Remodeling of the Rad51 DNA Strand-Exchange Protein by the Srs2 Helicase

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ABSTRACT Homologous recombination is associated with the dynamic assembly and disassembly of DNA–protein complexes. Assembly of a nucleoprotein filament comprising ssDNA and the RecA homolog, Rad51, is a key step required for homology search during recombination. The budding yeast Srs2 DNA translocase is known to dismantle Rad51 filament *in vitro*. However, there is limited evidence to support the dismantling activity of Srs2 *in vivo*. Here, we show that Srs2 indeed disrupts Rad51-containing complexes from chromosomes during meiosis. Overexpression of Srs2 during the meiotic prophase impairs meiotic recombination and removes Rad51 from meiotic chromosomes. This dismantling activity is specific for Rad51, as Srs2 Overexpression does not remove Dmc1 (a meiosis-specific Rad51 homolog), Rad52 (a Rad51 mediator), or replication protein A (RPA; a single-stranded DNA-binding protein). Rather, RPA replaces Rad51 under these conditions. A mutant Srs2 lacking helicase activity cannot remove Rad51 from meiotic chromosomes. Interestingly, the Rad51-binding domain of Srs2, which is critical for Rad51-dismantling activity *in vitro*, is not essential for this activity *in vivo*. Our results suggest that a precise level of Srs2, in the form of the Srs2 translocase, is required to appropriately regulate the Rad51 nucleoprotein filament dynamics during meiosis.

RECOMBINATION between homologous sequences promotes genomic stability. As such, a wide variety of DNA-associated proteins and protein complexes regulate the recombination process. Within chromatin, the chromosome structure probably also directly affects the assembly and disassembly of protein complexes involved in recombination. To understand the molecular mechanisms that govern assembly and disassembly of these complexes, homologous recombination during meiosis can be used as a model system.

Meiotic recombination is initiated when Spo11, a meiosis-specific topoisomerase-like protein, generates double-strand breaks (DSBs) (Keeney 2001). The 5' ends of these DSBs are quickly resected to produce a 3'-overhanging stretch of single-stranded DNA (ssDNA). The ssDNA is used to search for homologous double-stranded DNAs (dsDNAs). Once homology between the ss- and dsDNAs are matched, the ssDNA is invaded into the dsDNA. Strand invasion leads to DNA syn-

thesis with the 3' end of the invading strand as the primer. The resulting intermediate is then converted into the single-invasion intermediate (SEI) and then a DNA structure with double Holiday junctions (dHJ) (Schwacha and Kleckner 1994; Hunter and Kleckner 2001). Resolution of dHJ structures typically results in chromosomal crossover (CO) (Allers and Lichten 2001; Hunter and Kleckner 2001). Alternatively, newly synthesized DNA of the invading strand after the displacement can anneal with ssDNA from the other end of the DSB, which results in the formation of a noncrossover (NCO) product (through the synthesis-dependent strand-annealing pathway). In meiosis, the recombination occurs preferentially between homologous chromosomes rather than between sister chromatids.

After the resection of DSBs, Replication Protein A (RPA) binds to the ssDNA, followed by the binding of Rad51 (a homolog of bacterial RecA). Rad51 forms nucleoprotein filaments on ssDNA, thereby catalyzing the invasion of the ssDNA into a homologous DNA duplex (Ogawa *et al.* 1993; Sung 1994). Rad51 mediates the strand invasion during both mitosis and meiosis (Shinohara 1992), whereas Dmc1, another RecA homolog that also forms filaments on ssDNA, participates only in meiotic recombination (Bishop *et al.* 1992). The Rad51- and Dmc1-type filaments are structurally similar (Ogawa *et al.* 1993; Sheridan *et al.* 2008), but have very

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different *in vivo* functions. While, in mitosis, **Rad51** is essential for homology search and strand invasion, in meiosis, **Rad51** plays an accessory role for **Dmc1** (Cloud *et al.* 2012). The collaborative action of both **Rad51** and **Dmc1** ensures inter-homolog bias for the meiotic recombination.

In vivo assembly of a **Rad51**-ssDNA filament is a highly dynamic process and is extensively regulated. Several auxiliary proteins [e.g., **Rad52** and the **Rad55–Rad57** complex, the **Psy3–Csm2–Shu1–Shu2** (PCSS/Shu) complex] facilitate the loading of **Rad51** onto RPA-coated ssDNA (Sung 1997; Shinohara and Ogawa 1998; Liu *et al.* 2011; Sasanuma *et al.* 2013). **Rad54**, which is a **Swi2/Snf2**-like protein, subsequently promotes the postsynaptic function of **Rad51** (Solinger *et al.* 2001). In contrast, **Dmc1** assembly is regulated by a completely different set of proteins, including **Rad51** and the **Mei5–Sae3** complex (Hayase *et al.* 2004; Tsubouchi and Roeder 2004; Cloud *et al.* 2012). **Dmc1**-ssDNA filaments subsequently search for homologous stretches of DNA in conjunction with **Tid1/Rdh54** (a **Rad54** homolog) and **Mnd1/Hop2** (Shinohara *et al.* 1997; Tsubouchi and Roeder 2002). **Tid1/Rdh54** controls the assembly of **Dmc1** onto dsDNA by dismantling nonproductive **Dmc1** complexes (Holzen *et al.* 2006).

The assembly and activity of **Rad51** filaments are also negatively regulated. Several DNA helicases (including the highly conserved **Srs2** in *Saccharomyces cerevisiae*) downregulate **Rad51** function. **Srs2** is a 3'-to-5' superfamily 1 helicase that is related to the bacterial UvrD helicase (Marini and Krejci 2010). **Srs2** contains several distinct functional domains, including: (1) a DNA helicase domain that contains an ATP-binding/ATPase motif (Walker's A/B; residues 1–845), (2) a **Rad51**-interaction domain (residues 875–902), and (3) proliferating cell nuclear antigen (PCNA)- and SUMO (small-ubiquitin-like modifier)-interaction domains (residues 1036–1174) (Marini and Krejci 2010). **Srs2** is post-translationally modified by cyclin-dependent kinase (Cdk)-dependent phosphorylation and SUMOylation. During DNA repair, **Srs2** plays an important role in pathway choice. For example, DNA-repair deficits associated with the loss of post-replication repair genes (*i.e.*, **RAD6** and **RAD18**) are suppressed in the absence of **SRS2** (Schiestl *et al.* 1990; Schild 1995). During mitosis, a *srs2* mutant exhibits elevated levels of recombination (particularly CO), as repair events are preferentially channeled down the recombination pathway (Rong *et al.* 1991). This property of **Srs2** is called the antirecombinase function. An *srs2* mutant shows synthetic lethality when combined with various other mutants that are involved in DNA transactions (Marini and Krejci 2010). Importantly, synthetic lethality is seen when *srs2* is combined with a mutation in **SGS1** (Aboussekhras *et al.* 1992; Gangloff *et al.* 2000), which encodes a RecQ helicase that can resolve double-Holiday-junction structures into NCO events (Wu and Hickson 2003). Moreover, increased levels of **Srs2** decrease cell viability and reduce DNA-damage tolerance during mitosis (Kaytor *et al.* 1995; Mankouri *et al.* 2002). As such, **Srs2** plays multiple roles during the processing of DNA damage. Moreover, the *srs2* deletion is synthetically lethal with

deletion of the **RAD54** and this lethality is suppressed by a defect in early recombination, e.g., *rad51* deletion (Palladino and Klein 1992; Klein 2001). This suggests a role of **Srs2** in a step of postassembly of **Rad51** filament and this function is somehow redundant with **Rad54**.

Biochemical studies have characterized the antirecombinase activity of **Srs2**. By directly interacting with **Rad51**, **Srs2** dislodges **Rad51** from nucleoprotein filaments, thereby inhibiting **Rad51**-dependent formation of joint molecules and D-loop structures (Krejci *et al.* 2003; Veaute *et al.* 2003). In the presence of RPA, which competes with **Rad51** for ssDNA binding, purified **Srs2** efficiently removes **Rad51** from ssDNA *in vitro*. The ability of **Srs2** to dismantle **Rad51** from nucleoprotein filaments requires ATPase activity, which suggests that the **Srs2** helicase domain participates. Probably the **Srs2** helicase displaces **Rad51** as it travels along ssDNA. A recent study involving the **Rad51**-binding domain of **Srs2** (residues 875–905), however, provided an alternative mechanism for **Rad51** inhibition. The **Rad51**-binding domain of **Srs2** can stimulate an intrinsic **Rad51** ATPase activity, which causes **Rad51** to dissociate from its bound DNA (Antony *et al.* 2009); ADP-bound **Rad51** shows weaker DNA binding activity than ATP-bound form. The precise mechanism by which **Srs2** regulates **Rad51** activity *in vivo*, therefore, remains unclear.

For the study reported herein, to dissect an antirecombination role of **Srs2**, we studied the effect of **Srs2** overexpression on meiosis in *S. cerevisiae*. We found that increased levels of **Srs2** severely reduced DSB repair, the formation of CO/NCO structures, and spore viability, indicating that efficient meiotic recombination requires a specific amount of **Srs2**. Importantly, overexpression of **Srs2** during meiosis disrupted **Rad51**-containing foci. The disruption process required the ATPase activity of **Srs2**. The **Rad51**-interacting domain of **Srs2**, however, was not required to dismantle **Rad51** filaments *in vivo*. Finally, **Srs2** overexpression specifically affected **Rad51** as other proteins involved in recombination (e.g., **Dmc1**, **Rad52**, and RPA) were not affected. Our *in vivo* study demonstrates that **Srs2** remodels **Rad51** filaments via its translocase activity.

Materials and Methods

Yeast strains, plasmid construction, and culture conditions

All yeast strains were isogenic derivatives of *S. cerevisiae* SK1 and are listed in Table S1. Media and culture conditions regarding meiosis have been described (Sasanuma *et al.* 2008). The **DMC1**-promoter and **SRS2**-coding sequences were cloned into a *pAUR101* vector (Takara; *pYHS101*). *pYHS101* was then digested with *StuI* and integrated into the *aur1* locus in the HSY62 and HSY74 strains. Resulting transformants were validated using PCR and Southern blotting. To construct the strain in which **Srs2** was N terminally tagged with two hemagglutinin (2HA) moieties, we first deleted the **SRS2** promoter using the *KanMX6* marker (HSY543) and then integrated into

this site with the *TRP1* marker and two *HA* sequences (HSY575). The resulting *2HA-SRS2* strain exhibited normal spore viability and meiotic progression. To isolate *srs2-K41A* mutants, *Yplac211-srs2-K41A* (a gift from Dr. H. Klein) was integrated into the yeast genome and mutants were subsequently selected on 5-fluoroorotic acid (5-FOA) plates. The *srs2-Δ*(875–902) mutant was isolated via a one-step gene-replacement procedure that used a DNA fragment amplified from *pBS-srs2-Δ* (875–902) (a gift from H. Klein). To obtain an inducible strain, the native *SRS2* promoter was replaced with the *GAL1-10* promoter. Resulting transformants (*GALp-SRS2*) were crossed with HSY539, a *ura3::GPD1p-GAL4-ER::URA3* strain (Benjamin *et al.* 2003). To induce *SRS2* expression, β -estradiol (10 mM ethanol, Sigma, E8875) was added into sporulation medium (SPM) at 5 hr of meiosis (final concentration 1 μ M). For the *tid1Δ GALp-SRS2* diploid, β -estradiol (final concentration 200 nM) was added into SPS medium as the *tid1 srs2* diploid is lethal (Klein 1997). Cell precipitates were washed twice with distilled water at 30° to completely remove the β -estradiol. Cells were then suspended in sporulation medium that lacked β -estradiol. Strains with the *mnd2::kanMX6* and *CLB2p-SGS1::kanMX4* are generous gifts from Drs. Franz Klein and Neil Hunter.

For return-to-growth experiments at the *arg4* locus, cells from 0 and 5 hr of meiosis were diluted with water and plated onto SD-ARG and YPAD plates. The recombination frequency was calculated by counting the number of colonies on each plate.

Cytological analysis and antibodies

Immunostaining was conducted as described (Shinohara *et al.* 2000). Stained samples were observed using an epifluorescent microscope (BL51, Olympus with a 100 $\times$ objective (NA1.4). Images were captured by CCD camera (Cool Snap, Photometrics) at room temperature, and then processed using iVision (Sillicon) software. Pseudo-coloring was performed using Photoshop (Adobe) software. At each time point, more than 50 spreads were analyzed for focus counting. Primary antibodies directed against *Rad51* (guinea pig, 1:500 dilution), *Dmc1* (rabbit, 1:500 dilution), *Rad52* (rabbit, 1:300 dilution), and *Rfa2* (rabbit, 1:500 dilution) were used. Secondary antibodies (Alexa-fluor-488 and -594 goat, Molecular Probes) directed against primary antibodies from the different species were used at a 1:2000 dilution. Anti-*Rec8* antibody (Rao *et al.* 2011) was used at a 1:500 dilution. Anti-*Zip1* antibody (Shinohara *et al.* 2008) was used at a 1:500 dilution.

Southern, Northern, and Western blotting

Northern blotting and RNA preparation were used with previously described methods (Sasanuma *et al.* 2008). Probes were full-length *SRS2* (3525 bp) and nucleosides 881–1708 from *NDT80*.

Southern blotting was performed as described (Storlazzi *et al.* 1995; Shinohara *et al.* 2003). Genomic DNA was digested using *MluI* and *XhoI* (for CO and NCO) or *PstI*

(for meiotic DSB). Probes for Southern blotting were “pNKY155” for CO/NCO, and “pNKY291” for DSB detection (Xu *et al.* 1995). Image Gauge software (Fujifilm Co. Ltd., Japan) was used to quantify the R1, R3, and DSB-I bands.

For Western blotting, cell precipitates were washed twice with 20% (w/v) TCA and then disrupted using a bead beater (Yasui Kikai Co. Ltd., Japan) as described (Zhu *et al.* 2010). Precipitated proteins were recovered by centrifugation and then suspended in SDS-PAGE sample buffer. After adjusting the pH to 8.8, samples were incubated at 95° for 2 min and were subject to SDS-PAGE gel electrophoresis. Anti-*Srs2* (sc-11989) and Anti-*Cdc5* (sc-33625) antibodies (goat) are from Santa Cruz. Anti-*Rec8* antibody (Rao *et al.* 2011) was used at a 1:1000 dilution. A monoclonal antibody directed against the α -subunit of rat tubulin was also used (AbD Serotec, Oxford, UK).

Results

Srs2 is upregulated during meiosis

Srs2 clearly disrupts *Rad51* filaments *in vitro* (Krejci *et al.* 2003; Veaute *et al.* 2003), and the ability of *Srs2* to suppress recombination is supported by genetic experiments involving *srs2* mutant strains (Marini and Krejci 2010). Previous genetic studies (Marini and Krejci 2010) suggest that the amount of *Srs2* in mitotic cells is critical for the proper execution of recombination. The molecular function(s) of *Srs2* has not been well characterized *in vivo*, however, particularly during meiosis (Palladino and Klein 1992). As such, we first examined expression of *Srs2* during meiosis. SK1 strains that show synchronous meiosis were used in this study (Figure 1A). Northern blotting indicated that *SRS2* mRNA levels were relatively low and that its transcript was induced at 2–4 hr of meiosis (Figure S1A). These data are consistent with previous microarray data (Chu *et al.* 1998). We also analyzed *Srs2* levels by Western blotting. For these experiments, two copies of hemagglutinin (HA) were added at the N terminus of *Srs2*. The *2HA-SRS2* strain exhibited wild-type levels of spore viability and normal meiotic progression (Figure S1B). During meiosis, *2HA-Srs2* gradually accumulated, reaching maximum levels after 4–5 hr (Figure S1C); however, it is not detectable after 9 hr of meiosis when most of *Rec8* (a meiosis-specific component of cohesin) degraded. This indicates that *Srs2* disappearance occurs around the onset of meiosis I. Consistent with this result, *2HA-Srs2* does not disappear in *ndt80* mutants, which arrest at the midpachytene stage (Xu *et al.* 1995) (Figure S1D, left). *Srs2* degradation was not observed even in *ndt80 mnd2* double mutants, in which the anaphase-promoting complex/cyclosome is constitutively active during early prophase I (Oelschlaegel *et al.* 2005), indicating that the anaphase-promoting complex/cyclosome is not involved in the degradation of *Srs2* (Figure S1D, right). *Srs2* levels during meiosis were confirmed by Western blotting with an antibody directed against endogenous *Srs2* (Figure 1B).

Srs2 expression peaks at 5 hr, which is consistent with middle pachytene or meiosis I when *Cdc5*/polo kinase is induced and *Rec8* cleavage takes place (Chu *et al.* 1998).

Meiotic overexpression of *Srs2* results in dosage-dependent toxicity

During mitosis, overexpression of *Srs2* inhibits DNA repair and recombination in a dose-dependent manner (Kaytor *et al.* 1995; Mankouri *et al.* 2002; Leon Ortiz *et al.* 2011). Previous characterization of the *srs2* deletion mutant showed that *Srs2* is necessary for efficient formation of both cross-overs and noncrossovers during meiosis (Palladino and Klein 1992; Sasanuma *et al.* 2013). In addition, the *srs2* deletion mutation slightly reduces the steady levels of both *Rad51* and *Dmc1* foci (Sasanuma *et al.* 2013). These suggest a prorecombination role of *Srs2* protein in meiosis. To look for the *in vivo* antirecombination function of *Srs2* during meiosis, we constructed strains overexpressing *Srs2* that contained four copies of *SRS2*. In addition to expression from the native *SRS2* gene, *Srs2* expression was controlled by the *DMC1* promoter integrated into the *aur1* locus (*DMC1p-SRS2*). The *DMC1* promoter induces high levels of expression during the meiotic prophase (Bishop *et al.* 1992). In the *DMC1p-SRS2* strain we observed ~16-fold increase in *SRS2* mRNA expression levels during the prophase of meiosis I (Figure S1E). Induction of *Srs2* protein in the *DMC1p-SRS2* strains was confirmed by Western blotting (Figure 1C). Compared with the 0 hr of mitosis time point, an approximate fivefold increase in *Srs2* was seen at 4–8 hr of meiosis. Overexpressed *Srs2* generated a ladder on Western blotting, suggesting multiple post-translational modifications, *e.g.*, SUMOylation (Kolesar *et al.* 2012).

As reported previously (Palladino and Klein 1992; Sasanuma *et al.* 2013), the *srs2* deletion mutant exhibits reduced spore viability (36.8%), which indicates a critical role for this helicase in meiosis (Figure 1D). Spore viability of the *DMC1p-SRS2* strain was reduced to 27.4% (Figure 1D). Control cells with a marker

inserted at the *aur1* locus exhibited wild-type spore viability (98.4%), indicating that integration at this locus does not cause a relevant phenotype. Therefore, *Srs2* overexpression during meiosis inhibits the formation of viable spores. When a strain containing only one copy of the *DMC1p-SRS2* construct was examined, a mild reduction in spore viability was detected (74.7% viable), suggesting a dose-dependent effect caused by *Srs2* overexpression on meiosis (Figure 1D). The distribution of viable spores per tetrad was not biased, indicating that *Srs2* overexpression caused random lethal events in spores (Figure S2).

We next investigated the kinetics of meiotic division in *DMC1p-SRS2* cells using DAPI staining. In a control strain, meiosis I began at 4 hr of meiosis and was followed by meiosis II, which was completed at ~8 hr of meiosis (Figure 1, A and E). *DMC1p-SRS2* cells showed a 2.5-hr delay in meiosis I onset (Figure 1E) with some cells (~20%) arresting prior to meiosis I. *Srs2* overexpression was shown to delay prophase I by examining *Cdc5*/polo kinase and *Rec8* levels (Figure 1C). Upregulation of *Cdc5*/polo kinase, which normally occurs at the midpachytene stage (Clyne *et al.* 2003; Lee and Amon 2003), was delayed by ~2 hr in *DMC1p-SRS2* cells. In addition, *Rec8* degradation, which typically occurs at the onset of metaphase I (Buonomo *et al.* 2000), was delayed in *DMC1p-SRS2* cells (Figure 1C). When compared with control cells, *Srs2* overexpression also delayed meiosis II by 3 hr. When nuclear morphology in asci was examined with DAPI staining, a single spore from both *DMC1p-SRS2* and *srs2* deletion strains often contain multiple DAPI bodies (Figure 1F). In addition, as reported for a meiotic-null allele of the *SGS1* gene (Oh *et al.* 2008), which encodes a Bloom helicase necessary for multiple steps in meiotic recombination (Jessop *et al.* 2006; Oh *et al.* 2007), both *DMC1p-SRS2* and *srs2* deletion mutants show DAP staining outside of spore envelopes (Figure 1F). These suggest that proper amounts of *Srs2* are critical for chromosome segregation during meiosis.

Figure 1 Overexpression of *Srs2* protein reduces spore viability and meiotic recombination. (A) Schematic presentation on events during meiosis. (B and C) Expression pattern of *Srs2* protein in wild type (A, NKY1303/1543) and *DMC1p-SRS2* (B, HSY475/477) diploids. Western blotting analysis was conducted for whole-cell lysate prepared from meiotic diploid cells. *Rec8* and *Cdc5*/polo kinase were detected as a control for passage of meiosis I. Tubulin blot is a loading control. An amount of tubulin was slightly increased during meiosis. *Srs2* in *DMC1p-SRS2*, which is homologous for *DMC1p-SRS2* at the *aur1* locus, indicates multiple bands of post-translational modification. (D) Spore viability of various strains was measured by dissecting spores. Spores were incubated at 30° for 3 days. Each bar indicates percentage of spore viability and actual number of total dissected spores (parentheses). “*DMC1p-SRS2* hetero” and “*DMC1p-SRS2* homo” are strains heterologous and homologous for *DMC1p-SRS2* at the *aur1* locus, respectively. Wild type, NKY1303/1543; *DMC1p-SRS2* hetero, NKY1543/HSY477; *DMC1p-SRS2* homo, HSY475/477; *srs2* deletion homo, HSY310/315. (E) Meiotic cell divisions were analyzed by DAPI staining of wild type (left, NKY1303/1543) and *DMC1p-SRS2* (right, HSY475/477) cells. At least 150 cells were counted by DAPI staining for each time point. Meiosis I, open circles; meiosis II, solid circles. (F) DAPI staining of wild type, *DMC1p-SRS2*, *srs2*, and *CLB2p-SGS1* cells. After the incubation of 24 hr with SPM, cells were fixed and stained with DAPI. A representative image for each strain is shown. Arrowheads indicate DAPI bodies outside of spore membranes. Wild type, NKY1303/1543; *CLB2p-SGS1*, NHY2242; *DMC1p-SRS2* homo, HSY475/477; *srs2* deletion (*srs2* deletion homozygous), HSY310/315. (G) Schematic representation of *HIS4-LEU2* locus. Sizes of fragments for DSB and recombinant analysis are shown with lines below. (H and I) DSB and CO/NCO formation in wild type (left, NKY1303/1543) and the *DMC1p-SRS2* (right, HSY475/477) cells was analyzed by Southern blotting. DSB, bottom; CO/NCO, top. The bands of R1 (crossover, CO), R3 (noncrossover, NCO) and DSB were quantified (I). The experiments were independently performed several times and representative blots are shown. Error bars (for SD) in CO/NCO were obtained from three independent experiments. For DSB and CO/NCO assays, genomic DNAs were digested with *Pst*I and *Mlu*I and *Xho*I, respectively. In I, wild type (NKY1303/1543), open circles; *DMC1p-SRS2* (HSY475/477), solid circles.

DMC1p-SRS2 strains have defects in meiotic DSB repair

To examine the effect of *Srs2* overexpression on meiotic DSB repair, we physically characterized DSB repair and formation of recombinants (both CO and NCO) at the *HIS4-LEU2* locus, which is an artificial recombination hotspot on chromosome III (Figure 1G) (Storlazzi *et al.* 1995). In wild-type cells, DSB levels were highest at 4 hr of meiosis (~20% of the parental signal of site I; Figure 1, H and I, left graph). DSB levels then gradually decreased. In contrast, the R1 and R3 recombination products, which correspond to CO and NCO recombinants, respectively, reached maximum levels after 7 hr (Figure 1, H and I). In *DMC1p-SRS2* cells, we observed DSB accumulation and delayed resolution of these breaks (Figure 1I). Some DSBs in *DMC1p-SRS2* cells were more discrete than seen in controls. Other DSB signals, however, were extensively smeared in *DMC1p-SRS2* cells (4 hr in wild type vs. 5 hr in *DMC1p-SRS2*), suggesting defective transition of DSBs into subsequent intermediate DNA structures. Consistent with a delay in DSB repair, the appearance of R1 and R3 recombination products was delayed by 2 hr in the *DMC1p-SRS2* strain (Figure 1I). Compared with control cells, levels of R1 and R3 products in *DMC1p-SRS2* cells were diminished by 2- and 1.4-fold, respectively. These results indicate that increased levels of *Srs2* inhibit meiotic recombination. The frequency of intragenic recombination was determined by the return-to-growth assays using heteroalleles at the *ARG4* locus. Overexpression of *SRS2* from the *DMC1* promoter reduced Arg⁺ prototroph formation (*arg4-bgl/arg4-nsf*) to 49% of the wild-type frequency after 5 hr incubation with SPM ($5.9 \pm 1.5 \times 10^{-2}$ in wild type vs. $2.9 \pm 0.47 \times 10^{-2}$ in *DMC1p-SRS2*, $n = 3$), which supports the physical characterization presented above. At 0 hr, wild-type and *DMC1p-SRS2* strains show $1.2 \pm 0.72 \times 10^{-4}$ and $1.4 \pm 0.68 \times 10^{-4}$ for Arg⁺ prototroph formation, respectively. Taken together, these results suggest that a precise level of *Srs2* is required for meiotic recombination to proceed normally.

During meiotic prophase, homologous chromosomes are tightly linked to one another by a proteinaceous, zipper-like structure called the synaptonemal complex (SC). Meiotic recombination and SC assembly are related processes (Borner *et al.* 2004). Given that the *DMC1p-SRS2* strains exhibited defects in meiotic recombination, we hypothesized that these cells would also have aberrant SCs. To test this idea, *DMC1p-SRS2* cells were immunostained using a primary antibody directed against Zip1, which is a component of the SC central element (Sym *et al.* 1993). In wild-type cells, three classes of Zip1 staining were detected in chromosome spreads (Figure 2A, top). Punctate Zip1 staining (class I) was observed at early stages of meiosis (Figure 2B). These foci then expanded to form short lines (class II) and finally long lines that extended the length of the chromosome (class III). The Zip1 signal then quickly disappeared at the onset of MI. Greater than 70% of wild-type nuclei exhibited the class III Zip1 pattern at 4 hr of meiosis

(Figure 2B). In *DMC1p-SRS2* cells, however, 40% of the nuclei exhibited class I Zip1 staining at 4 hr, whereas the proportion of cells with the class III Zip1 pattern (*i.e.*, full synapsis) was significantly reduced compared with that for the control cells (30%; Figure 2B). Synapsis defect in the budding yeast is often associated with an assembly of the Zip1 aggregates referred to as a polycomplex (Sym and Roeder 1995). Formation of the Zip1 polycomplex is seen in a mutant defective in the meiotic recombination, *e.g.*, the *dmc1* mutant (Bishop *et al.* 1992). The number of *DMC1p-SRS2* cells containing a polycomplex (a Zip1 aggregate; Figure 2, A and C) (Sym *et al.* 1993) was dramatically increased to 75% (Figure 2C) compared to wild type of 5%. Interestingly, in *DMC1p-SRS2* cells, Zip1 foci formed at an earlier stage of meiosis than in wild-type cells. Consistent with these results, the loading of a chromosome axis protein, Rec8 (Klein *et al.* 1999), was observed slightly earlier in *DMC1p-SRS2* cells than in wild type (Figure 2, A and D). Finally, SCs (Zip1 and Rec8) were disassembled more slowly in *DMC1p-SRS2* than in wild-type cells (Figure 2, B and D). These results indicate that increased levels of *Srs2* compromise SC formation, an effect that may be an indirect consequence of impaired meiotic recombination.

Srs2 overexpression inhibits the formation of Rad51 foci

Srs2 functionally interacts with Rad51 during mitosis (Marini and Krejci 2010). Rad51 is known to facilitate the assembly of Dmc1 on meiotic chromosomes (Bishop 1994; Shinohara *et al.* 1997). To determine the effect of *Srs2* overexpression on Rad51 and Dmc1 assembly *in vivo*, we stained chromosome spreads for Rad51 and Dmc1 (Figure 2E). We measured kinetics of percentage of a chromosome spread with more than five foci (focus-positive nuclei; Figure 2F) as well as an average number of the foci per a focus-positive nucleus (Figure 2G). In wild-type cells, foci of Rad51 and Dmc1 were observed with levels peaking at 4 hr of meiosis as has been described (Bishop 1994; Shinohara *et al.* 1997) (Figure 2F). On average, 38.8 ± 13.0 Rad51 foci and 41.3 ± 13.6 Dmc1 foci were detected within each nuclear spread at this time point ($n = 146$; Figure 2G). In *DMC1p-SRS2* cells, the formation of Rad51 foci (positive cells) was slightly delayed compared with formation of Dmc1 foci (Figure 2F). Cells positive for Rad51 foci are slightly reduced in the strain compared to those for Dmc1 foci (Figure 2, E and F). Importantly, *DMC1p-SRS2* strain shows reduced number of Rad51 foci, but not of Dmc1 foci compared to wild type. At 4 hr of meiosis, *DMC1p-SRS2* nuclei had, on average, 10.9 ± 5.9 Rad51 foci and 42 ± 12.8 Dmc1 foci ($n = 83$; Figure 2G). The difference of Rad51 focus numbers between wild-type and *DMC1p-SRS2* strain is statistically significant (Mann-Whitney's *U*-test, $P < 0.01$). *Srs2* overexpression also delayed the turnover of these foci as they could still be detected during late meiosis. These results indicate that *Srs2* overexpression inhibits the assembly of Rad51 complexes on chromosomes, but does not significantly affect Dmc1 assembly.

Figure 2 *SRS2* overexpression leads to reduced Rad51 assembly and defective SC formation. (A) Immunostaining analysis of Zip1 (red) and Rec8 (green) for wild-type (NKY1303/1543) and *DMC1p-SRS2* (HSY475/477) strains was carried out. Representative images are shown for each strain. Bar indicates 2 μ m. (B) Zip1 staining in wild-type (left, NKY1303/1543) and *DMC1p-SRS2* (right, HSY475/477) strains was classified: dot (class I, blue), partial linear (class II, green), full SC (class III, red). More than 100 nuclei were counted at each time point. (C and D) Kinetics of Zip1-polycomplexes (C) and Rec8-positive cells (D). More than 100 nuclei were counted at each time point. Open circles, wild type (NKY1303/1543); solid circles, *DMC1p-SRS2* (HSY475/477). (E) Immunostaining analysis of Rad51 (green) and Dmc1 (red) for wild-type (NKY1303/1543) and *DMC1p-SRS2* (HSY475/477) strains was carried out. Bar indicates 2 μ m. (F) Kinetics of Rad51 (green) or Dmc1 (red)-focus-positive cells in wild type (left, NKY1303/1543) and *DMC1p-SRS2* (right, HSY475/477). A focus-positive cell was defined as a cell with more than five foci. More than 100 nuclei were counted at each time point. (G) An average number of foci of Rad51 (green) and Dmc1 (red) in wild-type (left, NKY1303/1543) and *DMC1p-SRS2* (right, HSY475/477) cells at 4 hr of meiosis. An average number of each focus is shown per a positive nucleus. Error bars show SD.

Srs2 disrupts the assembly of Rad51-containing nucleoprotein filaments

Our results demonstrate that *Srs2* overexpression reduced the number of Rad51 foci *in vivo*. As such, *Srs2* either inhibited the assembly of Rad51 complexes (a preassembly function) or disrupted Rad51 complexes once they were formed (a post-assembly function). To distinguish between these possibilities, we constructed a *mei5Δ GAL1p-SRS2* strain that expressed a Gal4-ER (estrogen receptor) fusion protein (Benjamin *et al.* 2003), thereby inducing *Srs2* expression when β -estradiol is added to the cells (Figure 3). In this *GAL1p-SRS2* strain, an endogenous promoter of the *SRS2* gene was replaced with the *GAL1/10* promoter. Therefore, in the strain, *Srs2* protein is expressed only from the *GAL1p-SRS2* locus. In addition, as *Mei5* facilitates the loading of *Dmc1* onto meiotic chromo-

somes (Hayase *et al.* 2004), the *mei5Δ* impairs recombination and leads to the accumulation of Rad51 on chromosomes (Hayase *et al.* 2004). We added β -estradiol to these cells at 5 hr of meiosis, which is after the accumulation of Rad51 foci in *mei5Δ* cells (Figure 3). The addition of β -estradiol clearly induced *SRS2* mRNA expression within 1 hr (Figure S3, A and B). Western blotting showed that 2 hr of induction had increased *Srs2* levels 40-fold compared with control levels (Figure 3A and Figure S3, C and D). In the *mei5Δ* mutant, Rad51 typically accumulates on meiotic chromosomes after 5 hr of meiosis, often forming large aggregates (Hayase *et al.* 2004) (Figure 3B). *Srs2* induction reduced the number of Rad51 foci and eliminated the large Rad51 aggregates (Figure 3B). Two hours of induction greatly reduced the number of Rad51 foci compared with those of the control cells. In fact, Rad51

Figure 3 Overexpression of Srs2 can remove Rad51 assembly on chromosomes. (A) The induction of *GALp-SRS2* during meiosis in the *mei5* cells (HSY781/783) was studied by Western blotting for Srs2 (top). β -Estradiol was added at 5 hr of meiosis. Tubulin is a loading control (bottom). (B) Nuclear spreads with (7 hr, after 2 hr induction) or without β -estradiol (5 and 7 hr) induction of the *mei5 GALp-SRS2 GAL4-BD-ER* diploid (HSY781/783) were stained with α -Rad51 (left), α -Rfa2 (middle) for RPA, and α -Rad52 (right). Merged images with DAPI (blue) are also shown (right columns of each staining). (C) Focus-positive *mei5* cells (HSY781/783) for Rad51, RPA (Rfa2), or Rad52 before and after induction of Srs2 were counted for >200 cells chosen randomly and focus-positive cells were counted at each time. Percentage of positive cells are shown. Open circles, without Srs2 induction; solid circles, with Srs2 induction by ER. (D) The number of Rad51 foci per a nuclear spread before and after induction of Srs2 were counted for randomly selected spreads and classified in the number of foci. Each bar shows percentage of cells with different number of foci for every 5 foci (0 to 60) per a spread from 0 to >60 and aggregates. "Aggregates" indicate a cell with fused foci, which are difficult to count. Open bars, without the ER induction (5, 7, and 9 hr, left three graphs); solid bars, with ER induction (7 and 9 hr, right two graphs).

foci were undetectable in 28.0% of the cells at that time (Figure 3, C and D). Although *Srs2* overexpression caused the rapid disassembly of *Rad51* complexes, residual levels of *Rad51* staining were always present. As such, there may be a *Rad51* species that is insensitive to *Srs2*, or the residual staining may represent ongoing assembly of *Rad51* complexes.

We also examined the effect of *Srs2* overexpression on the localization of *Rad52* (Shinohara and Ogawa 1998) and *Rfa2* (a subunit of the RPA complex; Figure 3, B and C). Following induction of *Srs2* expression, signal intensities associated with *Rfa2* foci were dramatically elevated (Figure 3B), suggesting that RPA was binding to sites formerly occupied by *Rad51*, which is consistent with *in vitro* results indicating that *Rad51* and RPA binding to ssDNA is competitive (Sugiyama *et al.* 1997). In contrast, the localization pattern of *Rad52*, which interacts with RPA (Shinohara *et al.* 1998), was not affected by *Srs2* overexpression, suggesting a rate-limiting event involved in the binding of *Rad52* to RPA-coated ssDNA (Miyazaki *et al.* 2004). Taken together, our results indicate that *Srs2* mediates the disassembly of *Rad51* complexes *in vivo* under the condition of overexpression of *Srs2* and can do so in the presence of *Rad51*-assembly factors, e.g., *Rad52*, *Rad54*, *Rad55*–*Rad57*, and PCSS.

Srs2* translocase activity is essential to remove *Rad51* from chromosomes *in vivo

Previous biochemical studies offered two models to explain *Srs2*-mediated removal of *Rad51* from chromosomes. Several reports suggested that the translocase activity of *Srs2* is critical for *Rad51* disassembly (Krejci *et al.* 2003; Veaute *et al.* 2003). It has also been proposed that the *Rad51*-binding domain of *Srs2* mediates *Rad51* disassembly by promoting the ATP-hydrolysis activity of *Rad51* (Antony *et al.* 2009). We used conditional induction of *Srs2* variants to distinguish between these two models. We expressed two versions of *Srs2*: (1) *Srs2*-K41A, which binds *Rad51* and lacks translocase activity (Van Komen *et al.* 2003; Krejci *et al.* 2004), and (2) *Srs2*- Δ (875–902), which contains the ATPase/helicase domain but lacks the *Rad51*-binding domain (Colavito *et al.* 2009). The induction protocol generated levels of *Srs2*-K41A and *Srs2*- Δ (875–902) that were similar to wild-type *Srs2* levels (Figure 4A and Figure S3C). As shown by Western blotting (Figure 4A and Figure S3C), expression of *Srs2*-K41A generated a more ladder of proteins than wild-type *Srs2* and *Srs2*- Δ (875–902) proteins, suggesting multiple post-translationally modified versions (e.g., SUMOylation) of *Srs2*-K41A. Expression of *Srs2*-K41A did not cause removal of *Rad51* from meiotic chromosomes (Figure 4, B and C). In contrast, *Srs2*- Δ (875–902) effectively disassembled *Rad51* complexes but exhibited decreased dismantling activity compared to wild-type *Srs2* (Figure 4, B and C). Therefore, the translocase activity of *Srs2* is key for *Rad51* disassembly *in vivo*, and the *Rad51*-binding domain is dispensable for this process.

***Srs2* specifically dismantles *Rad51* filaments**

Both *Rad51* and *Dmc1* form filamentous structures on ssDNA (Sheridan *et al.* 2008). It has been proposed that *Rad51* and *Dmc1* form completely independent nucleoprotein structures at DSB sites (Shinohara *et al.* 2000) rather than forming mixed coprotein filaments. We therefore asked if *Srs2* could dismantle *Dmc1* protein complexes. For this experiment, we incorporated an inducible *SRS2* construct into the *tid1/rdh54* strain (i.e., *tid1* Δ *GALp*-*SRS2*). Deletion of *TID1/RDH54* leads to the accumulation of both *Rad51* and *Dmc1* foci (Shinohara *et al.* 2000) (Figure 5, A and B). In the absence of β -estradiol, high levels of *Rad51* and *Dmc1* accumulated in *tid1* Δ *GALp*-*SRS2* cells (Figure 5, A and B) as reported (Shinohara *et al.* 2000). When expression of *Srs2* was induced at 5 hr of meiosis, *Rad51* foci were eliminated within 2 hr (as was seen in the *mei5* Δ mutant) (Figure 5A). *Dmc1* foci, however, remained on the meiotic chromosomes even after 2 hr of *Srs2* expression (Figure 5, A and B). The *Dmc1*-staining pattern also indicated that *Dmc1* did not expand into regions of ssDNA previously occupied by *Rad51* (i.e., once formed, *Dmc1* complexes were stable even in the absence of *Rad51* on the chromosomes). *In vivo*, therefore, *Srs2* specifically removed *Rad51* complexes from meiotic chromosomes and did not affect *Dmc1* assembly.

Discussion

Numerous studies, both *in vitro* and genetic, have firmly established that *Srs2* possesses antirecombinase activity (Marini and Krejci 2010). *In vitro*, the *Srs2* helicase acts to remove *Rad51* from ssDNAs. It is generally accepted that the translocase activity of *Srs2* is essential for its ability to dismantle *Rad51*-containing nucleoprotein filaments (Krejci *et al.* 2003; Veaute *et al.* 2003). However, a recent *in vitro* study has shown that a mutant *Srs2* that lacks the *Rad51*-binding domain, but retains the translocase domain, cannot disrupt *Rad51* filaments (Antony *et al.* 2009). This domain likely induces the ATP-hydrolytic activity of *Rad51*, which promotes *Rad51* dissociation from a chromosome (Antony *et al.* 2009). Moreover, PARI, the PCNA-associated recombination inhibitor, which is the human homolog of *Srs2*, lacks ATP-hydrolytic/helicase activities but can still disrupt *Rad51* filaments *in vitro* (Moldovan *et al.* 2012). These studies suggest a noncatalytic, stoichiometric role for *Srs2* in activating the intrinsic ATPase activity of *Rad51*, thereby forming ADP-bound *Rad51*, which has a weaker affinity for ssDNA than does ATP-bound *Rad51*.

There is also *in vivo* evidence to support the role for *Srs2* in regulating assembly of *Rad51* filaments. Cytological studies of an *srs2* deletion strain indicated that ionizing radiation (IR)-induced *Rad54*-GFP foci increase in the *srs2* mutant relative to wild-type cells (Burgess *et al.* 2009), suggesting a postassembly role for *Srs2* in regulating *Rad51* filaments. However, other scenarios can explain the persistence of these *Rad54*-GFP foci. This might be simply due to delayed repair events. Moreover, overexpression of *Srs2* decreases the *Rad54*-

Figure 4 Srs2-K41A protein is defective in dismantling of Rad51 *in vivo*. (A) The induction of *GALp-SRS2* (HSY781/783), mutant *GALp-srs2-K41A* (HSY1086/1088), and *GALp-srs2-Δ(875-902)* (HSY1064/1065) during meiosis in the *mei5* cells was studied by Western blotting. β-Estradiol (ER) was added at 5 hr of meiosis. Tubulin is a loading control. Srs2-K41A protein indicates more multiple bands of post-translational modification than wild-type Srs2 and Srs2-Δ(875-902) proteins. (B) Nuclear spreads with β-estradiol (+ER) or without β-estradiol (–ER) addition in *GALp-SRS2* (HSY781/783), mutant *GALp-srs2-K41A* (HSY1086/1088), and *GALp-srs2-Δ(875-902)* (HSY1064/1065) strains with the *mei5* mutation were stained with α-Rad51 (green) and DAPI (blue). Images after 2 hr induction of the proteins (7 hr in meiosis) are shown. (C) The number of Rad51 foci per a nuclear spread in overexpression of wild-type Srs2, Srs2-K41A, and Srs2-Δ(875-902) mutant proteins were counted for randomly selected spreads and classified in the number of foci. More than 100 nuclei were counted and the percentage of each class is shown as a graph. Open bars, without the induction; solid bars, with Srs2 induction.

GFP that binds to DSBs (Burgess *et al.* 2009), which indirectly supports this idea that Srs2 disrupts Rad51 on the mitotic DSB site, given that Rad54 binds to Rad51 ensembles (Colavito *et al.* 2009). For this study, we combined cytological characterization of chromosome spreads and genetic overexpression of Srs2 during meiosis to demonstrate that Srs2 could disrupt Rad51 filaments on chromosomes *in vivo*. This activity is specific for Rad51, as Srs2 did not disrupt RPA, Rad52, or Dmc1 foci. Furthermore, we found that the translocase activity of Srs2, rather than the Rad51-binding domain, was critical for the *in vivo* disruption of Rad51 filaments.

Srs2 removes Rad51 from chromosomes during meiotic recombination

Various factors positively and negatively regulate dynamics of Rad51 filaments, key protein ensembles for homology

search, and strand exchange (Krogh and Symington 2004). It is known that the Srs2 helicase plays a positive and negative role in the recombination. SRS2 deletion (Palladino and Klein 1992) and, as we have shown, Srs2 overexpression result in delays in DSB repair and recombination during meiosis, indicating that a precise amount of Srs2 is necessary for meiotic recombination to proceed normally, which is a conclusion consistent with previous studies that showed that different dosages of Srs2 are required during mitosis to repair damaged DNA and for recombination (Kaytor *et al.* 1995; Leon Ortiz *et al.* 2011).

We have now shown that Srs2 overexpression during meiosis disrupts the assembly of Rad51 filaments, whereas preexisting Dmc1 filaments are not affected. This is the first *in vivo* evidence that Srs2 can remove Rad51 filaments from

Figure 5 Dmc1 assembly is resistant to overexpression of Srs2. (A) Nuclear spreads with or without β -estradiol (ER) induction of wild-type Srs2 protein in the *tid1* deletion cells (*tid1 GALp-SRS2 GAL4-DB-ER*; HSY775/777) were stained with α -Rad51 (green) and α -Dmc1 (red). Images after 2-hr induction of the proteins (7 hr in meiosis) are shown. (B) The number of Rad51 and Dmc1 foci per a nuclear spreads of *tid1 GALp-SRS2* mutant (HSY775/777) cells were counted for randomly selected spreads and classified in the number of foci. Open bars, without the induction (5 and 7 hr); solid bars, with Srs2 induction (7 hr).

meiotic chromosomes as was clearly illustrated in mutants that exhibit impaired meiotic recombination (i.e., *mei5* and *tid1*). Overexpression of *Srs2* in wild-type cells also resulted in reduced levels of Rad51 foci (Figure 2). In *mei5* Δ or *tid1* Δ mutants, *Srs2* overexpression effectively removed Rad51 foci. It is likely that Rad51 filaments are particularly fragile in these contexts as factors that positively regulate Rad51 assembly are limited in number. Alternatively, *Srs2* may have access to Rad51-coated ssDNA because recombination is stalled. Interestingly, 2 hr after induction of *Srs2* expression, the Rad51 signal was completely eliminated from >25% of these cells. Once bound to DNA, *Srs2* efficiently removed Rad51 (including recombination-competent Rad51 foci) from DSB sites, suggesting that *Srs2* loading is rate limiting for its antirecombinase function.

Consistent with this suggestion, Rothstein and colleagues (Burgess *et al.* 2009) recently demonstrated that during mitosis the *in vivo* depletion of *Srs2* permits Rad54, thus possibly Rad51, loading onto chromatin, even in the absence of Rad52, indicating that *Srs2* depletion reduces the requirement for Rad52. Moreover, the deletion of the *SRS2* gene in mitosis suppresses DNA damage defect of the *rad55* or *rad57* mutants (Liu *et al.* 2011). The *PCSS/SHU* (*PSY3*, *CSM2*, *SHU1*, and *SHU2*) genes are also known to antagonize the *Srs2* function (Bernstein *et al.* 2011). However, deletion of *SRS2* does not dramatically suppress meiotic defects associated with the *rad55* mutation or mutations in components of the PCSS complex (Sasanuma *et al.* 2013). The assembly of Rad51 onto chromatin during meiosis, therefore, is more tightly regulated than during vegetative growth.

By characterizing the behavior of *Srs2*-K41A, we demonstrated that ATP-binding/hydrolysis was required for *Srs2* to displace *Rad51* *in vivo*. Surprisingly, the *Rad51*-binding domain of *Srs2* is less important *in vivo* than *in vitro* for this *Srs2* function. Therefore, the translocation of *Srs2* along ssDNA is probably necessary for disruption of *Rad51* complexes by the *Srs2* helicase. Given that the dismantling activity of *Srs2* is specific to *Rad51*, other *Srs2* domains may mediate this specificity.

Srs2* functions at postassembly stage of *Rad51

Previous studies showed that the *srs2* deletion mutant reduces both CO and NCO formation during meiosis (Sasanuma *et al.* 2013), suggesting a role of *Srs2* for efficient interhomolog recombination. Importantly, the mutant delays DSB repair with normal assembly of *Rad51*/*Dmc1* complexes on the chromosomes (Sasanuma *et al.* 2013). Thus, it is likely that *Srs2* helicase plays a role after the assembly of *Rad51*/*Dmc1*. This is consistent with the fact that the *srs2* mutation is synthetically lethal with a mutation of the *RAD54*, which acts in a late stage of the recombination, and that the *rad51* mutation can suppress the lethality of the *rad54 srs2* (Klein 2001). Moreover, the lethality of the *srs2* with the *sgs1* mutation is also suppressed by early recombination defects, *e.g.*, the *rad51* mutation. *Sgs1* has multiple functions including the dissolution of double Holliday structure into NCO products (Wu and Hickson 2003). In this study, overexpression of *Srs2* also decreases CO and NCO formation. Given the reduced *Rad51* function by the *Srs2* overexpression, the interhomolog recombination is channeled into the intersister recombination, resulting in reduced recombination. The reduced *Rad51* assembly in the mutant of *Rad51* mediators (*Rad52*, *Rad55–57*, and *PCSS*) in meiosis leads to an increase of intersister recombination (Schwacha and Kleckner 1997; Lao *et al.* 2008; Sasanuma *et al.* 2013). Recently, it is shown that *Rad55–57* protects *Rad51* filament from the *Srs2* (Liu *et al.* 2011). Taken together, these suggest that *Srs2* has at least two functions in meiotic recombination: a regulatory role for dynamics of *Rad51* assembly/disassembly (antirecombination) and a post-synaptic role for the processing of recombination intermediates (prorecombination).

Several DNA helicases suppress CO formation by disrupting the formation of D-loops by *Rad51*. These helicases include *Sgs1* in budding yeast (Blm in humans), FancM orthologs (*Mph1*) in various species, and RecQ5 and Rtel-1 in nematodes and humans (Shinohara *et al.* 2003; Hu *et al.* 2007; Prakash *et al.* 2009; Youds *et al.* 2010). These helicases promote the synthesis-dependent strand-annealing pathway, which results in NCO events. A similar role has been proposed for *Srs2*. Overexpression of *Srs2* during meiosis, however, decreased the formation of both CO and NCO events. Therefore, *Srs2* does not seem to function as either an anti-CO or a pro-NCO factor (at least during meiotic recombination). We speculate that *Srs2* controls various steps of the general recombination process.

***Srs2* affects *Rad51*- but not *Dmc1*-containing filaments**

The loading of *Rad51* and *Dmc1* onto meiotic chromosomes is likely regulated by distinct mechanisms (Bishop 1994; Shinohara *et al.* 1997), and we have now shown that *Srs2* negatively regulates the assembly of *Rad51* complexes but does not affect those of *Dmc1*. If co-complexes of *Dmc1* and *Rad51* were to form on ssDNA, *Srs2* overexpression would likely have affected assembly of both protein complexes. However, neither a reduction nor an increase in *Dmc1* foci was observed after *Srs2* overexpression. *Rad51* helps load *Dmc1* onto chromosomes (Bishop 1994; Shinohara *et al.* 1997; Gasior *et al.* 1998), and once loaded, *Dmc1* complexes are stably bound even in the absence of *Rad51*. At nonrecombinogenic sites, *Tid1/Rdh54* displaces *Dmc1* but not *Rad51* (Holzen *et al.* 2006), suggesting that *Tid1/Rdh54* specifically regulates *Dmc1*. Taken together, these results strongly suggest that *Rad51* and *Dmc1* form distinct protein complexes at recombination sites during meiosis. These distinct nucleoprotein filaments, along with their distinct regulatory networks, are key features of interhomolog recombination (Sheridan and Bishop 2006).

In conclusion, as described here, our cytological analysis combined with controlled expression of recombination regulators is a useful tool with which to dissect molecular functions of the protein *in vivo*.

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Supporting Information

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Remodeling of the Rad51 DNA Strand-Exchange Protein by the Srs2 Helicase

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Figure S1 Expression of Srs2 during meiosis.

- (A) *SRS2* and *NDT80* transcripts during meiosis in wild type cells (NKY1303/1543) were analyzed by Northern blotting. Blots for *SRS2* and *NDT80* as well as Ethidium bromide-stained gel image for loading control are shown (left). Quantification of signals on blots is shown in right. Relative intensities of amounts of *SRS2* mRNA at each time point are normalized against that at 0 hr and shown.
- (B) Meiosis progression of 2*HA-SRS2* strain (HSY560/562) was analyzed by DAPI staining. At least 150 cells were counted for each time point. Meiosis I, open circles; meiosis II, closed circles.
- (C) Expression of 2*HA-Srs2*, Rec8 proteins and tubulin during meiosis of 2*HA-SRS2* cells (HSY560/562) was analyzed by western blotting. Rec8 shows slower migrating species during meiosis by phosphorylation. An asterisk indicates non-specific signals.

- (D) Expression of HA-Srs2 protein during meiosis of the *ndt80* (HSY611/613) and *ndt80 mnd2* (HSY685/686) mutant cells were analyzed by western blotting. Rec8 and tubulin were analyzed as a control.
- (E) *SRS2* and *NDT80* transcripts during meiosis in *DMC1p-SRS2* cells (HSY475/477) were analyzed by Northern blotting. Blots for *SRS2* and *NDT80* as well as Ethidium bromide-stained gel image are shown (left). Quantification of signals on blots is shown in right. Relative intensities of amounts of *SRS2* mRNA at each time point are normalized against that at 0 hr and shown.

Figure S2 Overexpression of Srs2 reduces spore viabilities.

Distribution of number of viable spores per a tetrad is shown for wild type (left; NKY1303/1543), *DMC1p-SRS2* heterozygous (middle; NKY1303/HSY477) and homozygous (right; HSY474/477) cells. 96 tetrads were dissected for each strain.

Figure S3 Over-expression of Srs2 during meiosis.

(A, B) *SRS2* transcripts during meiosis in *mei5 GALp-SRS2* cells (HSY781/783) after the addition of β -estradiol at 5 hours of meiosis were analyzed by Northern blotting. Blots for *SRS2* as well as Ethidium bromide-stained gel image are shown (A). Quantification of *SRS2* signals on blots is shown in (B). Relative intensities of amounts of *SRS2* mRNA at each time point are normalized against that at 0 hr and shown.

(C) Induction of Srs2 protein in various strains was verified by western blotting. As a control, an amount of α -tubulin is also studied. Wild type, NKY1303/1543; *srs2*, HSY310/315; *GALp-SRS2*, HSY781/783; *GALp-srs2-K41A*, HSY1086/1088; *GALp-srs2-Δ(875-902)*; HSY1064/1065.

(D) Relative amounts of wild type Srs2 as well as tubulin were studied by western blotting after serial dilutions. Two-folds cell lysates from wild type cells (NKY1303/1543) at 4 hr (left lane) relative those from *GALp-SRS2* (HSY781/783) cells at 8 hr (3 hr induction, right lane) were analyzed.

Table S1 Strain list

Name	Genotype
NKY1303	<i>MATa ho::LYS2 ura3 leu2::hisG lys2 his4B-LEU2, arg4-bgl</i>
NKY1543	<i>MATα ho::LYS2 ura3 leu2::hisG, lys2 his4X-LEU2::BamHI-URA3 arg4-nsp</i>
HSY475	<i>MATα ho::LYS2 DMC1p-SRS2::aur, his4B-LEU2 arg4-bgl ho::LYS2 ura3 leu2::hisG lys2</i>
HSY477	<i>MATa ho::LYS2 DMC1p-SRS2::aur1 his4XLEU2-URA3 arg4-nsp ho::LYS2 ura3 leu2::hisG lys2</i>
HSY560	<i>MATa ho::LYS2 ho::LYS2 2HA-SRS2::TRP1 arg4-bgl ho::LYS2 ura3 leu2::hisG lys2</i>
HSY562	<i>MATα ho::LYS2 2HA-SRS2::TRP1 arg4-nsp ho::LYS2 ura3 leu2::hisG lys2</i>
HSY310	<i>MATa ho::LYS2 srs2::TRP1 his4B-LEU2</i>
HSY315	<i>MATα ho::LYS2 srs2::TRP1 his4X-LEU2::BamHI-URA3</i>
HSY516	<i>MATa ho::LYS2 pAUR101::aur1 his4B-LEU2 arg4-bgl ho::LYS2 ura3 leu2::hisG lys2</i>
HSY520	<i>MATα ho::LYS2 pAUR101::aur1 his4X-LEU2::BamHI-URA3 arg4-nsp ho::LYS2 ura3, leu2::hisG lys2</i>
HSY781	<i>MATa ho::LYS2 mei5::URA3 ura3::GPD1p-GAL4.ER::URA3 srs2Δ::GALp-SRS2::kanMX6 ho::LYS2 ura3 leu2::hisG, lys2</i>
HSY783	<i>MATα ho::LYS2 mei5::URA3 ura3:: GPD1p-GAL4.ER::URA3 srs2Δ:: kanMX6-GALp-SRS2 ho::LYS2 ura3 leu2::hisG lys2</i>
HSY775	<i>MATa ho::LYS2 tid1::LEU2 ura3:: GPD1p-GAL4.ER::URA3 srs2Δ:: kanMX6-GALp-SRS2 ho::LYS2 ura3 leu2::hisG lys2</i>
HSY777	<i>MATα ho::LYS2 tid1::LEU2 ura3::GPD1p-GAL4.ER::URA3 srs2Δ:: kanMX6- GALp-SRS2 ho::LYS2, ura3 leu2::hisG lys2</i>
HSY1086	<i>MATa ho::LYS2 mei5::URA3 ura3::GPD1p-GAL4.ER::URA3 srs2Δ:: kanMX6-GALp-srs2-K41A ho::LYS2 ura3 leu2::hisG lys2</i>
HSY1088	<i>MATα ho::LYS2 mei5::URA3 ura3::GPD1p-GAL4.ER::URA3 srs2Δ::kanMX6-GALp-srs2-K41A ho::LYS, ura3 leu2::hisG lys2</i>
HSY1064	<i>MATa ho::LYS2 mei5::URA3 ura3::GPD1p-GAL4.ER::URA3 srs2Δ::kanMX6-GALp-srs2-Δ(875–902)::hygMX6 ho::LYS2 ura3, leu2::hisG lys2</i>
HSY1065	<i>MATα ho::LYS2 mei5::URA3 ura3::GPD1p-GAL4.ER::URA3 srs2Δ:: kanMX6-GALp-srs2-Δ(875–902)::hygMX6 ho::LYS ura3 leu2::hisG lys2</i>
HSY611	<i>MATa ho::LYS2 2HA-SRS2::TRP1 ndt80::LEU2 leu2::hisG lys2</i>
HSY613	<i>MATα ho::LYS2 2HA-SRS2::TRP1 ndt80::LEU2 leu2 leu2::hisG lys2</i>
HSY685	<i>MATa ho::LYS2 2HA-SRS2::TRP1 ndt80::LEU2 mnd2::kanMX6 leu2::hisG lys2</i>
HSY586	<i>MATα ho::LYS2 2HA-SRS2::TRP1 ndt80::LEU2 mnd2::kanMX6 leu2::hisG lys2</i>
NHY2242	<i>MATa/α HIS4-LEU2-(BamHI)/his4X-LEU2-(NgoMV)I-URA3 pCLB2-SGS1::kanMX4/pCLB2-SGS1::kanMX4</i>