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DNA double-strand breaks in meiosis: Checking their formation, processing and repair

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ABSTRACT

DNA double-strand breaks (DSBs) are highly hazardous for genome integrity, but meiotic cells deliberately introduce them into their genome in order to initiate homologous recombination, which ensures proper homologous chromosome segregation. To minimize the risk of deleterious effects, meiotic DSB formation, processing and repair are tightly regulated in order to occur only at the right time and place. Furthermore, a highly conserved signal-transduction pathway, called meiotic recombination checkpoint, coordinates DSB repair with meiotic progression and promotes meiotic recombination.

1. Introduction

Meiosis is a specialized differentiation process that generates haploid gametes from diploid cells. Like mitosis, it begins with one round of DNA replication, thus producing a cell with four chromatids for each type of chromosome that have then to be properly distributed to four different nuclei. This is achieved by two subsequent rounds of chromosome segregation with no intervening DNA replication [1]. To both reduce chromosome number and ensure that gametes inherit a complete copy of the genome, maternal and paternal versions of each chromosome (homologous chromosomes) segregate in opposite directions at the first of the two meiotic divisions (meiosis I) (Fig. 1). Sister chromatids can then be segregated to the final haploid nuclei at the second meiotic division (meiosis II) (Fig. 1). For meiosis I to occur, homologs must pair and join prior to their segregation. Reciprocal recombination and the resulting chiasmata between homologous non-sister chromatids play an essential role in this linkage, and each chiasma results in genetic information exchange between maternal and paternal chromatids. By virtue of cohesion between sister chromatids, chiasmata provide physical connections between homologous chromosomes that allow them to align properly on the meiosis I spindle and to segregate accurately at the first meiotic division (Fig. 1) [1].

Chiasmata are generated by recombination events, which are initiated by the formation of self-inflicted DSBs made by the Spo11 protein in early meiotic prophase [2,3] (Fig. 2). After Spo11 removal, the 5′ ends are resected by 5′ to 3′ exonucleases, creating single stranded 3′ protruding overhangs on either side of the break. One 3′ protruding overhang invades a homologous non-sister chromatid (first end capture) and this invasion is crucial for generating exchanges between homologous chromosomes (Fig. 2) [4]. What causes 3′ overhangs to invade homologous non-sister chromatids rather than sister chromatids in most organisms is poorly understood. The invading 3′ end becomes paired with a complementary strand, thereby creating a template for repair synthesis. If the 3′ overhang at the other side of break is captured (second-end capture), subsequent extension and ligation result in the formation of a double Holliday junction (dHJ) [5,6]. Cleavage of these two HJs in either the same or opposite direction results in non-crossover (NCO) or crossover (CO) products, respectively (Fig. 2).

Indeed, homologous recombination during meiosis is radically different from somatic recombination. Self-inflicted DSB formation is an intrinsic part of the meiotic program and repair of DSBs can be considered a secondary goal of meiotic recombination, whose primary function is to facilitate pairing and generate chiasmata between homologous chromosomes. These unique features require a temporal coordination between meiotic recombination and progression, which allows meiotic DSBs to be generated only at the right time and place.

This review focuses on the molecular mechanisms controlling formation, processing and repair of programmed meiosis-specific DSBs, as well as on the surveillance mechanisms that couple their formation and repair to progression through meiosis. We will not only describe these control mechanisms in the budding yeast *Saccharomyces cerevisiae*, where the molecular details are best understood, but the evolutionary conservation of these regulatory processes will also be eventually highlighted.

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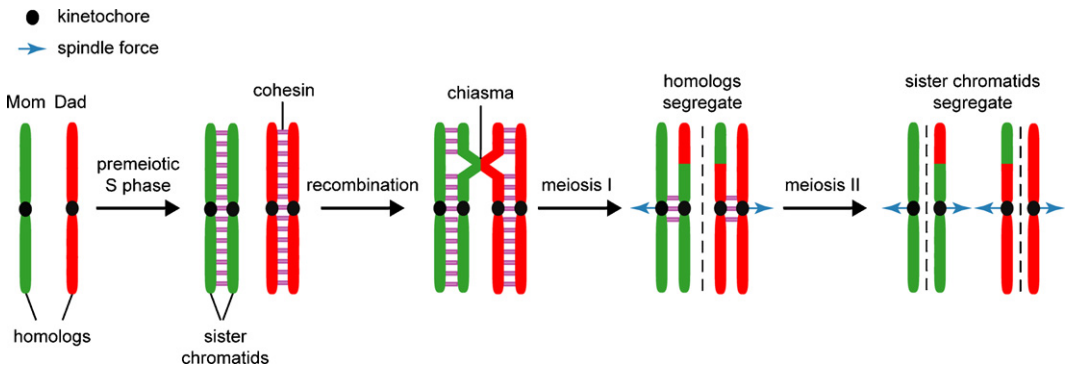

Fig. 1. Meiotic chromosome segregation. During premeiotic DNA replication, cohesion between sister chromatids is established. Following DNA replication, recombination between homologous maternal (green) and paternal (red) chromosomes generates chiasmata, which hold homologs together by virtue of cohesion between sister chromatids distal to the chiasmata. Following cohesin loss at chromosome arms distal to chiasmata during meiosis I, homologous chromosomes segregate to opposite poles, because mono-orientation of sister kinetochores allows maternal and paternal sister kinetochores to be pulled apart from each other in meiosis I. Cohesin present at centromeres is protected from cleavage and continues to hold sister centromeres together in order to allow sister chromatids biorientation and segregation in meiosis II.

2. Formation of meiotic DSBs

The initiation of meiotic recombination by programmed DSBs has been directly demonstrated in *S. cerevisiae* [2]. Breaks form during leptotene at specific sites and are catalyzed by the meiosis-specific protein Spo11, which shares sequence similarity with Top6A, the catalytic subunit of an archaebacterial type 2 topoisomerase [7,8]. A Spo11 dimer coordinately breaks both strands of a DNA molecule by generating a phosphodiester link between its catalytic tyrosine residue and the newly created 5′ DNA ends [2]. Yeast *spo11* null mutants do not undergo either meiotic DSBs or chiasmata, and therefore homologous chromosomes cannot be held together after premeiotic DNA replication, with consequent random segregation at meiosis I and production of aneuploid spores. The Spo11-dependent DSBs are not randomly located along budding yeast chromosomes, but occur mostly in intergenic promoter regions and preferentially in GC-rich chromosomal domains [9,10]. The requirement of Spo11 for the initiation of meiotic recombination is widely conserved, and Spo11 orthologs have been identified in many eukaryotes, including many fungi, nematodes, fruit flies, plants and mammals [11–16]. The isolation and analysis of *spo11* mutants in several model organisms have confirmed that Spo11 generates the DSBs initiating meiotic recombination, and also plays a critical role in ensuring proper meiotic progression.

However, Spo11 is not sufficient for DSB formation in vivo. Spo11-dependent DSB formation in *S. cerevisiae* requires the presence of at least nine other proteins, among which Mei4, Mer2, Rec102, Rec104 and Rec114 are meiosis-specific, whereas the remaining four proteins, Ski8, Mre11, Rad50 and Xrs2, also have roles in vegetative cells. In fact, Ski8, functions in RNA metabolism in mitotic cells [17–19], and the highly conserved MRX (Mre11–Rad50–Xrs2) complex has multiple functions in DSB repair, telomere maintenance and DNA damage checkpoint activation in both mitotic and meiotic cells [20]. Several lines of evidence indicate that the above proteins, rather than being part of a single DSB-forming holoenzyme of defined stoichiometry, form at least four functionally distinct subcomplexes, namely Rec102–Rec104, Mer2–Mei4–Rec114, Spo11–Ski8, and Mre11–Rad50–Xrs2 [21–28]. However, how these subcomplexes collaborate to promote Spo11-dependent DSB formation is not yet understood.

S. cerevisiae null mutants lacking any MRX complex component fail to generate meiotic DSBs [29–32]. Mre11 contains four conserved N-terminal phosphoesterase motifs, and both human and budding yeast Mre11 harbor single-strand DNA endonuclease, 3′–5′ double-strand DNA exonuclease and weak hairpin-opening activities [33]. However, *mre11* mutations impairing Mre11 nuclease activities allow Spo11-induced DSB formation, suggesting that the latter does not require per se these activities [29,34–37].

3. Temporal and spatial control of DSB formation

Because DSBs are highly hazardous for genome stability, the generation of Spo11-induced DSBs must be regulated in time and space so that DSBs are appropriately repaired. Furthermore, DSBs are necessary to generate chiasmata, each of them being a combination of local DNA exchanges between homologous non-sister chromatids plus higher order chromosome structure changes at the recombination sites (exchange of chromosome axes and local sister chromatid separation) [38]. Therefore, chiasmata formation requires a strict temporal order of meiotic chromosomal events, where recombination must initiate only after premeiotic DNA replication is completed and sister chromatid cohesion is established. Meiosis-specific transcription of genes involved in DSB formation might contribute to proper timing of these events.

Some studies suggest that DSB formation is coupled to the completion of premeiotic DNA replication. For example, DSB formation is prevented when premeiotic DNA replication is blocked, such as in the presence of the DNA synthesis inhibitor hydroxyurea (HU) [39]. However, interpretation of the above result is complex, because HU also blocks induction of early meiotic genes, including *SPO11* [40]. Other studies in budding and fission yeasts challenge the idea that recombination is absolutely dependent on premeiotic DNA replication. In fact, when significant amount of genome duplication is inhibited by inactivating the *S. cerevisiae* replication initiator factor Cdc6 [41] or its *S. pombe* ortholog Cdc18 [42], cells form nearly wild type levels of DSBs. Furthermore, inactivation of the *S. pombe* DNA replication checkpoint allows DSB formation even in the presence of HU [43,44], suggesting that a checkpoint mechanism can inhibit DSB formation when DNA replication is perturbed. Nevertheless, compelling evidence indicate that meiotic recombination initiates after local DNA replication. In fact, a delay in replication of one chromosome III arm selectively delays DSB formation along that arm without affecting its timing on the other arm of the same chromosome [39,45].

In eukaryotes, DNA replication depends on the highly conserved protein kinases CDK (cyclin-dependent kinase; Cdc28 in *S. cerevisiae*) and Cdc7 [46,47]. CDK associates with various cyclins, which activate the kinase and target it towards specific substrates. In particular, *S. cerevisiae* Cdc28, together with the cyclin partners Clb5 and Clb6, forms the S-phase CDKs (CDK-S) that control DNA replication. Similarly, Cdc7 kinase activity depends on its association with its accessory subunit Dbf4 in the DDK (Dbf4-dependent kinase) complex [48]. Both CDK-S and DDK are required for timely

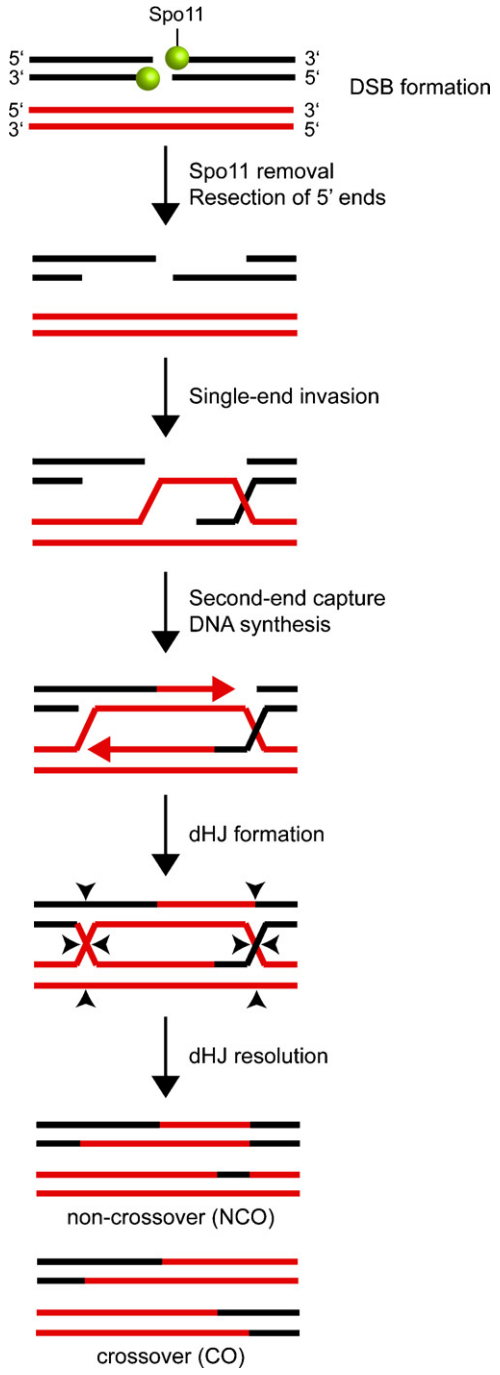

Fig. 2. The double-strand break repair model of meiotic recombination. Homologs are indicated in black (paternal) and red (maternal). Spo11 generates a DSB in one of the parental chromatids. After Spo11 removal, DSB ends are resected to generate 3′-ended ssDNA tails and one 3′-ended ssDNA tail invades the duplex homologous DNA sequence (red lines). Capture of the second ssDNA end and DNA synthesis create a double Holliday junction (dHJ), whose resolution can occur in either plane at both junctions (triangles) to generate crossover or non-crossover products. Red arrows indicate the 3′ ends of the newly synthesized strands.

and efficient premeiotic DNA replication and DSB formation in *S. cerevisiae* [49–54]. These findings raise the possibility that DSB formation is more directly dependent on the activity of CDK-S and/or DDK. Indeed, it has been shown that the CDK-S and DDK complexes can regulate DSB formation by phosphorylating the Mer2 protein, which collaborates with Spo11 to generate meiotic DSBs (see above) (Fig. 3) [55–57]. In fact, CDK-S inactivation by a specific inhibitor or by Clb5 and Clb6 elimination abrogates Mer2 phosphorylation. Moreover, CDK-S-dependent Mer2 phosphorylation occurs during early meiosis and does not require Spo11 or any other DSB proteins, and is therefore not a consequence of DSB formation [55]. Similarly, DSB formation is prevented and Mer2 phosphorylation is reduced when Cdc7 is inactivated in the *cdc7-as* mutant, where the enlarged ATP-binding pocket in the kinase-active site generates a variant that can be inactivated by addition of purine analogs [54,57]. Furthermore, both meiotic DSBs and Mer2 phosphorylation are defective when *CDC7* is deleted in the *bob1-1* background, which bypasses the essential requirement of Cdc7 for mitotic DNA replication [47,56].

Analysis of the Mer2 phosphorylation sites has revealed a complex pattern of partially interdependent regulation by CDK-S and DDK. Both the Ser30 and Ser271 residues are CDK-S targets, but only Ser30 is essential for DSB formation [55]. DDK phosphorylates multiple serine residues in the Mer2 N-terminal region. Among these, phosphorylation of Ser29 by DDK must be primed by prior action of CDK-S on Ser30, while other residues (Ser11, Ser15, Ser19, Ser22) are targeted by DDK independently of CDK-S [56,57]. The single substitutions of Ser11, Ser15, Ser19 and Ser22 with non-phosphorylatable residues confer little or no DSB defects, but double, triple or quadruple mutant combinations cause progressively more severe defects, suggesting that Mer2 function depends on cumulative phosphorylation events [56]. Importantly, substitution of Ser29 with alanine reduces Mer2 phosphorylation and confers a DSB defect similar to a *mer2* null mutant [56]. Thus, like CDK-S-targeted Ser30, phosphorylation of Ser29 by DDK is critical for DSB formation. Moreover, mutation of the Ser30 codon impairs protein–protein interactions with Mer2, Mei4, Rec114 and Xrs2 [55], whereas Cdc7-dependent phosphorylation of Mer2 facilitates the binding to a DSB of Rec114, Mei4 and Spo11 [56]. Taken together, these data indicate that both DDK and CDK-S act on Mer2 to promote DSB formation, possibly by controlling the loading of DSB proteins to chromatin (Fig. 3).

How does DNA replication normally precede DSB formation? CDK-S and DDK are involved both in DNA replication and DSB formation, but the two processes seem to be differently sensitive to DDK and/or CDK-S kinase activity. In fact, DSB formation, but not premeiotic DNA replication, is prevented by *cdc7ts* alleles at the restrictive temperature or by *cdc7-as* in the presence of the kinase inhibitor [49,54]. Based on these findings, it has been proposed that the threshold amount of DDK, and possibly of CDK-S, required for DNA replication might be reached before that required for DSB formation, thereby ensuring that DSB formation occurs only after DNA replication [38].

The involvement of CDK-S and DDK in both DNA replication and DSB formation may account also for DSB formation only occurring in replicated DNA sequences [39]. In fact, if CDK-S and/or DDK are physically associated with the replication forks, Mer2 would be preferentially phosphorylated locally and in a manner coupled to replication fork progression (Fig. 3). However, the finding that DSB formation is not prevented when significant amount of genome duplication is inhibited [41,42] implies that this local phosphorylation is not required for DSB formation when CDK-S and/or DDK activity is high enough to ensure Mer2 phosphorylation anyhow.

4. Control of DSB processing

Once Spo11 has catalyzed DSB formation, it remains covalently attached to the 5′ termini of each side of the break [58], from where it has to be removed in order to allow generation of single-stranded DNA overhangs that are essential for homologous pairing and strand exchange [59]. In both yeast and mouse, Spo11 removal is mediated by the formation of a single-strand DNA nick on each side of the break site, thereby liberating a Spo11-bound oligonucleotide [60]. Two discrete Spo11-oligonucleotide complexes are found in equal amounts, differing with respect to the length of the bound

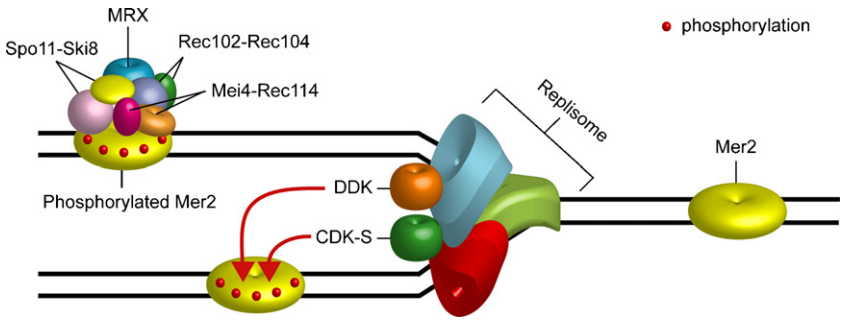

Fig. 3. Model for coupling DSB formation with DNA replication. Mer2 is preferentially phosphorylated by CDK-S and DDK complexes that are associated with the replication forks. Phosphorylated Mer2 is able to recruit proteins required to generate meiotic DSBs (see text for details).

DNA [60]. This suggests that Spo11 is released by asymmetrically placed endonuclease cleavages flanking each DSB end. Such Spo11 release may establish the proposed asymmetry in the double-strand break repair model, which envisages the strand exchange reaction as one DSB end invading the homologous duplex and the second being captured in a separate reaction [5].

It has been shown that *S. cerevisiae* Spo11 endonucleolytic cleavage requires both the Sae2 protein and the MRX complex. Budding yeast *sae2Δ* cells and *rad50s* separation-of-function mutants allow DSB formation, but are totally defective in Spo11 removal from DSB ends [29,32,34,58,61,62]. Seven out of nine *rad50s* reported mutations cluster on the Rad50 crystal structure to a narrow surface patch that has been proposed to form a protein interaction site [63]. This raises the possibility that Rad50s amino acid substitutions impair Sae2 function, possibly by disrupting Rad50–Sae2 interaction. Consistent with this hypothesis, Sae2 overproduction can partially rescue the single-strand annealing defects of *rad50s* mutants [64].

Similarly to *rad50s* and *sae2Δ* mutations, *mre11* alleles impairing Mre11 nuclease activity allow Spo11-induced DSB formation, but not Spo11 removal [35–37], suggesting that the latter may take place by Mre11-catalyzed endonucleolytic cleavage and that Sae2 participates in this process. As recently shown, Sae2 also exhibits an endonuclease activity on single-stranded DNA independently of MRX and cooperates with MRX in the processing of hairpin structures in vitro [65]. This finding suggests that Sae2, possibly in cooperation with MRX, may facilitate resection by mediating an endonucleolytic cleavage close to the DNA break, thus generating a clean end that can serve as an efficient substrate for nucleases such as MRX and Exo1.

Both MRX and Sae2 are involved in DSB processing also in mitotic cells. In fact, *SAE2* or *MRE11* deletion impairs DSB resection in vegetative *S. cerevisiae* cells [64,66,67]. Furthermore, Sae2 and MRX act in the same epistasis group to allow DSB resection in mitotic cells [67], and are required to ensure efficient repair by single-strand annealing (SSA) of both meiotic and mitotic DSBs [64,68]. Finally, they both participate in processing hairpin-containing DNA structures, and the Mre11 nuclease activity is essential for this process [69,70]. However, *SAE2* deletion only slows down resection at sites of clean DSBs in vegetative cells [64,66], whereas it completely impairs resection of Spo11-induced DSBs [34,58,61,62]. This is consistent with the hypothesis that Sae2 activity might be particularly important to initiate resection of DSB ends that are resistant to exonucleases because they bear protein–DNA crosslinks at their termini (such as Spo11-induced DSBs). It has been proposed recently that MRX and Sae2 catalyze a limited amount of DSB end resection. The 3′-ended DNA ends are then rapidly processed by either Exo1 or the RecQ helicase Sgs1, the latter acting in concert with the nuclease Dna2 [71,72,73]. If the same resection pathways contribute to the formation of ssDNA in meiotic cells remains to be determined.

Putative orthologs of *S. cerevisiae* Sae2 have been identified in other organisms like *Schizosaccharomyces pombe* (Ctp1/Nip1), *Arabidopsis thaliana* (Com1/Sae2), *Caenorhabditis elegans* (Com1/Sae2) and *Homo sapiens* (CtIP). In humans, CtIP was originally identified as an interactor of the transcriptional repressor CtBP [74]. It also interacts with the retinoblastoma protein RB [75] and the tumour suppressor protein BRCA1 [76,77]. Like *S. cerevisiae* Sae2, both *S. pombe* Ctp1/Nip1 and human CtIP facilitate ssDNA formation at DSB ends in mitotic cells [78,79], suggesting that they might play a role similar to that of *S. cerevisiae* Sae2 also in meiosis. Consistent with this hypothesis, the lack of Ctp1/Nip1 affects *S. pombe* spore viability [78] and causes accumulation of unrepaired meiotic DSBs [80]. Furthermore, *A. thaliana* and *C. elegans* Com1/Sae2 mutants are sterile, accumulate Spo11 during meiotic prophase and fail to form Rad51 foci despite the presence of unrepaired DSBs [81,82].

Spo11 removal and commitment to DSB resection should be highly regulated during meiosis in order to ensure meiotic DSB repair by HR. Effective DSB resection and HR in vegetative *S. cerevisiae* cells are promoted by CDK activity during the S and G2 phases of the cell cycle [83,84]. Because CDK activity is required to generate meiosis-specific DSBs, the CDK requirement for processing Spo11-induced DSBs is difficult to assess. One of the targets of the CDK-mediated regulation of DSB resection during a mitotic cell cycle is Sae2, which contains three potential CDK phosphorylation sites and is phosphorylated by CDK in vitro [85]. In particular, the Ser267 residue maps to the Sae2 region most highly conserved with its non-yeast orthologs, which include human CtIP, *C. elegans* Com1 and *A. thaliana* Com1. As recently shown, substitution of Sae2 Ser267 with a non-phosphorylatable residue causes phenotypes comparable to those of a *sae2* null mutant, including hypersensitivity to camptothecin, reduced hairpin-induced recombination and severely impaired DNA-end processing [85]. Furthermore, a Sae2 mutation mimicking constitutive Ser267 phosphorylation complements these phenotypes and overcomes the need of CDK activity for DSB resection [85]. These findings strongly suggest that cell-cycle control of DSB resection in vegetative *S. cerevisiae* cells results from the phosphorylation of Sae2 by CDK.

Sae2 phosphorylation by CDK is likely required also to allow Spo11 removal and subsequent resection of the meiotic DSB ends. In fact, substitution of Sae2 Ser267 with a non-phosphorylatable residue causes a strong reduction in spore viability [82,85]. Moreover, it prevents resection of meiosis-specific DSBs, whereas processing of Spo11-induced DSBs is allowed by a Sae2 mutation that mimics constitutive Ser267 phosphorylation [I. Guerini, N. Manfrini and M.P. Longhese, unpublished].

Interestingly, substitution with alanine of the Thr847 residue of human CtIP, which is surrounded by residues very similar to those surrounding Ser267 in Sae2, yields hypersensitivity to camptothecin [85], suggesting that CDK-control mechanisms for DSB resection might operate also in other organisms. One exception to this is likely to be represented by *S. pombe*, where Ctp1 lacks a CDK

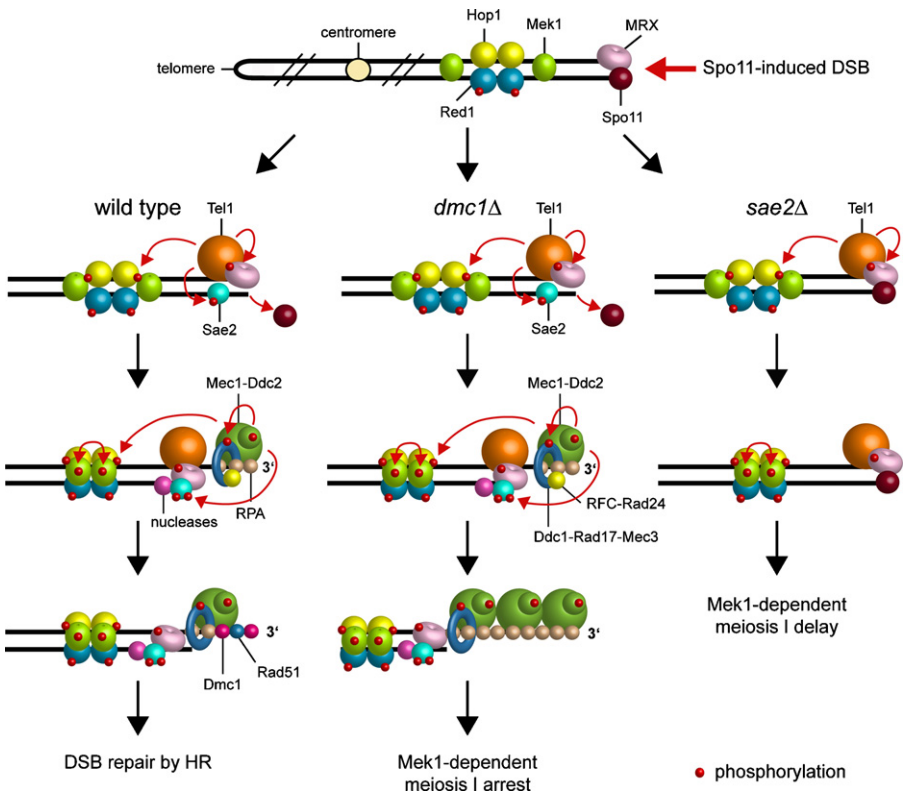

Fig. 4. The meiotic recombination checkpoint. Spo11, MRX and other proteins catalyze the formation of a DSB on the right arm of a yeast chromosome (top). Only one side of the DSB is shown. Hop1, Mek1 and phospho-Red1 are assembled onto DNA prior to DSB formation. In wild type cells (left panel) MRX allows checkpoint activation by recruiting Tel1, which in turn phosphorylates Sae2 and MRX. When Spo11 is removed from the DSB end, DSB resection by MRX, Sae2 and other nucleases generates 3′-ended ssDNA tails coated by RPA, Rad51 and/or Dmc1, which allow the loading of Mec1-Ddc2. Mec1 activation is also supported by independent loading of the Ddc1-Rad17-Mec3 complex by Rad24-RFC. Once loaded onto DNA, Mec1 and Tel1 trigger checkpoint activation by phosphorylating Hop1, which promotes Mek1 in trans autophosphorylation and activation. The absence of Dmc1 (middle panel) leads to accumulation of hyperesected meiotic DSBs, which trigger a Mec1-and Mek1-dependent meiosis I arrest. The absence of Sae2 (right panel) leads to accumulation of unprocessed meiotic DSBs, which trigger a Mek1-dependent slowing down of meiosis I that is primarily dependent of Tel1.

site analogous to Sae2 Ser267. However, the levels of *S. pombe* Ctp1 during the mitotic cell cycle are controlled at the transcriptional level by the CDK-regulated MBF transcription factor [86], being very low in G1 and increasing in the S, G2 and M phases [78]. Therefore, in this case, the CDK machinery seems to regulate Ctp1 levels, rather than Ctp1 phosphorylation.

After Spo11 is removed from the 5′ DNA ends by endonucleolytic cleavage, one or more nucleases resect the break to generate 3′-ended single-stranded DNA overhangs. The RecA-like strand exchange proteins Rad51 and Dmc1 bind these tails to form presynaptic nucleoprotein filaments, which engage in the search for homologous template, with a strong preference towards the homologous chromosome rather than the sister chromatid [87,88].

5. Coupling DSB repair with meiosis progression: the meiotic recombination checkpoint

5.1. Detection of meiosis-specific DSBs by the checkpoint machinery

Programmed DSB repair is coupled to cell cycle progression by a surveillance mechanism, named meiotic recombination checkpoint, which delays meiosis I until DSB repair is achieved (Fig. 4) [89]. Mechanistically, the meiotic recombination checkpoint is related to the DNA damage checkpoint, which senses and signals DSBs that arise at unpredictable locations as a consequence of DNA damage during both mitosis and meiosis. In both DNA damage and meiotic recombination checkpoints, DSB detection is accomplished by highly conserved protein kinases, including mammalian ATM (Ataxia Telangiectasia Mutated) and ATR (Ataxia Telangiectasia and RAD3-related), *S. cerevisiae* Tel1 and Mec1 and *S. pombe* Tel1 and Rad3 [90,91]. Both yeast Tel1 and human ATM interact with the MRX and MRN complexes, respectively [92,93], whereas Mec1, Rad3 and ATR function in a complex with Ddc2 [94], Rad26 [95] and ATRIP [96], respectively.

In both the DNA damage and the meiotic recombination checkpoints, Mec1/ATR ability to transmit and amplify the DNA damage signals is enhanced by a proliferating cell nuclear antigen (PCNA)-like complex called Ddc1-Rad17-Mec3 in budding yeast, and Rad9-Rad1-Hus1 in both mammals and *S. pombe* [97–101]. This complex is recruited to damaged DNA independently of Mec1 and ATR through a replication factor C (RF-C)-like complex, consisting of the four small RF-C subunits that interact with budding yeast Rad24, or its *S. pombe* and human ortholog Rad17 [102–104].

Several lines of evidence indicate that the signalling event for DSB-induced checkpoint activation in mitotic cells is the recruitment of the MRX complex to the break site. First, in both yeast and mammals, MRX binds directly to DNA and its initial recruitment to DSBs occurs independently of any other DNA damage response protein examined so far [105–107]. Moreover, it is required for Tel1/ATM association to DSB lesions through the interaction between the C-terminal motif of Xrs2/Nbs1 and Tel1/ATM [92,93,108]. Finally, both the hypermorphic *rad50s* allele and deletion of the *SAE2* gene prolong MRX occupancy at DSBs [67,106], and constitutively upregulate Tel1/ATM signalling [67,109]. The involvement of the MRX complex in activating the meiotic recombination checkpoint has not been clarified, because of its requirement for Spo11-induced DSB generation.

Tel1 and Mec1 detect unprogrammed and programmed DSBs in a similar manner, leading to a model for both DNA damage and meiotic recombination checkpoints (Fig. 4), in which Tel1/ATM can sense and signal unprocessed DSBs. Once DSB resection occurs, generation of 3′-ended ssDNA leads to Mec1/ATR recruitment and subsequent Mec1/ATR-dependent checkpoint activation. Specifically, during the mitotic cell cycle, Tel1/ATM appears to bind unprocessed DSBs via the MRX/MRN complex, and its signalling activity is disrupted when DSB termini are subjected to 5′–3′ exonucleolytic degradation [110]. Similarly, a recombination checkpoint that is primarily dependent of Tel1 is activated during meiosis in *S. cerevisiae sae2Δ* or *rad50s* mutants [109,111], which accumulate unprocessed meiosis-specific DSBs because Spo11 remains covalently attached to the DSB ends (Fig. 4) [29,32,34,58,61,62].

Unrepaired meiosis-specific DSBs with unusually long single-stranded tails are generated in *S. cerevisiae* cells lacking the strand exchange protein Dmc1. These cells are competent to remove Spo11 from DSB ends, but they accumulate large amounts of hyperesected DSBs due to their failure to engage in interhomolog repair [87]. Similarly to the DNA damage checkpoint, where 3′-ended ssDNA generation results in Mec1 recruitment and Mec1-dependent checkpoint activation [112], activation of the recombination checkpoint in *dmc1Δ* mutants is dependent on Mec1 and its regulators Rad24, Ddc1 and Rad17 (Fig. 4) [99,113]. It remains to be determined whether ssDNA-coated by RPA is the signalling event for Mec1 activation also in meiosis. Because RPA can directly compete with Rad51 and Dmc1 for binding to ssDNA, Rad51 and/or Dmc1 nucleoprotein filaments may also be a signal for Mec1.

Apparently, Tel1 has a very minor role in activating the recombination checkpoint in *dmc1Δ* cells [I. Guerini, H. Cartagena-Lirola and M.P. Longhese, unpublished]. However, because Tel1 appears to detect unprocessed DSBs in both mitosis and meiosis [109,110] and the meiotic-specific DSB ends are rapidly resected, the time window for Tel1 to sense and signal meiotic DSBs in *dmc1Δ* cells is transient and can be masked by the prevailing activity of Mec1. Alternatively, Tel1 can sense and signal meiotic DSBs less efficiently than Mec1. Consistent with the latter hypothesis, in mitotic cells Mec1 responds to a single unprogrammed DSB, whereas Tel1 signalling activity becomes apparent only when multiple DSBs are generated in the absence of Mec1 [110].

The meiotic recombination checkpoint has been extensively studied in *S. cerevisiae*, but it seems to operate also in *S. pombe*, worms, flies and mammals [114–120]. In contrast to budding yeast recombination mutants, which arrest permanently in prophase in a checkpoint-dependent manner [99,121], failure to repair meiotic DSBs in *S. pombe* does not cause meiosis I arrest. In fact, fission yeast cells defective in HR or homolog pairing undergo a modest delay in prophase [122,123] that is dependent on the DNA damage checkpoint components [122,124]. Furthermore, chemically induced DNA damage during meiotic S phase in *S. pombe* cells does not delay meiosis in a checkpoint-dependent manner [125], whereas it causes a checkpoint-dependent prophase arrest in *S. cerevisiae* cells [126]. This suggests that fission yeast meiosis is particularly tolerant to DNA damage (see below).

In mice, many mutations that affect DSB formation or repair result in arrest and/or programmed cell death during meiosis I prophase [127,128]. Examination of meiocyte development in mouse Spo11$^{-/-}$ and Dmc1$^{-/-}$ mutants has shown that oocytes and spermatocytes display distinct DNA damage-dependent and -independent responses [129,130]. In male mice, failure to initiate meiotic recombination (Spo11$^{-/-}$) or to repair Spo11-induced DSBs (Dmc1$^{-/-}$) cause spermatocyte apoptosis at the same point in meiotic prophase, equivalent to mid-pachynema in normal males [127,131]. Despite the similar timing of apoptosis, spermatocytes of these mutants appear to arrest at different stages of meiotic progression, such that Spo11$^{-/-}$ spermatocytes progress further than Dmc1$^{-/-}$ spermatocytes [129]. The apparently earlier arrest of Dmc1$^{-/-}$ spermatocytes is likely a response to unrepaired DSBs, since it is suppressed by eliminating DSB formation [129]. Although the timing of apoptosis in female mice is different from the male's one, also Spo11$^{-/-}$ oocytes progress further than Dmc1$^{-/-}$ oocytes [130], and the more severe oocyte loss in Dmc1$^{-/-}$ oocytes can be suppressed by eliminating DSB formation.

Thus, given that defects in DSB repair elicit DNA damage-dependent responses in mice, this suggests that a checkpoint mechanism monitoring DSB repair may exist also in mammalian meiotic cells. However, dissecting the roles of ATM and ATR in triggering meiosis arrest and/or cell death in mutants defective in DSB repair is hampered by the involvement of these proteins in meiotic progression and recombination. In fact, loss of ATM causes defects in meiotic prophase I progression [132–134]. Furthermore, Atm$^{-/-}$ spermatocytes and oocytes show similarities to Dmc1$^{-/-}$ meiocytes, and the severe meiocyte loss is a DSB-dependent response, since it can be suppressed by eliminating Spo11 [129,130]. Interestingly, Spo11 heterozygosity (which causes a reduction in the number of Spo11-induced DSBs) partially rescues Atm$^{-/-}$ meiotic defects in both males and females [135,136], whereas it does not suppress the meiotic phenotypes of Dmc1$^{-/-}$ meiocytes [Barchi M., Keeney S., Jasin M., personal communication]. This finding suggests that another protein kinase (e.g., ATR) can substitute for the lack of ATM in the presence of a reduced number of DSBs, whereas ATM becomes essential in meiotic DSB repair when the amount of DSBs exceeds a certain threshold. Indeed, one role of ATM in early meiotic prophase is to promote the phosphorylation of histone H2AX variant in response to Spo11-generated DSBs [129,136]. Because during a mitotic cell cycle H2AX phosphorylation may function to concentrate numerous DNA repair proteins in the vicinity of DNA lesions [137], ATM deficiency in meiosis may affect the functions of several proteins involved in the repair of meiotic DSBs.

5.2. Transduction of the checkpoint signals

Although Mec1/ATR and Tel1/ATM sense both unprogrammed and programmed DSBs, propagation of the checkpoint signals to the downstream targets occurs in two different ways, depending on whether the checkpoint response is elicited by unprogrammed or programmed DSBs. The Rad53 kinase and its mediator Rad9 are essential for transducing the DNA damage checkpoint signals in mitotic cells [138–140], where Rad9 first promotes Mec1–Rad53 interaction and Mec1-mediated Rad53 phosphorylation/activation [140], and then acts as a scaffold to facilitate in trans Rad53 autophosphorylation [139]. Despite their essential roles in activating the DNA damage checkpoint in response to mitotic DSBs, Rad9 and Rad53 are not involved in controlling meiosis I progression in response to meiotic programmed DSBs [87,99,126]. Activation of the meiotic recombination checkpoint requires instead the meiosis-specific proteins Mek1, Red1 and Hop1, which are major components of the chromosomal axes that assemble along homologs during meiotic prophase (Fig. 4) [141–143]. Mek1 is considered the meiotic paralog of Rad53, because they both are serine/threonine protein kinases and possess a phospho-specific FHA domain, which is required for their activation as kinases and mediates their interactions with Rad9 and Red1, respectively [142,144,145].

Activation of both Rad53 and Mek1 requires the formation of DSBs and the presence of Mec1 or Tel1 [111,138,146]. By contrast, Rad9 is not required for Mek1 activation, implying the existence of a meiosis-specific adaptor protein. Candidates for this role may be the meiosis-specific Red1 and Hop1 proteins, which are both required for Mek1 activation [142,147,148]. Hop1 is a DSB-dependent phosphoprotein with a putative oligomerization motif called HORMA domain, a central DNA binding zinc finger domain, and a C-terminal domain containing a predicted monopartite nuclear localization

signal [148]. Ectopic induced dimerization of Mek1 suppresses the effects of mutations in the Hop1 C-terminal domain, suggesting that Hop1 promotes Mek1 dimerization, which in turn enables its self-activation through in trans autophosphorylation [143,148]. Hop1 has been recently shown to contain a functional conserved motif that is a [S/T]Q cluster domain (SCD) comprising three adjacent sites (S298, S311, T318) that are targeted by the Mec1/Tel1 kinases [146]. Elimination of Mec1/Tel1-mediated phosphorylation within the Hop1 SCD (*hop1*^{SCD}) prevents chromosomal localization and phosphorylation of Mek1, consistent with Hop1 acting as an adaptor of Mek1 in the Mec1/Tel1 signalling pathway (Fig. 4) [146]. Still to be resolved in this scenario is the function of Red1, which interacts with Hop1 [149,150].

Although Hop1 orthologs have been identified in other organisms ranging from fission yeast to humans [151,152], the [S/T]Q sites important in budding yeast meiosis are only conserved among the fungal and plant orthologs [146]. Similarly, Mek1 is found only among fungal species, suggesting that the mechanism underlying transduction of the meiotic recombination checkpoint signal may vary among the organisms.

6. Mec1/Tel1-mediated regulation of meiotic recombination

In addition to monitoring the status of interhomolog repair, several lines of evidence indicate that Mec1 and Tel1, as well as their mammalian counterparts ATR and ATM, are involved in meiotic progression and recombination. Mec1 dysfunctions lead to a number of meiotic defects in budding yeast cells, including spore inviability, aberrant chromosome synapsis and reduced recombination frequency [153,154]. Mec1 and its regulators Rad17, Rad24 and Mec3 have been implicated in meiotic recombination partner choice. In fact, mutations in any of these genes cause a reduction in interhomolog recombination frequency, while increasing the frequency of both ectopic recombination and illegitimate repair from the sister chromatids [155–158].

Interhomolog bias during meiotic recombination requires also the axial element proteins Mek1, Hop1 and Red1 [121,141,148,156]. Conditional inhibition of Mek1 kinase activity impairs interhomolog recombination and allows DSB repair using sister chromatids in *dmc1Δ* cells, indicating that Mek1 activation as a kinase is important for this meiotic function [148]. Since Mec1 and Tel1 are required to activate Mek1, loss of interhomolog bias in *mec1*, *rad17*, *rad24* and *mec3* mutants may be due to their failure to activate Mek1.

In mammals, ATM deficient cells show aberrant synapsis and fragmented SCs (synaptonemal complexes), as well as high frequency of spontaneous chromosomal aberrations, intrachromosomal recombination and error-prone recombination during meiosis [132,133,159]. Moreover, ataxia telangiectasia (A-T) patients display gonadal atrophy and spermatogenetic failure, a phenotype that is mirrored by ATM-deficient mice [159,160]. Furthermore, mutations altering the *Drosophila* ATM ortholog Mei-41 reduce meiotic recombination frequency [161]. Finally, ATM has been recently shown to play a role in promoting the obligate crossover on the sex chromosomes and in controlling the number and distribution of crossovers on autosomes [135].

Budding yeast Mec1 and Tel1, like their mammalian counterparts ATR and ATM, are serine/threonine kinases [162,163]. They preferentially phosphorylate their substrates on serine or threonine residues that precede glutamine residue, the so-called [S/T]Q motifs [164]. The identification of *S. cerevisiae* Sae2 and Hop1 as targets of the Mec1 and Tel1 kinases has suggested that the latter can be involved at least in two early events of meiotic recombination: Spo11 removal from DSB ends by phosphorylating Sae2 [111] and interhomolog bias by phosphorylating Hop1 (Fig. 4) [146]. It is worth to point out that Sae2 is phosphorylated during an unperturbed S phase and in response to DNA damage during both mitosis and meiosis [111,165]. Mec1- and Tel1-mediated Sae2 phosphorylation during meiosis starts at the onset of premeiotic S phase, reaching the maximal peak at the time of meiotic DSB generation, and then decreasing when DSBs are repaired by homologous recombination [111]. Substitution with alanine of each serine and threonine residues (S73, T90, S249, T279, S289) located in Sae2 [S/T]Q motifs, which totally abrogates Sae2 mitotic functions [165], also leads to the accumulation of unprocessed Spo11-induced DSBs during meiosis, as does the simultaneous deletion of Mec1 and Tel1 [111]. This finding suggests that Tel1 and Mec1 might promote Spo11 removal from the DSB ends by phosphorylating Sae2.

Mec1/Tel1-dependent Sae2 phosphorylation may play a role in regulating Sae2 catalytic activities. In fact, the Sae2 variant carrying the substitution with alanines of the serines and threonines in the five Sae2 [S/T]Q motives, exhibits partial endonuclease activity on DNA duplexes containing ssDNA and fails to cleave hairpin structures cooperatively with MRX [65]. On the contrary, a Sae2 variant mimicking constitutive phosphorylation, because of the five phosphorylation sites changed to aspartates, shows wild type levels of hairpin endonuclease, flap endonuclease and DNA binding activity in vitro [65]. Checkpoint-mediated Sae2 phosphorylation is conserved throughout evolution. In fact, both Ctp1 and CtIP undergo ATM-dependent phosphorylation after DNA damage in mitotic cells [80,166], although the biological significance of their phosphorylation remains to be determined.

In addition to Sae2, Mec1 and Tel1 phosphorylate the meiosis-specific protein Hop1 [146]. The absence of Hop1 phosphorylation by Mec1/Tel1 does not affect formation or resection of meiotic DSBs. However, it leads to rapid meiotic DSB repair via a Dmc1-independent inter-sister repair pathway, resulting in diminished interhomolog crossing-over and spore viability [146]. These findings indicate that Mec1 and Tel1 promote meiotic interhomolog recombination by phosphorylating Hop1. Because Mec1/Tel1-mediated phosphorylation of Hop1 is required for Mek1 activation, which is in turn required for proper partner choice, Hop1 might prevent sister chromatids from acting as possible repair templates by activating Mek1.

7. Checkpoint kinase choice for signal transduction from meiosis-specific DSBs

As previously pointed out, the recombination checkpoint signal is propagated through the meiosis-specific proteins Mek1, Red1 and Hop1, whereas Rad53 and Rad9 mediate activation of the DNA damage checkpoint. How this specific use of Mek1–Red1–Hop1 instead of Rad53–Rad9 is achieved is currently unknown. Rad53 has been recently shown to undergo phosphorylation after generation of chemically induced DSBs during meiosis, indicating that Rad53 can be activated in response to DNA damage also during meiosis [126]. Nonetheless, neither Rad53 nor its activator Rad9 is phosphorylated and activated as soon as programmed meiosis-specific DSBs arise during meiosis I, even when their repair is prevented by the lack of Dmc1 or Sae2 [126]. Thus, Spo11-induced DSBs appear to avoid activation of the canonical Rad53-dependent DNA damage checkpoint machinery (Fig. 5).

Because Mec1 and Tel1 are responsible for the activation of both Mek1 and Rad53 in response to programmed or unprogrammed DSBs, respectively, the bias in using Mek1 instead of Rad53 is not likely to be exerted at the levels of DSB recognition. On the other hand, Mek1, Red1 and Hop1 are structural components of meiotic chromosomes [167]. Therefore, Mec1- and Tel1-dependent Rad53 activation in response to meiotic programmed DSBs might be prevented because Rad53 cannot access the meiotic recombination sites. Consistent with this possibility, targeting Rad53 to Mec1 by a

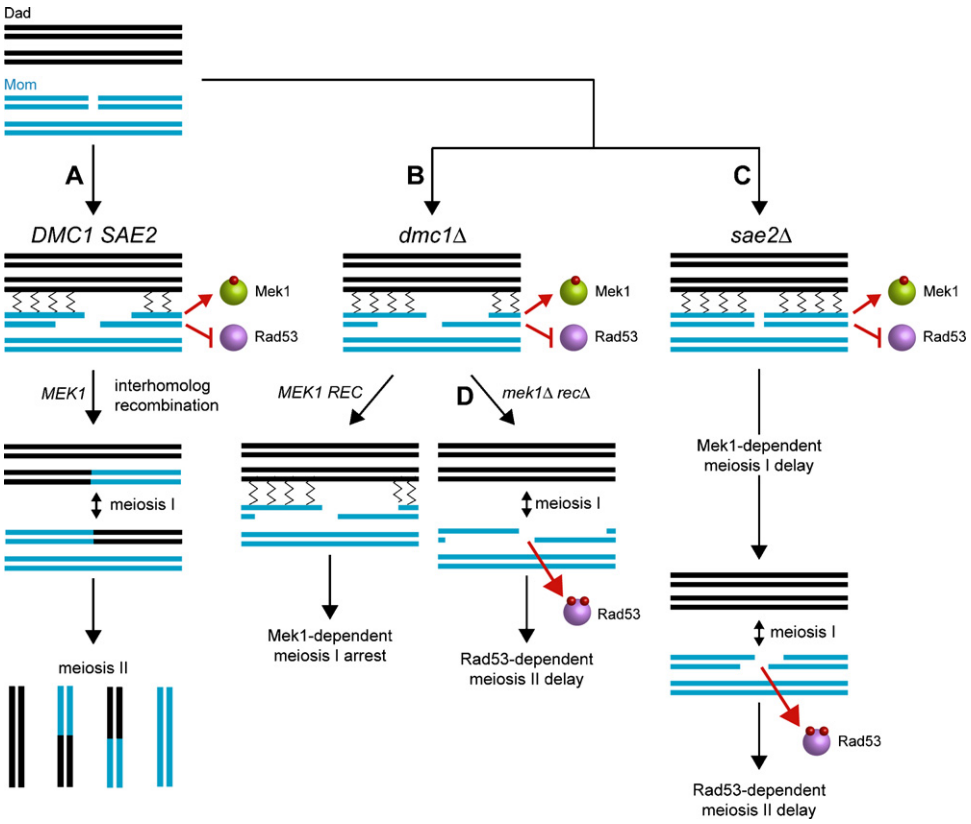

Fig. 5. Checkpoint activation by Spo11-induced DSBs. Homologs are indicated in black (paternal) and blue (maternal). Zig-zag lines represent the SC complex. (A) Formation of Spo11-induced DSBs in wild type cells triggers Mek1 phosphorylation/activation, which allows DSB repair via interhomolog recombination. (B) Accumulation of hyperesected meiotic DSBs in *dmc1Δ* cells leads to Mek1 phosphorylation/activation and Mek1-dependent meiosis I arrest. (C) Accumulation of unprocessed meiotic DSBs in *sae2Δ* cells leads to Mek1 phosphorylation/activation and a Mek1-dependent slowing down of meiosis I. Both hyperesected and unprocessed Spo11-induced DSBs fail to induce Rad53 phosphorylation/activation. When homologous chromosomes with unrepaired meiotic DSBs segregate from each other (C and D), these DSBs elicit a Rad53-dependent checkpoint that delays meiosis II.

Ddc2-Rad53 chimera allows Rad53 activation in response to meiotic programmed DSBs [126], suggesting that Rad53 and/or Rad9 are not reachable by Mec1 signalling from the meiotic recombination sites.

Why are self-inflicted DSBs in meiosis unable to trigger the canonical Rad53-dependent DNA damage checkpoint machinery? Whereas exogenous DSBs affect chromosomes at unpredictable locations, meiotic DSBs are highly integrated into large-scale chromosome structures and are part of a cellular program that serves the segregation of homologous chromosomes. Homologous chromosomes are joined via the SC at the meiosis I pachytene stage [168]. Along each side of the SC, each chromatid is organized into a linear array of chromatin loops, the bases of which define a geometric axis that is elaborated by various proteins into a structural "axis" (Fig. 6). Furthermore, sister chromatids are closely conjoined as parallel co-oriented linear loop-arrays, whose axes comprise a single morphological unit [169]. Because chromatin is organized along structural axes, exchange at the DNA/chromatin level must be accompanied by corresponding local exchange of chromosome axes [167]. This suggests that DSB-containing recombination complexes should be spatially associated with their chromosome axes. Although it is as yet unclear when and where this DSB/axis association is established, one possibility is that axes may hide Spo11-induced DSBs from being signalled as DNA damage to the Rad53 kinase, thus preventing activation of the Rad53-dependent checkpoint (Fig. 6). This hypothesis would explain why chemically induced DSBs, whose formation is not coordinated with the loop/axis configuration, can trigger Rad53 phosphorylation/activation during meiosis I (Fig. 6) [126].

This model predicts that disruption of chromosome axes without impairing Spo11-induced DSB formation should allow Spo11-induced DSBs to be monitored as DNA damage by the canonical Rad53-dependent checkpoint machinery. The observation that unrepaired meiosis-specific DSBs can elicit a Rad53-dependent checkpoint when homologous chromosomes segregate from each other and the SC is disassembled is consistent with this prediction (Fig. 5) [126]. In fact, Rad53 is phosphorylated in *dmc1Δ mek1Δ rad54Δ* cells, which fail to repair meiotic DSBs due to the absence of Rad54, but are allowed to segregate homologous chromosomes containing hyperesected DSBs due to the absence of Mek1. Moreover, Rad53 is phosphorylated in *sae2Δ* cells, which are known to perform anaphase I in the presence of unprocessed DSBs. This Rad53 phosphorylation and activation causes a delay of the second meiotic division (Fig. 5) [126], thus providing a salvage-mechanism preventing chromosome rearrangements and/or loss in the gametes even in the absence of the recombination checkpoint.

As previously mentioned, failure to repair DSBs in *S. pombe* meiosis does not result in checkpoint-mediated meiosis arrest [123,125], in contrast to *S. cerevisiae*, indicating that *S. pombe* cells evade checkpoint activation during meiosis. It is important to point out that one of the differences between budding and fission yeast meiosis is the absence of a classic SC complex in the latter [170]. Thus, if one of the SC functions is to hide meiosis-specific DSBs to be sensed as DNA damage, fission yeast cells may compensate for the lack of this function by elevating the DNA damage threshold for checkpoint activation relative to thresholds during the mitotic cell cycle.

The meiosis-specific large-scale structure should prevent neither sensing of meiotic programmed DSBs by Mec1, which

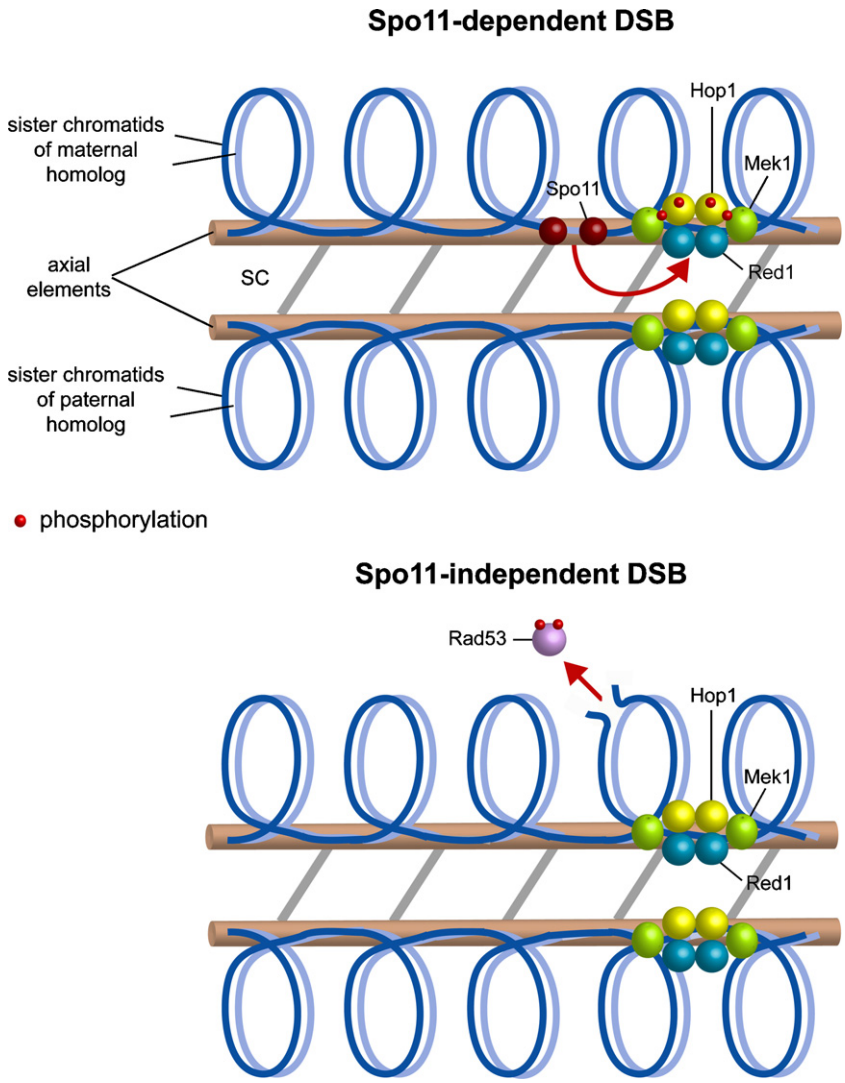

Fig. 6. Model for transduction of the DSB checkpoint signal in meiosis. A schematic model for loop/axis configuration is presented. Spo11-dependent DSBs may be spatially associated with their chromosome axes. This configuration may hide DSBs from being signalled as DNA damage to the Rad53 kinase. Mek1, Red1 and Hop1 can easily sense Spo11-induced DSBs because these proteins are structural components of chromosome axes. Spo11-independent DSBs can trigger Rad53 phosphorylation/activation during meiosis I, because their formation is not coordinated with the loop/axis configuration.

participates in meiotic progression and recombination [154], nor subsequent signalling to Mek1, Red1, Hop1, which are structural components of the meiotic chromosome axes (Fig. 6) [121,147,150].

Given that DSB-induced Mek1 activation is required to ensure the formation of interhomolog crossovers [148], the meiosis-specific propagation of the checkpoint signals through Mek1, Red1 and Hop1 instead of Rad53 is likely critical for the formation of genetically balanced gametes. In fact, if Spo11-induced DSBs would be sensed as DNA damage, cells would fail to activate Mek1, thereby impairing the correct repair partner choice and the formation of chiasmata, which are critical for proper meiotic chromosome segregation.

Conflict of interest

The authors declare that there are no conflicts of interest.

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