins required for Spo11-dependent DSBs: Rad50, Mre11, Xrs2, Mei4, Mer2, Rec102, Rec104, Rec114 and Ski8 [2]. However, relatively little was known about the functional interactions between Spo11 and these other proteins. We therefore carried out a systematic yeast two-hybrid analysis [24]. This work [24], in conjunction with other studies from several laboratories [25–30], defined a network of interactions that connects each of the DSB proteins to one another (Figure 2).

Importantly, this work [24] identified the WD repeat protein Ski8 as a direct partner of Spo11 (Figure 2). It had been puzzling how *SKI8* might be involved in DSB formation, because it was known to have cytoplasmic functions in non-meiotic cells: it inhibits translation of non-polyadenylated RNA and is required for 3′ → 5′ exonucleolytic RNA degradation [31–34]. *SKI8* (also known as *REC103*) was also found in a screen for meiotic recombination initiation mutations [35,36]. The meiotic role of *SKI8* appears to be conserved in some but not all organisms because Ski8 orthologues are required for meiotic recombination in *Schizosaccharomyces pombe* and *Sordaria macrospora* [21,22,37], but not in *Arabidopsis thaliana* [38]. In *S. cerevisiae*, we found that the roles of *SKI8* in RNA metabolism were genetically separable from its roles in meiotic recombination [24]. We also found that, depending on its interaction with Spo11, Ski8 relocalizes from the cytoplasm to the nucleus and associates with meiotic chromosomes, similar to the behaviour of the *Sordaria* homologue [22]. We also found that Ski8 in turn helps Spo11

interact with the Rec102 and Rec104 proteins and thereby recruit them to chromosomes (see Figure 2). These results show that Ski8 plays a direct role in DSB formation distinct from its function in RNA turnover.

Post-translational modifications of proteins important for DSB formation

In the course of analysing the behaviours of the yeast DSB proteins, we found that at least two of them (Rec104 and Mer2) are phosphorylated during meiosis (Figure 2) [29,39]. Mer2 is phosphorylated by the cyclin-dependent kinase Cdc28, in complex with a Clb (B-type cyclin; Clb5 or Clb6) [39]. Phosphorylation sites were identified and shown to be essential for modulating Mer2 interactions with other proteins and for DSB formation. These results suggest that the timing of DSB formation is controlled in part through the activity of Cdc28–cyclin complexes. These findings also provide a molecular explanation for previous studies demonstrating that DSB formation did not occur in the absence of Clb5 and Clb6 [40].

The kinase responsible for phosphorylating Rec104 is not yet known (Figure 2). Cdc28, Mek1 and Cdc7 kinases are not required, although the extent of phosphorylation is somewhat decreased when Cdc28 or Cdc7 are inhibited (K. Kee, J. Robbins and S. Keeney, unpublished work). Rec104 is phosphorylated normally in a *spo11*Δ mutant, revealing that this post-translational modification is not a consequence of DSB formation [29]. The modification does appear to be very specific however, because it is substantially decreased in a *rec102*Δ mutant [29]. Rec102 and Rec104 interact physically and functionally with each other, behaving as a single functional unit [27,29]. Mapping the phosphorylation site(s) on Rec104, identifying the kinase(s) responsible and determining the functional significance of this modification are important challenges for the future.

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