Dual regulation of Dmc1-driven DNA strand exchange by Swi5–Sfr1 activation and Rad22 inhibition

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Both ubiquitously expressed Rad51 and meiosis-specific Dmc1 are required for crossover production during meiotic recombination. The budding yeast Rad52 and its fission yeast ortholog, Rad22, are "mediators;" i.e., they help load Rad51 onto ssDNA coated with replication protein A (RPA). Here we show that the Swi5–Sfr1 complex from fission yeast is both a mediator that loads Dmc1 onto ssDNA and a direct "activator" of DNA strand exchange by Dmc1. In stark contrast, Rad22 inhibits Dmc1 action by competing for its binding to RPA-coated ssDNA. Thus, Rad22 plays dual roles in regulating meiotic recombination: activating Rad51 and inhibiting Dmc1.

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Homologous recombination (HR) not only produces genetic diversity but also plays a central role in HR-dependent DNA repair, leading to preserving genomic integrity. A central reaction in HR is the DNA strand exchange between homologous DNAs, which is promoted by evolutionarily conserved RecA family strand exchange proteins or simply so-called recombinases. In eukaryotes, there are two known classes of recombinases: Rad51 and Dmc1 (Masson and West 2001; Neale and Keeney 2006). Ubiquitously expressed Rad51 functions in both mitotic and meiotic HR and HR-mediated DNA repair, and meiotic-specifically expressed Dmc1 is important for meiotic recombination, especially for the production of crossover recombinants (San Filippo et al. 2008; Heyer et al. 2010; Holthausen et al. 2010; Krejci et al. 2012).

For DNA strand exchange, the RecA family proteins bind to ssDNA to form a right-handed nucleoprotein filament, which is also known as a presynaptic filament. Accessory proteins are known to regulate recombinase activity; they mostly stimulate the DNA strand exchange reaction promoted by recombinases (Heyer et al. 2010; Krejci et al. 2012). One of these is replication protein A (RPA), which plays both positive and negative roles (Sung et al. 2003; Sung and Klein 2006). In its positive role, RPA removes secondary structures formed on ssDNA that could impede presynaptic formation. In addition, RPA can assist recombinases by preventing reversal of the strand exchange reaction, in which the free ssDNA formed during the initial reaction might serve as a second DNA target for recombinases. In its negative role, the presence of RPA on ssDNA prevents recombinases from their nucleoprotein filament formation. This is a consequence of the higher affinity of RPA for ssDNA than that of recombinases. In living cells, however, RPA accumulates at recombinogenic sites before Rad51 accumulates there (Gasior et al. 1998; Lisby et al. 2004). Therefore, for strand exchange to be initiated, it is critical that RPA be replaced by recombinases. This replacement of RPA requires the involvement of a second group of auxiliary proteins, termed "recombination mediators," which help Rad51 bind to ssDNA already coated with RPA. Representative recombination mediators include Rad52 in the budding yeast *Saccharomyces cerevisiae*; its ortholog, Rad22, in the fission yeast *Schizosaccharomyces pombe*; and Brca2 in vertebrates (Sung and Klein 2006).

The third class of Rad51 auxiliary proteins includes the Rad55–Rad57 complex in *S. cerevisiae* (Sung and Klein 2006; Liu et al. 2011) and the Swi5–Sfr1 complex in *S. pombe*. These complexes are thought to stabilize and/or activate Rad51 filaments to promote the strand exchange reaction (Akamatsu et al. 2003, 2007; Ellermeier et al. 2004; Kurokawa et al. 2008). Notably, the Swi5–Sfr1 complex exhibits very low mediator activity (i.e., this complex does not significantly help Rad51 to bind ssDNA that is already coated with RPA), but additional information on this complex, which is required for full levels of recombination (Akamatsu et al. 2003; Ellermeier et al. 2004), is lacking, although its sharply kinked structure has been determined by X-ray crystallography (Kuwabara et al. 2012). Furthermore, in contrast to our relatively advanced knowledge regarding auxiliary proteins of Rad51 (described above), we currently know very little about the auxiliary proteins of Dmc1.

In this study, we address both of these deficiencies in our knowledge by a biochemical approach. We demonstrate that the Swi5–Sfr1 complex acts as both a mediator and an activator of Dmc1. In contrast, Rad22, although it helps load Rad51, inhibits Dmc1 action by competing for its binding to RPA-coated ssDNA. Thus, Rad22 plays dual roles in regulating meiotic recombination: activating Rad51 and inhibiting Dmc1. Rad22 is important during meiosis in *S. pombe* for intersister HR, which leads to noncrossover recombinants, presumably by promoting DNA double-strand break (DSB) repair (Cromie et al. 2006; Octobre et al. 2008). On the other hand, crossover

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recombinants (i.e., via interhomolog HR) are produced from DSB-cold regions in a Dmc1-dependent manner (Hyppa and Smith 2010). Both Rad51 and Dmc1 possess very similar biochemical properties (Masson and West 2001; Neale and Keeney 2006), but the impact of Rad22 shown in this study together with different polarities of Holliday junction branch migration (Murayama et al. 2008, 2011) are remarkable biochemical differences between Rad51 and Dmc1. Determination of the biochemical properties of these proteins, including those of the two recombinases Rad22 and Swi5–Sfr1, should provide insights into crossover production during meiosis in *S. pombe.*

Results and Discussion

The Swi5–Sfr1 complex robustly stimulates Dmc1-driven DNA strand exchange reaction

We previously reported that the Dmc1-driven three-strand exchange reaction is stimulated by the Swi5–Sfr1 complex at 37°C (Haruta et al. 2006). In the three-strand exchange reaction, a typical assay used to study recombinase activity, homologous DNA molecules of circular ssDNA (cssDNA) and linearized dsDNA (ldsDNA) are used as substrates (Fig. 1A). Paring yields joint molecules (JMs), and nicked circular DNA (NC) and linear ssDNA are produced as final products. Subsequent analyses showed that this reaction was much more efficient at 30°C, which is closer than 37°C to the optimal temperature of *S. pombe* meiosis (Li and Smith 1997; see also below). Next, we set up two types of three-strand exchange reactions at 30°C that differed with respect to the order of addition of the components (Fig. 1A). In the "Dmc1-start" reaction, a cssDNA was first incubated with Dmc1 and the Swi5–Sfr1 complex simultaneously, and subsequently, RPA was added to the mixture. The reaction was initiated by the addition of ldsDNA. In the "RPA-start" reaction, the cssDNA was first incubated with RPA, and subsequently, Dmc1 and the Swi5–Sfr1 complex were simultaneously added to the reaction. When the reactions were run in the absence of Swi5–Sfr1, neither reaction yielded detectable levels of JMs or the final products (Fig. 1B, left, lanes 2,9). The addition of the Swi5–Sfr1 complex dramatically stimulated both reactions (Fig. 1B, left, lanes 3–7,10–14): More than 85% of input ldsDNAs were converted to JMs and final products within 90 min in the Dmc1-start reactions, but only ~50% of input ldsDNAs were converted in the RPA-start reactions. The yields of both reactions were much higher than those at 37°C reported previously (Haruta et al. 2006).

The most effective concentration of Swi5–Sfr1 in the Dmc1-start reaction was only ~10%–20% of that of Dmc1 (5 μM). Higher concentrations of the Swi5–Sfr1 complex slightly reduced the formation of JMs and NCs (Fig. 1B, left gel image and graph), which is consistent with the results of previous studies (Haruta et al. 2006). In contrast, the RPA-start reaction formed JMs and NCs in a Swi5–Sfr1 concentration-dependent manner without inhibition at the highest concentration tested (3.5 μM) (Fig. 1B, right gel image and graph). A time-course experiment (Fig. 1C) confirmed this conclusion.

We noticed that Dmc1 was heat-denatured by a 15-min incubation at 37°C as judged by the loss of its ssDNA-dependent ATPase activity (Supplemental Fig. 1), which was not affected by Swi5–Sfr1. This suggests that inefficient strand exchange at 37°C is due to the heat lability of Dmc1.

The Swi5–Sfr1 complex stimulates Dmc1 loading onto ssDNA in the presence of RPA in an ATP-dependent manner

The effect of the Swi5–Sfr1 complex on loading of Dmc1 onto naked ssDNA was analyzed by performing a pull-down assay with cssDNA immobilized on magnetic beads (ssDNA beads), as shown in Figure 2A. The result demonstrated that ATP binding, but not ATP hydrolysis, was essential for ssDNA binding by Dmc1 because a non-hydrolyzable ATP analog, AMP-PNP, supported Dmc1 binding to ssDNA (Fig. 2B). The Swi5–Sfr1 complex enhanced ATP-dependent Dmc1 binding to naked ssDNA (~30% [Fig. 2B, lane 3] to ~50% [Fig. 2C, lane 3], an ~1.5-fold increase). On the other hand, the presence of saturating levels of RPA (1 μM) significantly decreased the amount of Dmc1 bound to ssDNA in the presence of ATP or AMP-PNP, indicating that RPA has a higher affinity for ssDNA than does Dmc1 (Fig. 2D). Importantly, the Swi5–Sfr1 complex increased the amounts of Dmc1 bound to ssDNA even in the presence of RPA in an ATP-dependent or AMP-PNP-dependent manner, clearly indicating that the Swi5–Sfr1 complex stimulates Dmc1 loading onto ssDNA in the presence of saturating levels of RPA (Fig. 2E).

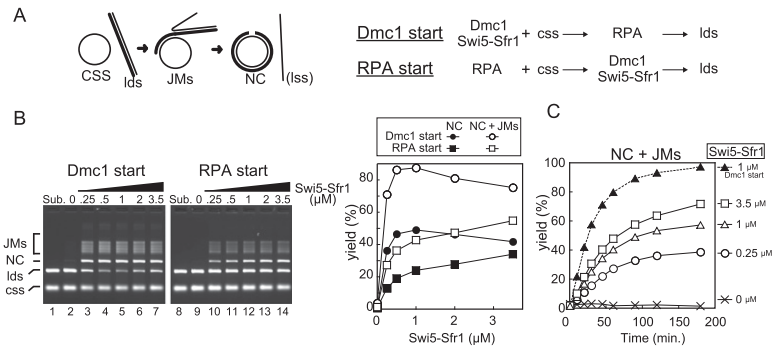

Figure 1. Swi5–Sfr1 stimulates the Dmc1-driven DNA three-strand exchange reaction. (*A*) Schematic of the three-strand exchange reaction. (*B*) Strand exchange in the Dmc1-start or RPA-start reactions with various concentrations of Swi5–Sfr1. The *right* graph shows quantification of results from the gels on the *left*. (*C*) Time-course experiment of Dmc1-driven strand exchange reaction. Large-volume reactions (50 μL) were carried out under standard conditions. Aliquots (6.5 μL) were taken at the indicated time points. Different concentrations of Swi5–Sfr1 were added to the RPA-start reaction (open symbols); closed triangles show the results of the Dmc1-start reaction in the presence of 1 μM Swi5–Sfr1. cssDNA (10 μM), ldsDNA (10 μM), Dmc1(5 μM), and RPA (1.5 μM) were included in each experiment.

The Swi5–Sfr1 complex is a canonical recombinase mediator for Dmc1

We next investigated whether Swi5–Sfr1 mediates Dmc1 loading onto ssDNA already bound by RPA. ssDNA beads were initially incubated with RPA to prepare RPA-coated ssDNA, and unbound RPA was washed out. Next, Dmc1 was mixed with the RPA-coated

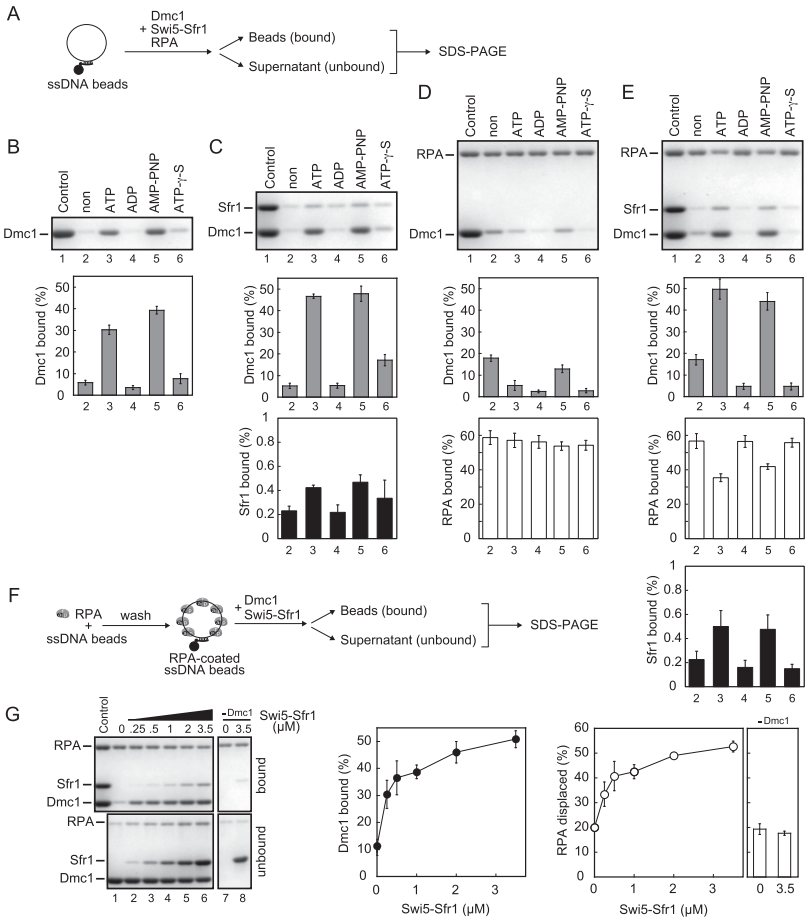

Figure 2. Swi5–Sfr1 facilitates Dmc1 loading onto ssDNA. (*A*) Schematic of ssDNA pull-down assay. Purified protein mixtures (5 μM Dmc1, 1 μM RPA, and 3.5 μM Swi5–Sfr1) were incubated with 10 μM ssDNA beads in the absence or presence of 1 mM various adenine nucleotise di- or triphosphates for 15 min at 30°C. The bead-bound fractions were pulled down using a magnetic stand and analyzed by SDS-PAGE. Proteins stained with Coomassie brilliant blue R-250 were quantified with an image analyzer. (*B*) Dmc1 only. (*C*) Dmc1 and Swi5–Sfr1. (*D*) Dmc1 and RPA. (*E*) Dmc1, Swi5–Sfr1, and RPA. When two or three proteins were incubated, a premix was prepared to add them simultaneously. (*F*) Schematic of ssDNA bead pull-down assay for Dmc1 loading onto RPA-coated ssDNA. RPA-coated ssDNA beads were prepared by washing the incubation mixture, which included 1 μM final concentration RPA and 10 μM ssDNA beads (in terms of total nucleotides) for 20 min at 30°C. Dmc1 (5 μM) and various concentrations of Swi5–Sfr1 were added to the RPA-coated ssDNA beads, and the bead-bound and supernatant (unbound) fractions were analyzed by SDS-PAGE. (*G*) An image of an SDS-PAGE gel of the pull-down assay (*left*) and graphic presentations of bound Dmc1 (*middle*) and displaced RPA (*right*) with values from three independent experiments (mean ± SD).

ssDNA beads in the absence or presence of the Swi5–Sfr1 complex (Fig. 2F). Without the Swi5–Sfr1 complex, Dmc1 was hardly loaded onto RPA-bound ssDNA (Fig. 2G, lane 1), but addition of Swi5–Sfr1 strongly increased, in a concentration-dependent manner, the amount of Dmc1 bound to the ssDNA beads (Fig. 2G; Supplemental Fig. 2). Concomitant with Dmc1 loading onto ssDNA beads, the amount of RPA in the unbound fraction was increased. These results demonstrate that the Swi5-Sfr1 complex mediates Dmc1 loading onto RPA-bound ssDNA and the removal of RPA from ssDNA. The Swi5–Sfr1 complex did not significantly affect the binding of RPA to ssDNA (Fig. 2G, lanes 7,8), suggesting that RPA is displaced by Dmc1, not by the Swi5-Sfr1 complex, but only in the presence of the Swi5–Sfr1 complex. Taken together, these data indicate that Swi5–Sfr1 is a canonical mediator of Dmc1 activity. Ferrari et al. (2009) demonstrated that the *S. cerevisiae* Sae3–Mei5 complex, a counterpart of the Swi5–Sfr1 complex, relieves the inhibition of the DNA-binding ability of Dmc1 by RPA. Notably, the Swi5–Sfr1 complex stimulates Rad51-driven strand exchange but does not act as a mediator of Rad51 loading onto RPA-bound ssDNA, as reported previously (Kurokawa et al. 2008).

We also found that Dmc1 physically interacted with RPA but with weak affinity, as revealed by the results of coimmunoprecipitation assays (Supplemental Figs. 3, 7). The higher interaction between Dmc1 and RPA was observed in the absence of adenine nucleoside di- or triphosphates compared with in the presence of the nucleotides (Supplemental Fig. 3). We suggest that Dmc1 binding to RPA, which is affected by the ATP-binding state and occurs without the help of a Rad52/Rad22-type mediator, is critical for Dmc1 recruitment to RPA-coated ssDNA for the formation of presynaptic filaments.

The Swi5–Sfr1 complex stabilizes the Dmc1 filament

Because the Swi5–Sfr1 complex stabilizes the Rad51 filament (Kurokawa et al. 2008; Kokabu et al. 2011; Kuwabara et al. 2012), we next investigated whether the Swi5–Sfr1 complex similarly stabilizes Dmc1 filaments. To this end, we first mixed Dmc1 (in the absence or presence of the Swi5–Sfr1 complex) with ssDNA beads to form Dmc1 filaments and then added RPA (Fig. 3A). As shown in Figure 3B, in the absence of the Swi5–Sfr1 complex, the amount of Dmc1 bound to ssDNA was dramatically decreased by the addition of 1 μM RPA (cf. lanes 1 and 5). In contrast, more than half of the input Dmc1 remained bound to ssDNA beads in the presence of the Swi5–Sfr1 complex, indicating that the Swi5–Sfr1 complex makes Dmc1 filaments resistant to disruption by RPA (Fig. 3B, lanes 5,8). We obtained essentially identical results using human RPA (hRPA) in place of fission yeast RPA (SpRPA) (Fig. 3B, lanes 9–12). These results strongly suggest that, as with Rad51 filaments, the Swi5–Sfr1 complex stabilizes Dmc1 filaments formed on ssDNA.

The Swi5–Sfr1 complex does not assist Dmc1 loading onto hRPA-coated ssDNA

Although the Swi5–Sfr1 complex mediated Dmc1 loading onto SpRPA-coated ssDNA and made Dmc1 filaments resistant to disruption by hRPA (Fig. 3B, right), the complex did not assist Dmc1 loading onto hRPA-coated ssDNA (Supplemental Fig. 4). Consistent with this, Dmc1-driven strand exchange by hRPA was greatly reduced in "hRPA-start" but not Dmc1-start reactions (Supplemental Fig. 5). An electrophoretic mobility

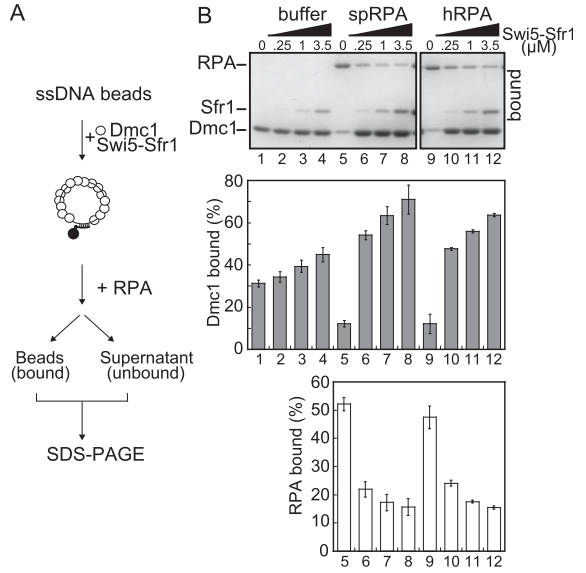

Figure 3. Swi5–Sfr1 stabilizes Dmc1 filaments. (*A*) Dmc1 filaments were prepared on ssDNA beads in the presence or absence of Swi5–Sfr1, and then RPA from *S. pombe* (Sp) or humans was added to the reaction. Bound and unbound proteins were analyzed by SDS-PAGE as in Figure 2. (*B*) SDS-PAGE image of the fractions (*top*) and graphic presentations of bound Dmc1 (*middle*) and displaced RPA (*bottom*) from three independent experiments (mean ± SD). cssDNA (10 μM), ldsDNA (10 μM), Dmc1 (5 μM), and RPA (1.5 μM) were added to each reaction.

shift assay showed that failure of strand exchange with hRPA-coated ssDNA could not be attributed to a difference in the ssDNA-binding affinities of the two RPAs (Supplemental Fig. 6). On the other hand, fission yeast Dmc1 physically interacts with SpRPA but not hRPA, as revealed by an immunoprecipitation assay (Supplemental Fig. 7), suggesting that protein–protein interactions between Dmc1 and RPA are pivotal for Dmc1 loading onto RPA-coated ssDNA.

Rad22 inhibits Dmc1-driven strand exchange

Rad22, a Rad52 ortholog in fission yeast, is a canonical mediator of Rad51 (Kurokawa et al. 2008). To investigate whether it also serves as a mediator for Dmc1, we monitored the effect of Rad22 on Dmc1-driven strand exchange in an RPA-start reaction (Fig. 4A). Rad22 was added to the reaction after RPA addition but before Dmc1/Swi5–Sfr1 addition, the optimal order for observing Dmc1-driven strand exchange. Surprisingly, Rad22 strongly decreased, in a concentration-dependent manner, the amount of strand exchange products formed by Dmc1.

Rad22 inhibits Dmc1 loading onto RPA-coated ssDNA

To understand how Rad22 inhibits Dmc1-driven strand exchange, we performed order of addition experiments (Fig. 4B). In an RPA-start reaction, the amount of strand exchange products was dramatically decreased when Rad22 was added to the reaction before the addition of Dmc1 (i.e., at time point A or B in Fig. 4B). However, the inhibitory effect of Rad22 was alleviated when Rad22 was added after the addition of Dmc1 (i.e., at time point C or D in Fig. 4B). In contrast, in the Dmc1-start reaction, no significant inhibition was observed when Rad22 was added at any time point, except for a slight inhibition at time point A (Fig. 4B). These results indicate that Rad22 inhibition occurs only when ssDNA, RPA, and Rad22 are mixed prior to the addition of Dmc1, suggesting that Dmc1 cannot be loaded onto RPA-coated ssDNA in the presence of Rad22.

Next, we examined directly whether Rad22 inhibits Dmc1 loading onto RPA-coated ssDNA. Dmc1, Swi5–Sfr1, and various amounts of Rad22 were mixed simultaneously with RPA-coated ssDNA beads, and proteins bound to ssDNA beads were analyzed by SDS-PAGE. Under these conditions, Rad22 decreased, in a concentration-dependent manner, the amount Dmc1 bound to the ssDNA beads and increased the amount of RPA that remained bound to the beads (Fig. 4C). Thus, we conclude that Rad22 inhibits Dmc1 loading onto RPA-coated ssDNA.

S. cerevisiae *Rad52 (ScRad52) has no inhibitory effect on Dmc1-driven strand exchange*

We also carried out the same assay using ScRad52 instead of Rad22, as species-specific interactions between Rad52 and RPA have been reported (Sugiyama et al. 1998). In contrast to Rad22, ScRad52 had no inhibitory effect on Dmc1-driven strand exchange or Dmc1 loading onto ssDNA (Supplemental Fig. 8). This finding suggests that physical and functional interactions between RPA and Rad22 are critical for the inhibition of Dmc1-driven strand exchange.

The Rad22–RPA interaction is primarily responsible for inhibiting Dmc1-driven stand exchange

We generated Rad22 mutant proteins defective in their interactions with RPA. Several proteins interact with RPA via acidic amino acid clusters (Ball et al. 2007); two such clusters located in the middle region of Rad22 (D240–E241 and E250–D251) are conserved among *Schizosaccharomyces* species (Fig. 4D). To determine which cluster mediated RPA interaction with Rad22, each cluster was mutated separately and used in GST pull-down assays with RPA. An internal region of Rad22 (amino acids 181–310) interacted with RPA, indicating that this region contained the RPA interaction domain (Fig. 4D), consistent with previous results (Seong et al. 2008). The E250A–D251A double mutant of this region interacted with RPA, but the D240A–E241A mutant did not detectably do so (Fig. 4D), indicating that D240 and E241 are important for Rad22 interaction with RPA.

We next purified and analyzed the properties of the $Rad22^{D240A–E241A}$ protein. Mutant $Rad22^{D240A–E241A}$ exhibited kinetics similar to that of wild-type Rad22 with respect to DNA binding (Supplemental Fig. 9). As expected, $Rad22^{D240A–E241A}$ did not stimulate the Rad51-driven strand exchange reaction in the RPA-start reaction (Supplemental Fig. 10), consistent with the idea that the physical interaction between Rad22 and RPA is necessary for Rad22 stimulation of Rad51-driven strand exchange (Plate et al. 2008). However, $Rad22^{D240A–E241A}$ did not inhibit either the Dmc1-driven strand exchange reaction (Fig. 4A; Supplemental Fig. 11) or Dmc1 loading onto RPA-coated ssDNA (Fig. 4C). These results clearly support the idea that the Rad22–RPA interaction is primarily responsible for inhibiting Dmc1 binding to RPA-bound ssDNA, which is weak and dependent on

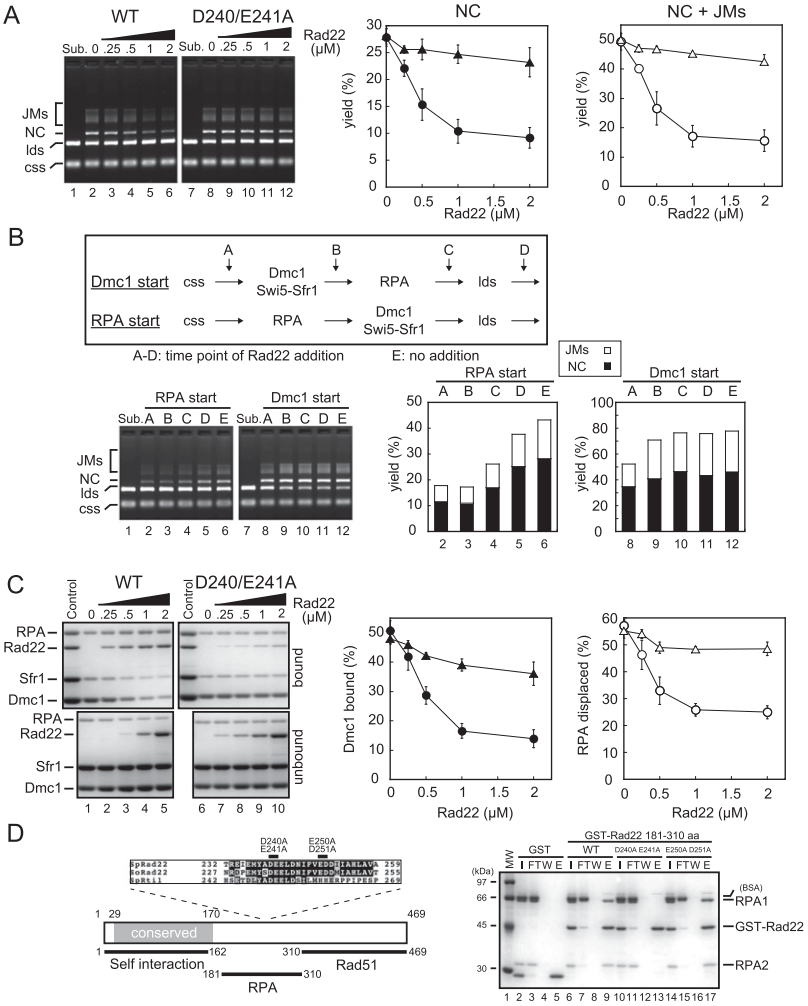

Figure 4. Effects of wild-type Rad22 (SpRad52) on Dmc1-driven strand exchange reaction. (*A*) Wild-type Rad22 protein (circles in the *right* panels), but not a Rad22 mutant defective in RPA binding (Rad22$^{D240A–E241A}$; triangles in the *right* panels), inhibits the RPA-start strand exchange reaction. Scheme is as in Figure 1, but Rad22 was added before Dmc1 and Swi5–Sfr1 (at time point B in *B*). (*B*) An order of addition experiment revealed that the reaction was inhibited by Rad22 before the addition of Dmc1 only under the RPA-start condition. (*C*) Wild-type, but not mutant, Rad22 protein inhibits Dmc1 loading onto RPA-coated ssDNA. Scheme is as in Figure 2A, but Rad22 was added simultaneously with Dmc1 and Swi5–Sfr1. (*D*) Rad22$^{D250–E251A}$ but not Rad22$^{D240A–E241A}$ binds to RPA, as judged by the GST pull-down assay. (I) Input; (FT) flow-through; (W) wash; (E) eluate fractions. Rad22 (1 μM) was added to the reaction 5 min after the addition of each reaction component at the indicated time points in *A–D*.

the ATP-unbound state of Dmc1 (Supplemental Figs. 3, 7). In other words, competition between Rad22 and Dmc1 for binding to RPA-bound ssDNA is the primary mechanism of inhibition by Rad22. Note that the Dmc1–Rad22 interaction was not detected under the same coimmunoprecipitation conditions as used in Supplemental Figure 3 (data not shown), suggesting that the physical interactions between Rad22 and Dmc1, if any, are very weak, although Rad51 shows a strong interaction with Rad22 (Kurokawa et al. 2008).

Conclusions and perspectives

The results presented in this study identify two separable functions for Swi5–Sfr1 in the Dmc1-driven strand exchange reaction. One function is that of canonical "mediator" of Dmc1. This is striking because Swi5–Sfr1 only weakly promotes Rad51 loading onto RPA-coated ssDNA and thus has very low mediator activity with respect to Rad51 (Kurokawa et al. 2008). The other function is that of stabilizer and activator of Dmc1, which are very similar to the functions it plays in Rad51-driven strand exchange. In addition, our data indicate that Rad22 plays a negative regulatory role in Dmc1-driven strand exchange in contrast to its well-known positive role in Rad51-driven HR. Octobre et al. (2008) have shown that Rad22 promotes intersister HR during meiosis in *S. pombe* and is not required for the production of interhomolog crossover recombinants. On the other hand, interhomolog recombinants are generated from DSB-cold regions in a Dmc1-dependent manner (Hyppa and Smith 2010). Furthermore, control of crossover production, termed crossover invariance, is affected by partner choice for DSB repair, which occurs predominantly via intersister HR repair independent of Dmc1 at DSB hot spots and via interhomolog HR dependent on Dmc1 in DSB-cold regions (Hyppa and Smith 2010). In DSB-cold regions, Rad22 may not be recruited, or the inhibitory activity of Rad22 may be abrogated to facilitate crossover formation by Dmc1. As Rad22 is SUMOylated (Ho et al. 2001), we speculate that the inhibitory function of Rad22 is alleviated by its SUMOylation. The balance between Swi5–Sfr1-mediated positive effects and regulation of Rad22 actions may determine the DSB repair modes, which include choice of sister chromatids or homologs as repair templates, leading to noncrossover or crossover production during meiosis in *S. pombe*.

Lao et al. (2008) showed that assembly of Rad51 foci in *S. cerevisiae* is strictly Rad52-dependent, whereas the assembly of Dmc1 foci is decreased only twofold by the deletion of Rad52. They also showed significant levels of crossover production in *rad52*Δ cells, whereas crossover production was almost abolished in *DMC1*Δ cells. Although Dmc1 foci assemble with normal timing in *rad52*Δ cells, their disassembly is severely delayed in *rad52*Δ cells (Lao et al. 2008). These observations are in accord with our results showing that Rad22 inhibits Dmc1 loading onto ssDNA. In addition, Lao et al. (2008) also reported that the interhomolog bias for DSB repair in wild-type cells is changed to intersister bias in *rad52Δ* cells. Therefore, although the roles of Rad52 and Rad22 in the two yeasts are ostensibly opposite (in *S. cerevisiae* for interhomolog and in *S. pombe* for intersister HR), crossing over—involving crossover homeostasis in *S. cerevisiae* (Martini et al. 2006) and crossover invariance in *S. pombe* (Hyppa and Smith 2010)—appears to be controlled by the same underlying mechanism; i.e., whether the DNA repair template is chosen by Rad22/Rad52, Rad51, or Dmc1. Previous results and ours also indicate that the inhibitory

role of Rad22 (Rad52) extends to very distantly related species and may be widespread among eukaryotes.

Both Rad51 and Dmc1 possess very similar biochemical properties (Masson and West 2001; Neale and Keeney 2006), but the impact of Rad22 shown here is a remarkable, newly recognized characteristic. We also showed that the preferential polarities of Holliday junction branch migration driven by Rad51 and Dmc1 are different (Murayama et al. 2008, 2011). These differences provide important clues to understand how Dmc1 is involved in crossover production. Further investigations into the differences between Rad51 and Dmc1 recombinases and into the positive and negative effects of Rad22 on these recombinases will increase our knowledge of crossover production during meiosis.

Materials and methods

The three-strand exchange reaction was carried out essentially as described (Murayama et al. 2008). In the Dmc1-start reaction, 10 μM pSKsxAS cssDNA was mixed with 5 μM Dmc1 and Swi5–Sfr1 and incubated for 10 min at 30 °C. RPA (1.5 μM) was added to the mixture, which was then further incubated for 10 min at 30°C. The reaction was initiated by addition of 10 μM EcoRI-linearized pSKsxAS (ldsDNA) and further incubated for 90 min. In the case of the RPA-start reaction, cssDNA was initially incubated with RPA, followed by addition of Dmc1 and Swi5–Sfr1. For details, see the Supplemental Material.

Other information on materials and methods is described in the Supplemental Material.

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