Published in final edited form as:
Mol Cell. 2012 April 13; 46(1): 43–53. doi:10.1016/j.molcel.2012.02.020.

BLM helicase ortholog Sgs1 is a central regulator of meiotic recombination intermediate metabolism

Arnaud De Muyt[1], **Lea Jessop**[2], **Elizabeth Kolar**[3], **Anuradha Sourirajan**[4], **Jianhong Chen**, **Yaron Dayani**, and **Michael Lichten**[*]
Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA

Summary

The BLM helicase has been shown to maintain genome stability by preventing accumulation of aberrant recombination intermediates. We show here that the *Saccharomyces cerevisiae* BLM ortholog, Sgs1, plays an integral role in normal meiotic recombination, beyond its documented activity limiting aberrant recombination intermediates. In wild type meiosis, temporally and mechanistically distinct pathways produce crossover and noncrossover recombinants. Crossovers form late in meiosis I prophase, by polo kinase-triggered resolution of Holliday junction (HJ) intermediates. Noncrossovers form earlier, via processes that do not involve stable HJ intermediates. In contrast, *sgs1* mutants abolish early noncrossover formation. Instead, both noncrossovers and crossovers form by late HJ intermediate resolution, using an alternate pathway requiring the overlapping activities of Mus81-Mms4, Yen1, and Slx1–Slx4, nucleases with minor roles in wild-type meiosis. We conclude that Sgs1 is a primary regulator of recombination pathway choice during meiosis, and suggest a similar function in the mitotic cell cycle.

Introduction

Homologous recombination is critical to the successful division of the diploid genome among haploid gametes during meiosis. The crossover (CO) products of recombination, visible at the chromosome level as chiasmata, provide stable connections between homologous chromosomes of different parental origin (homologs), and these connections are required for accurate homolog segregation at meiosis I, the first division of meiosis (Bascom-Slack et al., 1997)). In many organisms, early inter-homolog (IH) recombination intermediates create reversible contacts important for homolog association, alignment and pairing during meiosis I prophase (Bhalla and Dernburg, 2008). These early events occur in excess over COs, and many are resolved without exchange of flanking sequences as noncrossover (NCO) recombinants. Since excessive COs adversely affect homolog segregation (Koehler et al., 1996), it is of considerable interest to understand the mechanisms that distinguish CO and NCO recombination during meiosis.

[*]Correspondence: lichten@helix.nih.gov.
[1]Current address: Institut de Génétique et Microbiologie, UMR 8621, Université Paris-Sud, 91405 Orsay, France
[2]Current address: Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892, USA
[3]Current address: Graduate Program in Biochemistry, Cellular and Molecular Biology, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA
[4]Current address: Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh, India

Meiotic recombination is initiated by programmed double strand breaks (DSBs) produced by the Spo11 transesterase (Keeney, 2007). DSB ends are resected to produce 3' overhangs, which initiate strand invasion of a homologous donor (Figure 1A). These initial strand invasion intermediates can be further processed in different ways, with different recombination product outcomes. For example, if a single DSB end, after priming DNA synthesis, is displaced and anneals with the other DSB end, a NCO is produced in a process called synthesis-dependent strand-annealing (SDSA, Figure S1A; Paques and Haber, 1999). Alternatively, stabilization of strand invasion intermediates, followed by capture of the second DSB end, can create a double Holliday junction joint molecule (dHJ-JM) intermediate (Schwacha and Kleckner, 1995; Szostak et al., 1983), which can be resolved by multiple mechanisms (Youds and Boulton, 2011). Coupled helicase and topoisomerase activities can disassemble dHJ-JMs, in a process called dissolution, to produce only NCOs (Figure S1B). dHJ-JMs can also be resolved by endonuclease cleavage of the two HJs to produce either a CO or a NCO, depending upon the relative orientation of the two cleavage events (Figure S1C).

In budding yeast, where meiotic recombination has been best characterized at the molecular level, NCOs and COs have been shown to form by distinct mechanisms (Bishop and Zickler, 2004). NCOs and JMs appear at the same time in meiosis, but COs appear only later, when JMs resolve, indicating that only COs are formed by the resolution of stable JMs (Allers and Lichten, 2001). In addition, several classes of mutants confer meiotic CO and JM defects without reducing NCOs. Mutants lacking the yeast polo kinase, Cdc5, or Ndt80, a transcription factor that drives meiotic Cdc5 expression (Chu and Herskowitz, 1998), show normal NCO formation but markedly reduced JM resolution and CO production, and ectopic Cdc5 expression in *ndt80* mutants restores JM resolution and COs without additional NCO formation (Allers and Lichten, 2001; Clyne et al., 2003; Sourirajan and Lichten, 2008; Xu et al., 1995). A second class of mutants, lacking members of the ZMM (Zip1/2/3 Msh4/5 Mer3 Spo16/22) protein family, show diminished JMs and COs but normal NCO formation (Börner et al., 2004; Lynn et al., 2007). ZMM proteins are components of the synaptonemal complex (SC), a tripartite protein structure that pairs and aligns homologs at the pachytene stage of meiosis (Page and Hawley, 2003), and many ZMM proteins form foci at sites of IH recombination (Lynn et al., 2007). Mutants lacking MutL homologs Mlh1 or Mlh3, or lacking exonuclease I (Exo1), also show reduced COs, but available data suggest that NCOs and JMs are not similarly affected (Argueso et al., 2004; Khazanehdari and Borts, 2000; Wang et al., 1999; Zakharyevich et al., 2010; Zakharyevich et al., 2012). Taken together, these observations suggest that most meiotic NCOs are not derived from stable JMs, but instead are formed by SDSA or dissolution. COs, on the other hand, are produced by polo kinase-triggered, biased resolution of JMs that are stabilized by the ZMM proteins (Figure 1A, Figure S1C). In addition, a minor fraction of COs are produced by ZMM-independent processes (alt-CO in Figure 1A), as inferred from findings that COs are still present at reduced levels in *zmm* mutants (Argueso et al., 2004; de los Santos et al., 2003). However, the identity of factors and activities responsible for directing meiotic recombination events amongst these three pathways remains elusive.

One enzyme complex with the potential to regulate meiotic recombination pathway choice is the RecQ family helicase BLM (Sgs1 in budding yeast) and its partners topoisomerase III (Top3) and Rmi1, called BLAP75 in mammals (Bernstein et al., 2010). The BLM/Sgs1 complex has two *in vitro* activities that might promote NCOs at the expense of COs *in vivo*. First, BLM disassembles D-loops structures, analogous to early strand invasion intermediates, and thus could promote NCO formation by SDSA (Adams et al., 2003; Bachrati et al., 2006; McVey et al., 2004b; van Brabant et al., 2000). Second, the BLM/Sgs1 complex can drive dHJ dissolution *in vitro*, producing NCOs (Cejka et al., 2010; Wu and Hickson, 2003). Consistent with these *in vitro* activities, *sgs1* mutants show increased

mitotic JMs and COs (Bzymek et al., 2010; Ira et al., 2003), and mutants lacking BLM homologs show increased mitotic recombination in a variety of multicellular organisms (Bernstein et al., 2010). Thus, the BLM/Sgs1 complex appears to play an important role in regulating recombination during the mitotic cell cycle.

Evidence for a similar role in meiosis is limited, as *sgs1* mutants produce COs and NCOs at near wild type levels (Jessop et al., 2006; Oh et al., 2007; Rockmill et al., 2003). JMs do accumulate at modestly elevated levels in *sgs1* mutants, with particular increases in JMs involving sister chromatids and in JMs containing three or four chromosomes (multichromatid JMs, MC-JMs), but these JMs are resolved efficiently and with normal timing (Jessop et al., 2006; Oh et al., 2007). *sgs1* mutation also partially suppresses the meiotic JM and CO defects of *zmm* mutants and the CO defects of *mlh3* mutants (Jessop et al., 2006; Oh et al., 2007; Rockmill et al., 2003). These findings have been taken to indicate that Sgs1 acts as a recombination chaperone during meiosis, by disassembling aberrant recombination intermediates that form outside the context of the SC.

Similar uncertainty exists regarding the nucleases that participate in meiotic JM resolution. Biochemical studies have identified three structure-selective nucleases with potential JM-resolving activity (Schwartz and Heyer, 2011): XPF ortholog Mus81, which partners with Mms4 (Eme1 in some organisms); XPG ortholog Yen1(Gen1 in other organisms); and GIY-domain nuclease Slx1 and its partner Slx4 (BTBD12, HIM-18 and MUS312 in other organisms). Evidence for these nucleases being meiotic JM resolvases is incomplete and varied. *Drosophila* mutants lacking Slx4 homolog MUS312 show marked CO defects (Yildiz et al., 2002), but only minor defects are seen in analogous nematode and mouse mutants (Holloway et al., 2011; Saito et al., 2009). Fission yeast *mus81* mutants show marked CO defects (Smith et al., 2003), but only minor meiotic CO defects are seen in budding yeast, *Arabidopsis*, *Drosophila* and mouse *mus81* or *mms4* mutants, primarily in ZMM-independent COs (Argueso et al., 2004; Berchowitz et al., 2007; de los Santos et al., 2003; Higgins et al., 2008; Holloway et al., 2008; Jessop and Lichten, 2008; Oh et al., 2008; Trowbridge et al., 2007). Budding yeast *mus81* and *mms4* mutants display limited meiotic chromosome segregation defects, but most JMs resolve, indicating that Mus81-Mms4 is required for timely resolution of a minor fraction of JMs (de los Santos et al., 2003; Jessop and Lichten, 2008; Matos et al., 2011; Oh et al., 2008). A recent study reported more severe segregation defects in *mus81 yen1* double mutants, suggesting a greater JM resolution defect, but the extent of the resolution defect was not quantified (Matos et al., 2011). This study also showed that Mms4, Yen1, and Slx1 undergo programmed modification at the end of meiosis I prophase, with Mms4 and Slx1 being phosphorylated, and Yen1 being dephosphorylated, and that Cdc5 is the kinase that phosphorylates Mms4, thereby stimulating Mus81-Mms4 nuclease activity.

These findings identify Mus81-Mms4 as a Cdc5-stimulated nuclease that acts redundantly with other nucleases to resolve meiotic JMs, but also suggest that it acts mainly in secondary CO-forming processes. This suggestion is supported by the finding that Mus81-Mms4 is both necessary and sufficient to resolve many of the aberrant meiotic JMs that form in the absence of Sgs1 (Jessop and Lichten, 2008; Oh et al., 2008), indicating that Sgs1 and Mus81-Mms4 collaborate to prevent the accumulation of aberrant recombination intermediates that form outside of the primary pathways for meiotic recombination. However, because meiotic CO and NCO levels were not markedly altered in *sgs1* single mutants, and because NCOs form at normal levels in *sgs1 mus81* double mutants, it was not anticipated that Sgs1 would determine the outcome of the majority of meiotic recombination events.

We present here data indicating that, to the contrary, Sgs1 is a central regulator of most of the recombination events that occur during budding yeast meiosis. We show that, during normal meiosis, Sgs1 is responsible for directing meiotic recombination towards the alternate formation of either early NCOs or JMs, the latter being subsequently resolved as COs in a Mus81-Mms4, Yen1, and Slx1–Slx4 independent manner. In contrast, in *sgs1* mutants, early NCO formation is abolished, and most meiotic recombination events form JMs that are later resolved as both COs and NCOs, by mechanisms that require Mus81-Mms4, Yen1, or Slx1–Slx4. Remarkably, Cdc5 promotes JM resolution under all circumstances, regardless of whether JMs are formed in the presence or absence of Sgs1, and regardless of whether resolution primarily produces CO, or both COs and NCOs.

Results

Sgs1 is required for early NCO formation during meiosis

NCOs form before COs during wild-type meiosis, consistent with NCO- and CO-forming processes diverging early in meiosis I prophase (Figure 1A; Allers and Lichten, 2001; Börner et al., 2004; Hunter and Kleckner, 2001). Using a recombination reporter (Jessop et al., 2005; Figure 1B), we asked if NCO and CO formation showed similar differential timing in *sgs1* mutants. To avoid *sgs1* mitotic growth defects, we used an *sgs1 meiotic null* (*sgs1-mn*) mutant (Jessop et al., 2006; Oh et al., 2008), in which *SGS1* is transcribed from a *CLB2* promoter that is active during the mitotic cell cycle and inactive during meiosis. As expected, NCOs preceded COs by about 45 min in wild-type cells. However, NCO formation was delayed in *sgs1-mn*, and COs and NCOs appeared at the same time (Figure 1C). Thus, Sgs1 is responsible for early NCO formation during wild-type meiosis. In addition, cotemporaneous formation of NCOs and COs in *sgs1-mn* suggests that, in the absence of Sgs1, NCOs and COs might be produced by resolution of a common JM precursor.

Sgs1-independent JMs resolve to form both COs and NCOs

In wild type, JM resolution is triggered by the Cdc5 polo kinase, and the vast majority of JMs resolve as COs (Clyne et al., 2003; Sourirajan and Lichten, 2008). To test the hypothesis that JMs formed in *sgs1-mn* cells resolve as both NCOs and COs, we asked if resolution of these JMs requires Cdc5, and if so, what type of recombinants are produced. We used meiotic *CDC5* expression-defective *ndt80*Δ mutants and a β-estradiol (ED)-inducible *CDC5* allele (*CDC5-IN*; Sourirajan and Lichten, 2008) to allow controlled Cdc5 expression late in meiosis I prophase. In cells where Sgs1 was active (*SGS1 ndt80*Δ), NCOs formed normally, JMs accumulated, and COs were greatly reduced (Allers and Lichten, 2001; Figure 2A, C, −ED); these JMs resolve as COs without additional NCO formation when *CDC5* is expressed (Sourirajan and Lichten, 2008; Figure 2A, C, +ED). In contrast, in *sgs1-mn ndt80*Δ cells, when Cdc5 was not expressed (Figure 2B, D, −ED), NCOs were greatly reduced, and JMs accumulated to levels roughly twice that seen in *SGS1 ndt80*Δ controls. This confirms that Sgs1 directs some meiotic recombination events towards NCOs and away from JM formation. Subsequent *CDC5* expression triggered efficient JM resolution, but unlike in *SGS1 ndt80*Δ, both NCOs and COs were produced (Figure 2B, D, +ED). Thus, JMs that form in the absence of Sgs1 differ from JMs that form in wild type, in that the former resolve as both COs and NCOs, while the latter predominantly produce COs.

Mus81-Mms4, Yen1 and Slx1–Slx4 resolve a minor fraction of JMs in wild-type meiosis but have redundant roles in resolving the majority of JMs that form in the absence of Sgs1

The different product spectra seen upon resolution of JMs formed in the absence or presence of Sgs1 suggests that these two classes of JMs are resolved by different nucleases. We therefore examined the contribution of three candidate HJ-resolving nucleases, Mus81-

Mms4, Yen1, and Slx1–Slx4, to JM resolution and recombinant product formation in wild type and in *sgs1-mn* cells lacking one or more of these nucleases. We first examined wild type meiosis. All single nuclease mutants completed meiotic recombination and resolved the vast majority of JMs (Figure S2A). Thus, none of these putative resolvases are essential for JM resolution during wild-type meiosis.

Previous studies had suggested redundant roles for Mus81-Mms4 and Yen1 during the mitotic cell cycle and during meiosis (Agmon et al., 2011; Blanco et al., 2010; Ho et al., 2010; Matos et al., 2011; Tay and Wu, 2010). We therefore examined meiotic recombination in *mms4 yen1* double mutants, using a meiotic-null *mms4-mn* allele to avoid the marked growth defects seen in their combined absence (Agmon et al., 2011; L. J., E. K. and M. L., unpublished observations). As reported previously (Matos et al., 2011), *mms4-mn yen1*Δ mutant cells underwent meiotic catastrophe, failing to divide nuclei, even though spindle pole bodies separated and meiotic spindles assembled with normal timing (Figure 3A, B and data not shown). Instead, nuclei displayed transient nuclear stretching (Figure S2C and data not shown), a phenotype seen in cells that enter meiosis I with unresolved JMs (Jessop and Lichten, 2008; Oh et al., 2008). However, molecular analysis showed that only a minor fraction of JMs were not resolved (about 10–20% of total JMs formed, compare Figures 3D and 2C), with a corresponding modest decrease in COs (Figure 3C, D). These data indicate that Mus81-Mms4 and Yen1 are required for resolution of only a subset of JMs that form during normal meiosis. They also indicate that a small number of unresolved JMs is sufficient to block nuclear division. Consistent with this latter conclusion, a DSB/recombination-null *spo11 mms4-mn yen1*Δ mutant strain underwent efficient meiotic division (Figure S2B).

The finding that most JMs resolve in *mms4-mn yen1*Δ mutants prompted us to ask if the other candidate resolvase, Slx1–Slx4, acts redundantly with Yen1 or Mu81-Mms4. *mms4-mn slx1*Δ and *yen1*Δ *slx1*Δ strains displayed efficient nuclear division, JM resolution, and CO formation (Figure S2A), and *mms4-mn yen1*Δ *slx1*Δ mutants displayed no defects beyond those seen in *mms4-mn yen1*Δ (Figure 3C, D). Similar results were obtained when *slx4*Δ was used in place of *slx1*Δ (Figure S2A and data not shown). Therefore, activities other than Mus81-Mms4, Yen1 and Slx1–Slx4 must resolve the majority of JMs during wild-type meiosis.

A very different picture emerged when resolution of JMs formed in *sgs1* mutants was examined. Previous studies have shown that Mus81-Mms4 is required to resolve a substantial fraction of the JMs that form in the absence of Sgs1 (Jessop and Lichten, 2008; Oh et al., 2008). To ask if Yen1 and Slx1–Slx4 also resolved some of these JMs, we examined meiotic progression and monitored recombination in *sgs1-mn* mutants lacking one or more of these nucleases. Consistent with previous findings, *sgs1-mn mms4-mn* double mutants failed to separate nuclei at meiosis I; a similar phenotype was seen in *sgs1-mn slx1*Δ and in *sgs1-mn slx4*Δ (Figures 4A and S3A). All three strains accumulated unresolved JMs, consistent with a role for all three nucleases in resolving JMs that form in the absence of Sgs1 (Figures 4C and S3A). All three strains also displayed reductions in both COs and NCOs (Figures 4D and S3A), consistent with the conclusion that JMs that form in the absence of Sgs1 resolve as both COs and NCOs. Eliminating meiotic recombination restored nuclear division (Figure S3B; Jessop and Lichten, 2008), indicating that even the low level of unresolved JMs seen in *sgs1-mn slx1*Δ can completely block nuclear division. In contrast, *sgs1-mn yen1*Δ double mutants were similar to *sgs1-mn YEN1* strains in terms of progression, JM resolution, and recombinant product formation (Figure 4). Multiple mutant analyses revealed limited redundancy between the three nucleases. *sgs1-mn* mutants lacking two of the three nucleases displayed greater levels of unresolved JMs and lower levels of COs and NCOs than did *sgs1-mn* strains lacking any single nuclease, and *sgs1-mn* mutants

lacking all three nucleases (*sgs1-mn mms4-mn yen1*Δ *slx1*Δ) accumulated the greatest level of unresolved JMs and the lowest levels of CO and NCO recombinants (Figure 4, Figure S3A). Thus, when Sgs1 is absent, most meiotic JM resolution requires Mus81-Mms4, Yen1 and Slx1–Slx4.

Taken together, these data indicate that a minor fraction of the JMs that form during wild type meiosis are resolved by the three candidate Holliday junction resolvases identified to date, Mus81-Mms4, Yen1 and Slx1–Slx4, while most of the JMs that form in the absence of Sgs1 are resolved by the combined activity of these three nucleases.

ZMM-independent JMs are resolved by Mus81-Mms4 and Yen1

Mus81-Mms4 is necessary for many of the residual genetic crossovers recovered from yeast *zmm* mutants (Argueso et al., 2004; de los Santos et al., 2003), suggesting that, like JMs that form in the absence of Sgs1, ZMM-independent JMs are resolved by Mus81-Mms4 and other resolvases. However, this suggestion has never been directly tested. We therefore examined the effect of *mms4-mn yen1*Δ on meiotic recombination in a *msh4*Δ background (Figure 5).

The vast majority of *msh4*Δ single mutant cells underwent nuclear division and distributed nuclear DNA among four spores (Figure 5 and data not shown). In contrast, *msh4*Δ *mms4-mn yen1*Δ triple mutants suffered nuclear division failure similar to that seen in *mms4-mn yen1*Δ, with a single unsegregated DNA mass that was excluded from spores (Figure 5A and data not shown). Nuclear division failure was accompanied by an accumulation of unresolved JMs, to levels similar to those seen in *msh4*Δ *ndt80*Δ mutants, where all JM resolution is blocked (Figure 5B, C). These data are consistent with the suggestion that most of the JMs formed in the absence of Msh4 are resolved by Mus81-Mms4 and Yen1.

Discussion

Sgs1 directs events towards resolvase-independent NCO formation and towards ZMM-dependent CO formation

We have examined contributions of the budding yeast BLM helicase homolog, Sgs1, and of the Mus81-Mms4, Yen1 and Slx1–Slx4 nucleases, to meiotic recombination intermediate metabolism and recombinant product formation. Sgs1 was identified as a regulator of meiotic recombination in previous studies (Jessop et al., 2006; Oh et al., 2007; Rockmill et al., 2003), but these had focused on Sgs1 activity in limiting intersister- and multichromatid-JMs during wild type meiosis, and in limiting all JM and CO formation in *zmm* mutants. In particular, the observation of similar NCO and CO levels in *sgs1* mutants and in wild type seemed to indicate a limited role for Sgs1 in the majority of meiotic recombination events.

Our current findings indicate that, despite the numerical similarity in NCOs and COs, meiotic recombination differs in fundamental aspects in *SGS1* and in *sgs1-mn* cells. Early, Cdc5-independent NCO formation does not occur in *sgs1-mn* mutants (Figure 1), and cumulative JM levels are roughly doubled relative to wild type (Figure 2). In addition, while most JMs that form in *SGS1* cells are resolved as COs without contributions from Mus81-Mms4, Yen1, or Slx1–Slx4 (Figure 3), resolution of JMs that form in *sgs1* mutants produces both COs and NCOs (Figure 2), and JM resolution is strongly dependent upon the Mus81-Mms4, Yen1 and Slx1–Slx4 resolvases (Figure 4). Similar observations are reported by Zakharyevich and coworkers (Zakharyevich et al., 2012).

On the basis of these and previous findings, we suggest that Sgs1 functions as a central regulator that impacts virtually all meiotic recombination (Figure 6). In the absence of Sgs1 activity, the majority of events form JMs in an unregulated manner and outside of the

normal meiotic chromosomal context. Inter-sister and multichromatid JMs are frequently produced, in addition to biparental interhomolog JMs. Furthermore, these JMs are resolved in an unbiased manner to produce both COs and NCOs. In contrast, in wild type cells, Sgs1 directs about half of events towards NCO formation before they can form stable JMs, and directs most of the remaining events towards ZMM protein-associated, interhomolog biparental JMs, which undergo biased resolution as COs when Cdc5 triggers exit from pachytene.

Both of these Sgs1 functions can be explained by suggesting that the BLM/Sgs1 helicase complex has the potential to disassemble all of the branched recombination intermediates that form during meiosis (Figure 6A). We suggest that the D-loop unwinding activity of BLM/Sgs1 (Figure S1A) disassembles most early interhomolog strand invasion intermediates before they can capture a second DSB end, maintaining DSBs in a state of dynamic instability between strand invasion and free DSB ends. Events can escape BLM/Sgs1-mediated disassembly when break ends anneal to form an unbranched NCO *via* SDSA (McMahill et al., 2007; McVey et al., 2004a). Alternatively, when strand invasion intermediates are captured by the ZMM proteins and thus protected from BLM/Sgs1, they are stabilized for enough time to allow second end-capture, forming JMs that are later resolved as COs. On occasion, ZMM-independent JMs can form when strand invasion intermediates capture a second end before BLM/Sgs1 disassembles them. Because the ZMM proteins do not protect these intermediates, they are substrates for BLM/Sgs1-Top3-Rmi1 complex-mediated dissolution to form NCOs (Figure S1B).

Dissolution has been identified as a prominent mechanism for JM resolution during the mitotic cell cycle (Dayani et al., 2011), and studies have identified marker segregation patterns in some tetrads that are consistent with dHJ dissolution (Gilbertson and Stahl, 1996; Martini et al., 2011). However, because dissolution produces only fully duplex NCOs (Figure S1B), it cannot direct events towards subsequent ZMM-dependent JM formation. We therefore believe that mechanisms such as D-loop disassembly must also be involved in regulating meiotic recombination intermediate metabolism. Current data do not distinguish between these two possible BLM/Sgs1 activities, and addressing this issue is an important goal for ongoing research.

Mus81-Mms4, Yen1, and Slx1–Slx4 are not the major JM resolvase in normal meiosis

Studies of repair and recombination in budding yeast have suggested redundant roles for Mus81-Mms4 and Yen1 in JM resolution during mitotic cell cycle (Agmon et al., 2011; Blanco et al., 2010; Ho et al., 2010; Tay and Wu, 2010), while the role of Slx1–Slx4 has not been fully evaluated. Our data, and the data of others, indicate that none of these nucleases, either singly or in combination, are the main JM-resolving activity during budding yeast meiosis, since most meiotic JMs still are resolved in *mms4-mn yen1*Δ *slx1*Δ triple mutants (Figures 3 and S2; Zakharyevich et al., 2012). It is likely that the limited number of JMs that remain unresolved in *mms4-mn yen1*Δ *slx1*Δ triple mutants represent ZMM-independent JMs that escape Sgs1-mediated disassembly, and that these JMs contribute to what was previously described as a ZMM-independent, Mus81-Mms4-dependent “alternative” recombination pathway (Argueso et al., 2004; de los Santos et al., 2003; Figure 1A). Consistent with this suggestion, unresolved JMs are seen at similar levels in *msh4*Δ *ndt80*Δ and in *msh4*Δ *mms4-mn yen1*Δ cells (Figure 5). While this alternative pathway is usually described as producing COs, data from *sgs1* mutants make it likely that it also produces NCOs (see below).

Mus81-Mms4, Yen1, and Slx1–Slx4 resolve JMs that form in the absence of Sgs1

Unlike in wild-type meiosis, Mus81-Mms4, Yen1 and Slx1–Slx4 play important roles in resolving the meiotic JMs that form in the absence of Sgs1, since JMs persist at high levels in *sgs1-mn mms4-mn yen1*Δ *slx1*Δ cells (Figure 4; Zakharyevich et al., 2012). As in wild type, these JMs are resolved by Cdc5-dependent mechanisms (Figure 2). Our data thus provide *in vivo* confirmation of recent reports that the HJ-resolving activity of Mus81-Mms4 is activated by the Cdc5-catalyzed phosphorylation (Matos et al., 2011). In addition, unresolved JM levels increase as *sgs1-mn mms4-mn* is combined with *yen1*Δ, *slx1*Δ or *slx4*Δ, and a further increase is seen in *sgs1-mn mms4-mn yen1*Δ *slx1*Δ cells (Figure 4, Figure S3; Zakharyevich et al., 2012). These synthetic JM resolution defects confirm that both Yen1 and Slx1–Slx4 can resolve meiotic JMs *in vivo*, that Mus81-Mms4 and Yen1 act redundantly (Matos et al., 2011), and also suggest that, like Mus81-Mms4, Yen1 and Slx1–Slx4 are activated in a Cdc5-dependent manner during meiosis. Furthermore, the increase in unresolved JMs in *sgs1-mn* strains lacking one or more of these nucleases is associated with a reduction in both NCOs and COs (Figure 4), indicating that the three nucleases resolve dHJ-JMs in an unbiased manner. This is incompatible with a class of models (Whitby, 2005) in which Mus81-Mms4 or other nucleases cleave nascent recombination intermediates in a manner that generates only COs.

Concluding remarks

We have shown here that Sgs1, the budding yeast BLM helicase ortholog, controls meiotic recombination by preventing accumulation of unregulated JMs, which in the absence of Sgs1 comprise the default recombination intermediate. Sgs1 most likely does so, either alone or in complex with Top3 and Rmi1, by disassembling all unprotected branched DNA structures (Figure S1), thus channeling events both towards NCOs (which lack branched structures), and towards JMs that are protected by SC-associated ZMM proteins (Figure 6). These ZMM-protected JMs are later resolved, at the end of pachytene, by an as yet uncharacterized, Cdc5-activated resolvase. The nuclease(s) that resolve ZMM-dependent JMs remain to be identified. We suggest that ZMM-dependent JMs undergo biased resolution as COs because they reside in a structurally coordinated context that ensures cleavage of the two HJs in opposite orientations (Figure S1C). Mlh1, Mlh3, and Exo1, which are not required for JM formation or resolution but which are necessary for full CO formation, may provide this structural context, and possibly the HJ cleavage activity itself (Zakharyevich et al., 2010; Zakharyevich et al., 2012).

JMs that form in the absence of Sgs1, or that escape Sgs1 surveillance in wild type cells, also are resolved by Cdc5-triggered mechanisms, but these involve known nucleases (Mus81-Mms4, Yen1 and Slx1–Slx4) that cleave JMs in an unbiased manner, forming both COs and NCOs as predicted by the original DSBR model (Szostak et al., 1983). Our data indicate that this alternative mode of JM resolution acts in a minor fraction of the interhomolog recombination events that occur during wild-type meiosis. However, this alternative pathway contains sufficient JM resolution capacity to resolve most of the events that occur in the absence of Sgs1, thus revealing a remarkable robustness and flexibility in the budding yeast meiotic recombination program.

Studies in several other organisms, including mouse and *Arabidopsis*, have also suggested a dual contribution to crossover recombination, with ZMM-dependent processes being responsible for most crossovers, and with minor contributions from ZMM-independent processes involving nucleases such as Mus81-Mms4 (Berchowitz et al., 2007; Holloway et al., 2008). In other organisms, such as *C. elegans*, CO recombination is almost completely ZMM-dependent (Zetka, 2009). Finally, in organisms that lack ZMM protein homologs, such as *S. pombe* and *Drosophila*, CO recombination is almost completely dependent on

structure-selective nucleases (Radford et al., 2005; Smith et al., 2003; Yildiz et al., 2002). We suggest that variation between organisms, in terms of dependence upon structure selective nucleases for JM resolution, reflects the relative efficiency with which helicases disassemble early recombination intermediates. It will be of considerable interest to test this suggestion in different organisms, to determine if requirements for alternative JM resolution activities become greater during meiosis in the absence of BLM helicase activity. It is important to note that, while Sgs1 appears to be the dominant helicase regulating meiotic recombination in budding yeast, other helicases have the potential to regulate meiotic recombination in multicellular organisms; these include the Srs2 homolog RTEL-1, the Fanconi anemia complementation group M (FANCM) protein, as well as other RecQ helicase homologs (Barber et al., 2008; Higgins et al., 2011; Whitby, 2010). Testing the impact of these helicases on meiotic recombination will be an important subject for future research.

Finally, our findings suggest a striking parallel between recombination that occurs during the mitotic cell cycle and the ZMM protein-independent recombination that occurs during meiosis, in that both types of recombination are regulated by Sgs1 to frequently form NCOs, rarely form JMs, and both use known structure-selective nucleases to resolve the JMs that do form in an unbiased manner (Figure S4). In this view, the recombination events that occur during meiosis proceed through a combination of two independent pathways. In one, the synaptonemal complex and associated proteins provide a structural context that stabilizes JMs and directs their resolution as COs, thus promoting homolog disjunction at the first meiotic division. In the second, additional interhomolog intermediates, needed to promote the earlier events of homolog pairing and synapsis, are disassembled or resolved by the same mechanisms that function during the mitotic cell cycle. Our data indicate that Sgs1 plays an important role in partitioning meiotic recombination between these coexisting processes.

Experimental Procedures

Yeast strains

Strains (Table S1) were derived from the haploid parents of MJL2984 (Jessop et al., 2005) by transformation or genetic crosses. Construction details are given in the legend to Table S1.

Sporulation

Yeast strains were grown in buffered liquid presporulation medium and shifted to sporulation medium (supplemented 1% potassium acetate) with vigorous aeration to induce meiosis, as described (Jessop et al., 2006). For experiments where *CDC5* expression was induced, sporulation cultures were split at the indicated time, β-estradiol or vehicle were added as described (Sourirajan and Lichten, 2008), and aeration was continued.

DNA extraction and analysis

DNA was prepared using a CTAB extraction procedure that stabilizes joint molecule intermediates, and was analyzed on Southern blots of one-dimensional agarose gels as described, using electrophoresis conditions that stabilize joint molecules (Allers and Lichten, 2000, 2001; Jessop and Lichten, 2008). *Xho*I and *Xmn*I digests, to score DSBs and JMs respectively, were probed with *ARG4* coding sequences (+165 to +1413). *Xho*I/*Eco*RI double digests, to score NCO and CO recombinants, were probed with *HIS4* coding sequences (+538 to +718).

Cytology

Nuclear morphology was scored by DAPI staining (Goyon and Lichten, 1993); cells with a stretched single nucleus or with more than one nucleus were scored as having initiated meiosis I. Progression through meiosis was monitored by scoring spindle pole body/spindle morphology, by immunostaining for β-tubulin as described (Jessop and Lichten, 2008); cells with a single monopolar spindle were scored as not having exited prophase I.

Highlights

- Sgs1 is required for normal noncrossover and crossover formation during meiosis
- Noncrossovers and crossovers form via different pathways in wild-type.
- Noncrossovers and crossovers form via a common pathway in *sgs1* mutants.
- Polo kinase Cdc5 triggers joint molecule resolution in both wild type and *sgs1*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Neil Hunter for communicating unpublished data, and Valérie Borde, Dhruba Chattoraj, Mathilde Grelon, Neil Hunter, Raphaël Mercier, Yikang Rong, and Denise Zickler for helpful discussions. This work was supported by the Intramural Research Program of the National Institutes of Health, through the Center for Cancer Research of the National Cancer Institute.

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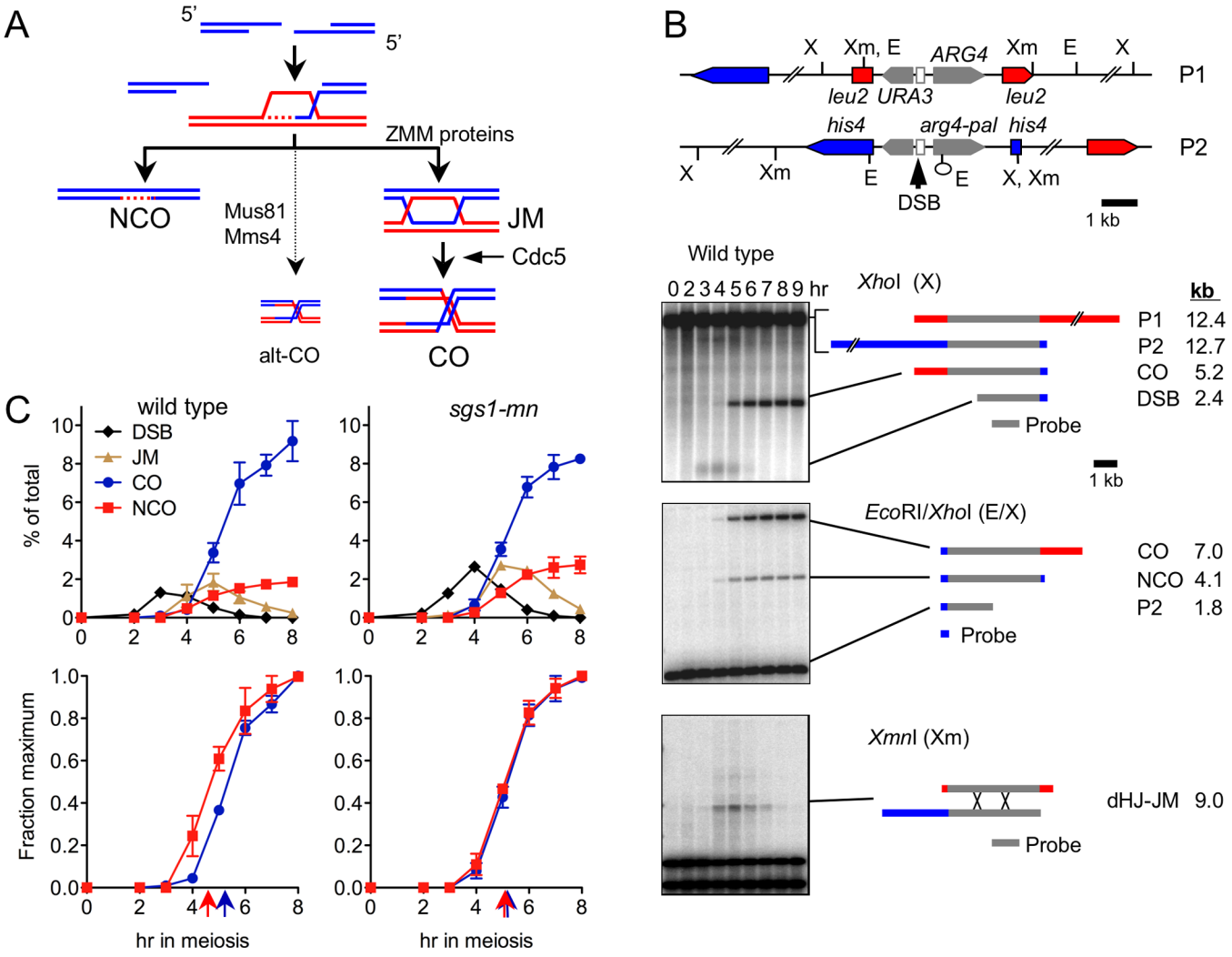

Figure 1. Sgs1 is involved in NCO formation

(A) Early crossover decision model for meiotic recombination (Bishop and Zickler, 2004). A double-strand break (DSB) is resected to expose 3'-ended single-strand tails, which invade the homolog and initiate DNA synthesis, forming an early D-loop intermediate. D-loop disassembly (left) creates a noncrossover by annealing with the two DSB ends; early intermediate stabilization by synaptonemal complex components (ZMM proteins, right) leads to JM formation by capture of the other DSB end. At exit from pachytene, triggered by polo kinase Cdc5, JMs are resolved in a biased manner to produce crossovers. A minor fraction of crossovers are produced by an alternative, ZMM-independent mechanism that involves the Mus81-Mms4 nuclease (alt-CO, center).

(B) Recombination reporter system used to detect intermediates and products (Jessop et al., 2005). A 3.5 kb insert with *URA3* and *ARG4* genes (grey arrows) contains a strong meiotic DSB site (open box), and is inserted at *LEU2* (red) on one chromosome *III* homolog and at *HIS4* (blue) on the other. A short palindrome with an *Eco*RI site (lollipop) creates the *arg4-pal* allele. Restriction sites: Xm—*Xmn*I; X—*Xho*I; E—*Eco*RI. *Xmn*I digests probed with *ARG4* sequences (grey bar) detect dHJ-JMs. *Xho*I digests probed with the same sequences detect DSBs and COs. *Eco*RI/*Xho*I double digests, probed with *HIS4* sequences (blue bar), detect NCOs where *arg4-pal* is converted to *ARG4* (full conversion shown), as well as a subset of COs. Representative Southern blots are shown.

(C) Recombination intermediates and products in wild type (MJL2984) and *sgs1-mn* (MJL3166). Top—DSB (black), JM (tan), CO (blue), and NCO (red) signals from southern blots. Bottom—COs and NCOs, expressed as a fraction of maximum levels. Arrows indicate times of half-maxima. Values are from two independent experiments; error bars indicate S.E.M.

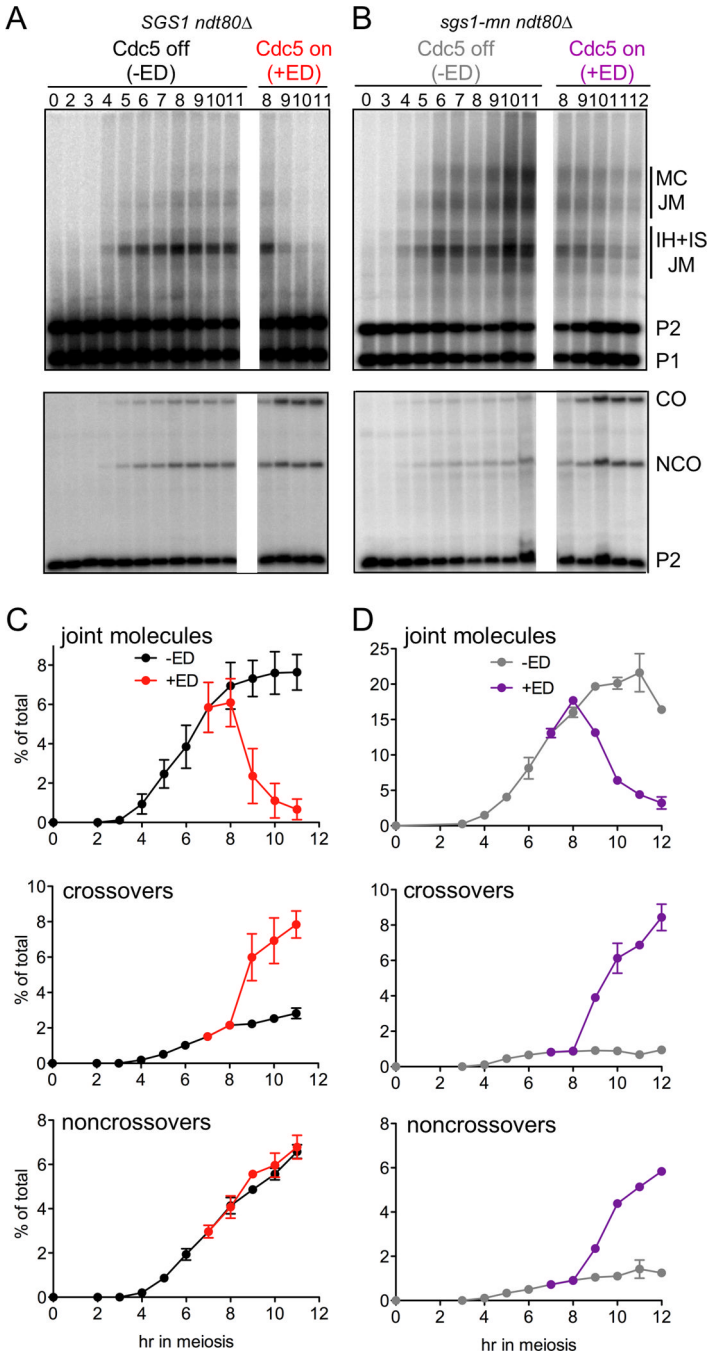

Figure 2. Polo kinase Cdc5 triggers JM resolution as COs and NCOs in *sgs1-mn*

SGS1 ndt80Δ CDC5-IN cells (MJL3553) and *sgs1-mn ndt80Δ CDC5-IN* cells (MJL3557) were sporulated for 7h, and the culture was divided into two portions: uninduced (no β-estradiol added; CDC5 off; -ED), and induced (β-estradiol added to 1μM at 7h; CDC5 on; +ED).

(A, B) Southern blot detection of intermediates and products in *SGS1* (A) and *sgs1-mn* (B). Top—*Xmn*I digest to detect bimolecular interhomolog and intersister chromatid intermediates (IH + IS JM) and multichromatid JMs composed of 3 or 4 chromosomes (MC JM). Bottom—*Eco*RI/*Xho*I digest to detect CO and NCO recombinants. See Figure 1B for details.

(C, D) Frequencies of JMs (IH+IS+MC), COs, and NCOs, in *SGS1* (C) and *sgs1-mn* (D), plotted as a percentage of total lane signal. Values are from two independent experiments; error bars indicate S.E.M.

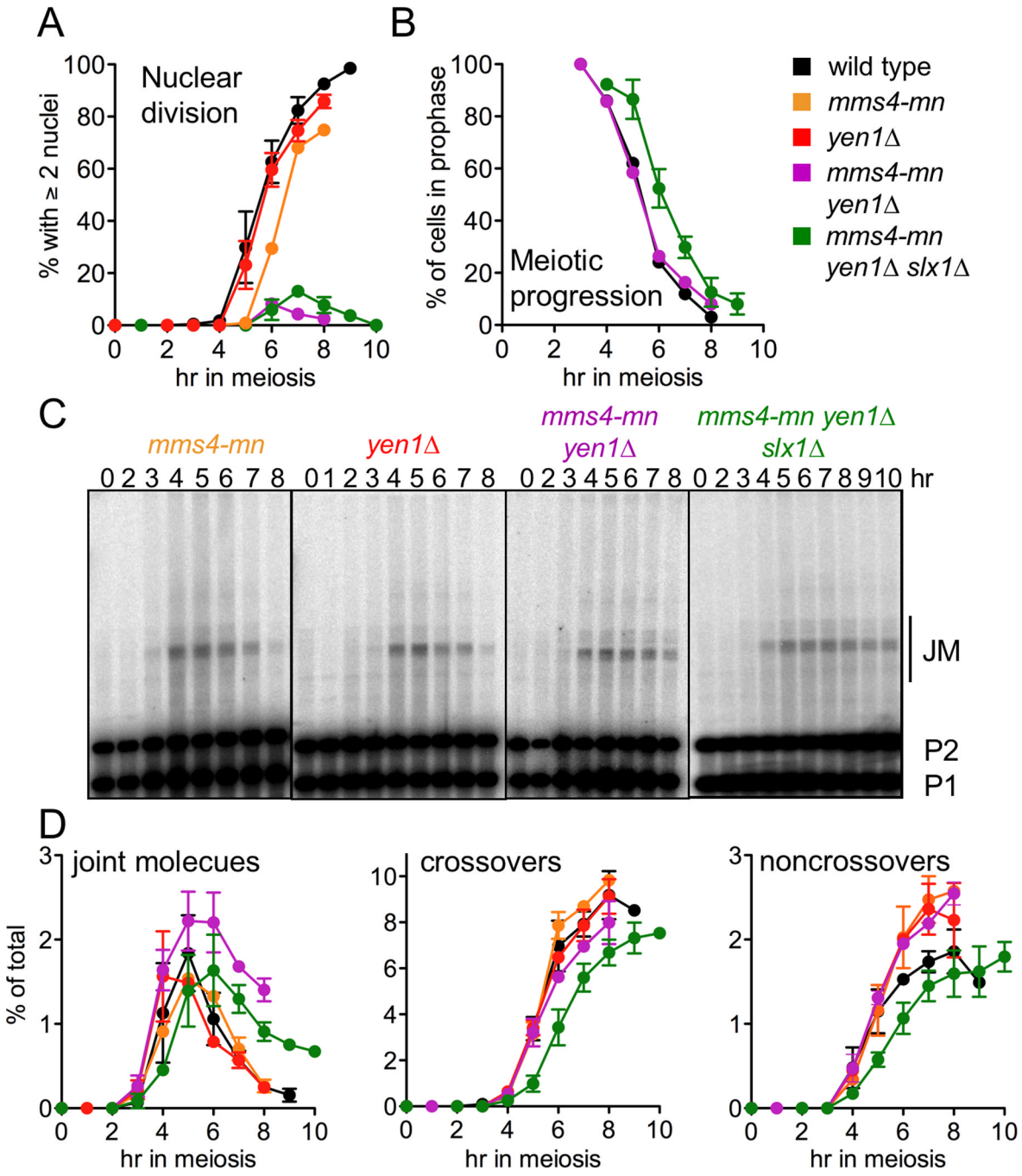

Figure 3. Mus81-Mms4, Yen1 and Slx1–Slx4 resolve a minor fraction of JMs in wild type meiosis

(A) Fraction of cells undergoing the first meiotic nuclear division, scored as cells with two or more nuclei, including cells where 2 nuclei are connected by DNA bridges. Wild-type (black, MJL2984), *yen1*Δ (red, MJL3441), *mms4-mn yen1*Δ (purple, MJL3390) and *mms4-mn yen1*Δ *slx1*Δ (green, MJL3491) values are from two independent experiments; for *mms4-mn* (orange, MJL3172), a single experiment. Error bars indicate S.E.M.

(B) Meiotic progression. Cells with a single spindle pole body were scored as remaining in meiosis I prophase. Values for *mms4-mn yen1*Δ *slx1*Δ are from two experiments; error bars indicate S.E.M. Values for other strains are from a single experiment.

(C) Representative Southern blots used to detect JMs.

(D) Frequencies of JMs, COs, and NCOs, plotted as a percentage of total lane signal. JMs were quantified using *Xmn*I digests; COs and NCOs were quantified using *Xho*I/*Eco*RI digests (see figure 1B). Values are from two independent experiments; error bars indicate S.E.M.

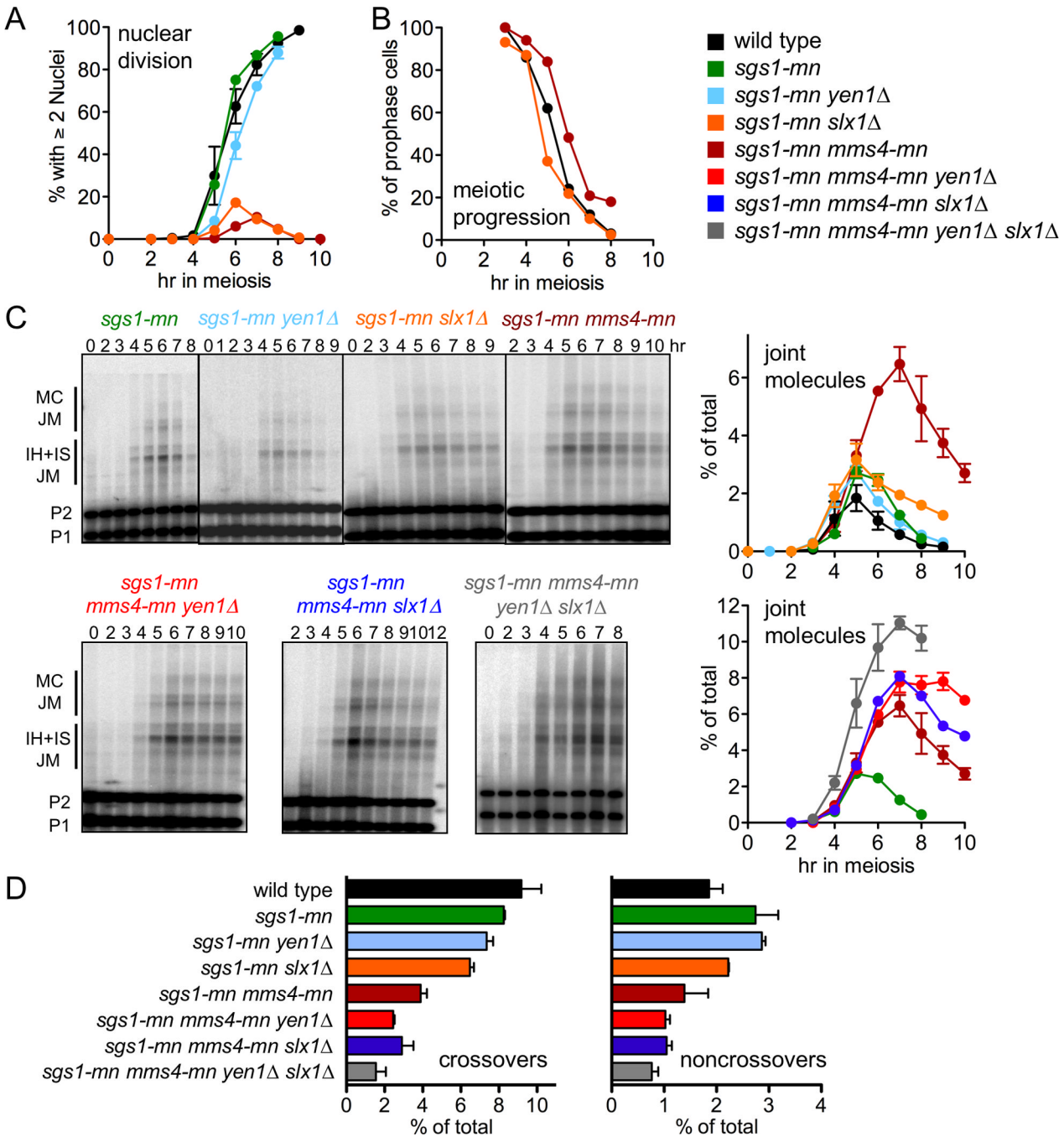

Figure 4. Mus81-Mms4, Yen1 and Slx1–Slx4 have a major role in JM resolution during meiosis in the absence of Sgs1

(A) Fraction of cells undergoing the first meiotic nuclear division, scored as cells with two or more nuclei, including cells where nuclei are connected by DNA bridges. Wild type (black, MJL2984), *sgs1-mn* (green, MJL3166) and *sgs1-mn yen1*Δ (light blue, MJL3363) values are from two independent experiments; error bars indicate S.E.M. Values for *sgs1-mn slx1*Δ (orange, MJL3467) and *sgs1-mn mms4-mn* (brown, MJL3171) are from a single experiment.

(B) Meiotic progression. Cells with a single spindle pole body were scored as remaining in meiosis I prophase. All values are from a single experiment.

(C) Left—representative Southern blots used to detect JMs. Additional strains are *sgs1-mn mms4-mn yen1*Δ (red, MJL3436), *sgs1-mn mms4-mn slx1*Δ (dark blue, MJL3544), and *sgs1-mn mms4-mn yen1*Δ *slx1*Δ (grey, MJL3582). Right—Total JM frequencies, plotted as percentage of total lane signal. Values are from two independent experiments; error bars indicate S.E.M.

(D) CO and NCO frequencies, 8 hr values, plotted as percent of total lane signal, from Southern blots of *Xho*I/*Eco*RI digests (see figure 1B). Values are from two independent experiments; error bars indicate S.E.M.

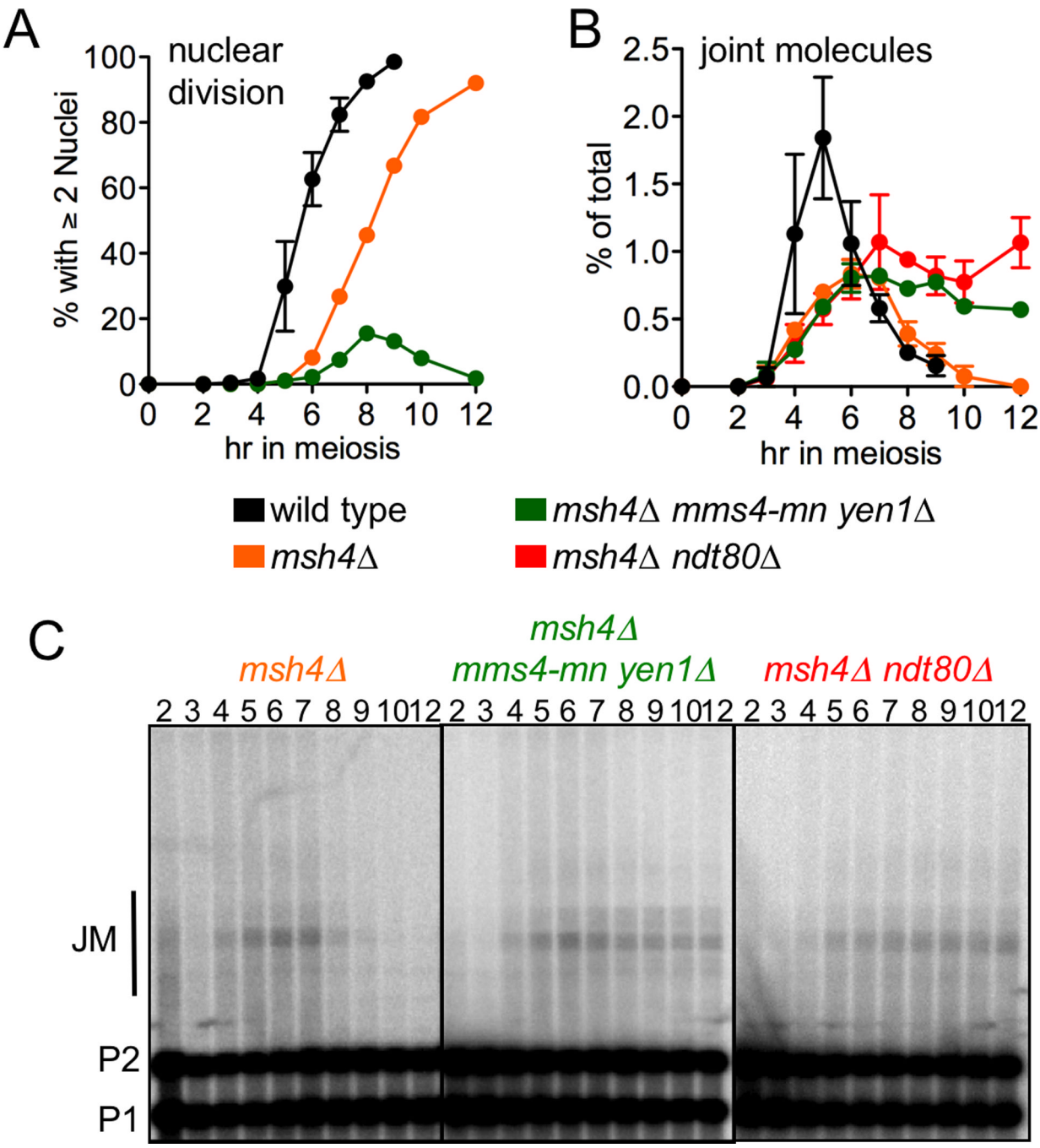

Figure 5. *MSH4*-independent JMs are not resolved in *mms4-mn yen1*Δ double mutants

(A) Fraction of cells undergoing meiosis I nuclear division, scored as cells with two or more nuclei, including cells where 2 nuclei are by DNA bridges, in wild-type (black, MJL2984), *msh4*Δ (orange, MJL3020), and *msh4*Δ *mms4-mn yen1*Δ (green, MJL3489). Wild-type values are from two independent experiments; error bars indicate S.E.M. Other values are from a single experiment.

(B) JM frequencies, plotted as a percentage of total lane signal. Values are from two independent experiments; error bars indicate S.E.M.

(C) Representative Southern blots of *Xmn*I digests used to detect JMs.

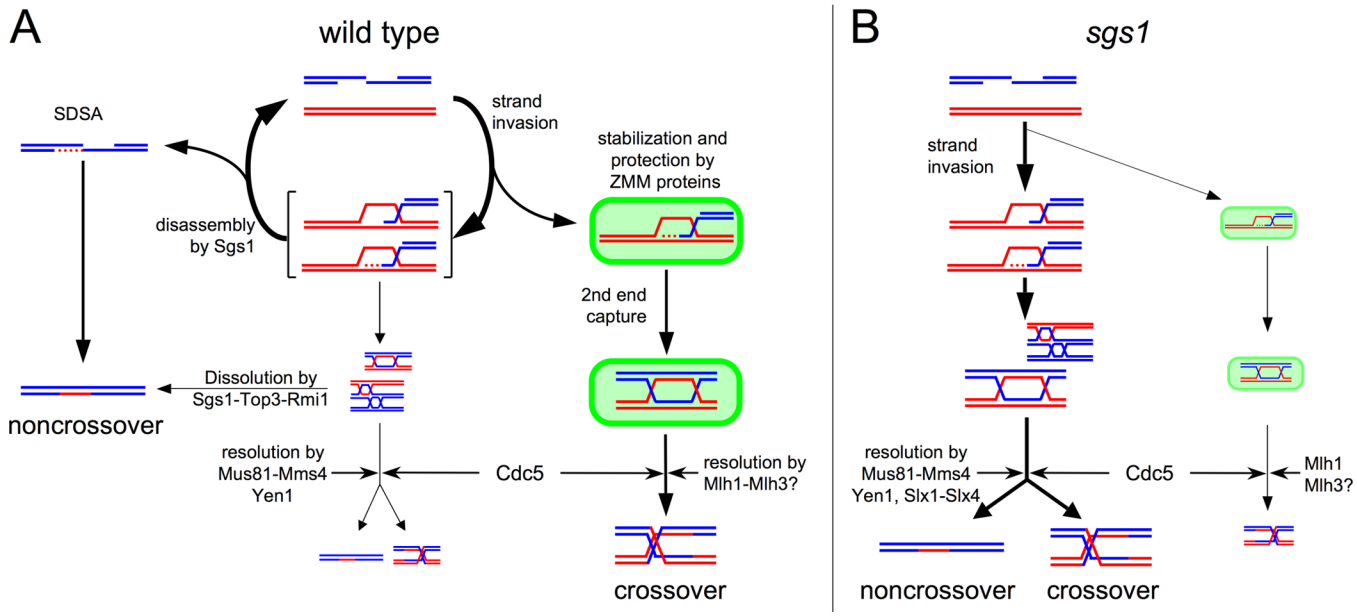

Figure 6. Model of how Sgs1 regulates meiotic recombination intermediate metabolism

(A) During wild type meiosis, nascent recombination intermediates that contain branched DNA structures are disassembled by Sgs1 helicase. Disassembly of unprotected strand invasion intermediates can drive events towards strand annealing with the other DSB end to form NCOs (SDSA, left) or can return molecules to the broken state. Strand invasion events that are captured by ZMM proteins (right) are stabilized and protected from Sgs1-mediated disassembly, allowing second end capture and dHJ-JM formation. Branched intermediates that escape Sgs1 helicase can form both dHJ and multichromatid JMs (center), which are further vulnerable to Sgs1/Top3/Rmi1-mediated dissolution to form NCOs. JMs that are protected by ZMM proteins are designated by Mlh1-Mlh3 to be resolved as crossovers in a Cdc5-triggered process; ZMM-independent JMs that escape Sgs1 disassembly and dissolution undergo Cdc5-triggered resolution by Mus81-Mms4 and Yen1 to form both NCOs and COs. Strand invasion events involving two sister chromatids also occur, but are not illustrated here.

(B) In *sgs1* mutant cells, most strand invasion recombination intermediates proceed directly to form JMs (both dHJ and multichromatid) without ZMM protein involvement, with a consequent reduction in ZMM-associated JMs. All JMs persist until Cdc5 triggers JM resolution. Most JMs are resolved by Mus81-Mms4, Yen1, and Slx1–Slx4 to form both COs and NCOs, with a minor contribution from the ZMM-dependent, CO forming processes that dominate in wild type. It is possible that minor fraction of NCOs are still formed by SDSA; these are not illustrated here.