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Crossing and zipping: molecular duties of the ZMM proteins in meiosis

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Abstract

1 Accurate segregation of homologous chromosomes during meiosis depends on the ability
2 of meiotic cells to promote reciprocal exchanges between parental DNA strands, known
3 as crossovers (COs). For most organisms, including budding yeast and other
4 fungi, mammals, nematodes and plants, the major CO pathway depends on ZMM proteins,
5 a set of molecular actors specifically devoted to recognize and stabilize CO-specific DNA
6 intermediates that are formed during homologous recombination. The progressive
7 implementation of ZMM-dependent COs takes place within the context of the
8 synaptonemal complex (SC), a proteinaceous structure that polymerizes between
9 homologs and participates in close homolog juxtaposition during prophase I of meiosis.
10 While SC polymerization starts from ZMM-bound sites and ZMM proteins are required for
11 SC polymerization in budding yeast and the fungus *Sordaria*, other organisms differ in
12 their requirement for ZMM in SC elongation. This review provides an overview of ZMM
13 functions and discusses their collaborative tasks for CO formation and SC assembly, based
14 on recent findings and on a comparison of different model organisms.
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Introduction

1 During meiosis, chromosomes undergo a series of large-scale structural changes as well
2 as localized genetic exchanges by homologous recombination that contribute to the
3 accurate segregation of homologs at the first meiotic division. Chromosome
4 morphogenesis and the molecular processes of recombination are intimately linked at all
5 stages of the recombination process, although the underlying mechanisms of these links
6 are not fully understood.
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12 Recombination is initiated by programmed DNA double-strand break (DSB) formation by
13 the topoisomerase VI-like Spo11 protein together with a number of protein partners (Lam
14 and Keeney 2014; Robert et al. 2016). DSB formation takes place when sister chromatids
15 are condensed into an array of chromatin loops anchored at their bases to the
16 chromosome axis (Zickler and Kleckner 1999). The axis comprises several proteins,
17 including the cohesin complex containing at least one meiosis-specific kleisin subunit,
18 Rec8, and the proteins that will later form the lateral element of the synaptonemal
19 complex (SC) (e.g. Red1 and Hop1 in budding yeast and SYCP2 and SYCP3 in mammals).
20 An intriguing feature of meiotic recombination is that DSB formation occurs in chromatin
21 loop sequences located distal from the protein axis, whereas proteins required for DSB
22 formation are located on the chromosome axis (Blat et al. 2002; Pan et al. 2011; Panizza
23 et al. 2011). This has led to the proposal that DSB-prone sequences transiently interact
24 with the chromosome axis as a prerequisite for cleavage by Spo11 (Blat et al. 2002;
25 Miyoshi et al. 2012; Panizza et al. 2011). The chromosome axis is believed to play a role
26 in controlling DSB numbers and distribution through the activities of the Tel1^{ATM} kinase
27 (Cooper et al. 2016; Garcia et al. 2015; Joyce et al. 2011; Lange et al. 2016; Mohibullah and
28 Keeney 2017; Zhang et al. 2011). It also plays a role in the repair template choice, i.e.,
29 whether the sister chromatid or a chromatid of the homolog is the template for repair.
30 This role is exerted through in particular the local action of the axis-associated Mek1
31 kinase, which phosphorylates proteins involved in inhibiting inter-sister recombination
32 and favoring interhomolog repair of meiotic DSBs (Callender et al. 2016; Hollingsworth
33 and Gagliano 2019; Humphries and Hochwagen 2014).
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After Spo11 removal and DSB 5' end resection by Mre11 and Exo1 proteins (Cannavo and
Cejka 2014; Garcia et al. 2011; Mimitou et al. 2017; Neale et al. 2005; Zakharyevich et al.
2010), strand invasion takes place and a D-loop is formed, which is a common
intermediate to all types of meiotic DSB repair (Bishop and Zickler 2004) (Figure 1). D-

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loop formation is highly dynamic and reversible due to perpetual conflicts between activities that promote and inhibit DNA strand invasion (De Muyt et al. 2012). Non-crossovers (NCOs) can be formed by synthesis-dependent strand annealing (SDSA) when the D-loop is dismantled after DNA synthesis has extended the invading strand (Allers and Lichten 2001; Hunter and Kleckner 2001). The newly synthesized end then anneals to the 3' end on the other side of the DSB to repair the break without an exchange of chromosome arms (Figure 1). Alternatively, DNA synthesis extends the D-loop region, thereby providing a single stranded site for annealing of the DSB second end, a process referred to as second-end capture (Lao et al. 2008). Ligation of DNA ends leads to the formation of a double Holliday junction (dHJ) (Schwacha and Kleckner 1995) which may after cleavage give rise to either a NCO or a CO, depending on the cleavage orientation of each Holliday junction (Figure 1). The factors that control the fate of a D-loop intermediate to give a NCO or a CO have been well described and mostly result from two opposite activities that are well conserved during evolution: dismantling of the intermediate by a helicase (the Sgs1–Top3–Rmi1 (STR) complex in *S. cerevisiae*) or stabilization by a group of proteins referred to as “the ZMMs” (an acronym for Zip1-4, Msh4-5, Mer3, Spo16) (Börner et al. 2004; Jessop et al. 2006; Kaur et al. 2015; Oh et al. 2007; Tang et al. 2015). In budding yeast, most of D-loops stabilized by ZMMs are processed as COs. However, ZMM foci outnumber the COs in several other species, suggesting that the D-loops bound by ZMM proteins are not exclusively processed as COs and can still form NCOs (De Muyt et al. 2014; Edelmann et al. 1999; Higgins et al. 2008) (Figure 1). In addition, a minor fraction of the formed D-loops escapes these two pathways, and forms joint molecules that can be cleaved as COs or NCOs by the structure-specific nucleases (SSN), Mus81/Yen1/Slx1 (De Muyt et al. 2012; Zakharyevich et al. 2012) (Figure 1). ZMM-dependent COs rarely form in close vicinity of each other and are more evenly spaced than would be expected from a random distribution (Muller 1916), reviewed in (Berchowitz and Copenhaver 2010). These COs are referred to as interfering or “type I” crossovers. They represent the major fraction, ranging from 75 to 100 % of the totality of crossovers (Figure 1). By contrast, crossovers produced by the SSNs do not show interference, and are called “type II” crossovers.

The canonical budding yeast “zmm” phenotype

In the absence of ZMM genes in budding yeast, less SEI and dHJ intermediates are formed, meiotic progression is delayed, spore viability is decreased and fewer COs occur at least

1 when recombination is monitored at hotspots along yeast chromosome III (Börner et al.
2 2004; Jessop et al. 2006). These effects are more pronounced at high temperature (Börner
3 et al. 2004). The COs occurring in these mutants are non-interfering and depend on the
4 SSNs (Argueso et al. 2004; de los Santos et al. 2003; Novak et al. 2001; Shinohara et al.
5 2008; Sym and Roeder 1994). However, the original phenotype of *zmm* mutants has
6 evolved with the use of other approaches that led to the discovery of feedback
7 mechanisms that increase the frequency of DSBs and some effects varying with
8 chromosome size (see below).

9 According to genetic and molecular studies, the mechanism of action of ZMM begins after
10 DNA strand invasion (Figure 1) (Börner et al. 2004). Binding of ZMMs is proposed to
11 stabilize D-loops and convert them into a more stable intermediate called single-end
12 invasion (SEI) that can be detected on two-dimensional gels (Hunter and Kleckner 2001).
13 This intermediate is the precursor of COs. This stabilized intermediate is then processed
14 into a protected dHJ that will be exclusively resolved as a CO by the endonuclease activity
15 of the MutL (Mlh1-Mlh3) complex (Nishant et al. 2008; Zakharyevich et al. 2012).

16 It is difficult to estimate accurately CO frequencies in *zmm* mutants genetically, since their
17 spore viability is reduced, selecting for those events that resulted in viable spores. Using
18 genome-wide approaches in budding yeast hybrids, several studies found a reduction of
19 COs ranging from 34 to 60 % (one exception was for the *zip1Δ*, see below), and confirmed
20 that the COs were non-interfering (Chen et al. 2008; Mancera et al. 2008; Marsolier-
21 Kergoat et al. 2018; Oke et al. 2014; Sym and Roeder 1994). However, the reduction in
22 COs is not equal on all chromosomes and varies depending on chromosome size.
23 Accordingly, COs are more reduced on small chromosomes (such as chromosome III) than
24 on large chromosomes (such as chromosome VIII), where CO frequencies in certain *zmm*
25 mutants may exceed those observed in the wild type (Chen et al. 2015; Serrentino et al.
26 2013). One hypothesis is that failure to synapse results in continued Spo11 activity
27 disproportionately increasing DSBs along larger chromosomes (Keeney et al. 2014;
28 Subramanian et al. 2019; Thacker et al. 2014). Approximately half of these breaks can be
29 repaired as COs via the SSNs and they differ from ZMM COs in that they do not interfere.
30 Therefore, caution is needed when analyzing CO frequencies on only one chromosome
31 since the effects can vary according to chromosome size. By contrast, NCO numbers are
32 not decreased in *zmm* mutants, but actually increased, which may be due to a combination
33 of feedback mechanisms that increase DSB frequency in response to the defect in
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1 homologous chromosomes juxtaposition and an increase in the SDSA pathway promoted
2 by helicase (Sgs1 in budding yeast) (Börner et al. 2004; Chen et al. 2015; Keeney et al.
3 2014; Thacker et al. 2014).

4
5 In addition to their recombination function, all ZMM proteins are required to promote
6 synaptonemal complex formation in budding yeast, but not in plants and, for some cases,
7 in the mouse (Adelman and Petrini 2008; Chelysheva et al. 2007; de Vries et al. 1999;
8 Edelman et al. 1999; Guiraldelli et al. 2018; Higgins et al. 2004; Higgins et al. 2008;
9 Hunter 2015; Kneitz et al. 2000; Macaisne et al. 2008; Mercier et al. 2005; Mercier et al.
10 2015; Shen et al. 2012; Shinohara et al. 2008; Wang et al. 2009; Yang et al. 2008; Zhang et
11 al. 2014b; Zhang et al. 2019; Zhang et al. 2018b).

20 **The ZMM group of proteins: roles and known molecular functions**

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22 It becomes clear that ZMM proteins do not work independently on early steps of meiotic
23 recombination but form subcomplexes that recognize particular DNA structures and act
24 collectively, probably through transient protein-protein interactions, to shape DNA
25 branched molecules and form the appropriate precursor cleaved by the Mutly complex to
26 make an interfering CO. In addition, the stabilization of CO precursors by ZMMs is
27 connected to SC dynamics and, as suggested by fungal and mouse studies, ZMM proteins
28 help to bring chromosomes axes closer together and start SC polymerization.
29 Furthermore, direct protein-protein interactions are being uncovered between ZMM and
30 components of the SC.

31 ○ **The synaptonemal complex transverse filament Zip1**

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33 The budding yeast Zip1 protein is a coiled-coil protein with two terminal globular
34 domains, which forms the transverse filaments that span the central region of the SC. Zip1
35 is required for synapsis (Sym et al. 1993; Tung and Roeder 1998). No Zip1 protein can be
36 identified in other organisms by simple sequence homology analysis, underlining the high
37 divergence of SC proteins during evolution (Fraune et al. 2016; Gao and Colaiacovo 2018;
38 Page and Hawley 2004). However, transverse filament proteins in many organisms share
39 structural similarities with Zip1, including a long coiled-coil domain flanked by globular
40 domains, as well as the localization to the central region of the SC. These proteins include
41 SYCP1 in mammals, ZEP1 in rice, two closely related proteins ZYP1a and ZYP1b in *A.*
42 *thaliana*, SYP-1 and SYP-2 in *C. elegans*, C(3)G in *D. melanogaster* and Sme4/Zip1 in
43 *Sordaria* (Colaiácovo et al. 2003; de Vries et al. 2005; Espagne et al. 2011; Higgins et al.
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1 2005; Meuwissen et al. 1992; Page and Hawley 2001; Wang et al. 2010) (Table 1). In these
2 organisms as well, except in plants, mutation of the Zip1 functional homolog prevents
3 both synapsis and normal crossover formation (Colaiácovo et al. 2003; de Vries et al.
4 2005; Espagne et al. 2011; Higgins et al. 2005; MacQueen et al. 2002; Page and Hawley
5 2001), indicating that the “ZMM” function of Zip1 is mostly conserved. Within the
6 polymerized SC, Zip1 is in the form of head-to-head dimers that assemble in a ladder-like
7 structure with the N-termini in the center of the SC and the C-termini associated with the
8 axes of the homologous partners (Dong and Roeder 2000).

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14 It was initially thought that Zip1 would play a “late” role among the ZMM proteins because
15 all *ZMMs* are necessary for SC polymerization in budding yeast (Agarwal and Roeder
16 2000; Chua and Roeder 1998; Shinohara et al. 2008; Tsubouchi et al. 2006). Consistently,
17 *zip1Δ* seems less affected than the other *zmm* for formation of the SEI and dHJ
18 intermediates, although their formation is strongly delayed (Börner et al. 2004).
19 Moreover, ZMM proteins still localize on chromosomes in the absence of Zip1 relatives in
20 the mouse, rice and *Sordaria*, suggesting a later function of Zip1 and its relatives (de Vries
21 et al. 2005; Espagne et al. 2011; Wang et al. 2010). However, recent studies in budding
22 yeast suggest that Zip1 has an early role during CO biochemistry by triggering, right after
23 being recruited to the DSBs, the other ZMM assembly/activation. For instance, the
24 localization of Zip3, another ZMM, to DSB sites is dependent on *ZIP1* (Serrentino et al.
25 2013), and in several *zmm* mutants, Zip1 shows residual loading to chromosomes as seen
26 by immunostaining, but with a dotted pattern (Agarwal and Roeder 2000; Chen et al. 2015;
27 Chua and Roeder 1998; Shinohara et al. 2008; Storlazzi et al. 1996; Tsubouchi et al. 2006)
28 (Figure 2a).

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43 Zip1 is recruited to chromosomes via its C-terminal region requiring DSB formation
44 (Henderson and Keeney 2004; Tung and Roeder 1998). The Zip1 C-terminus is then
45 phosphorylated after DSB formation by the DDK (DBF4-dependent kinase) cell cycle
46 kinase and this phosphorylation is required for crossover recombination (Chen et al.
47 2015). After its recruitment presumably to DSB sites, Zip1 phosphorylation would create
48 a patch of negative charges that helps promote its interaction with other ZMM proteins
49 (Chen et al. 2015), including Zip2 and Zip3 to form the “synapsis initiation complex” (SIC)
50 (Fung et al. 2004). The resulting SIC then initiates SC polymerization to allow synapsis.
51 This function may be conserved, since the C-terminal region of Zip1 phosphorylated in
52 yeast is conserved in species that use recombination for synapsis like yeast and mice
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1 (Chen et al. 2015). Interestingly, recent analyses showed that although Zip1 is necessary
2 to promote *ZMM*-dependent crossovers, its polymerization into the SC is not. A *K. Lactis*
3 yeast version of *ZIP1* used to replace the *S. cerevisiae ZIP1* is not able to form SC, but
4 complements *zip1Δ* for crossover recombination, albeit with reduced interference
5 (Voelkel-Meiman et al. 2015). Furthermore, the region of *S. cerevisiae* Zip1 important for
6 SC and synapsis but not crossover resides in the 21 to 162 residues region (Voelkel-
7 Meiman et al. 2016). Thus, Zip1 seems to have separable functions: one early to assemble
8 a proper and functional set of *ZMM* proteins at recombination sites (Chen et al. 2015), and
9 the other one later to elongate the synaptonemal complex (Voelkel-Meiman et al. 2016).

16 ○ **The D-loop binding Mer3 helicase**

18 The *MER3* gene was discovered in budding yeast as a mutant defective in meiotic
19 recombination and encodes a DNA helicase with a 3' to 5' polarity (Nakagawa et al. 2001;
20 Nakagawa and Ogawa 1999). Mer3 has orthologs in other fungi, in plants and mammals,
21 and its inactivation leads to a similar CO and, with the exception of plants, synapsis defect
22 as in budding yeast, indicating that its function is mostly conserved (Guiraldelli et al.
23 2013; Mercier et al. 2005; Storlazzi et al. 2010; Wang et al. 2009) (Table 1). Furthermore,
24 *hfm1* (the mammalian name for *MER3*) mutations are found in human patients with
25 azoospermia or primary ovarian insufficiency syndromes, illustrating its essential role for
26 human fertility (Wang et al. 2014; Zhang et al. 2017).

27 The genetic dependencies for Mer3 association with meiotic chromosomes have not been
28 fully determined. However, although *mer3* mutants clearly have a “*zmm*” phenotype, there
29 are several indications that it associates to DSB sites early in the recombination process.
30 First, in *Sordaria*, Mer3 localizes as pairs of foci already at the leptotene stage, with one
31 focus on each homolog, suggesting that Mer3 binds the two DSB ends, or the two sides of
32 a recombination intermediate (Storlazzi et al. 2010). In addition, Mer3 focus numbers,
33 where examined, are much higher than the observed CO numbers (Chen et al. 2005; Rao
34 et al. 2017; Storlazzi et al. 2010; Wang et al. 2009). Finally, a role of *MER3*, independent of
35 its role in crossover formation, was recently described, which affects DNA synthesis
36 during CO and NCO formation (Duroc et al. 2017). In budding yeast, Mer3 specifically
37 interacts with and recruits a mismatch repair-related heterodimer, MutLβ (Mlh1-Mlh2 in
38 budding yeast, MLH1-PMS1 in mammals) to recombination hotspots, to limit the
39 extension of recombination intermediates by DNA synthesis, and thus the length of gene
40 conversion tracts (Duroc et al. 2017). Strikingly, this function is independent of Mer3's
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1 helicase activity and does not influence CO frequency (Duroc et al. 2017). In accordance
2 with this early role, Mer3 has a preference *in vitro* for binding model D-loop intermediates
3 that are formed early during recombination (Duroc et al. 2017). The Mer3/MutL β
4 interaction seems conserved in the mouse as shown by two-hybrid interaction between
5 HFM1 and MutL β (Duroc et al. 2017). The fact that MutL β is “linked” to the ZMM pathway
6 is illustrated by a study that examined the gains and losses of ZMM proteins during yeast
7 evolution (Vakirlis et al. 2016). Strikingly, Mlh2 was lost concomitantly with the ZMM
8 proteins in the *Lachancea* budding yeast clade, consistent with a functional connection
9 between them (Vakirlis et al. 2016). Intriguingly, MutL β is absent from plants genomes,
10 although plants have the Mer3 protein (Campbell et al. 2014). Therefore, it is not clear if
11 the function of MutL β is taken over by another actor in these organisms. The biological
12 meaning of limiting the extent of DNA synthesis at recombination sites is not known, but
13 it may have an evolutionary advantage in preserving ancestral allelic combinations from
14 extensive gene conversion (Duroc et al. 2017).
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16 Mer3 is able to unwind D-loops, Holliday junctions and substrates with a 3' overhang *in*
17 *vitro* (Duroc et al. 2017; Nakagawa et al. 2001; Nakagawa and Kolodner 2002a). It was
18 further shown that Mer3 is able to stimulate Rad51-mediated strand exchange activity
19 and D-loop extension in the direction opposite to DNA synthesis (Mazina et al. 2004). This
20 *in vitro* function might *in vivo* stabilize the nascent recombination intermediates into a
21 more stable intermediate, which would correspond to the SEI, specific to the ZMM
22 crossover pathway (Börner et al. 2004; Hunter and Kleckner 2001; Mazina et al. 2004)
23 (Figure 1). However, Mer3 helicase activity is only partly responsible for Mer3's ZMM
24 function, since a helicase-dead mutant is less affected for meiotic progression, spore
25 viability and crossover frequency than a null mutant (Duroc et al. 2017; Nakagawa and
26 Kolodner 2002b; Storlazzi et al. 2010). It is possible that Mer3's binding to D-loops is
27 sufficient to stabilize them and allow stronger subsequent or parallel stabilization by the
28 other ZMMs (see below). The helicase activity would only reinforce this stabilization.
29

30 In rice, MER3 localization on chromosomes is dependent of the ZMM proteins ZIP4 and
31 MSH4, suggesting that it needs other members of the ZMM group to be recruited or
32 stabilized (Shen et al. 2012; Zhang et al. 2014b). However, no physical interactions with
33 other ZMM proteins have been described (Figure 2b). Mer3 is therefore not in a stable
34 complex with other ZMMs, but may interact with them only transiently.
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36 ○ **The Zip2-Zip4-Spo16 “ZZS” complex**

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ZIP2, *ZIP4* and *SPO16* had been inferred to work collectively to promote the formation of interfering COs (Chua and Roeder 1998; Perry et al. 2005; Shinohara et al. 2008; Tsubouchi et al. 2006). Recently, these proteins have been shown to form a functional complex (ZZS, for *Zip2-Zip4-Spo16*) (De Muyt et al. 2018). Within this complex, Zip2 and Spo16 share homology with the XPF/ERCC1 family of structure-selective endonucleases, although Zip2 does not contain the canonical motif required for endonuclease activity of most XPF domain proteins (Arora and Corbett 2019; De Muyt et al. 2018; Macaisne et al. 2008). Consistently, the Zip2 XPF domain, in complex with Spo16, lacks endonuclease activity towards branched DNA molecules *in vitro* (De Muyt et al. 2018). However, the Zip2 XPF domain is required for ZZS pro-CO activity, and the Zip2 XPF-Spo16 subcomplex binds preferentially DNA branched structures (D-loops and Holliday junctions), suggesting that it acts as a recognition module to attract the whole ZZS complex at specific joint molecules that will be prone to be converted into CO (Arora and Corbett 2019; De Muyt et al. 2018).

Zip4 is a large TPR (tetratricopeptide repeat) protein (Perry et al. 2005; Tsubouchi et al. 2006). TPR motifs are known to mediate protein-protein interaction and the assembly of multiprotein complexes (D'Andrea and Regan 2003). Consistent with this inference, Zip4 is proposed to coordinate several meiotic processes by acting as a hub through physical interactions with components of the chromosome axis (Red1 and Hop1) and other ZMMs (Zip3 and Msh4-5) (De Muyt et al. 2018). In addition, Zip4 is important for Zip2 protein stability, suggesting that Zip4 might also work as a chaperone besides its function as a scaffold protein (De Muyt et al. 2018).

The Zip2 and Zip4 orthologs, mZIP2/SHOC1 and TEX11/AtZIP4, have been identified in mammals and plants, respectively, and are required for normal levels of interfering COs and fertility, suggesting the conservation of this function of the ZZS complex (Table 1) (Adelman and Petrini 2008; Chelysheva et al. 2007; Guiraldelli et al. 2018; Macaisne et al. 2008; Shen et al. 2012; Yang et al. 2008). Interestingly, mutations in the *TEX11* gene in humans is associated with non-obstructive azoospermia, suggesting the importance of the ZZS complex for CO formation and human fertility (Sha et al. 2018; Yang et al. 2015; Yatsenko et al. 2015). In *Arabidopsis*, the ERCC1-like protein PTD interacts with the XPF domain protein SHOC1 and is required for meiotic COs, suggesting that a Zip2-Spo16 equivalent might be present in plants (Macaisne et al. 2011; Wijeratne et al. 2006). A Spo16/PTD-relative has recently been identified in the mouse. mSpo16^{-/-} mice are sterile

1 and show a strong reduction of MLH1 foci and SYCP1 signal, suggesting a role of mSPO16
2 in CO and SC assembly and a conservation of the entire ZZS complex between yeast, plants
3 and mammals (Zhang et al. 2019).
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5 ○ **The Zip3 and HEI10 E3 ligases**
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7 DNA repair processes are coordinated by multiple post-translational modifications,
8 including SUMOylation (Psakhye and Jentsch 2012). This may also be the case during
9 meiotic recombination, since SUMOylation/ubiquitylation has been observed along
10 chromosome axes during prophase I of meiosis in yeast, fungi, worm and mouse (Bhalla
11 et al. 2008; De Muyt et al. 2014; Hooker and Roeder 2006; Rao et al. 2017). In addition,
12 the budding yeast E3 ligase Zip3, potentially involved in SUMOylation/ubiquitylation, is
13 one of the ZMM proteins (Agarwal and Roeder 2000). Zip3 is recruited right after DSB
14 processing by the DNA damage response complex 9-1-1 and further stabilized on
15 recombination intermediates after strand invasion (Serrentino et al. 2013; Shinohara et
16 al. 2015). *ZIP3* is required for the association of other ZMM proteins to chromosomes and
17 for SC elongation (Figure 2a) (Shinohara et al. 2008). Zip3, like the other E3 ligases,
18 possesses a C3HC4 zinc finger RING domain, which is required for Zip3 E3 SUMO ligase
19 activity *in vitro* (Cheng et al. 2006). This domain generally promotes protein interactions
20 to recruit E2 conjugating enzymes to transfer ubiquitin/SUMO to its ultimate target and
21 provides substrates specificity (Kerscher et al. 2006). In budding yeast, this domain is
22 required for Zip3 binding to chromosomes, meiotic progression and SC polymerization,
23 indicating that post-translational modifications carried out by Zip3 E3 ligase are
24 important for meiotic recombination/chromosome structure processes (Cheng et al.
25 2006; Serrentino et al. 2013).
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27 Zip3 orthologs have been discovered in other organisms and it emerges that two
28 subgroups exist within the Zip3 family: the Zip3/RNF212 group and the HEI10 group
29 (Chelysheva et al. 2012; De Muyt et al. 2014) (Table 1). Plants, fungi, *C. elegans* and
30 *Drosophila* possess a single RNF212 or HEI10 ortholog (Bhalla et al. 2008; Chelysheva et
31 al. 2012; De Muyt et al. 2014; Jantsch et al. 2004; Lake et al. 2015; Wang et al. 2012),
32 whereas vertebrates encode separate RNF212 and HEI10 proteins that function as
33 antagonistic SUMO and ubiquitin E3 ligases, respectively (Gray and Cohen 2016; Qiao et
34 al. 2014; Rao et al. 2017; Reynolds et al. 2013; Ward et al. 2007). Moreover, the presence
35 of several Zip3-related proteins within the same organism, for instance the mammalian
36 RNF212 ortholog (RNF212B), provides an additional layer of complexity in the
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1 recombination control by E3-ligase activities (Kong et al. 2008; Reynolds et al. 2013).
2 Similarly, the *C. elegans* genome, which lacks an obvious HEI10 ortholog, encodes at least
3 four RING finger E3 ligase proteins, ZHP-1, ZHP-2, ZHP-3 and ZHP-4 that act together to
4 promote a spatio-temporal accumulation of pro-CO factors (Nguyen et al. 2018; Zhang et
5 al. 2018b).
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9 The function of *ZIP3*-like genes can differ between species. For instance, the Zip3-related
10 Vilya, beyond its role in crossover formation, participates in DSB fate determination in
11 *Drosophila* female by interacting directly with Mei-P22, a DSB protein partner of Spo11,
12 and is required for DSB formation (Lake et al. 2015). The role of *ZIP3*-like genes can vary
13 between genders. In mouse, RNF212 is involved in a female-specific mechanism of pre-
14 follicle quality control that guides oocytes with unrepaired DNA damage towards
15 apoptotic pathways (Qiao et al. 2018).
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18 The biochemical distinction between Ubiquitin and SUMO E3 ligases is difficult.
19 Therefore, it remains unclear what the exact enzymatic activity of the Zip3 family is.
20 Zip3/RNF212 group members appear to act solely as SUMO E3 ligases, from biochemical
21 and genetic studies performed in budding yeast and *C. elegans* (Bhalla et al. 2008; Cheng
22 et al. 2006). By contrast, some members of the HEI10 group appear to possess ubiquitin
23 E3 ligase activity (Toby et al. 2003), even if studies suggest that mouse HEI10 may also
24 function as a SUMO E3 ligase (Strong and Schimenti 2010).
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27 The function of Zip3-mediated SUMO/ubiquitin post-translational modification is also
28 obscure. One possible mechanism might be to locally SUMOylate proteins, which would
29 act like a glue to consolidate multiple interactions between partners and help to promote
30 CO at DSB repair sites (De Muyt et al. 2018; De Muyt et al. 2014). Such a role for Zip3 and
31 its relatives would be consistent with the presence of recombination nodules at
32 recombination sites, visible by electron microscopy as highly dense chromosome
33 structures (Zickler and Kleckner 1999), which might reflect a high concentration of DNA
34 repair/pro-CO proteins as was described for the progressive accumulation of HEI10
35 proteins observed at the future CO sites (Chelysheva et al. 2012; De Muyt et al. 2014; Qiao
36 et al. 2014). In addition, it was suggested that Zip1 associates with SUMO conjugates (e.g.
37 Red1) in order to form the SC (Cheng et al. 2006).
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40 Direct SUMO/ubiquitin targets of the Zip3 family have not been identified. Yeast Zip3
41 interacts with Zip1, ZZS members (Zip2 and Zip4) and the ZMM Msh4 (Figure 2b)
42 (Agarwal and Roeder 2000; De Muyt et al. 2018), suggesting that CO formation may occur
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1 via post-translational modification of the ZMM proteins. Consistently, it has been
2 suggested that mouse RNF212 and HEI10 promote crossovers by stabilizing meiosis-
3 specific recombination factors, such as the ZMM MSH4-MSH5 (MutSy) heterodimer, on
4 recombining meiotic chromosomes via a SUMO-ubiquitin relay, which would promote
5 subsequent recruitment of the proteins involved in crossover formation, including MLH1-
6 MLH3 (MutLy) (Gray and Cohen 2016; Qiao et al. 2014; Rao et al. 2017; Reynolds et al.
7 2013; Ward et al. 2007). Similarly, rice HEI10 can interact with MSH5, suggesting that a
8 similar mechanism occurs in plants. These observations suggest that the relationship
9 between SUMOylation and ubiquitylation of proteins at recombination sites progressively
10 determines which DSBs become competent for CO (Qiao et al. 2014).

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18 ○ **The MutSy Msh4-Msh5 heterodimer**

19 Msh4 and Msh5, two other ZMMs, share homology with DNA mismatch recognition factor
20 MutS of the bacterial mismatch repair (MMR) and form the MutSy heterodimer
21 (Hollingsworth et al. 1995; Pochart et al. 1997; Ross-Macdonald and Roeder 1994). Msh4
22 and Msh5 are conserved among fungi, plants and mammals, and are also present in the
23 worm *C. elegans* (Table 1). Msh4 and Msh5 exhibit structural homology with four of the
24 five domains of MutS homolog proteins, including the ATP-binding motif and the
25 heterodimerization domain, but lack the first domain critical for DNA mismatch binding
26 and have no role in MMR (Manhart and Alani 2016). In mice, MutSy is essential for
27 chromosomal synapsis, crossover formation and thus fertility, the *null* mutant mouse
28 being sterile (de Vries et al. 1999; Kneitz et al. 2000). In *Arabidopsis* and rice, null mutants
29 of MutSy homologs also have decreased fertility and less crossovers, and chromosome
30 synapsis is slightly affected (Higgins et al. 2004; Higgins et al. 2008; Zhang et al. 2014b).
31 In *C. elegans*, the ortholog of Msh4, HIM-14, and MSH-5 play a critical role in CO formation
32 (Kelly et al. 2000; Zalevsky et al. 1999). In *Sordaria*, Msh4 plays a role in chromosome
33 recognition and pairing, thus before its supposed role in the recombination process
34 (Storlazzi et al. 2010). The human recombinant heterodimer specifically binds D-loops
35 and Holliday junctions *in vitro*. After DNA binding, MSH4-MSH5 is converted into a sliding
36 clamp in an ATP-dependent manner, which diffuses away from the junction while
37 embracing two homologous DNA-duplex arms (Snowden et al. 2004). A recent study in *C.*
38 *elegans* showed that two MSH-5 doublets are present at CO sites (Woglar and Villeneuve
39 2018). Although this result needs biochemical support, it suggests that two populations
40 of MutSy complexes encircle and accumulate on each DNA duplex located in the interval
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1 between the two Holliday junctions (Figure 3). The binding and stabilization of dHJs by
2 MutSy are assumed to protect them *in vivo* from dissolution by anti-recombinases such as
3 Sgs1/BLM (Jessop et al. 2006; Oh et al. 2007). In addition to this “ZMM” function, it has
4 been proposed, by homology to the MMR, that MutSy recruits and activates the CO-biased
5 JM resolving heterodimer MutLy in order to favor the resolution of the stabilized dHJs in
6 a CO (Gray and Cohen 2016). In support of this hypothesis, MSH4-MSH5 interacts with
7 MutLy in mice testes (Santucci-Darmanin et al. 2002).

8
9 Strikingly, as seen by cytology in *Sordaria*, *C. elegans*, plants and the mouse, the initial
10 number of MutSy foci is greater than the final number of crossovers and most foci are lost
11 during prophase while the remaining MutSy foci correspond to future CO sites (De Muyt
12 et al. 2014; de Vries et al. 1999; Edelmann et al. 1999; Higgins et al. 2008; Kneitz et al.
13 2000; Yokoo et al. 2012; Zhang et al. 2014b). The removal of Msh4-Msh5 depends on the
14 ZMM ZHP-1/2/RNF212/HEI10 E3 ligases protein family (Qiao et al. 2014; Reynolds et al.
15 2013; Zhang et al. 2018a). In mice, it has been suggested that the balance of RNF212-
16 mediated SUMOylation and HEI10-mediated ubiquitylation determines the stability of
17 MutSy complex by protecting it from proteolysis at CO-specific DNA intermediates (Qiao
18 et al. 2014). Interestingly, a recent study in budding yeast indicates that another type of
19 post-translational modification is important for MutSy complex stability (He et al. 2018).
20 This study showed that MutSy is intrinsically unstable, unless its N-terminal degra-
21 sequence is phosphorylated by DDK. The phosphorylation-defective *msh4-6A* mutant
22 retains the chromosome binding function and is only mildly affected for SEI and dHJ
23 formation and synapsis. However, CO numbers in *msh4-6A* strain are reduced like in the
24 *msh4Δ* mutant while NCO numbers are not. Therefore, phosphorylation of Msh4 could
25 ensure the protection of dHJs against Sgs1-Top3-Rmi1 activity and favor their biased
26 resolution towards COs versus NCOs. In a proposed model, sliding clamps of MutSy would
27 need to accumulate above a threshold in order to facilitate COs, which is only possible
28 when Msh4 is stabilized by phosphorylation (He et al. 2018). When not stabilized by
29 phosphorylation, MutSy is lost from recombination sites, due to the abundance of SC-
30 associated proteasomes (Ahuja et al. 2017; Rao et al. 2017). Msh4 phosphorylation by
31 DDK observed in budding yeast may be conserved among other organisms, and
32 consequently the regulation pathway of crossover formation, since the DDK consensus
33 S/T enriched region is conserved in all Msh4 homologs (He et al. 2018).
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New additions to the ZMM group

1 So far, the ZMM group comprises eight proteins (or protein family for the E3 ligases Zip3
2 and HEI10 relatives) that direct the fate of recombination intermediates towards COs.
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4 However, other proteins with pro-CO activity have been recently identified that could also
5
6 be considered as members of the ZMM group. Besides its role in centromere pairing, the
7
8 budding yeast phosphatase PP4 (Pph3/Psy2) is important for joint molecule stability
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10 (SEIs and dHJs) and for CO formation, possibly by antagonizing the checkpoint kinase
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12 Mec1^{ATR}-dependent phosphorylation of the N-terminal region of Zip1 (Falk et al. 2010).
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14 Also in budding yeast, the alpha 3 subunit of the proteasome's core particle, Pre9, has been
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16 identified through a screen for transposon insertion mutations that conferred a
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18 temperature-dependent defect in spore formation, like *zmm* mutants (Ahuja et al. 2017).
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20 *pre9Δ* possesses features of a *zmm* mutant, as defined by the reduction of CO level due to
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22 the inability to stabilize SEIs and by the reduction of SC formation (Ahuja et al. 2017). This
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24 function of the proteasome seems evolutionary conserved, since components of the
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26 proteasome are observed on meiotic chromosomes in budding yeast, but also in mouse
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28 and *C. elegans* (Ahuja et al. 2017; Rao et al. 2017). It has been proposed that the
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30 proteasome is recruited to the chromosome axis and regulates the turnover of
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32 recombination factors, including the ZMMs HFM1/Mer3 and TEX11/Zip4, through HEI10-
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34 mediated ubiquitylation (Rao et al. 2017).

35 Interestingly, the HEI10 protein might not work alone during ubiquitylation processes. In
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37 plants, the newly identified HEI10-interacting protein, HEIP1, is necessary for the
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39 formation of interfering COs and colocalizes with the site of COs represented by late
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41 HEI10 foci (Li et al. 2018). Besides its interaction with HEI10, HEIP1 interacts with both
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43 ZIP4 and MSH5, suggesting that this new putative ZMM member may regulate CO
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45 formation by bridging multiple pro-CO factors in order to modify them by ubiquitin
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47 activities and progressively shape meiotic recombination intermediates towards the pro-
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49 CO state (Li et al. 2018). Similarly, the *Tetrahymena* protein Sa15 interacts with Zhp3, the
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51 Zip3 ortholog, and is required for wild-type CO levels and localizes to meiotic
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53 chromosomes (Shodhan et al. 2017). However, this protein has no conserved domain and
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55 does not seem to be evolutionarily conserved.

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57 **ZMM proteins influence SC formation, and the SC *per se* is not strictly required for**
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59 **ZMM-dependent CO formation, but influences their distribution**
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1 During early meiotic prophase, meiotic recombination and SC assembly are temporally
2 and spatially closely associated. In several organisms, including budding yeast, plants,
3 *Sordaria* and mice, normal SC assembly requires initiation of meiotic recombination and
4 Rad51/Dmc1-mediated stable strand exchange activity (Baudat et al. 2000; Cahoon and
5 Hawley 2016; De Muyt et al. 2009; Espagne et al. 2011; Henderson and Keeney 2004;
6 Mercier et al. 2015; Pittman et al. 1998; Romanienko and Camerini-Otero 2000; Vignard
7 et al. 2007; Yoshida et al. 1998). ZMM proteins also participate in SC elongation. The
8 presence of ZMM proteins on chromosome axes might reflect their early loading at the
9 time when DSBs are processed towards CO-competent DNA intermediates, as it is
10 observed by the early recruitment of Zip3 and Mer3 to chromosome axes in budding yeast
11 and *Sordaria*, respectively, or by the physical interaction between Zip4/TEX11 and axis
12 components, Red1 and SYCP2, in yeast and mouse, respectively (De Muyt et al. 2018;
13 Serrentino et al. 2013; Storlazzi et al. 2010; Yang et al. 2008).

14 In budding yeast and *Sordaria zmm* mutants, axial elements are formed normally but the
15 central region fails to polymerize normally at the leptotene-zygotene transition (Börner
16 et al. 2004; Espagne et al. 2011; Storlazzi et al. 2010). Interestingly, *zmm* mutants can be
17 distinguished according to their ability to polymerize the SC. The absence of *ZIP2*, *ZIP4* or
18 *SPO16* abolishes SC assembly in budding yeast, while in the lack of MutS γ (*MSH4-MSH5*)
19 or *ZIP3*, SC is incomplete but not absent (Agarwal and Roeder 2000; Börner et al. 2004;
20 Chua and Roeder 1998; Humphryes et al. 2013; Novak et al. 2001; Tsubouchi et al. 2006).
21 Functions of ZMM proteins for normal SC assembly have also been observed in other
22 organisms. In the mouse, the chromosome axes form normally in mutants lacking
23 *Hfm1/Mer3*, *Msh4* or *Msh5*, but the SC central element SYCP1 fails to assemble correctly
24 (de Vries et al. 1999; Edelmann et al. 1999; Guiraldelli et al. 2013; Kneitz et al. 2000).
25 Curiously, *Tex11^{-/-}* and hypomorph *Shoc1/Mzip2^{-/-}* mutant mice lacking intact *ZIP4* and
26 *ZIP2*-relatives, respectively, show a much less severe phenotype as shown by normal
27 synapsis and nearly wild type levels of MLH1 foci (which mark CO sites) (Adelman and
28 Petrini 2008; Guiraldelli et al. 2018; Yang et al. 2008). However, a recent report found a
29 strong decrease in MLH1 foci and synapsis defects in a *Mzip2*-null mouse, suggesting that
30 the role of SHOC1/MZIP2, and possibly TEX11, for CO formation and SC assembly has
31 been underestimated (Zhang et al. 2018b). By contrast, the Zip3-relatives, RNF212 and
32 HEI10, are not important for synapsis despite being required for CO formation (De Muyt
33 et al. 2014; Reynolds et al. 2013; Ward et al. 2007). In plants, SC polymerization relies on

1 the production of interhomolog recombination intermediates but not on the ZMM
2 proteins (Mercier et al. 2015). However, in rice, synapsis is affected in a mutant combining
3 both *zip4* and *mer3* mutations, suggesting that these ZMM genes might have redundant
4 roles for SC loading in plants (Shen et al. 2012).
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7 Early studies in several organisms suggested correlations between CO and sites of
8 synapsis initiation (Maguire 1972; Nur 1968; Stack and Soulliere 1984). More recent
9 analyses in budding yeast support this hypothesis by showing that the number of
10 interstitial SC initiation sites corresponds well to CO numbers (Agarwal and Roeder 2000;
11 Henderson and Keeney 2004; Tsubouchi et al. 2006; Zickler 2006). However, in other
12 organisms such as the mouse, plants and *Sordaria*, there are more ZMM foci than CO
13 numbers (De Muyt et al. 2014; Edelman et al. 1999; Higgins et al. 2008). In *Sordaria*, SC
14 nucleation seems to occur both at CO sites and at a subset of other recombination sites
15 that are matured to NCOs, despite initial loading by ZMM proteins (Zhang et al. 2014a).
16 Thus, recombination intermediates may be reversed to NCO products after ZMM loading,
17 possibly at a later step than initial D-loop formation (as shown in Figure 1), that may
18 involve a dissolution activity of recombination intermediates by helicase complex such as
19 STR. In both cases, SC initiation sites often colocalize with ZMM proteins and decrease in
20 number in mutants with decreased CO numbers, suggesting that SC formation starts at
21 sites where ZMMs nucleate, also called SICs (for “Synapsis Initiation Complex”)
22 (Tsubouchi et al. 2006). The specific pattern of ZMM/SC installation is dependent on DSB
23 numbers. In hypomorphic *spo11* mutants of budding yeast, there are only a few Zip3 foci,
24 from where only partial SC elongation occurs (Henderson and Keeney 2004). Similarly, in
25 mouse or *Sordaria*, few SC initiation sites and incomplete polymerization are observed
26 when DSBs are reduced, suggesting that a minimum number of nucleation of ZMM/SC
27 formation sites is required for full homolog synapsis (Kauppi et al. 2013; Tessé et al. 2017;
28 Tessé et al. 2003). These nucleation sites are suggested to be sites for a crosstalk between
29 ZMM proteins, chromosome axis juxtaposition and SC polymerization (Zhang et al.
30 2014b). Consistent with this inference, localization studies in *Sordaria* suggest that ZMM
31 components (at least Zip2, Zip4, Mer3 and Msh4) would help to bring “miniatures axes”,
32 known as bridges, from the parental chromosome axes into the inter-axis region (Dubois
33 et al. 2019) (see below) (Figure 3). SC installation would then nucleate at these sites of
34 parental bridges when bridges reach a length of precisely 100 nm, which corresponds to
35 the space between homologs during synapsis.
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1 Although *per se*, the SC is not required for interfering COs, several pieces of evidence
2 suggest that it plays a regulatory role in the distribution of interfering CO. In budding
3 yeast, the absence of the SC central element proteins, Ecm11 and Gmc2, or of the N-
4 terminal domain (21-163) of Zip1 does not have apparent meiotic defects, as suggested
5 by wild-type spore viability (Humphryes et al. 2013). However, these mutants show
6 increased CO frequency on certain chromosomes, although COs are still dependent on
7 *MSH4*, suggesting that the SC plays an inhibitory role that limits ZMM-dependent CO
8 formation (Voelkel-Meiman et al. 2016). Likewise, decrease of the transverse filament
9 protein in rice and *C. elegans* results in an increase of CO markers and an attenuation of
10 CO interference indicating that negative regulation of interfering CO by the SC is
11 conserved (Hayashi et al. 2010; Libuda et al. 2013; Wang et al. 2010). The mechanism
12 behind this control remains unclear. It is possible that the SC central element somehow
13 promotes CO interference. An alternative explanation could be that higher CO frequency
14 might be partly due to the formation of *de novo* DSBs when homologous chromosomes fail
15 to correctly synapse (Kauppi et al. 2013; Thacker et al. 2014).
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29 **Integrated functions of ZMM proteins in promoting and coordinating crossover** 30 **formation and synapsis**

31 Distinct protein-protein interactions and biochemical activities identified among ZMM
32 proteins suggest that they work at different steps of CO maturation, namely D-loop
33 stabilization (step a, Figure 3), SEI formation (step b, Figure 3), second-end capture and
34 dHJ stabilization (step c, Figure 3). We therefore propose the following integrated model
35 for their action. In budding yeast, an epistatic study made between *zip1-4A* mutant and
36 the other *ZMMs* suggested that Zip1 is one of the earliest pro-CO factors to act during
37 meiotic recombination, possibly by recruiting other *ZMMs* through the acidic patch
38 resulting from DDK-dependent phosphorylation of the Zip1 C-terminus (Chen et al. 2015).
39 The ZZS complex and Mer3 might be recruited to DSB sites in a Zip1-dependent manner.
40 Indeed, genetic, biochemical and cytological studies suggest that these proteins are
41 recruited early to DSBs sites, presumably during D-loop formation (De Muyt et al. 2018;
42 Duroc et al. 2017; Storlazzi et al. 2010), to perform two distinct biochemical activities: the
43 ZZS complex directly binds branched DNA molecules, through its XPF-ERCC1-like domain
44 (De Muyt et al. 2018), while the helicase Mer3 binds D-loops, and promotes heteroduplex
45 extension in a 3' to 5' polarity (in the opposite direction to DNA synthesis), resulting in D-
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1 loop stabilization (Mazina et al. 2004; Nakagawa and Kolodner 2002a) (Figure 3a). The
2 ZZS and Mer3 activities may therefore synergize to stabilize joint molecules and
3 antagonize the anti-recombination effect of STR. In budding yeast, the E3 ligase Zip3
4 interacts with the ZZS complex and is important for the recruitment of most of the ZMM
5 proteins (Figure 2b), suggesting that it also acts early on D-loops by stabilizing newly
6 recruited ZMM proteins presumably via SUMO/Ubiquitin post-translational
7 modifications (Figure 3a).
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12 Meanwhile, the ZZS complex, in particular Zip4, interacts with the SC axial element
13 component, Red1 (Figure 2b). One attractive possibility is that the physical association
14 between the ZZS complex and Red1 could bring closer together the parental
15 chromosomes axes during the homology search and the binding of recombination
16 intermediates by ZMMs. In this model, the mechanical imprinting provided by the transit
17 of the axis-associated ZZS complex from the parental chromosome axes into the inter-axis
18 region would lead to the formation of chromosomes axis bridges that progressively align
19 and tether parental chromosomes (Figure 3(i)). This hypothesis would fit well with the
20 observation that the ZZS complex co-purifies with the axial component Red1, although it
21 does not strongly associate with the DNA attached to the axis (De Muyt et al. 2018).
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31 The Msh4-Msh5 heterodimer interacts directly with DNA and exhibits biochemical
32 activities that could promote or stabilize stable strand invasion by forming a sliding clamp
33 on recombination intermediates (Snowden et al. 2004). Although the prediction of the
34 cavity size formed by the Msh4-Msh5 heterodimer suggests that it is large enough to
35 embrace a whole unfolded Holliday junction (Rakshambikai et al. 2013), recent high-
36 resolution cytological studies in *C. elegans* are compatible with the MutS γ complex
37 binding only one heteroduplex DNA at a time (Woglar and Villeneuve 2018) (Figure 3b-
38 c). Msh4-Msh5 and ZZS complexes bind independently from each other to chromosomes
39 (De Muyt et al. 2018; Shinohara et al. 2008) (Figure 2a). However, the physical interaction
40 observed between Zip4 and Msh5 suggests that these complexes, probably along with
41 Mer3, act synergistically on DNA intermediates to promote stable SEI intermediate
42 formation (Figure 3b-c). During this process, SEI will be progressively stabilized by the
43 MutS γ heterodimer and the phosphorylation of Msh4 protein (He et al. 2018). The
44 physical link observed between Zip3 and the MutS γ complex suggests that Zip3
45 consolidates the mechanism of CO maturation by further stabilizing this complex
46 (Agarwal and Roeder 2000) (Figure 2b).
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1 Msh5, like Zip4, physically interacts with the axis protein Red1 in budding yeast (De Muyt
2 et al. 2018) and Msh4 primarily localizes on chromosome axes before relocating to central
3 region of the SC in *Sordaria* (Storlazzi et al. 2010), suggesting that Msh4-Msh5
4 heterodimer could act in concert with the ZZS complex to bring chromosome axes at a
5 100 nm distance, which allows initiation of the central region of the SC polymerization.
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7 Based on cytological and molecular results, Tsubouchi and colleagues proposed a
8 mechanism of polymerization of the SC from SIC sites, in which the central element
9 Ecm11-Gmc2 complex is recruited to SIC sites and facilitates, along with the SIC, the initial
10 polymerization of Zip1 (Humphryes et al. 2013) (Figure 3(i)). This Zip1 assembly along
11 the chromosomes is then consolidated by the SUMOylation of Ecm11 protein,
12 independently of the Zip3 SUMO ligase activity (but dependent of both E3 ligases Siz1 and
13 Siz2) (Leung et al. 2015).
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16 During the SEI-to-dHJ transition, the displaced 3' strand of an SEI undergoes annealing to
17 the second DSB end (Lao et al. 2008) (Figure 3c). Interestingly, in the *zip3Δ* mutant gene
18 conversion tracts associated with COs are slightly longer, which may result from a defect
19 in the second-end capture (Oke et al. 2014). In addition, the interaction observed between
20 Zip4 and Zip3 suggests that the ZZS complex could cooperate with Zip3 in this process,
21 possibly by interacting and stabilizing DNA branched molecules that are formed during
22 this post-invasion step of meiotic DSBs repair (De Muyt et al. 2018)(Figure 3c). After dHJ
23 formation, the SC is fully polymerized along the parental chromosomes, forming the
24 characteristic tripartite structure visible by electron microscopy (Figure 3(ii)). Finally, in
25 budding yeast, the exit from pachytene stage is controlled by Ndt80, a transcription factor
26 that activates transcription of the *CDC5* polo-like kinase, which triggers both resolution of
27 dHJs as interfering COs by the Mutly complex (Mlh1-Mlh3) and SC breakdown (Prugar et
28 al. 2017; Sourirajan and Lichten 2008), leading to homologous chromosomes that are only
29 linked by COs and sister chromatid cohesion (Figure 3d).
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49 **Perspectives**

50 By means of many studies performed in multiple organisms, our understanding of the role
51 of ZMM proteins during homologous recombination has greatly improved. Still, several
52 important points about ZMM functions remain to be clarified:
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(i) The biochemical activities of ZMM proteins are still poorly understood, in particular their roles, synergistic or not, to shape early recombination intermediates toward CO fate or their antagonistic interplay with the Sgs1-Top3-Rmi1 anti-recombination activities.

(ii) The identification of new pro-CO proteins, such as HEI10 or HEIP1, indicates that the number of actors required for formation of interfering COs is still increasing and calls for further exploration of not yet-identified pro-CO genes.

(iii) Even though there is significant evidence that ZMM proteins play a predominant role in SC formation in budding yeast, *Sordaria* and, to a lesser extent, in the mouse, the molecular mechanism that coordinates CO and SC formation remains to be elucidated. Moreover, both the assembly and disassembly of the SC is likely achieved through multiple layers of regulation (Gao and Colaiacovo 2018). Therefore, it will be important to determine what governs the coordination between SC dynamics and the homologous recombination process.

(iv) Interference between COs implies the existence of a signal along the chromosome that disfavors the appearance of a second CO if a CO is already formed nearby. The absence of ZMM results in non-interfering COs, whereas the absence of SC central element proteins influences the distribution of interfering CO. It is not clear whether the coupling between SC assembly and CO formation plays a role in CO interference and how this connection could be integrated with the implementation CO interference by DNA topology (Zhang et al. 2014c).

In conclusion, further investigation of the ZMM-dependent CO formation and its relationship with the SC dynamics in different model organisms will be needed to uncover both their conserved as well as distinct features and reveal how it could impact human fertility. The identification of causal genetic variants by whole genome/exome sequencing and their association to diseases or disorders is a rapidly growing field. The combination with molecular studies of recombination proteins should help to identify the regions or nucleotides of genes that are critical for fertility and might improve diagnosis of sterile patients. Reciprocally, the emergence of new genome editing technologies will open new areas to test candidate variants and improve our understanding of the molecular function of recombination proteins, including ZMMs.

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1 **Table and figure Legends**

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4 **Table 1: Homologs of ZMM proteins identified in model organisms and**
5 **corresponding references.**
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10 **Figure 1: Model of meiotic recombination with corresponding changes in**
11 **chromosome structure**
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14 Left: Representation of the three main pathways of meiotic recombination. Parental
15 double-stranded DNA molecules are depicted in blue and red. Dashed lines indicate newly
16 synthesized DNA. Meiotic double-strand breaks (DSBs) formed by Spo11 can be repaired
17 as two types of recombination products: crossovers (COs) and non-crossovers (NCOs),
18 which correspond to DSB repair products with and without exchange of flanking
19 sequences, respectively. COs produced by the ZMM pathway are formed via stabilized D-
20 Loop (the single-end invasion (SEI)) and double-Holliday-junction (dHJ) intermediates,
21 and exhibit interference. Nevertheless, some CO-specific recombination intermediates
22 may be redirected towards NCO products following ZMM loading (dashed arrow).
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26 By contrast to ZMM-dependent COs, the non-interfering COs are randomly distributed
27 along the chromosomes. Non-interfering COs are produced simultaneously with NCOs by
28 the resolution by structure-specific nucleases of DNA intermediates that have escaped
29 from helicases or ZMM activities. The estimated percentage of interfering COs over the
30 total number of COs in different organisms is shown: *S.c.*: *Saccharomyces cerevisiae*; *M.m.*:
31 *Mus musculus*; *A.t.*: *Arabidopsis thaliana*; *C.e.*: *Caenorhabditis elegans* (Serrentino and
32 Borde 2012). The disassembly of DNA intermediates by helicase(s) leads to NCO products.
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34 Right: Illustrations of the corresponding changes in chromosome structure are shown for
35 a single pair of homologous chromosomes (red and blue lines).
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49 **Figure 2: Relationships between ZMM proteins in *S. cerevisiae***
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51 (a) Table indicating the interdependency between ZMM proteins for localization on
52 chromosomes. Green cells indicate the presence of ZMM proteins while red cells indicate
53 abolished or altered binding of ZMM proteins to chromosomes. Not tested dependency is
54 indicated by a grey cell. Different types of Zip1 staining (dots or lines) are specified. PC:
55 polycomplex. References are also indicated: (1) (Chua and Roeder 1998); (2) (Tsubouchi
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1 et al. 2006); (3) (Shinohara et al. 2008); (4) (Nishant et al. 2010) (5) (Serrentino et al.
2 2013); (6) (De Muyt et al. 2018). Underlined and non-underlined references refer to
3 chromatin immunoprecipitation (ChIP) and immunolocalization data, respectively.
4

5 (b) Physical interactions observed among ZMM proteins. Interactions between ZMM
6 members, the MutL β complex and the synaptonemal complex are also showed. Red and
7 black arrows correspond to interactions identified by yeast two-hybrid and co-
8 immunoprecipitation, respectively. References for yeast two-hybrid: (Agarwal and
9 Roeder 2000; Cheng et al. 2006; De Muyt et al. 2018; Duroc et al. 2017; Hollingsworth and
10 Ponte 1997; Humphryes et al. 2013; Pochart et al. 1997). References for co-
11 immunoprecipitation: (Agarwal and Roeder 2000; Cheng et al. 2006; De Muyt et al. 2018;
12 Duroc et al. 2017; Humphryes et al. 2013; Shinohara et al. 2008; Woltering et al. 2000).
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22 **Figure 3: Proposed model for ZMM-dependent formation of interfering CO and** 23 **polymerization of the synaptonemal complex.**

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26 Left: progressive implementation of interfering CO by the ZMM proteins. Budding yeast
27 proteins are shown. Details are in the text. For simplicity, phosphorylated Zip1 protein is
28 not shown on recombination intermediate of steps a-c. Recombination steps b and c
29 consider the binding of Msh4-5 complexes on duplex DNA but does not exclude the
30 possibility that they entrap the entire dHJ.
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35 Right: ZMM-mediated recruitment of SC central components (orange rods).

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37 Chromosome bridges are formed during the transit of ZMM proteins from the
38 chromosome axis to the inter-axis space. SC nucleation then occurs which brings axes
39 closer together, allowing SC polymerization initiation. See also the text for more details.
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41 The electron microscopy image in (i) represents axes association via interhomolog
42 bridges (Albini and Jones 1987). The SC polymerizes and chromosomes are synapsed at a
43 precise distance of 100 nm. The electron microscopy image in (ii) represents a pair of
44 synapsed chromosomes with a recombination site also known as a recombination nodule
45 (RN) (From (del Cacho et al. 2005)). See also (Zickler and Kleckner 2015) for more details
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<i>S.cerevisiae</i>	Molecular function	<i>S. macrospora</i>	<i>M.musculus</i>	<i>A. thaliana</i>	<i>C.elegans</i>	<i>D.melanogaster</i>	References
Zip1	Coiled-coil protein Polymerizes to form the central element of the SC Regulated by phosphorylation of the C-terminal part Recruited prior to other ZMMs	Sme4	SYCP1	ZYP1a and ZYP1b	SYP-1 and SYP-2	c3G	Meuwissen et al. 1992 Sym et al. 1993 Page and Hawley 2001 MacQueen et al. 2002 Colaiácovo et al. 2003 Higgins et al. 2005 Espagne et al. 2011
Mer3	DNA Helicase Recruits MutLβ (Mlh1-Mlh2) to limit D-loop extension during DNA synthesis	Mer3	HFM1	AtMER3/ Rock-N-Rollers	-	-	Nakagawa et al. 1999 Chen et al. 2005 Mercier et al. 2005 Storlazzi et al. 2010 Guiraldelli et al. 2013
Zip2	XPF protein forming an XPF-ERCC1-like complex with Spo16 which binds to branched DNA structures <i>in vitro</i> Part of the Zip2-Zip4-Spo16 (ZZS) complex	Zip2	SHOC1/MZIP2	SHOC1	-	-	Chua and Roeder 1998 Macaisne et al. 2008 De Muyt et al. 2018 Guiraldelli et al. 2018 Dubois et al 2019
Spo16	ERCC1-like protein forming an XPF-ERCC1-like complex with Zip2 which binds to branched DNA structures <i>in vitro</i> Part of the ZZS complex	-	SPO16	PTD	-	-	Wijeratne et al. 2006 Shinohara et al. 2008 Macaisne et al. 2011 De Muyt et al. 2018 Zhang et al 2019
Zip4	TPR motif protein Part of the ZZS complex Scaffold protein that has multiple protein-protein interactions with ZMM and axis proteins	Zip4	TEX11	Zip4	-	-	Tsubouchi et al. 2006 Chelysheva et al. 2007 Adelman et al. 2008 Yang et al. 2008 De Muyt et al. 2014
Zip3	RING finger protein Probably involved in SUMO and/or ubiquitylation (precise targets are unknown)	Hei10	HEI10 and RNF212	HEI10	ZHP-3	Vilya	Agarwal and Roeder 2000 Jantsch et al. 2004 Ward et al. 2007 Chelysheva et al. 2012 Reynolds et al. 2013 Qiao et al. 2014 Lake et al. 2015 De Muyt et al. 2014
Msh4	Forms a complex with Msh5 (MutSy complex) in order to bind recombination intermediates such as D-loops and HJs Stabilized at future CO sites by phosphorylation of its N-terminal region	Msh4	MSH4	AtMSH4	HIM-14/MSH-4	-	Ross-MacDonald and Roeder 1994 Zalevsky et al. 1999 Kneitz et al. 2000 Higgins et al. 2004 Storlazzi et al. 2010
Msh5	Forms a complex with Msh5 (MutSy complex) that binds <i>in vitro</i> to recombination intermediates such as D-loops and HJs	-	MSH5	AtMSH5	MSH-5	-	Hollingsworth et al. 1995 Edelmann et al. 1999 De Vries et al. 1999 Kelly et al. 2000 Higgins et al. 2008

Table 1

Figure 1

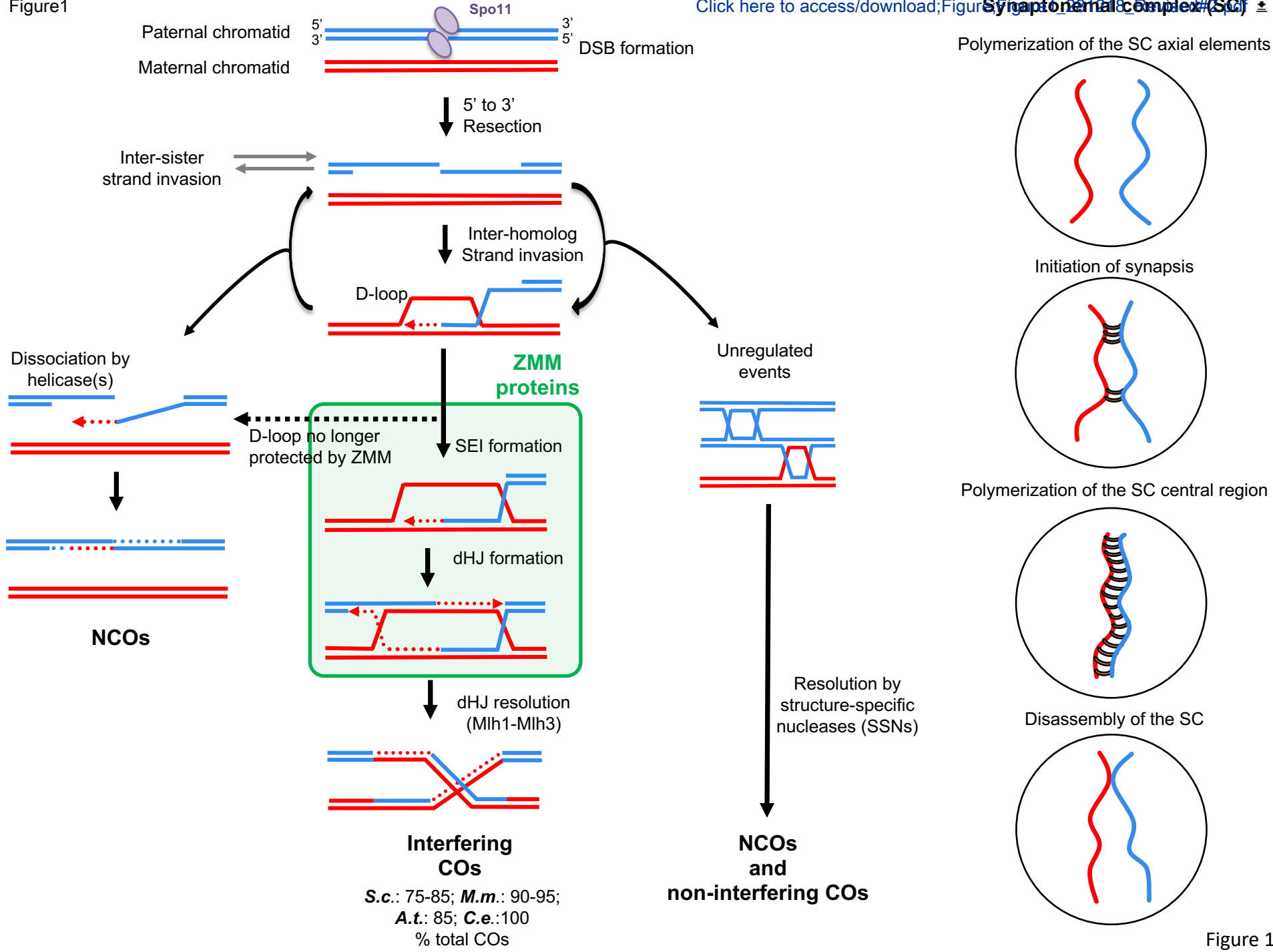


Figure 1

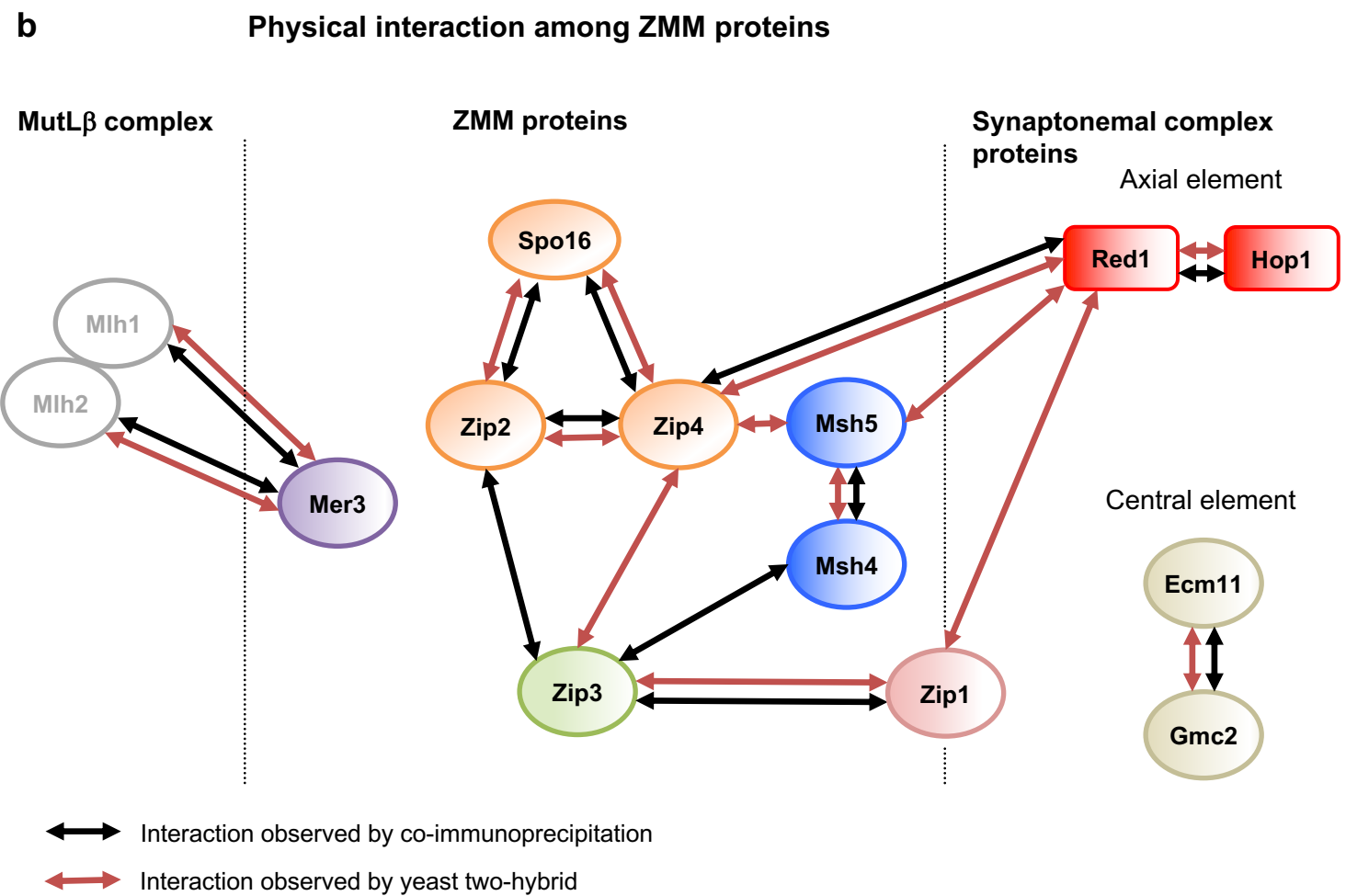
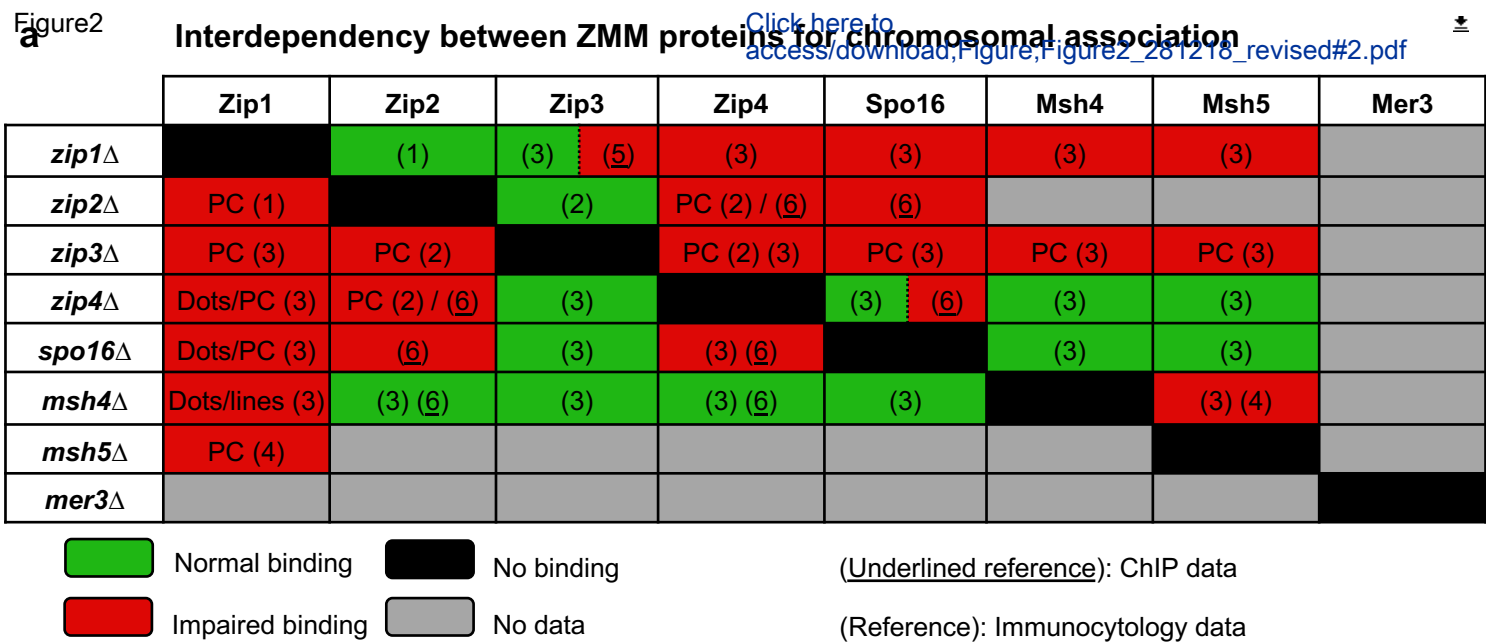


Figure 2

