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The Mei5-Sae3 Protein Complex Mediates Dmc1 Activity in *Saccharomyces cerevisiae*[*] [S]

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Susan R. Ferrari[‡§], Jennifer Grubb[§], and Douglas K. Bishop[‡§¶‖1]

From the [‡]Committee on Cancer Biology, [¶]Committee on Genetics, [‖]Department of Molecular Genetics and Cell Biology, and [§]Department of Radiation and Cellular Oncology, University of Chicago, Chicago, Illinois 60637

During homologous recombination, a number of proteins cooperate to catalyze the loading of recombinases onto single-stranded DNA. Single-stranded DNA-binding proteins stimulate recombination by coating single-stranded DNA and keeping it free of secondary structure; however, in order for recombinases to load on single-stranded-DNA-binding protein-coated DNA, the activity of a class of proteins known as recombination mediators is required. Mediator proteins coordinate the handoff of single-stranded DNA from single-stranded DNA-binding protein to recombinase. Here we show that a complex of Mei5 and Sae3 from *Saccharomyces cerevisiae* preferentially binds single-stranded DNA and relieves the inhibition of the strand assimilation and DNA binding abilities of the meiotic recombinase Dmc1 imposed by the single-stranded DNA-binding protein replication protein A. Additionally, we demonstrate the physical interaction of Mei5-Sae3 with replication protein A. Our results, together with previous *in vivo* studies, indicate that Mei5-Sae3 is a mediator of Dmc1 assembly during meiotic recombination in *S. cerevisiae*.

During meiosis, recombination between homologous chromosomes ensures proper segregation into haploid products. Recombination events are initiated by the formation of double strand breaks (DSBs)[2] in DNA (1). This is followed by resection of free DNA ends to yield 3′ single-stranded tails, upon which recombinase assembles to form nucleoprotein filaments. Following recombinase assembly, the nucleoprotein filament engages a donor chromatid, searches for homologous DNA sequences on that chromatid, and promotes strand exchange to yield a heteroduplex DNA intermediate often referred to as a joint molecule. Although recombinase alone is capable of promoting homology search and strand exchange *in vitro*, genetic and biochemical studies have demonstrated that normal recombinase function *in vivo* requires the activity of a number of accessory factors (2). These factors enhance the assembly of nucleoprotein filaments, target capture, homology search, and dissociation of recombinase from duplex DNA.

Most eukaryotes possess two recombinases, both homologues of the *Escherichia coli* recombinase RecA: Rad51, which is the major recombinase in mitotic cells and is also important during meiotic recombination, and Dmc1, which functions only in meiosis. Dmc1 and Rad51 have been shown to assemble at DSBs by immunofluorescence and chromatin immunoprecipitation (3–6), and both proteins oligomerize on single-stranded DNA (ssDNA) to form nucleofilaments that catalyze strand invasion (7–9).

A number of biochemical studies have defined the role of accessory factors in stimulating the activity of Rad51 (10–12). Replication protein A (RPA), the yeast ssDNA-binding protein (SSB), removes secondary structure in ssDNA that otherwise prevents formation of fully functional nucleoprotein filaments (13). Both Rad52 protein (11, 12) and the heterodimeric protein Rad55/Rad57 (14) can overcome the inhibitory effect of RPA on Rad51 nucleoprotein filament formation in purified systems, mediating a handoff between RPA and Rad51. It is thought that the mechanism for the mediator activity of Rad52 involves Rad52 recognizing and binding to RPA-coated ssDNA, where it provides nucleation sites for the recruitment of free molecules of Rad51 (15). The tumor suppressor protein BRCA2 also serves as an assembly factor for Rad51 during mitosis in a variety of species that encode orthologues of this protein, including mice (16), corn smut (17), and humans (18).

The meiosis-specific recombinase Dmc1 is stimulated by a distinct set of accessory factors. Immunostaining studies suggest that the Rad51 mediators Rad52 and Rad55/Rad57 are not required for assembly of Dmc1 foci *in vivo*, although Rad51 itself promotes Dmc1 foci (19–21). More recently, immunostaining and chromatin immunoprecipitation experiments demonstrated a role for the Mei5 and Sae3 proteins of *Saccharomyces cerevisiae* in assembly of Dmc1 at sites of DSBs *in vivo* (22, 23). Consistent with these observations, *mei5* and *sae3* mutants display markedly similar meiotic defects as compared with *dmc1* mutants, including defects in sporulation, spore viability, crossing over, DSB repair, progression through meiosis, and synaptonemal complex formation (19, 22–24). Finally, the three proteins have been shown to physically interact; Mei5 and Sae3 have been co-purified and co-immunoprecipitated, and an N-terminal portion of Mei5 has been shown to interact with Dmc1 in a two-hybrid assay (22).

The fission yeast *Schizosaccharomyces pombe* encodes two proteins, Swi5 and Sfr1, which share sequence homology with Sae3 and Mei5, respectively (22). Swi5 and Sfr1 have been shown to stimulate the strand exchange activity of Rhp51 (the *S. pombe* Rad51 homologue) and Dmc1 (25). Although some results indicate functional similarity of Swi5-Sfr1 and Mei5-Sae3, there are also clear differences. The Mei5-Sae3 complex

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental "Experimental Procedures" and two supplemental figures.

[1] To whom correspondence should be addressed: University of Chicago, Cummings Life Science Center, 920 E. 58th St., Chicago, IL 60637. Tel.: 773-702-9211; Fax: 773-834-9064; E-mail: dbishop@midway.uchicago.edu.

[2] The abbreviations used are: DSB, double strand break; SSB, single-stranded DNA-binding protein; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; RPA, replication protein A; RF II, replicative form II; EMSA, electrophoretic mobility shift assay; nt, nucleotides; DTT, dithiothreitol; BSA, bovine serum albumin.

of budding yeast is expressed solely during meiosis, and no mitotic phenotypes have been reported for *mei5* or *sae3* mutants (22, 24, 26). In contrast, the Swi5-Sfr1 complex of fission yeast is expressed in mitotic and meiotic cells, and mutations in *SWI5* have been shown to cause defects in mitotic recombination (27). Furthermore, although *mei5* and *sae3* mutants are phenotypically similar to *dmc1* mutants, *swi5* and *sfr1* mutants display more severe meiotic defects during fission yeast meiosis than do *dmc1* mutants (27–29). These data suggest that although Swi5-Sfr1 clearly contributes to Rad51 activity in fission yeast, it is possible that the activity of Mei5-Sae3 is restricted to stimulating Dmc1 in budding yeast.

In this study, a biochemical approach is used to test the budding yeast Mei5-Sae3 complex for properties expected of a recombinase assembly mediator. We show that Mei5-Sae3 binds both ssDNA and double-stranded DNA (dsDNA) but binds ssDNA preferentially. We also show that Mei5-Sae3 can overcome the inhibitory effects of RPA on the ssDNA binding and strand assimilation activities of Dmc1. Finally, we show that Mei5-Sae3 and RPA bind one another directly. These results indicate that Mei5-Sae3 acts directly as a mediator protein for assembly of Dmc1.

EXPERIMENTAL PROCEDURES

DNA Substrates—The plasmid pRS306 has previously been described (30). Preparation of 531-nt ssDNA substrates is detailed in the supplemental materials. Bacteriophage ϕX virion and RF II DNA were purchased from New England Biolabs.

Protein Expression and Purification—Expression and purification of all proteins used in these assays are detailed in the supplemental materials.

Electrophoretic Mobility Shift Assay (EMSA)—Mei5-Sae3 was incubated with 10 μM ϕX virion (ssDNA) or RF II (relaxed dsDNA) in EMSA buffer (20 mM HEPES, pH 7.5, 1 mM DTT, 1 mM MgOAc, 2 mM ATP) for 5 min at 37 °C. DNA-protein complexes were resolved on a 0.8% agarose gel in 1× TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 7.5) and detected by staining with SYBR Gold (Invitrogen).

Strand Assimilation Assays—RPA (0.033 μM) was preincubated with ^{32}P-labeled 531-nt ssDNA substrate (1 μM nt) in Strand Assimilation buffer (20 mM HEPES, pH 7.5, 1 mM DTT, 1 mM $CaCl_2$, 2 mM ATP), after which Mei5-Sae3 and finally Dmc1 (0.3 μM) were added. Following preincubation, pRS306 (7.9 μM bp) was added along with sufficient calcium chloride to give a final concentration of 10 mM. The reactions were incubated at 37 °C and stopped by the addition of SDS to a final concentration of 0.08%. After digestion with proteinase K, DNA from reaction mixtures was resolved on a 1.2% agarose gel in 1× TAE buffer. Gels were dried on Whatman paper and analyzed by phosphorimaging.

DNA Pulldown Assay—The magnetic bead-bound 531-nt ssDNA substrate described above (1 μM nt) was incubated with RPA (0.2 μM), Mei5-Sae3 (0.03 μM-0.5 μM), and/or Dmc1 (0.08 μM) in DNA Binding buffer (20 mM HEPES, pH 7.5, 2 mM ATP, 150 mM KCl, 1 mM DTT, 1 mM MgOAc, 0.375 mg/ml BSA). After the addition of Dmc1, MgOAc was added to a final concentration of 10 mM. The beads were collected and washed twice with Wash buffer (20 mM HEPES, pH 7.5, 150 mM KCl, 1 mM DTT, 1 mM MgOAc, 0.375 mg/ml BSA, 0.01% Nonidet P-40 (v/v)) before being resuspended in SDS sample buffer and boiled. The proteins associated with the supernatant and with the beads were analyzed by SDS-PAGE and Western blot against RPA and Dmc1.

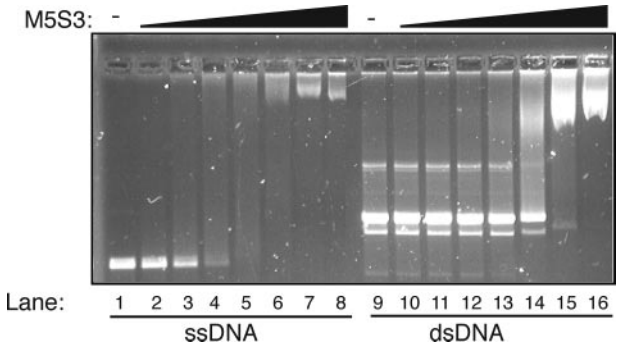

FIGURE 1. **DNA binding activity of Mei5-Sae3 assayed by electrophoretic mobility shift.** 10 μM ϕX virion (ssDNA) *lanes 1–8* or 10 μM ϕX RF II (dsDNA) *lanes 9–16* was mixed with varying concentrations of Mei5-Sae3 as follows: 0 μM (*lanes 1 + 9*), 0.04 μM (*lanes 2 + 10*), 0.08 μM (*lanes 3 + 11*), 0.16 μM (*lanes 4 + 12*), 0.32 μM (*lanes 5 + 13*), 0.625 μM (*lanes 6 + 14*), 1.25 μM (*lanes 7 + 15*), and 2.5 μM (*lanes 8 + 16*). Bound and unbound DNA was resolved on an agarose gel.

Immunoprecipitation Assays—Protein-G-coupled magnetic beads (DYNAL) were bound to anti-FLAG (Sigma), anti-RFA2, or anti-Dmc1 antibodies per the manufacturer's instructions in Buffer D (1× phosphate-buffered saline, 0.1% BSA). Mei5-Sae3, RPA, and/or Dmc1 were incubated in Strand Assimilation buffer for 5 min at 37 °C, after which they were added to the bead-bound antibodies and incubated overnight at 4 °C with rotation. The beads were washed once with Buffer D with 0.5 M NaCl and five times with Buffer D. After the final wash, the remaining protein was recovered by boiling beads in SDS sample buffer. Fractions were then analyzed by SDS-PAGE and Western blotting.

RESULTS AND DISCUSSION

Purification of Mei5-Sae3—Our initial attempts to purify Mei5 following expression in *E. coli* yielded insoluble protein (data not shown), as reported previously (22). Therefore, we used the pET-Duet system (Novagen) to co-express Mei5 and Sae3. A hexahistidine tag was added to the N terminus of Mei5, and a FLAG tag was added to the C terminus of Sae3 to facilitate purification of the proteins over affinity media. Tandem T7 expression of His_6-Mei5 and Sae3-FLAG yielded soluble protein, which was purified by affinity chromatography on nickel-Sepharose 6 Fast Flow (GE Healthcare) followed by anion exchange (supplemental Fig. 1). Sae3 co-purified with Mei5 on nickel-Sepharose (supplemental Fig. 1, *lane Ni*), whereas Mei5 co-purified with Sae3 on α-FLAG resin (data not shown) and co-immunoprecipitated with Sae3 (see Fig. 3*A*, *top panel*), consistent with a direct interaction between Mei5 and Sae3.

DNA Binding Activity of Mei5-Sae3—Mei5-Sae3 had previously been shown to form DSB-dependent foci (22, 23), suggesting that it might bind directly to DNA. To test this possibility, we used an EMSA. We incubated ϕX virion (ssDNA, 10 μM nt) or ϕX RF II (dsDNA, 10 μM bp) with a range of concentrations of Mei5-Sae3 and resolved protein-DNA structures by

agarose gel electrophoresis (Fig. 1). We found that Mei5-Sae3 reduced the mobility of both ssDNA and dsDNA. The binding to ssDNA was observed at lower concentrations than for dsDNA, with the mobility of about half of the ssDNA shifted at 0.16 μM Mei5-Sae3 (*lane 4*) and half the dsDNA shifted between 0.625 and 1.25 μM Mei5-Sae3 (*lanes 14* and *15*). These results indicate that Mei5-Sae3, like other mediator proteins (14, 31, 32), displays a preference for binding ssDNA over dsDNA.

Inhibition of the Strand Assimilation Activity of Dmc1 by RPA and Restoration by Mei5-Sae3—The homology-dependent incorporation of ssDNA into homologous duplex DNA is an essential feature of homologous recombination, and this reaction can be catalyzed *in vitro* by recombinases. The ability of Mei5-Sae3 to act as a mediator for Dmc1 was tested in the strand assimilation or "D-loop" assay (8). The product of the assimilation of a radiolabeled ssDNA into a homologous target is termed a D-loop and can be deproteinized, resolved with agarose gel electrophoresis, and visualized by phosphorimaging. We first asked whether RPA inhibits the ability of Dmc1 to catalyze the formation of D-loops. RPA was titrated into reaction mixtures at the filament formation step, allowing it to bind the single-stranded substrate before the addition of Dmc1. This accurately recapitulates the order of protein binding *in vivo*, as RPA has been shown to localize to sites of DSBs before recombinase assembly (33, 34). Once RPA binds DNA, recombinases are unable to displace it unless mediator proteins are present (11, 12, 14, 35). The addition of RPA inhibited the production of Dmc1-dependent D-loop products in a concentration-dependent manner, with full inhibition occurring at a concentration of 0.033 μM RPA (supplemental Fig. 2, *lane 6*). Given that the binding site size of a single RPA heterotrimer is ~45 nt (36) and the dissociation constant for RPA from ssDNA is in the range of 10^{-9} M (37), the concentration of RPA required for full inhibition corresponds to the minimal amount of RPA required to fully coat the ssDNA substrate. Having established the concentration of RPA necessary to inhibit D-loop formation, we sought to restore D-loop formation by adding Mei5-Sae3 (Fig. 2*A*). Mei5-Sae3 was added after the addition of RPA but before the addition of Dmc1. Under these conditions, Mei5-Sae3 counteracted the inhibitory activity of RPA. At optimal concentration (Fig. 2*A*, *lane 10*), Mei5-Sae3 stimulated the yield of D-loops 6–10-fold over the level seen in the presence of both Dmc1 and RPA and 2-fold over the level seen in the presence of Dmc1 alone (Fig. 2*A*, *lane 4*). Maximum stimulation of the D-loop activity of Dmc1 was observed at 0.125 μM Mei5-Sae3: a molar ratio of ~1 Mei5-Sae3 to 2.4 Dmc1p. Under these conditions, Mei5-Sae3 did not stimulate the ability of Dmc1 to promote strand assimilation of the 531-nt ssDNA substrate in the absence of RPA; in fact, the addition of Mei5-Sae3 to the reaction in the absence of RPA inhibited the D-loop activity of Dmc1. However, additional experiments assessing the ability of Mei5-Sae3 to stimulate Dmc1-mediated assimilation of a shorter (55 nt) ssDNA indicated that Mei5-Sae3 did not significantly affect the ability of Dmc1 to catalyze D-loop formation (data not shown). This finding may reflect a greater tendency of the 531-nt substrate to form secondary structures in the absence of RPA. Any paired regions formed in the long substrate could be bound by Mei5-Sae3 or Dmc1, forming a complex incapable of assimilation. Alternatively, regions of secondary structure could prevent the formation of plectonemic joints between the ssDNA and the target plasmid given that previous studies suggest that paranemic joints do not survive the deproteinization step of the strand assimilation assay (38). The fact that maximal stimulation of the strand assimilation of Dmc1 by Mei5-Sae3 requires the presence of RPA is consistent with the critical role of RPA in homologous recombination.

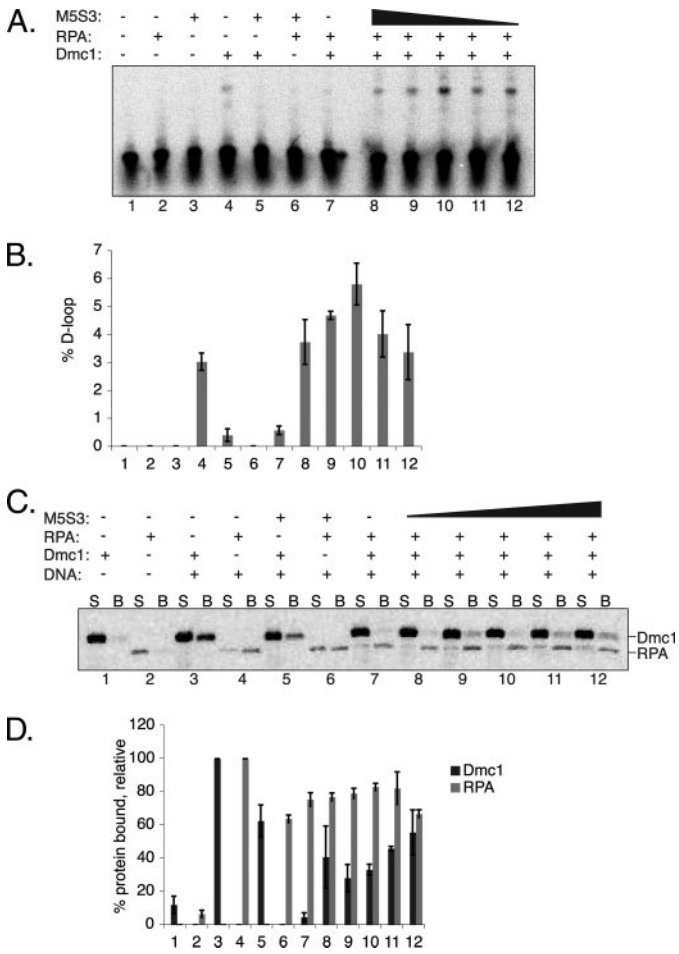

FIGURE 2. **Relief of RPA-mediated inhibition of Dmc1 by Mei5-Sae3.** *A*, strand assimilation assays using a 531-nt ssDNA substrate as follows: reaction containing no protein (*lane 1*), 0.033 μM RPA alone (*lane 2*), 0.5 μM Mei5-Sae3 alone (*lane 3*), 0.3 μM Dmc1 alone (*lane 4*), Mei5-Sae3 and Dmc1 (*lane 5*), Mei5-Sae3 and RPA (*lane 6*), RPA and Dmc1 (*lane 7*), and RPA, Dmc1, and 0.5, 0.25, 0.125, 0.0625, or 0.0313 μM Mei5-Sae3 (*lanes 8–12*). *B*, data from three independent runs of the experiment in *panel A* were plotted. *Error bars* indicate S.E. *C*, DNA pulldown assay. Empty magnetic beads were incubated with Dmc1 (0.08 μm) (*lane 1*) or RPA (0.03 μm) (*lane 2*), or magnetic beads coupled to 531-nt ssDNA (1 μM nt) substrate were incubated with Dmc1 alone (*lane 3*); RPA alone (*lane 4*); Mei5-Sae3 (0.5uM) and Dmc1 (*lane 5*); RPA and Mei5-Sae3 (*lane 6*); RPA and Dmc1 (*lane 7*); or RPA, 0.03, 0.06, 0.12, 0.25, or 0.5 μM Mei5-Sae3, and Dmc1 (*lanes 8–11*). Supernatant (*S*) and bead-bound (*B*) fractions are displayed for each lane. The beads were collected and washed, and Dmc1 and RPA were detected by SDS-PAGE and Western blotting. Supernatant and bead loadings represent 12.5% of each fraction. In all reactions, the order of addition was DNA, RPA, Mei5-Sae3, Dmc1. *D*, data from three independent runs of the experiment in *panel B* were plotted. All values for Dmc1 and RPA were renormalized with the amount of protein bound when the protein was incubated alone with DNA being set as 100%; for Dmc1, this reference value was 25.7% bound (6.43% of the quantity required to saturate the ssDNA substrate), and for RPA, it was 87% bound (87% saturating). *Error bars* indicate S.E.

Inhibition of the DNA Binding Activity of Dmc1 by RPA and Restoration by Mei5-Sae3—Previous studies of mediator proteins indicate that they function by promoting the assembly of

recombinase filaments on RPA-coated ssDNA. To determine whether Mei5-Sae3 overcomes RPA inhibition of Dmc1 by a similar mechanism, we measured the DNA binding activity of Dmc1 by attaching the 531-nt ssDNA substrate to magnetic beads. The same order of the addition of proteins was used in this experiment as in the D-loop assay above. The amount of protein bound to DNA was determined by Western blot. We found that, in the absence of Mei5-Sae3 and RPA, roughly 26% of Dmc1 was bound to the DNA (Fig. 2*C*, *lane 3*). As expected, RPA substantially blocked the binding of Dmc1 to DNA (Fig. 2*C*, *lane 7*). The addition of Mei5-Sae3 restored binding of Dmc1 to DNA in the presence of RPA (Fig. 2*C*, *lanes 8–11*). The Mei5-Sae3-dependent increase in binding of Dmc1 to RPA-coated ssDNA was about 3–9.5-fold. Thus, despite some differences in reaction conditions necessary to avoid nonspecific binding of Dmc1 to beads (see "Experimental Procedures"), the maximal effect of Mei5-Sae3 in the DNA binding assay (9.5-fold enhancement) is consistent with the results observed in the D-loop assay (10-fold enhancement). Interestingly, the maximum amount of Dmc1 bound to DNA in the presence of RPA and Mei5-Sae3 was significantly less that than seen in the absence of these two proteins. This is in contrast to the D-loop assay, where maximum levels of activity were observed in the presence of all three recombination proteins. These findings raise the possibility that under the conditions examined, RPA and Mei5-Sae3 promoted a mode of Dmc1-ssDNA binding that was more active in the D-loop reaction than that which occurred in the absence of RPA and Mei5-Sae3. One possible explanation for this is that a smaller fraction of the Dmc1-DNA complexes formed in the absence of accessory factors consisted of active helical filaments as opposed to defective filaments or less organized aggregates. Taken together with the D-loop data (Fig. 2*A*), these findings support the view that Mei5-Sae3 promotes the D-loop activity of Dmc1 by allowing active ssDNA-Dmc1 filaments to assemble on RPA-coated ssDNA substrate.

The mechanism by which Mei5-Sae3 promotes Dmc1 assembly in the presence of RPA remains to be determined. Both Mei5-Sae3 (Fig. 2*C*, *lane 6*) and Dmc1 (Fig. 2*C*, *lane 7*) appear to compete with RPA for binding to ssDNA, with Mei5-Sae3 being a slightly more effective competitor, although RPA remains associated with DNA even when Mei5-Sae3 mediates Dmc1 assembly. The continued presence of RPA is unsurprising in light of evidence from related recombination systems. *E. coli* SSB has been shown to remain associated with ssDNA even after RecA has nucleated onto the DNA(39), a phenomenon thought to reflect a modification of SSB-ssDNA binding or an association of SSB with the RecA filament. In both the *E. coli* and the T4 phage systems, models of modifiable SSB binding have been proposed. In *E. coli*, the relationship between RecA binding and SSB binding appears to be competitive at low concentrations of divalent cation, whereas at higher concentrations, such as those that promote strand exchange, saturating levels of both RecA and SSB were retained on ssDNA (39–41). In T4 phage, the mediator UvsY appears to interact competitively with the SSB gp32 in low salt conditions, whereas in high salt, a co-filament containing both UvsY and gp32 may be observed (42). The retention of SSBs, such as RPA, with other recombination proteins during the process of nucleofilament formation by recombinases may be a physiologically relevant observation.

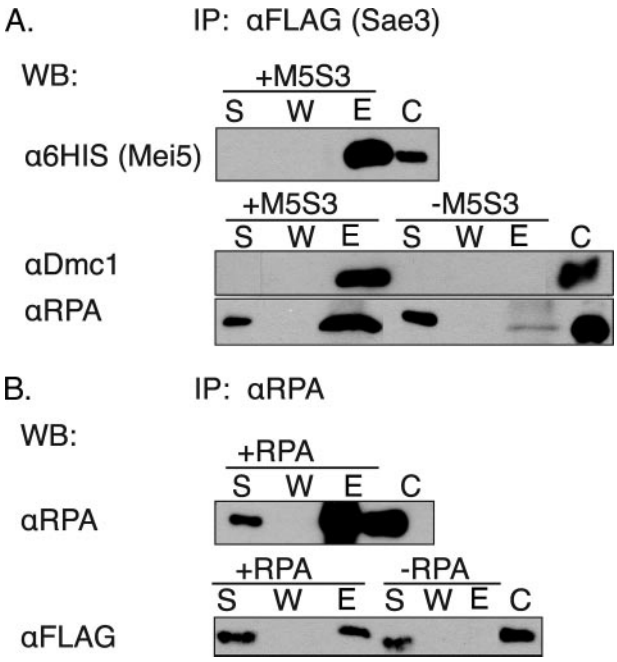

FIGURE 3. **Direct interactions between Mei5-Sae3 and RPA.** Mei5-Sae3, Dmc1, and/or RPA were incubated together at 37 °C. Reaction mixtures were then subjected to pulldown with antibody-coupled magnetic beads. The supernatants were collected, and the beads were washed with buffer and treated with SDS to elute bound protein. Supernatants (*S*), washes (*W*), and SDS-eluates (*E*) were analyzed by SDS-PAGE and Western blot (*WB*), with 50 ng of purified protein run on the gel as a control (*C*). *A*, proteins immunoprecipitated (*IP*) with anti-FLAG monoclonal antibody (against Sae3-FLAG). *Top panel*, Mei5-Sae3 was immunoprecipitated, and the precipitated fraction was probed with anti-His_6 monoclonal antibody to confirm the presence of Mei5. *Middle panel*, Dmc1 was incubated with or without Mei5-Sae3; the reactions were pulled down with anti-FLAG and analyzed by Western blot against Dmc1. *Third panel*, RPA was incubated with or without Mei5-Sae3, pulled down with anti-FLAG, and subsequently analyzed by Western blot against RPA. *B*, protein mixtures were subjected to pulldown with anti-RPA2 polyclonal antibody, resolved by SDS-PAGE, and immunoblotted. *Top panel*, RPA was immunoprecipitated, and the precipitated fractions were analyzed by Western blot against RPA. *Bottom panel*, Mei5-Sae3 was incubated with or without RPA and pulled down with anti-RPA, and precipitated fractions were analyzed by Western blot against Sae3-FLAG (*lower panel*). Supernatant and wash loadings represent 2% of each fraction; bead loadings represent 10%.

Co-immunoprecipitation of Mei5-Sae3 with RPA—To provide insight into the mechanism by which Mei5-Sae3 catalyzes the binding of Dmc1 to RPA-coated ssDNA, we sought to determine whether Mei5-Sae3 and RPA interact directly. We incubated purified recombinant Mei5-Sae3, RPA, and/or Dmc1 in buffer conditions identical to those in the D-loop reaction. Proteins were specifically recovered from these mixtures by incubation with magnetic bead-coupled antibodies. The protein bound beads were washed and eluted with SDS treatment, and the supernatants, washes, and eluates analyzed by SDS-PAGE and Western blot. Mei5-Sae3 was precipitated with anti-FLAG monoclonal antibody (Fig. 3*A*, *top panel*) and RPA with anti-RFA2 polyclonal antibody (Fig. 3*B*, *top panel*). As a control, we confirmed that Dmc1 specifically co-immunoprecipitates with Mei5-Sae3 (22) (Fig. 3*A*, *middle panel*). Under the same conditions, we found that RPA could be co-immunoprecipitated with Mei5-Sae3 using anti-FLAG (Fig. 3*A*, *bottom panel*) and that Mei5-Sae3 co-immunoprecipitated with RPA

(Fig. 3*B*, *bottom panel*). These data indicate that Mei5-Sae3 binds RPA directly.

Our finding that Mei5-Sae3 binds RPA as well as Dmc1 is not the first demonstration that a recombination mediator can bind directly to both a recombinase and an ssDNA-binding protein. In eukaryotic mitotic recombination, as well as in bacterial recombination, physical interactions between mediator proteins and SSBs have been shown to be essential for mediator activity. Rad52 has been shown to bind both Rad51 and RPA directly (43, 44), and it is thought to promote Rad51 filament assembly by binding to RPA-coated ssDNA and providing sites of nucleation for free molecules of Rad51 (15). Furthermore, physical interaction between Rad52 and RPA has been shown to be required for homologous recombination (45, 46). Similarly, interactions between the *E. coli* RecFOR complex and SSB are essential for recombination. RecF (or RecFR) binds to ssDNA-dsDNA junctions and recruits RecOR (or RecO); the RecFOR complex then either displaces SSB or alters the conformation of SSB-DNA interactions, permitting the nucleation of RecA (47). As in yeast mitotic repair, a mediator protein directly interacts with an ssDNA-binding protein; in this example, it is RecO (48), which physically interacts with SSB, and mutations to a putative RecO-interaction site on SSB disrupt the mediator activity of RecOR (49). In T4 phage, the mediator protein UvsY and the ssDNA-binding protein gp32 have been shown to physically interact (50), although these interactions were found to be dispensable for mediator activity *in vitro*. This finding suggested that UvsY might be able to promote assembly of UvsX by displacing gp32 via alteration of the conformation of ssDNA (51). The mechanistic role of the physical interactions we observe between Mei5-Sae3 and RPA has yet to be determined.

The data presented here support the idea that Mei5-Sae3 is a mediator of Dmc1 assembly. In this respect, it is similar to its *S. pombe* homologue Swi5-Sfr1, although the Mei5-Sae3 and Swi5-Sfr1 complexes display strikingly different mitotic phenotypes, suggesting that their roles may diverge in some way. In this study, we have also demonstrated an interaction between Mei5-Sae3 and RPA. It remains to be seen whether this interaction is also present between Swi5-Sfr1 and RPA.

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