BRCA2 regulates DMC1-mediated recombination through the BRC repeats

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In somatic cells, BRCA2 is needed for RAD51-mediated homologous recombination. The meiosis-specific DNA strand exchange protein, DMC1, promotes the formation of DNA strand invasion products (joint molecules) between homologous molecules in a fashion similar to RAD51. BRCA2 interacts directly with both human RAD51 and DMC1; in the case of RAD51, this interaction results in stimulation of RAD51-promoted DNA strand exchange. However, for DMC1, little is known regarding the basis and functional consequences of its interaction with BRCA2. Here we report that human DMC1 interacts directly with each of the BRC repeats of BRCA2, albeit most tightly with repeats 1–3 and 6–8. However, BRC1–3 bind with higher affinity to RAD51 than to DMC1, whereas BRC6–8 bind with higher affinity to DMC1, providing potential spatial organization to nascent filament formation. With the exception of BRC4, each BRC repeat stimulates joint molecule formation by DMC1. The basis for this stimulation is an enhancement of DMC1–ssDNA complex formation by the stimulatory BRC repeats. Lastly, we demonstrate that full-length BRCA2 protein stimulates DMC1-mediated DNA strand exchange between RPA–ssDNA complexes and duplex DNA, thus identifying BRCA2 as a mediator of DMC1 recombination function. Collectively, our results suggest unique and specialized functions for the BRC motifs of BRCA2 in promoting homologous recombination in meiotic and mitotic cells.

BRCA2 | DMC1 | RAD51 | mediator | meiosis

The breast cancer susceptibility protein 2, BRCA2, regulates RAD51-mediated homologous recombination (HR) (1–3). Both RAD51, a DNA strand exchange protein, and its meiotic counterpart, DMC1 (disrupted meiotic cDNA 1 or DNA meiotic recombinase 1), promote HR through the formation of a nucleoprotein filament on ssDNA (4). This filament finds and invades a homologous template, resulting in a DNA strand invasion product called a joint molecule or a displacement-loop (D-loop). The joint molecule provides a primer template for the new DNA synthesis required to repair the DNA double strand break (DSB).

The first evidence implicating BRCA2 in meiosis came from studies in *Ustilago maydis*, where strains lacking the BRCA2 ortholog, Brh2, resulted in absence of meiotic products (5). Shortly thereafter, mouse BRCA2 was inferred to coordinate the activities of RAD51 and DMC1 (6). The first direct interaction between BRCA2 and DMC1 was observed in plants (7) and later in humans (8). In the plant, *Arabidopsis thaliana*, the interaction between Brca2 and Dmc1 was mapped to the BRC repeats (9), a highly conserved motif comprising a sequence of ∼35 amino acids that is present at least once in all BRCA2-like proteins (10). In humans, BRCA2 contains eight BRC repeats that bind with different affinities to RAD51, and they segregate into two functional classes (11). Within a BRC repeat, two motifs that bind RAD51 have been identified: one comprising the consensus sequence FxxA that mimics the oligomerization interface (12) and contacts the catalytic domain of RAD51; the other binding module comprises the alpha-helical region of the BRC repeat, contains the consensus sequence LFDE, and binds to RAD51 through a different hydrophobic pocket (13).

Importantly, loss of Brca2 in plants causes chromosomal aberrations during meiosis (14). In humans, GST-pull down assays using peptide fragments of BRCA2 mapped a unique DMC1 interacting site to residues 2386–2411 (8). However, in mouse, mutation of a key residue (Phe-2406) within this site, which had been shown to disrupt the interaction of BRCA2 with DMC1 by peptide array analysis, had no effect in meiosis (15), suggesting that another site or sites in BRCA2 provide the functions needed during meiosis in this organism (6). A direct physical interaction was indeed established for purified full-length human BRCA2 and DMC1 (2), but the functional relevance of this interaction was not elaborated.

We have previously shown that the BRC repeats of BRCA2 modulate the DNA binding selectivity of RAD51 to stimulate the assembly on ssDNA by inhibiting its ATP hydrolysis and preventing its association with dsDNA (2, 11, 16); as a result, BRCA2 catalyzes the recombination activity of RAD51 (17).

A comprehensive analysis of aligned sequences of RAD51 orthologs and human RAD51 paralogues suggested that most eukaryotic RAD51 proteins, including DMC1, could interact with the BRC repeats, at least in principle (10). Here we investigate whether and how BRCA2 modulates DMC1-mediated recombination.

Results

The BRC Repeats of BRCA2 Bind DMC1. DMC1 forms a helical structure on ssDNA similar to that of RAD51 (18). A superposition

Significance

The function of BRCA2 (breast cancer susceptibility protein 2) in mitotic homologous recombination is well established; this role for BRCA2 includes promotion of RAD51 recombination function by regulating its assembly on DNA. However, loss of BRCA2 also leads to aberrant meiosis. Even though interaction of BRCA2 and the meiotic counterpart of RAD51, DMC1 (DNA meiotic recombinase 1), was reported, the specific role of BRCA2 in meiotic recombination is unknown. We demonstrate that the BRC repeats interact with DMC1 to stimulate both association with ssDNA and homologous DNA pairing. Furthermore, we demonstrate strong stimulation by BRCA2 of DNA strand exchange promoted by DMC1, even using ssDNA complexed with RPA (replication protein-A). These findings define BRCA2 as a mediator of meiotic recombination.

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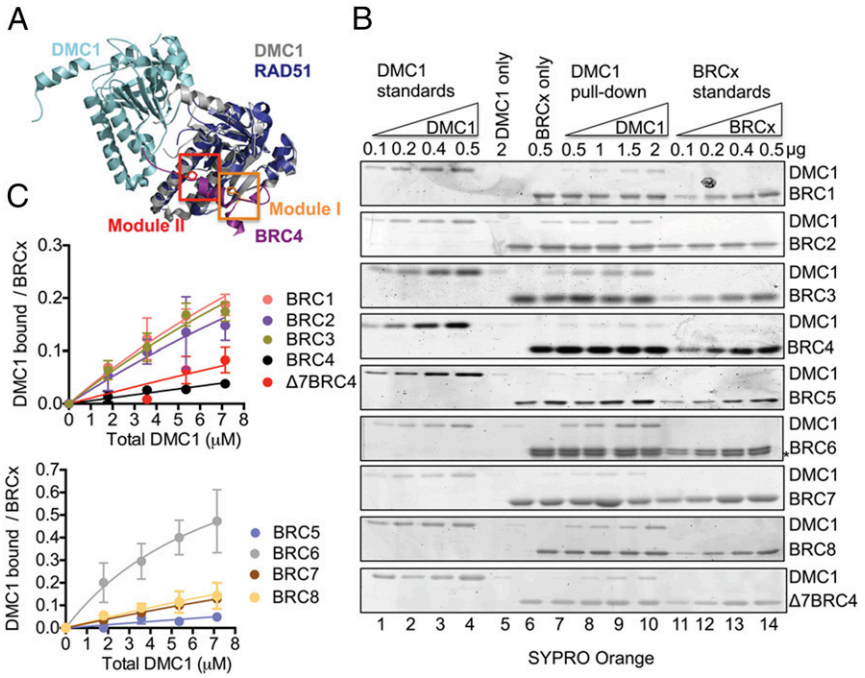

Fig. 1. The BRC repeats bind to DMC1 in vitro. (*A*) Structural conservation of DMC1 and RAD51. Superposition of DMC1 (1V5W) onto RAD51-BRC4 interface (1N0W) showing the structural conservation of DMC1 and RAD51 at the subunit interface which is shown in the complex with the BRC4 repeat (purple). The conserved Phe of the FxxA motif (module I) and Phe of LFDE motif (module II) are highlighted in orange and red, respectively (sticks). (*B*) SDS/PAGE gel showing a pull down experiment with each GST-tagged BRC peptide or BRC4 mutant (Δ7BRC4) (0.4 μM) and increasing concentrations (0.3, 0.6, 1, 1.3 μM) of DMC1. The asterisk denotes contaminating GST. (*C*) The data from *B* was fitted to a single-site binding curve. Error bars represent the SD for three or more independent experiments.

of DMC1 on the RAD51–BRC4 structure reveals that the critical residues involved in RAD51 oligomerization and in BRC-binding are also conserved in DMC1 (Fig. 1*A*).

To test whether the BRC repeats bind DMC1, we used purified His-tagged DMC1 (hereafter referred to as DMC1) and GST-tagged BRC1, -2, -3, -4, -5, -6, -7, and -8. In addition, we examined a BRC4 mutant, Δ7BRC4, which lacks seven conserved amino acid residues (1524-FHTASGK-1530) that are necessary to bind RAD51 (16) (Fig. 1*A*, module I). Each GST-BRC fusion was immobilized on glutathione beads (Fig. 1*B*, lane 6) and was tested individually for interaction with DMC1 (Fig. 1*B*, lanes 7–10). Using known concentrations of purified DMC1 (Fig. 1*B*, lanes 1–4) and each GST–BRC fusion (Fig. 1*B*, lanes 11–14) as a calibration standard, the binding of increasing concentrations of DMC1 to a fixed concentration of BRC peptide was quantified to generate binding curves (Fig. 1*C*). The control lane with DMC1 alone was subtracted as background for the calculations (Fig. 1*B*, lane 5). All of the BRC repeats and Δ7BRC4 showed binding to DMC1 to different extents. The strongest affinity for DMC1 was manifest by BRC6 (apparent K_d = 8 ± 0.8 μM; Table S1), with significant affinities also manifest by BRC1, -2, -3, -7, and -8.

Unexpectedly, DMC1 displayed one of the lowest affinities for BRC4 (apparent K_d estimated to be ~172 ± 14 μM) (Fig. 1*C* and Table S1), whereas our previous studies with RAD51 revealed this interaction to be one of the strongest for RAD51. Moreover, the mutant Δ7BRC4, which is unable to bind RAD51 (16), showed a slightly higher (approximately twofold) affinity for DMC1 relative to BRC4 (Table S1 and Fig. 1*C*). These results suggest that the interaction with DMC1 involves a C-terminal motif of the BRC repeats (Fig. 1*A*, module II) different from the one essential for binding to RAD51 and its oligomerization (Fig. 1*A*, module I).

Most BRC Repeats Stimulate DMC1-Mediated Joint Molecule Formation. To test the relevance of the BRC–DMC1 interaction with respect to the recombination activity of DMC1, we analyzed the effect of each BRC repeat on DMC1-mediated joint molecule formation in vitro. We used a saturating concentration of DMC1 (75 nM) relative to the ssDNA (225 nM nt) (19) and a molar excess of DMC1–ssDNA filaments (ssDNA at 2.5 nM molecule) over plasmid scDNA (0.8 nM molecule). Except for BRC4, the other BRC repeats increased joint molecule formation by DMC1 to some extent, up to as much as ~threefold (Fig. 2 *A* and *B*). The stimulation generally began to plateau at between 1 and 2.5 μM, depending on the BRC repeat (Fig. 2*B* and Fig. S1 *A* and *B*). BRC1, -2, -3, -6, and -8 showed the highest stimulation, whereas BRC4 inhibited the reaction, as did BRC6 at higher concentrations; the inhibition by BRC6 is more evident at higher BRC6 concentrations (Fig. S1 *A* and *B*). Compared with BRC4, the Δ7BRC4 mutant stimulated joint molecule formation approximately threefold, showing that the deleted FHTASGK sequence of module I is responsible for the inhibition (Fig. 2 *A* and *B*).

The Same BRC Repeats That Stimulate DMC1-Mediated Joint Molecule Formation Also Stimulate Association of DMC1 with ssDNA. To obtain mechanistic insight into the stimulatory effect exerted by the BRC repeats in joint molecule formation, we tested whether the repeats could stabilize DMC1–ssDNA complexes, as it is the case for RAD51 (11, 16). An electrophoretic mobility shift assay (EMSA) was used with the same ssDNA oligonucleotide used in

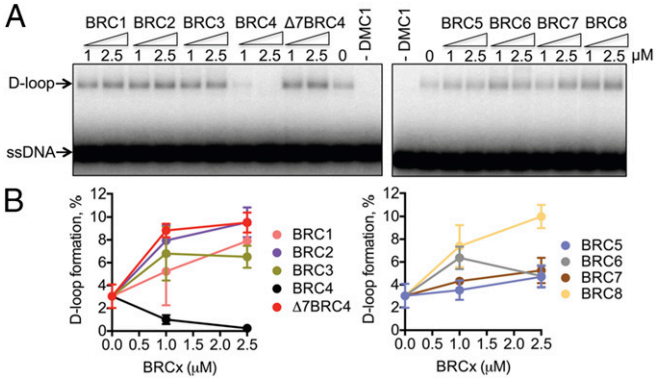

Fig. 2. The BRC repeats enhance joint molecule formation by DMC1. (*A*) DMC1 (75 nM) and the indicated concentrations of each BRC peptide, Δ7BRC4, were preincubated with a 5′-end ^{32}P-labeled 90-mer ssDNA [oAC203, 0.2 μM nt; (2.4 nM, molecule)] for 10 min at 37 °C and the supercoiled DNA (scDNA), (pUC19, 0.8 nM, molecule) was added last to start the reaction. The mix was incubated at 37 °C for 30 min and the products were resolved on a 1% agarose gel. (*B*) Quantification of joint molecule formation from *A*. Error bars, s.d. (n = 3).

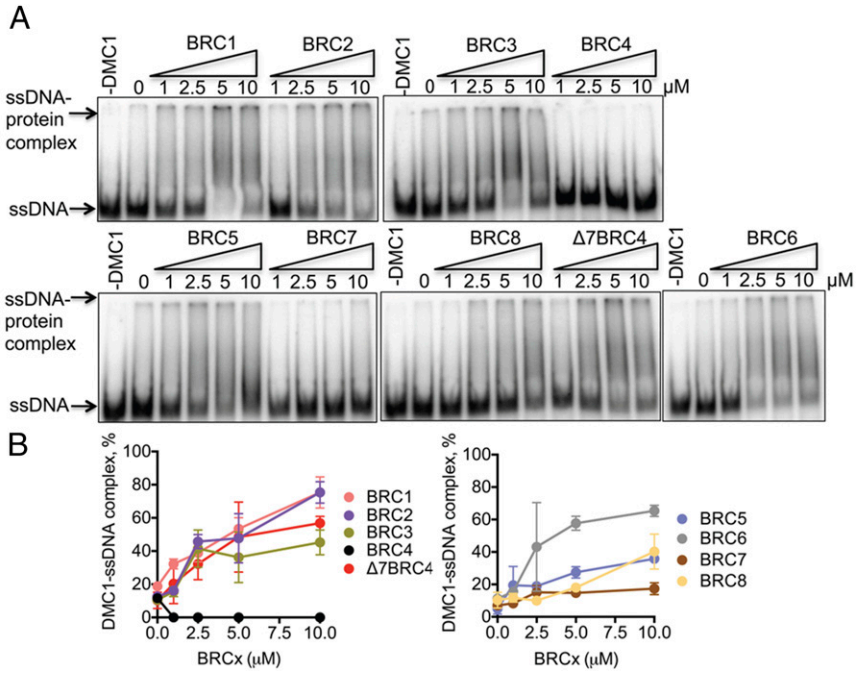

Fig. 3. BRC1, -2, -3, -5, -6, -8, and Δ7BRC4 enhance DMC1 assembly on ssDNA. (*A*) DMC1 (25 nM) was incubated with the individual BRC repeats for 15 min at 37 °C before addition of 5′-end ^{32}P-labeled ssDNA (oAC203, 0.2 μM nt) and further incubation for 1 h. The complexes were analyzed by PAGE and visualized by autoradiography. (*B*) Quantification of the EMSA from *A*. Error bars, s.d. (n = 3).

the D-loop reactions and the same assay conditions to determine whether the BRC repeats could stimulate DMC1 assembly on ssDNA. DMC1 (25 nM) was incubated with each of the individual BRC repeats, followed by addition of ssDNA. We note, as previously reported (11, 16), that none of the BRC repeats alone bind DNA (Fig. S2). We found that BRC1, -2, -3, -5, -6, -8, and Δ7BRC4 stimulated formation of a slower migrating species by up to sixfold, suggesting enhancement or stabilization of the DMC1–ssDNA complex by these repeats. BRC7 showed a marginal effect, but BRC4 clearly inhibited ssDNA binding by DMC1 (Fig. 3), providing an explanation for its inhibition of joint molecule formation seen in Fig. 2. The stimulation of ssDNA binding correlated exactly with stimulation of joint molecule formation, suggesting that enhancement of DMC1–ssDNA complexes formation is at least one basis for joint molecule stimulation. To verify that the BRC repeats also stimulated joint molecule formation at the lower DMC1 concentration used in the EMSA experiments, D-loop assays were conducted at 25 nM DMC1; at this concentration, the yields were low, but consistent behavior was detected (Fig. S1 *C* and *D*).

Most BRC Repeats Do Not Affect DNA-Dependent ATP Hydrolysis by DMC1. We previously showed that the BRC repeats segregate in two groups, based on the mechanism by which they stabilize RAD51–ssDNA complexes: BRC1–4 acts by decreasing the ssDNA-dependent ATPase activity of RAD51, a behavior that is also manifest by full-length BRCA2 (2, 11, 16). Consequently, we tested the effect of the BRC repeats on the ATPase activity of DMC1. None of the BRC repeats possess ATPase activity (Fig. S3). The ssDNA-dependent ATPase activity of DMC1 was examined in the presence of increasing concentrations of the BRC peptides using the conditions of the joint molecule formation assays. BRC4 reduced ssDNA-dependent ATP hydrolysis by DMC1, consistent with its reduction of ssDNA binding by DMC1. BRC6 and Δ7BRC4 stimulated the reaction up to 1.8-fold (Fig. 4 *A* and *B*); however, neither BRC1, -2, -3, -5, -7, nor -8 (Fig. 4 *A* and *B*) detectably altered the ATPase activity of DMC1.

Most of the BRC Repeats Do Not Alter Association of DMC1 with dsDNA. Because BRC1–4 and BRCA2 limit nucleation of RAD51 onto dsDNA, favoring its binding to ssDNA (2, 11, 16), we tested the effect of the BRC repeats on the formation of dsDNA–DMC1 complexes using EMSA as above. In the presence of ATP, Mg^{+2} and Ca^{+2}, ∼75% of a short duplex DNA, dT_{40}•dA_{40}, was bound by 0.6 μM DMC1 (Fig. 5). BRC4 reduced the DMC1-dsDNA complexes by sevenfold, in a concentration dependent manner (Fig. 5 *A* and *B*). Under the same conditions, none of the other BRC peptides or the Δ7BRC4 mutant significantly affected DMC1–dsDNA complex formation. The increased binding of DMC1 to dsDNA (Fig. 5*B*) compared with ssDNA (Fig. 3*B*) is due to the higher protein concentration (600 nM) used in the dsDNA binding experiments relative to the ssDNA binding experiments (25 nM DMC1). To detect a possible stimulation of dsDNA binding by the BRC repeats, we decreased DMC1 concentration to 300 nM, at which only ∼5% of dsDNA was bound by DMC1 (Fig. 5*C* and Fig. S4), whereupon only BRC5 and BRC6 modestly stimulated binding of DMC1 (Fig. 5C).

Purified Full-Length BRCA2 Stimulates DMC1-Mediated Joint Molecule Formation. Next, to determine whether our observed stimulation of DMC1-mediated joint molecule formation by the BRC repeats is

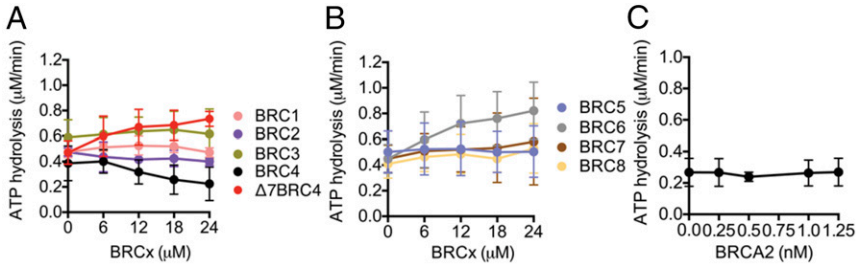

Fig. 4. Only BRC4 and BRC6, but not BRCA2, marginally alter the ssDNA-dependent ATPase activity of DMC1. DMC1 (3 μM) was incubated with increasing concentrations of GST-BRC peptide or BRCA2, as indicated, before addition of 90-mer ssDNA [oAC203, 9 μM (nt)] and was further incubated for 1 h in the presence of 1 mM $MgCl_2$ and 2 mM ATP. (*A* and *B*) Quantification of ATP hydrolysis by DMC1 as a function of BRC peptide. (*C*) Quantification of ATP hydrolysis by DMC1 as a function of BRCA2 concentration. (Error bars, SD; n > 3.)

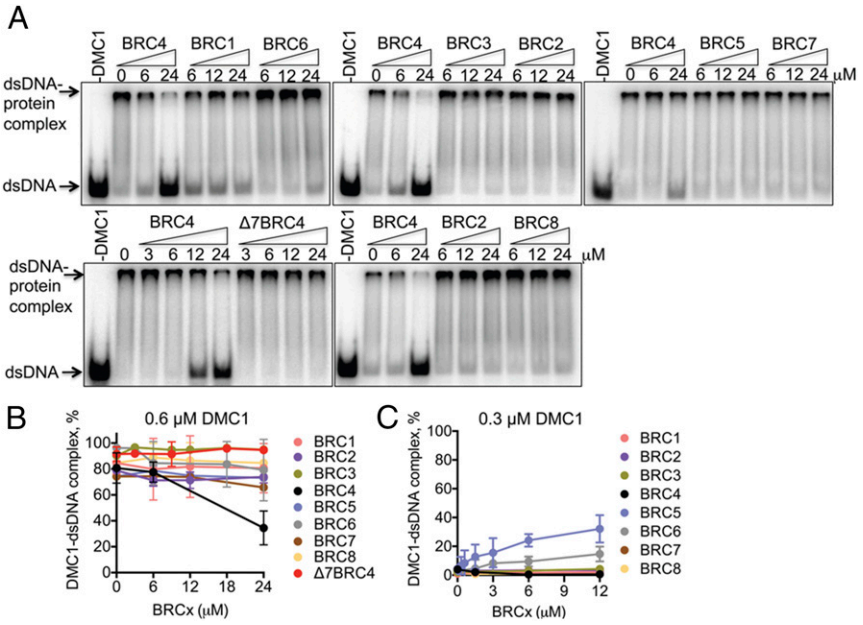

Fig. 5. Only BRC4 reduces DMC1 assembly on dsDNA. (*A*) dsDNA binding assay (EMSA) where DMC1 (0.6 μM) was mixed with each BRC repeat before addition of 5′-end ^{32}P-labeled dsDNA [dT_{40}•dA_{40}, 3 μM (bp)] and further incubated for 1 h. The complexes were analyzed by PAGE and visualized by autoradiography. (*B*) Quantification of *A*. (*C*) Quantification of EMSA as in *A* but with 0.3 μM DMC1. (Error bars, SD; $n > 3$.)

also manifest by full-length BRCA2 protein; we purified a GFP-MBP-tagged BRCA2 (Fig. S5*A*) using a protocol that was modified slightly from the one we described previously for 2xMBP-tagged BRCA2 (12) (hereafter referred to as BRCA2). We tested the effect of BRCA2 on DMC1-mediated joint molecule formation at the same conditions that we had used with the BRC repeats, i.e., a saturating concentration of DMC1 (75 nM) relative to the ssDNA (225 nM nt) and a molar excess of DMC1–ssDNA filaments (ssDNA at 2.5 nM molecule) over plasmid scDNA (0.8 nM molecule). BRCA2 stimulated joint molecule formation by up to 2.8-fold at the maximum attainable concentration of (10 nM), whereas BRCA2 alone did not promote joint molecule formation (Fig. 6). The effect of BRCA2 on ATP hydrolysis by DMC1 was also examined (Fig. 4C); in contrast to what had been observed with RAD51 (2), but in agreement with our observations on the BRC repeats (Fig. 4 *A* and *B*), BRCA2 did not alter the ATPase activity of DMC1.

BRCA2 Stimulates the DNA Strand Exchange by DMC1, Overcoming the Inhibition Posed by RPA. Both in meiosis and mitosis, after resection of a DSB, RPA binds rapidly to the newly generated ssDNA (4). Subsequently, displacement of the RPA by RAD51 is slow, unless catalyzed by a mediator protein (20). This particular ability to counteract the inhibitory effect of RPA to permit formation of a nucleoprotein filament defines a mediator protein, which, in the case of RAD51, is BRCA2 (2, 3). Consequently, we next tested whether BRCA2 could stimulate DNA strand exchange by DMC1, using ssDNA that was saturated with RPA (Fig. 7*A*). For this experiment, a more concentrated preparation of BRCA2 was needed; hence, the previously published 2XMBP-BRCA2 was used (2). RPA was preincubated with the ssDNA before addition of DMC1 or RAD51 and BRCA2, and then complementary dsDNA was added to initiate DNA strand exchange. As expected, RPA inhibited the RAD51-mediated DNA strand exchange (Fig. 7*B*, compare lanes 2 and 3, and Fig. 7*C*); BRCA2 overcame this inhibition and stimulated RAD51 by ∼6.5-fold (Fig. 7*B*, lanes 4–6 and Fig. 7*C*). RPA also inhibited DMC1-dependent DNA strand exchange (Fig. 7*B*, compare lanes 8 and 9, and Fig. 7*C*). BRCA2 overcame this inhibition, stimulating DNA strand exchange in a BRCA2 concentration-dependent manner to a nearly comparable yield of product (Fig. 7*B*, lanes 10–12, and Fig. 7*C*). These results demonstrate that BRCA2 is a mediator protein for DMC1-promoted recombination.

Discussion

RAD51 and DMC1 share similar structure (Fig. 1*A*) and biochemical function (4, 18). Because BRCA2 has been implicated in meiosis in multiple organisms through direct protein-protein interaction (5, 7–9), we set out to investigate the functional relevance of BRCA2–DMC1 binding and whether or not the BRC repeats, interaction sites for RAD51, bind DMC1. Our results show that all BRC repeats bind DMC1 to some extent (Fig. 1), although with affinities that in some cases are 10-fold weaker than for RAD51 (Table S1), in agreement with a previous report (8).

To investigate whether the interaction observed with the BRC repeats had functional significance, we tested both the BRC repeats and full-length BRCA2 on joint molecule formation mediated by DMC1. Remarkably, all BRC peptides except BRC4 stimulated DMC1-mediated joint molecule formation to a different extent, up to threefold (Fig. 2). In agreement with these results, BRCA2 stimulated DMC1-mediated joint molecule formation by up to 2.8-fold (Fig. 6). Interestingly, although the magnitude of joint molecule stimulation achieved by using BRCA2 was similar to that of the BRC repeats, the concentration of BRCA2 required for this

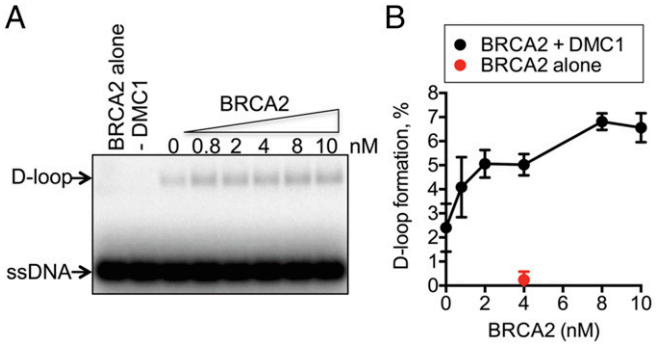

Fig. 6. Purified BRCA2 stimulates DMC1-dependent DNA pairing. (*A*) BRCA2 alone (0.5 nM), DMC1 (75 nM) alone, or DMC1 plus the indicated concentrations of GFP-MBP-BRCA2 were preincubated with the 5′-end ^{32}P-labeled 90-mer ssDNA (oAC203, 0.2 μM nt; 2.4 nM molecule) for 10 min at 37 °C, (left to right, respectively); scDNA (pUC19, 0.8 nM molecule) was added last to start the reaction. The mix was incubated at 37 °C for 30 min and the products resolved on 1% agarose gel. (*B*) Quantification of D-loop formation from *A*. (Error bars, SD; $n = 3$.)

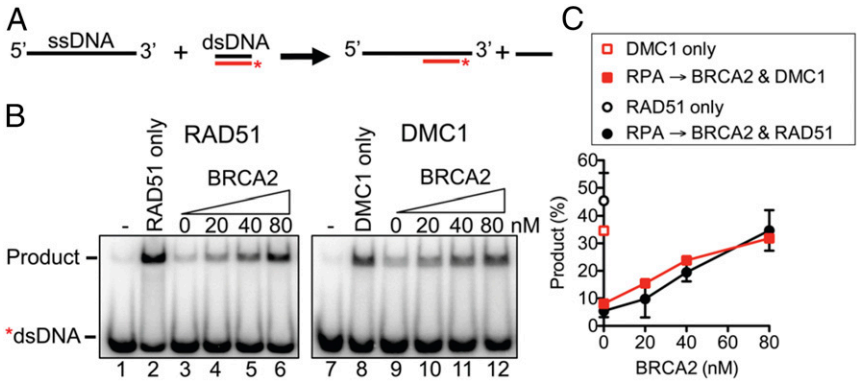

Fig. 7. BRCA2 stimulates DMC1-promoted DNA strand exchange. (*A*) Scheme of the DNA strand exchange reaction. (*B*) Gel showing a DNA strand exchange reaction where RPA (25 nM) was first incubated with an ssDNA substrate (167-mer, 4 nM molecule) for 5 min at 37 °C. Then, RAD51 or DMC1 (0.22 μM) and increasing concentrations of 2xMBP-BRCA2 (20–80 nM) were added and incubated for 5 min at 37 °C. A 5′-end ^{32}P-labeled 40 bp duplex DNA (4 nM molecule) complementary to the ssDNA was added last, and the reaction was further incubated for 30 min at 37 °C. (*C*) Quantification of the DNA strand exchange product formation shown in *B*. (Error bars, range; $n = 2$.)

stimulation was 250-fold lower, suggesting cooperation between the DMC1-interacting sites (BRC repeats) and the ssDNA binding domain of BRCA2; a similar stimulatory differential was seen for RAD51 (2, 11). Most importantly, we also established that BRCA2 stimulated DNA strand exchange by DMC1 involving RPA–ssDNA complexes, qualifying BRCA2 as a bona fide mediator of meiotic recombination (Fig. 7 *B* and *C*).

We previously reported that the BRC repeats of BRCA2 can be categorized in two classes (11): class I (BRC1, -2, -3, -4) stimulates assembly of RAD51 onto ssDNA by limiting its ATPase activity, reduces dsDNA-RAD51 complex formation, and stimulates DNA strand exchange; class II (BRC5, -6, -7, -8) stimulates assembly of RAD51-ssDNA filaments to a greater extent than class I without altering the ATPase activity but does not affect the dsDNA binding or the DNA strand exchange activity of RAD51. In the case of DMC1, we discovered that all of the BRC repeats that stimulated joint molecule formation also stimulated DMC1–ssDNA assembly (Fig. 4). Whereas BRC4, which decreased the yield of joint molecules, inhibited DMC1–ssDNA complex formation, implying that stabilization of DMC1–ssDNA filaments is the mechanism underlying stimulation of joint molecule formation. As with class II BRC repeats in the case of RAD51, here we observe that most of the BRC repeats stimulate the ssDNA binding activity of DMC1 without altering its ATPase activity (Fig. 4) but, in contrast to RAD51, this effect correlates with enhancement of joint molecule formation by DMC1, suggesting a different mechanism of action. For RAD51, BRC1–4 impeded filament formation on dsDNA, but for DMC1, only BRC4 decreased complex formation on dsDNA (Fig. 5*B*). However, disruption of DMC1 binding to dsDNA by BRC4 does not represent a stimulatory component of joint molecule formation, because BRC4 reduces joint molecule formation. Therefore, we propose that the major mechanism by which the BRC repeats, and by inference BRCA2, stimulate DMC1 recombination activity is likely a stabilization of the DMC1–ssDNA complex.

One unexpected result from this study is that BRC6 shows the strongest affinity for DMC1 (unlike for RAD51) and that the Δ7BRC4 mutant, which is unable to bind RAD51 (11), exhibits increased affinity for DMC1 relative to BRC4, and readily stabilizes the DMC1–ssDNA complex and stimulates joint molecule formation. This result indicates that the RAD51 oligomerization interface (Fig. 1*A*, module I), which is mimicked by BRC4 to bind RAD51 (12, 13), is dispensable for interaction with DMC1. Furthermore, it is possible that the conserved C-terminal LFDE motif (Fig. 1*A*, module II) of the BRC repeats could be the main site of interaction for DMC1. If so, it is tempting to speculate that in the context of meiosis, BRCA2 could accommodate binding of both DMC1 and RAD51.

The combined facts that a mutation disrupting a previously described site of interaction with DMC1, which mapped outside of the BRC repeat region of BRCA2, does not have a meiotic phenotype in mice (15) and the results reported here lead us to propose that the BRC repeats are directly involved in regulating the homologous recombination function of DMC1.

Work in *Saccharomyces cerevisiae* and *Arabidopsis* has established that Rad51 is needed for Dmc1 filament formation, and one model posits that Rad51 serves to initiate a Dmc1 filament (4, 21, 22). In our previous work, we showed that BRC1–4 bind with high affinity to free RAD51, enhancing ssDNA assembly and limiting its nucleation on dsDNA, resulting in a stimulation of DNA strand exchange. In contrast, BRC5–8 were shown to not bind free RAD51 but rather to stabilize preexisting filaments on ssDNA without altering the ATPase activity or dsDNA assembly of RAD51 and with no effect on DNA strand exchange (11). Our combined findings establish that free RAD51 binds to BRC1–5 with a 10- to 100-fold higher affinity than DMC1, but that free DMC1 binds to BRC6–8 with an uninhibited affinity because free RAD51 has no measurable affinity for these three BRC repeats. If arranged in a linear fashion in the BRCA2 structure, this arrangement of repeats thereby provides a bipartite sequence for ordering RAD51 and DMC1 monomers within the organization of a nascent filament. Putting together our previous results and this work in the context of DMC1, we therefore propose a model (Fig. 8) in which BRCA2 would bind free RAD51 through BRC1–5, and free DMC1 through BRC6–8 (Fig. 8*A*). Consequently, through its interaction with BRC repeats 1–5 and stabilized by the local inhibition of ATP hydrolysis, a nascent RAD51 homofilament would constitute a nucleus on ssDNA for a

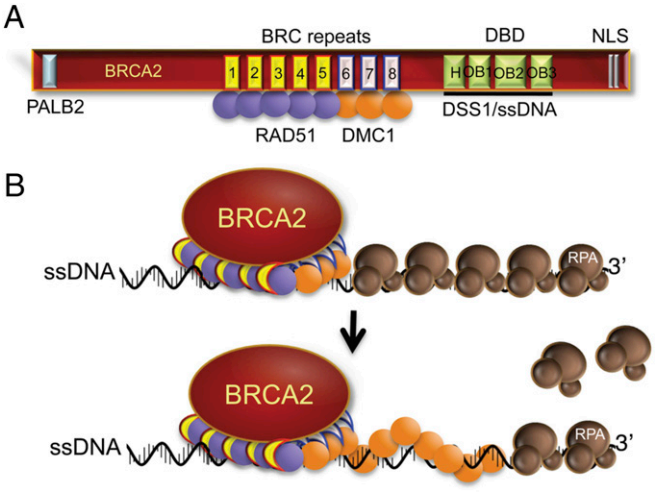

Fig. 8. Proposed model for BRCA2 function in meiotic recombination. (*A*) Schematic of BRCA2 primary structure showing the BRC repeats that would preferentially bind to free RAD51 (BRC1–5) or to free DMC1 (BRC6–8). (*B*) Hypothetical scheme showing BRCA2 binding ssDNA, displacing RPA, and delivering a stable nucleus of RAD51 and DMC1 to the ssDNA. The DMC1 nucleus enables growth of the nascent DMC1 filament away from the BRCA2 heteronucleus and concomitant displacement of RPA.

DMC1 homofilament assembly; up to three monomers of DMC1 would bind to BRCA2 adjacent to the RAD51 filament. BRCA2 would then deliver this nascent heterofilament to ssDNA, displacing RPA in the process of binding and enhancing DMC1 assembly on ssDNA. In principle, the DMC1 filament could grow from the DMC1 side of the heterofilament to form a DMC1 homofilament of sufficient length to promote DNA strand invasion and joint molecule formation (Fig. 8*B*).

Although it remains to be established how the multitude of accessory factors, mediators, and recombination proteins coordinate their activities to promote meiosis, our work identifies BRCA2 as a mediator for DMC1 recombination activity, implicates the BRC motifs in this function, and points to distinct motifs and mechanisms for the BRC repeats in mediating mitotic and meiotic recombination.

Materials and Methods

Protein Expression and Purification. The GST-tagged BRC repeats, the Δ7BRC4 mutant, RAD51, RPA, and 2xMBP-BRCA2 were purified as described (2, 11, 16). The GST-tag was purified as the GST-tagged BRC repeats. Human DMC1 was purified as detailed in *SI Materials and Methods* and manifested the expected biochemical activity (Fig. S6).

Clonogenic Survival Assay. The generation of stable clones with GFP-MBP-BRCA2 construct or the vector containing GFP-MBP tag in *brca2*-deficient hamster cells (VC8) and the clonogenic survival was done as described for 2xMBP-BRCA2 (2).

GST Pull Down Assay. The pull down assays were performed as described for RAD51 (11). Details can be found in *SI Materials and Methods*.

Joint Molecule (D-Loop) Assay. DMC1 protein (75 nM) was mixed with 5′-end ^{32}P-labeled oligonucleotide oAC203 (90-mer oligonucleotide complementary to pUC19), at a saturating concentration of 2.5 nM molecule (225 nM nt) (3:1 ssDNA:DMC1 ratio) or at 25 nM DMC1 and 2.4 nM molecule (200 nM nt) ssDNA (8:1 ssDNA:DMC1) in buffer B [25 mM Tris acetate (pH 7.5), 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mg/mL BSA, 2 mM ATP, and 1 mM DTT], in the absence or presence of each BRC peptide (0.25–2.5 μM) or BRCA2 (0.8–10 nM), and the mixture was incubated for 10 min at 37 °C. Joint molecule formation was initiated by addition of supercoiled pUC19 dsDNA (scDNA) prepared by detergent lysis at 0.8 nM molecules (when using 75 nM DMC1) or 0.26 nM molecules (when using 25 nM DMC1) and further incubated for 30 min at 37 °C. All reactions were terminated by Proteinase K (Roche) treatment, followed by incubation for 10 min at 37 °C. The samples were analyzed by electrophoresis on a 1% agarose gel in 1× TAE run at 120 V for 1.5 h. Joint molecule yield is expressed as a percentage of the total label concentration and multiplied by 3 to get the yield with respect to the limiting scDNA.

DNA Strand Exchange Assay. The reactions and the generation of substrates were carried out essentially as described (2). Details can be found in *SI Materials and Methods*.

ATP Hydrolysis Assay. The experiments were carried out as described (11), except for the DNA substrate used. Details are found in *SI Materials and Methods*.

EMSA. The procedure was essentially as described (11). Details are found in *SI Materials and Methods*.

Analysis. In all graphs, error bars represent the SD derived from at least three independent experiments and, in some cases, error bars are smaller than the symbol; all analyses were conducted by using GraphPad Prism (version Mac 6.0e).

The structure superposition of DMC1 (1V5W) onto RAD51–BRC4 interface (1N0W) in Fig. 1*A* was generated using by PyMol Molecular Graphics version 1.3.

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